Molecular stressors underlying exercise training-induced improvements in K+ regulation during exercise and Na+,K+-ATPase adaptation in human skeletal muscle

Title

Molecular mechanisms underlying improvements in K^+ regulation in human skeletal muscle: A basis for optimising exercise training prescription

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Short title

Molecular basis of improved K^+ handling

Abstract

Despite substantial progress made towards a better understanding of the importance of skeletal muscle K^+ regulation for human physical function and its association with several disease states (e.g. type-II diabetes and hypertension), the molecular basis underpinning adaptations in K^+ regulation to various stimuli, including exercise training, remains inadequately explored in humans. In this review, the molecular mechanisms essential for enhancing skeletal muscle K^+ regulation and its key determinants, including Na^+,K^+-ATPase function and expression, are examined. Special attention is paid to the following molecular stressors and signaling proteins: oxygenation, redox balance, hypoxia, reactive oxygen species, antioxidant function, Na^+, K^+, and Ca^{2+} concentrations, anaerobic ATP turnover, AMPK, lactate, and mRNA expression. On this basis, an update on the effects of different types of exercise training on K^+ regulation in humans is provided, focusing on recent discoveries about the muscle fibre-type-dependent regulation of Na^+,K^+-ATPase-isoform expression. Further, with special emphasis on blood-flow-restricted exercise as an exemplary model to modulate the key molecular mechanisms identified, it is discussed how training prescription may be optimised to maximise improvements in K^+ regulation in humans. The novel insights gained from this review may help us to better understand how exercise training and other strategies, such as pharmacological interventions, may be best designed to enhance K^+ regulation and thus the physical function in humans.

Keywords

Ion transport, molecular mechanisms, Na^+-K^+-ATPase, reactive oxygen species, training adaptation, human skeletal muscle

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Introduction

Many important scientific contributions to the topic of potassium ion (K^+) regulation by skeletal muscle have emerged since the discovery in 1938 that the loss of K^+ from excited myocytes is related to altered electrical activity [1]. Many experiments using animals [2-5], and a substantial number of human studies over the years [6-9], have consistently shown that the ability to maintain K^+ homeostasis in skeletal muscle is essential for physical function. This is underpinned by observations in both animals and humans that the muscle's capacity for K^+ regulation can be improved by exercise training [7,9] and is reduced in various disease states, including diabetes [10-15], heart failure [16], hypertension [17,18], McArdle disease [19], and osteoarthritis [20], as well as with age [21-23], inactivity [24,25], obesity [13], and caloric restriction [26]. Despite this evidence, the molecular mechanisms by which enhancements in skeletal muscle K^+ regulation are mediated have been inadequately addressed in the literature. Identifying these mechanisms is essential, because as the above evidence suggests they could serve as targets for potential disease-preventive interventions, including pharmacological manipulation, but this information may also be of potential ergogenic value to athletes and their coaches.

Over the past three decades, several excellent reviews have been published highlighting the involvement of K^+ regulation in regulating muscle contractile function, intense exercise performance, as well as its coupling to various disease states [2,4,14,16,22,27-38]. However, most of these reviews go >15 years back and thus lack essential novel inputs, specifically about how different types of exercise training (e.g. interval-endurance, sprint-interval and resistance training regimens), may affect K^+ regulation and its key determinants, including Na^+,K^+-ATPase function, content, and isoform expression, in humans. In line with findings in animals [5,39], a number of recent discoveries point to a different regulation of Na^+,K^+-ATPase function and expression by exercise training between different human skeletal muscle fibre types [24,40-44]. However, the potential implications of this fibre type-dependent regulation have been scarcely examined. Further, a number of recent improvements in methodology for muscle protein analysis have been used to investigate changes in Na^+,K^+-ATPase-isoform abundance; experiments that have provided important new insights about the relationship between adaptations in Na^+,K^+-ATPase expression and K^+ regulation in humans that need to be considered.

In this review, I discuss the key molecular mechanisms underpinning improvements in the capacity for K^+ regulation in humans, focusing on skeletal muscle. Then, I provide an update on the effects of different types of exercise training on the skeletal muscle capacity for K^+ regulation and its key determinants, including Na^+,K^+-ATPase function, content, and isoform expression, in humans, with emphasis on fibre-type-dependent adaptations. This is followed by a discussion of how changes in these variables with different types of training may associate with exercise-induced adaptations in

mRNA content. On this basis, I examine how to maximise training-induced adaptations in key determinants of K^+ regulation, focusing on blood-flow-restricted exercise as an exemplary model to manipulate the molecular stressors likely required to enhance this capacity in humans.

The synthesis of literature for this review was based on searches in several databases, including PubMed, Web of Science, SportDiscus®, MEDLINE, and Google Scholar. Key search terms used were K^+ regulation, Na^+,K^+-ATPase, FXYD, phospholemman, training adaptation, skeletal muscle, cell culture, myocytes, reactive oxygen species, ion homeostasis, ionic mechanisms, redox state, antioxidant, metabolic stress, mRNA expression, calcium signaling, AMP-activated protein kinase (AMPK). In addition, manual searches were performed using reference lists from retrieved original studies and review papers.

Sites in skeletal muscle affected by perturbed K^+ homeostasis

At the onset of muscle contraction, rapid and marked perturbations in muscle intracellular and interstitial concentrations of ions (K^+, Na^+, Ca^{2+}, Cl^-, H^+, lac^-) occur, which can both augment and restrict muscle contractile activity [32,45-49]. Strenuous physical exertion raises muscle interstitial K^+ concentration ($[K^+]_{int}$) by up to 2.5 to 3 fold in humans, which has been linked with impaired muscle force development [6,7,9,46,50,51]. This may be caused in part by depolarisation of the cell membrane [30,51-53] and altered sarcoplasmic reticulum (SR) Ca^{2+} kinetics (i.e. decline in rate of SR Ca^{2+} uptake and increased SR Ca^{2+} release) [54], but may also be caused by increased activation of group III/IV afferent nerve fibres by K^+ in the interstitial space, resulting in diminished central motor drive to contracting muscle fibres [55,56] (Fig. 1). K^+ efflux from exercising muscles to the bloodstream may also impair myocardial excitation [57]. On the other hand, K^+ acts as an arteriolar vasodilator e.g. via insulin action [15,58], which may elevate blood perfusion of muscle fibres under certain conditions (*in vitro*) [59]. Increased $[K^+]_{int}$ has also been shown to activate the exercise pressor reflex by stimulating afferent nerve fibres, resulting in an elevated heart rate and rate of ventilation [32,60]. These actions can both aid the delivery of substrate for metabolism and the removal of metabolic by-products from exercising or recovering muscle fibres. Altered cytosolic K^+ concentration has also been shown to contribute to the control of mitochondrial ATP synthesis, acting through K^+-selective channels localized in the outer and inner mitochondrial membranes, although the exact role of K^+ in the control of mitochondrial oxidative phosphorylation remains debatable [61]. Further, the electrogenic movement of K^+ from the intracellular space to extracellular compartments (interstitium and bloodstream) via voltage-gated K^+ channels likely impacts the transmembrane movement of other ions, including Ca^{2+}, H^+, Na^+, and Cl^- [62]. For example, membrane depolarization invoked by elevated $[K^+]_{int}$ inactivates

Na^+-channels, resulting in lowered action potential amplitude [30]. On this basis, proper regulation of K^+ concentrations is essential for maintenance of metabolic homeostasis and contractile function of skeletal muscle and thus physical performance. An overview of the described myocellular sites affected by perturbed K^+ homeostasis and the associated implications for muscle contractile function is provided in Fig. 1.

Role of Na^+,K^+-ATPase isoforms in regulating muscle contractile function and adaptation

Intense exercise elicits marked increases (up to ~12 $mmol \cdot L^{-1}$) in muscle $[K^+]_{int}$ [6,8] and intramyocellular Na^+ concentration [3], both of which have been linked to muscle fatigue, both directly or indirectly through interactions with other ionic processes [33]. These ionic (Na^+ and K^+) perturbations are primarily counteracted by increasing the activity of the Na^+,K^+-ATPase, which actively transports three Na^+ out and two K^+ into the muscle fibres for each ATP molecule hydrolysed [3,63] (Fig. 1). The Na^+,K^+-ATPase is thus critical for maintenance of muscle Na^+ and K^+ homeostasis, excitability, and contractile function [30]. The Na^+,K^+-ATPase consists of a catalytic α subunit, a structural and regulatory β subunit, and an accessory γ subunit, named phospholemman (FXYD), which is coexpressed with the α subunit and is required for basal Na^+,K^+-ATPase function [64-66]. In human skeletal muscle, each of the α and β subunits exists as three different isoforms (α_{1-3} and β_{1-3}) [40], whereas FXYD1 is the only isoform of the γ subunit expressed in this tissue [67]. The total capacity for Na^+ and K^+ transport by the Na^+,K^+-ATPase is in part determined by the number of active αβ-heterodimer complexes at the cell surface [68]. But the relative recruitment of different α isoforms may also be influential, because of the distinct ion transport properties of these isoforms [69,70]. Over the past decades, it has been recognized that FXYD also participates in the complex regulation of Na^+,K^+-ATPase function by protecting this system against oxidative damage [71] and by modulating Na^+,K^+-ATPase Na^+ affinity [65,72]. Thus, each of these subunits appears to be functionally relevant and their recruitment important for the net transport of K^+ and Na^+ across the plasma and T-tubular membranes. In addition, the functional Na^+,K^+-ATPase protein complex and its specific isoforms play essential roles in regulating cell volume [73,74] and signaling transduction underlying hypertrophy [75-77], gene transcription, and protein synthesis [78], making the Na^+,K^+-ATPase an interesting molecular target in disease therapy, as well as in the context of performance optimization.

Molecular stressors underlying improvements in the skeletal muscle capacity for K^+ regulation

Exercise demands muscle fibres to contract in a coordinated fashion. This process is mediated by action potential (AP) propagation along sarcolemma and down transverse tubules, where activation of voltage-sensors enables release of Ca^{2+} from the sarcoplasmic reticulum (SR) and resultant initialisation of the excitation-contraction coupling. AP propagation is mediated by Na^+ influx, which causes membrane depolarisation and K^+ efflux. During the repolarisation phase, K^+ is taken up by the fibres, while influx of chloride ions (Cl-) may also participate in this phase [30,79]. Thus, contracting muscle fibres are exposed to constant perturbations in ion homeostasis. In addition to these perturbations, contracting fibres are under a constant redox disequilibrium due to contraction-induced changes in transmural pressure, resulting in episodes of ischaemia (or anoxia) separated by periods with reoxygenation. These oscillations in redox homeostasis create a favourable environment for the production of free radicals [80,81], classified as molecules that contain one or more unpaired electrons [82]. While an imbalance in both ion and redox homeostasis and free radical production have been implicated in the aetiology of muscle fatigue [79,83], involvement of these processes in training adaptation is an emerging area of research in humans. In this section, the role of these processes in muscle adaptation specific to K^+ regulation, including alterations in expression of Na^+,K^+-ATPase isoforms, will be discussed. Most of our current knowledge on this topic stems from experiments *in vitro* using cell cultures and animal tissue. These experiments will thus be the center of the following discussion. However, human studies will be included where possible.

Oxygenation and redox balance

The severity of fluctuations in local oxygen levels in contracting muscle fibres varies with exercise duration and intensity and partly determine the amount of free radicals that are formed in exercising muscles [80,84]. These redox fluctuations may also exacerbate perturbations in ion homeostasis [85]. Both free radicals [86,87] and disturbance of ion (e.g. Ca^{2+} and K^+) homeostasis [88,89] have been associated with upregulation of Na^+,K^+-ATPase-isoform expression *in vitro*. Redox fluctuations could thus be a central determinant of exercise training-induced increases in Na^+,K^+-ATPase abundance and thus K^+ regulation. In rabbit kidney cells, selectively increasing the oxygen level of the cell bathing solution caused an increase in α_1 [90] and β_1 [90-92] mRNA expression independent of ROS. In another experiment using lung tissue of piglets, increases in both global Na^+,K^+-ATPase mRNA and protein content were evident after breathing hyperoxic gas (inspired oxygen fraction = 0.96) [93]. Consistent with the observations *in vitro*, we have shown that exercise training with reduced muscle blood flow, which substantially raise muscle oxygen perfusion (>3 fold) in the recovery from each exercise bout (as assessed *in vivo* by ultrasound Doppler), augmented training-induced increases

in muscle Na^+,K^+-ATPase-isoform abundance (β_1 in type I, α_1 in type II, and FXYD1 in both fibre types) concomitant with a reduced net thigh K^+ release during near-maximal exercise in humans [44]. Together, this evidence suggests that increased oxygen perfusion of contracting muscle fibres may be a key stimulus underlying increases in Na^+,K^+-ATPase expression and the capacity for K^+ regulation in human skeletal muscle. In support, an increased oxygen level facilitates transcription of Na^+,K^+-ATPase isoforms in cell culture [90-92] by activating binding of oxygen-sensing transcription factors (specificity protein 1 and 3; Sp1 and Sp3), to promoter regions on Na^+,K^+-ATPase mRNA transcripts [94,95]. However, it should be noted that upregulation of mRNA may be one amongst several factors that may enhance the potential for an increased net protein turnover of Na^+,K^+-ATPase isoforms in human skeletal muscle, as highlighted later in this review.

