



**VICTORIA UNIVERSITY**  
MELBOURNE AUSTRALIA

*Exercise and fatigue: integrating the role of K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> in the regulation of sarcolemmal excitability of skeletal muscle*

This is the Published version of the following publication

Renaud, Jean-Marc, Ørtenblad, Niels, McKenna, Michael and Overgaard, Kristian (2023) Exercise and fatigue: integrating the role of K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup> in the regulation of sarcolemmal excitability of skeletal muscle. *Eur J Appl Physiol*. ISSN 1439-6319

The publisher's official version can be found at  
<https://link.springer.com/article/10.1007/s00421-023-05270-9>  
Note that access to this version may require subscription.

Downloaded from VU Research Repository <https://vuir.vu.edu.au/46898/>



# Exercise and fatigue: integrating the role of $K^+$ , $Na^+$ and $Cl^-$ in the regulation of sarcolemmal excitability of skeletal muscle

Jean-Marc Renaud<sup>1</sup> · Niels Ørtenblad<sup>2</sup> · Michael J. McKenna<sup>3,4,5</sup> · Kristian Overgaard<sup>6</sup>

Received: 7 February 2023 / Accepted: 29 June 2023  
© The Author(s) 2023

## Abstract

Perturbations in  $K^+$  have long been considered a key factor in skeletal muscle fatigue. However, the exercise-induced changes in  $K^+$  intra-to-extracellular gradient is by itself insufficiently large to be a major cause for the force decrease during fatigue unless combined to other ion gradient changes such as for  $Na^+$ . Whilst several studies described  $K^+$ -induced force depression at high extracellular  $[K^+]_e$  ( $[K^+]_e$ ), others reported that small increases in  $[K^+]_e$  induced potentiation during submaximal activation frequencies, a finding that has mostly been ignored. There is evidence for decreased  $Cl^-$  CIC-1 channel activity at muscle activity onset, which may limit  $K^+$ -induced force depression, and large increases in CIC-1 channel activity during metabolic stress that may enhance  $K^+$  induced force depression. The ATP-sensitive  $K^+$  channel ( $K_{ATP}$  channel) is also activated during metabolic stress to lower sarcolemmal excitability. Taking into account all these findings, we propose a revised concept in which  $K^+$  has two physiological roles: (1)  $K^+$ -induced potentiation and (2)  $K^+$ -induced force depression. During low-moderate intensity muscle contractions, the  $K^+$ -induced force depression associated with increased  $[K^+]_e$  is prevented by concomitant decreased CIC-1 channel activity, allowing  $K^+$ -induced potentiation of sub-maximal tetanic contractions to dominate, thereby optimizing muscle performance. When ATP demand exceeds supply, creating metabolic stress, both  $K_{ATP}$  and CIC-1 channels are activated.  $K_{ATP}$  channels contribute to force reductions by lowering sarcolemmal generation of action potentials, whilst CIC-1 channel enhances the force-depressing effects of  $K^+$ , thereby triggering fatigue. The ultimate function of these changes is to preserve the remaining ATP to prevent damaging ATP depletion.

**Keywords** CIC-1 channel ·  $K_{ATP}$  channel · Membrane excitability · Force potentiation · Force depression · Metabolic stress

## Abbreviations

9-AC	9-Anthracene carboxylic acid
$[Cl^-]_e$	Extracellular $[Cl^-]$
$[K^+]_e$	Extracellular $[K^+]$
$[Na^+]_e$	Extracellular $[Na^+]$
$[Ca^{2+}]_i$	Intracellular $[Ca^{2+}]$
$[K^+]_{int}$	Interstitial $[K^+]$
$[Na^+]_{int}$	Interstitial $[Na^+]$
$[Cl^-]_i$	Intracellular $[Cl^-]$
$[K^+]_i$	Intracellular $[K^+]$
$[Na^+]_i$	Intracellular $[Na^+]$
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AMPK	AMP kinase
AP	Action potential
ADR	Adrenergic receptor
ATP	Adenosine triphosphate
$Ca_v1.1$	Voltage-sensitive $Ca^{2+}$ channel 1.1
CGRP	Calcitonin gene related peptide
CIC-1	$Cl^-$ channel 1

Communicated by Michael I Lindinger.

✉ Jean-Marc Renaud  
jmrenaud@uottawa.ca

<sup>1</sup> Faculty of Medicine, Department of Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Rd., Ottawa, ON K1H 8M5, Canada

<sup>2</sup> Department of Sports Science and Clinical Biomechanics, University of Southern Denmark, Odense, Denmark

<sup>3</sup> Institute for Health and Sport, Victoria University, Melbourne, VIC 8001, Australia

<sup>4</sup> College of Physical Education, Southwest University, Chongqing, China

<sup>5</sup> College of Sport Science, Zhuhai College of Science and Technology, Zhuhai, China

<sup>6</sup> Exercise Biology, Department of Public Health, Aarhus University, Aarhus, Denmark

$E_{Cl}$	$Cl^-$ equilibrium potential
E-C-R	Excitation–contraction–relaxation
EDL	Extensor digitorum longus
$E_K$	$K^+$ equilibrium potential
$E_M$	Membrane potential
EMG	Electromyogram
FDB	Flexor digitorum brevis
HEK-293	Human embryonic kidney 293
Hz	Hertz
$G_{Cl}$	$Cl^-$ conductance
$G_K$	$K^+$ conductance
$G_{Na}$	$Na^+$ conductance
IMP	Inosine monophosphate
$K_{ATP}$ channel	ATP-sensitive $K^+$ channel
Kir6.2	Inward $K^+$ rectifier 6.2
Kir6.2 <sup>-/-</sup>	Knockout for the $K_{ATP}$ channel subunit Kir6.2
$K_V$	Voltage-sensitive $K^+$ channel
mM	Millimolar
ms	Millisecond
mV	Millivolt
$Na_V$	Voltage-sensitive $Na^+$ channel
$Na_V1.4$	Voltage-sensitive $Na^+$ channel 1.4
NKA	$Na^+,K^+$ -ATPase, $Na^+,K^+$ -pump
PCr	Phosphocreatine
PKC	Protein kinase C
$pH_e$	Extracellular pH
$pH_i$	Intracellular pH
Resting $E_M$	Resting membrane potential
SR	Sarcoplasmic reticulum
TPS	Train per s
t-tubule	Transverse tubule
TTX	Tetrodotoxin
W	Watt
$\mu M$	Micromolar

## Introduction: the initial concept about $K^+$ and fatigue

Muscle fatigue is defined as a transient decrease in the capacity of skeletal muscles to generate force or do work when repetitively activated. Fenn and colleagues in the 1930's documented  $K^+$  loss,  $Na^+$  and water gain in contracting skeletal muscle (Fenn 1938, 1937; Fenn and Cobb 1936). A very large number of reports that followed these studies were on muscle contraction-induced changes in plasma, muscle interstitial and intracellular  $[K^+]$  (see the extensive companion review by McKenna et al. (2023)). Although the mechanism for the  $K^+$  loss was not understood at the time, Fenn proposed that a progressive  $K^+$  loss could cause the simultaneously observed loss of contractile force (Fenn 1940). Later on, the ionic mechanisms underlying the

resting membrane potential (resting  $E_M$ ) and action potential (AP) were elucidated, which allowed further development of the concept of a central role for  $K^+$  in muscle fatigue. In brief, the  $K^+$  loss from muscle fibers occurs over several APs due to the  $K^+$  efflux associated with the AP repolarization phase, resulting in an increased extracellular  $[K^+]$  ( $[K^+]_e$ ) and lowered intracellular  $[K^+]$  ( $[K^+]_i$ ). As the  $[K^+]$  gradient across the sarcolemma decreases, resting  $E_M$  depolarizes (for details see next section entitled “Sarcolemmal resting  $E_M$  and action potentials”). As a consequence of the depolarization, voltage sensitive  $Na^+$  channels ( $Na_V$  channels) become inactivated, reducing AP amplitude and slowing AP propagation along the sarcolemma and into t-tubules. For this review, any physiological conditions leading to either a reduction in AP amplitude or a complete loss in the capacity of the sarcolemma to generate an AP compared to AP measured in unfatigued and normal physiological conditions is considered a decrease in membrane excitability. As membrane excitability is reduced due to the  $K^+$ -induced depolarization, less  $Ca^{2+}$  is released by sarcoplasmic reticulum (SR) resulting in reduced muscular force or work, i.e. fatigue.

Over the years, this concept gained tremendous support and  $K^+$  shifts are still considered an important factor in the mechanism of muscle fatigue. However, studies have demonstrated that: (1) by itself, the lower intra-to-extracellular  $K^+$  gradient, and hence depolarization, may in many cases not be sufficient to explain the contraction-induced decrease in membrane excitability and force; (2) moderate increases in  $[K^+]_e$  actually potentiates sub-tetanic force; and (3) changes in  $Cl^-$  conductance ( $G_{Cl}$ ) may be crucial in determining when  $K^+$  can either potentiate or depress force. A first objective is to review how perturbations in  $K^+$ ,  $Na^+$  and  $Cl^-$  gradients across the muscle membrane during muscle activity affect membrane excitability and force and to discuss whether the changes in gradients are sufficient to contribute to the decrease in membrane excitability and force as originally conceptualized. A second objective is to discuss the long-ignored phenomenon of  $K^+$ -induced force potentiation and consider its physiological role at the onset of moderate exercise. A third objective is to discuss the link between metabolism and membrane excitability. The fourth and final objective is to propose an evolved concept integrating the dual roles of  $K^+$  (i.e., potentiation and depression) during muscle activity and fatigue and how they are modulated or controlled by  $Na^+$ ,  $Cl^-$   $ClC-1$  channels,  $K_{ATP}$  channels and  $Na^+,K^+$ -ATPase ( $Na^+,K^+$ -pump, NKA).

## Sarcolemmal resting $E_M$ and action potentials

### Resting $E_M$

$E_M$  is a diffusion potential that is created when ions cross the sarcolemma through their channels. Thus, the sarcolemma must be permeable to the ions, which must have a concentration gradient across the sarcolemma to contribute to  $E_M$ . In resting skeletal muscle,  $[K^+]_i$ , measured in different species and muscles, is approximately 160 mM (ranging between 90 and 190 mM), whilst the interstitial  $[K^+]$  ( $[K^+]_{int}$ ), measured mostly in human muscles, is typically just over 4 mM (Table 1, also see review by (McKenna et al. 2023)). This large  $K^+$  gradient favors muscle  $K^+$  efflux making  $E_M$  more negative. Early studies using

amphibian muscles demonstrated that resting  $E_M$  mostly behaves like a  $K^+$  electrode (Hodgkin and Horowicz 1959; Adrian 1956). This conclusion was based on the fact that the steady state resting  $E_M$  became depolarized when  $[K^+]_e$  is increased and hyperpolarized with a decreased  $[K^+]_e$ ; the new steady state resting  $E_M$  values being close to the expected values calculated from the Nernst equation. However, close examination of the data reveals that the resting  $E_M$ - $[K^+]_e$  slope in these studies was not completely identical to the expected Nernst potential (Table 2). For amphibian muscles, the calculated slope is 40 mV per decade change in  $[K^+]_e$  instead of 59 mV as predicted by the Nernst equation in the Adrian (1956) study. In the Hodgkin and Horowicz (1959) study, the slope was 51 mV. Notably, the differences in the measured and expected slope are even greater for mammalian muscles (Table 2). At 25 °C and 37 °C, the expected slopes are 59 and 62 mV,

**Table 1** Changes in interstitial and intracellular  $K^+$  concentrations at rest and after fatigue

Species	Muscle	Rest (R)	Fatigue (F)	$\frac{[K^+]_E}{[K^+]_R}$	References
<b><math>[K^+]_{int}</math> (mM)</b>					
Human	Gastrocnemius	4.1	7.5	1.8	(Green et al. 1999)
Human	Gastrocnemius	4.4	11.8	2.7	(Green et al. 2000)
Human	Vastus lateralis	4.2	9.0	2.1	(Juel et al. 2000)
Human	Vastus lateralis	4.4	10.3	2.3	(Nielsen et al. 2004a)
Human	Vastus lateralis	4.4	13.7	3.1	(Street et al. 2005)
Human	Vastus lateralis	4.1	11.4	2.8	(Mohr et al. 2004)
Human	Vastus lateralis	4.2	11.9	2.8	(Nordsborg et al. 2003)
Human	Vastus lateralis <sup>1</sup>	3.9	5.5	1.4	(Lott et al. 2001)
Human	Vastus lateralis	4.0	10.5	2.6	(Gunnarsson et al. 2013)
Cat	Triceps surae <sup>2</sup>	4.0	4.8	1.2	(MacLean et al. 1998)
Mouse	Gastrocnemius	5.2	8.8	1.7	(Radzyukevich et al. 2009)
<b><math>[K^+]_i</math> (mM)</b>					
Frog	Semitendinosus	142	97	0.68	(Balog and Fitts 1999)
Human	Vastus lateralis	161	141	0.88	(Sjøgaard 1983)
Human	Vastus lateralis	162	129	0.80	(Sjøgaard et al. 1985)
Human	Vastus lateralis	125	110	0.88	(Gunnarsson et al. 2013)
Mouse	EDL	182	134	0.74	(Juel 1986)
	soleus	168	136	0.81	
Mouse	Soleus	174	145	0.83	(Juel 1988)
Rat	EDL	150	85	0.57	(Nagaoka et al. 1994)
	soleus	131	96	0.73	
Rat <sup>3</sup>	Soleus	90	64	0.71	(Lindinger and Heigenhauser 1987)
	plantaris	109	93	0.85	
	white gastrocnemius	125	86	0.70	
	red gastrocnemius	114	87	0.76	
Rat <sup>4</sup>	Soleus	118	112	0.95	(Lindinger et al. 1987)
	plantaris	141	122	0.87	
	white gastrocnemius	143	130	0.91	
	red gastrocnemius	147	128	0.87	

Concentration values are the reported mean using the unit as per each study.  $[K^+]_{int}$  and  $[K^+]_i$  were reported after a fatigue bout, except for two studies: <sup>1</sup>Exercise at 60%  $VO_{2max}$ . <sup>2</sup>Twitch contractions at 5 Hz. For  $[K^+]_i$  measurements, fatigue was elicited in vitro by field stimulation with two exceptions; <sup>3</sup>muscles were stimulated in situ with intact blood flow and <sup>4</sup> $[K^+]_i$  was measured following swimming

**Table 2**  $[K^+]$ -resting  $E_M$  relationship in unfatigued skeletal muscles

Species	Muscle	Temperature	$[K^+]$ (mM)	$\Delta$ Resting $E_M / \Delta$ decade $[K^+]$ (mV)	References
From change in $[K^+]_e$					
Frog	Sartorius	13–23 °C	0.5–5.0	40 <sup>C</sup>	(Adrian 1956)
Frog	Semitendinosus	20 °C	5.0–50	51 <sup>C</sup>	(Hodgkin and Horowicz 1959)
			0.5–10 <sup>1</sup>	34 <sup>C</sup>	
			10–100 <sup>1</sup>	55 <sup>C</sup>	
Frog	Sartorius	25 °C	3–12	59	(Renaud and Light 1992)
Mouse	EDL soleus	25 °C	4–14	51 <sup>C</sup>	(Cairns et al. 1997)
			4–14	48 <sup>C</sup>	
Mouse	EDL soleus	25 °C	4–11	45 <sup>C</sup>	(Cairns and Borrani 2015)
				48 <sup>C</sup>	
Mouse	Red sternomastoid	37 °C	3–100	28	(Dulhunty 1977)
Mouse	EDL soleus	37 °C	4–11	37	(Yensen et al. 2002)
			4–11	45	
Mouse	EDL soleus diaphragm	37 °C	4–15	46	(Ammar et al. 2015)
			4–15	47	
			4–15	44	
Mouse	Soleus	37 °C	4–10	24	(Uwera et al. 2020)
Rat	Soleus	30 °C	4–14	39 <sup>C</sup>	(Cairns et al. 1995)
From change in $[K^+]_i$					
Frog	Sartorius	13–23 °C	110–190	50	(Adrian 1956)

Resting  $E_M$  was determined after manipulating either  $[K^+]_e$  or  $[K^+]_i$  over the range of  $[K^+]$  as indicated in the table. The change in resting  $E_M$  per decade change in  $[K^+]$  are as reported or calculated (C) using the data from individual resting  $E_M$  and  $[K^+]$  values. <sup>1</sup>Relationship obtained in  $Cl^-$  free solutions

respectively, whereas the measured slopes ranged from 28 to 47 mV. This deviation of the slope from expected values involves effects from  $Na^+$ ,  $Cl^-$  and electrogenic contribution of NKA.

In the resting state, the sarcolemmal permeability to  $Na^+$  is very small, which was estimated to be 1% of the  $K^+$  permeability (Hodgkin and Horowicz 1959). This  $Na^+$  permeability is due to a very small proportion of  $Na_V$  channels being open at rest; a fact that is confirmed in some studies by small hyperpolarization in the presence of tetrodotoxin (TTX) or in  $Na^+$  free saline solutions (Yensen et al. 2002; Nastuk and Hodgkin 1950; Huxley and Stampfli 1951) but not all (Cairns et al. 2003; Overgaard et al. 1997). As demonstrated by Hodgkin and Horowicz (1959),  $Na^+$  causes only small resting  $E_M$  depolarization when  $[K^+]_e$  ranged between 2 and 10 mM.

$Cl^-$  is the third ion of importance affecting resting  $E_M$ . Contrary to  $K^+$  and  $Na^+$ , the  $Cl^-$  distribution across the sarcolemma is in equilibrium with the resting  $E_M$  in amphibian muscles; i.e., the  $Cl^-$  equilibrium potential ( $E_{Cl}$ ) and resting  $E_M$  are the same (Hodgkin and Horowicz 1959). As a consequence, there is no net  $Cl^-$  flux at rest. For mammalian muscles, some studies also reported an equilibrium for  $Cl^-$  with resting  $E_M$  similar to amphibian muscles, while others reported an  $E_{Cl}$  that was less negative than resting  $E_M$  (Aickin et al. 1989; Donaldson and Leader 1984; Dulhunty

1978; Geukes Foppen 2004). As recently reviewed (Pedersen et al. 2016), studies reporting an  $E_{Cl}$  less negative than resting  $E_M$  may have used experimental conditions, such as hypertonic extracellular solutions, that increase the activity of some active  $Cl^-$  transport, such as the secondary active  $Na^+$ ,  $K^+$ ,  $Cl^-$  co-transporter, allowing an accumulation of intracellular  $Cl^-$ . As a consequence of this  $Cl^-$  accumulation, a net  $Cl^-$  efflux occurs, which results in small  $Cl^-$ -induced depolarization. In another study, intracellular  $[Cl^-]$  ( $[Cl^-]_i$ ) was measured under several conditions involving changes in  $[Cl^-]_e$  and  $[K^+]_e$  either with or without a constant  $[K^+]_e$   $[Cl^-]_e$  product; in all cases the changes in  $[Cl^-]_i$  were as predicted for passive  $Cl^-$  distribution across the sarcolemma (McCaig and Leader 1984). Thus, it is likely that in mammalian muscles  $E_{Cl}$  and resting  $E_M$  are the same.

Contrary to the  $K^+$  effect, changes in  $[Cl^-]_e$  only cause transient changes in resting  $E_M$  (Hodgkin and Horowicz 1959; Cairns et al. 2004). For example, lowering  $[Cl^-]_e$  from 120 to 30 mM caused a depolarization from  $-99$  to  $-78$  mV as  $Cl^-$  left the sarcoplasm, followed by a hyperpolarization back to  $-99$  mV as  $[Cl^-]_i$  decreased (Hodgkin and Horowicz 1959). A major  $Cl^-$  effect is the stabilization of resting  $E_M$  from two points of view. First, it reduces the extent and rate of depolarization when  $[K^+]_e$  is increased. For example, exposing red sternomastoid fibers to 60 mM  $K^+$  resulted in a 25 mV depolarization within 3 min in the presence of

$\text{Cl}^-$  compared to a 32 mV depolarization within 1 min when  $\text{Cl}^-$  was replaced by sulfate (Dulhunty 1978). Second, in the absence of  $\text{Cl}^-$ , muscle fibers become myotonic; i.e., they spontaneously generate AP in the absence of any stimulation (Bretag 1987; Lehmann-Horn and Jurkat-Rott 1999).

Thus, while changes in  $[\text{Cl}^-]_e$  only causes transient change in resting  $E_M$ , the  $\text{Cl}^-$  effect on reducing the  $\text{K}^+$ -induced depolarization is another factor that reduces the  $[\text{K}^+]_e$ - $E_M$  slope from the expected Nernst potential.

Most of the measured  $[\text{K}^+]_e$ - $E_M$  slopes in Table 2 are less than the expected slope calculated from the Nernst potential. The mean difference ( $\pm$  standard error) between measured and expected slopes is  $10.2 \pm 4.7$  mV for amphibian muscles (13–25 °C), which is not that different from  $11.8 \pm 2.0$  mV for mammalian muscles (25 °C). However, the mean difference is higher in mammalian muscles at 37 °C, being  $22.3 \pm 3.5$  mV. This greater difference may partially be related to a greater contribution of the electrogenic NKA as it transports three  $\text{Na}^+$  out of and two  $\text{K}^+$  into the sarcolemma as well as a greater activity at higher temperatures. Indeed, the NKA electrogenic contribution to resting  $E_M$  increases from 10 mV at 19 °C (Hicks and McComas 1989) to 15–20 mV at 37 °C (Ammar et al. 2015; Chibalin et al. 2012).

Overall, resting  $E_M$  depends primarily on the distribution of three ions and the activity of one active transport. The sarcolemma behave as a  $\text{K}^+$  electrode, for which any changes in the  $[\text{K}^+]_e$  gradient result in a new steady state resting  $E_M$ . Changes in  $[\text{Cl}^-]_e$  gradient, on the other hand, causes transient change in resting  $E_M$  while  $\text{Na}^+$  has a very small effect as the sarcolemma is almost impermeable to this ion at rest. NKA also contributes to resting  $E_M$  because of (i) its electrogenic nature and (ii) the maintenance of the  $[\text{Na}^+]_e$  and  $[\text{K}^+]_e$  gradients.

## Action potentials

APs in muscle fibers are triggered when sarcolemmal  $E_M$  reaches a threshold, defined as the  $E_M$  at which  $\text{Na}_v$  channels start to open resulting in a subsequent increase in  $\text{Na}^+$  conductance ( $G_{\text{Na}}$ ) allowing large  $\text{Na}^+$  influx that rapidly depolarizes the sarcolemma from  $-70$  mV to  $+30$  mV. The repolarization back to  $-70$  mV depends on (i)  $\text{Na}_v$  channel fast inactivation to stop the depolarization and (ii) the activation of voltage-sensitive  $\text{K}^+$  channels ( $\text{K}_v$ ) that increases  $G_{\text{K}}$  and  $\text{K}^+$  efflux, allowing  $E_M$  to return to its original resting level (Hodgkin and Huxley 1952).

Although it is well established that AP kinetics depend primarily on  $\text{Na}_v$  and  $\text{K}_v$  channel characteristics, a  $\text{Cl}^-$  effect should not be ignored. The role of  $\text{Cl}^-$  in membrane excitability has been extensively reviewed (Pedersen et al. 2016), so here the role of  $\text{Cl}^-$  on sarcolemmal and t-tubular excitability is briefly discussed. Skeletal muscle expresses the

$\text{Cl}^-$  CIC-1 channel, a member of the large CIC family of  $\text{Cl}^-$  channels (Jentsch et al. 2002). CIC-1 channels are active at rest providing a  $G_{\text{Cl}}$  that is 2 to 4-times greater than the  $\text{K}^+$  conductance ( $G_{\text{K}}$ ) in amphibian muscles and 5 to 9-times greater than  $G_{\text{K}}$  in mammalian muscles (Pedersen et al. 2009b; Hodgkin and Horowitz 1959; Sperelakis 1969; Dulhunty 1979). The fraction of open CIC-1 channels is high at rest and remains nearly constant during a single AP. This is because AP only lasts 1–2 ms while the depolarization-induced activation of CIC-1 channels has time constants of 40 and 450 ms (Weiss and Magleby 1990; Fahlke and Rüdell 1995).

CIC-1 channels allow for  $\text{Cl}^-$  influx during both depolarization and repolarization phases of the AP because as soon as  $E_M$  depolarizes it becomes less negative than  $E_{\text{Cl}}$ , which favors a net  $\text{Cl}^-$  influx (Pedersen et al. 2016). This is supported by at least three studies. First, current–voltage relationship using rat psoas muscles show an outward  $\text{Cl}^-$  current upon membrane depolarization, which indicate a  $\text{Cl}^-$  influx (Fahlke and Rüdell 1995). Second, removing  $\text{Cl}^-$  from the bathing solutions or exposing mechanically rat EDL skinned fibers to 9-anthracene carboxylic acid (9-AC), a CIC-1 channel blocker, result in more negative AP threshold; this suggests that the  $\text{Cl}^-$  influx provide an outward current counteracting any depolarizing stimulus to trigger an AP, at least in t-tubules (Dutka et al. 2008). Third, removing  $\text{Cl}^-$  or the presence of 9-AC also prolong the AP repolarization phase in the t-tubules of frog semitendinosus muscle and in rat EDL t-tubules providing evidence for  $\text{Cl}^-$  influx that can contribute to the repolarization phase with  $\text{K}^+$  (Heiny et al. 1990; Dutka et al. 2008). However, under normal conditions with normal resting  $[\text{K}^+]_e$ , reducing  $[\text{Cl}^-]_e$  from 120 to 10 mM has little impact on AP kinetics (Cairns et al. 2004), possibly because the increase in  $G_{\text{Na}}$  is such that the  $\text{Na}^+$  inward current overwhelmingly exceeds the  $\text{Cl}^-$  outward current that opposes the  $\text{Na}^+$ -induced depolarization. However, this is not the case under conditions of high  $[\text{K}^+]_e$  (see section on “*Modulation of the  $\text{K}^+$ -induced force depression by changes in  $G_{\text{Cl}}$* ”).

A major consequence of the  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  fluxes during APs are changes in both intra- and extracellular  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  concentrations, which then affect resting  $E_M$ , membrane excitability and consequently force. To fully understand these processes, we must first look at the magnitude of these ionic changes during muscle activity and fatigue.

## Ionic disturbances during muscle contractions and fatigue

Initially, plasma ion concentrations were measured in the venous blood derived from contracting muscles. The advent of the microdialysis technique, however, has

enabled the determination of the interstitial ion concentrations. Notably, during fatiguing exercise increases in  $[K^+]_{int}$  are 4–7 mM greater than increases in venous plasma  $[K^+]$  (Green et al. 2000; Juel et al. 2000; Nielsen et al. 2004a; Street et al. 2005). The changes in plasma  $[K^+]$ , skeletal muscle  $[K^+]_{int}$  and  $[K^+]_i$  with exercise have recently been extensively reviewed (McKenna et al. 2023). So here, we briefly summarize changes in interstitial and intracellular ion concentrations, being closest to the sarcolemma and being the ion concentrations affecting resting  $E_M$  and AP.

