

REVIEW

Regulation of pH in human skeletal muscle: adaptations to physical activity

C. Juel

Copenhagen Muscle Research Centre, Department of Molecular Biology, University of Copenhagen, Copenhagen, Denmark

Received 27 September 2007,
revision requested 31 October
2007,
final revision received 13 January
2008,
accepted 1 February 2008
Correspondence: C. Juel,
Copenhagen Muscle Research
Centre, August Krogh Building,
Department of Molecular Biology,
University of Copenhagen,
Universitetsparken 13, DK 2100,
Copenhagen NV, Denmark.
E-mail: cjuel@aki.ku.dk

Abstract

Regulation of pH in skeletal muscle is the sum of mechanisms involved in maintaining intracellular pH within the normal range. Aspects of pH regulation in human skeletal muscle have been studied with various techniques from analysis of membrane proteins, microdialysis, and the nuclear magnetic resonance technique to exercise experiments including blood sampling and muscle biopsies. The present review characterizes the cellular buffering system as well as the most important membrane transport systems involved (Na^+/H^+ exchange, Na-bicarbonate co-transport and lactate/ H^+ co-transport) and describes the contribution of each transport system in pH regulation at rest and during muscle activity. It is reported that the mechanisms involved in pH regulation can undergo adaptational changes in association with physical activity and that these changes are of functional importance.

Keywords adaptations, cellular pH, membrane transport, physiochemical buffering.

A restriction in the ability to generate muscle force limits physical exercise capacity. In some situations, the ability to generate absolute power output is limiting, whereas in other situations, muscular or cardiovascular endurance is important. Repetitive activation of skeletal muscle challenges the mechanisms that regulate energy metabolism and ion homeostasis, of which pH regulation is an important part, indicating that regulation of cellular pH may be a limiting factor to physical exercise. It is not surprising that pH regulation adapts to exercise training and that this adaptation is important to performance. This review describes the mechanisms underlying pH regulation in the skeletal muscle and adaptations that help improve physical performance.

Mechanisms affecting cellular pH at rest

H⁺ distribution and the membrane potential

The distribution of the cation H^+ is influenced by a negative membrane potential, which tends to cause H^+ accumulation in the cell. Consequently, allowing H^+ to

equilibrate acidifies the cell interior. The Nernst equation shows that with a membrane potential of -61 mV the internal H^+ concentration at equilibrium will be 10 times higher than the external H^+ concentration, i.e. the internal pH will be one unit lower than the external pH. This suggests that as the membrane potential in skeletal muscle is -60 to -80 mV, the internal pH with H^+ equilibrated would be more than one unit lower than the external pH.

Several mechanisms are responsible for the tendency to accumulate H^+ in the muscle cell. H^+ is released from the metabolic pathways, and H^+ can enter the cell from outside. H^+ alone can only enter by protein-mediated membrane transport because the phospholipid membrane is naturally impermeable to H^+ . However, fluxes of, for instance, amino acids and ammonia can influence the H^+ load. In addition, the internal H^+ concentration is affected by the rapid diffusion of CO_2 /bicarbonate.

Physiochemical buffering

The cellular buffering involves sequestering of H^+ resulting in a reduced number of free H^+ . The number

of H^+ ions and the buffering capacity therefore determine cellular pH. The presence of a buffer increases the amount of acid that can be added to obtain a given change in pH; a good buffering therefore reduces the fluctuations in cellular pH, for instance during muscle activity. The buffer capacity in a muscle homogenate can be estimated by titration with a strong acid or base and is defined from the amount of acid (or base) needed to change pH one unit per wet weight or per dry weight of muscle. The total cellular buffer capacity is the sum of contributions from all buffering compounds, which include proteins, small peptides, bicarbonate and free phosphate, but it does not include the main part of the bicarbonate system (because CO_2 disappears during the homogenization).

Adaptations to physical activity might include changes in buffer capacity. The *in vitro* buffer capacity can theoretically be modified in two ways: if the amount of buffer compounds per wet weight of muscle is increased (more protein per wet weight) or if changes per dry weight are obtained (the quality of the buffer compounds is improved). Some studies have suggested a correlation between training, *in vitro* buffer capacity and performance (Parkhouse & McKenzie 1984, Mannion *et al.* 1994, Weston *et al.* 1997); however, some of these results seem not to be consistent. The buffer action of the dipeptide carnosine has been given special attention; carnosine is present in human skeletal muscle and the concentration of carnosine is correlated with work capacity (Hill *et al.* 2007).

It has also been reported that the physiochemical buffer capacity can be acutely decreased immediately after one bout of exercise (Bishop *et al.* 2007). However, a dramatic reduction in cellular protein content during one bout of exercise is unrealistic; the explanation is more likely that the estimated buffer capacity is influenced by chemical changes during homogenization, and that these changes are affected by whether the muscle samples were obtained at rest or after exercise.