Hypoxia

Because oxygen deficiency (hypoxia) in recruited muscle fibres is an inevitable consequence of changes in transmural pressure occurring secondary to muscle contractions, it is also relevant to consider hypoxia as a possible contributory signal to enhancement of K^+ regulation in skeletal muscle. It is well-known that periods with lowered tissue oxygenation can promote ROS production [80,96-98], whereas facilitated ROS formation has been linked to increased Na^+,K^+-ATPase expression [86] and K^+ regulation in humans [99,100]. Further, hypoxia enhances anaerobic ATP turnover during exercise [85], and thereby could facilitate perturbations in ion homeostasis, which may favor Na^+,K^+-ATPase-isoform synthesis [85,88]. Given these observations, It may seem surprising that different types of hypoxic training concepts, including exercising in normobaric, systemic hypoxia [101,102] and living at high and training at low altitude (LHTL) [103-105], either decreased or had no effect on, respectively, muscle Na^+,K^+-ATPase-isoform abundance or plasma K^+ concentration during exercise in humans. We recently assessed in humans the role of muscle hypoxic level *per se* on the molecular signaling events thought to be involved in improving muscle K^+ regulation by exercise [85]. In this study, we compared changes in mRNA and activation of signaling proteins (i.e. AMPK and CaMKII) to exercise sessions performed with reduced muscle blood flow (blood flow restriction; BFR) and in systemic hypoxia (~3250 m altitude). Key observations were that BFR augmented exercise-induced increases in FXYD1 mRNA content, type-I fibre AMPK downstream signaling (increased ACC phosphorylation), and in markers of oxidative stress [85], consistent with an elevated FXYD1 protein abundance and a reduced net thigh K^+ release (i.e. improved K^+ regulation) during exercise following six weeks of blood-flow-restricted training [44]. In contrast, the session in systemic hypoxia did not result in selective changes in levels of Na^+,K^+-ATPase-isoform mRNA transcripts, AMPK or CaMKII downstream signaling, or oxidative stress, despite a similar level of muscle hypoxia (as assessed *in vivo* by near-infrared spectroscopy; NIRS) compared to the session with BFR [85]. Importantly, the

measurement of muscle oxygen level in the latter study likely accounted for a possible compensatory rise in muscle oxygen perfusion that is likely to occur during exercise at the chosen intensity in systemic hypoxia (i.e. arterial hypoxaemia) [106], but not to the same extent by exercising with BFR, where blood flow is mechanically restricted by a cuff. Together, these findings suggest that hypoxia *per se* is not an essential signal for increasing Na^+,K^+-ATPase expression and K^+ regulation in human skeletal muscle. Nevertheless, it should be noted that local spatial and temporal oscillations in oxygen perfusion that are likely to take place in exercising muscles are not considered by measuring muscle deoxygenation by NIRS [107].

Reactive oxygen species

Reactive oxygen species (ROS) is a broad term used to classify oxygen-centered molecules that contain one or more unpaired electrons, but also includes reactive derivates of oxygen such as hydrogenperoxide (H_2O_2). ROS is one of two subcategories of free radicals. The other subcategory is reactive nitrogen species (RNS), which refers to free radicals for which nitrogen is the reactive center [82]. For a thorough inspection of the different types and sources of ROS in skeletal muscle, the reader is referred to the reviews by Powers, Ji, Kavazis, Jackson [82] and Jackson, Pye, Palomero [108]. It is now well-established that chronic increases in ROS levels are involved in the aetiology of many pathological conditions, including type-II diabetes [109] and peripheral artery disease [110,111]. Conversely, transient increases in ROS levels that are rapidly reversible are central to the regulation of normal contractile function [112], as well as signaling transduction underlying training adaptation [113,114]. The latter roles of ROS in relation to K^+ regulation will be the focus of the following discussion.

Exercise elicits marked increases in ROS concentrations in skeletal muscle, which can modulate muscle force development in both a time- and dose-dependent manner [115]. High doses of ROS have been shown to impair myocytic force development by perturbing ion (e.g. Ca^{2+} and K^+) homeostasis [112,116]. Accordingly, antioxidant treatment in humans attenuated exercise-induced increases in arterialised-venous K^+ concentration [99,100], and thigh K^+ release [44], indicating ROS may affect plasma and muscle K^+ homeostasis in exercising humans. Disturbance of K^+ homeostasis due to ROS accumulation is likely mediated via oxidative modifications to, and thereby dysfunction, of K^+ channels and transport systems, including the Na^+,K^+-ATPase [84,112,117-119]. One type of modification is the formation of disulphide bonds between glutathione and reactive cysteine thiols on amino acid structures (S-glutathionylation), i.e. oxidative damage [120,121]. Na^+,K^+-ATPase dysfunction induced by severe ROS formation has been demonstrated in cell culture preparations [122-125], and this has been confirmed by observations of an inverse relationship between the degree of glutathionylation of Na^+,K^+-ATPase subunits and Na^+,K^+-ATPase activity in rat skeletal muscles [120] and findings of increased β-subunit glutathionylation coinciding with fatigue during intense exercise in humans [121].

However, the observation that Na^+,K^+-ATPase is under redox control is not new. The first evidence for such regulation was published in the sixties, where hydrogen peroxide (H_2O_2) treatment markedly depressed Na^+,K^+-ATPase activity in electrical eels [126]. Later, this was confirmed in several other tissues, including the brain [127], kidney [128,129], and myocardium [130,131] by actions of the hypochlorous and hyperchlorite anions, hydroxyl radicals, superoxide, and singlet oxygen. Around the same time, it was shown that Na^+,K^+-ATPase activity is inhibited by tert-butyl hydroperoxide at high, but not at low concentrations [132], and a dose-dependent (inverse U-shaped) effect of ROS on Na^+,K^+-ATPase function was documented in rat cerebellar granule cells a decade later [119]. This relationship is strikingly similar to that reported for muscle contractile function by Lamb, Westerblad [112]. Thus, tight control of ROS levels seems necessary to preserve K^+ homeostasis and contractile performance of skeletal muscle. But equally important, this evidence underscores that the degree and pattern of ROS accumulation in contracting fibres could play a central role in regulating adaptations specific to K^+ regulation in part by affecting ion homeostasis.

In addition to their acute impact on ion (e.g. K^+, Na^+ and Ca^{2+}) handling systems, ROS may be involved in the long-term (chronic) regulation of muscle Na^+,K^+-ATPase expression by exercise training via actions as signalling transducers for mRNA transcription and protein synthesis [86,113,133]. In humans, involvement of ROS in regulating the turnover of mRNAs of importance to ion transport function has, to my knowledge, only been investigated (indirectly) in a single study. In this study, Murphy, Medved, Brown, Cameron-Smith, McKenna [86] found that intravenous infusion with the multiple ROS scavenger, N-acetylcysteine, blunted the rise in Na^+-K^+-ATPase α_2-isoform mRNA during the recovery from 45 min of cycling at 71% VO_{2max}. Further, they observed that pre-incubation of rat EDL muscle with N-acetylcysteine abolished the increase in Na^+-K^+-ATPase α_1, α_2 and α_3 mRNA induced by electrical stimulations *in vitro*. Although the impact of N-acetylcysteine on muscle antioxidant status was not determined in that study, it demonstrates that ROS accumulation may be a critical determinant of exercise-induced increases in mRNA content of catalytic Na^+-K^+-ATPase isoforms in mammalian muscles. In agreement, long-term treatment of kidney cells with H_2O_2 resulted in upregulation of Na^+-K^+-ATPase expression and activity, whereas adding a bolus of the antioxidant apocynin abolished these effects *in vitro* [87]. In extension of these results, blood flow restriction has been shown to augment increases in oxidative damage to a single exercise session [85], as well as facilitate training-induced improvements in Na^+-K^+-ATPase-isoform protein abundance and K^+ regulation in human skeletal muscle [44]. Adding to this point, rapid and marked perturbations in redox homeostasis effectively increases ROS levels [134-139], whereas a single bout of post-exercise cold-water immersion, which is likely to temporarily perturb muscle redox state, caused a selective increase in Na^+-K^+-ATPase α_2 isoform mRNA expression in skeletal muscle of recreationally-active men [43].

Thus, increased muscle ROS production and resultant oxidative stress during training sessions may be an important stimulus for training-induced enhancements in skeletal muscle Na^+,K^+-ATPase-isoform content and its capacity for K^+ regulation in humans. This conclusion is underlined by observations that oxidative damage to ion channels and transport systems, such as the Na^+,K^+-ATPase, exacerbates disturbances in ion homeostasis [71,117,125], which has been coupled to elevated expression of Na^+,K^+-ATPase isoforms [88,89]. Accordingly, in humans, greater exercise-induced changes in venous plasma K^+ concentration were associated with a more pronounced mRNA and signaling response underlying adaptations specific to K^+ regulation [85].

Antioxidant function

To counteract oxidative damage during periods of elevated ROS exposure, muscle fibres contain a network of strategically-located ROS scavenging systems, including enzymatic and non-enzymatic antioxidants, defined as substances that either delay or hinder oxidation of a substrate [82]. The primary antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). These enzymes catalyse the degradation of highly-reactive ROS into less-reactive molecules. Non-enzymatic antioxidants also provide a significant reservoir for ROS scavenging and this term covers substances such as glutathione, uric acid, bilirubin, biliverdin, and carnosine, among others [140-143]. The content and function of these scavengers are typically altered by exercise training. In humans, for example, exercise training regardless of the type being performed (e.g. endurance, resistance, or a combination) has been reported to increase the activity and/or expression of SOD, GPx, glutathione:glutathione disulphide ratio, total antioxidant capacity, and/or decrease oxidative damage in blood, erythrocytes, or skeletal muscles [144-147]. Antioxidant treatment acutely reversed ROS-induced oxidative inhibition of ion channels and transport systems *in vitro* [117], whereas an improved K^+ regulation after a period of blood-flow-restricted training was temporally linked with altered muscle antioxidant capacity in humans [44]. Further, expression of the cytosolic (copper/zinc) isoform of the antioxidant enzyme succinate dehydrogenase (SOD1) increased, concomitant with increases in Na^+,K^+-ATPase α_1, β_1, and FXYD1 abundance, in the vastus lateralis muscle of humans after 15 weeks of swimming [148]. These findings support that antioxidant function could serve as a fine-tuning system in the regulation of K^+ homeostasis in skeletal muscle by controlling levels of ROS and this possible interaction may be altered by exercise training. More research in humans is required to shed more light on this topic. Given ROS have been linked with the aetiology of several diseases, including diabetes [109] and peripheral artery disease [111], an essential question that remains to be answered in this regard is whether an impaired capacity for K^+ regulation associated with such diseases [12,149] may be circumvented by modulating antioxidant function.

In summary of the last two sections, the level of ROS appears to be a critical factor in both the acute and long-term regulation of K^+ transport function and Na^+,K^+-ATPase expression in skeletal muscle. As only few studies have been undertaking using human subjects and these did not directly assess ROS levels, present conclusions concerning a role of ROS in regulating adaptations specific to K^+ regulation in humans are currently restricted to indirect measurements. Further, enhancements in antioxidant function may be a contributory explanation for exercise training-induced enhancement (or fine-tuning) of K^+ regulation in humans. Future human studies are warranted to elucidate the extend to which different sources of ROS and antioxidants may play a role in the adaptive response related to altered K^+ regulation by exercise training (and other interventions) in human skeletal muscle.

Intracellular Na^+

The first evidence to support a role of intracellular Na^+ ($[Na^+]_i$) in mediating increases in the abundance of Na^+-K^+-ATPase subunits stems from studies using cultured rat cardiac and vascular smooth muscle cells published in the early nineties. In these reports, a rise in $[Na^+]_i$ facilitated by culture incubation with aldosterone [150], thyroid hormone [151], ouabain [152] and veratridine [153] induced a 2 to 7-fold increase in the mRNA expression of the Na^+-K^+-ATPase isoforms. A potent role of $[Na^+]_i$ was later confirmed by findings of increased α_1 and β_1 mRNA in rat kidney epithelial cells and astrocytes incubated with ouabain [154,155]. At this time, it therefore seemed plausible that $[Na^+]_i$ could be a key initiator of Na^+-K^+-ATPase gene transcription. But Murphy, Macdonald, McKenna, Clausen [88] later observed that incubation with ouabain (2 h), veratridine (30 min), or monensin, a Na^+ ionophore (30 min), abolished the increase in Na^+-K^+-ATPase α_1, α_3, β_1 and β_3 mRNA in rat EDL muscle after intermittent electrical stimulations *in vitro*. They also observed a decline in α_1 and β_2 mRNA content with ouabain and veratridine, whilst all incubations caused a downregulation of β_3. Thus, a chronic high $[Na^+]_i$ might not be beneficial, and could even be detrimental, to the adaptability of the Na^+-K^+-ATPase genes in mammalian skeletal muscle. In contrast, the intermittent electrical stimulations increased the catalytic isoforms (α_1, α_2 and α_3) [88]. This suggests that an oscillatory nature of $[Na^+]_i$ could be a potent stimulus for elevating Na^+-K^+-ATPase mRNA expression. This is in agreement with a 1.4 to 3.4-fold increase in the α_1-, α_2- and α_3-isoform expression in human muscle in recovery from repeated intense contractions [156,157], which is known to induce drastic fluctuations in $[Na^+]_i$. In addition, Na^+-induced Na^+-K^+-ATPase signal transduction is linked to elevated ROS production [78], which could facilitate Na^+-K^+-ATPase-isoform mRNA transcription[86], highlighting a possible mechanistic link between increased $[Na^+]_i$ and Na^+-K^+-ATPase expression. More research into the possible role of $[Na^+]_I$ in regulating adaptations in Na^+-K^+-ATPase expression and K^+ transport function in human muscle is clearly required.

Extracellular K^+ and membrane depolarisation

In isolated rat EDL muscle, increasing [K^+] (13 mM) of the muscle bathing solution invoked an increase (160%) in Na^+-K^+-ATPase α_1 mRNA content [88]. This suggests that membrane depolarization, as a result of the increased extracellular [K^+], may be a potent stimulus for increasing muscle α_1 mRNA content. In agreement, inhibition of Na^+-K^+-ATPase activity by adding 0.5 to 1.0 mM ouabain, which increases extracellular [K^+] [3], raised α_1 and β_1 mRNA content in astrocytes *in vitro*. Similar effects of depressed Na^+-K^+-ATPase activity have been observed in humans, where increases in α_1 (1.5 fold) and α_2 mRNA (2.5 fold) after fatiguing knee-extensor exercise were inversely correlated with the change in 3-O-MFPase activity from rest to exhaustion ($r = -0.60$ in both cases; $p < 0.05$) [158]. Consistent with this, more pronounced changes in venous plasma K^+ concentration during training sessions caused by blood flow restriction were associated with greater training-induced improvements in skeletal muscle K^+ regulation and exercise performance in humans [44,85]. Taken together, these observations indicate that extracellular K^+ accumulation and resultant membrane depolarisation positively regulates Na^+-K^+-ATPase-isoform expression and K^+ regulation in the musculature, although more studies are necessary to confirm the scarce number of observations in human skeletal muscle.