Muscle  $[K^+]_{int}$  have been primarily measured in human studies, albeit there are two studies with animal muscles. In resting muscles,  $[K^+]_{int}$  varies between 4.1 and 4.4 mM, increasing to peak value of 7.5 to 13.7 mM during or immediately after fatiguing muscle contractions, while less intense muscle contractions resulted in smaller increases (Table 1). Muscle  $[K^+]_i$  values have, in contrast, mostly been reported in studies of isolated muscles from rats, mice or frogs and vary largely among studies; e.g., resting values range from 90 to 182 mM. Although the extent of the decrease in  $[K^+]_i$  with fatigue was quite variable, most studies (~80% in Table 1) reported decreases in  $[K^+]_i$  ranging from 1.1- to 1.5-fold. In line with this, in two human studies, vastus lateralis muscle  $[K^+]_i$  decreased from 161 to 129 mM in one study and to 141 mM in the other following exhaustive exercise (Sjøgaard 1983; Sjøgaard et al. 1985).

While both plasma and  $[K^+]_{int}$  are increased substantially during muscle contractions (Table 2, McKenna et al. 2023), the situation is different for  $Na^+$ . For example, one study in exercising humans reported a 15 mM decrease in  $[Na^+]_{int}$ , whilst venous  $[Na^+]$  increased by 8 mM (Street et al. 2005). The increase in venous  $[Na^+]$  occurs because of greater water than  $Na^+$  flux from plasma into muscle interstitial fluid (Sjøgaard et al. 1985; Lindinger et al. 1994), whilst the decrease in  $[Na^+]_{int}$  is because of this interstitial fluid influx, together with lower water than  $Na^+$  shift from muscle interstitium to fiber intracellular space. At rest,  $[Na^+]_i$  ranged between 10 and 29 mM and increased with fatigue in most studies, with values ranging from no change to a threefold increase (Table 3). Although there is a  $Cl^-$  influx during APs, the few reports of activity-induced changes in  $[Cl^-]_i$  in mammalian muscles are inconsistent (Table 4).

## Contribution of the ionic disturbances to changes in resting $E_M$ and action potential

### Resting $E_m$

$E_M$  measurements in muscle fibers are complicated by muscle movements during contraction often causing microelectrode damage or dislodgment out of the fiber. Very few studies continuously recorded  $E_M$  during the fatigue bout; most

**Table 3** Changes in interstitial and intracellular  $Na^+$  concentrations at rest and after fatigue

Species	Muscle	Rest (R)	Fatigue (F)	$\frac{[Na^+]_F}{[Na^+]_R}$	References
$[Na^+]_{int}$ (mM)					
Human	Vastus lateralis	143	128	-0.1	(Street et al. 2005)
$[Na^+]_i$ (mM)					
Frog	Semitendinosus	16	49	3.1	(Balog and Fitts 1996)
Human	Vastus lateralis	22	24	1.1	(Sjøgaard 1983)
Human	Vastus lateralis	13	23	1.8	(Sjøgaard et al. 1985)
Mouse	Soleus	13	23	1.8	(Juel 1986)
Mouse	Soleus	11	15	1.4	(Juel 1988)
Rat	EDL	18	66	3.7	(Nagaoka et al. 1994)
	soleus	28	62	2.2	
Rat	EDL	19	34	1.8	(Everts et al. 1993)
	soleus	29	36	1.9	
	tibialis	29	36	1.2	
Rat <sup>1</sup>	Soleus	28	27	0.96	(Lindinger and Heigenhauser 1987)
	plantaris	24	27	1.1	
	white gastrocnemius	12	16	1.3	
	red gastrocnemius	23	29	1.3	
Rat <sup>2</sup>	Soleus	26	33	1.3	(Lindinger et al. 1987)
	plantaris	11	14	1.3	
	white gastrocnemius	14	16	1.2	
	red gastrocnemius	10	13	1.3	

Concentration values are the reported mean as per each study. For  $[Na^+]_i$  measurements, fatigue was elicited in vitro by field stimulation with two exception; <sup>1</sup>muscles were stimulated in situ with intact blood flow and <sup>2</sup> $[Na^+]_i$  was measured following swimming

**Table 4** Changes in intracellular  $\text{Cl}^-$  concentration at rest and after fatigue

Species	Muscle	Rest (R)	Fatigue (F)	$\frac{[\text{Cl}^-]_F}{[\text{Cl}^-]_R}$	References
$[\text{Cl}^-]_i$ (mM)					
Human	Quadriceps femoris	21.9	25.8	1.2	(Sahlin et al. 1978)
Human	Quadriceps femoris	15.1	28.0	1.9	(Bergström et al. 1971)
Human	Quadriceps femoris	8.8	9.1	1.1	(Kowalchuk et al. 1988)
Rat <sup>1</sup>	Soleus	24	23	0.96	(Lindinger and Heigenhauser 1987)
	plantaris	17	26	1.5	
	white gastrocnemius	11	11	1.0	
Rat <sup>2</sup>	red gastrocnemius	15	23	1.5	(Lindinger et al. 1987)
	Soleus	11	13	1.2	
	plantaris	7	9	1.3	
Rat <sup>1</sup>	white gastrocnemius	9	11	1.2	(Lindinger and Heigenhauser 1988)
	red gastrocnemius	6.4	9.5	1.5	
	Soleus	13.5	22.3	1.7	
	plantaris	8.6	23.1	2.7	
Rat <sup>1</sup>	white gastrocnemius	7.7	12.0	1.6	(Lindinger and Heigenhauser 1988)
	red gastrocnemius	9.7	13.1	1.4	

Concentration values are the reported mean as per each study. Fatigue was elicited as follows: <sup>1</sup>perfused muscles were stimulated in situ; <sup>2</sup> $[\text{Cl}^-]_i$  was measured following swimming

studies measured  $E_M$  after fatigue. Among the latter, several  $E_M$  measurements were carried out at various times during the recovery allowing an extrapolation of  $E_M$  back to the last contraction of the fatigue bout. However, for studies in which data is only provided for the period after fatigue, one must take into account that some recovery may have resulted in some underestimation of the  $E_M$  changes during fatigue.

Some studies reported no sarcolemmal depolarization in human intercostal, rat intercostal and extensor carpi radialis longus muscles, when continuously stimulated at 10 Hz for 30 min (Hanson 1974a, b) (Table 5, bottom section). However, for both studies, twitch force either did not decrease (human muscle) or increased (rat muscle); so based on this, it is unlikely that there was any large extent of fatigue. In another study (Hicks and McComas 1989), for which changes in force was not reported, a 12 mV hyperpolarization was observed in rat soleus muscle stimulated with 4 s long train of 20 Hz pulses every 5 s for 5 min. When NKA activity was reduced with either an exposure to the specific inhibitor ouabain or with a reduction in temperature from 37 °C to 19 °C, resting  $E_M$  then depolarized by 4–5 mV during muscle activity. The authors concluded that the hyperpolarization during muscle activity under control conditions was because of an increased NKA activity contributing to resting  $E_M$ .

In contrast to the abovementioned studies, large sarcolemmal depolarization in both amphibian and mammalian muscles has been observed when there were large decreases in force; i.e., evidence of fatigue (Table 5, top section). The mean depolarization from all these studies is 16 mV, with a range from 7 to 35 mV. Changes in  $[\text{K}^+]_i$  gradient across the sarcolemma is most likely the major component responsible

for the depolarization. However, since most studies did not concomitantly measure changes in resting  $E_M$ ,  $[\text{K}^+]_{\text{int}}$  and  $[\text{K}^+]_i$ , here we estimate the extent of the  $\text{K}^+$  contribution to the depolarization in mammalian muscles by using mean values ( $\pm$  standard error) from Table 2. Mean  $[\text{K}^+]_{\text{int}}$ , from human studies, and  $[\text{K}^+]_i$ , from mostly animal studies, are in resting muscles respectively  $4.3 \pm 0.1$  and  $134.1 \pm 6.8$  mM, giving rise to a Nernst potential for  $\text{K}^+$  ( $E_K$ ) of -92 mV. After fatigue, the values are respectively  $10.1 \pm 0.9$  mM,  $108.1 \pm 5.9$  mM and -63 mV. This implies that mean  $E_K$  decreases by 29 mV; mostly from the increase in  $[\text{K}^+]_{\text{int}}$  (~23 mV, 79%) compared to the decrease in  $[\text{K}^+]_i$  (~6 mV, 21%). More importantly, the 29 mV decrease in  $E_K$  is almost twice as large as the 16 mV resting  $E_M$  depolarization. This is further supported by one study in which resting  $E_M$ ,  $[\text{K}^+]_{\text{int}}$  and  $[\text{K}^+]_i$  were concomitantly measured reporting a 24 mV decrease in  $E_K$  versus a 12 mV resting  $E_M$  depolarization (Juel 1986). Thus, changes in  $[\text{K}^+]_i$  gradient contribute to the sarcolemmal depolarization, but the extent of the depolarization is considerably less than that of  $E_K$ . This is most likely because of the NKA electrogenic contribution (Juel 1986; Hicks and McComas 1989) and the  $\text{Cl}^-$  stabilizing effect on resting  $E_M$  that reduces the  $\text{K}^+$ -induced depolarization as discussed above under resting conditions.

## Action potentials

The above studies for which there was no force loss and depolarization with muscle activity (Table 5 bottom), also reported no change in AP overshoot (the term overshoot was originally used to reflect the fact that during an AP the  $E_M$  peak became positive; i.e., the overshoot normally

**Table 5** Resting  $E_M$  and AP overshoot at rest and post stimulation

Species	Muscle	Electrical stimulation	Change in force	°C	Resting $E_M$ (mV)		Overshoot (mV)		References
					Rest	Post	Rest	Post	
Substantial fatigue									
Frog	Lumbrical <sup>1</sup>	C: 70 Hz, 30 s	-89%	22 °C	-85	-50	20		(Lannergren and Westerblad 1986)
Frog	Lumbrical I <sup>1</sup>	C: 70 Hz, 30 s	-60%	22 °C	-90	-75			(Westerblad and Lannergren 1986)
	II		-60%		-89	-65			
	III		-60%		-85	-70			
Frog	Lumbrical <sup>1</sup> Detubulated	C: 70 Hz, 30 s	Not measured	22 °C	-79	-46	32	-11	(Lannergren and Westerblad 1987)
Frog	Sartorius <sup>2</sup>	I: 80 Hz, 1TPS, 3 min	-82%	22 °C	-82	-68	32	30	(Renaud and Mainwood 1985a)
Frog	Sartorius <sup>2</sup>	I: 80 Hz, 1TPS, 3 min	See Note #4	22 °C	-87	-68			(Renaud and Mainwood 1985b)
Frog	Sartorius <sup>2</sup>	I: 80 Hz, 1TPS, 3 min	-81%	22 °C	-85	-71			(Renaud 1989)
Frog	Semitendinosus <sup>2</sup>	I: 150 Hz, 1TPS, 5 min	-90%	22 °C	-84	-77	19	10	(Balog et al. 1994)
Frog	Semitendinosus <sup>2</sup>	I: 150 Hz, 1TPS, 5 min	See Note #5	22 °C	-83	-74	20	7	(Balog and Fitts 1996)
Frog	Sartorius <sup>3</sup>	I: 200 Hz, 1TPS, 3 min	-89%	22 °C	-86	-78	26	24	(Light et al. 1994)
Mouse	EDL <sup>3</sup>	I: 40 Hz, 1TPS, 1 min	-90%	37 °C	-75	-56			(Juel 1986)
	Soleus <sup>3</sup>		-71%		-70	-58			
Mouse	Soleus <sup>3</sup>	I: 140 Hz, 1TPS, 3 min	-70%	37 °C	-78	-83			(Matar et al. 2000)
Mouse	FDB <sup>1</sup>	I: 140 Hz, 1TPS, 3 min	See Note #6	37 °C	-82	-58			(Cifelli et al. 2008)
Mouse	Soleus <sup>2</sup>	I: 40 Hz, 0.3 TPS, 5 min	-60%	35 °C	-70	-57			(Juel 1988)
Rat	Plantaris <sup>2</sup>	I:50 Hz, 0.4 TPS, 60 min	-71%	37 °C	-79	-71			(Karelis et al. 2005)
No fatigue									
Frog	EDL <sup>3</sup>	C: 10 Hz, 60 s	180%	20–24 °C			17	28	(Hanson and Persson 1971)
Human	Intercostal <sup>3</sup>	C: 10 Hz, 30 s	0%	37 °C	-80	-81	24	22	(Hanson 1974a)
Rat	Intercostal <sup>3</sup>	C: 10 Hz, 30 s	174% <sup>C</sup>	37 °C	-77	-75	30	24	(Hanson 1974a)
Rat	Extensor carpi radialis longus <sup>3</sup>	C: 10 Hz, 30 s	157% <sup>C</sup>	37 °C	-77	-73	26	25	(Hanson 1974b)
Rat	Soleus <sup>2</sup>	I:20 Hz, 0.8 TPS, 5 min	Not measured	37 °C	-80	-92	3	6	(Hicks and McComas 1989)
				19 °C	-80	-80	18		

The table top part reports changes in resting  $E_M$  and overshoot following substantial fatigue; i.e., when the decrease in force was at least 60%. The bottom portion reports changes in resting  $E_M$  and overshoot when there was either no or increase in twitch force. Muscles were either 'C' continuously stimulated at the indicated frequency and duration or 'I' intermittently stimulated to trigger tetanic contractions with the indicated frequency during the train in Hz, the number of train per sec (TPS) and fatigue period in min. <sup>1</sup> $E_M$  measurements were continuously carried out during the fatigue bout. <sup>2</sup> $E_M$  measurements were carried out before and at various times after the fatigue bout allowing an extrapolation of  $E_M$  data to the time of the last contraction. <sup>3</sup> $E_M$  measurements were carried out before and after the fatigue bout. <sup>4</sup>Tetanic forces were reported in the accompanying paper (Renaud and Mainwood 1985a) <sup>5</sup>Force was not measured in the study but authors referred to an earlier study by Balog et al. (1994). <sup>6</sup> Tetanic  $[Ca^{2+}]_i$  but not force was measured

represents the peak  $E_M$  above 0 mV. For this review the term overshoot will refer to the AP  $E_M$  peak value even when under some conditions it remains negative). Studies describing large resting  $E_M$  depolarization and force decrease also reported decreases in AP overshoot, from as small as 2 mV to as large as 43 mV (Table 5, top). Two mechanisms detailed below are involved in the decrease in

AP overshoot: (i) the decrease in  $[Na^+]$  gradient and (ii) a depolarization-induced  $Na_V$  channel inactivation.

A decrease in  $[Na^+]$  gradient reduces the  $Na^+$  current during the depolarization phase leading to smaller AP overshoot. For most experiments presented in Table 3 (15 out of 19 measures), the  $[Na^+]$  gradient decreased by 1.1 to 1.8-fold and for a minority of studies (4 out of 19 measures)

the decrease exceeded twofold. Increasing  $[Na^+]_i$  (Desmedt 1953) or decreasing  $[Na^+]_e$  (Nastuk and Hodgkin 1950) in amphibian muscles to mimic changes in  $[Na^+]$  gradient, resulted in lower AP overshoot. In mouse soleus muscle, 1.5 and 2.5-fold decreases in  $[Na^+]_e$  had little effect on resting  $E_M$  while it reduced AP overshoot by 10 and 20 mV, respectively (Cairns et al. 2003). These studies then support the notion that decreases in  $[Na^+]$  gradient can contribute to a decrease in AP overshoot.

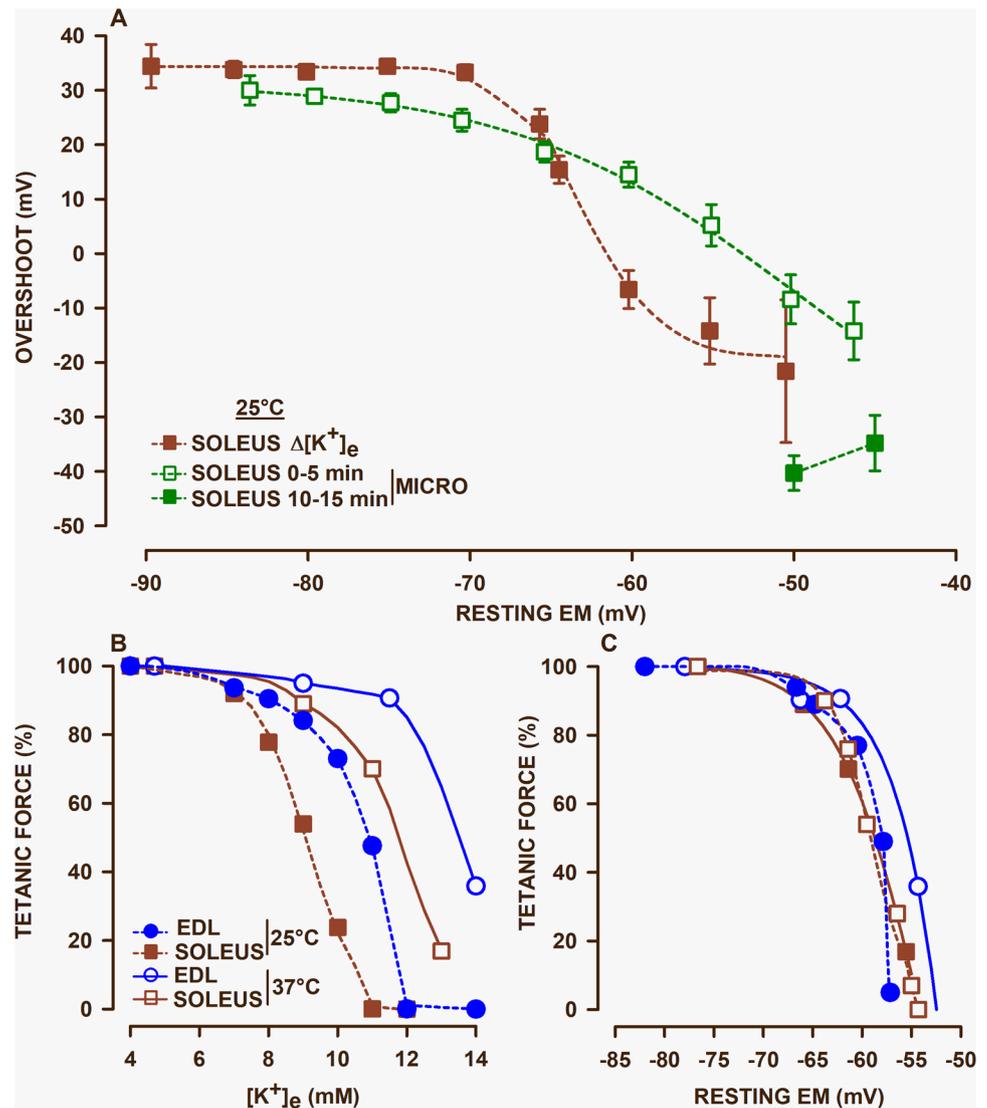
Depolarization-induced  $Na_V$  channel inactivation, fast and slow, is a second mechanism for overshoot depression during fatigue. Fast inactivation occurring in ms is crucial for (i) stopping the depolarization phase so that the subsequent  $K^+$  efflux can repolarize the membrane and (ii) allowing the unidirectional AP propagation. Studies on steady state fast inactivation reported that 10% of  $Na_V$  channels are fast inactivated when resting  $E_M$  is on average  $-90$  mV (range between studies from  $-105$  to  $-70$  mV); a value increasing to 50% at  $-67$  mV (range from  $-87$  to  $-50$  mV) and with all  $Na_V$  channels fast inactivated at  $-40$  mV (range from  $-60$  to  $-20$  mV) (Cummins and Sigworth 1996; Cummins et al. 1993; Bendahhou et al. 2002, 1995, 1999; Hayward et al. 1996, 1997, 1999; Webb et al. 2009; Wu et al. 2014; Kuzmenkin et al. 2003; Rojas et al. 1999). Slow inactivation occurs over seconds to minutes and is believed to be an important regulator of  $Na_V$  channel activity during muscle activity. Studies on steady state slow inactivation, in which  $Na_V1.4$  channel, the  $Na_V$  channel expressed in skeletal muscle, is transfected in HEK-293 cells and oocytes, reported that 10% of  $Na_V1.4$  channels were slow inactivated at  $-90$  mV, being 50% at an average of  $-63$  mV (range between studies from  $-70$  to  $-60$  mV) and with all  $Na_V1.4$  channels being slow inactivated at  $-10$  mV (from  $-20$  mV to  $0$  mV). Other studies using skeletal muscle fibers reported more hyperpolarized steady state slow inactivation; i.e., 10% decrease at  $-107$  mV (range from  $-120$  to  $-90$  mV), 50% decrease at  $-90$  mV (range from  $-110$  to  $-70$  mV) and no current at  $-60$  mV (range  $-80$  to  $-50$  mV) (Featherstone et al. 1996; Ruff 1996, 1999; Ruff et al. 1988; Kirsch and Anderson 1986; Simoncini and Stuhmer 1987). Ruff (Ruff et al. 1988) has proposed that slow inactivation of  $Na^+$  channels is important in the decreased sarcolemmal excitability with fatigue. If depolarization-induced slow as well as fast inactivation plays a role in the decrease in AP amplitude, then the  $E_M$  range for which AP amplitude decreases should correspond to the  $E_M$  range for which fast and slow inactivation occurs.

One approach to induce membrane depolarization is to increase  $[K^+]_e$ . For mouse soleus and extensor digitorum longus (EDL) muscles, AP amplitude decreased by 50% at resting  $E_M$  of  $-70$  and  $-60$  mV, respectively (Ammar et al. 2015; Wang et al. 2022). Maximum rate of depolarization, often used as an index of  $Na^+$  current (Hodgkin and Katz

1949), decreased by 50% at  $-64$  mV (Uwera et al. 2020). Thus, the  $E_M$  range at which AP amplitude and  $Na^+$  current is reduced by 50% correspond to the  $E_M$  range at which fast inactivation reaches 50% as mentioned above. It also corresponds to the  $E_M$  range for 50% slow inactivation when  $Na_V1.4$  channels are expressed in HEK-293 cells and oocytes. Similarly, muscle fibers do not generate AP when resting  $E_M$  becomes less negative than  $-50$  mV (Ammar et al. 2015; Wang et al. 2022; Uwera et al. 2020) corresponding to the  $E_M$  range at which there is complete  $Na_V1.4$  channel fast inactivation. Taken together, these studies supports the notion that decreases in AP amplitude/overshoot during fatigue are in part due to the sarcolemmal depolarization triggering  $Na^+$  channel inactivation.

One issue with the  $K^+$ -induced depolarization approach is the long time required for tetanic force to reach a new steady state following an increase in  $[K^+]_e$ , i.e. up to 30–40 min. Usually AP are captured in fibers for which resting  $E_M$  remains stable upon microelectrode penetration. However, in a recent study (Cairns et al. 2022), APs were also measured in fibers exposed to 4 mM  $[K^+]_e$  that did not maintain a constant resting  $E_M$  upon microelectrode penetration. The depolarization lasted  $\sim 5$  min before staying constant over the next 10 min, the latter suggesting that the initial five min depolarization was due to some shifts in  $[K^+]$  and  $[Cl^-]$  gradients as opposed to microelectrode-induced sarcolemmal damages that would have caused much greater depolarization. Figure 1A compares the overshoot-resting  $E_M$  relationship measured during the short depolarization period (i.e., 0 to 5 min microelectrode-induced depolarization) and the prolonged depolarization ( $K^+$ -induced depolarization). Mean overshoots were quite similar between the two measurements for resting  $E_M$  from  $-90$  to  $-67$  mV. At resting  $E_M$  less negative than  $-67$  mV, overshoot depression was greater with the  $K^+$ -induced than the microelectrode-induced depolarization. Despite a stable resting  $E_M$  10–15 min after the microelectrode penetration, overshoot continued to decrease, reaching values that were even less negative than those measured at elevated  $[K^+]_e$ . For example, at resting  $E_M$  of  $-50$  mV, mean AP overshoot was  $-8$  mV after 5 min of depolarization further decreasing to  $-40$  mV for the 10–15 min period during which resting  $E_M$  remained at  $-50$  mV. The most likely mechanism responsible for the further decrease in overshoot while resting  $E_M$  remained is slow inactivation that can take up to 20 min to reach steady state (Webb et al. 2009). Cairns et al. (2022) also reported another important observation; that is a complete lack of inexcitable fibers at all resting  $E_M$  during the microelectrode-induced depolarization compared to several fibers becoming inexcitable for the  $K^+$ -induced depolarization. Considering that most fatigue protocols involve stimulation periods of 5 min or less, the Cairns et al. (2022) study raises the possibility that the resting  $E_M$  at which large decreases in AP

**Fig. 1** **A** Action potential overshoot-resting  $E_M$  relationship, **B** mean tetanic force- $[K^+]_e$  relationship and **C** mean tetanic force-mean resting  $E_M$  relationship in mouse EDL and soleus muscles. **A** Resting  $E_M$  were measured at different ( $\Delta$ )  $[K^+]_e$  after tetanic force had reached a steady state (over a 30–40 min period) or at 4 mM  $K^+$  but in fibers for which resting  $E_M$  depolarized over a 5 min period before remaining stable over another 10 min period following microelectrode (micro) penetration. Resting  $E_M$  were separated in bin of 5 mV and overshoot were averaged (vertical bars being standard error; not visible if smaller than symbols); data from (Cairns et al. 2022). **B** and **C** Data at 25 °C from Cairns et al. 1997; data at 37 °C from Ammar et al. 2015



amplitude, and thus force, most likely occur at more depolarized resting  $E_M$  than what we have so far estimated from  $K^+$ -induced depolarization as shown in Fig. 1C. Nevertheless, all these studies still support fast/slow inactivation as a second mechanism responsible for the decrease in AP overshoot during fatigue.

In summary, resting  $E_M$  depolarizes when there is evidence of fatigue; i.e., there is a decrease in force and this depolarization is primarily the result of a reduced  $[K^+]_e$  gradient. As a consequence of the depolarization, the extent of  $Na^+$  channel inactivation increases, resulting in lower AP overshoot/amplitude, which is further lowered by a reduced  $[Na^+]_e$  gradient. There is now abundant evidence for a decrease in SR  $Ca^{2+}$  release and reduced active  $[Ca^{2+}]_i$  ( $[Ca^{2+}]_i$ ), defined as the  $[Ca^{2+}]_i$  during contraction, as a major mechanism by which force decreases during fatigue (Allen et al. 1989, 2008a; Lee et al. 1991; Westerblad and Allen 1991). The question is whether the  $K^+$  and  $Na^+$

changes associated with muscle APs are large enough to be a major cause for the reduced  $Ca^{2+}$  release and force during fatigue.