***In vivo* buffering**

The buffer capacity defined above is called the *in vitro* buffer capacity. This parameter is unaffected by exchange of substances across the muscle membrane. If the purpose is to characterize the overall ability of a cell to regulate pH, the removal of H^+ mediated by membrane transport systems and metabolic processes must also be taken into consideration. To this end, another parameter, the *in vivo* buffer capacity, can be defined from the ratio between changes in muscle lactate and changes in muscle pH during exercise (Sahlin & Henriksson 1984). It must be emphasized that this is a functional and dynamic measure dependent

on the presence of the removal mechanisms for H^+ and lactate, which are activated during muscle activity. A third variant is to determine the changes in H^+ concentration per work performed (McKenna *et al.* 1996). Again, this measure is dependent on the H^+ removal capacity and duration. The *in vivo* buffer capacity can be used to characterize changes in a group of subjects, but it is difficult to compare studies because of differences in work duration and intensity. Training-induced changes in *in vivo* buffer capacity could be mediated by adaptations in membrane transport systems for H^+ , which is discussed below.

pH regulation

As noted, if H^+ is equilibrated across the muscle membrane, the internal pH would be more than one pH unit lower than the external pH; however, this is never seen in the intact cell. In contrast, the external pH in the body remains relatively constant near 7.4, whereas the internal pH at rest is about 7.2. The small external-to-internal pH difference indicates that H^+ is not equilibrated across the muscle membrane, as the internal H^+ concentration remains low because H^+ is transported against the electrochemical gradient. A number of mechanisms contribute to the decrease in the internal H^+ concentration; the identified mechanisms include H^+ transport and physiochemical buffering, and the collective mechanism is termed pH regulation.

pH regulation in resting human muscle

Transport systems involved

Na⁺/H⁺ exchange. The Na^+/H^+ exchange system, which in muscle is represented by the isoform NHE1, is the classical pH-regulating transport system present in many cell types. This system has been found in most cell types to have a set point close to the normal internal pH of 7.2, and acidification of the cell activates this system, which tends to normalize internal pH. In the skeletal muscle cell, the exchange system seems to be half-activated at normal pH (Juel 1998) and to be activated further at lower pH. The reason for this pH sensitivity in muscle is unclear but might be related to the need for activity at neutral pH because of H^+ release by the metabolic pathway. The Na^+/H^+ exchange system is most abundant in glycolytic fibres (Juel 2000).

Na⁺/bicarbonate co-transport. Although Na^+/H^+ exchange is considered the most important system in regulating pH, bicarbonate transport is probably also involved. It was previously assumed that the bicarbonate-dependent aspect of pH regulation is mediated by $Cl^-/bicarbonate$ exchange (Aickin & Thomas 1977,

Grossie *et al.* 1988). However, the passive distribution of Cl^- in resting muscle makes it difficult to see how H^+ could be transported against the electrochemical gradient. In addition, muscle activity is associated with a depolarization mainly resulting from K^+ displacements. This may create an inward electrochemical Cl^- gradient, which may result in a bicarbonate efflux not helpful in pH regulation.

More recent experiments have identified the wide distribution of Na/bicarbonate co-transporters (NBCs). Human skeletal muscle possesses two variants of the sodium/bicarbonate co-transporter isoforms, called NBCe1 and NBCe2 (Kristensen *et al.* 2004). However, these transport systems have only been identified in western blots and their efficiency and contribution to pH regulation has not been quantified, nor has the regulation of these transport systems in human skeletal muscle been described. However, both transport systems are dependent on the Na^+ gradient, which is known to change during high-intensity muscle activity; this may affect the efficiency of these pH-regulating transport systems.

Cost of pH regulation: the Na^+/K^+ -pump in pH regulation. As mentioned above, in resting skeletal muscle pH regulation is mediated by the Na^+ -dependent transport system and involves Na^+/H^+ exchange and Na^+ /bicarbonate co-transport. The activity of these systems brings Na^+ into the cell and therefore places an extra load on the Na^+/K^+ -pump, which is activated when internal Na^+ is increased. Consequently, the flux of H^+ and the use of energy for pH regulation can be indirectly quantified from the extra activity of the Na^+/K^+ -pump. In rat skeletal muscle, inhibition of the Na^+/H^+ exchange and bicarbonate transport reduces the pump-mediated K^+ influx by 40%, indicating that pH regulation places a considerable load on the pump.

Lactate/ H^+ co-transport. Lactate transport in human skeletal muscle is mediated by two proteins, called MCT1 and MCT4 (for monocarboxylate transporter), which co-transport H^+ (for a review, see Juel 1997). The stoichiometry is always one lactate ion to one H^+ ion. The isoform MCT1 is found mainly in oxidative fibres, whereas MCT4 is found in all fibres. It has been argued that MCT1 transports lactate for oxidation, although the transporters can move lactate and H^+ in both directions, and the direction is dependent only on the gradients. In addition to the MCT transporters, the muscle membrane is to some degree permeable to undissociated lactic acid. Therefore, all movements of lactate ions in the body take place together with H^+ with a ratio of 1:1, and consequently, all movements of lactate ions influence pH through coupled H^+ transport.