Cytosolic Ca^{2+}

In mouse muscle, it has been shown that Ca^{2+} is released from SR in an exercise-intensity dependent manner, which appears to be tightly coupled to activation of the Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in intact muscle fibres [159]. Exercise-induced induction of some Na^+-K^+-ATPase mRNA transcripts is positively associated with exercise intensity (e.g. isoform α_1, Fig. 2). Thus, it is possible that fluctuations in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_{cyt}$) and altered activation of CaMKII could play a role in exercise-induced changes in Na^+-K^+-ATPase-isoform mRNA content in skeletal muscle fibres. This was investigated by Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]. Based on rat muscle incubations *in vitro*, they found the Na^+-K^+-ATPase α_1-isoform to be regulated by Ca^{2+} signalling pathways (CaMK and calcineurin). In another *in vitro* rat experiment, a rise in $[Ca^{2+}]_{cyt}$ induced by incubation with the Ca^{2+} ionophore A-23187 elevated the Na^+-K^+-ATPase α_3 mRNA (1.2 fold), but reduced β_1 (0.8 fold). Thus, despite a scarcity of published research, it appears likely that increases in $[Ca^{2+}]_{cyt}$ and resultant signalling transduction through either CaMKII, calcineurin, or both, may be involved in exercise-induced regulation of Na^+-K^+-ATPase-isoform mRNA levels in skeletal muscle. However, exercise-induced modulation of the degree of phosphorylation at Thr^{287}, and thus autonomous activity [161-163], of CaMKII was dissociated from increases in Na^+-K^+-ATPase α_2 and FXYD1 mRNA in human skeletal muscle [85], indicating signaling via CaMKII is not required for exercise-induced increases in the levels of some Na^+,K^+-ATPase

mRNA transcripts in human skeletal muscle. Nevertheless, CaMKII activity has been shown to be upregulated at the onset of exercise, from where it may gradually decrease towards resting level during sustained moderate-intensity exercise [162]. As such, the timing of the muscle biopsy after the end of the exercise session in the latter study may not have been optimal for detecting changes in CaMKII phosphorylation (and activity). Thus, further experiments in humans are warranted to clarify the involvement of altered $[Ca^{2+}]_{cyt}$ and activation of Ca^{2+} signaling proteins in mediating Na^+-K^+-ATPase adaptation.

Anaerobic ATP turnover and the 5'AMP-activated protein kinase (AMPK)

Amongst the few published human studies, there is consensus that exercising with a high, compared to a lower, relative intensity yields a more powerful stimulus for induction of ion transport genes [156,160,164]. This is supported by the positive relationship (r = 0.85) between exercise intensity and fold-increases in muscle Na^+-K^+-ATPase α_1-isoform mRNA in trained humans (Fig. 2). Sustained exercise at a high intensity requires a high anaerobic energy turnover, which results in accumulation of H^+ (i.e. decline in pH) in exercising muscles. In turn, this has been shown to impair the function of K^+ and Ca^{2+} transport systems, including the Na^+-K^+-ATPase and SR Ca^{2+}-ATPase (SERCA) [165,166], thereby exacerbating perturbations in ion homeostasis in the exercising musculature. However, this pH dependency remains to be shown in human skeletal muscle within the physiological range of fluctuations in muscle (or intracellular) pH. Furthermore, a marked increase in anaerobic glycolytic flux raises the availability of NAD(P)H for ROS production and exacerbate fluctuations in redox homeostasis [167]. On this basis, the degree of anaerobic energy turnover appears to be an important determinant of the effects of a given exercise intervention on the expression of, at least some, Na^+-K^+-ATPase isoforms. Accordingly, decreasing the relative exercise intensity by performing the same exercise session after compared to before a period of intense training attenuated the exercise-induced increase (3 fold) in α_1 mRNA observed before training [156]. However, several other factors might be involved in this regulation. For example, exercise has been shown to activate AMPK in an intensity-dependent manner [168], whereas increased activity of AMPK has been linked with transcription of both the Na^+-K^+-ATPase β_1 isoform and FXYD1 *in vitro* [169]. In support of these results, the degree of AMPK downstream signaling (i.e. phosphorylation of the Acetyl-CoA carboxylase, ACC) was positively associated with upregulation of FXYD1 mRNA in human skeletal muscle [85]. This provides an indication of involvement of AMPK in mediating adaptations in expression of FXYD1 in human muscle. However, although phosphorylation of ACC strongly reflects AMPK activity [170], it may be elevated by factors other than AMPK. Thus, at present, it cannot be unequivocally stated that AMPK is involved in regulating FXYD1 expression in human muscle. Further studies are warranted

to determine the relationship between exercise-stimulated changes in muscle AMPK activity and FXYD1 abundance in humans.

Lactate

The concentration of lactate, a surrogate marker of anaerobic ATP consumption, has been shown to regulate the expression of several mRNA transcripts involved in various cellular functions, including PGC-1α, a regulator of mitochondrial content [171]. We recently examined whether lactate accumulation could be a regulator of the acute molecular response underpinning adaptations in K^+ regulation to exercise in humans [85]. This was done by modulating muscle lactate concentration during exercise using blood flow restriction and systemic hypoxia, both types of exercise shown to invoke drastic increases in muscle lactate concentration [172,173]. Despite similar muscle lactate concentration, the exercise sessions differed with respect to alterations in expression of Na^+-K^+-ATPase mRNA transcripts [85], indicating elevated muscle lactate does not play a direct role in regulating exercise-induced adaptations in mRNA expression of Na^+-K^+-ATPase isoforms in human skeletal muscle. This finding is limited to the mRNA level, thus further research is required to clarify if lactate accumulation during training sessions is an important determinant of (long-term) adaptations in the function and content of the Na^+-K^+-ATPase, and ultimately of improvements in muscle K^+ regulation, in humans, although this would seem unlikely based on the existing evidence.

Summary

In Table 1, an overview of the molecular stressors potentially involved in mediating adaptations specific to K^+ regulation in skeletal muscle is provided, along with an indication of the empirical support from animal/cell culture (*in vitro*) and human experiments provided to each of these stressors about their involvement in mediating these adaptations. In summary, improvements in K^+ regulation and increases in expression of Na^+-K^+-ATPase isoforms in human skeletal muscle are likely initiated by transient perturbations in redox, ionic, and metabolic state, whilst increased oxygenation, ROS levels, extracellular [K^+], and anaerobic ATP turnover may be of particular importance to this regulation. In contrast, the level of muscle hypoxia and lactate concentration *per se* do not appear to be essential to these adaptations in humans. At present, the involvement of alterations in $[Na^+]_I$ and $[Ca^{2+}]_{cyt}$ is unclear, although evidence *in vitro* point to a role of transient shifts in the concentrations of these ions in increasing Na^+-K^+-ATPase expression. Little is currently understood about how the identified key molecular stressors may be conveying the signal(s) for adaptation in the capacity for K^+ regulation in human skeletal muscle, although some evidence points to a contributory role of AMPK and oxygen-sensing transcription factors.

An update on the effects of exercise training on K^+ regulation, and Na^+,K^+-ATPase function, content, and isoform abundance, in human skeletal muscle

In the previous section, the key molecular stressors underpinning improvements in the capacity for K^+ regulation in humans were reviewed. It is obvious to speculate that strategies, such as exercise training, that have the potential to substantially promote these stressors could be useful to enhance K^+ regulation and physical function in humans. In the next sections, an update on the effects of different types of exercise training on key determinants of K^+ regulation in humans, including plasma K^+ concentration, thigh K^+ release, and skeletal muscle Na^+,K^+-ATPase activity, content, and isoform abundance, is provided, and findings on the fibre-type-dependent regulation of Na^+,K^+-ATPase-isoform abundance will be discussed. Then, it is examined how changes in these variables with different types of training may relate to exercise-induced adaptations in mRNA expression.

From the cross-sectional data in Table 2 and the visual summary provided in Fig. 3, it is clear that exercise training, regardless of whether it is performed at/below (primarily aerobic; AEH) or above (primarily anaerobic; ANH) the intensity eliciting VO_{2max}, is an effective stimulus to increase both Na^+,K^+-ATPase content (11–15%; as determined by [^{3}H]-ouabain binding) and isoform abundance (5–44%; as quantified using western blotting) in humans. Training with a high aerobic energy component (AEH) has also been shown to increase Na^+,K^+-ATPase maximal *in vitro* activity (4%; as assessed by the 3-O-MFPase method), whereas the effects of ANH training modalities on this variable remain to be examined. The functional relevance of increases in Na^+,K^+-ATPase content, isoform abundance, and/or activity is evidenced by findings of concomitant reductions (5–8 %) in K^+ concentration in the bloodstream or in the muscle interstitial space during exercise, and simultaneous improvements (14–16%) in physical performance in many studies (Table 2). These adaptations may occur rapidly, with decreases in venous blood [K^+] (5%) and increases in Na^+,K^+-ATPase activity (41%), content (9–14 %), and isoform abundance (27–113%, α_1, α_2 and β_1), reported after only six to ten days of training [174-176]. However, changes in functional variables, such as thigh K^+ release, venous [K^+], and Na^+,K^+-ATPase activity, with both one type or different types of training appear to be dissociated from those of Na^+,K^+-ATPase content or isoform abundance measured in whole-muscle (fibre-type-heterogeneous) samples in a substantial number of studies [7,157,177-179]. For example, three consecutive days of continuous training (2 hour cycling at 60% VO_{2max}) increased (12%) [^{3}H]-ouabain binding site content, but decreased (34%) 3-O-MFPase activity in recreationally-active subjects [177]. In similarly trained men, muscle abundance of Na^+,K^+-ATPase α_1 (29%) and α_2 (15%) increased and $[K^+]_{int}$ decreased (-27%), whereas K^+ release from exercising muscles remained unaltered after seven weeks of intense interval training (15 x 1 min at 150% of leg VO_{2max})

[7]. Further, six to eleven weeks of anaerobic training resulted in an increased β_1 abundance (39 to 58%), despite a higher (14%) venous blood [K^+] during exercise in highly-trained runners [179].

Thus, amongst the published human studies to date, there is a consensus that exercise training, regardless of whether it is performed below/at or above the intensity eliciting VO_{2max}, is a powerful stimulus to enhance K^+ regulation, as well as Na^+,K^+-ATPase function, content, and isoform abundance. Further, increases in these variables are often temporally associated with improvements in one or more aspects of physical performance following a period of intense training. However, training-induced alterations in blood [K^+] or Na^+,K^+-ATPase activity were dissociated from those of Na^+,K^+-ATPase (isoform) expression in a substantial number of studies. Based on the above evidence, it remains unclear what is the optimal training stimulus for improving skeletal muscle K^+ regulation in humans.

Fibre-type-dependent regulation of muscle Na^+,K^+-ATPase-isoform abundance by different types of exercise training in humans

Human skeletal muscle is a heterogeneous tissue consisting of fibres with distinct metabolic and ionic properties. Due to these differences, fibres can be defined according to their content of proteins with different functions. For example, fibres may be characterised by their content of myosin heavy chain (MHC) isoforms as type I, IIa, IIx, or hybrid if containing multiple MHC isoforms (e.g. I/IIa or IIa/IIx). In comparison to type II (fast-twitch) fibres, type I (slow-twitch) fibres have a slower rate of force development and SR Ca^{2+} release and uptake kinetics, altered glycogen utilisation, possess more mitochondria, and are more fatigue resistant [180-185]. In animals, differences in the capacity for Na^+/K^+ handling among different skeletal muscles have also been observed, and this has been associated with a different expression of Na^+,K^+-ATPase isoforms between different fibre types [39,186,187]. In humans, α_2 abundance was found to be higher in type II compared to type I skeletal muscle fibres in recreationally-active men [188]. Furthermore, FXYD1 was more highly expressed in type I compared to type II muscle fibres in sedentary rats [65,187], and in humans its phosphorylation state was rapidly increased in type II but not in type I muscle fibres after a single session of intense exercise [188]. Collectively, these studies suggest that expression of Na^+,K^+-ATPase isoforms and FXYD1 activation (by phosphorylation) may be altered by exercise in a fibre-type-dependent manner, which may significantly impact K^+ regulation in the musculature. Nevertheless, in most human training studies, Na^+,K^+-ATPase-isoform abundance was quantified in fibre-type heterogeneous (whole-muscle) samples (Table 2), indicating that important changes in isoform levels could have been overlooked. Another concern is that protein abundance was quantified using fractionated samples in many previous studies [7,176,189-192]. This is an issue, because a proportion of the protein being analysed may be inadvertently lost by fractionation [193]. In addition, some of the studies did not

take into consideration blot linearity, making it impossible to know if protein bands were saturated and thus should be excluded from analysis [194]. Moreover, few studies validated their antibodies, for example by loading positive and/or negative control tissues. Limitations in methodology for protein quantification could thus be, at least, a contributory explanation for the dissociation between changes in expression of Na^+,K^+-ATPase isoforms in whole muscle samples and those of the capacity for K^+ regulation observed in the literature (Table 2), as highlighted in the previous section. With the use of improved methodology (i.e. no fractionation, normalization to a standard curve, and for some antibody validation), a number of recent human studies have investigated the effects of different types of exercise training on the expression of Na^+,K^+-ATPase isoforms in type I and II skeletal muscle fibres. The results from these studies are summarised in Table 3 and will be discussed in the following.

α-isoform abundance

In one of our recent studies, six weeks of sprint-interval training increased (210 to 330%) α_1 abundance in both type I and II fibres in the skeletal muscle of recreationally-active men [43]. In agreement, four weeks of sprint-interval training resulted in an elevated (29%) α_1 abundance in whole-muscle samples from similarly trained subjects [189]. In contrast, in two other studies, interval-endurance training, characterized by a high rate of aerobic energy consumption, was without effect on α_1 abundance in type I and II fibres [41,44]. However, performing the same type of training with reduced muscle blood flow, thereby increasing rate of anaerobic ATP production, caused a ~50% higher abundance of α_1 in both fibre types [44]. Furthermore, resistance training, characterised by repeated, near-maximal efforts interspersed by several minutes of rest, was found to increase (79%) α_1 abundance in type II fibres [24] and in whole-muscle samples [195] in sedentary individuals. Collectively, these results support that training above the intensity eliciting VO_{2max}, and thus the degree of metabolic and ionic stress in exercising muscles [85], is important for training-induced increases in α_1-isoform abundance in both human muscle fibre types. This is consistent with the observation made in the first part of this review that drastic perturbations in both metabolic and ionic homeostasis are likely essential signals underlying elevated Na^+,K^+-ATPase expression. Moreover, the duration of intense exercise bouts, and thus the time spent with perturbed ion homeostasis, could also be decisive. For example, 4 to 8 weeks of sprint-interval training with a shorter sprint duration (4 to 6 seconds) compared to the above studies (30 seconds) had no effect on α_1 abundance in either type I or II fibres [40] or in whole-muscle homogenates [178] in recreationally-active humans. Together, this evidence supports that repeated, near-maximal exercise bouts of substantial duration (at least 30 seconds) punctuated by several minutes of rest is an effective training approach to increase α_1 abundance in both muscle fibre types in humans.