### The $K^+$ -induced force depression

Decreases in active  $[Ca^{2+}]_i$  have been demonstrated in frog semitendinosus and mouse FDB muscle fibers with concomitant decrease in shortening capacity in mouse FDB when  $[K^+]_e$  is increased (Lucas et al. 2014; Quinonez et al. 2010). More importantly, a recent study has clearly demonstrated a relationship between AP overshoot and active  $[Ca^{2+}]_i$  in which active  $[Ca^{2+}]_i$  remains stable from +30 to 0 mV, decreasing rapidly as AP peak became less than 0 mV, with no  $Ca^{2+}$  release by -40 mV (Wang et al. 2022). The changes in and effects of  $K^+$  on resting  $E_M$  and AP and the relationship between AP and  $Ca^{2+}$  release strongly support a

role for  $K^+$  in the decrease in force during fatigue. However, the  $K^+$ -force relationships for twitch and tetanic contractions are more complicated than originally thought. For tetanic contractions, there is a range of  $[K^+]_e$  for which tetanic force remains close to maximal ( $\geq 90\%$ ) despite significant resting  $E_M$  depolarization and lower overshoot, until a  $[K^+]_e$  is reached above which force declines abruptly. For twitch and sub-maximal tetanic contractions, small increases in  $[K^+]_e$  actually potentiate force, while at higher  $[K^+]_e$ , twitch and sub-maximal tetanic force are decreased. In this section, we discuss the  $K^+$ -induced force depression on tetanic force and how it is modulated by changes in  $[Na^+]$  gradient,  $G_{Cl}$  and NKA activity, then followed by a subsequent section discussing  $K^+$ -induced potentiation.

### Tetanic force- $[K^+]_e$ relationship

The maximum force a muscle can generate is measured during a completely fused tetanus. For frog sartorius and mouse EDL muscles at 25 °C, peak tetanic force decreases by less than 10–15% when  $[K^+]_e$  is raised from 4 to 9 mM and is completely abolished at 12 mM  $[K^+]$  (Fig. 1B) (Renaud and Light 1992; Cairns et al. 1997). The critical  $[K^+]_e$ , defined as the  $[K^+]_e$  above which tetanic force drops abruptly, is 9 mM for these two muscles. Notably, mouse soleus is more sensitive to raised  $[K^+]_e$  as the critical  $[K^+]_e$  is only 7 mM and no force is generated at 11 mM (Fig. 1B). The differences between mouse soleus and EDL tetanic force- $[K^+]_e$  relationships persist at 37 °C, but with higher critical  $[K^+]_e$ , being 9 and 12 mM for soleus and EDL, respectively (Fig. 1B) (Ammar et al. 2015). Similarly for rat muscles at 30 °C, the critical  $[K^+]_e$  for soleus and EDL were 9 and 11 mM, respectively (Hansen et al. 2005; Pedersen et al. 2003; Cairns et al. 1995). Soleus is known as a slow-twitch fatigue resistant muscle primarily composed of type I fibers, being 87% of all fibers in rat and 67% in mouse; the remaining fibers being type IIA fibers (Banas et al. 2011; Armstrong and Phelps 1984). EDL, on the other hand, is a fast twitch fatigable muscle with fibers being composed primarily of type IIB (56–57% in rat and mouse) and IIX (46% in mouse). Therefore, the most fatigue resistant type I and IIA fibers have lower critical  $[K^+]_e$  values than the fatigable type IIB and IIX fibers.

Another observation from the above studies was that the higher the experimental temperature the higher the critical  $[K^+]_e$ . This was especially confirmed in rat soleus as the critical  $[K^+]_e$  values were 8, 9 and 10 mM at 20°, 30° and 35 °C, respectively (Pedersen et al. 2003). There is one study reporting faster and greater  $K^+$ -induced force depression at 37 °C than at 25 °C when mouse soleus were exposed to 10 mM  $[K^+]_e$  (Cairns et al. 2011). It is more than likely, however, that the critical  $[K^+]_e$  increases with temperature from two points of view. First, the  $K^+$ -induced

depolarization is less at 37 °C than at 25 °C (Table 2). For mouse EDL, the depolarization per decade change in  $[K^+]_e$  is 48 mV at 25 °C and 42 mV at 37 °C; for soleus, the values are respectively 45 and 37 mV. Thus, one should expect that less depolarization at 37 °C results in a higher critical  $[K^+]_e$ . Second, as discussed above, the NKA electrogenic contribution is greater at 37 °C, being 15–20 mV, than at 19 °C, being 10 mV (Hicks and McComas 1989; Ammar et al. 2015; Juel 1986; Chibalin et al. 2012). More importantly, if NKA activation with salbutamol reduces the rate and extent of the force loss at high  $[K^+]_e$  and increases the critical  $[K^+]_e$  (Andersen and Clausen 1993; Clausen et al. 1993; Clausen and Everts 1991; Pedersen et al. 2003), then greater NKA activity at 37 °C should give rise to slower and smaller force loss at 37 °C than at 25 °C for a given increase in  $[K^+]_e$ .

The most important aspect to consider is the tetanic force-resting  $E_M$  relationship because the  $K^+$  effect is not direct but via a depolarization of the sarcolemma. Muscle  $[K^+]_{int}$  have been primarily measured in human studies. For frog sartorius, mouse EDL and soleus muscles at 25 °C, tetanic force remains constant from -95 to -70 mV, drops by about 10% from -70 to -65 mV and reaches zero between -60 and -55 mV (Fig. 1C) (Renaud and Light 1992; Cairns et al. 1997, 2022); i.e., 90% of the force loss occurs over a 5–10 mV range. Notably, the differences between EDL and soleus for the tetanic force- $[K^+]_e$  relationship shown in Fig. 1B are no longer observed for the tetanic force-resting  $E_M$  relationship as shown in Fig. 1C; i.e., the greater  $K^+$  sensitivity of soleus is due to greater depolarization at a given  $[K^+]_e$ . The tetanic force-resting  $E_M$  relationship of mouse soleus is basically the same at 25 °C and 37 °C, while it is slightly shifted toward less negative resting  $E_M$  for EDL. The small difference in tetanic force-resting  $E_M$  relationship between EDL and soleus at 37 °C may be related to smaller decreases in overshoot when resting  $E_M$  becomes less negative than -70 mV (Ammar et al. 2015).

It is important to note that the tetanic force-resting  $E_M$  relationships in Fig. 1C are derived from studies using  $K^+$ -induced depolarization. If we now take into consideration the Cairns et al. 2022 study discussed above, the critical resting  $E_M$  at which force decreases abruptly may be more depolarized than what is shown in Fig. 1C. That is, from the AP overshoot-resting  $E_M$  relationship obtained with  $K^+$ -induced depolarization a sudden drop in AP peak to -10 mV occurred when resting  $E_M$  dropped from -65 to -60 mV (Fig. 1A). Under the same conditions, the critical resting  $E_M$  for the abrupt tetanic force decrease occurred between -60 mV and -55 mV (Fig. 1C) suggesting that it occurs when AP peak is -10 mV or more negative. For the shorter microelectrode-induced depolarization, a decrease in AP peak to -10 mV occurred at -50 mV (Fig. 1A) representing a 10 mV shift toward less negative

resting  $E_M$  compared to the  $K^+$ -induced depolarization. Assuming that tetanic force abruptly declines once AP peak becomes  $-10$  mV or more negative, then for shorter depolarization period the critical resting  $E_M$  for abrupt tetanic force loss would be  $-50$  mV instead of  $-60$  mV. Thus, to fully understand the role of  $K^+$  in fatigue, future studies are necessary to determine the full time course of the AP depression following increases in  $[K^+]_e$  as opposed to when tetanic force reaches a steady state after more than 30 min.

The next question is whether the changes in  $[K^+]_e$  and  $[K^+]_i$  during fatigue by itself can be considered a major factor in the mechanism of fatigue. In comparing the relationships between  $[K^+]_e$ , resting  $E_M$ , AP and force in frog sartorius, Light et al. (1992) demonstrated that while an increase in  $[K^+]_e$  to 7 mM in unfatigued sartorius muscles mimicked changes in resting  $E_M$  and AP during fatigue, the increased  $[K^+]_e$  had little effect on tetanic force of unfatigued muscle. Furthermore, increasing  $[K^+]_e$  to 7.5 mM immediately after a fatigue bout did not reduce recovery of force after fatigue despite preventing a recovery of resting  $E_M$  (Comtois et al. 1994). In humans, during 30 min moderate (30 Watts, W) knee extension exercise, mean  $[K^+]_{int}$  increased to 10 mM within 5 min but then decreased to a steady state level ranging between 7 and 9 mM; mean  $[K^+]_{int}$  reached 9.7 mM during exhaustive exercise (Nielsen et al. 2004a). Thus, the difference in peak  $[K^+]_{int}$  between a non- exhausting and exhausting exercise was not only small, but slightly less in exhaustive exercise. Most studies in which  $[K^+]_{int}$  was measured by microdialysis in human reported a mean value not exceeding 12 mM (Table 1). Although more studies are needed for  $[K^+]_{int}$  in animal muscles, changes during fatigue in  $[K^+]_i$  of human vastus lateralis muscles are within the range reported for mouse and rat muscles (Table 1). So, if the changes during fatigue in  $[K^+]_{int}$  (measured in human muscles) and if the critical  $[K^+]_e$  (measured in mouse and rat muscles) are representative of the situation in human, mouse and rat muscles, then 12 mM  $[K^+]_{int}$  at 37 °C is expected to reduce tetanic force by 50% in fatigue resistant muscles such as the soleus, but having little effect in fatigable muscles such as the EDL. Human muscle has a mixed fiber type composition, so the expected effects would be intermediate between these two different types of muscles. Overall, these results do not strongly support the concept that perturbations in  $K^+$  (i.e., both  $[K^+]_{int}$  and  $[K^+]_e$ ) per se are not a major mechanism for the decrease in force during fatigue at least in muscles with low fatigue resistance. Further studies are necessary to determine  $[K^+]_{int}$  in animal models as well as the critical  $[K^+]_e$  in human muscles. However, as discussed below, one cannot exclude  $K^+$  as a potential factor in muscle fatigue without looking at an interaction with  $Na^+$ ,  $Cl^-$  and NKA.

### **Modulation of the $K^+$ -induced force depression by $Na^+$**

Although studies reported faster and greater extent of force decrease when amphibian and mammalian muscles were fatigued at lowered extracellular  $[Na^+]_e$  ( $[Na^+]_e$ ) compared to control conditions (Cairns et al. 2003; Bezanilla et al. 1972), one must also determine if the  $[Na^+]_e$  and  $[Na^+]_i$  changes during fatigue significantly affect force in unfatigued muscles. In frog sartorius, mimicking a reduction in  $[Na^+]_e$  gradient observed with fatigue (Table 3) by lowering  $[Na^+]_e$  by 1.2 and twofold (i.e., from 120 to 100 and 60 mM) reduced peak force by 10% and 30%, respectively (Boucllin et al. 1995). Mammalian muscles are more resistant to a decrease in  $[Na^+]_e$ . In rat soleus and mouse EDL and soleus, a twofold reduction in  $[Na^+]_e$  from 147 to 75 mM had no effect on tetanic force as significant decreases in tetanic force occurred at  $[Na^+]_e$  below 40 mM, i.e., a 3.8-fold decrease in  $[Na^+]_e$  gradient (Overgaard et al. 1997, 1999; Cairns et al. 2003). Considering that most studies report a less than twofold decrease in  $[Na^+]_e$  gradient during fatigue (Table 3), it would appear that the change in  $[Na^+]_e$  gradient is by itself in most cases too small to be of any major importance in the force decrease during fatigue, despite its effects on AP as discussed above.

Concomitant changes in  $Na^+$  and  $K^+$  gradients, on the other hand, have a synergistic depressive effect on tetanic force; i.e., their combined effects are greater than their additive effects. In frog sartorius, tetanic force decreased by about 8% when either  $[K^+]_e$  was increased from 3 to 7 mM to mimic a 2.3-fold decrease in  $[K^+]_e$  gradient, or when  $[Na^+]_e$  was decreased from 120 to 110 mM to mimic a 1.2-fold decrease in  $[Na^+]_e$  gradient. If the  $Na^+$  and  $K^+$  effects were additive, the concomitant change in  $Na^+$  and  $K^+$  gradient should lower force by 15% whereas a much greater 31% decrease was actually observed (Boucllin et al. 1995). In rat soleus muscle, tetanic force decreased by 10% when  $[K^+]_e$  was increased from 4 to 9 mM (2.3-fold reduction in the  $[K^+]_e$  gradient) and remained constant when  $[Na^+]_e$  was reduced from 147 to 85 mM (1.7-fold in the  $[Na^+]_e$  gradient); concomitant changes of both gradients resulted in a 50% force reduction (Overgaard et al. 1999). Finally, in mouse soleus, a 2.0-fold increase in  $[K^+]_e$  from 4 to 8 mM decreased tetanic force by 9% and a 1.5-fold decrease in  $[Na^+]_e$  (from 147 to 100 mM) reduced force by 3%, while concomitant changes in  $[K^+]_e$  and  $[Na^+]_e$  resulted in a force depression of 40%, more than threefold greater than a calculated additive effect of 12% (Cairns et al. 2022). Noticeably, Cairns et al (2022) reported that a similar concomitant change in  $[K^+]_e$  and  $[Na^+]_e$  had an additive and not a synergistic depressive effect on single AP, albeit the effect may be different for a train of APs. Furthermore, they reported that 15% of soleus fibers became inexcitable when  $[K^+]_e$  was increased from 4 to 8 mM while

a decrease in  $[\text{Na}^+]_e$  from 147 to 100 mM had no effect; concomitant changes in both  $[\text{Na}^+]_e$  and  $[\text{K}^+]_e$  resulted in 20% of fibers becoming inexcitable suggesting that  $\text{Na}^+$  and  $\text{K}^+$  have a small synergistic effect on sarcolemmal excitability. Thus, reductions in either  $[\text{Na}^+]$  or  $[\text{K}^+]$  gradients observed during fatigue have by themselves limited adverse impact on tetanic force, whereas concomitant reductions in  $[\text{K}^+]$  and  $[\text{Na}^+]$  gradients result in tetanic force decreases that are large enough to suggest that the combined changes in  $[\text{K}^+]$  and  $[\text{Na}^+]$  gradient are important in the mechanism of muscle fatigue.

### **Modulation of the $\text{K}^+$ induced force depression by changes in $G_{\text{Cl}}$**

As discussed in the section on AP, there is a net  $\text{Cl}^-$  influx during both AP depolarization and repolarization phases (Dutka et al. 2008; Heiny et al. 1990; Fahlke and Rüdell 1995). Under normal resting  $[\text{K}^+]_e$  of ~4 mM and  $[\text{Na}^+]_e$  of ~147 mM, reducing  $[\text{Cl}^-]_e$  to 10 mM had no long-lasting effect on resting  $E_M$ , AP or tetanic force in unfatigued mouse soleus, while it increased the rate of fatigue (Cairns et al. 2004). As discussed in the section on resting  $E_M$ , a major effect of  $\text{Cl}^-$  is a slower and lower extent of the  $\text{K}^+$ -induced membrane depolarization; this effect implies that a decrease in  $[\text{Cl}^-]_e$  or of  $G_{\text{Cl}}$  should increase the  $\text{K}^+$ -induced force depression and thus the rate of fatigue. However, a series of studies demonstrated that the  $\text{Cl}^-$  effects are more complex.

In one study, an increase of  $[\text{K}^+]_e$  to 11 mM at a normal  $\text{pH}_e$  of 7.4 reduced tetanic force and M-wave (an extracellular measurement of APs from the muscle surface) to 20–25% of the initial values measured at 4 mM (Pedersen et al. 2005). The extracellular pH ( $\text{pH}_e$ ) was then lowered from 7.4 to 6.8 by raising  $\text{CO}_2$  in the gas phase from 5 to 24%, in order to reduce  $G_{\text{Cl}}$ , as  $\text{Cl}^-$   $\text{ClC-1}$  channels are pH-sensitive (Hutter and Warner 1967; Palade and Barchi 1977). Following the decrease in  $\text{pH}_e$  to 6.8, both tetanic force and M-wave area increased to 80–90% of initial values at 4 mM  $[\text{K}^+]_e$  and  $\text{pH}_e$  7.4. Likewise, at 9 mM  $[\text{K}^+]_e$ , the same decrease in  $\text{pH}_e$  increased the number of excitable fibers from 48 to 94% and AP overshoot by 10 mV (Pedersen et al. 2005). Accordingly, lowering  $\text{pH}_e$  from 7.4 to 6.8 shifted the tetanic force- $[\text{K}^+]_e$  relationship by 2 mM toward higher  $[\text{K}^+]_e$ . These acidic  $\text{pH}_e$  effects were associated with a 46% reduction in  $G_{\text{Cl}}$  (with no effect on  $G_{\text{K}}$ ). Mimicking the reduction in  $G_{\text{Cl}}$  by lowering  $[\text{Cl}^-]_e$  as well as by exposing soleus to 9-AC at  $\text{pH}_e$  7.4 had the same effect on force and M-wave as the low  $\text{pH}_e$ . The authors concluded that a partial decrease in  $G_{\text{Cl}}$  is the mechanism by which acidic  $\text{pH}_e$  caused an increase in tetanic force and M-wave area during the  $\text{K}^+$ -induced depolarization.

The above conclusion was further supported by another study in which mechanically skinned fibers with intact

t-tubules were exposed to various  $[\text{K}^+]_i$  in order to alter t-tubular resting  $E_M$  (Pedersen et al. 2004). When contractions were elicited with electrical stimulations to trigger APs in t-tubules, a decrease in intracellular pH ( $\text{pH}_i$ ) from 7.1 to 6.6 shifted the force- $[\text{K}^+]_i$  relationship toward lower  $[\text{K}^+]_i$ , i.e., more depolarized t-tubules. A similar shift was not observed when i)  $\text{Cl}^-$  was removed from the bathing solution and ii) when contractions were elicited via an activation of the voltage sensor (also known as  $\text{Ca}_v1.1$  channel or dihydropyridine receptor). The authors concluded that greater t-tubular depolarization was necessary to induce force loss in the presence of  $\text{Cl}^-$  (or  $G_{\text{Cl}}$ ) in acidic than in normal  $\text{pH}_i$ .

In a third study (de Paoli et al. 2013), rat soleus muscles were stimulated for 30 s train at 60 Hz. Under those conditions, force reached a plateau in about 2 s and decreased constantly thereafter. The extent of the depolarization between APs became greater as  $[\text{Cl}^-]_e$  was decreased stepwise from 127 to 0 mM. Despite greater depolarization, the rate at which force decreased became slower when  $[\text{Cl}^-]_e$  was lowered from 127 to 60 mM (to lower  $G_{\text{Cl}}$ ) and then became faster from 60 to 0 mM  $\text{Cl}^-$ . The authors concluded that any decrease in  $G_{\text{Cl}}$  worsens the  $\text{K}^+$ -induced depolarization whereas small decrease in  $G_{\text{Cl}}$  improves membrane excitability and tetanic force while large decrease in  $G_{\text{Cl}}$  worsens the  $\text{K}^+$ -induced decrease in membrane excitability and force by mechanisms explain below.

The mechanism of action by which decreases in  $G_{\text{Cl}}$  affects membrane excitability and tetanic force as  $[\text{K}^+]_e$  increases have been extensively reviewed (Nielsen et al. 2017; Pedersen et al. 2016). Briefly, three issues must be taken into account. First, when a stimulation, either electrical during an experiment or at the neuromuscular junction following acetylcholine binding to its receptor, depolarizes the membrane toward AP threshold, there is a constant  $\text{Cl}^-$  influx that counteracts the stimulation-induced depolarization. AP threshold becomes less negative following prolonged depolarization, induced either by higher  $[\text{K}^+]_e$  or continuous stimulations as  $\text{Na}_v$  channels become inactivated. As a consequence of a less negative threshold, greater stimulation current is needed to reach it. Lowering  $G_{\text{Cl}}$  reduces the  $\text{Cl}^-$  influx that opposes the stimulatory depolarization. This explains why the number of excitable fibers increases at 9 mM  $\text{K}^+$  when  $G_{\text{Cl}}$  is lowered by decreasing  $\text{pH}_e$ . Second, as discussed in the section on AP, there is a constant  $\text{Cl}^-$  influx during AP depolarization and repolarization phases. Under normal conditions,  $G_{\text{Na}}$  during the AP depolarization is substantial and largely overwhelms the counteracting  $\text{Cl}^-$  current that opposes the depolarization; i.e., the  $G_{\text{Na}}:G_{\text{Cl}}$  ratio is very high. This is no longer the case when  $\text{Na}_v$  channels are inactivated by prolonged membrane depolarization. However, small decreases in  $G_{\text{Cl}}$  has two opposing effects: it allows (i) for greater  $\text{K}^+$ -induced depolarization and (ii) greater  $G_{\text{Na}}:G_{\text{Cl}}$  ratio. If small decrease in

$G_{Cl}$  improves AP amplitude and force at raised  $[K^+]_e$ , then one can suggest that the increase in AP amplitude due to greater  $G_{Na}:G_{Cl}$  ratio largely overcomes the expected lower AP amplitude due to the greater  $K^+$ -induced depolarization. Third, there is an optimum decrease in  $G_{Cl}$  for which the extent of the depressive effects of any depolarizations on excitability and force is at its lowest. As shown by de Paoli et al. (2013), small decreases in  $G_{Cl}$ , induced by decreases in  $[Cl^-]_e$  from 127 to 60 mM, reduce the extent of the depressive effects of any membrane depolarization because the increased  $G_{Na}:G_{Cl}$  ratio improves AP threshold and allows greater AP depolarization. Further decreases in  $G_{Cl}$  not only worsen the  $K^+$ -induced depolarization but it may do it to the point at which the depolarization depressive effects as  $Na_v$  channel inactivation becomes too great resulting in further decrease in membrane excitability and force.

### **Modulation of the $K^+$ -induced force depression by NKA**

NKA is largely responsible for the maintenance of the  $[Na^+]_i$  and  $[K^+]_i$  gradients across the muscle membrane. The NKA mechanisms of action, molecular isoforms and activity regulation in muscle have been extensively reviewed (Pirkmajer and Chibalin 2016; Clausen 2003, 2013; McKenna et al. 2023). Here, we briefly discuss how NKA, its activation and inhibition, modulates the  $K^+$  effects on force depression. Exposing unfatigued soleus muscles to 12.5 mM  $K^+$  reduced tetanic force to zero within 20 min, while in the presence of 10  $\mu$ M ouabain, a NKA-specific inhibitor, the decrease occurred in only 2 min; conversely, NKA activation with 10  $\mu$ M salbutamol, a  $\beta_2$ -adrenergic receptor agonist, reduced the rate of force decrease, reaching zero after 40 min (Clausen and Everts 1991). Slower force decrease also occurred when NKA was activated by insulin, epinephrine and calcitonin gene related peptide (CGRP) (Andersen and Clausen 1993; Clausen and Everts 1991; Clausen et al. 1993; Clausen and Flatman 1977). Furthermore, activating NKA after force had decreased to a steady level at elevated  $[K^+]_e$  or after a concomitant increase in  $[Na^+]_i$  and decrease in  $[K^+]_i$  allowed for large force recovery (Andersen and Clausen 1993; Clausen et al. 1993; Macdonald et al. 2005; Pedersen et al. 2003). Improvement of tetanic force in the presence of salbutamol correlated with improvement of M-waves, which suggest an improvement of membrane excitability (Overgaard et al. 1999). Thus, activation of NKA has the capacity to reduce the rate and extent of the  $K^+$ -induced force depression in unfatigued skeletal muscle.

NKA activity increases during muscle activity (see review by (McKenna et al. 2023)). This for example was shown as 2 Hz stimulation for 10 min or 60–120 Hz stimulation for 10 s increased ouabain-suppressible  $Na^+$  efflux and  $K^+$  influx in rat soleus muscle (Everts and Clausen 1994; Nielsen and

Clausen 1997). More importantly, stimulating soleus muscle with 1.5–2 s long 30 Hz tetanic contractions every min after force had been depressed at 10 mM  $K^+$  allowed for full force recovery; for soleus exposed to 12.5 mM  $K^+$  the stimulation allowed for a partial recovery (Overgaard and Nielsen 2001; Nielsen et al. 1998). The force recovery was associated with a partial recovery of resting  $E_M$  and membrane excitability, with the latter determined by M-waves. Furthermore, Nielsen et al. (1998) provided evidence that resting  $E_M$  and force recovery were associated with increases in NKA activity brought about by the release of CGRP from neurons innervating skeletal muscle. Salbutamol, epinephrine, insulin and CGRP, all NKA activators, substantially reduced the rate at which force declined when rat soleus was continuously stimulated at 60 Hz for min while exposed at various  $[K^+]_e$  (Clausen and Nielsen 2007). Finally, when soleus muscles were stimulated with 400 ms long tetanic stimulation at 40 Hz every 3rd s for 5 min and compared to control, 10  $\mu$ M, terbutaline, a  $\beta_2$ -adrenergic receptor agonist, reduced the extent of the resting  $E_M$  depolarization by 35%, the  $[K^+]_i$  decrease by 31%, the  $[Na^+]_i$  increase by 25% and the force decrease by 10% (Juel 1988). Juel (1988) suggested that the terbutaline effects involved a NKA activation. Thus, muscle contractions induce NKA activation, which then has the capacity to minimize perturbations in muscle  $E_M$ ,  $[K^+]_i$ ,  $[Na^+]_i$  and force.

In resting unfatigued skeletal muscle, the electrogenic NKA contribution to resting  $E_M$  under normal  $[K^+]_e$  conditions (i.e., 4–5 mM  $K^+$ ) is 12–20 mV in EDL, soleus and diaphragm muscles (Ammar et al. 2015; Chibalin et al. 2012; Clausen and Flatman 1977). Furthermore, several studies have reported that under normal  $[K^+]_e$  conditions and in the resting state, a 3 to 9 mV hyperpolarization occurs when NKA is activated by  $\beta_2$ -adrenergic receptor agonists or insulin (Clausen and Flatman 1977; Kuba 1970; Kuba et al. 1978; Kuba and Nohmi 1987; van Mil et al. 1995; Juel 1988). Finally as discussed above, muscle contractions increase NKA activity, which then modulates resting  $E_M$  and membrane excitability (Juel 1988; Hicks and McComas 1989; Nielsen et al. 1998; Overgaard and Nielsen 2001). Thus, one mechanism of action for NKA is via its electrogenic effects making resting  $E_M$  more negative and counteracting the  $K^+$ -induced depolarization and the subsequent decrease in force.