Interaction between systems

The pH-regulating transport systems do not function independently. For instance, lactate transport mediated by MCT1 is facilitated by the activity of NBC (Becker *et al.* 2004), and the presence of extracellular carbonic anhydrase (carbonic anhydrase isoforms IV and XIV) facilitates lactate/ H^+ transport in rat skeletal muscle (Wetzel *et al.* 2001). A probable general mechanism is that the transporters that transport H^+ out are facilitated by mechanisms that remove H^+ and thereby stabilize the H^+ gradient across the membrane; this mechanism might also operate in the opposite direction.

pH changes and regulation during muscle activity

Time course of pH changes in blood and in the muscle interstitium

At rest, H^+ accumulation and transport are balanced, whereas during exercise, cellular acid production and net H^+ release increase markedly. In active skeletal muscle, H^+ and lactate ions accumulate in nearly equimolar ratios; this is a consequence of the formation of dissociated carboxylate anions further up the Embden-Meyerhoff pathways. Clearly, the rate of H^+ production can exceed the rate of release, which leads to cellular accumulation of H^+ . There is usually a linear relationship between lactate (+pyruvate) accumulation and muscle pH.

Analysis of muscle biopsies shows that the accumulation of lactate is dependent on the exercise intensity and may reach 40 mmol kg^{-1} muscle w.w. (Ahlborg *et al.* 1972). At the same time, pH decreases and might reach 6.5 in exhausted muscle. After muscle activity, some lactate and H^+ are removed by metabolism and released to the interstitium and blood. The reported half-time for recovery of muscle pH is about 4.9 min when obtained by measuring pH in homogenized biopsies obtained at different times after exercise, 4 min for H^+ release and 5 min for lactate release (Juel *et al.* 1990, Bangsbo *et al.* 1993, Juel 1998). A similar time course is seen for lactate and H^+ accumulation in the blood after exercise. The microdialysis technique has been applied to the measurement of interstitial pH in human skeletal muscle, and a good time resolution has been obtained for the pH changes in the interstitium during and after exercise (Street *et al.* 2001). The interstitial acidification is nearly linearly related to power output; the lowest interstitial pH is obtained 1–2 min after exercise, and recovery occurs with a half-time of 5.2 min. The reason for the transient decrease in interstitial pH after exercise probably relates to phosphocreatine resynthesis, which results in a net release of

H⁺. Thus, there is a good agreement between the time course for recovery of muscle pH and interstitial pH. At rest the pH in blood and in the interstitial space is similar. However, during intense exercise, interstitial pH can be lower than blood pH, suggesting a pH gradient from the interstitium to the blood, but at all exercise intensities with a larger intracellular to interstitial pH gradient.

The integrated system: pH regulation during muscle activity

The H⁺ transport mediated by the Na⁺/H⁺ exchanger and by Na⁺/bicarbonate co-transport cannot be distinguished in human experiments, whereas the H⁺ transporting activity of the H⁺/lactate (1 : 1) co-transporters can be quantified from the movements of lactate. pH regulation in skeletal muscle can therefore be divided into lactate-dependent and lactate-independent H⁺ transport.

Release of H⁺ to the blood can also be evaluated from changes in blood pH. However, because the buffer capacity is influenced by pH, changes in pH are not always proportional to the amount of H⁺. The quantitative changes in blood H⁺ can be evaluated from the actual base excess calculated from blood pH, and the concentrations of HCO₃⁻ and haemoglobin using the method of Siggaard-Andersen (1974).

Figure 1 compares lactate-dependent and lactate-independent H⁺ release from active human knee extensor muscle at moderate and high-intensity exercise. At low-intensity exercise, lactate formation is low and the lactate-dependent H⁺ removal makes up a small fraction so that the main part of H⁺ removal is mediated by lactate-independent mechanisms. During high-intensity

exercise, both systems increase, but lactate-independent H⁺ removal can only double, whereas lactate-dependent H⁺ removal can increase by at least five times. Therefore, the lactate/H⁺ co-transporters seem to be the most important systems to remove H⁺ during high-intensity exercise. The K_m for lactate transport is high compared with its cellular concentration, and a saturating efflux is therefore not seen in human experiments.