Exercise training comprising a high exercise volume has been demonstrated in several studies to be an effective stimulus to increase α_2 abundance in the skeletal muscle of both untrained [7,174,176] and recreationally active humans [177,178]. Accordingly, a large training volume appears to be required to elicit changes in muscle α_2 abundance at the fibre-type level. For example, an increase in α_2 abundance in type II fibres (30%) was observed after 576 minutes of moderate-intensity interval training performed over twelve weeks [41], whereas 324 minutes of similar training over six weeks was insufficient to alter α_2 abundance in type I and II fibres [44]. Further, no significant change in α_2 abundance was detected in both fibre types after a period of sprint-interval training with a low (45 min) exercise volume [43]. In addition, whole-muscle α_2 abundance was elevated after six [195], but not four weeks [24] of resistance training in untrained subjects. However, a training-induced increase (76%) in α_2 abundance was evident in type I fibres in the latter study [24], indicating six weeks of sprint-interval training is sufficient to invoke fibre type-specific changes in α_2 abundance. In contrast, in another study, six weeks of sprint-interval training did not result in significant increases in α_2 abundance in any fibre type. However, in the latter study, a quantitatively higher α_2 abundance was observed in around three quarters of type I and II fibres, indicating a small sample size and great inter-subject variability prevented a statistically significant result (i.e. a statistical type-II error). Moreover, a non-significant increase (30%) in α_2 abundance was evident in type-II fibres after training with reduced muscle blood flow [44]. This is in line with the concept that a greater proportion of type-II fibres must be recruited to sustain power output during continuous exercise when oxygen supply to skeletal muscle is compromised [196]. Together, above findings indicate that training volume and relative exercise intensity are important determinants of the fibre type-dependent regulation of α_2 abundance in human skeletal muscle. In keeping with this, the demand for Na^+/K^+ transport invoked by training on each fibre type could be influential, consistent with the finding *in vitro* that the major role of α_2 in skeletal muscle is to assure that contraction-stimulated increases in demand for Na^+/K^+ transport are met [70].

The abundance of the α_3 isoform remained unchanged with both interval-endurance [44] and sprint-interval training [43] in both muscle fibre types in humans. This is consistent with observations in whole-muscle samples of unchanged α_3 abundance after three weeks of interval cycling with a high aerobic component (8 x 5-min at 85% VO_{2max}) in well-trained men [157]. Although only semi-quantitative, western blots for α_3 indicate that this isoform may be lowly expressed at the protein level in human skeletal muscle, which is in accordance with the low α_3 mRNA expression detected previously in the same tissue [157]. These observations downplay the functional importance of α_3 for the muscle's contractile function in humans, which could be one explanation for the lack of change in α_3 abundance in both fibre types with the different types of training studied. In contrast to these observations, α_3 abundance increased (31%) in whole-muscle samples after three consecutive days

with 2 hours of cycling at 60% VO_{2max} per day [177], indicating exercise training, in some cases, can increase α_3 abundance in human muscle, although the physiological reason(s) for the latter finding is unclear.

β-isoform abundance

In one human study, four weeks of sprint-interval training increased the abundance of β_1 in type II, but not in type I fibres (identified in individual fibre segments) [40]. In agreement, we have recently found that β_1 abundance was selectively increased (44%) in type II fibres after six weeks of sprint-interval training in humans [43]. The α-isoform abundance was also raised in the same fibre type in the latter study, suggesting that intense training requires a high functional capacity of the Na^+,K^+-ATPase in type II fibres. In support, in rat gastrocnemius muscle, higher Na^+,K^+-ATPase hydrolytic activity was reported in membrane vesicles with a reduced (50 %) molar α_2/β_1 ratio caused by higher β_1 content, relative to vesicles with a greater ratio (1.0) [63]. In contrast to the selective increase in type-II fibre β_1 abundance by sprint-interval training, β_1 abundance was reported to decrease by 18% in type I fibres after six weeks of interval-endurance training [44]. Whilst the decrease is likely unrelated to method variation, because technical variability in measurement was low (<12%), it might be explained by a possible detraining effect given that the subjects in the latter study was used to train at a substantially higher intensity. Accordingly, increasing relative intensity by reducing muscle blood flow during training attenuated the decline in type-I fibre β_1 abundance with the training period [44]. Collectively, these results suggest that β_1 is regulated in a fibre type-specific manner to some types of exercise training (and detraining) in human skeletal muscle. This regulation appears to be dependent on relative training intensity and thus the demands imposed by training on transmembrane Na^+/K^+ transport in the different muscle fibre types.

Findings from our lab indicate that β_2 is lowly expressed in human vastus lateralis muscle [43], suggesting even large changes in β_2 abundance in one type or both fibre types with training may be of small relevance for the skeletal muscle ion transport function in humans. Accordingly, neither sprint-interval [40] nor resistance training [24] resulted in altered β_2 abundance in type I or II fibres in humans. However, a higher (27%) β_2 abundance has been observed in type II compared to type I fibres identified in individual fibre segments from human skeletal muscle [40]; a finding we have recently confirmed in separate pools of type I and II fibres using a modified method for fibre-type-specific protein analysis [43]. These results highlight that β_2, although not altered in any fibre type by several types of intense training (Table 3), is expressed in a fibre type-specific manner in human skeletal muscle. Whilst it might be suggested that this pattern of expression might be functionally relevant, supported by the observation in rat skeletal muscle that the K_m for Na^+ of α/β_2 heterodimers

(7.5-13 mM) is higher than the corresponding K_m for α/β_1 complexes (4-5.5 mM) [186], this remains to be elucidated in humans.

Na^+,K^+-ATPase β_3 abundance has been shown to be elevated in type I (1 fold) and II (3 fold) muscle fibres, and in whole-muscle homogenates (2.5 fold), with age in humans [21,197]. In rat skeletal muscles, a similar age-associated increase in β_3 abundance was reversed by fourteen weeks of endurance training [198]. Thus, regular continuous muscle activity potently attenuates age-induced increases in β_3 abundance in human muscle. In contrast, sprint-interval training increased β_3 abundance by more than two fold in both human muscle fibre types [43]. Together, these findings underline that β_3 abundance is similarly regulated in type I and II muscle fibres in humans and that this regulation appears to be dependent on the type of muscle activity (or lack thereof) regularly undertaken. In one of the human studies, the increase in β_3 abundance with sprint-interval training occurred concomitant with an increase in α_1 abundance [43], suggesting an enhanced potential for α_1/β_3 complex assembly in both fibre types after the training period. This supports that the β_3 isoform could take part in maintenance of resting membrane potential in both fibre types, in line with the ion transport function of the α_1 isoform [70]. However, this warrants further investigation.

FXYD1 abundance

Previous human studies using whole-muscle samples reported no alterations in FXYD1 protein abundance following 10 days to 8 weeks of intense training [104,174,190,199]. In contrast, we have recently shown that six weeks of sprint-interval training decreased FXYD1 abundance by 33 % in type I fibres [43]. FXYD1 abundance remained unchanged in type II fibres in the same study, highlighting FXYD1 abundance is regulated in a fibre type-dependent manner by intense training in human skeletal muscle. As FXYD1 may regulate Na^+,K^+-ATPase function in multiple ways (e.g. by control of oxidation [71] and Na^+ affinity [200]), this indicates that physiologically relevant adaptations could be overlooked by the use of whole-muscle homogenate for protein analyses. Other methodological steps should also be avoided, including sample fractionation, i.e. removal of an indefinite amount of protein [193]. In another human study, FXYD1 abundance was higher in both type I and II fibres after interval-endurance training with compared to without reduced muscle blood flow [44], indicating relative exercise intensity is important for alterations in FXYD1 abundance at the fibre-type level with training in humans. The different regulation of FXYD1 abundance in type I fibres with training in the latter two studies is likely explained by differences in work-to-rest ratio, and/or training duration (approximately 45 vs. 324 minutes).

Summary

In summary of the scarce number of published human studies on this topic, the abundance of Na^+,K^+-ATPase α_1 and β_3 appears to be similarly altered in type I and II muscle fibres by different types of training in humans. Furthermore, increases in both α_1, α_2, and β_1 abundance by exercise training are likely training-intensity-dependent, consistent with the role of both metabolic, ion and redox perturbations in regulating Na^+,K^+-ATPase expression. The abundance of α_2 may also be regulated according to training volume. The regulation of the α_2 isoform to different types of training appears to occur in a fibre-type dependent manner and this may be a result of the extent to which the different fibre types are recruited while training. Both the isoforms α_3 and β_2 may be lowly expressed in human skeletal muscle and the expression of α_3 hardly altered at the fibre type level by training in humans. The existing evidence suggests that changes in the abundance of α_3 and β_2 may be of little functional importance for the ion transport capacity of human skeletal muscle. Moreover, FXYD1 is upregulated in both muscle fibre types to certain types of intense training (i.e. blood-flow-restricted training), but not in response to other types (e.g. sprint-interval) in human skeletal muscle. The regulation of FXYD1 likely depends on training intensity, work:rest ratio, exercise duration, or a combination of these factors.

Implications of fibre type-specific adaptations in Na^+,K^+-ATPase-isoform expression for K^+ regulation in humans: Novel insights from blood flow-restricted training

In most published studies that have examined training-induced effects on Na^+,K^+-ATPase abundance in type I and II muscle fibres in humans, measurement of K^+ regulation was either not included [40] or K^+ concentration was measured in venous plasma [24,41], which poorly reflects K^+ homeostasis at the muscle level [7,9]. In a recent experiment, we assessed the effects of exercise training with and without blood flow restriction (BFR) on Na^+,K^+-ATPase-isoform abundance at the fibre-type level, together with measurement of net thigh K^+ release during isolated work with the quadriceps muscle [44]. A novel observation was that higher abundance of β_1 (33%) and FXYD1 (108%) in type I, and α_1 (51%) and FXYD1 (60%) in type II fibres occurred concomitant with a reduced net rate of thigh K^+ release during intense exercise after training with BFR. Furthermore, in the three subjects where α_2 abundance was determined, a large effect for an increase in α_2 was evident in both type I (38%, d = 0.8) and type II fibres (38%, d = 0.7) after the training period with BFR. In contrast, simultaneous training of the contralateral leg without BFR neither increased isoform levels nor altered thigh K^+ release [44]. Thus, adaptations in the expression of Na^+,K^+-ATPase isoforms at the fibre-type level appear to be important for muscle K^+ regulation during exercise in humans. In

support, an increased expression of these isoforms would inevitably improve the potential for assembly of more Na^+,K^+-ATPase complexes at the cell surface, thereby increasing the capacity for K^+ re-uptake [33]. Moreover, consistent with previous observations in humans [6,8], enhancement of muscle K^+ regulation in the study highlighted above was temporally associated with an improved exercise tolerance after training with BFR (11% greater vs. control leg) [44], providing support for a positive association between locomotor muscle K^+ regulation and exercise performance.

Regulation of Na^+,K^+-ATPase-isoform mRNA in human skeletal muscle by a single exercise session

Changes in steady-state protein abundance may often be partly determined by variance in mRNA levels [201]. A single exercise session increases the mRNA of a growing number of genes [202-204], and these increases may often occur prior to upregulation of protein content in human skeletal muscle [205]. Thus, measurement of mRNA responses to a single exercise session can often provide valuable insights into the potential of a given training strategy to modulate abundance of proteins and ultimately their function. However, this assumption should be carefully regarded, because of the complex process that underpins upregulation of protein levels. Increases in protein are not exclusively a result of elevated mRNA availability, but also depends on efficient mRNA translation and protein stabilisation (synthesis relative to degradation). Further complicating interpretation, mRNA availability is rapidly altered according to mRNA transcription, alternative splicing, synthesis, decay, and translation [206].

The cross-sectional data summarised in Table 4 indicate that Na^+,K^+-ATPase isoforms (α_{1-3} and β_{1-3}) may be partly regulated at the mRNA level by exercise in humans. By comparing the responses in mRNA expression to a single exercise session (Table 4) with corresponding changes in protein content to a period of exercise training (Table 2), it could be argued that effects of an exercise session on Na^+,K^+-ATPase mRNA transcript levels reflect changes in corresponding isoform protein abundance after a period of the same type of training. For example, an increase (3 fold) in muscle α_1 mRNA was observed in recovery from a single session consisting of fifteen 1-min exercise bouts at 150% of leg VO_{2max} separated by 3 min of rest [156], whereas the same training protocol performed 3 to 5 times per week for seven weeks resulted in an elevated (29%) α_1 protein abundance in what appears to be the same individuals [7]. Further, increases in α_1 (2 fold) [207], α_2 (1.8 fold), and α_3 mRNA (3.3 fold) [86] have been reported after a session of continuous aerobic exercise (45 to 55 minutes at 71 to 75% of VO_{2max}). In comparison, 6 days to 11 weeks of regularly performing continuous aerobic training (2 hours at 60 to 65% VO_{2max}) resulted in elevated α_1 (16%) and α_2 (9%) protein abundance and [^{3}H]-ouabain binding site content (9 to 14%) [175,176,208]. Further, an increase in [^{3}H]-ouabain

binding (14%) was reported after several weeks of intense aerobic interval training with one leg (5 x 3-min of unilateral cycling at 100% of pre-train aerobic peak power), whereas one session with the same type of exercise (5 x 2-5 min at 56 ± 5 W and 60 kick/min separated by 3 min of rest) elevated the mRNA levels of α_1 (3.8 fold) and α_2 (2.4 fold) in recreationally-active subjects. In another study, a session of sprint-interval exercise increased both α_1 and β_3 mRNA content, whereas six weeks of performing the same type of training caused an elevated protein abundance of these isoforms in muscle of recreationally-active men [43]. In addition, six weeks of interval-endurance training with BFR resulted in elevated FXYD1 protein abundance in both muscle fibre types. Accordingly, a single session comprising of the same type of training increased FXYD1 mRNA content in human skeletal muscle [85]. However, an association between mRNA and protein adaptations is not a universal finding. For example, no change in α_2 mRNA was observed after a session of intense interval exercise [156], despite an increase in α_2 protein (15%) after seven weeks of regularly performing the same type of exercise [7]. Similarly, a selective increase in α_2 mRNA after a single session of post-exercise cold-water immersion was observed, despite no change at the protein level with six weeks of training, where each training session were concluded with cold-water immersion [43]. These observations in human muscle are supported by a similar dissociation between responses of α_2 mRNA and protein to thyroid hormone in cultured skeletal muscle cells [209] and to sprint-interval training in rat EDL and soleus muscles [164].