It is important to note, however, that while NKA activation during muscle activity with or without an exposure to catecholamines leads to more negative resting  $E_M$  and smaller force loss, the same does not always apply when resting muscles are exposed to elevated  $[K^+]_e$ . First, some studies reported that in rat diaphragm, mouse soleus and lumbrical muscles the extent of the catecholamine-induced hyperpolarization decreased as  $[K^+]_e$  was increased; the hyperpolarization near 0 mM  $[K^+]_e$  being 10–20 mV and

becoming zero at 10 mM  $[K^+]_e$  (Uwera et al. 2020; Kuba and Nohmi 1987; van Mil et al. 1995). In the study of Uwera et al. (2020), salbutamol triggered an increase in tetanic force at 10 mM  $[K^+]_e$  in soleus muscle despite no effect on resting  $E_M$ . Second, an exposure of resting muscle under normal  $[K^+]_e$  conditions (4–5 mM) to catecholamines/agonists results in a hyperpolarization (Clausen and Flatman 1977; Kuba 1970; Kuba et al. 1978; Kuba and Nohmi 1987; van Mil et al. 1995; Juel 1988) and increase in twitch force (Holmberg and Waldeck 1980; Reading et al. 2003; Cairns et al. 1995, 1993; Bowman and Zaimis 1958; Cairns and Dulhunty 1993a, b). However, the hyperpolarization cannot be the mechanism by which twitch force increases because the changes in resting  $E_M$  are not within the range that affects twitch force; i.e., under normal conditions resting  $E_M$  is more negative than  $-75$  mV while twitch force depression occurs when resting  $E_M$  becomes less negative than  $-60$  mV. Together these results suggest that the mechanism of action by which catecholamine improves force, regardless of  $[K^+]_e$ , cannot be solely due to an effect on resting  $E_M$ . Indeed, catecholamines also increases  $Ca^{2+}$  release. This mechanism involves (i) phosphorylation of SR  $Ca^{2+}$  release channels, known as the ryanodine receptors (RyR1) and (ii) in some muscles, such as diaphragm and amphibian muscle but not mammalian limb muscles, a phosphorylation of the t-tubular voltage sensor/ $Ca^{2+}$   $Ca_v1.1$  channels (for more details see review by Cairns and Borrani 2015).

Overall, NKA activation by muscle contraction, catecholamines and CGRP is most likely crucial at protecting skeletal muscle from the  $K^+$ -induced force depression. As discussed in greater detail in the section below entitled “*A new perspective about the role of  $K^+$ ,  $Na^+$  and  $Cl^-$  on muscle performance from the onset of exercise to fatigue*”, this protection is important at the onset of, or during mild exercise, when  $[K^+]_{int}$  is high but there is no metabolic stress triggering fatigue.

## The $K^+$ -induced force potentiation

The observation that increased  $[K^+]_e$  can potentiate twitch force of skeletal muscle was made as early as 1935, where Anna Baetjer reported an increase in twitch force in cat muscle upon arterial infusion of  $K^+$ -enriched Ringer, an effect that was attributed to an increase in muscle rather than nerve function as it was present also in curarized muscle (Baetjer 1935). In a subsequent paper, which described the phenomenon of post-tetanic potentiation, Brown and Euler performed experiments in which arterial KCl infusion of cat tibialis anterior muscle induced either increased or decreased twitch force depending on the  $K^+$  dose delivered (Brown and von Euler 1938). Thus, the basic observation of a biphasic concentration dependent effect of  $K^+$  on

twitch force was present in the literature already more than 80 years ago. Since then, sporadic reports of  $K^+$ -induced force potentiation have appeared in the literature (Walker 1948; Holmberg and Waldeck 1980; Cairns et al. 1997, 2011; Renaud and Light 1992; Pedersen et al. 2019; Olesen et al. 2021; Yensen et al. 2002; Overgaard et al. 2022; Lannergren and Westerblad 1986) where the phenomenon has been observed in many vertebrate animal species, including cats, mice, rats, frogs, guinea pigs and also humans (Grob et al. 1957). The magnitude of  $K^+$  potentiation depends on  $[K^+]_e$  and is observed between 6 and 14 mM in various preparations (Pedersen et al. 2019; Olesen et al. 2021; Lannergren and Westerblad 1986; Yensen et al. 2002). The fiber type of the preparation may be an important determinant of the magnitude of force potentiation and the degree of  $[K^+]_e$ -elevation needed to evoke the response as shown by Pedersen et al. 2019, where  $[K^+]_e$  elevation from 4 to 8 mM provided a maximal response of 17% twitch potentiation in slow twitch dominant rat soleus muscles, while an elevation to 11 mM was needed for full twitch potentiation (60% increase) in fast twitch dominant rat EDL muscles. Similarly, the  $K^+$ -induced twitch potentiation was more pronounced in EDL than in soleus muscles of guinea pigs (Holmberg and Waldeck 1980). However, in mice, EDL and soleus muscles showed approximately the same capacity for twitch potentiation (Yensen et al. 2002). In all three species, the EDL muscles reached maximal twitch potentiation and exhibited potentiation at higher  $[K^+]_e$  compared to soleus, indicating a fiber type effect on the dose–response relationship between twitch force and  $[K^+]_e$ .

Another notable feature of the  $K^+$ -potentiation phenomenon is the reliance on stimulation frequency. As such,  $K^+$ -induced potentiation is most prominent in twitch contractions evoked by a single stimulus, where relative improvements of up to 100% have been observed. Further,  $[K^+]_e$ -induced potentiation has also been observed to increase maximal force of partially fused tetanic contractions evoked by low frequency stimulation (Pedersen et al. 2019; Olesen et al. 2021; Holmberg and Waldeck 1980). In contrast, the  $[K^+]_e$  that lead to  $K^+$ -induced twitch potentiation invariably do not potentiate maximal force of fully fused tetanic contractions evoked at high frequencies;  $K^+$ -induced twitch potentiation may even occur concomitantly with small depression of tetanic force (Pedersen et al. 2019; Overgaard et al. 2022; Olesen et al. 2021). Interestingly, however, recent reports suggest that rate of force development in high-frequency tetanic contractions may be enhanced by moderate elevations of  $[K^+]_e$  (e.g., to 7–10 mM) (Overgaard et al. 2022; Olesen et al. 2021) and  $[K^+]_e$  increase may also enhance peak force of doublet-induced contractions with a short interspike interval corresponding to up to 300 Hz in rat muscle (Olesen et al. 2021) but not in mouse muscle (Overgaard et al. 2022). Furthermore, moderate  $[K^+]_e$ -elevations

have been shown to potentiate power in dynamic contractions of rat muscle (Pedersen et al. 2019). Taken together, moderate  $[K^+]_e$  elevation broadly enhances several important aspects of muscle contractile function and may therefore be considered as a potentially important positive modulator of muscle contractile function during exercise.

The possible mechanisms behind  $K^+$ -induced potentiation have been investigated in only a few studies. In 2002, Yensen et al. considered the possibility that the increased force was a consequence of a broadened AP seen during elevation of  $[K^+]_e$ . However, the authors dismissed this possibility since an experimentally induced non- $K^+$  related broadening of the AP did not lead to potentiation of twitch-responses in mouse muscle. In accordance, Wang et al. (2022) found that increasing  $[K^+]_e$  to 16 mM induced an initial increase in  $Ca^{2+}$  release and twitch force followed by a decrease in both variables; the increase in  $Ca^{2+}$  release occurs as the sarcolemmal depolarized from  $-80$  to  $-65$  mV while the subsequent decrease started once resting  $E_M$  fell to less than  $-65$  mV. However, changes in the time integral of the AP was not related to the initial increases in force and  $Ca^{2+}$ , but did correlate strongly with the subsequent reduction in force and  $Ca^{2+}$  release.

In a recent report, Overgaard et al. (2022) re-addressed an old notion originally proposed more than 80 years ago by Brown & Euler (1938) that  $K^+$ -potentiation shared mechanistic pathways with post-tetanic potentiation. More recent knowledge links post-tetanic potentiation to phosphorylation of the regulatory light chain of myosin, but since mouse muscles devoid of the enzyme that phosphorylates myosin light chain could still produce  $K^+$ -induced potentiation of a normal magnitude and since  $K^+$ -induced potentiation and post-tetanic potentiation were additive, it was concluded that the two potentiation phenomena were mechanistically distinct (Overgaard et al. 2022). The most convincing clues to the mechanism behind  $K^+$ -induced potentiation comes from studies that link the contractile potentiation to an increase in intracellular  $Ca^{2+}$  transients, which again is consequent to membrane-depolarization (Quinonez et al. 2010; Pedersen et al. 2019). The proposal that  $K^+$  induced potentiation is related to an increase in  $Ca^{2+}$  transients fits well with the frequency dependence of  $K^+$ -potentiation, where potentiation is observed in contractions evoked by a single stimulus (twitch) or by a low-frequency train, both of which fall on the steep portion of the  $Ca^{2+}$ -tension relationship. In contrast, there is no potentiation during fully fused contractions, which lie on the plateau of the  $Ca^{2+}$ -tension relationship.

The link between depolarization at rest and increased  $Ca^{2+}$  transients during activation, is however, still not elucidated. Possibly the small increase in resting  $[Ca^{2+}]_i$  that is observed following  $[K^+]_e$  elevation could be involved in the pathway (Quinonez et al. 2010), since higher resting  $[Ca^{2+}]_i$  would enhance the  $Ca^{2+}$  binding of intracellular  $Ca^{2+}$  buffers

and pave the way for a faster rise in free active  $[Ca^{2+}]_i$  when SR  $Ca^{2+}$  release channels are activated. However, such a link has yet to be proven (Pedersen et al. 2019).

In studies demonstrating  $K^+$ -potentiation the effect typically develops within 2–10 min of placing a muscle in increased  $[K^+]_e$  (Pedersen et al. 2019; Overgaard et al. 2022). It seems likely this time-course corresponds to the development of depolarization, which depends on the size of the muscle preparation and, hence, the diffusion distance to the center of the preparation (Cairns et al. 1995). However Yensen et al., (2002) observed a more gradual development of potentiation over 90 min with small increases in  $[K^+]_e$  and other studies have shown that the time-course for the decrease in force following large increases in  $[K^+]_e$  may require up to 30–90 min to reach a new steady state. A major reason for such slow development is that depolarization is delayed by the membrane potential-clamping effect of a large  $G_{Cl}$  (de Paoli et al. 2013; Dulhunty 1978). A functional implication of this could be that during moderate intensity exercise, when  $[K^+]_{int}$  is accumulated gradually, potentiation may be the dominant effect of elevated  $K^+$  early in an exercise session, while  $K^+$ -induced force depression will occur later because it requires further  $K^+$  accumulation and full development of the associated depolarization to elicit reduction in excitability.

## Modulators of $K^+$ -induced potentiation

It is of interest to determine whether various “exercise factors” could modulate the magnitude of  $K^+$ -induced potentiation as was the case for the  $K^+$ -induced force depression. Temperature is one such factor. For mouse muscles, twitch potentiation reached a maximum of 20% at 25 °C (Cairns et al. 1997) while an increase of almost 100% was observed at 37 °C (Yensen et al. 2002). Although twitch force is smaller at 37 °C than at 25 °C so that relative change appears greater at 37 °C, it can be concluded that at physiologically relevant temperatures for mammalian muscles,  $K^+$ -induced twitch potentiation is quite substantial (Olesen et al. 2021; Yensen et al. 2002).

Another exercise factor, lactic acid, reduces the  $Cl^-$  conductance but did not alter the degree of  $K^+$ -induced potentiation in rat muscle (Olesen et al. 2021). Furthermore, NKA activation via  $\beta_2$ -agonists, gave rise to further enhancement of twitch force in EDL muscles already potentiated by high  $[K^+]_e$ , but had no effect on soleus twitch force (Olesen et al. 2021).

The  $Na^+$  gradient may decrease during exercise, but so far,  $K^+$ -induced potentiation has not been studied in conjunction with a reduced  $Na^+$  gradient. Interestingly, however, lowering the  $Na^+$  gradient by itself was observed to give rise to a small increase in twitch force in rat muscles (Overgaard et al. 1999), but not in mouse muscle

(Cairns et al. 2003). Taken together, these abovementioned results demonstrate that exercise factors heat, lactic acid and adrenaline do not attenuate the  $K^+$ -induced potentiation, although these factors are all known to affect the  $K^+$ -induced force depression.

### Muscle metabolic links to sarcolemmal excitability

So far, we discussed how changes in ion gradients across the muscle membrane during fatigue affects its excitability. As discussed in the section entitled “*A new perspective for the role of  $K^+$ ,  $Na^+$  and  $Cl^-$  on muscle performance from the onset of exercise to fatigue*”, fatigue is likely a protective mechanism that prevents damaging ATP depletion. Thus, it is of vital importance for the muscle to keep the balance between ATP-utilization and ATP-production in order to maintain [ATP] within a narrow range and avoid an irreversible deleterious ATP depletion (Hochachka and Matheson 1992). A link between metabolic capacity and muscle excitability may be a feed-forward signal, lowering membrane excitability and thereby restraining the muscle energy turn-over (Ørtenblad and Nielsen 2015). To support such notion we discuss three examples of how changes in metabolic status affect the activity of NKA, the ATP sensitive  $K^+$  channel ( $K_{ATP}$  channel) and the  $Cl^-$  CIC-1 channel, being an ion pump and two ion channels that affect membrane excitability.

Cells must continually regenerate ATP to keep up with demand. This is achieved by an integration of the various energy pathways and by efficient regulatory systems, ensuring that rates of ATP resynthesis are balanced with the ATP demands of exercise. Such a tight balance between ATP production and resynthesis rates is of vital importance for all cells, but particularly so in excitable cells due to their high and fluctuating energy turnover. It is remarkable how skeletal muscle fibers can instantly adjust to provide the necessary energy during exercise, where a several-fold elevated energy turnover can be sustained for hours or a more than a 100-fold increase in turn-over is obtained for short time. A number of the steps in muscle excitation–contraction–relaxation (E-C-R) coupling are either directly (ATPases) or indirectly (ion channels) dependent on muscle energy status. In contracting skeletal muscle fibers, the three main ATP consuming processes are the myosin ATPases, the SR  $Ca^{2+}$  ATPases and the  $Na^+,K^+$ -ATPases (NKA), which respectively consume approximately 50–60%, 40–50% and 5–10% of the energy during exercise (Clausen et al. 1991; Rolfe and Brown 1997; Ørtenblad et al. 2009). Thus, the  $Na^+,K^+$ -ATPase has a relatively low energy turn-over in skeletal muscle during exercise with high absolute ATPase activity.

### Exercise metabolism and ion homeostasis

Already during the early 1900's it was established that both fat and carbohydrate can be used as fuel for metabolism during exercise and that the relative contributions of the fuels during lower exercise intensities mainly is determined by the diet (Christensen and Hansen 1939; Frenzler and Reach 1901; Krogh and Lindhard 1920; Zuntz 1896). There was also a clear knowledge of carbohydrate being the main source of substrate when exercising at high intensities, with a relationship between exercise intensity and carbohydrate metabolism. At the same time, seminal studies established the foundations of our understanding of metabolic pathways in general and herein ATP production in contracting muscle. Different glycolytic enzymes from muscle tissue were extracted and combined to artificially establish a pathway from glycogen to lactic acid (Meyerhof 1942), and with this the full glycolytic (Embden-Meyerhof) pathway, from glycogen to lactate, was elaborated by the early 1940s see (Kresge et al. 2005). Although these early studies had documented the glycolytic pathway, that carbohydrate is a major substrate during exercise and that the diet plays an important role in endurance capacity, it was not until after the introduction of the needle biopsy technique in the 1960s, that it was demonstrated that prolonged exercise performance is highly correlated with muscle glycogen contents and that exhaustion is associated with low skeletal muscle glycogen contents (Bergström et al. 1967). At present, we still have limited explanations for the mechanisms linking metabolism and muscle glycogen to muscle function. In addition to the effects of  $K^+$ ,  $Na^+$  and  $Cl^-$  discussed above, there are numerous other factors affecting muscle excitability and none has been more agreed on than metabolically derived factors affecting NKA and ion channel (i.e.,  $K_{ATP}$  and CIC-1 channel) activity. It is now well established that NKA and  $K_{ATP}$  channels are primarily fueled by glycolytically-derived ATP with direct effect of muscle glycogen on muscle excitability (Dhar-Chowdhury et al. 2007).

### A causal link between metabolism and muscle excitability

The causal link between energy availability or glycogen depletion and depressed muscle function is most likely multifactorial, but studies on isolated muscles from rodents (Chin and Allen 1997) and amphibians (Stephenson et al. 1999) suggest a local factor within the muscle E-C-R coupling. The association between low muscle glycogen content and depressed contractile function was proposed that low glycogen causes a slowed glycogenolytic and glycolytic flux, compromising the required rate of ATP regeneration to sustain muscle function during exercise, referred to as the ‘energy crisis’ theory (Green 1991; Sahlin et al. 1998).

Consequently, adequate ATP supply to one or more of the processes involved in E-C-R coupling cannot be maintained, leading to depressed muscle function and fatigue. The energy crisis theory is supported by observations of phosphocreatine (PCr) decreases along with increases in free ADP and IMP contents in muscles following prolonged, glycogen-depleting exercise (Norman et al. 1988; Sahlin et al. 1997).

However, the energy crisis theory is challenged by both *in vitro* and *in vivo* studies. First, a number of studies at both the cellular and whole muscle level demonstrate a strong association between low glycogen and decreased muscle function even after recovery periods, where ATP concentration would be expected to be normal (Bangsbo et al. 1992; Chin and Allen 1997). Second, muscular fatigue is also observed even when glycogen is far from depleted, or when glycogen is lowered prior to the start of exercise (Duhamel et al. 2006a, b; Ørtenblad et al. 2011). Also, intramuscular concentrations of ATP are in the range of 5–9 mM (Hargreaves and Spriet 2018; Greiner and Glonek 2021) and NKA has a high affinity for ATP with a  $K_{1/2}$  less than 0.5 mM (Blanco and Mercer 1998) and the  $\text{Na}^+$ -stimulated NKA activity is saturated at 0.5 mM ATP (Walas and Juel 2012), suggesting that the cellular global [ATP] *per se* does not directly affect NKA activity (Ewart and Klip 1995). Finally, low glycogen contents also affect muscle function in the mechanically skinned fiber preparation where cellular global ATP and PCr are maintained high during contractile activity (Kabbara et al. 2000; Nielsen et al. 2009; Stephenson et al. 1999). Collectively, these series of experiments do not provide experimental support for the energy crisis hypothesis, at least at the whole cellular level.

On the other hand, one cannot exclude metabolic effects within the compartmentalised muscle fibre as [ATP] at the subcellular level inside cells may not be uniform (Jones 1986). The highly organized muscle cell forms many compartments, and hence microenvironments with high ATPase activity and restricted diffusional access of metabolites; observations on experiments utilizing whole muscles or intact fibers do not rule out a metabolic role in maintaining muscle fiber excitability. Such a functional compartmentalization of glycolytic metabolism is known in a variety of tissues, with a possible role of ATP and other metabolites regulating key steps in the muscle E-C-R coupling by delivering ATP in microenvironment of the fiber (Han et al. 1992; Korge and Campbell 1995). This would be particularly noteworthy in the muscle triad junction between the transverse tubular-system and the SR, with a diffusional restricted space around 12 nm wide, and with a high metabolic activity (Dulhunty 1984). An important conceptualization of this idea is that most of the glycolytic enzymes are connected with membranes of intracellular compartments such as the SR (Dhar-Chowdhury et al. 2007; Xu and Becker 1998) and

the existence of a glycogenolytic complex associated with the SR is now well established (Entman et al. 1980; Wanson and Drochmans 1972; Xu and Becker 1998). In line with this, transmission electron microscopy imaging has revealed the existence of a heterogenic subcellular distribution of three distinct glycogen pools in skeletal muscle, indicating a requirement for distinct subcellular energy stores (Nielsen and Ørtenblad 2013). This organization places the energy stores in close vicinity to the position of utilization and provides support for the concept for highly compartmentalized energetic networks. Interestingly, it has been demonstrated that the three main energy-consuming ATPases in skeletal muscles ( $\text{Na}^+$ ,  $\text{K}^+$ -,  $\text{Ca}^{2+}$ -, and myosin- ATPases) utilize different local pools of glycogen, clearly demonstrating compartmentalized glycogen metabolism and emphasize that spatially distinct pools of glycogen are used for separated energy requiring processes (Nielsen et al. 2022). Physiologically, this ensures an effective energy transfer and regulation of energy production and utilization in the restricted cellular compartments (Nielsen and Ørtenblad 2013; Korge and Campbell 1995; Saks et al. 2008). In line with this, it has become increasingly clear that glycolytic intermediates and end-products by themselves also regulate the activity of membrane ion channels and pumps, coupling muscle energy metabolism to the regulation of ion homeostasis and cellular excitability. In this context, NKA was one of the first membrane proteins to be described to be preferentially regulated by glycolytically-derived ATP (Mercer and Dunham 1981). This concept of the fueling of the NKA by glycolytically derived ATP has later found ample support in the literature (Dhar-Chowdhury et al. 2007).

### Fueling of NKA and role in muscle excitability

There is a reasonably well established association between glycolytically-derived ATP and NKA activity and strong evidence to support the concept that glycolysis and NKA are functionally coupled. This seems to be an evolutionary conserved coupling and has been observed in several tissue types, including mammalian erythrocytes (Kennedy et al. 1986; Mercer and Dunham 1981; Schrier 1966), axons (Caldwell et al. 1960); brain synaptosomes (Erecińska and Dagoni 1990), kidney cells (Lynch and Balaban 1987), smooth muscle (Campbell and Paul 1992), cardiac myocytes (Hasin and Barry 1984; MacLeod 1989; Philipson and Nishimoto 1983) and skeletal muscles (Clausen 1965; James et al. 1999a; Jensen et al. 2020; Okamoto et al. 2001). This is supported by the observation that a number of tissue types generate both pyruvate and lactate under primarily aerobic conditions in a process linking glycolytic ATP supply to NKA activity (Brooks 1986; Dhar-Chowdhury et al. 2007). This occurs in resting, well oxygenated skeletal muscles, and is closely linked to NKA stimulation by epinephrine

(James et al. 1996, 1999a, 1999b; Bundgaard et al. 2003; Levy et al. 2005).

Such a tight coupling between the glycogenolytic rate and NKA activity is demonstrated by the observation that  $[\text{Na}^+]$  decreases if glycogen breakdown is stimulated with epinephrine at rest, whilst ouabain attenuates glycogen utilization (James et al. 1999b). Also, experiments using ouabain and measurements of myoplasmic high-energy phosphates, in resting rat EDL muscles, demonstrated that NKA activity is only suppressed when glycolysis is inhibited and not affected by inhibition of oxidative phosphorylation; this suggests that normal glycolysis is the predominant source of the fuel for NKA (Okamoto et al. 2001). In support, an early study by Clausen was one of the first to demonstrate a link between muscle glycogenolysis and NKA activity, showing that glycogen utilization in resting muscle was decreased when muscle NKA activity was blocked by ouabain (Clausen 1965). Moreover, there was an apparent lactic acid production in proportion to NKA activation. It might appear that the glycolytically-derived ATP may be an inefficient metabolic pathway, as aerobic glycolysis is an inefficient means of generating ATP per unit of glucose, compared to the amount obtained by mitochondrial respiration (Vander Heiden et al. 2009; Locasale and Cantley 2011). However, the rate of glucose metabolism through aerobic glycolysis is substantially higher, with a 10–100 times faster production of lactate than the full oxidation of glucose in the mitochondria. Thus, the amount of ATP synthesized over any given period of time is comparable when either form of glucose metabolism is utilized (Shestov et al. 2014).

Also, in cell cultures, inhibition or activation of different cell membrane active pumps (such as NKA, P-glycoprotein pump, pumps involved in osmoregulation) led to reduction or increase in glycolysis, respectively, while the oxidative phosphorylation remained constant (Epstein et al. 2014). Together, available data strongly suggest that in skeletal muscle, glycolysis is the predominant source of the fuel for NKA, with a clear association of glycogenolytic/ glycolytically derived ATP on ion transport across muscle membranes. Functionally, a decreased glycogenolysis/glycolytic rate will therefore potentially adversely affect muscle excitability during contractions. Indeed, a direct link between energy state and excitability of the muscle was confirmed by blocking cross-bridge cycling and SR  $\text{Ca}^{2+}$  release with the cross-bridge cycling blockers N-benzyl-p-toluene sulfonamide and dantrolene, respectively, thereby conserving energy during repeated electrical stimulations, which in turn reduced the extent of muscle excitability loss during high-frequency stimulation (Macdonald et al. 2007).

The essential role of glycogenolytically-derived ATP on muscle excitability is substantiated in experiments using mechanically skinned fibers, enabling the maintenance of a high and constant global [ATP] during experiments

under different metabolic conditions. Using this muscle fiber preparation, fatigability induced by repeated contractions is associated with lowered glycogen contents in most experiments during both AP stimulation (Nielsen et al. 2009) and voltage sensor activation (Barnes et al. 2001; Stephenson et al. 1999), but not in all studies (Goodman et al. 2005). Further, enzymatically lowering glycogen by 70% led to a reduction in both voltage sensor activated- and AP-induced forces in skinned fibers, with larger decrease in AP-induced force by lowering glycogen (Watanabe and Wada 2019). Together, these data suggest that low glycogen and glycogenolytic rate affects the t-system polarization and excitability, as the voltage sensor inactivation is displaced to markedly more positive  $E_M$  values compared with AP ( $\text{Na}^+$  channel) inactivation (Ørtenblad and Stephenson 2003; Nielsen et al. 2004b). Taken together, studies with the mechanically skinned fiber preparation strongly demonstrate that glycogenolytically-derived energy is associated with fiber contractile endurance and excitability, irrespective of the global fiber [ATP].

Direct in-vivo or in-situ  $\text{Na}^+/\text{K}^+$  fluxes and ATP hydrolysis rate measures to determine NKA activity are difficult to obtain and are mostly done before or after muscle activity (see concurrent review by (McKenna et al. 2023). Alternatively NKA function can be estimated indirectly in an in vivo setting by estimating the muscle fiber membrane's ability to respond to two closely spaced AP and hence the repriming time, define as the time interval for the second pulse to generates an AP, depends in part on NKA activation. With this measure of NKA activity, a depolarization of the t-system increases the repriming time as expected, however, the addition of phosphoenolpyruvate, which increases glycolytic ATP resynthesis, decreases the repriming period (Dutka and Lamb 2007a, b). When glycogen is enzymatically lowered with glucoamylase treatment, the repriming period increases (Watanabe and Wada 2019). The role of glycogenolytically-derived ATP was studied by the use of glycogen phosphorylase inhibitors and glycogen lowering treatment in mechanically skinned fibers, which invariably prolonged repriming time, strongly indicating an attenuated NKA activity (Jensen et al. 2020), further supporting the concept of a direct role of glycogenolytically-derived ATP on NKA activity in skeletal muscle, irrespective of bulk [ATP].

In summary, substantial evidence indicates a tight coupling between metabolism, via the glycogenolytic-glycolytic-derived ATP production, and NKA in muscle. As a consequence of lower glycogen and lower derived ATP production, a subsequent attenuation of increases in NKA activity can then result in greater  $\text{K}^+$ -induced loss of membrane excitability and force as discussed in the section above entitled “*Modulation of the  $\text{K}^+$ -induced force depression by NKA*”.