Although in most situations, lactate and H⁺ move in the same direction, it is possible to create an experiment in which the two ions move in opposite directions. In these experiments, the subjects exercise submaximally using the knee extensors, which produces a moderate net lactate and H⁺ release from the legs. However, adding intense arm exercise releases a large amount of lactate and H⁺. Under this condition, there is a net lactate uptake into the exercising leg, but still there is a net H⁺ release. The uptake is mediated by lactate and H⁺ through the MCT transporters, but at the same time, the H⁺ uptake mediated by this lactate/H⁺ co-transport is exceeded by the H⁺ release mediated by Na⁺/H⁺ exchange and bicarbonate transport. Thus, lactate and H⁺ net fluxes can be dissociated and opposite, although this is a rare phenomenon (Bangsbo *et al.* 1997).

In conclusion, at rest and at low-intensity exercise, the main fraction of H⁺ release is mediated by Na⁺/H⁺ exchange and Na⁺/bicarbonate co-transport, and lactate-coupled H⁺ transport plays a minor role because lactate production is low. During intense exercise, the non-lactate-coupled transporters seems to reach their maximal capacity, and the main part of H⁺ release is mediated by the lactate/H⁺ co-transporters, which are stimulated by the high lactate gradient.

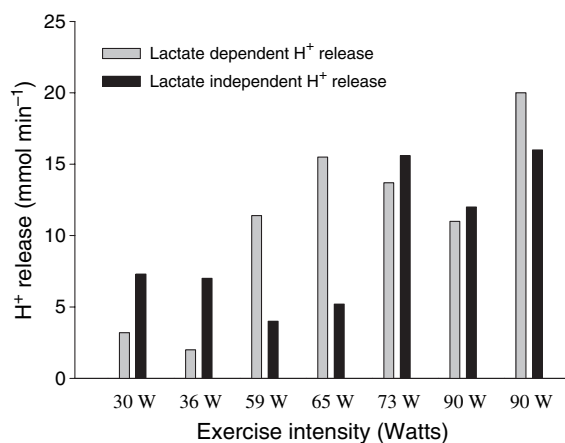


Figure 1 H⁺ release during one-legged knee extensor exercise. H⁺ release is divided into H⁺ release associated with a coupled release of lactate ions and H⁺ release not associated with lactate release. The absolute exercise intensity is given in watts (W) (data from Juel *et al.* 1990, 2004a, Bangsbo *et al.* 1993, 1997, Pilegaard *et al.* 1999).

pH regulation, adaptations and training

The pH-regulating system in blood and skeletal muscle can adapt in response to exercise training and other stimuli. A stay at high altitude can change the protein expression of transporters in blood and skeletal muscle (Juel *et al.* 2003). In erythrocytes, the increase in H⁺-transporting transport systems may be a side effect to the improvements in haematological parameters (e.g. a higher fraction of new erythrocytes), which also occur with high altitude training. The underlying mechanism may be a release of erythropoietin (EPO) induced by high altitude. In line with this suggestion, EPO injections in humans increase the protein expression of a number of transporters in red blood cells (Rentsch *et al.* 2006). A similar effect has not been found in human skeletal muscle (Juel *et al.* 2007). In fact, the only change with EPO was a decrease in protein expression of the Na⁺/K⁺-pump.

The beneficial effect of training on the capacity to regulate pH in human skeletal muscle has been

described both at the protein level and at the functional level. Sarcolemmal vesicles as a model system show a correlation between training status of human subjects and the capacity to transport lactate and H^+ (Pilegaard *et al.* 1994). Later, after the cloning of the proteins, western blotting showed that training can increase the density of the lactate/ H^+ co-transporter proteins MCT1 and MCT4 (Bonen *et al.* 1998, Pilegaard *et al.* 1999, Juel *et al.* 2004a,b). Of these proteins, MCT1 seems to be easiest to increase and its density increases the most. Also, the amount of the Na^+/H^+ exchanger protein NHE1 increases with training in humans (Juel *et al.* 2004a), and the Na^+ /bicarbonate transporters can undergo large changes (Thomas *et al.* 2007).

At the functional level, the effects of training on pH regulation have been studied in blood samples and muscle biopsies. Figure 2 shows data from knee extensor exercise obtained before and after 7–8 weeks of high-