In summary, there are some indications that the accumulative effects of repeated exercise-induced changes in mRNA is an important determinant of net protein turnover of most Na^+,K^+-ATPase isoforms after several weeks of training. However, it is clear from the current evidence that variance in mRNA is likely one of several factors that control protein levels of these isoforms in human skeletal muscle following a period of exercise training; indicating altered mRNA to a single training session is not a solid marker of potential protein outcomes for these isoforms after several weeks of training.

An alternative approach to designing exercise training interventions to maximise improvements in K^+ regulation

Based on the previous sections of this review, a key question that remains to be answered is what strategy should be chosen to most effectively enhance (i.e. within shortest time with maximal benefit) K^+ regulation and thus fatigue tolerance during intense exercise in humans. In recent years, we in our lab have focused on developing a training strategy to maximally stimulate the molecular stressors identified in cell culture and animal models (*in vitro*) to be prerequisite to beneficial adaptations specific to K^+ regulation, with the aim to maximise training adaptation. This alternative approach to optimising training has with success been centered on the use of blood flow restriction (BFR) to

manipulate blood perfusion of exercising muscles [44,85]. With emphasis on this training strategy, the aim of this section is to provide the reader with an alternative view on how training interventions may be designed to maximise skeletal muscle adaptation in humans.

Blood flow restriction (BFR) typically involves inflation of a pneumatic tourniquet (or cuff) around the most proximal portion of the limbs, thereby reducing muscle blood perfusion (Fig. 4). This strategy has been applied during various types of exercise, including walking, cycling, running, and resistance training [85,210-212]. Superimposition of BFR during exercise leads to premature fatigue [213]. Although evidence in humans is lacking, this appears to relate, at least partly, to malfunction of ion channels and transport systems [33,80,117]. For example, inactivation of the Na^+-K^+-ATPase may occur earlier during exercise with than without BFR due, in part, to inhibition of its primary energy pathway (anaerobic glycolysis), promoted by intramuscular acidification [33]. BFR exercise may also inactivate the Na^+-K^+-ATPase by increasing the formation of ROS and oxidative damage [80,85,99]. Inhibition of the Na^+-K^+-ATPase leads to an increase in $[Na^+]_i$ and a concomitant rise in $[Ca^{2+}]_i$ via excitation of the Na^+/Ca^{2+} exchanger [214]. Accumulation of $[Ca^{2+}]_i$ may also be augmented by ROS formation due, in part, to their capability to inhibit the sarcoplasmic reticulum calcium ATPase (SERCA) [117]. Thus, $[Na^+]_i$ and $[Ca^{2+}]_i$ could both be amplified by exercising with BFR. A rise in the inward osmotic pressure gradient accompanying BFR, forcing fluid to accumulate in the intracellular compartment, may further magnify the ionic perturbations during exercise, for example, by increasing the intracellular dilution space for K^+ [52]. In humans, post-exercise muscle oxygenation, a key regulator of Na^+-K^+-ATPase expression *in vitro* [90,92,93], has been shown to be augmented by exercising with BFR [215,216]. In summary, several key stressors involved in enhancing K^+ regulation, which were identified earlier in this review, could be augmented by exercising with BFR. Accordingly, we have provided evidence that BFR augments the exercise-induced molecular signaling response underlying enhancement of K^+ regulation in humans [85], and cycling for six weeks with compared to without BFR caused superior improvements in performance and K^+ regulation during intense exercise in recreationally-active men [44].

Prior to achieving these successful outcomes, it was carefully considered how to best design the BFR-training protocol. With primary basis in experiments *in vitro*, a hypothetical model of fluctuations in skeletal muscle oxygen level and ROS production during BFR exercise and subsequent reperfusion rest was drawn (Fig. 5). According to this model, the availability of ROS substrate (i.e. oxygen and NAD(P)H) mainly dictates the amount of ROS that are formed [80,81]. Further, ROS production peaks during the reperfusion phase, where the convective oxygen delivery to tissues is maximal [80,81,135,137,217]. Accordingly, we have shown that the skeletal muscle blood flow reaches its maximum within the first minute after the end of BFR-exercise, where the tourniquet is deflated (Fig.

4). At this point, it was therefore considered that repeated exercise bouts with BFR separated by few minutes with intact blood flow to exercised muscles would be the most effective BFR strategy to augment perturbations in muscle oxygenation, ROS accumulation, and ionic stress. In support, others have found that BFR applied during exercise amplifies metabolic by-product accumulation [218,219], leading to a more hypoxic and acidic intramuscular environment [220,221]. However, too severe an intracellular acidosis may be detrimental to the post-exercise increase in mRNA levels [160] and adaptability of the ion transport systems to exercise training [222]. Thus, it was considered that exercise bouts with BFR preferably should be several minutes in length to markedly, but only transiently perturb ion (and redox) homeostasis [223]. Nevertheless, existence of a threshold of exercise bout duration at which perturbations in redox, ion, and metabolic state may become detrimental to training adaptation remains to be elucidated. Moreover, a moderate exercise intensity may preferably be chosen to avoid premature fatigue caused by BFR. Given these considerations, the BFR-training protocol that we have successfully used in our human experiments consisted of three series of 3 x 2-min exercise bouts performed at ~60 to 80% of maximal aerobic power, with 1 and 5 min of recovery between bouts and series, respectively. The tourniquet was inflated 10 s prior to and deflated immediately after each exercise bout. The pressure of the tourniquet was determined in a pilot study, where several exercise sessions were completed with varying degree of BFR (pressure range: 100 to 250 mmHg; n=2 subjects), whereby the highest tolerable pressure, by which the exercise protocol could be completed, was chosen for our experiments (~175 mmHg) [85].

In summary, exercising with BFR is a potent training concept to promote the molecular mechanisms underlying adaptations in the capacity for K^+ regulation in humans. On this basis, it is proposed that optimising exercise training prescription, in part, by studying outcomes from animal and cell culture research about key mechanisms involved in cell adaptation is a successful approach to increase the outcome of exercise training on human physical function. In addition, BFR-exercise is an excellent research model to evaluate what molecular signals may drive skeletal muscle adaptation to various stimuli, including physical activity and disease, in humans.

Conclusion and perspectives

In humans, improvements in the capacity for K^+ regulation likely result from transient perturbations in both redox, ion, and metabolic homeostasis. Specifically, increases in local oxygen level, ROS production, extracellular [K^+], rate of anaerobic ATP generation, and mRNA expression appear to be key stressors underlying adaptations specific to K^+ regulation, including increases in expression of different Na^+,K^+-ATPase isoforms. In contrast, the level of muscle hypoxia and lactate accumulation *per se* do not seem essential to these adaptations in humans. The involvement of alterations in $[Na^+]_i$ and $[Ca^{2+}]_{cyt}$ in mediating these adaptations is presently unclear, although evidence *in vitro* points to a role of transient oscillations in the concentrations of these ions in regulating Na^+-K^+-ATPase expression. Little is currently understood about how the identified key molecular stressors may be transducing the signal(s) for adaptation in the capacity for K^+ regulation in human skeletal muscle, although a contributory role of AMPK and oxygen-sensing transcription factors has been suggested. Furthermore, enhancement of K^+ regulation by exercise training in humans probably takes place as a result of coordinated increases in expression of catalytic Na^+,K^+-ATPase isoforms and FXYD1 among different fibre types, which appears to be dependent on relative exercise intensity and/or volume, as well as the demand for Na^+/K^+ transport imposed on each fibre type while training. In addition, by the use of blood-flow-restricted exercise as a model to manipulate the molecular stressors underlying training adaptation, it was highlighted how mechanistic insights from animal and cell culture research may be preferably used to make informed decisions about how to optimise exercise training prescription to maximise improvements in K^+ regulation in humans. The novel information provided in this review paves the way for a better understanding of how to develop interventions, such as exercise training and pharmacological therapies, to improve physical function and potentially hinder the progression of several life-style-related diseases in humans.

Conflict of interests

The author has no conflict of interest that relates to the content of this article.

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Figures

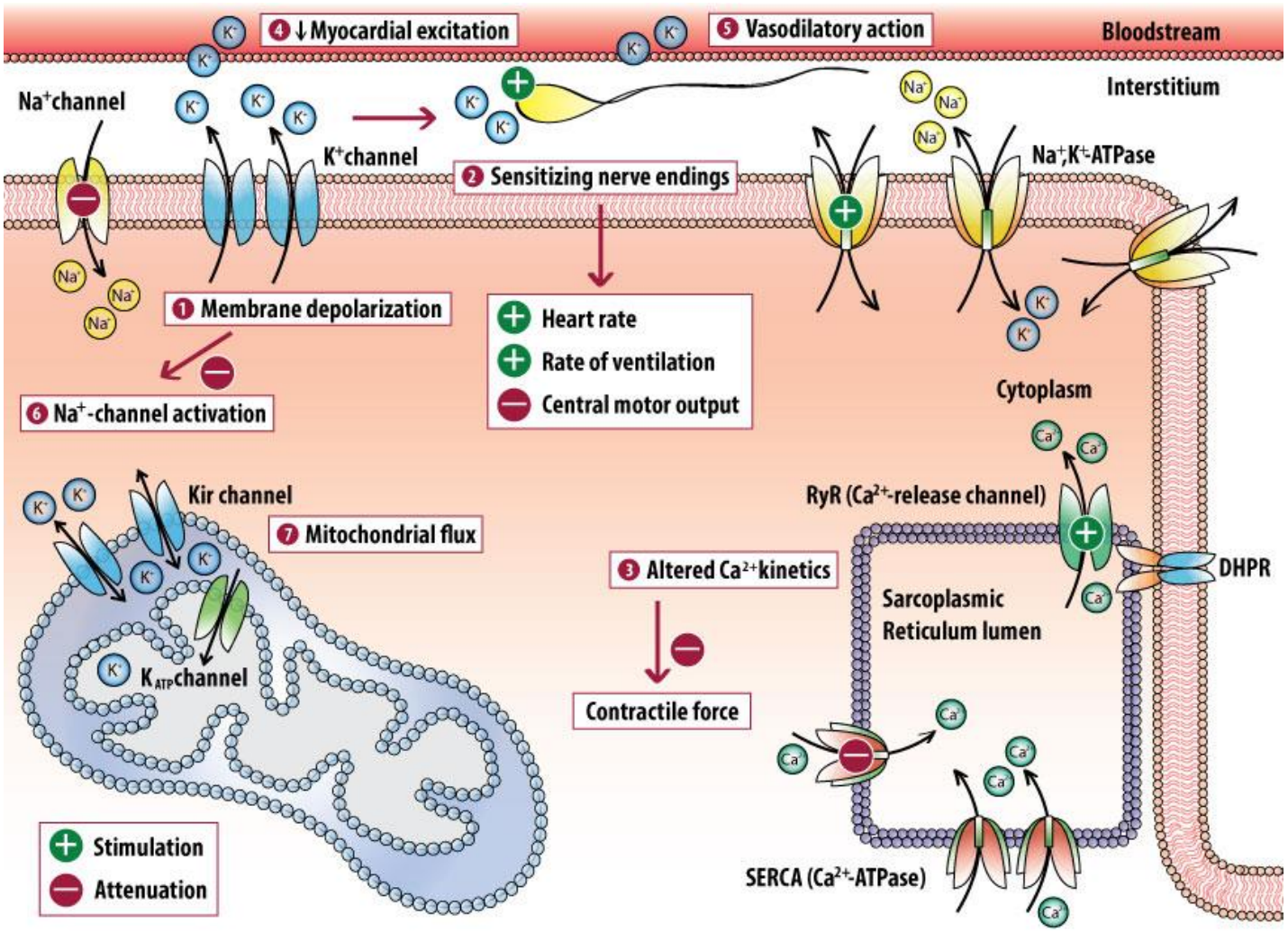

Figure 1. Sites in skeletal muscle influenced by elevated interstitial K^+ concentration ($[K^+]_{int}$). Accumulation of K^+ in the interstitial space causes membrane depolarization (1), stimulates group III/IV nerve endings, resulting in decreased central motor output and increased heart and ventilation rate (2), facilitates cytosolic Ca^{2+} accumulation by impairing SR Ca^{2+} re-uptake and enhancing release (3). Increased $[K^+]_{int}$ may also affect myocardial excitation by increasing plasma K^+ concentration (4) and contribute to local vasodilation (5). Further, altered K^+ concentration is known to inactivate Na^+-channels, which is likely to reduce action potential amplitude (6) and impact rate of mitochondrial ATP generation (flux) (7), although its exact role in this matter remains unknown. RyR = ryanodine receptor; DHPR: dihydropyridine receptor.

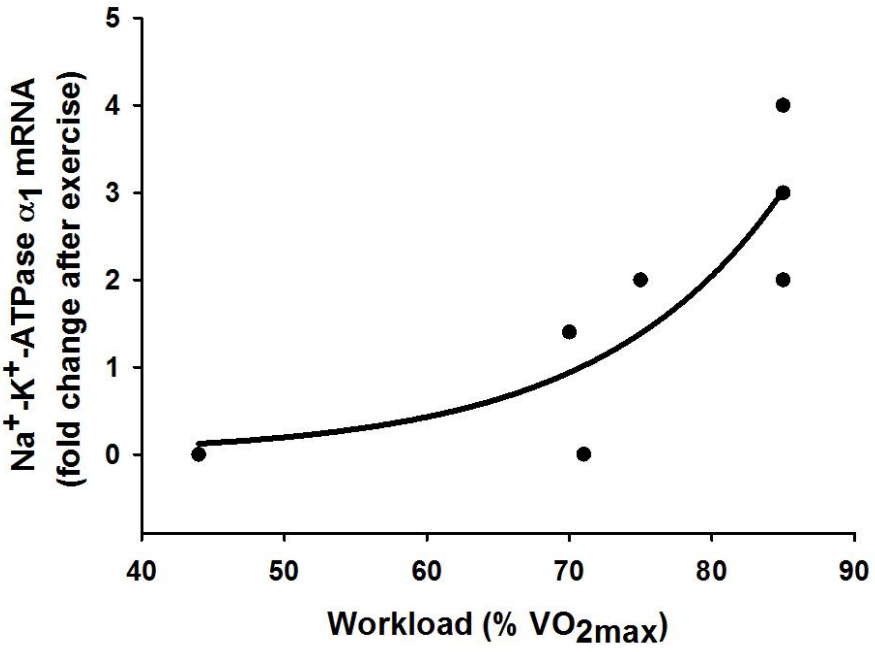

Figure 2. Effect of exercise intensity on change in Na^+-K^+-ATPase α_1 mRNA in response to a single session of exercise in human skeletal muscle. The figure is based on data from Aughey, Murphy, Clark, Garnham, Snow, Cameron-Smith, Hawley, McKenna [157] and Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [89] that used similar cohorts (trained humans) and exercise modality (cycling). A single, two-parameter exponential fit [f=a(exp(bx))] provided the strongest relationship between the two factors (r = 0.85).