## **K<sub>ATP</sub> channels: a second metabolism-excitability link**

The regulation of the K<sub>ATP</sub> channel is a second mechanism that links energy metabolism to membrane excitability. In this section, we briefly discuss the channel activation properties followed by another section on its physiological role. The molecular structure, regulation, pharmacological properties and physiological roles of K<sub>ATP</sub> channels in various tissues are detailed in other reviews (Aguilar-Bryan and Bryan 1999; Inagaki et al. 1996; Babenko et al. 1998; Foster and Coetzee 2016; Seino 1999). In skeletal muscle, K<sub>ATP</sub> channel is composed of two subunits: Kir6.2, a weak inward K<sup>+</sup> rectifier with four ATP binding sites, and SUR2A, a regulatory subunit with ATP/ADP binding sites. ATP closes the channel in the  $\mu\text{M}$  range when it is bound to the Kir6.2 subunit (Barrett-Jolley et al. 1996; Noma 1983; Vivaudou et al. 1991). Thus, one expects that K<sub>ATP</sub> channels are closed in resting muscle as ATP levels are about 5–9 mM (Greiner and Glonek 2021; Hargreaves and Spriet 2018). Indeed, *in vitro* studies demonstrated that K<sub>ATP</sub> channels are closed under voltage clamp conditions with mM ATP levels on the sarcolemmal cytoplasmic side (Vivaudou et al. 1991). However, two studies using *in-situ* skeletal muscle preparations demonstrated that a small number of K<sub>ATP</sub> channels are open at rest, because glibenclamide, a K<sub>ATP</sub> channel antagonist, reduced  $[\text{K}^+]_{\text{int}}$  in human muscle (Nielsen et al. 2003) and lowered K<sup>+</sup> efflux to almost zero in perfused rabbit muscle (Lindinger et al. 2001). Thus, other factors must be modulating K<sub>ATP</sub> channel activity in resting skeletal muscle under *in-situ* conditions, one possible factor being insulin (Tricarico et al. 1997, 1999).

Electrically stimulating mouse tibialis muscle at 1 Hz resulted in sarcolemmal hyperpolarization by 10 mV, reduction in AP overshoot by 14 mV, as well as reduction in SR Ca<sup>2+</sup> release (Zhu et al. 2014). Zhu et al. (2014) further showed that none of these changes were observed in K<sub>ATP</sub> channel deficient muscles; i.e., tibialis muscle from Kir6.2 knockout (Kir6.2<sup>-/-</sup>) mouse model. The authors concluded that 1 Hz stimulation resulted in an activation of K<sub>ATP</sub> channels. Exposing skeletal muscles to ischemia or chemical metabolic inhibition causes large K<sub>ATP</sub> channel activity (Gramolini and Renaud 1997; Castle and Haylett 1987; Allard et al. 1995; Pang et al. 1997). Importantly, K<sub>ATP</sub> channels are activated during fatiguing contractions. Stimulating rat soleus muscle with 3.5-s AP trains at 15 Hz repeatedly every 7 s eventually caused a 14-fold increase in G<sub>K</sub>, which was blocked by glibenclamide, a K<sub>ATP</sub> channel blocker. The authors suggested that the increased G<sub>K</sub> occurred as metabolic stress triggers fatigue and was due to an increased K<sub>ATP</sub> channel activity (Pedersen et al. 2009a). Furthermore, large excitability, contractile and metabolic dysfunctions as well as fiber damage occur in the absence of K<sub>ATP</sub> channel activity during fatigue (see section entitled

“K<sub>ATP</sub> channels: another physiological function for K<sup>+</sup> during fatigue”). Together, these results strongly suggests that not only are K<sub>ATP</sub> channels activated during fatigue but that they are crucial at protecting muscles from deleterious exhaustion of [ATP].

The mechanism by which K<sub>ATP</sub> channels are activated during fatigue is still not well understood. Firstly, the decreases in bulk [ATP] during or at the end of a fatigue bout range between 10 and 50% (Scott et al. 2016; Nagesser et al. 1993; Meyer and Terjung 1979; Whitlock and Terjung 1987; Mainwood et al. 1972; Dawson et al. 1978); i.e., ATP does not fall to the  $\mu\text{M}$  range necessary to activate the channel, raising the question as to whether low ATP itself is the main activator of K<sub>ATP</sub> channels during fatigue. However, similar to NKA, the ATP that blocks K<sub>ATP</sub> channel is primarily provided by glycolysis, at least in cardiac muscle, and there is close physical association and functional interaction between glycolytic enzymes and K<sub>ATP</sub> channels (Weiss and Lamp 1989; Hong et al. 2011; Dhar-Chowdhury et al. 2005). It is therefore possible that the sub-sarcolemmal [ATP] becomes much lower than the bulk [ATP] allowing an activation of K<sub>ATP</sub> channels, especially when glycogen is low, as discussed above for NKA. There are also other metabolites that reduce the ATP inhibition of K<sub>ATP</sub> channels; including an increase in ADP (Vivaudou et al. 1991), decrease in intracellular pH as observed during fatigue (Davies et al. 1992; Standen et al. 1992; Allard et al. 1995) and increases in extracellular adenosine via its A1 receptor (Barrett-Jolley et al. 1996).

Taken together these studies have long suggested that K<sub>ATP</sub> channels link energy metabolism to membrane excitability. That is, decreases in [ATP] as well as other metabolite changes during any metabolic stress result in the ATP dissociation from the channel, and as the channel opens it lowers AP amplitude and membrane excitability to ultimately reduce Ca<sup>2+</sup> release and force generated or work done by muscle. This may in turn prevent damaging ATP depletion, under conditions with a high ATP turnover.

## **K<sub>ATP</sub> channels: another physiological function for K<sup>+</sup> during fatigue**

To study the physiological role of K<sub>ATP</sub> channels, one must take a similar approach to that used for studying the role of any proteins, which is to determine the physiological response during fatiguing stimulation while the channel is either activated or blocked. Activating K<sub>ATP</sub> channels in mouse EDL and soleus muscles with pinacidil lowered AP overshoot in unfatigued muscle (Gong et al. 2003). Although there is evidence for K<sub>ATP</sub> channel activation during fatigue (Pedersen et al. 2009a), pinacidil further increased, compared to control, K<sup>+</sup> efflux as well as the rate at which M-wave area and tetanic force decreased in mouse EDL

and soleus muscle when fatigue was triggered with one tetanic contraction every sec for 3 min (Gong et al. 2003; Matar et al. 2000). Pinacidil had none of these effects in Kir6.2<sup>-/-</sup> muscles, which do not express functional K<sub>ATP</sub> channels. This suggests that not all K<sub>ATP</sub> channels are activated during fatigue in control conditions and that the mechanism of action of the K<sub>ATP</sub> channel involves an increased outward K<sup>+</sup> current, which counteracts the Na<sup>+</sup> inward current resulting in less depolarization and smaller AP in resting muscles and during fatigue. Furthermore, decreases in AP amplitude has been shown to reduce the SR Ca<sup>2+</sup> release (Zhu et al. 2014; Wang et al. 2022). Considering the activation of K<sub>ATP</sub> channels during fatigue (Pedersen et al. 2009a) and its link to metabolic state, it is more than likely that one essential function of the channel is to lower membrane excitability in order to prevent damaging ATP depletion by reducing Ca<sup>2+</sup> release and force so that ATP utilization by SR Ca<sup>2+</sup>- and myosin- ATPases are reduced when ATP production is no longer sufficient to meet the demand.

Accordingly, one should expect that the absence of K<sub>ATP</sub> channel activity leads to an impairment of muscle function. To test this, two approaches has been used to abolish K<sub>ATP</sub> channel activity in skeletal muscle: (i) pharmacologically exposing wild type muscles to glibenclamide, a K<sub>ATP</sub> channel blocker, and (ii) genetically using a knockout model in which the Kir6.2 subunit is no longer expressed; i.e., muscles from Kir6.2<sup>-/-</sup> mice. Notably, in all the studies described here, the effects of abolishing K<sub>ATP</sub> channel activity were quantitatively the same for the pharmacological and genetic approaches. Furthermore, glibenclamide had no effect in Kir6.2<sup>-/-</sup> skeletal muscles (Cifelli et al. 2007). These results suggest that the different physiological responses between normal and K<sub>ATP</sub> channel deficient muscles were due to a lack of channel activity and neither to some non-specific glibenclamide effect nor to other effects associated with a lack Kir6.2 expression. Experiments *in vitro* were initially carried out with mouse EDL and soleus with a fatigue protocol consisting of one 200 ms long tetanic contractions every s for 3 min (Matar et al. 2000; Gong et al. 2000). The major impact of blocking K<sub>ATP</sub> channels were slightly faster decrease in tetanic force, greater increase in unstimulated force, defined as the force between contractions, and a reduced capacity to recover force following fatigue.

Experiments were then repeated using smaller muscle preparations, i.e., FDB muscle bundles and single fibers (Cifelli et al. 2008, 2007; Selvin and Renaud 2015). Compared to wild type FDB muscles, the lack of K<sub>ATP</sub> channel activity led to four major impairments during fatiguing contractions. (1) The decreases in active [Ca<sup>2+</sup>]<sub>i</sub> and tetanic force were faster and the final extent of the decrease greater. (2) The increases in unstimulated [Ca<sup>2+</sup>]<sub>i</sub> and force, measured between contractions, were greater. In some cases the increase in unstimulated [Ca<sup>2+</sup>]<sub>i</sub> was so large that unattached

single fibers supercontracted from an elongated fiber to a very small square structure. (3) Resting E<sub>M</sub> of several fibers depolarized to greater extent from a mean -80 mV to -30 mV (compared to just -60 mV for control conditions). Large depolarizations during fatigue were also reported in rat EDL exposed to glibenclamide (Pedersen et al. 2009b). (4) The capacity to recover tetanic force following fatigue was largely reduced. The authors suggested that the absence of K<sub>ATP</sub> channel activity first lead to an excitability impairment as the large depolarization results in greater Na<sub>v</sub>1.4 channel inactivation compared to control contributing to greater decreases in AP amplitude, active [Ca<sup>2+</sup>]<sub>i</sub> and tetanic force. The depolarization was also large enough to cause the opening of t-tubular Ca<sub>v</sub>1.1 channels allowing for large Ca<sup>2+</sup> release between contractions. This was confirmed by exposing FDB to 0.6 mM Ca<sup>2+</sup> (vs. 2.3 mM) or to 1 μM verapamil, a Ca<sub>v</sub>1.1 channel blocker at a concentration that had no effect on the pre-fatigue tetanic contraction of Kir6.2<sup>-/-</sup> FDB. Both lower [Ca<sup>2+</sup>]<sub>e</sub> and verapamil significantly reduced during fatigue the rate at which tetanic force decreased and the extent of the increase in unstimulated force, while it fully restored the capacity to recover force following fatigue in K<sub>ATP</sub> channel deficient muscle. Based on these results, the authors suggested that the contractile impairment in regard to tetanic force during fatigue and recovery in K<sub>ATP</sub> channel deficient FDB were in part due to some cellular damages possibly caused by the high unstimulated [Ca<sup>2+</sup>]<sub>i</sub>.

Notably, experiments with treadmill running further confirmed the lower fatigue resistance and the appearance of fiber damage in active Kir6.2<sup>-/-</sup> muscles (Thabet et al. 2005). In regard to lower fatigue resistance, Kir6.2<sup>-/-</sup> mice ran shorter distances when elicited to run on treadmill at 24 m/min with 20° inclination. On the first day of running wild type mice ran 2 km before they could no longer maintain the speed whereas Kir6.2<sup>-/-</sup> mice only ran 0.5 km. After five consecutive running days, wild type mice had increased their running distance to 5 km while it only increased to 1.5 km for Kir6.2<sup>-/-</sup> mice. In regard to fiber damage, 12% of plantaris and EDL muscle fibers of Kir6.2<sup>-/-</sup> mice had centrally located nuclei, which occurs when a fiber had been damaged and regenerated by satellite cells; a value that was 25% in tibialis anterior. Less than 0.5% of fibers had central nuclei in the same muscles of wild type mice. Fiber damage occurred primarily in type IIB Kir6.2<sup>-/-</sup> fibers. Finally, severe fiber damage with no evidence of fiber regeneration was also reported for Kir6.2<sup>-/-</sup> diaphragm.

A last series of experiments demonstrated several metabolic dysfunctions in Kir6.2<sup>-/-</sup> compared to wild type FDB (Scott et al. 2016). (1) ATP content decreased by about 5 μmoles/g wet weight during the first 30 s of fatigue in both wild type and Kir6.2<sup>-/-</sup> FDB bundles. Thereafter, it slowly returned back to pre-fatigue levels by the second min of the

3 min fatigue bout in wild type but not in Kir6.2<sup>-/-</sup> FDB. (2) At the end of the fatigue bout, there was a net loss of total adenylates (ATP+ADP+AMP contents). (3) Compared to wild type, Kir6.2<sup>-/-</sup> FDB had greater glucose uptake, similar glycogen mobilization and greater glucose oxidation during the first min that resulted in a 3.5-fold greater ATP production. (4) However, during the remaining 2 min of the fatigue period glycogen was no longer mobilized and oxidative phosphorylation stopped. The decrease in ATP production and the large increases in unstimulated  $[Ca^{2+}]_i$  and force between contractions increases the ATP demand by  $Ca^{2+}$  and myosin ATPases, which most likely were the cause for the lack of increase in ATP content in Kir6.2<sup>-/-</sup> FDB that occurred in wild type FDB during the final two min of the fatigue bout.

In summary,  $K_{ATP}$  channels are activated during fatigue. They contribute to the reduction of AP amplitude, which then lowers SR  $Ca^{2+}$  release and force to preserve ATP as it reduces the ATP demand by  $Ca^{2+}$  ATPase and myosin ATPase. The lack of  $K_{ATP}$  channel activity during fatigue causes: (i) fiber damage and (ii) major excitability, contractility and metabolic dysfunctions suggesting that the channel is crucial in terms of myoprotection. That is, once activated, the channel prevents massive sarcolemmal depolarization and the subsequent large increases in unstimulated  $[Ca^{2+}]_i$  and force. The apparent faster fatigue rate and lower force recovery in  $K_{ATP}$  channel deficient muscles, using either glibenclamide in wild type muscles or Kir6.2<sup>-/-</sup> muscles, is most likely because of (i)  $Na_v$  channel inactivation associated with the large depolarization as well as (ii) fiber damage associated with greater increase in unstimulated  $[Ca^{2+}]_i$  and ATP depletion.

### **CLC-1 $Cl^-$ channel: a third potential metabolism-excitability link**

As discussed above in the section entitled “*Modulation of the  $K^+$ -induced force depression by changes in  $G_{Cl}$* ”, changes in CLC-1 channel activity or  $G_{Cl}$  significantly alter how changes in  $[K^+]_e$  affect membrane excitability and force in resting, unfatigued muscles. For these observations to be of any physiological significance, one then expects changes in  $G_{Cl}$  during muscle activity and fatigue. In fact, there is evidence for the activation of CLC-1 channels during metabolic stress such as metabolic inhibition and fatigue (Fink and Luttgau 1976; Pedersen et al. 2009a, 2009b). In other words, CLC-1 channels may be a third membrane component for which its activity is regulated in part by the fiber metabolic status.

In two studies (Pedersen et al. 2009b, 2009a), 3.5 s long trains of APs at 15 Hz were triggered every seven seconds in rat/mouse EDL and rat soleus muscle fibers. At the onset of stimulation,  $G_{Cl}$  decreased by ~70% due to phosphorylation of CLC-1 channels by PKC while  $G_K$  increased

very slightly; this period was called phase 1. Notably, there is no sign of any decreases in AP amplitude during phase 1. In rat EDL muscle, both  $G_{Cl}$  and  $G_K$  increased drastically after almost 2000 APs, this period was called phase 2. The increase in  $G_{Cl}$  was ~threefold above pre-stimulation levels while the increase in  $G_K$  was ~14-fold and was entirely due to activation of  $K_{ATP}$  channels. Two other studies then reported similar phases 1 and 2 in mouse EDL and human abdominal and intercostal skeletal muscles; the decrease in  $G_{Cl}$  during phase 1 in human muscles was also due to CLC-1 channel phosphorylation by PKC (Riisager et al. 2014, 2016). Pedersen et al. (2009a) suggested that phase 2 was associated with substantial reduction in muscle fiber energetic state, based on the following evidence.

First, metabolic poisoning results in substantial increases in both  $G_{Cl}$  and  $G_K$ , the latter being related to an activation of  $K_{ATP}$  channels (Fink and Luttgau 1976; Gramolini and Renaud 1997; Allard et al. 1995). Second, CLC-1 channels are ATP sensitive. Decrease in [ATP] shifts the steady state activation kinetics of CLC-1 channels toward more negative resting  $E_M$  (Tseng et al. 2007, 2011; Zhang et al. 2008); i.e., the shift allows for greater CLC-1 activity at a given resting  $E_M$ . Third, that both CLC-1 and  $K_{ATP}$  channel activity increase simultaneously during phase 2, strongly supports the concept that this phase occurs when there is a metabolic stress. Fourth, contrary to rat EDL muscle, a glycolytic and fatigable muscle, in rat soleus, an oxidative and fatigue resistant muscle, phase 2 was not observed even after 15,000 APs (Pedersen et al. 2009b). Fifth, in EDL muscle phase 2 is triggered sooner and  $G_M$  increases to a greater extent in the absence than in the presence of glucose in the extracellular milieu (Pedersen et al. 2009b).

Three major facts can be made from all the studies discussed so far. First, it has long been known that large changes in plasma, interstitial and intracellular concentrations of  $K^+$  and  $Na^+$  observed during fatigue have the capability to affect sarcolemmal excitability and thus SR  $Ca^{2+}$  release and force/work in skeletal muscle. Second, observations of both the  $K^+$ -induced force potentiation and depression appeared early last century in the literature. However,  $K^+$ -induced potentiation has been much less prominently featured, often receiving only brief mention in reviews concerning the role of  $K^+$  effects in fatigue (Sejersted and Sjøgaard 2000; Cairns and Lindinger 2008; McKenna et al. 2008) while in two reviews a  $K^+$  role in muscle fatigue was questioned (Sjøgaard 1991; Allen et al. 2008b). Third, there is now evidence for a link between sarcolemmal excitability and muscle energy status involving at least three sarcolemmal components: NKA,  $K_{ATP}$  and CLC-1 channels.

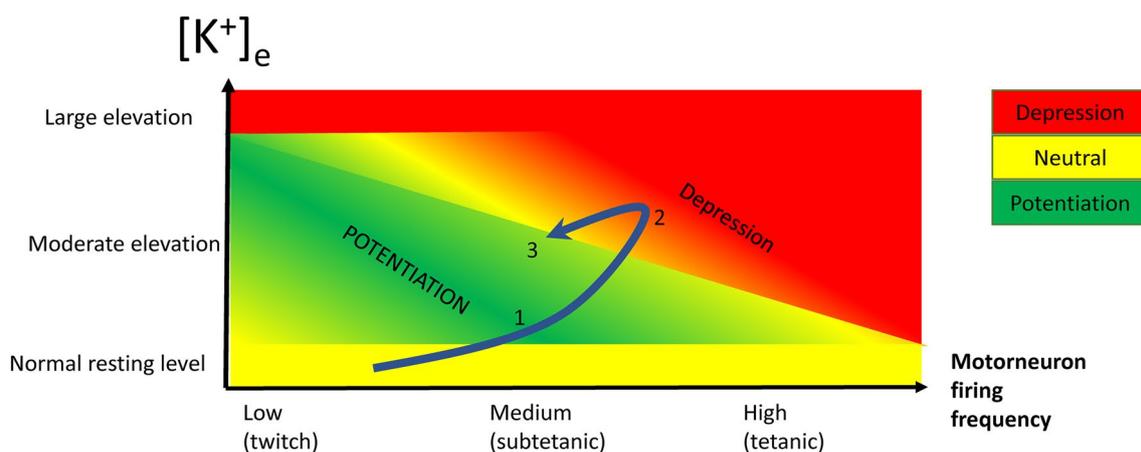
## A new perspective for the role of $K^+$ , $Na^+$ and $Cl^-$ on muscle performance from the onset of exercise to fatigue

The documented contrasting effects of  $K^+$  on contractile performance begs the question as to when  $[K^+]_e$  is a positive or negative modulator of performance during stimulated muscle contractions or during exercise in humans. This question has been discussed in at least two previous reviews (Renaud 2002; McKenna et al. 2008). Renaud (2002) emphasized that  $K^+$  has in fact several physiological roles during muscle activity and first proposed that  $K^+$  optimizes muscle performance at the onset of muscle activity and in the absence of metabolic stress. That is,  $K^+$  (i) potentiates twitch and sub-tetanic force, (ii) augments blood flow by triggering blood vessel vasodilation (Wilson et al. 1994; Knot et al. 1996; Armstrong et al. 2007), and (iii) activates muscle pressor reflex that increases heart rate and arterial blood pressure (MacLean et al. 2001; Rybicki et al. 1984) possibly via an activation of muscle metabosensitive afferent fibers (Laurin et al. 2010). Further details about how increases in plasma  $[K^+]$  affects the respiratory and cardiovascular function can be found elsewhere (Paterson 1996). Then, when a metabolic stress occurs as ATP demand exceeds ATP supply, fatigue is triggered in which the  $K^+$  effect switches from force potentiation to depression in order to reduce ATP utilization and prevent potential damaging ATP depletion. At the time, some of the factors that modulate the  $K^+$  effects on force were unknown. With the knowledge of how changes in  $G_{Cl}$  modulate the  $K^+$  effects and how it changes during muscle activity we now further elaborate the concept first

proposed by Renaud (2002). Three factors are taken into consideration: (i)  $[K^+]_e$  itself, (ii) stimulation frequency and (iii) muscle physiological/metabolic state as regulators of NKA activity,  $G_{Cl}$  (ClC-1 channels) and  $G_K$  ( $K_{ATP}$  channels) during exercise and fatigue.

### $[K^+]_e$ and stimulation frequency

The first two factors are interrelated as *in-vivo* stimulating frequencies directly affect increases in  $[K^+]_e$ . Considering the range of known activation frequencies (Hennig and Lomo 1985; Enoka and Fuglevand 2001) and the observed  $[K^+]_i$  and  $[K^+]_{int}$  during both maximal and submaximal exercise (McKenna et al. 2023), it is conceivable that  $K^+$  may exert both positive and negative effects depending on the exercise intensity and duration, and may even be involved in regulating exercise intensity by exerting these effects within a given exercise session. These impacts are conceptualized in Fig. 2. Consider an individual who embarks on a run at an initial slow pace. Here, muscle  $[K^+]_{int}$  will increase to a moderate degree and motor units will be activated mainly with low or moderate activation frequencies, rendering the influence of  $K^+$  positive, due to its location on the  $[K^+]_e$  –motorneuron firing frequency continuum (point “1” in Fig. 2). Increasing speed of running will require a higher motorneuron firing frequency, leading to further increase in  $[K^+]_{int}$ . If  $[K^+]_{int}$  is sufficiently high to move into the red zone (point “2” in Fig. 2) then fatigue inevitably ensues. However, it is known that during fatiguing contractions, the motor unit motorneuron firing frequency diminishes (Bigland-Ritchie et al. 1983). Furthermore, such reduction in stimulation frequencies allows for a decreased loss or even recovery of force (Jones et al. 1979), partially because it



**Fig. 2** Effect of  $[K^+]_e$  and motorneuron firing frequency on contractile performance: a balance between positive and negative effects of muscle  $[K^+]_e$ . Diagram depicting the proposed effects of  $[K^+]_e$  on muscle force at various levels of  $[K^+]_e$  increase and at various activation frequencies. The colors indicate potentiation (green) or depres-

sion (red) of force. The arrow and numbered points refer to various situations during exercise as explained in the text. Note that a given moderate elevation of  $[K^+]_e$  can give rise to either potentiation or depression of force depending on the motorneuron firing frequency

allows more time for the membrane to repolarize between APs and partially because it reduces cellular  $K^+$  loss and subsequently  $[K^+]_{int}$  with the end result of rendering the  $K^+$  effects to potentiating again (point “3” in Fig. 2). Furthermore, as exercise continues, muscle  $[K^+]_{int}$  can actually decline from an early peak of  $> 10$  mM even with unchanged exercise intensity (Nielsen et al. 2004a), presumably due to factors such as increased muscle NKA activity, elevated blood flow, increased oxidative energy supply and possibly reduced motor unit motorneuron firing frequency. Thus, the influence of elevated  $[K^+]_e$  on contractile performance may be continuously fluctuating between potentiation and fatigue effects and thereby both aid initial or submaximal exercise performance, but also help determine the limits for muscular performance during intense activities, in a regulated balance involving motor output from the central nervous system as well as various hormonal factors (catecholamines and CGRP) that modulate the  $K^+$  effect. Nevertheless, it should be noted that during non-fatiguing moderate exercise, where activation frequencies are sub-maximum and increased  $[K^+]_e$  are low/moderate, it seems reasonable to consider  $K^+$  to be a primarily positive regulator of contractile performance in muscles.