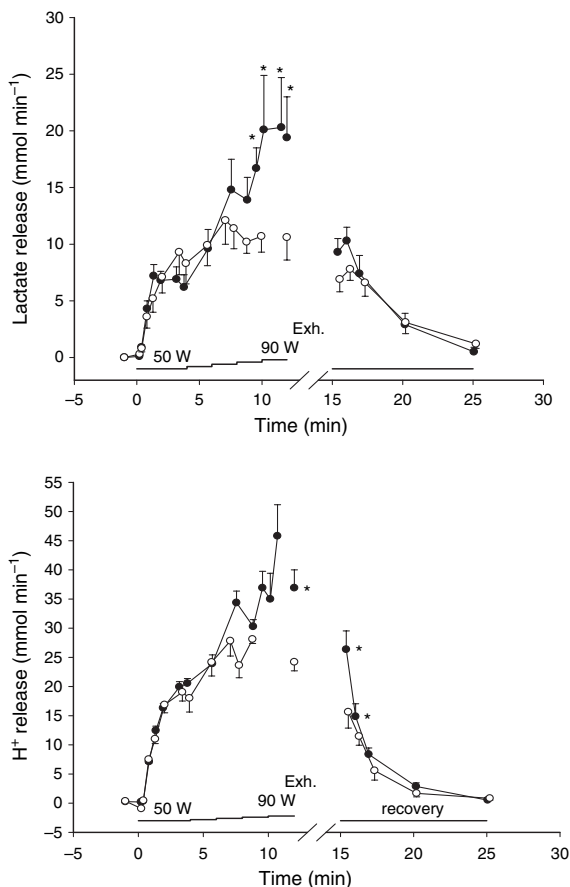


Figure 2 Lactate release (above) and H^+ release (below) during high-intensity exercise. The power output started at 50 W, and the work rate was increased by 10 W every 2 min until exhaustion (connected data points). Time to exhaustion was 8.2 min before training (open symbols) and 10.6 min after 7–8 weeks of training (closed symbols). EXH, value at exhaustion. Values are expressed as the mean from six subjects (reproduced from Juel *et al.* 2004a, with permission).

intensity intermittent training (Juel *et al.* 2004a,b). Training increased both lactate and H^+ release considerably, and the time to fatigue in the incremental test increased after training. The same study demonstrated that the densities of the lactate/ H^+ co-transporter protein MCT1 and the Na^+/H^+ exchanger protein NHE1 increased by 15% and 16%, respectively, after training. It is tempting to relate the increased release of H^+ and lactate to the training-induced increase in membrane proteins. Although this mechanism probably contributed, other underlying mechanisms might also be involved. In the same study, training increased the number of capillaries per muscle fibre by as much as 41% (Jensen *et al.* 2004). Improved blood flow and thereby better washout of lactate and H^+ probably also contributed to the increased release. The involvement of increased blood flow is consistent with the finding that increased release can occur with smaller muscle-to-arterial concentration gradients for lactate and H^+ after training. The changes in H^+ and lactate release might be partly responsible for the improvement in performance after training, although other mechanisms might also contribute. For example, Na^+/K^+ homeostasis also improved in the trained subjects, which might be another underlying mechanism (McKenna *et al.* 1996, Nielsen *et al.* 2004). This example illustrates the complexity of the adaptive modifications to training, which involve both changes in membrane proteins and structural changes of importance for blood flow.

There is also a positive correlation between work capacity and other proteins. In a human test one study found both a positive correlation between the concentration of the membrane bound carbonic anhydrase CAIV and total work performed, and a positive correlation between the concentrations of the two cytosolic carbonic anhydrases CAII and CAIII and pH changes (Messonnier *et al.* 2007).

The Peter Stewart approach

This review describes pH regulation in a mechanistic way. In this model, pH in a compartment is dependent on production and consumption of H^+ , and the dynamic of a number of membrane transport systems that mediate the flux of H^+ . This view contrasts with the physicochemical approach described by Stewart (1983). Today, few physiologists continue to support this approach despite continued enthusiastic support by some (Lindinger *et al.* 2005). The physicochemical approach is not used in other areas, such as in the description of ion changes in kidney and pancreas.

According to this approach, H^+ and HCO_3^- are dependent variables determined by three independent variables. Thus, the concentration of H^+ can be calculated from the concentrations of the independent

variables: the strong ion difference or SID, which equals $(\text{Na} + \text{K}) - (\text{lac} + \text{Cl})$, the total concentration of weak acids (A_{tot}) and PCO_2 . H^+ is not consumed or produced by chemical reactions and changes in H^+ can never indicate how much H^+ has been moved in or out of the solution. This approach takes a different view on membrane transport and cannot be combined with the traditional view on H^+ transport. One consequence of the Stewart approach is that H^+ is not believed to be transported by the traditional transport systems (H^+ /lactate co-transport, Na^+/H^+ exchange, H^+ /bicarbonate co-transport). Although the authors who advocate the Stewart approach question that co-transfer of H^+ takes place, they have never clearly stated what the nature of these systems are, whether they exist and whether they transport only Na^+ , lac^- or HCO_3^- in an electrogenic manner. This situation is illustrated by Putman *et al.* (2003) who mentioned ‘independent movements of lac^- ’ but did not define the nature of this mechanism.