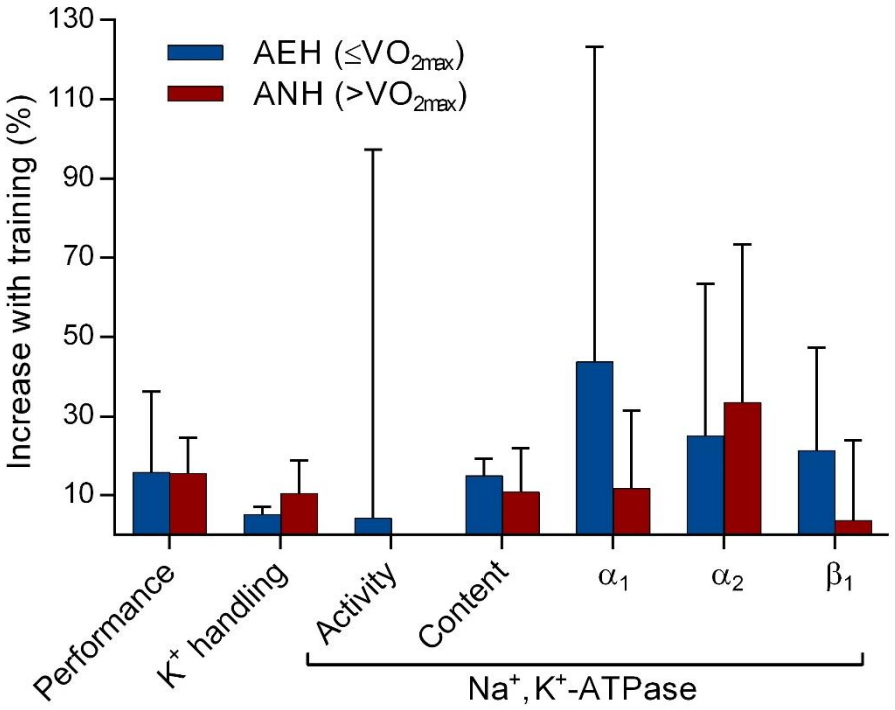

Figure 3. Effects of different types of exercise training on intense exercise performance, K^+ regulation (i.e. plasma blood K^+ concentration, thigh K^+ release, or muscle interstitial K^+ accumulation), and skeletal muscle Na^+,K^+-ATPase function, content, and isoform (α_1, α_2, β_1) abundance in humans. AEH = Training at intensities ≤ VO_{2max} (*n* = 13 interventions) [101,157,174-177,208,224-226], ANH = Training at intensities > VO_{2max} (*n* = 8 interventions) [7,12,178,189,191,227,228]. Note that measurement of Na^+,K^+-ATPase activity was not included in studies on ANH training. Data are expressed as means + 95% confidence intervals.

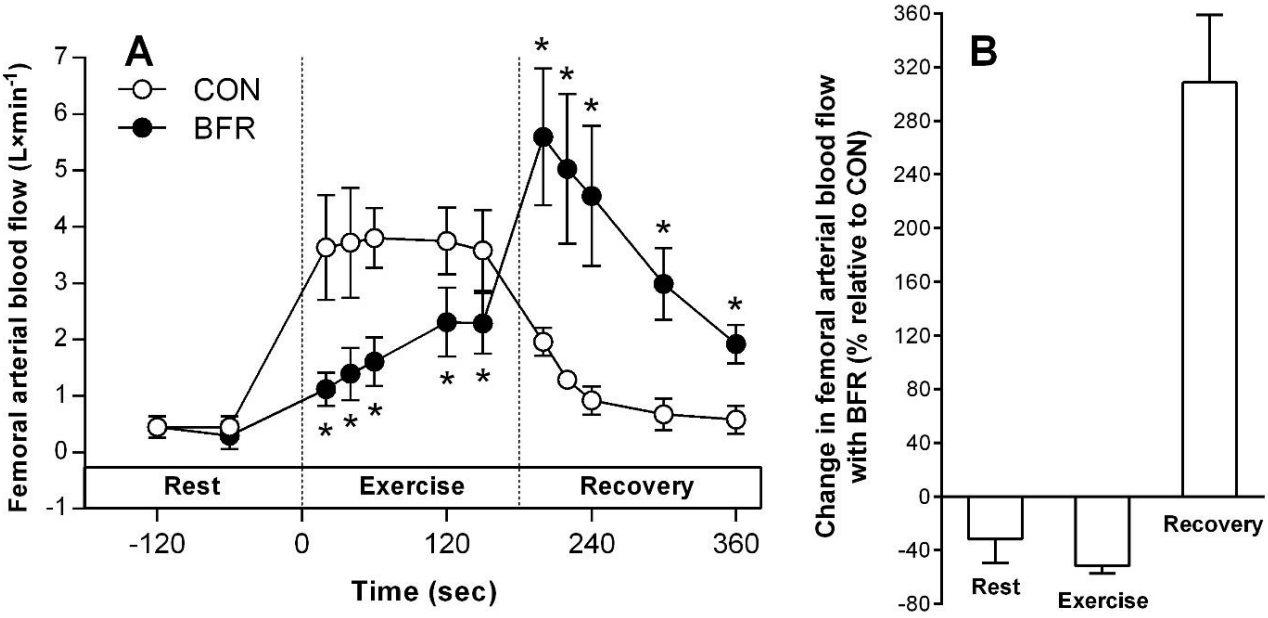

Figure 4. Absolute (A) and relative (B) fluctuations in thigh blood flow during and in recovery from moderate-intensity (12 W), single-leg, knee-extensor exercise without (CON) or with blood flow restriction (BFR; ~175 mmHg). Data are expressed as means ± SD. This figure was reproduced from a previous paper with permission from the authors.

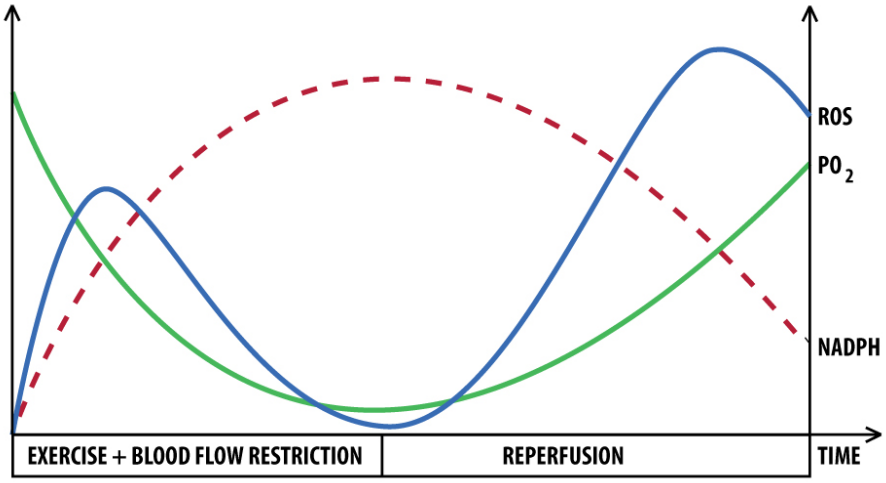

Figure 5. Proposed model for fluctuations in reactive oxygen species (ROS) formation, anaerobic glycolytic substrate production for ROS synthesis, and oxygen partial pressure, in skeletal muscle during exercise with blood flow restriction and during the subsequent recovery with intact blood circulation (reperfusion). ROS: blue; PO_2: green; NADPH: hatched red.

Tables

Table 1. Summary of the likely involvement of key molecular stressors in mediating adaptations specific to K^+ regulation in human skeletal muscle

Molecular signal	Interaction with other key signal mechanisms	Effect on adaptation in K^+ regulation or Na^+,K^+-ATPase expression	Evidence in animals or cell culture	Supported by findings in humans
Perturbations in redox state	↑ ROS, altered ion homeostasis	+	Yes	Yes
↑ oxygenation	↑ Sp1/Sp3 activation; ↑ mRNA transcription	+	Yes	(Yes)
Hypoxia	Redox homeostasis	–	Yes	Yes
↑ ROS levels	↑AMPK activation, perturbed K^+, Ca^{2+}, Na^+ homeostasis	+	Yes	Yes
↑ Antioxidant function	↓ ROS	–	Yes	Yes
Intracellular Na^+	Ion homeostasis, ↑ ROS	?	Conflicting	n/a
Interstitial K^+ accumulation (membrane depolarization)	Ion homeostasis, ↑ ROS	+	Yes	No
Cytosolic Ca^{2+}	Ion and redox homeostasis, CaMKII activation	+	Yes	No
Anaerobic ATP turnover	AMPK, perturbed ion and redox homeostasis, ↑ ROS	+	No	(Yes)
Lactate	Metabolic homeostasis	–	No	Yes

↑ and ↓ denote potentiation and attenuation, respectively. + and – denote a positive and negative effect, respectively. ? denotes unknown and brackets denote weak support.

Table 2 Effects of different types of exercise training on exercise performance, K^+ regulation, and skeletal muscle Na^+,K^+-ATPase function, content and (whole-muscle) isoform expression in humans

Study	Subjects	Training	Performance	K^+ regulation (% decline vs. pre training)	Na^+,K^+-ATPase activity	[^{3}H]ouabain binding site content	Na^+,K^+-ATPase-isoform abundance
Aughey, Murphy, Clark, Garnham, Snow, Cameron-Smith, Hawley, McKenna [157]	Male cyclists or triathletes (n=12) 65 mL/kg/min	2–3 sessions/wk for 3 weeks 8 x 5-min cycling at 80 % peak power output (1 min cycling at 100 W)	Peak power during incremental cycling to exhaustion ↑3%		3-O-MFPase activity ↑5%	0%	α_1, α_2, α_3: 0% β_1, β_2, β_3: 0%
Edge, Eynon, McKenna, Goodman, Harris, Bishop [224]	Recreationally-active women (n=6) 46 mL/kg/min	3 sessions/wk for 5 weeks 6-10 x 2 min at 140-190 % LT (1 min rest)	Power at VO_{2peak} ↑12%			↑22%	
Edge, Eynon, McKenna, Goodman, Harris, Bishop [224]	Recreationally-active women (n=6) 46 mL/kg/min	3 sessions/wk for 5 weeks 6-10 x 2 min at 140-190 % LT (3 min rest)	Power at VO_{2peak} ↑9%			↑26%	
Green, Chin, Ball-Burnett, Ranney [175]	Untrained men (n=9) 48 mL/kg/min	6 sessions over 6 days 2-h cycling at 60-65 % VO2max	Incremental cycling test to exhaustion (VO2max) ↑7%	Venous plasma [K^+] ↓5%		↑14%	
Green, Dahly, Shoemaker, Goreham, Bombardier, Ball-Burnett [208]	Healthy and untrained subjects (n=7), sex n/a 44.4 mL/kg/min	5–6 sessions/wk for 11 weeks 2-h cycling at 68 % VO2max	Incremental cycling test to exhaustion (VO2max) ↑15%			↑22%	
Green, Barr, Fowles, Sandiford, Ouyang [176]	Untrained men (n=7) 46 mL/kg/min	6 sessions over 6 days 2-h cycling at 60-65 % VO2max			3-O-MFPase activity ↑41%	↑9%	α_1: ↑16% α_2: ↑9% β_1: ↑39%
Green, Duhamel, Stewart, Tupling, Ouyang [177]	Recreationally-active men (n=6) and women (n=6) 45 mL/kg/min	3 sessions over 3 days 2 h cycling at 60 % VO2max			3-O-MFPase activity ↑34%	↑12%	α_1: ↑46% α_2: ↑42% α_3: ↑31% β_1: ↑19%, β_2: ↑28% β_3: : ↑20%
Madsen, Franch, Clausen [225]	Male runners (n=42) 55 mL/kg/min	3 sessions/wk for 6 weeks 25 min running at 93% HRmax	Running at pre-train 86 % VO2max to exhaustion (TTE) ↑75%			↑15%	
Benziane, Widegren, Pirkmajer, Henriksson, Stepto, Chibalin [174]	Healthy men (n=8) 61 mL/kg/min	4 sessions over 10 days 45-90 min cycling at 75 % VO2max and 6 x 5 min cycling at 90-100 % VO2max with 2 min cycling <40 % VO2max between bouts on alternate days		Venous plasma [K+] ↓5%			α_1: ↑113% α_2: ↑49% α_3: 0% β_1: ↑27%, β_2: 0%

Table 2 Continued from previous page.