### Metabolic state sarcolemmal excitability link: role of NKA, $K_{ATP}$ and CLC-1 channel

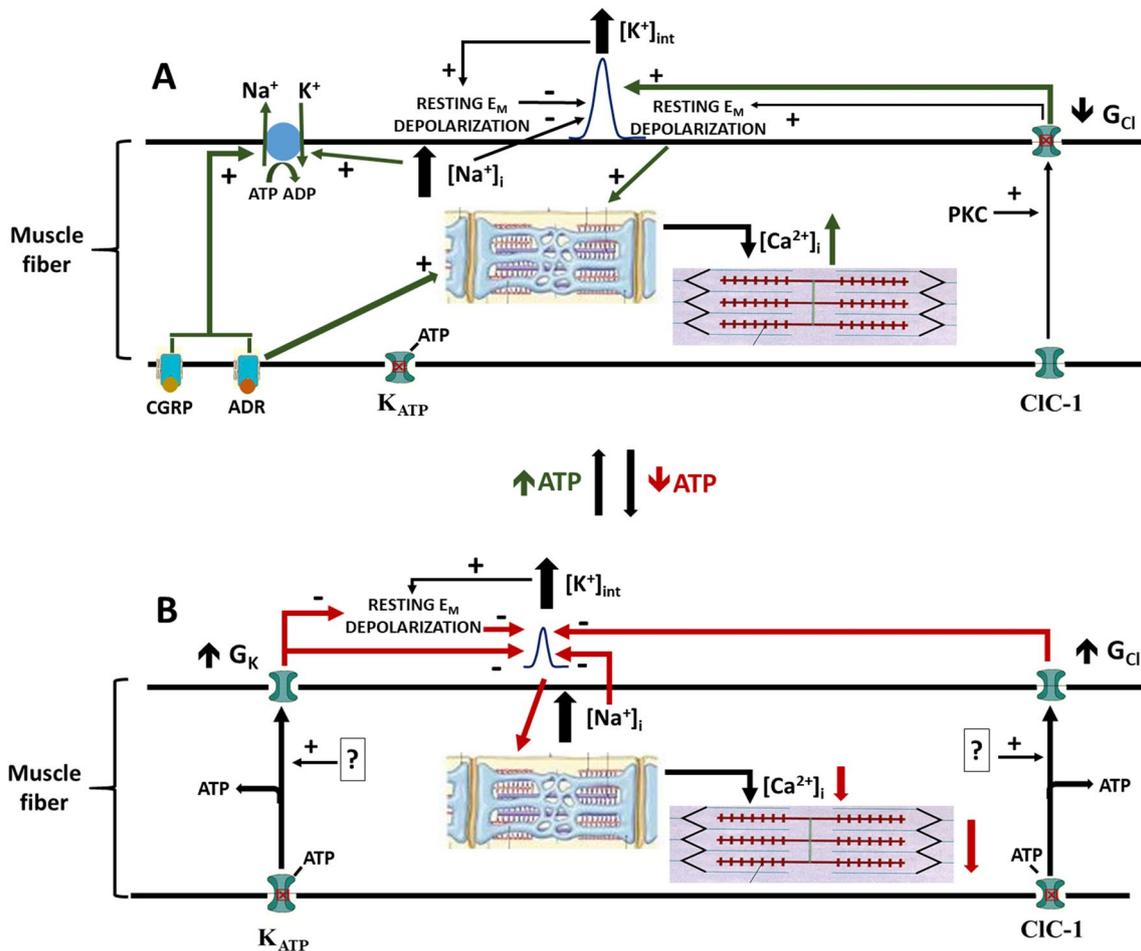
At the onset of any muscle activity, one expects that ATP demands are met by adequate ATP production so that there is no metabolic stress related to an energy deficit. Four major events occur under this metabolic/energy condition (Fig. 3A); (i) large increases in  $[K^+]_{int}$  that can reach 10–12 mM as observed even in moderate 30 W knee extension exercise (Nielsen et al. 2004a), (ii) increases in  $[Na^+]_i$ , (iii) increases in NKA activity due to increases in  $[Na^+]_i$ , activation by catecholamines and CGRP, and iv) decreases in  $G_{Cl}$  as CLC-1 channels close following phosphorylation by PKC (as observed during phase 1 in the Pedersen et al. studies (2009). The expected high  $[K^+]_{int}$ - and  $[Na^+]_i$ -induced loss of membrane excitability and thus force as discussed above is counteracted by the increased in NKA activity and closure of CLC-1  $Cl^-$  channels. This occurs because under those conditions the critical  $[K^+]_e$  that causes force depression is shifted to higher  $[K^+]_e$ , which also shifts the critical resting  $E_M$  to less negative potential. Furthermore, sub-maximal tetanic forces are potentiated by the increased  $[K^+]_{int}$  as well as by catecholamines increasing SR  $Ca^{2+}$  release. The duration of this condition (i.e., phase 1) lasts as long as there is no or minimal metabolic stress becoming shorter as the muscular activity becomes more intense.

When muscle ATP does not meet the demand, especially in restricted areas of the muscle fiber, the ATP depletion triggers phase 2. Phase 2 involves large increases in  $G_{Cl}$  and

$G_K$  as CLC-1 and  $K_{ATP}$  channels are activated in part by the decrease in ATP concentration allowing the ATP dissociation from the channels and in part by intracellular signaling pathway(s) that has yet to be identified (Fig. 3B). The increases in CLC-1 channel activity or  $G_{Cl}$  and in  $K_{ATP}$  channel activity or  $G_K$  directly reduce sarcolemmal excitability by providing an outward  $Cl^-$  and  $K^+$  outward currents that (i) move the action potential threshold toward less negative  $E_M$  and (ii) counteract the  $Na^+$  inward current during the depolarization reducing action potential amplitude. Although the effect of an increase in  $G_{Cl}$  on membrane excitability and force generation has not been studied directly due to the lack of a CLC-1 channel opener, one can expect that the increased  $G_{Cl}$  lowers the critical  $[K^+]_e$  switching the  $K^+$  effect from potentiation to depression.

These physiological responses during phase 2 are critical to prevent damaging ATP depletion as well as excitability, contractile and metabolic dysfunctions that occur during fatigue in  $K_{ATP}$  channel deficient muscles, as discussed above in the section entitled “ *$K_{ATP}$  channels: another physiological function for  $K^+$  during fatigue*”. Finally, as recently reviewed, after intense muscle activity plasma  $[K^+]$  falls rapidly and can decline to below pre-exercise levels indicating ongoing elevated muscle NKA activity. (McKenna et al. 2023). This suggests that local and circulating factors (e.g., CGRP and catecholamines, respectively) that augment NKA activity during exercise may thereby also contribute to preventing excessive increases in muscle  $[K^+]_{int}$  and  $[Na^+]_i$  that might otherwise completely paralyze the contracting muscle.

The model proposed in Fig. 3 is based mostly on results obtained from animal studies. So, one can raise the issue as to whether it applies to human skeletal muscle. On the one hand, considering i) that a few studies reported similar changes in  $[K^+]_i$ ,  $[Na^+]_{int}$  and  $[Na^+]_i$  between animal and human muscles, together with the larger number of reports on human muscle  $[K^+]_{int}$  (Tables 1 and 3), one can expect that the model in Fig. 3 also applies to human muscles. Furthermore, the changes in  $G_{Cl}$  (i.e., phases 1 and 2) during repetitive AP firing that have been reported in rat and mouse EDL also occur in human skeletal muscles (Riisager et al. 2014, 2016; Pedersen et al. 2009b, 2009a). On the other hand, a decreased sarcolemmal excitability in human muscle during fatigue is controversial because most studies reported no change or increases in M-wave amplitude during fatigue in human muscles as previously reviewed (Allen et al. 2008b). Interestingly, it has recently been proposed that increases in M-waves amplitude are in fact evidence of excitability disruption, especially AP propagation (Rodriguez-Falces and Place 2018). This is because the M-wave has two components: (i) a propagating positive signal from AP and (ii) a non-propagating negative signal from the AP termination at the tendon. They discuss the possibility that under normal unfatigued



**Fig. 3** A modified concept for the physiological roles of  $K^+$  in combination with combined effects of  $Na^+$ ,  $Cl^-$ , NKA activity, and CIC-1 and  $K_{ATP}$  channels, during muscle activity in the absence and presence (i.e., fatigue) of metabolic stress. **A** In the absence of metabolic stress, the expected high  $[K^+]_{int}$  and  $[Na^+]_i$ -induced loss of membrane excitability due to increases in  $[Na^+]_i$  and  $[K^+]_{int}$  and thus force is counteracted by the closure of CIC-1  $Cl^-$  channels following phosphorylation by PKC and by an increased NKA activity due to its activation by catecholamines (via their adrenergic receptors, ADR), CGRP as well as elevation in  $[Na^+]_i$ . This occurs because under those conditions the critical  $[K^+]_{int}$  that causes force depression is shifted to higher  $[K^+]_{int}$ , which also shifts the critical resting  $E_M$  to less negative potential. Furthermore, sub-maximal tetanic forces are potentiated

by the increased  $[K^+]_{int}$  as well as by catecholamines increasing SR  $Ca^{2+}$  release. **B** During metabolic stress, CIC-1 and  $K_{ATP}$  channels are activated in part by the decrease in ATP concentration allowing ATP dissociation from the channels and in part by intracellular signaling pathway(s) that has yet to be identified. The increases in CIC-1 channel activity ( $G_{Cl}$ ) and in  $K_{ATP}$  channel activity ( $G_K$ ) directly reduce sarcolemmal excitability by providing an outward  $Cl^-$  and  $K^+$  currents that (i) move the action potential threshold toward less negative  $E_M$  and (ii) counteract the  $Na^+$  inward current during the depolarization, thereby reducing action potential amplitude. The increase in  $G_{Cl}$  also lowers the critical  $[K^+]_e$  switching the  $K^+$  effect from force potentiation to depression

conditions, the non-propagating negative signal is close to the propagating positive signal with the net effect of reducing the amplitude of the former. As fatigue occurs and as AP propagation slows down, the distance between the two signals increases resulting in less counteraction of the negative signal, resulting in greater amplitude of the positive signal. Thus, future studies will be necessary to better understand the significance of the M-wave signals and to determine if the model in Fig. 3 also applies to human muscles.

## Conclusions

$K^+$  disturbances in muscle have long been considered a factor in the mechanism by which force/work decreases during fatigue in skeletal muscle. Studies have questioned this potential role based on (i) the increase in muscle  $[K^+]_e$  during fatigue not being high enough by itself to induce force depression during fatigue, or actually declining during continued exercise and ii) the fact that for submaximal

tetanic contraction, small increases in  $[K^+]_e$  actually potentiate muscle force. This review emphasizes that  $K^+$  exerts dual roles. A first role is one of force potentiation during low-to-moderate exercise intensities, as part of several mechanisms that optimize muscle contraction. A second role is one of force depression that occurs during a metabolic stress that restricts ATP availability. At the onset of muscle activity and during moderate muscle activity, the  $K^+$ -induced potentiation predominates while the  $K^+$ -induced depression is prevented, primarily because of NKA activation and a reduced  $G_{Cl}$  as CIC-1 channels close. The  $K^+$ -induced force depression in muscle occurs when metabolic stress/energy deficit occurs and leads to the activation of CIC-1 and  $K_{ATP}$  channels, which reduces sarcolemmal excitability and thus force. The  $K^+$ -induced force depression is further enhanced by the synergistic depressive effect of reduced  $Na^+$  and  $K^+$  gradients.

## Future studies

Although the decrease in  $G_{Cl}$  in phase 1 involves CIC-1 channel phosphorylation by PKC, the mechanism by which metabolic stress activates both CIC-1 and  $K_{ATP}$  channels remains to be elucidated. Although ATP modulates the activity of both channels, where decreases in ATP result in greater channel activity, it is more than likely that intracellular signaling pathways are implicated. For example, the adenosine A1-receptor activates  $K_{ATP}$  channels under patch clamp conditions (Barrett-Jolley et al. 1996) while AMP kinase (AMPK), a well-known intracellular cell energy sensor, activates cardiac  $K_{ATP}$  channels during metabolic stress (Yoshida et al. 2012). Furthermore, it is important to bear in mind that the majority of mechanistic studies on muscle ion regulation and excitability are conducted in rodent muscles, with few studies in humans. Also, studies measuring compound action potentials (EMG) inherently only estimate muscle surface AP propagation and not t-tubule excitability, representing the larger part of the muscle membrane. In fact, little is known about t-tubule excitability even in rodent muscle. Thus, a better understanding of the importance of the t-system excitability in determining the force response under physiologically relevant conditions in human as well as animal skeletal muscles is requisite.

**Author contributions** J-M Renaud wrote most of the manuscript. KO wrote the section entitled “The  $K^+$ -induced force potentiation” and with J-M Renaud wrote the section entitled “A new perspective for the role of  $K^+$ ,  $Na^+$  and  $Cl^-$  on muscle performance from the onset of exercise to fatigue” NØ wrote the first three subsections of the section entitled “Muscle metabolic links to sarcolemmal excitability”. MM commented on the manuscript as well as every other co-authors.

**Funding** This study was funded by Natural Sciences and Engineering of Canada, 153604, Jean-Marc Renaud.

**Data availability** All data discussed in this review comes from published work. There was no new data for this review.

**Declaration**

**Conflict of interest** None of the authors had a conflict of interests in regard to this review.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## References

- Adrian RH (1956) The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J Physiol* 133(3):631–658. <https://doi.org/10.1113/jphysiol.1956.sp005615>
- Aguilar-Bryan L, Bryan J (1999) Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Rev* 20:101–135
- Aickin CC, Betz WJ, Harris GL (1989) Intracellular chloride and the mechanism for its accumulation in rat lumbrical muscle. *J Physiol* 411:437–455
- Allard B, Lazdunski M, Rougier O (1995) Activation of ATP-dependent  $K^+$  channels by metabolic poisoning in adult mouse skeletal muscle: role of intracellular  $Mg^{2+}$  and pH. *J Physiol* 485(Pt 2):283–296. <https://doi.org/10.1113/jphysiol.1995.sp020730>
- Allen DG, Lee JA, Westerblad H (1989) Intracellular calcium and tension during fatigue in isolated single muscle fibres from *Xenopus laevis*. *J Physiol (lond)* 415:433–458
- Allen DG, Lamb GD, Westerblad H (2008a) Impaired calcium release during fatigue. *J Appl Physiol* 104(1):296–305. <https://doi.org/10.1152/jappphysiol.00908.2007>. (00908.2007[pii])
- Allen DG, Lamb GD, Westerblad H (2008b) Skeletal muscle fatigue: cellular mechanisms. *Physiol Rev* 88(1):287–332. <https://doi.org/10.1152/physrev.00015.2007>. (88/1/287[pii])
- Ammar T, Lin W, Higgins A, Hayward LJ, Renaud JM (2015) Understanding the physiology of the asymptomatic diaphragm of the M1592V of the hyperkalemic periodic paralysis mouse. *J Gen Physiol* 146:509–525
- Andersen SLV, Clausen T (1993) Calcitonin gene-related peptide stimulates active  $Na^+$ - $K^+$  transport in rat soleus muscle. *Am J Physiol* 264:C419–C429
- Armstrong RB, Phelps RO (1984) Muscle fiber type composition of the rat hindlimb. *Am J Anat* 171(3):259–272. <https://doi.org/10.1002/aja.1001710303>
- Armstrong ML, Dua AK, Murrant CL (2007) Potassium initiates vasodilatation induced by a single skeletal muscle contraction in hamster cremaster muscle. *J Physiol* 581(Pt 2):841–852. <https://doi.org/10.1113/jphysiol.2007.130013>

- Babenko AP, Aguilar-Bryan L, Bryan J (1998) A view of SUR/Kir6.X  $K_{ATP}$  channels. *Annu Rev Physiol* 60:667–687
- Baetjer AM (1935) The effect of potassium (and calcium) on the contractions of mammalian skeletal muscle. *Am J Physiol* 112(1):147–151
- Balog EM, Fitts RH (1996) Effects of fatiguing stimulation on intracellular  $Na^+$  and  $K^+$  in frog skeletal muscle. *J Appl Physiol* (1985) 81(2):679–685. <https://doi.org/10.1152/jappl.1996.81.2.679>
- Balog EM, Thompson LV, Fitts RH (1994) Role of sarcolemma action potentials and excitability in muscle fatigue. *J Appl Physiol* (1985) 76(5):2157–2162. <https://doi.org/10.1152/jappl.1994.76.5.2157>
- Banas K, Clow C, Jasmin BJ, Renaud JM (2011) The  $K_{ATP}$  channel Kir6.2 subunit protein content is higher in glycolytic than in oxidative skeletal muscle fibers. *Am J Physiol* 301:R916–R925
- Bangsbo J, Graham TE, Kiens B, Saltin B (1992) Elevated muscle glycogen and anaerobic energy production during exhaustive exercise in man. *J Physiol (lond)* 451:205–227
- Barnes M, Gibson LM, Stephenson DG (2001) Increased muscle glycogen content is associated with increased capacity to respond to T-system depolarisation in mechanically skinned skeletal muscle fibres from the rat. *Pflügers Arch* 442(1):101–106. <https://doi.org/10.1007/s004240000510>
- Barrett-Jolley R, Comtois A, Davies NW, Stanfield PR, Standen NB (1996) Effect of adenosine and intracellular GTP on  $K_{ATP}$  channels of mammalian skeletal muscle. *J Membr Biol* 152:111–116
- Bendahhou S, Cummins TR, Potts JF, Tong J, Agnew WS (1995) Serine-1321-independent regulation of the  $\mu 1$  adult skeletal muscle  $Na^+$  channel by protein kinase C. *Proc Natl Acad Sci U S A* 92(26):12003–12007
- Bendahhou S, Cummins TR, Tawil R, Waxman SG, Ptacek LJ (1999) Activation and inactivation of the voltage-gated sodium channel: role of segment S5 revealed by a novel hyperkalaemic periodic paralysis mutation. *J Neurosci* 19(12):4762–4771
- Bendahhou S, Cummins TR, Kula RW, Fu YH, Ptacek LJ (2002) Impairment of slow inactivation as a common mechanism for periodic paralysis in DIIS4-S5. *Neurology* 58(8):1266–1272
- Bergström J, Hermansen L, Hultman E, Saltin B (1967) Diet, muscle glycogen and physical performance. *Acta Physiol Scand* 71(2):140–150. <https://doi.org/10.1111/j.1748-1716.1967.tb03720.x>
- Bergström J, Guarnieri G, Hultman E (1971) Carbohydrate metabolism and electrolyte changes in human muscle tissue during heavy work. *J Appl Physiol* 30(1):122–125. <https://doi.org/10.1152/jappl.1971.30.1.122>
- Bezaniilla F, Caputo C, Gonzales-Serratos H, Venosa RA (1972) Sodium dependence of the inward spread of activation in isolated twitch muscle fibres of the frogs. *J Physiol (lond)* 223:507–523
- Bigland-Ritchie B, Johansson R, Lippold OC, Smith S, Woods JJ (1983) Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J Physiol* 340:335–346. <https://doi.org/10.1113/jphysiol.1983.sp014765>
- Blanco G, Mercer RW (1998) Isozymes of the Na-K-ATPase: heterogeneity in structure, diversity in function. *Am J Physiol* 275(Pt 2):F633–F650
- Bouclin R, Charbonneau E, Renaud JM (1995)  $Na^+$  and  $K^+$  effect on contractility of frog sartorius muscle: implication for the mechanism of fatigue. *Am J Physiol* 268:C1528–C1536
- Bowman WC, Zaimis E (1958) The effects of adrenaline, noradrenaline and isoprenaline on skeletal muscle contractions in the cat. *J Physiol* 144(1):92–107
- Bretag AH (1987) Muscle chloride channels. *Physiol Rev* 67:618–724
- Brooks GA (1986) Lactate production under fully aerobic conditions: the lactate shuttle during rest and exercise. *Fed Proc* 45(13):2924–2929
- Brown GL, von Euler US (1938) The after effects of a tetanus on mammalian muscle. *J Physiol (lond)* 93:39–60
- Bundgaard H, Kjeldsen K, Suarez Krabbe K, van Hall G, Simonsen L, Qvist J, Hansen CM, Moller K, Fonsmark L, Lav Madsen P, Klarlund Pedersen B (2003) Endotoxemia stimulates skeletal muscle  $Na^+$ - $K^+$ -ATPase and raises blood lactate under aerobic conditions in humans. *Am J Physiol Heart Circ Physiol* 284(3):H1028–1034. <https://doi.org/10.1152/ajpheart.00639.2002>
- Cairns SP, Borrani F (2015) beta-Adrenergic modulation of skeletal muscle contraction: key role of excitation-contraction coupling. *J Physiol* 593(21):4713–4727. <https://doi.org/10.1113/JP270909>
- Cairns SP, Dulhunty AF (1993a) Beta-adrenergic potentiation of E-C coupling increases force in rat skeletal muscle. *Muscle Nerve* 16(12):1317–1325. <https://doi.org/10.1002/mus.880161208>
- Cairns SP, Dulhunty AF (1993b) The effects of beta-adrenoceptor activation on contraction in isolated fast- and slow-twitch skeletal muscle fibres of the rat. *Br J Pharmacol* 110(3):1133–1141
- Cairns SP, Lindinger MI (2008) Do multiple ionic interactions contribute to skeletal muscle fatigue? *J Physiol* 586(17):4039–4054. <https://doi.org/10.1113/jphysiol.2008.155424>
- Cairns SP, Westerblad H, Allen DG (1993) Changes of tension and  $[Ca^{2+}]_i$  during beta-adrenoceptor activation of single, intact fibres from mouse skeletal muscle. *Pflügers Arch* 425(1–2):150–155. <https://doi.org/10.1007/bf00374515>
- Cairns SP, Flatman JA, Clausen T (1995) Relation between extracellular  $[K^+]_o$ , membrane potential and contraction in rat soleus muscle: modulation by the  $Na^+$ - $K^+$  pump. *Pflügers Arch* 430(6):909–915. <https://doi.org/10.1007/BF01837404>
- Cairns SP, Hing WA, Slack JR, Mills RG, Loiselle DS (1997) Different effects of raised  $[K^+]_o$  on membrane potential and contraction in mouse fast- and slow-twitch muscle. *Am J Physiol* 273(Pt 1):C598–611. <https://doi.org/10.1152/ajpcell.1997.273.2.C598>
- Cairns SP, Buller SJ, Loiselle DS, Renaud JM (2003) Changes of action potentials and force at lowered  $[Na^+]_o$  in mouse skeletal muscle: implication for fatigue. *Am J Physiol Cell Physiol* 285:C1131–C1141
- Cairns SP, Ruzhynsky V, Renaud JM (2004) Protective role of extracellular chloride in fatigue of isolated mammalian skeletal muscle. *Am J Physiol* 287:C762–C770
- Cairns SP, Leader JP, Loiselle DS (2011) Exacerbated potassium-induced paralysis of mouse soleus muscle at 37°C vis-a-vis 25°C: implications for fatigue. *Pflügers Arch* 461:469–479
- Cairns SP, Leader JP, Loiselle DS, Higgins A, Lin W, Renaud JM (2015) Extracellular  $Ca^{2+}$ -induced force restoration in  $K^+$ -depressed skeletal muscle of the mouse involves an elevation of  $[K^+]_i$ : implications for fatigue. *J Appl Physiol* (1985) 118(6):662–674. <https://doi.org/10.1152/japplphysiol.00705.2013>
- Cairns SP, Leader JP, Higgins A, Renaud JM (2022) The peak force-resting membrane potential relationships of mouse fast- and slow-twitch muscle. *Am J Physiol Cell Physiol* 322(6):C1151–C1165. <https://doi.org/10.1152/ajpcell.00401.2021>
- Caldwell PC, Hodgkin AL, Keynes RD, Shaw TL (1960) The effects of injecting ‘energy-rich’ phosphate compounds on the active transport of ions in the giant axons of Loligo. *J Physiol* 152(3):561–590. <https://doi.org/10.1113/jphysiol.1960.sp006509>
- Campbell JD, Paul RJ (1992) The nature of fuel provision for the  $Na^+$ ,  $K^+$ -ATPase in porcine vascular smooth muscle. *J Physiol* 447:67–82. <https://doi.org/10.1113/jphysiol.1992.sp018991>
- Castle NA, Haylett DG (1987) Effect of channel blockers on potassium efflux from metabolically exhausted frog skeletal muscle. *J Physiol (lond)* 383:31–43
- Chibalin AV, Heiny JA, Benziane B, Prokofiev AV, Vasiliev AV, Kravtsova VV, Krivoi II (2012) Chronic nicotine modifies skeletal muscle Na, K-ATPase activity through its interaction with the