Changes in pH: implications for blood flow and fatigue development

pH and blood flow regulation

Changes in pH are thought to be involved in the regulation of blood flow. However, most of the arguments are based on indirect evidence. In a number of studies, the pH recovery after exercise has a time course similar to that of the decrease in blood flow. At first glance, it seems likely that the decrease in pH is involved in blood flow regulation. However, a closer inspection shows a faster increase in blood flow at the onset of exercise, whereas interstitial pH declines gradually and more slowly. In addition, studies with a good time resolution show that the lowest blood and interstitial pH values are obtained 1–2 min after exercise (Street *et al.* 2001), whereas the gradual reduction in blood flow starts immediately after exercise. This observation seems to exclude pH as a dominant factor in blood flow regulation, but it is still possible that changes in pH modify other vasodilatory mechanisms. Thus, it is generally believed that acidosis is associated with vasodilatation by mediating a decrease in intracellular Ca^{++} . The underlying mechanism might be that changes in pH activate ATP-sensitive K^+ channels, which then modify the membrane potential (Aalkjær & Peng 1997), although direct proof, especially in humans, is difficult to obtain. It has also been suggested that changes in pH influence the activity of nitric oxide synthase, which determines the formation of nitric oxide, a potent vasodilatory compound. In addition, changes in pH may indirectly affect blood flow by modifying the distribution of K^+ .

Interaction between pH and interstitial K^+

The vasodilatory responses to K^+ and pH might not be independent. In one human experiment involving intense arm exercise to increase the leg lactate content and decrease leg pH, K^+ release from the leg during moderate-intensity exercise was greater than that in the control experiments without arm exercise (Bangsbo *et al.* 1996). This finding suggests that K^+ release is affected by pH. A more direct experiment manipulated plasma pH by citrate ingestion (Street *et al.* 2005), which caused less decrease in the interstitial pH during exercise compared with the placebo-treated control trial. At the same time, the interstitial K^+ concentration was lower in the citrate-ingesting group than in the control group. Sostaric *et al.* (2006) found that bicarbonate-induced whole-body alkalosis was associated with lower circulating K^+ concentration and increased K^+ uptake, suggesting that the smaller effect on exercise-induced membrane depolarization that accompanies alkalosis is important for improving performance. These studies suggest a link between interstitial H^+ and K^+ accumulation. This link might have two important implications of physiological significance. First, the debate about the involvement of H^+ in muscle fatigue (Bangsbo & Juel 2006) must take into account the indirect effects of H^+ on the muscle K^+ balance. Second, the lower pH might have an indirect effect on blood flow and might be mediated by modifications in the K^+ balance.

pH changes and fatigue

Low intracellular muscle pH has traditionally been considered the dominant factor causing fatigue, and acidosis caused by the accumulation of lactic acid was thought to cause fatigue in active skeletal muscle. First, this is incorrect from a biochemical viewpoint because H^+ and lactate ions are created during different biochemical events. However, because lactate and H^+ accumulate at nearly the same rate during muscle activity, accumulation of lactate ions can be used as a marker of H^+ accumulation. Second, the idea that lactate and H^+ accumulation cause fatigue was based mainly on the similar time courses of H^+ and lactate accumulation and fatigue in the intact organism and on experiments with isolated muscle studies at temperatures below the normal body temperature. Later experiments demonstrated little or no effect of low pH at normal body temperature (Westerblad *et al.* 1997). Nielsen *et al.* (2001) reported that changes in pH have a beneficial effect on force development in isolated rat muscle incubated with high potassium concentration. The underlying recovery of excitability has been associated with pH-dependent changes in chloride

conductance (Pedersen *et al.* 2004). The latter two studies both concluded that a lower pH is also beneficial to muscle function in the intact organism. The last interpretation has been questioned. Although the experiments by Nielsen *et al.* (2001) in isolated rat muscle incubated in high potassium can be reproduced easily, rat muscles stimulated to develop fatigue have a slower fatigue development if pre-incubated with lactic acid (Kristensen *et al.* 2005). It was argued that the conclusion of Nielsen *et al.* (2001) cannot be extended to the intact organism and that there is no proof of a protective role of lactic acid in human muscle during exercise. In contrast, a study of leg exercise in humans demonstrated shorter endurance time if the lactate concentration in the legs was increased by previous arm exercise (Bangsbo *et al.* 1996). Thus, lactic acid still seems to have a negative effect on the intact organism, indicating that a powerful pH-regulating system is beneficial to exercising muscle, which is consistent with the improvement in pH regulation after training. One underlying mechanism might be the influence of pH on potassium distribution described above.

Conflict of interest

There is no conflict of interest.