Study	Subjects	Training	Performance	K^+ regulation (% decline vs. pre training)	Na^+,K^+-ATPase activity	[^{3}H]ouabain binding site content	Na^+,K^+-ATPase-isoform abundance
Evertsen, Medbo, Jebens, Nicolaysen [226]	Male (n=11) and female (n=9) cross country skiers 73 and 58 mL/kg/min	7 sessions/wk for 20 weeks Running, roller- or cross-country skiing at moderate (40 min to 3 h at 60-70% VO2max) (86%) and high intensity (40 s to 7 min at 80-90% 2max for 10 min to 2 h) (14%)	20-min running time trial ↑2%			↑16%	
Evertsen, Medbo, Jebens, Nicolaysen [226]	Male (n=11) and female (n=9) cross country skiers 73 and 58 mL/kg/min	7 sessions/wk for 20 weeks Running, roller- or cross-country skiing at 80-90 % VO2max (10 min to 2 h) or 40 s to 7 min at similar intensity (83%) or at 60-70 % VO2max (17%)	20-min running time trial ↑4%			↑16%	
Green, MacDougall, Tarnopolsky, Melissa [101]	Healthy men (n=9) VO_{2max} n/a	3 sessions/wk for 8 weeks 30-min one-legged cycling at 75-87% peak power output (first 6 weeks) and 5 x 3-min cycling at 100% pre-train peak power output (final 2 weeks)	Unilateral cycling to fatigue at 95 % VO2max ↑400%			↑14%	
Green, MacDougall, Tarnopolsky, Melissa [101]	Healthy men (n=9) VO_{2max} n/a	3 sessions/wk for 8 weeks 30-min one-legged cycling at 75-87% peak power output (first 6 weeks) and 5 x 3-min cycling at 100% pre-train peak power output (final 2 weeks) in normobaric systemic hypoxia	Unilateral cycling to fatigue at 95 % VO2max ↑510%			↑14%	
Bangsbo, Gunnarsson, Wendell, Nybo, Thomassen [227]	Endurance-trained men (n=17) 63 mL/kg/min	2–3 sessions/wk for 6–9 weeks Addition of sessions of 8-12 x 30-s at 95% maximal speed (3 min rest)	Exhaustive supramaximal treadmill run at 130% VO2peak ↑36%	Venous plasma [K+] ↓6%			α_1: 0%, α_2: ↑68% β_1: 0%

Table 2 Continued from previous page.

Study	Subjects	Training	Performance	K^+ regulation (% decline vs. pre training)	Na^+,K^+-ATPase activity	[^{3}H]ouabain binding site content	Na^+,K^+-ATPase-isoform abundance
Gunnarsson, Christensen, Holse, Christiansen, Bangsbo [191]	Danish 2nd Division male soccer players (n=16) 61 mL/kg/min	1 additional session/wk for 5 weeks Addition of a session of 6-9 x 30-s runs at 90-95 % maximal intensity (3 min)	incremental running time to exhaustion ↑11%				α_1: 0%, α_2: 0% β_1: ↓13%
Harmer, Ruell, McKenna, Chisholm, Hunter, Thom, Morris, Flack [12]	type-1 diabetic men (n=5) and women (n=3) 3300 mL/min	3 sessions/wk for 7 weeks 4-10 x 30-s 'all-out' cycling sprints, 3-4 min rest	Incremental cycling test to exhaustion (VO2max and peak power) ↑11%	Venous plasma [K+] ↓7%		↑8%	
Harmer, Ruell, McKenna, Chisholm, Hunter, Thom, Morris, Flack [12]	Healthy men (n=4) and women (n=3) 3170 mL/min	3 sessions/wk for 7 weeks 4-10 x 30-s 'all-out' cycling sprints, 3-4 min rest	Incremental cycling test to exhaustion (VO2max and peak power) ↑11%	Venous plasma [K+] ↓7%		↑8%	
Iaia, Thomassen, Kolding, Gunnarsson, Wendell, Rostgaard, Nordsborg, Krustrup, Nybo, Hellsten, Bangsbo [189]	Male runners (n=8) 56 mL/kg/min	3–4 sessions/wk for 4 weeks 8-12 x 30-s running bouts at 90-95 % of the max. speed attained during a 30-s 'all-out' run (3 min rest bt sprints)	running to fatigue at 130 % VO2max and incremental test to exhaustion ↑5%	Venous plasma [K+] ↓8%			α_1: ↑29% α_2: ↑16% β_1: 0%
McKenna, Schmidt, Hargreaves, Cameron, Skinner, Kjeldsen [228]	Healthy, untrained men (n=6) 51 mL/kg/min	3 sessions/wk for 7 weeks 4-10 x 30-s maximal-intensity cycling sprints (2.5-4 min rest)	4 maximal 30-s sprints ↑6%	Venous plasma [K+] ↓19%		↑16%	
Mohr, Krustrup, Nielsen, Nybo, Rasmussen, Juel, Bangsbo [178]	Healthy men (n=7) 49 mL/kg/min	3–6 sessions/wk for 8 weeks 8 x 30-s runs at 130 % VO2max, 1.5 min rest	incremental exercise to exhaustion ↑15%	Venous plasma [K+] ↓0%			α_1: 0% α_2: ↑68% β_1: ↑31%
Dela, Holten, Juel [195]	Healthy subjects (n=7) VO_{2max} n/a	3 sessions/wk for 6 weeks 3-4 sets of 10-12 reps at 50-80% 1RM, 1.5-2 min rest	Maximal leg press and knee-extensor force ↑77% and ↑29%				α_1: ↑37% α_2: ↑22% β_1: ↑33%
Dela, Holten, Juel [195]	Type-II diabetes patients (n=10) VO_{2max} n/a	3 sessions/wk for 6 weeks 3-4 sets of 10-12 reps at 50-80% 1RM, 1.5-2 min rest	Maximal leg press and knee-extensor force ↑75% and ↑42%				α_1: ↑45% α_2: ↑41% β_1: ↑47%
Green, Dahly, Shoemaker, Goreham, Bombardier, Ball-Burnett [208]	Healthy and untrained subjects (n=9), sex n/a 45 mL/kg/min	3 sessions/wk for 12 weeks Resistance training: 3 x 6-8 1RM of squats, leg press, leg extensions	Incremental cycling test to exhaustion (VO2max) ↑0%			↑16%	

Table 2 Continued from previous page.

Study	Subjects	Training	Performance	K^+ regulation (% decline vs. pre training)	Na^+,K^+-ATPase activity	[^{3}H]ouabain binding site content	Na^+,K^+-ATPase-isoform abundance
Medbo, Jebens, Vikne, Refsnes, Gramvik [229]	Male athletes (n=23) VO_{2max} n/a	1–3 sessions/wk for 12 weeks 5 x 4 squats at 50% 1RM (concentric phase) and 110-135 % 1RM (eccentric phase)	1RM squat and Number of squats at 70 % 1RM to exhaustion ↑7% and ↑29%			↑15%	
Perry, Wyckelsma, Murphy, Steward, Anderson, Levinger, Petersen, McKenna [24]	Sedentary, healthy men (n=4) and women (n=2) 46 mL/kg/min	3 sessions/wk for 4 weeks 3-4 sets of 8-12 reps of leg-press, knee-extension, hamstring curls, and calf raises at 65-70 % 1RM (1 min rest)	Time to fatigue cycling at 85 % leg VO2 peak ↑31%	Venous plasma [K+] and Δ[K+]/work ratio ↓0%		0%	α_1, α_2, α_3: 0% β_1, β_2, β_3: 0%
Skovgaard, Christensen, Larsen, Rostgaard Andersen, Thomassen, Bangsbo [230]	Endurance-trained male runners (n=12) 59 mL/kg/min	4 sessions/wk for 8 weeks Two sessions of 4-12 x 30-s 'all-out' (3 min rest) and two sessions of resistance training (3 sets of 8 reps at 15RM to 4 sets of 4 reps at 4 RM, squats, deadlift, leg press)	10-km run, 1500 m run, Yo-Yo IR2, VO2max ↑4%, ↑6%, ↑44% and ↑0%				α_1, α_2: 0% β_1: 0%
Skovgaard, Almquist, Bangsbo [179]	Endurance-trained male and female runners (n=11) 59 and 50 mL/kg/min	6 sessions/wk for 6 wk Maintained high-frequency SET (four sessions of 8-12 x 30-s 'all-out' runs, 3.5 min rest) and moderate-intensity running (two sessions of 30-60 min running at 60-80 % HRmax)	TTE during intense exercise ↑12%	Venous plasma [K+] ↓0%			α_1, α_2: 0% β_1: ↑39%
Skovgaard, Almquist, Bangsbo [179]	Endurance-trained male and female runners (n=7) 59 and 50 mL/kg/min	4 sessions/wk for 12 weeks Maintained low-frequency SET (two sessions of 10 x 30-s 'all-out' runs, 3.5 min rest) and moderate-intensity running (two sessions of 30-60 min running at 60-80 % HRmax)	TTE during intense exercise ↑16%	Venous plasma [K+] ↓14%			α_1, α_2: 0% β_1: ↑58%
Skovgaard, Almquist, Bangsbo [231]	Endurance-trained male runners (n=8) 59 mL/kg/min	4 sessions/wk for 6 weeks Two sessions of 4-12 x 30-s 'all-out' (3 min rest) and two sessions of running for 30-60 min at 60-80 % HRmax	Incremental running to exhaustion (VO2max) ↑20%				α_1, α_2: 0% β_1: 0%

Table 2 Continued from previous page.

Study	Subjects	Training	Performance	K^+ regulation (% decline vs. pre training)	Na^+,K^+-ATPase activity	[^{3}H]ouabain binding site content	Na^+,K^+-ATPase-isoform abundance
Thomassen, Christensen, Gunnarsson, Nybo, Bangsbo [190]	Elite soccer players (n=18) 55 mL/kg/min	4.5 sessions/wk for 2 weeks Small-sided games (8 x 2 min at 88 % HRmax, 1 min rest) and 10-12 x 25-30-s 'all-out' runs and 16 x 40-60-s runs at 84 % HRmax, 40-60-s rest	RSA, total sprint time, fastest sprint time, Yo-Yo IR2 ↑3%, ↑2%, ↑0%, and ↑0%				α_1: 0%, α_2: ↑15% β_1: 0%
Nielsen, Mohr, Klarskov, Kristensen, Krustrup, Juel, Bangsbo [7]	Healthy men (n=6) 50 mL/kg/min	3–5 sessions/wk for 7 weeks 15 x 1 min at 150% thigh VO2peak (3 min rest)	Incremental exercise to exhaustion ↑28%	Muscle interstitial [K+] ↓27%			α_1: ↑29%, α_2: ↑15% β_1: 0%
Nielsen, Mohr, Klarskov, Kristensen, Krustrup, Juel, Bangsbo [7]	Healthy men (n=6) 50 mL/kg/min	3–5 sessions/wk for 7 weeks 15 x 1 min at 150% thigh VO2peak (3 min rest)	Incremental exercise to exhaustion ↑28%	Leg K+ release ↓0%			α_1: ↑29%, α_2: ↑15% β_1: 0%
Mohr, Krustrup, Nielsen, Nybo, Rasmussen, Juel, Bangsbo [178]	Healthy men (n=6) 50 mL/kg/min	3–6 sessions/wk for 8 weeks 15 x 1 min at 150% thigh VO2peak (3 min rest)	Incremental exercise to exhaustion ↑28%	Venous plasma [K+] ↓0%			α_1: 0%, α_2: 0% β_1: ↑34%

Thirty human training studies included measurement of two or more of the following factors: Na^+,K^+-ATPase activity, content, whole-muscle isoform abundance, and K^+ regulation. Two studies were excluded as their main focus was on the effect of the environment (altitude exposure) rather than training [103,104], and one because training sessions were performed on a single day [232]. Searches were conducted on Pubmed, Science Direct, Google Scholar, and Web of Science using words such as: Na^+,K^+-ATPase, performance, training, human muscle. Manual searches were also performed using reference lists of published studies.

Table 3 Fibre type-specific changes in Na^+,K^+-ATPase-isoform abundance with exercise training in human skeletal muscle

Study	Subjects	Training			Exercise performance	Measurement of K^+ handling	Whole-muscle Na^+,K^+-ATPase content	Na^+,K^+-ATPase-isoform abundance	
		Frequency and duration	*Protocol*	*Type*				*Type I fibres*	*Type II fibres*
Wyckelsma, McKenna, Serpiello, Lamboley, Aughey, Stepto, Bishop, Murphy [40]	Recreationally active men (n=4) and women (n=5) 42 mL/kg/min	3 sessions/wk for 4 weeks	Three sets of 5 x 4-s all-out running sprints separated by 20 s of rest, with 4.5 min of rest between sets	Sprint-interval	n/a	n/a	n/a	α_1 α_2 α_3 ↔ β_1 β_2 β_3 ↔	β_1 ↑ 33% α_1 α_2 α_3 ↔ β_2 β_3 ↔
Perry, Wyckelsma, Murphy, Steward, Anderson, Levinger, Petersen, McKenna [24]	Sedentary, healthy men (n=4) and women (n=2) 46 mL/kg/min	3 sessions/wk for 4 weeks	Three to four sets of 8-12 repetitions at 65-70% 1RM, with 1 min rest between sets (Leg-press, knee-extension, hamstring curl, and calf raise)	Resistance	Cycling time to exhaustion at 85% leg VO_{2max} ↑31%	Venous plasma $[K^+]$ ↔ $\Delta[K^+]$/work ratio ↔	[^{3}H]ouabain binding ↔ α_1 α_2 α_3 ↔ β_1 β_2 β_3 ↔	α_2 ↑ 76% α_3 ↑ 143% α_1 β_1 β_2 β_3 ↔	α_1 ↑ 79% β_1 ↑ 35% α_2 α_3 β_2 β_3 ↔
Wyckelsma, Levinger, Murphy, Petersen, Perry, Hedges, Anderson, McKenna [41]	Recreationally active older adults (69 years) men (n=6) and women (n=2)	3 sessions/wk for 12 weeks	4 x 4-min cycling bouts at 90-95% HR_{peak} separated by 4 min recovery at 50-60% HR_{peak}	Aerobic-interval	Aerobic peak power output during cycling ↑ 25%	Peak venous plasma $[K^+]$ ↑ (p=0.07) $\Delta[K^+]$/work ratio ↔	[^{3}H]ouabain binding ↑ 11% α_1 α_2 β_1 ↔	α_1 α_2 β_1 ↔	α_2 ↑ 30% α_1 β_1 ↔
Christiansen, Bangsbo [44]	Recreationally active men (n=10) 50 mL/kg/min	3 sessions/wk for 6 weeks	Three series of 3 x 2-min cycling bouts at 60-80% peak aerobic power (1 and 2 min rest between bouts and series)	Aerobic-interval	Aerobic peak power output during cycling ↑ 11%	Arterial + Venous plasma $[K^+]$ ↓ a-v-K^+ difference ↔ thigh K^+ release ↔	n/a	β_1 ↓18% α_1 α_2 ↔ FXYD1 ↔	α_1 α_2 β_1 ↔ FXYD1 ↔
Christiansen, Bangsbo [44]	Recreationally active men (n=10) 50 mL/kg/min	3 sessions/wk for 6 weeks	Three series of 3 x 2-min cycling bouts at 60-80% peak aerobic power (1 and 2 min rest between bouts and series) + blood flow restriction	Aerobic-interval + blood flow restriction	Aerobic peak power output during cycling ↑ 23%	Arterial + Venous plasma $[K^+]$ ↓ a-v-K^+ difference ↓ thigh K^+ release ↓	n/a	α_1 ↑ 46% (p=0.075) FXYD1 ↑ 108% α_2 β_1 ↔	α_1 ↑51% FXYD1 ↑ 60% α_2 β_1 ↔
Christiansen, Bishop, Broatch, Bangsbo, McKenna, Murphy [43]	Recreationally active men (n=7) 45 mL/kg/min	3 sessions/wk for 6 weeks	4-6 x 30-s all-out cycling sprints separated by 4 min of rest	Sprint-interval	Aerobic peak power output during cycling ↑ 6% Incremental 2-km cycling time trial ↑ 3.3% 20-km cycling time trial ↔	n/a	n/a	α_1 ↑ 210% β_3 ↑ 300% FXYD1 ↓33% α_2 α_3 β_1 ↔	α_1 ↑ 270% β_1 ↑ 44% β_3 ↑ 410% α_2 α_3 FXYD1 ↔