- nicotinic acetylcholine receptor and phospholemman. *Plos One* 7(3):e33719. <https://doi.org/10.1371/journal.pone.0033719>. (PONE-D-11-22631 [pii])
- Chin ER, Allen DG (1997) Effects of reduced muscle glycogen concentration on force,  $\text{Ca}^{2+}$  release and contractile protein function in intact mouse skeletal muscle. *J Physiol (lond)* 498:17–29
- Christensen EH, Hansen O (1939) III. Arbeitsfähigkeit und Ernährung I. Skandinavisches Archiv für Physiologie 81(1):160–171
- Cifelli C, Bourassa F, Gariépy L, Banas K, Benkhalti M, Renaud JM (2007)  $\text{K}_{\text{ATP}}$  channel deficiency in mouse flexor digitorum brevis causes fiber damage and impairs  $\text{Ca}^{2+}$  and force development during fatigue in vitro. *J Physiol (lond)* 582:843–857
- Cifelli C, Boudreault L, Gong B, Bercier JP, Renaud JM (2008) Contractile dysfunctions in  $\text{K}_{\text{ATP}}$  channel mouse FDB during fatigue involves  $\text{Ca}^{2+}$  through L-type  $\text{Ca}^{2+}$  channels. *Exp Physiol* 93:1126–1138
- Clausen T (1965) The relationship between the transport of glucose and cations across cell membranes in isolated tissues. I. Stimulation of glycogen deposition and inhibition of lactic acid production in diaphragm, induced by ouabain. *Biochim Biophys Acta* 109(1):164–171. [https://doi.org/10.1016/0926-6585\(65\)90100-7](https://doi.org/10.1016/0926-6585(65)90100-7)
- Clausen T (2003)  $\text{Na}^{+}$ - $\text{K}^{+}$  pump regulation and skeletal muscle contractility. *Physiol Rev* 83:1269–1324
- Clausen T (2013) Quantification of  $\text{Na}^{+}$ ,  $\text{K}^{+}$  pumps and their transport rate in skeletal muscle: functional significance. *J Gen Physiol* 142(4):327–345. <https://doi.org/10.1085/jgp.201310980>
- Clausen T, Everts ME (1991)  $\text{K}^{+}$ -Induced inhibition of contractile force in rat skeletal muscle: role of active  $\text{Na}^{+}$ - $\text{K}^{+}$  transport. *Am J Physiol Cell Physiol* 261:C799–C807
- Clausen T, Flatman JA (1977) The effect of catecholamines on  $\text{Na}$ - $\text{K}$  transport and membrane potential in rat soleus muscle. *J Physiol (lond)* 270:383–414
- Clausen T, Nielsen OB (2007) Potassium,  $\text{Na}^{+}$ ,  $\text{K}^{+}$ -pumps and fatigue in rat muscle. *J Physiol* 584(Pt 1):295–304. <https://doi.org/10.1113/jphysiol.2007.136044>
- Clausen T, Van Hardevelde C, Everts ME (1991) Significance of cation transport in control of energy metabolism and thermogenesis. *Physiol Rev* 71(3):733–774. <https://doi.org/10.1152/physrev.1991.71.3.733>
- Clausen T, Andersen SLV, Flatman JA (1993)  $\text{Na}^{+}$ - $\text{K}^{+}$  pump stimulation elicits recovery of contractility in  $\text{K}^{+}$ -paralysed rat muscle. *J Physiol (lond)* 472:521–536
- Comtois A, Sinderby C, Comtois N, Grassino A, Renaud JM (1994) An ATP-sensitive potassium channel blocker decreases diaphragmatic circulation in anesthetized dogs. *J Appl Physiol* 77:127–134
- Cummins TR, Sigworth FJ (1996) Impaired slow inactivation in mutant sodium channels. *Biophys J* 71(1):227–236. [https://doi.org/10.1016/S0006-3495\(96\)79219-6](https://doi.org/10.1016/S0006-3495(96)79219-6)
- Cummins TR, Zhou J, Sigworth FJ, Ukomadu C, Stephan M, Ptáček LJ, Agnew WS (1993) Functional consequences of a  $\text{Na}^{+}$  channel mutation causing hyperkalemic periodic paralysis. *Neuron* 10:667–678
- Davies NW, Standen NB, Stanfield PR (1992) The effect of intracellular pH on ATP-dependent potassium channels of frog skeletal muscle. *J Physiol (lond)* 445:549–568
- Dawson MJ, Gadian DG, Wilkie DR (1978) Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature* 274:861–866
- de Paoli FV, Broch-Lips M, Pedersen TH, Nielsen OB (2013) Relationship between membrane  $\text{Cl}^{-}$  conductance and contractile endurance in isolated rat muscles. *J Physiol* 591(Pt 2):531–545. <https://doi.org/10.1113/jphysiol.2012.243246>. (jphysiol.2012.243246[pii])
- Desmedt JE (1953) Electrical activity and intracellular sodium concentration in frog muscle. *J Physiol* 121(1):191–205. <https://doi.org/10.1113/jphysiol.1953.sp004940>
- Dhar-Chowdhury P, Harrell MD, Han SY, Jankowska D, Parachuru L, Morrissey A, Srivastava S, Liu W, Malester B, Yoshida H, Coetzee WA (2005) The glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, triose-phosphate isomerase, and pyruvate kinase are components of the  $\text{K}(\text{ATP})$  channel macromolecular complex and regulate its function. *J Biol Chem* 280(46):38464–38470. <https://doi.org/10.1074/jbc.M508744200>. (M508744200[pii])
- Dhar-Chowdhury P, Malester B, Rajacic P, Coetzee WA (2007) The regulation of ion channels and transporters by glycolytically derived ATP. *Cell Mol Life Sci* 64(23):3069–3083. <https://doi.org/10.1007/s00018-007-7332-3>
- Donaldson PJ, Leader JP (1984) Intracellular ionic activities in the EDL muscle of the mouse. *Pflugers Arch* 400(2):166–170. <https://doi.org/10.1007/bf00585034>
- Duhamel TA, Green HJ, Perco JG, Ouyang J (2006a) Comparative effects of a low-carbohydrate diet and exercise plus a low-carbohydrate diet on muscle sarcoplasmic reticulum responses in males. *Am J Physiol Cell Physiol* 291(4):C607–617. <https://doi.org/10.1152/ajpcell.00643.2005>
- Duhamel TA, Green HJ, Perco JG, Ouyang J (2006b) Effects of prior exercise and a low-carbohydrate diet on muscle sarcoplasmic reticulum function during cycling in women. *J Appl Physiol* (1985) 101(3):695–706. <https://doi.org/10.1152/jappphysiol.00052.2006>
- Dulhunty A (1977)  $\text{K}$ -contractures and membrane potential in mammalian skeletal muscle. *Nature* 266(5597):75–78. <https://doi.org/10.1038/266075a0>
- Dulhunty AF (1978) The dependence of membrane potential on extracellular chloride concentration in mammalian skeletal muscle fibres. *J Physiol* 276:67–82. <https://doi.org/10.1113/jphysiol.1978.sp012220>
- Dulhunty AF (1979) Distribution of potassium and chloride permeability over the surface and t-tubule membranes of mammalian skeletal muscle. *J Membr Biol* 45:293–310
- Dulhunty AF (1984) Heterogeneity of T-tubule geometry in vertebrate skeletal muscle fibres. *J Muscle Res Cell Motil* 5(3):333–347. <https://doi.org/10.1007/bf00713111>
- Dutka TL, Lamb GD (2007a)  $\text{Na}^{+}$ - $\text{K}^{+}$  pumps in the transverse tubular system of skeletal muscle fibers preferentially use ATP from glycolysis. *Am J Physiol Cell Physiol* 293(3):C967–977. <https://doi.org/10.1152/ajpcell.00132.2007>
- Dutka TL, Lamb GD (2007b) Transverse tubular system depolarization reduces tetanic force in rat skeletal muscle fibers by impairing action potential repriming. *Am J Physiol Cell Physiol* 292(6):C2112–2121. <https://doi.org/10.1152/ajpcell.00006.2007>
- Dutka TL, Murphy RM, Stephenson DG, Lamb GD (2008) Chloride conductance in the transverse tubular system of rat skeletal muscle fibres: importance in excitation-contraction coupling and fatigue. *J Physiol* 586(3):875–887. <https://doi.org/10.1113/jphysiol.2007.144667>
- Enoka RM, Fuglevand AJ (2001) Motor unit physiology: some unresolved issues. *Muscle Nerve* 24(1):4–17. [https://doi.org/10.1002/1097-4598\(200101\)24:1%3c4::aid-mus13%3e3.0.co;2-f](https://doi.org/10.1002/1097-4598(200101)24:1%3c4::aid-mus13%3e3.0.co;2-f)
- Entman ML, Keslensky SS, Chu A, Van Winkle WB (1980) The sarcoplasmic reticulum-glycogenolytic complex in mammalian fast twitch skeletal muscle. Proposed in vitro counterpart of the contraction-activated glycogenolytic pool. *J Biol Chem* 255(13):6245–6252
- Epstein T, Xu L, Gillies RJ, Gatenby RA (2014) Separation of metabolic supply and demand: aerobic glycolysis as a normal physiological response to fluctuating energetic demands

- in the membrane. *Cancer Metab* 2:7. <https://doi.org/10.1186/2049-3002-2-7>
- Erecińska M, Dagani F (1990) Relationships between the neuronal sodium/potassium pump and energy metabolism. Effects of K<sup>+</sup>, Na<sup>+</sup>, and adenosine triphosphate in isolated brain synaptosomes. *J Gen Physiol* 95(4):591–616. <https://doi.org/10.1085/jgp.95.4.591>
- Everts ME, Clausen T (1994) Excitation-induced activation of the Na<sup>+</sup>-K<sup>+</sup> pump in rat skeletal muscle. *Am J Physiol* 266:C925–C934
- Everts ME, Lomo T, Clausen T (1993) Changes in K<sup>+</sup>, Na<sup>+</sup> and calcium contents during in vivo stimulation of rat skeletal muscle. *Acta Physiol Scand* 147(4):357–368. <https://doi.org/10.1111/j.1748-1716.1993.tb09512.x>
- Ewart HS, Klip A (1995) Hormonal regulation of the Na<sup>(+)</sup>-K<sup>(+)</sup>-ATPase: mechanisms underlying rapid and sustained changes in pump activity. *Am J Physiol* 269(2 Pt 1):C295–311. <https://doi.org/10.1152/ajpcell.1995.269.2.C295>
- Fahlke C, Rüdell R (1995) Chloride currents across the membrane of mammalian skeletal muscle fibres. *J Physiol (lond)* 484:355–368
- Featherstone DE, Richmond JE, Ruben PC (1996) Interaction between fast and slow inactivation in Skm1 sodium channels. *Biophys J* 71(6):3098–3109. [https://doi.org/10.1016/S0006-3495\(96\)79504-8](https://doi.org/10.1016/S0006-3495(96)79504-8)
- Fenn WO (1937) Loss of potassium in voluntary contraction. *Am J Physiol* 120:675–680
- Fenn WO (1938) Factors affecting the loss of potassium from stimulated muscles. *Am J Physiol* 124:213–227
- Fenn WO (1940) The role of potassium in physiological processes. *Physiol Rev* 20:377–415
- Fenn WO, Cobb DM (1936) Electrolyte changes in muscle during activity. *Am J Physiol* 115(2):345–356
- Fink R, Luttgau HC (1976) An evaluation of the membrane constants and the potassium conductance in the metabolically exhausted muscle fibers. *J Physiol (lond)* 263:215–238
- Foster MN, Coetzee WA (2016) KATP channels in the cardiovascular system. *Physiol Rev* 96(1):177–252. <https://doi.org/10.1152/physrev.00003.2015>
- Frentzel J, Reach F (1901) Untersuchungen zur frage nach der quelle der muskelkraft. *Archiv Für Die Gesamte Physiologie Des Menschen Und Der Tiere* 83(10):477–508
- Geukes Foppen RJ (2004) In skeletal muscle the relaxation of the resting membrane potential induced by K<sup>+</sup> permeability changes depends on Cl<sup>-</sup> transport. *Pflugers Arch* 447:416–425
- Gong B, Miki T, Seino S, Renaud JM (2000) A K<sup>+</sup><sub>(ATP)</sub> deficiency affects resting tension not contractile force during fatigue in skeletal muscle. *Am J Physiol* 279:C1351–C1358
- Gong B, Legault D, Miki T, Seino S, Renaud JM (2003) K<sub>ATP</sub> channels depress force by reducing action potential amplitude in mouse EDL and soleus. *Am J Physiol Cell Physiol* 285:C1464–C1474
- Goodman C, Blazev R, Stephenson G (2005) Glycogen content and contractile responsiveness to T-system depolarization in skinned muscle fibres of the rat. *Clin Exp Pharmacol Physiol* 32(9):749–756. <https://doi.org/10.1111/j.1440-1681.2005.04260.x>
- Gramolini A, Renaud JM (1997) Blocking ATP-sensitive K<sup>+</sup> channel during metabolic inhibition impairs muscle contractility. *Am J Physiol Cell Physiol* 41:C936–C946
- Green HJ (1991) How important is endogenous muscle glycogen to fatigue in prolonged exercise. *Can J Physiol Pharmacol* 69:290–297
- Green S, Bulow J, Saltin B (1999) Microdialysis and the measurement of muscle interstitial K<sup>+</sup> during rest and exercise in humans. *J Appl Physiol* (1985) 87(1):460–464. <https://doi.org/10.1152/jappl.1999.87.1.460>
- Green S, Langberg H, Skovgaard D, Bulow J, Kjaer M (2000) Interstitial and arterial-venous [K<sup>+</sup>] in human calf muscle during dynamic exercise: effect of ischaemia and relation to muscle pain. *J Physiol* 529(Pt 3):849–861. <https://doi.org/10.1111/j.1469-7793.2000.00849.x>
- Greiner JV, Glonek T (2021) Intracellular ATP concentration and implication for cellular evolution. *Biology (Basel)* 10(11):1166. <https://doi.org/10.3390/biology10111166>
- Grob D, Liljestrand A, Johns RJ (1957) Potassium movement in normal subjects: effect on muscle function. *Am J Med* 23(3):340–355. [https://doi.org/10.1016/0002-9343\(57\)90315-7](https://doi.org/10.1016/0002-9343(57)90315-7)
- Gunnarsson TP, Christensen PM, Thomassen M, Nielsen LR, Bangsbo J (2013) Effect of intensified training on muscle ion kinetics, fatigue development, and repeated short-term performance in endurance-trained cyclists. *Am J Physiol Regul Integr Comp Physiol* 305(7):R811–821. <https://doi.org/10.1152/ajpregu.00467.2012>
- Han J-W, Thieleczek R, Varsanyi M, Heilmeyer LMG (1992) Compartmentalized ATP synthesis in skeletal muscle triads. *Biochemistry* 31:377–384
- Hansen AK, Clausen T, Nielsen OB (2005) Effects of lactic acid and catecholamines on contractility in fast-twitch muscles exposed to hyperkalemia. *Am J Physiol Cell Physiol* 289(1):C104–112. <https://doi.org/10.1152/ajpcell.00600.2004>
- Hanson J (1974a) Effects of repetitive stimulation on membrane potentials and twitch in human and rat intercostal muscle fibres. *Acta Physiol Scand* 92(2):238–248. <https://doi.org/10.1111/j.1748-1716.1974.tb05741.x>
- Hanson J (1974b) The effects of repetitive stimulation on the action potential and the twitch of rat muscle. *Acta Physiol Scand* 90(2):387–400. <https://doi.org/10.1111/j.1748-1716.1974.tb05600.x>
- Hanson J, Persson A (1971) Changes in the action potential and contraction of isolated frog muscle after repetitive stimulation. *Acta Physiol Scand* 81(3):340–348. <https://doi.org/10.1111/j.1748-1716.1971.tb04908.x>
- Hargreaves M, Spriet LL (2018) Exercise metabolism: fuels for the fire. *Cold Spring Harb Perspect Med*. <https://doi.org/10.1101/cshperspect.a029744>
- Hasin Y, Barry WH (1984) Myocardial metabolic inhibition and membrane potential, contraction, and potassium uptake. *Am J Physiol* 247(2 Pt 2):H322–329. <https://doi.org/10.1152/ajpheart.1984.247.2.H322>
- Hayward LJ, Brown RH Jr, Cannon SC (1996) Inactivation defects caused by myotonia-associated mutations in the sodium channel III-IV linker. *J Gen Physiol* 107(5):559–576. <https://doi.org/10.1085/jgp.107.5.559>
- Hayward LJ, Brown RH Jr., Cannon SC (1997) Slow inactivation differs among mutant Na channels associated with myotonia and periodic paralysis. *Biophys J* 72(3):1204–1219. [https://doi.org/10.1016/S0006-3495\(97\)78768-X](https://doi.org/10.1016/S0006-3495(97)78768-X)
- Hayward LJ, Sandoval GM, Cannon SC (1999) Defective slow inactivation of sodium channels contributes to familial periodic paralysis. *Neurology* 52(7):1447–1453. <https://doi.org/10.1212/wnl.52.7.1447>
- Heiny JA, Valle JR, Bryant SH (1990) Optical evidence for a chloride conductance in the T-system of frog skeletal muscle. *Pflugers Arch* 416(3):288–295. <https://doi.org/10.1007/BF00392065>
- Hennig R, Lomo T (1985) Firing patterns of motor units in normal rats. *Nature* 314:164–166
- Hicks A, McComas AJ (1989) Increased sodium pump activity following repetitive stimulation of rat soleus muscles. *J Physiol* 414:337–349. <https://doi.org/10.1113/jphysiol.1989.sp017691>
- Hochachka PW, Matheson GO (1992) Regulating ATP turnover rates over broad dynamic work ranges in skeletal muscles. *J Appl Physiol* 73:1697–1703
- Hodgkin AL, Horowicz P (1959) The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J*

- Physiol 148:127–160. <https://doi.org/10.1113/jphysiol.1959.sp006278>
- Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. *J Physiol* 117(4):500–544. <https://doi.org/10.1113/jphysiol.1952.sp004764>
- Hodgkin AL, Katz B (1949) The effect of sodium ions on the electrical activity of giant axon of the squid. *J Physiol* 108(1):37–77. <https://doi.org/10.1113/jphysiol.1949.sp004310>
- Holmberg E, Waldeck B (1980) On the possible role of potassium ions in the action of terbutaline on skeletal muscle contractions. *Acta Pharmacol Toxicol* 46:141–149
- Hong M, Kefaloyianni E, Bao L, Malester B, Delaroché D, Neubert TA, Coetzee WA (2011) Cardiac ATP-sensitive K<sup>+</sup> channel associates with the glycolytic enzyme complex. *FASEB J* 25(7):2456–2467. <https://doi.org/10.1096/fj.10-176669>
- Hutter OF, Warner AE (1967) The pH sensitivity of the chloride conductance of frog skeletal muscle. *J Physiol (Lond)* 189:403–425
- Huxley AF, Stampfli R (1951) Effect of potassium and sodium on resting and action potentials of single myelinated nerve fibers. *J Physiol* 112(3–4):496–508. <https://doi.org/10.1113/jphysiol.1951.sp004546>
- Inagaki N, Gonoi T, Clement JP IV, Wang C-Z, Aguilar-Bryan L, Bryan J, Seino S (1996) A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K<sup>+</sup> channels. *Neuron* 16:1011–1017
- James J, Fang C, Schrantz SJ, Hasselgren P, Paul RJ, Fischer J (1996) Linkage of aerobic glycolysis to sodium-potassium transport in rat skeletal muscle. *J Clin Invest* 98:2388–2397
- James JH, Luchette FA, McCarter FD, Fischer JE (1999a) Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. *Lancet* 354(9177):505–508. [https://doi.org/10.1016/s0140-6736\(98\)91132-1](https://doi.org/10.1016/s0140-6736(98)91132-1)
- James JH, Wagner KR, King JK, Leffler RE, Uppaturi RK, Balasubramanian A, Friend LA, Shelly DA, Paul RJ, Fischer JE (1999b) Stimulation of both aerobic glycolysis and Na(+)-K(+)-ATPase activity in skeletal muscle by epinephrine or amylin. *Am J Physiol* 277(1 Pt 1):E176–186
- Jensen R, Nielsen J, Ørtenblad N (2020) Inhibition of glycogenolysis prolongs action potential repriming period and impairs muscle function in rat skeletal muscle. *J Physiol* 598(4):789–803. <https://doi.org/10.1113/JP278543>
- Jentsch TJ, Stein V, Weinreich F, Zdebik AA (2002) Molecular structure and physiological function of chloride channels. *Physiol Rev* 82(2):503–568. <https://doi.org/10.1152/physrev.00029.2001>
- Jones DP (1986) Intracellular diffusion gradients of O<sub>2</sub> and ATP. *Am J Physiol* 250(5 Pt 1):C663–675. <https://doi.org/10.1152/ajpcell.1986.250.5.C663>
- Jones DA, Bigland-Ritchie B, Edwards RH (1979) Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. *Exp Neurol* 64(2):401–413. [https://doi.org/10.1016/0014-4886\(79\)90279-6](https://doi.org/10.1016/0014-4886(79)90279-6)
- Juel C (1986) Potassium and sodium shifts during in vitro isometric muscle contraction, and the time course of the ion-gradient recovery. *Pflügers Arch* 406(5):458–463. <https://doi.org/10.1007/bf00583367>
- Juel C (1988) The effect of beta 2-adrenoceptor activation on ion-shifts and fatigue in mouse soleus muscles stimulated in vitro. *Acta Physiol Scand* 134:209–216
- Juel C, Pilegaard H, Nielsen JJ, Bangsbo J (2000) Interstitial K<sup>(+)</sup> in human skeletal muscle during and after dynamic graded exercise determined by microdialysis. *Am J Physiol Regul Integr Comp Physiol* 278(2):R400–406. <https://doi.org/10.1152/ajpregu.2000.278.2.R400>
- Kabbara AA, Nguyen LT, Stephenson GM, Allen DG (2000) Intracellular calcium during fatigue of cane toad skeletal muscle in the absence of glucose. *J Muscle Res Cell Motil* 21(5):481–489
- Karelis AD, Peronnet F, Gardiner PF (2005) Resting membrane potential of rat plantaris muscle fibers after prolonged indirect stimulation in situ: effect of glucose infusion. *Can J Appl Physiol* 30(1):105–112. <https://doi.org/10.1139/h05-108>
- Kennedy BG, Lunn G, Hoffman JF (1986) Effects of altering the ATP/ADP ratio on pump-mediated Na/K and Na/Na exchanges in resealed human red blood cell ghosts. *J Gen Physiol* 87(1):47–72. <https://doi.org/10.1085/jgp.87.1.47>
- Kirsch GE, Anderson MF (1986) Sodium channel kinetics in normal and denervated rabbit muscle membrane. *Muscle Nerve* 9(8):738–747. <https://doi.org/10.1002/mus.880090810>
- Knot HJ, Zimmermann PA, Nelson MT (1996) Extracellular K<sup>+</sup>-induced hyperpolarizations and dilatations of rat coronary and cerebral arteries involve inward rectifier K<sup>+</sup> channels. *J Physiol (Lond)* 492:419–430
- Korge P, Campbell KB (1995) The importance of ATPase microenvironment in muscle fatigue: a hypothesis. *Int J Sports Med* 16(3):172–179. <https://doi.org/10.1055/s-2007-972987>
- Kowalchuk JM, Heigenhauser GJ, Lindinger MI, Sutton JR, Jones NL (1988) Factors influencing hydrogen ion concentration in muscle after intense exercise. *J Appl Physiol Respir Environ Exercise Physiol* 65(5):2080–2089
- Kresge N, Simoni RD, Hill RL (2005) Otto Fritz Meyerhof and the elucidation of the glycolytic pathway. *J Biol Chem* 280(4):e3
- Krogh A, Lindhard J (1920) The relative value of fat and carbohydrate as sources of muscular energy: with appendices on the correlation between standard metabolism and the respiratory quotient during rest and work. *Biochem J* 14(3–4):290–363. <https://doi.org/10.1042/bj0140290>
- Kuba K (1970) Effects of catecholamines on the neuromuscular junction in the rat diaphragm. *J Physiol* 211(3):551–570
- Kuba K, Nohmi M (1987) Role of ion conductance changes and of the sodium-pump in adrenaline-induced hyperpolarization of rat diaphragm muscle fibres. *Br J Pharmacol* 91(3):671–681
- Kuba K, Kuba M, Koketsu K (1978) Adrenaline hyperpolarization in rat diaphragm muscle fibers. *Nihon Seirigaku Zasshi* 40(10):377–380
- Kuzmenkin A, Jurkat-Rott K, Lehmann-Horn F, Mitrovic N (2003) Impaired slow inactivation due to a polymorphism and substitutions of Ser-906 in the II-III loop of the human Nav1.4 channel. *Pflügers Arch* 447(1):71–77. <https://doi.org/10.1007/s00424-003-1137-5>
- Lannergren J, Westerblad H (1986) Force and membrane potential during and after fatiguing, continuous high-frequency stimulation of single *Xenopus* muscle fibres. *Acta Physiol Scand* 128(3):359–368. <https://doi.org/10.1111/j.1748-1716.1986.tb07989.x>
- Lannergren J, Westerblad H (1987) Action potential fatigue in single skeletal muscle fibres of *Xenopus*. *Acta Physiol Scand* 129(3):311–318. <https://doi.org/10.1111/j.1748-1716.1987.tb08074.x>
- Laurin J, Dousset E, Decherchi P (2010) Modulation of the spinal excitability by muscle metabosensitive afferent fibers. *J Neurosci Res* 88(12):2755–2764. <https://doi.org/10.1002/jnr.22432>
- Lee JA, Westerblad H, Allen DG (1991) Changes in tetanic and resting [Ca<sup>2+</sup>]<sub>i</sub> during fatigue and recovery of single muscle fibres from *Xenopus laevis*. *J Physiol (Lond)* 433:307–326
- Lehmann-Horn F, Jurkat-Rott K (1999) Voltage-gated ion channels and hereditary disease. *Physiol Rev* 79:1317–1372
- Levy B, Gibot S, Franck P, Cravoisy A, Bollaert PE (2005) Relation between muscle Na<sup>+</sup>K<sup>+</sup> ATPase activity and raised lactate concentrations in septic shock: a prospective study. *Lancet* 365(9462):871–875. [https://doi.org/10.1016/s0140-6736\(05\)71045-x](https://doi.org/10.1016/s0140-6736(05)71045-x)

- Light PE, Comtois AS, Renaud JM (1994) The effect of glibenclamide on frog skeletal muscle: evidence for  $K^+$  ATP channel activation during fatigue. *J Physiol (lond)* 475:495–507
- Lindinger MI, Heigenhauser GJ (1987) Intracellular ion content of skeletal muscle measured by instrumental neutron activation analysis. *J Appl Physiol* (1985) 63(1):426–433. <https://doi.org/10.1152/jappl.1987.63.1.426>
- Lindinger MI, Heigenhauser GJ (1988) Ion fluxes during tetanic stimulation in isolated perfused rat hindlimb. *Am J Physiol* 254(1 Pt 2):R117–126. <https://doi.org/10.1152/ajpregu.1988.254.1.R117>
- Lindinger MI, Heigenhauser GJ, Spriet LL (1987) Effects of intense swimming and tetanic electrical stimulation on skeletal muscle ions and metabolites. *J Appl Physiol* (1985) 63(6):2331–2339. <https://doi.org/10.1152/jappl.1987.63.6.2331>
- Lindinger MI, Spriet LL, Hultman E, Putman T, McKelvie RS, Lands LC, Jones NL, Heigenhauser GJ (1994) Plasma volume and ion regulation during exercise after low- and high-carbohydrate diets. *Am J Physiol* 266(6 Pt 2):R1896–1906. <https://doi.org/10.1152/ajpregu.1994.266.6.R1896>
- Lindinger MI, Hawke TJ, Vickery L, Bradford L, Lipskie SL (2001) An integrative, in situ approach to examining  $K^+$  flux in resting skeletal muscle. *Can J Physiol Pharmacol* 79:996–1006
- Locasale JW, Cantley LC (2011) Metabolic flux and the regulation of mammalian cell growth. *Cell Metab* 14(4):443–451. <https://doi.org/10.1016/j.cmet.2011.07.014>
- Lott ME, Hogeman CS, Vickery L, Kunselman AR, Sinoway LI, MacLean DA (2001) Effects of dynamic exercise on mean blood velocity and muscle interstitial metabolite responses in humans. *Am J Physiol Heart Circ Physiol* 281(4):H1734–1741. <https://doi.org/10.1152/ajpheart.2001.281.4.H1734>
- Lucas B, Ammar T, Khogali S, DeJong D, Barbalinardo M, Nishi C, Hayward LJ, Renaud JM (2014) Contractile abnormalities of mouse muscles expressing hyperkalemic periodic paralysis mutant  $NaV1.4$  channels do not correlate with  $Na^+$  influx or channel content. *Physiol Genomics* 46(11):385–397. <https://doi.org/10.1152/physiolgenomics.00166.2013>
- Lynch RM, Balaban RS (1987) Coupling of aerobic glycolysis and  $Na^+$ - $K^+$ -ATPase in renal cell line MDCK. *Am J Physiol* 253(2 Pt 1):C269–276. <https://doi.org/10.1152/ajpcell.1987.253.2.C269>
- Macdonald WA, Nielsen OB, Clausen T (2005)  $Na^+$ - $K^+$  pump stimulation restores carbacholine-induced loss of excitability and contractility in rat skeletal muscle. *J Physiol (lond)* 563:459–469
- Macdonald WA, Ørtenblad N, Nielsen OB (2007) Energy conservation attenuates the loss of skeletal muscle excitability during intense contractions. *Am J Physiol Endocrinol Metab* 292(3):E771–778. <https://doi.org/10.1152/ajpendo.00378.2006>
- MacLean DA, LaNoue KF, Gray KS, Sinoway LI (1998) Effects of hindlimb contraction on pressor and muscle interstitial metabolite responses in the cat. *J Appl Physiol* (1985) 85(4):1583–1592. <https://doi.org/10.1152/jappl.1998.85.4.1583>
- MacLean DA, Vickery LM, Sinoway LI (2001) Elevated interstitial adenosine concentrations do not activate the muscle reflex. *Am J Physiol Heart Circ Physiol* 280(2):H546–553. <https://doi.org/10.1152/ajpheart.2001.280.2.H546>
- MacLeod KT (1989) Effects of hypoxia and metabolic inhibition on the intracellular sodium activity of mammalian ventricular muscle. *J Physiol* 416:455–468. <https://doi.org/10.1113/jphysiol.1989.sp017771>
- Mainwood GW, Worsley-Brown P, Paterson RA (1972) The metabolic changes in frog sartorius muscles during recovery from fatigue at different external bicarbonate concentrations. *Can J Physiol Pharmacol* 50(2):143–155. <https://doi.org/10.1139/y72-021>
- Matar W, Nosek TM, Wong D, Renaud JM (2000) Pinacidil suppresses contractility and preserves energy but glibenclamide has no effect during fatigue in skeletal muscle. *Am J Physiol* 278:C404–C416
- McCaig D, Leader JP (1984) Intracellular chloride activity in the extensor digitorum longus (EDL) muscle of the rat. *J Membr Biol* 81(1):9–17. <https://doi.org/10.1007/BF01868805>
- McKenna MJ, Bangsbo J, Renaud JM (2008) Muscle  $K^+$ ,  $Na^+$ , and  $Cl^-$  disturbances and  $Na^+$ - $K^+$  pump inactivation: implications for fatigue. *J Appl Physiol* 104:288–295
- McKenna MJ, Ørtenblad N, Overgaard K, Renaud JM (2023) A century of exercise physiology. Effects of muscle contraction and exercise on skeletal muscle  $Na^+$  and  $K^+$  ions,  $Na^+$ , $K^+$ -ATPase and plasma  $K^+$ : historical development. *European Journal of Applied Physiology* Submitted
- Mercer RW, Dunham PB (1981) Membrane-bound ATP fuels the  $Na/K$  pump. *J Gen Physiol* 78:547–568
- Meyer RA, Terjung RL (1979) Differences in ammonia and adenylate metabolism in contracting fast and slow muscle. *Am J Physiol* 237(3):C111–118. <https://doi.org/10.1152/ajpcell.1979.237.3.C111>
- Meyerhof O, Symposium on respiratory enzymes (1942) In: Symposium on the respiratory enzymes (1941): University of Wisconsin. University of Wisconsin Press
- Mohr M, Nordsborg N, Nielsen JJ, Pedersen LD, Fischer C, Krstrup P, Bangsbo J (2004) Potassium kinetics in human muscle interstitium during repeated intense exercise in relation to fatigue. *Pflügers Arch* 448(4):452–456. <https://doi.org/10.1007/s00424-004-1257-6>
- Nagaoka R, Yamashita S, Mizuno M, Akaike N (1994) Intracellular  $Na^+$  and  $K^+$  shifts induced by contractile activities of rat skeletal muscles. *Comp Biochem Physiol A Physiol* 109(4):957–965. [https://doi.org/10.1016/0300-9629\(94\)90244-5](https://doi.org/10.1016/0300-9629(94)90244-5)
- Nagesser AS, Van der Laarse WJ, Elzinga G (1993) ATP formation and ATP hydrolysis during fatiguing, intermittent stimulation of different types of single muscle fibres from *Xenopus laevis*. *J Muscle Res Cell Motil* 14:608–618
- Nastuk WL, Hodgkin AL (1950) The electrical activity of single muscle fibers. *J Cell Comp Physiol* 35:39–73
- Nielsen OB, Clausen T (1997) Regulation of  $Na^+$ - $K^+$  pump activity in contracting rat muscle. *J Physiol (lond)* 503:571–581
- Nielsen J, Ørtenblad N (2013) Physiological aspects of the subcellular localization of glycogen in skeletal muscle. *Appl Physiol Nutr Metab* 38(2):91–99. <https://doi.org/10.1139/apnm-2012-0184>
- Nielsen OB, Hilsted L, Clausen T (1998) Excitation-induced force recovery in potassium-inhibited rat soleus muscle. *J Physiol* 512(Pt 3):819–829. <https://doi.org/10.1111/j.1469-7793.1998.819bd.x>
- Nielsen JJ, Kristensen M, Hellsten Y, Bangsbo J, Juel C (2003) Localization and function of ATP-sensitive potassium channels in human skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 284(2):R558–R563. [https://doi.org/10.1152/ajpregu.00303.2002.\(00303.2002\[pii\]\)](https://doi.org/10.1152/ajpregu.00303.2002.(00303.2002[pii]))
- Nielsen JJ, Mohr M, Klarskov C, Kristensen M, Krstrup P, Juel C, Bangsbo J (2004a) Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *J Physiol* 554(Pt 3):857–870. <https://doi.org/10.1113/jphysiol.2003.050658>
- Nielsen OB, Ørtenblad N, Lamb GD, Stephenson DG (2004b) Excitability of the t-tubular system in rat skeletal muscle: roles of  $K^+$  and  $Na^+$  gradients and  $Na^+$ - $K^+$  pump activity. *J Physiol (lond)* 557:133–146
- Nielsen J, Schroder HD, Rix CG, Ortenblad N (2009) Distinct effects of subcellular glycogen localization on tetanic relaxation time and endurance in mechanically skinned rat skeletal muscle fibers. *J Physiol (lond)* 587:3679–3690
- Nielsen OB, de Paoli FV, Riisager A, Pedersen TH (2017) Chloride channels take center stage in acute regulation of excitability in