References

- Aalkjær, C. & Peng, H.L. 1997. pH and smooth muscle. *Acta Physiol Scand* **161**, 557–566.
- Ahlborg, B., Bergström, J., Ekelund, L.-G., Guarnieri, G., Harris, R.C., Hultman, E. & Nordesjö, L.-O. 1972. Muscle metabolism during isometric exercise performed at constant force. *J Appl Physiol* **33**, 224–228.
- Aickin, C.C. & Thomas, R.C. 1977. An investigation of the ionic mechanism of intracellular pH regulation in mouse soleus muscle fibres. *J Physiol* **273**, 295–316.
- Bangsbo, J. & Juel, C. 2006. Counterpoint: lactic acid accumulation is a disadvantage during muscle activity. *J Appl Physiol* **101**, 1412–1413.
- Bangsbo, J., Johansen, L., Graham, T. & Saltin, B. 1993. Lactate and H⁺ efflux from human skeletal muscle during intense, dynamic exercise. *J Physiol* **462**, 115–133.
- Bangsbo, J., Madsen, K., Kiens, B. & Richter, E.A. 1996. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol* **495**, 587–596.
- Bangsbo, J., Juel, C., Hellsten, Y. & Saltin, B. 1997. Dissociation between lactate and proton exchange in muscle during intense exercise in man. *J Physiol* **504**, 489–499.
- Becker, H.M., Broer, S. & Dietmer, J.W. 2004. Facilitated lactate transport by MCT1 when coexpressed with the sodium bicarbonate cotransporter (NBC) in *Xenopus* oocytes. *Biophys J* **86**, 235–247.
- Bishop, D., Edge, J., Thomas, C. & Mercier, J. 2007. High-intensity exercise acutely decreases the membrane content of MCT1 and MCT4 and buffer capacity in human skeletal muscle. *J Appl Physiol* **102**, 616–621.
- Bonen, A., McGullagh, K.J.A., Putman, C.T., Hultman, E., Jones, N.L. & Heigenhauser, G.J.F. 1998. Short-term training increases human MCT1 and femoral venous lactate in relation to muscle lactate. *Am J Physiol Endocrinol Metab* **274**, E102–E107.
- Grossie, J., Collins, C. & Julian, M. 1988. Bicarbonate and fast twitch muscle: evidence for a major role in pH regulation. *J Membr Biol* **105**, 265–272.
- Hill, C.A., Harris, R.C., Kim, H.J., Harris, B.D., Sale, C., Boobis, L.H., Kim, C.K. & Wise, J.A. 2007. Influence of beta-alanine supplementation on skeletal muscle carnosine concentrations and high intensity cycling capacity. *Amino Acids* **32**, 225–233.
- Jensen, L., Bangsbo, J. & Hellsten, Y. 2004. Effect of high intensity training on capillarization and presence of angiogenic factors in human skeletal muscle. *J Physiol* **557**, 571–582.
- Juel, C. 1997. Lactate-proton cotransport in skeletal muscle. *Physiol Rev* **77**, 321–358.
- Juel, C. 1998. Skeletal muscle Na⁺/H⁺ exchange in rats: pH dependency and the effect of training. *Acta Physiol Scand* **164**, 135–140.
- Juel, C. 2000. Expression of the Na⁺/H⁺ exchanger isoform NHE1 in rat skeletal muscle and effect of training. *Acta Physiol Scand* **170**, 59–63.
- Juel, C., Bangsbo, J., Graham, T. & Saltin, B. 1990. Lactate and potassium fluxes from human skeletal muscle during and after intense, dynamic, knee-extensor exercise. *Acta Physiol Scand* **140**, 147–159.
- Juel, C., Lundby, C., Sander, M., Calbet, J.A. & Hall, G. 2003. Human skeletal muscle and erythrocyte proteins involved in acid-base homeostasis: adaptations to chronic hypoxia. *J Physiol* **548**, 639–648.
- Juel, C., Holten, M.K. & Dela, F. 2004a. Effects of strength training on muscle lactate release and MCT1 and MCT4 content in healthy and type 2 diabetic humans. *J Physiol* **556**, 297–304.
- Juel, C., Klarskov, C., Nielsen, J.J., Krstrup, P., Mohr, M. & Bangsbo, J. 2004b. Effect of high-intensity intermittent training on lactate and H⁺ release from human skeletal muscle. *Am J Physiol Endocrinol Metab* **286**, E245–E251.
- Juel, C., Thomsen, J.J., Rentsch, R.L. & Lundby, C. 2007. Effects of prolonged recombinant human erythropoietin administration on muscle membrane transport systems and metabolic marker enzymes. *Eur J Appl Physiol* **102**, 41–44.
- Kristensen, J.M., Kristensen, M. & Juel, C. 2004. Expression of Na⁺/HCO₃⁻ co-transporter proteins (NBCs) in rat and human skeletal muscle. *Acta Physiol Scand* **182**, 69–76.
- Kristensen, M., Albertsen, J., Rentsch, M. & Juel, C. 2005. Lactate and force production in skeletal muscle. *J Physiol* **562**, 521–526.
- Lindinger, M.I., Kowalchuk, J.M. & Heigenhauser, G.J.F. 2005. Applying physicochemical principles to skeletal muscle acid-base status. *Am J Physiol Regul Integr Comp Physiol* **289**, R891–R894.
- Mannion, A.F., Jakeman, P.M. & Willan, P.L.T. 1994. Effects of isokinetic training of the knee extensors on high-intensity