Christiansen, Bishop, Broatch, Bangsbo, McKenna, Murphy [43]	Recreationally active men (n=5) 45 mL/kg/min	3 sessions/wk for 6 weeks	4-6 x 30-s all-out cycling sprints separated by 4 min of rest + Cold-water immersion	Sprint-interval + Cold-water immersion	Aerobic peak power output during cycling ↑ 6% 2-km cycling time trial ↑ 2.4% 20-km cycling time trial ↔	n/a	n/a	α_1 ↑ 330% β_3 ↑ 540% FXYD1 ↓33% α_2 α_3 β_1 ↔	α_1 ↑ 260% β_1 ↑ 44% β_3 ↑ 400% α_2 α_3 ↔ FXYD1 ↔

Table 4 Literature summary of the effects of a single exercise session on mRNA content of Na^+,K^+-ATPase isoforms in human skeletal muscle

Study	Subjects	Exercise protocol	Metabolic changes	Fold-changes in mRNA content of Na^+,K^+-ATPase isoforms						
				α_1	α_2	α_3	β_1	β_2	β_3	FXYD1
Christiansen, Murphy, Bangsbo, Stathis, Bishop [85]	Recreationally active (trained) men (n=8) 57 mL/kg/min	Three sets of 3 x 2 min running bouts at 105% lactate threshold (~12 km/h), with bouts separated by 1 min and sets by 5 min. Blood flow restriction during bouts: ~175 mmHg.	Peak muscle lactate: ~50 mmol/kg d.w. muscle Peak blood lactate: ~6.7 mmol/L Minimum blood pH: ~7.2 Peak blood K^+: ~5.1 mmol/L	3 h: →	3 h: ~1.6↑	3 h: →	3 h: →	3 h: →	3 h: →	3 h: ~2.7↑
Christiansen, Murphy, Bangsbo, Stathis, Bishop [85]	Recreationally active (trained) men (n=8) 57 mL/kg/min	Three sets of 3 x 2 min running bouts at 105% lactate threshold (~12 km/h), with bouts separated by 1 min and sets by 5 min. Systemic hypoxia during bouts: ~3250 m altitude.	Peak muscle lactate: ~42 mmol/kg d.w. muscle Peak blood lactate: ~6.4 mmol/L Minimum blood pH: ~7.2 Peak blood K^+: ~5.1 mmol/L	3 h: →	3 h: ~1.6↑	3 h: →	3 h: ~1.7↑	3 h: →	3 h: →	3 h: →
Christiansen, Murphy, Bangsbo, Stathis, Bishop [85]	Recreationally active (trained) men (n=8) 57 mL/kg/min	Three sets of 3 x 2 min running bouts at 105% lactate threshold (~12 km/h), with bouts separated by 1 min and sets by 5 min.	Peak muscle lactate: ~28 mmol/kg d.w. muscle Peak blood lactate: ~3.5 mmol/L Minimum blood pH: ~7.25 Peak blood K^+: ~5.0 mmol/L	3 h: →	3 h: →	3 h: →	3 h: →	3 h: →	3 h: →	3 h: →
Christiansen, Bishop, Broatch, Bangsbo, McKenna, Murphy [43]	Recreationally active men (n=10) 45 mL/kg/min	Four 30-s 'all-out' cycling sprints separated by 4 min of rest.	n/a	0 h: → 3 h: ~2↑	0 h: → 3 h: →	0 h: → 3 h: →	0 h: → 3 h: →	0 h: → 3 h: ↓ to 0.7	0 h: → 3 h: ~2↑	0 h: → 3 h: →
Christiansen, Bishop, Broatch, Bangsbo, McKenna, Murphy [43]	Recreationally active men (n=9) 45 mL/kg/min	Four 30-s 'all-out' cycling sprints separated by 4 min of rest with post-exercise cold-water immersion (15 min at 10°C).	n/a	0 h: → 3 h: ~2↑	0 h: ~2.1↑ 3 h: →	0 h: → 3 h: →	0 h: → 3 h: →	0 h: → 3 h: ↓ to 0.7	0 h: → 3 h: ~2.5↑	0 h: → 3 h: →

Table 4 Continued from previous page

Study	Subjects	Exercise protocol	Metabolic changes	Fold-changes in mRNA content of Na^+,K^+-ATPase isoforms						
				α_1	α_2	α_3	β_1	β_2	β_3	FXYD1
Nordsborg, Bangsbo, Pilegaard [156]	Healthy males (n=6) *Trained leg* 50 mL/kg/min	15 x 1 min at 150 % leg VO_{2peak} separated by 3 min of rest	n/a	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	n/a	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	n/a	n/a	n/a
Nordsborg, Thomassen, Lundby, Pilegaard, Bangsbo [233]	Recreationally active males (n=8) VO_{2max} n/a	5 x 2-5 min at 56 ± 5 W and 60 kick/min separated by 3 min of rest	Blood lactate = 4.2 ± 2.2 mM Epinephrine: 0.3 ± 0.1 nM Norepinephrine: 0.8 ± 0.3 nM	0 h: → 1 h: ~3.8↑ 3 h: ~3.5↑ 5 h: ~2.5↑	0 h: → 1 h: ~2.1↑ 3 h: ~2.4↑ 5 h: ~1.9↑	n/a	0 h: → 1 h: ~2.8↑ 3 h: ~2.7↑ 5 h: ~2.2↑	0 h: → 1 h: → 3 h: → 5 h: →	0 h: ~1.9↑ 1 h: ~2.2↑ 3 h: ~3.1↑ 5 h: ~2↑	n/a
Nordsborg, Thomassen, Lundby, Pilegaard, Bangsbo [233]	Recreationally active males (n=8) VO_{2max} n/a	5 x 2-5 min at 56 ± 5 W and 60 kick/min separated by 3 min of rest	Blood lactate = 11.4 ± 4.2 mM Epinephrine: 0.75 ± 0.45 nM Norepinephrine: 7.5 ± 5.5 nM	0 h: → 1 h: ~3.0↑ 3 h: ~3.8↑ 5 h: ~2.5↑	0 h: → 1 h: ~1.9↑ 3 h: ~1.9↑ 5 h: ~1.9↑	n/a	0 h: → 1 h: ~2↑ 3 h: ~2.1↑ 5 h: →	0 h: → 1 h: → 3 h: → 5 h: →	0 h: ~2.0↑ 1 h: ~2.0↑ 3 h: ~2.2↑ 5 h: ~2.0↑	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Untrained (n=8) 44 mL/kg/min	4 x 4 min at 83 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 10.8 ± 0.5 mM (arm vein)	1+3 h: ~2.0↑	1+3 h: →	n/a	1+3 h: ~2.1↑	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=10) 55 mL/kg/min	4 x 4 min at 85 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 10.1 ± 0.4 mM (arm vein)	1+3 h: ~2.0↑	1+3 h: →	n/a	1+3 h: ~1.7↑	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=6) 55 mL/kg/min	4 x 4 min at 70 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 3.1 ± 0.7 mM (arm vein)	1+3 h: ~1.4↑	1+3 h: →	n/a	1+3 h: →	n/a	n/a	n/a

Table 4 Continued from previous page

Study	Subjects	Exercise protocol	Metabolic changes	Fold-changes in mRNA content of Na^+,K^+-ATPase isoforms						
				α_1	α_2	α_3	β_1	β_2	β_3	FXYD1
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Untrained (n=6) 44 mL/kg/min	~3 h 20 min at 44 ± 1% VO_{2peak} (exhaustion)	n/a	1 h: ~3.0↑	1 h: ~1.8↑	n/a	1 h: →	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=6) 55 mL/kg/min	~3 h 45 min at 44 ± 1% VO_{2peak} (exhaustion)	n/a	1 h: →	1 h: →	n/a	1 h: →	n/a	n/a	n/a
Petersen, Murphy, Snow, Leppik, Aughey, Garnham, Cameron-Smith, McKenna [158]	Healthy males (n=8) and females (n=7) 51 mL/kg/min	5 min 52 s ± 4 min 27 s at 40% of total work (exhaustion)	Plasma volume: ~3.3-12.3 % ↓ Plasma $[K^+]$: ↑ from 3.9 (rest) to 4.3 (exercise peak) mmol/L	0 h: ~1.5↑ Mean of 0+3+24 h: ~1.5↑	0 h: ~2.5↑ Mean of 0+3+24 h: ~3.3↑	0 h: ~2.4↑ Mean of 0+3+24 h: ~1.4↑	0 h: → Mean of 0+3+24 h: ~1.1↑	0 h: ~1.7↑ Mean of 0+3+24 h: ~2.0↑	0 h: → Mean of 0+3+24 h: ~1.0↑	n/a
Nordsborg, Bangsbo, Pilegaard [156]	Healthy males (n=6) *Trained leg* 50 mL/kg/min	15 x 1 min at 150 % leg VO_{2peak} separated by 3 min of rest	n/a	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	n/a	0 h: → 1 h: → 3 h: → 5 h: → 24 h: →	n/a	n/a	n/a
Nordsborg, Thomassen, Lundby, Pilegaard, Bangsbo [233]	Recreationally active males (n=8) VO_{2max} n/a	5 x 2-5 min at 56 ± 5 W and 60 kick/min separated by 3 min of rest	Blood lactate = 4.2 ± 2.2 mM Epinephrine: 0.3 ± 0.1 nM Norepinephrine: 0.8 ± 0.3 nM	0 h: → 1 h: ~3.8↑ 3 h: ~3.5↑ 5 h: ~2.5↑	0 h: → 1 h: ~2.1↑ 3 h: ~2.4↑ 5 h: ~1.9↑	n/a	0 h: → 1 h: ~2.8↑ 3 h: ~2.7↑ 5 h: ~2.2↑	0 h: → 1 h: → 3 h: → 5 h: →	0 h: ~1.9↑ 1 h: ~2.2↑ 3 h: ~3.1↑ 5 h: ~2↑	n/a
Nordsborg, Thomassen, Lundby, Pilegaard, Bangsbo [233]	Recreationally active males (n=8) VO_{2max} n/a	5 x 2-5 min at 56 ± 5 W and 60 kick/min separated by 3 min of rest	Blood lactate = 11.4 ± 4.2 mM Epinephrine: 0.75 ± 0.45 nM Norepinephrine: 7.5 ± 5.5 nM	0 h: → 1 h: ~3.0↑ 3 h: ~3.8↑ 5 h: ~2.5↑	0 h: → 1 h: ~1.9↑ 3 h: ~1.9↑ 5 h: ~1.9↑	n/a	0 h: → 1 h: ~2↑ 3 h: ~2.1↑ 5 h: →	0 h: → 1 h: → 3 h: → 5 h: →	0 h: ~2.0↑ 1 h: ~2.0↑ 3 h: ~2.2↑ 5 h: ~2.0↑	n/a

Table 4 Continued from previous page

Study	Subjects	Exercise protocol	Metabolic changes	Fold-changes in mRNA content of Na^+,K^+-ATPase isoforms						
				α_1	α_2	α_3	β_1	β_2	β_3	FXYD1
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Untrained (n=8) 44 mL/kg/min	4 x 4 min at 83 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 10.8 ± 0.5 mM (arm vein)	1+3 h: ~2.0↑	1+3 h: →	n/a	1+3 h: ~2.1↑	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=10) 55 mL/kg/min	4 x 4 min at 85 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 10.1 ± 0.4 mM (arm vein)	1+3 h: ~2.0↑	1+3 h: →	n/a	1+3 h: ~1.7↑	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=6) 55 mL/kg/min	4 x 4 min at 70 ± 2% VO_{2peak} separated by 3 min of rest	Plasma lactate = 3.1 ± 0.7 mM (arm vein)	1+3 h: ~1.4↑	1+3 h: →	n/a	1+3 h: →	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Untrained (n=6) 44 mL/kg/min	~3 h 20 min at 44 ± 1% VO_{2peak} (exhaustion)	n/a	1 h: ~3.0↑	1 h: ~1.8↑	n/a	1 h: →	n/a	n/a	n/a
Nordsborg, Kusuhara, Hellsten, Lyngby, Lundby, Madsen, Pilegaard [160]	Trained males (n=6) 55 mL/kg/min	~3 h 45 min at 44 ± 1% VO_{2peak} (exhaustion)	n/a	1 h: →	1 h: →	n/a	1 h: →	n/a	n/a	n/a
Petersen, Murphy, Snow, Leppik, Aughey, Garnham, Cameron-Smith, McKenna [158]	Healthy males (n=8) and females (n=7) 51 mL/kg/min	5 min 52 s ± 4 min 27 s at 40% of total work (exhaustion)	Plasma volume: ~3.3-12.3 % ↓ Plasma $[K^+]$: ↑ from 3.9 (rest) to 4.3 (exercise peak) mmol/L	0 h: ~1.5↑ Mean of 0+3+24 h: ~1.5↑	0 h: ~2.5↑ Mean of 0+3+24 h: ~3.3↑	0 h: ~2.4↑ Mean of 0+3+24 h: ~1.4↑	0 h: → Mean of 0+3+24 h: ~1.1↑	0 h: ~1.7↑ Mean of 0+3+24 h: ~2.0↑	0 h: → Mean of 0+3+24 h: ~1.0↑	n/a

↓ = decrease; ↑ increase; → no change. Fold-changes are relative to baseline (i.e. rest and/or before exercise)

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