- skeletal muscle: implications for fatigue. *Physiology* (Bethesda) 32(6):425–434. <https://doi.org/10.1152/physiol.00006.2015>
- Nielsen J, Dubillot P, Stausholm MH, Ørtenblad N (2022) Specific ATPases drive compartmentalized glycogen utilization in rat skeletal muscle. *J Gen Physiol*. <https://doi.org/10.1085/jgp.202113071>
- Noma A (1983) ATP-regulated K<sup>+</sup> channels in cardiac muscle. *Nature* 305:147–148
- Nordsborg N, Mohr M, Pedersen LD, Nielsen JJ, Langberg H, Bangsbo J (2003) Muscle interstitial potassium kinetics during intense exhaustive exercise: effect of previous arm exercise. *Am J Physiol Regul Integr Comp Physiol* 285(1):R143–148. <https://doi.org/10.1152/ajpregu.00029.2003>
- Norman B, Sollevi A, Jansson E (1988) Increased IMP content in glycogen-depleted muscle fibres during submaximal exercise in man. *Acta Physiol Scand* 133(1):97–100. <https://doi.org/10.1111/j.1748-1716.1988.tb08385.x>
- Okamoto K, Wang W, Rounds J, Chambers EA, Jacobs DO (2001) ATP from glycolysis is required for normal sodium homeostasis in resting fast-twitch rodent skeletal muscle. *Am J Physiol Endocrinol Metab* 281(3):E479–488. <https://doi.org/10.1152/ajpendo.2001.281.3.E479>
- Olesen JH, Herskind J, Pedersen KK, Overgaard K (2021) Potassium-induced potentiation of subtetanic force in rat skeletal muscles: influences of beta2-activation, lactic acid, and temperature. *Am J Physiol Cell Physiol* 321(5):C884–C896. <https://doi.org/10.1152/ajpcell.00120.2021>
- Ørtenblad N, Nielsen J (2015) Muscle glycogen and cell function—Location, location, location. *Scand J Med Sci Sports* 25(Suppl 4):34–40. <https://doi.org/10.1111/sms.12599>
- Ørtenblad N, Stephenson DG (2003) A novel signalling pathway originating in mitochondria modulates rat skeletal muscle membrane excitability. *J Physiol (Lond)* 548:139–145
- Ørtenblad N, Macdonald WA, Sahlin K (2009) Glycolysis in contracting rat skeletal muscle is controlled by factors related to energy state. *Biochem J* 420(2):161–168. <https://doi.org/10.1042/bj20082135>
- Ørtenblad N, Nielsen J, Saltin B, Holmberg HC (2011) Role of glycogen availability in sarcoplasmic reticulum Ca<sup>2+</sup> kinetics in human skeletal muscle. *J Physiol* 589(Pt 3):711–725. <https://doi.org/10.1113/jphysiol.2010.195982>
- Overgaard K, Nielsen OB (2001) Activity-induced recovery of excitability in K<sup>+</sup>-depressed rat soleus muscle. *Am J Physiol* 280:R48–R55
- Overgaard K, Nielsen OB, Clausen T (1997) Effects of reduced electrochemical Na<sup>+</sup> gradient on contractility in skeletal muscle: role of the Na<sup>+</sup>-K<sup>+</sup> pump. *Pflügers Arch* 434(4):457–465. <https://doi.org/10.1007/s004240050421>
- Overgaard K, Nielsen OB, Flatman JA, Clausen T (1999) Relations between excitability and contractility in rat soleus muscle: role of the Na<sup>+</sup>-K<sup>+</sup> pump and Na<sup>+</sup>/K<sup>+</sup> gradients. *J Physiol* 518(Pt 1):215–225. <https://doi.org/10.1111/j.1469-7793.1999.0215r.x>
- Overgaard K, Gittings W, Vandenboom R (2022) Potentiation of force by extracellular potassium and posttetanic potentiation are additive in mouse fast-twitch muscle *in vitro*. *Pflügers Arch* 474(6):637–646. <https://doi.org/10.1007/s00424-022-02681-z>
- Palade PT, Barchi RL (1977) Characteristics of the chloride conductance in muscle fibers of the rat diaphragm. *J Gen Physiol* 69(3):325–342
- Pang CY, Neligan P, Xu H, He W, Zhong A, Hopper R, Forrest CR (1997) Role of ATP-sensitive K<sup>+</sup> channels in ischemic preconditioning of skeletal muscle against infarction. *Am J Physiol* 273(1 Pt 2):H44–51. <https://doi.org/10.1152/ajpheart.1997.273.1.H44>
- Paterson DJ (1996) Role of potassium in the regulation of systemic physiological function during exercise. *Acta Physiol Scand* 156(3):287–294. <https://doi.org/10.1046/j.1365-201X.1996.190000.x>
- Pedersen TH, Clausen T, Nielsen OB (2003) Loss of force induced by high extracellular [K<sup>+</sup>] in rat muscle: effect of temperature, lactic acid and beta2-agonist. *J Physiol* 551(Pt 1):277–286. <https://doi.org/10.1113/jphysiol.2003.041418>
- Pedersen TH, Nielsen OB, Lamb GD, Stephenson DG (2004) Intracellular acidosis enhances the excitability of working muscle. *Science* 305(5687):1144–1147. <https://doi.org/10.1126/science.1101141>
- Pedersen TH, de Paoli F, Nielsen OB (2005) Increased excitability of acidified skeletal muscle: role of chloride conductance. *J Gen Physiol* 125(2):237–246. <https://doi.org/10.1085/jgp.200409173>
- Pedersen TH, de Paoli FV, Flatman JA, Nielsen OB (2009a) Regulation of CLC-1 and K<sub>ATP</sub> channels in action potential-firing fast-twitch muscle fibers. *J Gen Physiol* 134:309–322
- Pedersen TH, Macdonald WA, de Paoli FV, Gurung IS, Nielsen OB (2009b) Comparison of regulated passive membrane conductance in action potential-firing fast- and slow-twitch muscle. *J Gen Physiol* 134:323–337
- Pedersen TH, Riisager A, de Paoli FV, Chen TY, Nielsen OB (2016) Role of physiological ClC-1 Cl<sup>-</sup> ion channel regulation for the excitability and function of working skeletal muscle. *J Gen Physiol* 147(4):291–308. <https://doi.org/10.1085/jgp.201611582>
- Pedersen KK, Cheng AJ, Westerblad H, Olesen JH, Overgaard K (2019) Moderately elevated extracellular [K<sup>+</sup>] potentiates submaximal force and power in skeletal muscle via increased [Ca<sup>2+</sup>]<sub>i</sub> during contractions. *Am J Physiol Cell Physiol* 317(5):C900–C909. <https://doi.org/10.1152/ajpcell.00104.2019>
- Philipson KD, Nishimoto AY (1983) ATP-dependent Na<sup>+</sup> transport in cardiac sarcolemmal vesicles. *Biochim Biophys Acta* 733(1):133–141. [https://doi.org/10.1016/0005-2736\(83\)90099-8](https://doi.org/10.1016/0005-2736(83)90099-8)
- Pirkmajer S, Chibalin AV (2016) Na, K-ATPase regulation in skeletal muscle. *Am J Physiol Endocrinol Metab* 311(1):E1–E31. <https://doi.org/10.1152/ajpendo.00539.2015>
- Quinonez M, Gonzalez F, Morgado-Valle C, DiFranco M (2010) Effects of membrane depolarization and changes in extracellular [K<sup>+</sup>] on the Ca (2+) transients of fast skeletal muscle fibers. Implications for muscle fatigue. *J Muscle Res Cell Motil* 31(1):13–33. <https://doi.org/10.1007/s10974-009-9195-8>
- Radzyukevich TL, Lingrel JB, Heiny JA (2009) The cardiac glycoside binding site on the Na, K-ATPase alpha2 isoform plays a role in the dynamic regulation of active transport in skeletal muscle. *Proc Natl Acad Sci U S A* 106(8):2565–2570. <https://doi.org/10.1073/pnas.0804150106>
- Reading SA, Murrant CL, Barclay JK (2003) Increased cAMP as a positive inotropic factor for mammalian skeletal muscle *in vitro*. *Can J Physiol Pharmacol* 81(10):986–996. <https://doi.org/10.1139/y03-104>
- Renaud JM (1989) The effect of lactate on intracellular pH and force recovery of fatigued sartorius muscles of the frog, *Rana pipiens*. *J Physiol* 416:31–47. <https://doi.org/10.1113/jphysiol.1989.sp017747>
- Renaud JM (2002) Modulation of force development by Na<sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup> K<sup>+</sup> pump and K<sub>ATP</sub> channel during muscular activity. *Can J Appl Physiol* 27:296–315
- Renaud JM, Light P (1992) Effects of K<sup>+</sup> on the twitch and tetanic contraction in the sartorius muscle of the frog, *Rana pipiens*. Implication for fatigue *in vivo*. *Can J Physiol Pharmacol* 70:1236–1246
- Renaud JM, Mainwood GW (1985a) The effects of pH on the kinetics of fatigue and recovery in frog sartorius muscle. *Can J Physiol Pharmacol* 63:1435–1443
- Renaud JM, Mainwood GW (1985b) The interactive effect of fatigue and pH on the ionic conductance of frog sartorius muscle fibers. *Can J Physiol Pharmacol* 63:1444–1453

- Riisager A, Duehmke R, Nielsen OB, Huang CL, Pedersen TH (2014) Determination of cable parameters in skeletal muscle fibres during repetitive firing of action potentials. *J Physiol* 592(20):4417–4429. <https://doi.org/10.1113/jphysiol.2014.280529>
- Riisager A, de Paoli FV, Yu WP, Pedersen TH, Chen TY, Nielsen OB (2016) Protein kinase C-dependent regulation of ClC-1 channels in active human muscle and its effect on fast and slow gating. *J Physiol* 594(12):3391–3406. <https://doi.org/10.1113/JP271556>
- Rodriguez-Falces J, Place N (2018) Determinants, analysis and interpretation of the muscle compound action potential (M wave) in humans: implications for the study of muscle fatigue. *Eur J Appl Physiol* 118(3):501–521. <https://doi.org/10.1007/s00421-017-3788-5>
- Rojas CV, Neely A, Velasco-Loyden G, Palma V, Kukuljan M (1999) Hyperkalemic periodic paralysis M1592V mutation modifies activation in human skeletal muscle Na<sup>+</sup> channel. *Am J Physiol* 276:C259–C266
- Rolfe DF, Brown GC (1997) Cellular energy utilization and molecular origin of standard metabolic rate in mammals. *Physiol Rev* 77(3):731–758. <https://doi.org/10.1152/physrev.1997.77.3.731>
- Ruff RL (1996) Sodium channel slow inactivation and the distribution of sodium channels on skeletal muscle fibres enable the performance properties of different skeletal muscle fibre types. *Acta Physiol Scand* 156(3):159–168. <https://doi.org/10.1046/j.1365-201X.1996.189000.x>
- Ruff RL (1999) Effects of temperature on slow and fast inactivation of rat skeletal muscle Na<sup>+</sup> channels. *Am J Physiol* 277(5):C937–947. <https://doi.org/10.1152/ajpcell.1999.277.5.C937>
- Ruff RL, Simoncini L, Stuhmer W (1988) Slow sodium channel inactivation in mammalian muscle: a possible role in regulating excitability. *Muscle Nerve* 11(5):502–510. <https://doi.org/10.1002/mus.880110514>
- Rybicki KJ, Kaufman MP, Kenyon JL, Mitchell JH (1984) Arterial pressure responses to increasing interstitial potassium in hindlimb muscle of dogs. *Am J Physiol* 247(4 Pt 2):R717–721. <https://doi.org/10.1152/ajpregu.1984.247.4.R717>
- Sahlin K, Alvestrand A, Brandt R, Hultman E (1978) Intracellular pH and bicarbonate concentration in human muscle during recovery from exercise. *J Appl Physiol Respir Environ Exerc Physiol* 45(3):474–480. <https://doi.org/10.1152/jappl.1978.45.3.474>
- Sahlin K, Söderlund K, Tonkonogi M, Hiraokoba K (1997) Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise. *Am J Physiol* 273(1 Pt 1):C172–178. <https://doi.org/10.1152/ajpcell.1997.273.1.C172>
- Sahlin K, Tonkonogi M, Söderlund K (1998) Energy supply and muscle fatigue in humans. *Acta Physiol Scand* 162(3):261–266. <https://doi.org/10.1046/j.1365-201X.1998.0298f.x>
- Saks V, Beraud N, Wallimann T (2008) Metabolic compartmentation - a system level property of muscle cells: real problems of diffusion in living cells. *Int J Mol Sci* 9(5):751–767. <https://doi.org/10.3390/ijms9050751>
- Schrier SL (1966) Organization of enzymes in human erythrocyte membranes. *Am J Physiol* 210(1):139–145. <https://doi.org/10.1152/ajplegacy.1966.210.1.139>
- Scott K, Benkhalti M, Calvert ND, Paquette M, Zhen L, Harper ME, Al-Dirbashi OY, Renaud JM (2016) KATP channel deficiency in mouse FDB causes an impairment of energy metabolism during fatigue. *Am J Physiol Cell Physiol* 311(4):C559–C571. <https://doi.org/10.1152/ajpcell.00137.2015>
- Seino S (1999) ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 61:637–662
- Sejersted OM, Sjøgaard G (2000) Dynamics and consequences of potassium shifts in skeletal muscle and heart during exercise. *Physiol Rev* 80:1411–1481
- Selvin D, Renaud JM (2015) Changes in myoplasmic Ca<sup>2+</sup> during fatigue differ between FDB fibers, between glibenclamide-exposed and Kir6.2<sup>-/-</sup> fibers and are further modulated by verapamil. *Physiol Rep*. <https://doi.org/10.14814/phy2.12303>
- Shestov AA, Liu X, Ser Z, Cluntun AA, Hung YP, Huang L, Kim D, Le A, Yellen G, Albeck JG, Locasale JW (2014) Quantitative determinants of aerobic glycolysis identify flux through the enzyme GAPDH as a limiting step. *Elife*. <https://doi.org/10.7554/eLife.03342>
- Simoncini L, Stuhmer W (1987) Slow sodium channel inactivation in rat fast-twitch muscle. *J Physiol* 383:327–337. <https://doi.org/10.1113/jphysiol.1987.sp016411>
- Sjøgaard G (1983) Electrolytes in slow and fast muscle fibers of humans at rest and with dynamic exercise. *Am J Physiol* 245(1):R25–31. <https://doi.org/10.1152/ajpregu.1983.245.1.R25>
- Sjøgaard G (1991) Role of exercise-induced potassium fluxes underlying muscle fatigue: a brief review. *Can J Physiol Pharmacol* 69(2):238–245. <https://doi.org/10.1139/y91-037>
- Sjøgaard G, Adams RP, Saltin B (1985) Water and ion shifts in skeletal muscle of humans with intense dynamic knee extension. *Am J Physiol* 248(2 Pt 2):R190–196. <https://doi.org/10.1152/ajpregu.1985.248.2.R190>
- Sperelakis N (1969) Changes in conductances of frog sartorius fibers produced by CO<sub>2</sub>, ReO<sub>4</sub><sup>-</sup>, and temperature. *Am J Physiol* 217(4):1069–1075. <https://doi.org/10.1152/ajplegacy.1969.217.4.1069>
- Standen NB, Pettit AI, Davies NW, Stanfield PR (1992) Activation of ATP-dependent K<sup>+</sup> currents in intact skeletal muscle fibres by reduced intracellular pH. *Proc R Soc Lond B* 247:195–198
- Stephenson DG, Nguyen LT, Stephenson GM (1999) Glycogen content and excitation-contraction coupling in mechanically skinned muscle fibres of the cane toad. *J Physiol* 519(1):177–187. <https://doi.org/10.1111/j.1469-7793.1999.01770.x>
- Street D, Nielsen JJ, Bangsbo J, Juel C (2005) Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle interstitium. *J Physiol* 566(Pt 2):481–489. <https://doi.org/10.1113/jphysiol.2005.086801>
- Thabet M, Miki T, Seino S, Renaud JM (2005) Treadmill running causes significant damage in skeletal muscle K<sub>ATP</sub> channel deficient mice. *Physiol Gen* 22:204–212
- Tricarico D, Mallamaci R, Barbieri M, Camerino DC (1997) Modulation of ATP-sensitive K<sup>+</sup> channel by insulin in rat skeletal muscle fibers. *Biochem Biophys Res Commun* 232:536–539
- Tricarico D, Servidei S, Tonali P, Jurkat-Rott K, Camerino DC (1999) Impairment of skeletal muscle adenosine triphosphate-sensitive K<sup>+</sup> channels in patients with hypokalemic periodic paralysis. *J Clin Invest* 103:675–682
- Tseng PY, Bennetts B, Chen TY (2007) Cytoplasmic ATP inhibition of CLC-1 is enhanced by low pH. *J Gen Physiol* 130:217–221
- Tseng PY, Yu WP, Liu HY, Zhang XD, Zou X (2011) Binding of ATP to the CBS domains in the C-terminal region of the CLC-1. *J Gen Physiol* 137:357–368
- Uwera F, Ammar T, McRae C, Hayward LJ, Renaud JM (2020) Lower Ca<sup>2+</sup> enhances the K<sup>+</sup>-induced force depression in normal and HyperKPP mouse muscles. *J Gen Physiol*. <https://doi.org/10.1085/jgp.201912511>
- van Mil HG, Kerkhof CJ, Siegenbeek van Heukelum J (1995) Modulation of the isoprenaline-induced membrane hyperpolarization of mouse skeletal muscle cells. *Br J Pharmacol* 116(7):2881–2888
- Vander Heiden MG, Cantley LC, Thompson CB (2009) Understanding the warburg effect: the metabolic requirements of cell proliferation. *Science* 324(5930):1029–1033. <https://doi.org/10.1126/science.1160809>
- Vivaudou MB, Arnoult C, Villaz M (1991) Skeletal muscle ATP-sensitive K<sup>+</sup> channels recorded from sarcolemmal blebs of split fibers:

- ATP inhibition is reduced by magnesium and ADP. *J Membr Biol* 122:165–175
- Walas H, Juel C (2012) Purigenic activation of rat skeletal muscle membranes increases  $V_{max}$  and  $Na^+$  affinity of the  $Na^+$ ,  $K^+$ -ATPase and phosphorylates phospholemman and  $\alpha 1$  subunits. *Pflugers Arch* 463:319–326
- Walker SM (1948) Action potentials in rat muscle with twitch tension potentiated by KCl treatment, adrenalectomy, tetanus and treppe. *Am J Physiol* 154:63–72
- Wang X, Nawaz M, DuPont C, Myers JH, Burke SR, Bannister RA, Foy BD, Voss AA, Rich MM (2022) The role of action potential changes in depolarization-induced failure of excitation-contraction coupling in mouse skeletal muscle. *Elife*. <https://doi.org/10.7554/eLife.71588>
- Wanson JC, Drochmans P (1972) Role of the sarcoplasmic reticulum in glycogen metabolism. Binding of phosphorylase, phosphorylase kinase, and primer complexes to the sarcovesicles of rabbit skeletal muscle. *J Cell Biol* 54(2):206–224. <https://doi.org/10.1083/jcb.54.2.206>
- Watanabe D, Wada M (2019) Effects of reduced muscle glycogen on excitation-contraction coupling in rat fast-twitch muscle: a glycogen removal study. *J Muscle Res Cell Motil* 40(3–4):353–364. <https://doi.org/10.1007/s10974-019-09524-y>
- Webb J, Wu FF, Cannon SC (2009) Slow inactivation of the  $NaV1.4$  sodium channel in mammalian cells is impeded by co-expression of the  $\beta 1$  subunit. *Pflugers Arch* 457(6):1253–1263. <https://doi.org/10.1007/s00424-008-0600-8>
- Weiss JN, Lamp ST (1989) Cardiac ATP-sensitive  $K^+$  channels. Evidence for preferential regulation by glycolysis. *J Gen Physiol* 94:911–935
- Weiss DS, Magleby KL (1990) Voltage dependence and stability of the gating kinetics of the fast chloride channel from rat skeletal muscle. *J Physiol (Lond)* 426:145–176
- Westerblad H, Allen DG (1991) Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J Gen Physiol* 98:615–635
- Westerblad H, Lannergren J (1986) Force and membrane potential during and after fatiguing, intermittent tetanic stimulation of single *Xenopus* muscle fibres. *Acta Physiol Scand* 128(3):369–378. <https://doi.org/10.1111/j.1748-1716.1986.tb07990.x>
- Whitlock DM, Terjung RL (1987) ATP depletion in slow-twitch red muscle of rat. *Am J Physiol* 253:C426–C432
- Wilson JR, Kapoor SC, Krishna GG (1994) Contribution of potassium to exercise-induced vasodilation in humans. *J Appl Physiol* (1985) 77(6):2552–2557. <https://doi.org/10.1152/jappl.1994.77.6.2552>
- Wu L, Zhang B, Kang Y, Wu W (2014) Enhanced slow inactivation of the human skeletal muscle sodium channel causing normokalemic periodic paralysis. *Cell Mol Neurobiol* 34(5):707–714. <https://doi.org/10.1007/s10571-014-0052-y>
- Xu KY, Becker LC (1998) Ultrastructural localization of glycolytic enzymes on sarcoplasmic reticulum vesicles. *J Histochem Cytochem* 46(4):419–427. <https://doi.org/10.1177/002215549804600401>
- Yensen C, Matar W, Renaud JM (2002) The  $K^+$ -induced twitch potentiation is not due to longer action potential. *Am J Physiol* 283:C169–C177
- Yoshida H, Kefaloyianni E, Taskin E, Okorie U, Hong M, Dhar-Chowdhury P, Kaneko M, Coetzee WA (2012) AMP-activated protein kinase connects cellular energy metabolism to  $K_{ATP}$  channel function. *J Mol Cell Card* 52:410–418
- Zhang XD, Tseng PY, Chen TY (2008) ATP inhibition of  $CLC-1$  is controlled by oxidation and reduction. *J Gen Physiol* 132(4):421–428. <https://doi.org/10.1085/jgp.200810023>. (jgp.200810023[pii])
- Zhu Z, Sierra A, Burnett CM, Chen B, Subbotina E, Koganti SR, Gao Z, Wu Y, Anderson ME, Song LS, Goldhamer DJ, Coetzee WA, Hodgson-Zingman DM, Zingman LV (2014) Sarcolemmal ATP-sensitive potassium channels modulate skeletal muscle function under low-intensity workloads. *J Gen Physiol* 143(1):119–134. <https://doi.org/10.1085/jgp.201311063>. (jgp.201311063[pii])
- Zuntz N (1896) Über die Rolle des Zuckers im tierischen Stoffwechsel. *Arch Physiol*, 538–542

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.