- exercise performance and skeletal muscle buffering. *Eur J Physiol* 68, 356–361.
- McKenna, M.J., Harmer, A.R., Fraser, S.F. & Li, J.L. 1996. Effects of training on potassium, calcium and hydrogen ion regulation in skeletal muscle and blood during exercise. *Acta Physiol Scand* 156, 335–346.
- Messonier, L., Kristensen, M., Juel, C. & Denis, C. 2007. Importance of pH regulation and lactate/H⁺ transport capacity for work production during supramaximal exercise in humans. *J Appl Physiol* 102, 1936–1944.
- Nielsen, O.B., Paoli, F. & Overgaard, K. 2001. Protective effects of lactic acid on force production in rat skeletal muscle. *J Physiol* 536, 161–166.
- Nielsen, J.J., Mohr, M., Klarskov, C., Kristensen, M., Krusturup, P., Juel, C. & Bangsbo, J. 2004. Effects of high-intensity intermittent training on potassium kinetics and performance in human skeletal muscle. *J Physiol* 554, 857–870.
- Parkhouse, W.S. & McKenzie, D.C. 1984. Possible contribution of skeletal muscle buffer to enhanced anaerobic performance: a brief review. *Med Sci Sports Exer* 16, 328–338.
- Pedersen, T.H., Nielsen, O.B., Lamb, G.D. & Stephenson, G.D. 2004. Intracellular acidosis enhances the excitability of working muscle. *Science* 305, 1144–1147.
- Pilegaard, H., Bangsbo, J., Richter, E.A. & Juel, C. 1994. Lactate transport studied in sarcolemmal giant vesicles from human skeletal muscle biopsies: relation to training status. *J Appl Physiol* 77, 1858–1862.
- Pilegaard, H., Domino, K., Noland, T., Juel, C., Hellsten, Y., Halestrap, A.P. & Bangsbo, J. 1999. Effect of high-intensity exercise training on lactate/H⁺ transport capacity in human skeletal muscle. *Am J Physiol Endocrinol Metab* 276, E255–E261.
- Putman, C.T., Jones, N.L. & Heigenhauser, G.J.F. 2003. Effects of short-term training on plasma acid-base balance during incremental exercise in man. *J Physiol* 550, 585–603.
- Rentsch, R.L., Damsgaard, R., Lundby, C. & Juel, C. 2006. Effects of darbepoetin injections on erythrocyte membrane transporter protein expression in humans. *J Appl Physiol* 101, 164–168.
- Sahlin, K. & Henriksson, J. 1984. Buffer capacity and lactate accumulation in skeletal muscle of trained and untrained men. *Acta Physiol Scand* 122, 331–339.
- Siggaard-Andersen, O. 1974. *The Acid-Base Status of the Blood*. Munksgaard, Copenhagen.
- Sostaric, S.M., Skinner, S.L., Brown, M.J., Sangkabutra, T., Medved, I., Medley, T., Selig, S.E., Fairweather, I., Rutar, D. & McKenna, M.J. 2006. Alkalosis increases muscle K⁺ release, but lowers plasma [K⁺] and delays fatigue during dynamic forearm exercise. *J Physiol* 570, 185–205.
- Stewart, P.A. 1983. Modern quantitative acid-base chemistry. *Can J Physiol Pharmacol* 61, 1444–1461.
- Street, D., Bangsbo, J. & Juel, C. 2001. Interstitial pH in human skeletal muscle during and after dynamic graded exercise. *J Physiol* 537, 993–998.
- Street, D., Nielsen, J.J., Bangsbo, J. & Juel, C. 2005. Metabolic alkalosis reduces exercise-induced acidosis and potassium accumulation in human skeletal muscle. *J Physiol* 566, 481–489.
- Thomas, C., Bishop, D.J., Moore-Morris, T. & Mercier, J. 2007. Effects of high-intensity training on MCT1, MCT4 and NBC expression in rat skeletal muscle: influence of chronic metabolic alkalosis. *Am J Physiol* 293, E916–E922.
- Westerblad, H., Bruton, J.D. & Lannergren, H. 1997. The effect of intracellular pH of contractile function in intact, single fibers of mouse muscle declines with increasing temperature. *J Physiol* 500, 193–204.
- Weston, A.R., Myburgh, K.H., Lindsay, F.H., Dennis, S.C., Noakes, T.D. & Hawley, J.A. 1997. Skeletal muscle buffer capacity and endurance performance after high-intensity interval training by well-trained cyclists. *Eur J Appl Physiol* 75, 7–13.
- Wetzel, P., Hasse, A., Papadopoulos, S., Voipoi, J., Kaila, K. & Gros, G. 2001. Extracellular carbonic anhydrase activity facilitates lactic acid transport in rat skeletal muscle fibres. *J Physiol* 531, 743–756.