High-intensity exercise decreases muscle buffer capacity via a decrease in protein buffering in human skeletal muscle

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Abstract We have previously reported an acute decrease in muscle buffer capacity ($\beta m_{in\ vitro}$) following high-intensity exercise. The aim of this study was to identify which muscle buffers are affected by acute exercise and the effects of exercise type and a training intervention on these changes. Whole muscle and non-protein $\beta m_{in\ vitro}$ were measured in male endurance athletes (VO_{2max} = 59.8 ± 5.8 mL kg^{-1} min^{-1}), and before and after training in male, team-sport athletes (VO_{2max} = 55.6 ± 5.5 mL kg^{-1} min^{-1}). Biopsies were obtained at rest and immediately after either time-to-fatigue at 120% VO_{2max} (endurance athletes) or repeated sprints (team-sport athletes). High-intensity exercise was associated with a significant decrease in $\beta m_{in\ vitro}$ in endurance-trained males (146 ± 9 to 138 ± 7 mmol $H^+ \cdot kg\ d.w.^{-1} \cdot pH^{-1}$), and in male team-sport athletes both before (139 ± 9 to 131 ± 7 mmol $H^+ \cdot kg\ d.w.^{-1} \cdot pH^{-1}$) and after training (152 ± 11 to 142 ± 9 mmol $H^+ \cdot kg\ d.w.^{-1} \cdot pH^{-1}$). There were no acute changes in non-protein buffering capacity. There was a significant increase in $\beta m_{in\ vitro}$ following training, but this did not alter the post-exercise decrease in $\beta m_{in\ vitro}$. In conclusion, high-intensity exercise decreased $\beta m_{in\ vitro}$ independent of exercise type or an interval-training intervention; this was largely explained by a decrease in protein buffering. These findings have important implications when examining training-induced changes in $\beta m_{in\ vitro}$. Resting and post-exercise muscle samples cannot be used interchangeably to determine $\beta m_{in\ vitro}$, and researchers must ensure that post-training measurements of $\beta m_{in\ vitro}$ are not influenced by an acute decrease caused by the final training bout.

Keywords Buffer Capacity · Lactate transport · Hydrogen ions · Acidosis · Exercise · Muscle · Activity · Fatigue

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Introduction

Intense muscle contractions result in large ionic changes and an increased non-mitochondrial adenosine triphosphate (ATP) turnover, contributing to the accumulation of hydrogen ions (H^+). While recent findings indicate that the role of H^+ accumulation during the fatigue process of mammalian muscle fibres may be limited [32], the

accumulation of H^+ has been shown to affect oxidative phosphorylation, enzyme activity and ion regulation during some exercise tasks [15, 22, 38, 40]. The removal of H^+ during intense skeletal muscle contractions occurs via a number of different transport systems and via muscle buffering [4, 23, 24]. Muscle buffer capacity ($\beta m_{in\ vitro}$) is typically estimated by titrating a muscle homogenate with a fixed acid and measures the contribution to physicochemical buffering by protein-bound histidine residues, imidazole-containing dipeptides and phosphates within the muscle but excludes 'dynamic' metabolic buffering such as the rephosphorylation of adenosine diphosphate (ADP) by phosphocreatine (PCr).

It was originally believed that $\beta m_{in\ vitro}$ was fixed and unaffected by acute exercise. However, unaccustomed eccentric exercise has been reported to acutely decrease $\beta m_{in\ vitro}$ in rats [34]. In addition, we have reported that high-intensity exercise is associated with an acute decrease in $\beta m_{in\ vitro}$ in moderately-trained females [3]. As this was the first study in humans, it is important to verify these results and to determine if this acute decrease in $\beta m_{in\ vitro}$ is a common response to different types of high-intensity exercise and to determine whether this response is seen in all subjects (i.e. males and females, the trained and untrained). It is known that metabolic perturbations in response to high-intensity exercise differ between males and females [13] and between untrained and trained subjects [18]. Therefore, acute, exercise-induced changes in $\beta m_{in\ vitro}$ may also differ in these populations and be influenced by training.

It is also of interest to determine which of the muscle buffers that are measured using the in vitro titration technique (i.e. intramuscular phosphates, protein-bound histidine and dipeptides) are responsible for these acute decreases in $\beta m_{in\ vitro}$. Due to its capacity to buffer H^+ within the typical physiological range experienced during high-intensity exercise (pKa=6.83), carnosine (*N*-β-alanyl-L-histidine) is an important muscle buffer, and any loss from the muscle will cause a decrease in $\beta m_{in\ vitro}$. However, using the Hendersson–Hasselbach equation, it can be calculated that our previously reported decrease in $\beta m_{in\ vitro}$ [3] would necessitate a loss of ~25 mmol·kg d.w.$^{-1}$ of carnosine from the muscle (i.e. an amount greater than that typically reported in muscle [19, 27, 31]). Consistent with this, our subsequent research [12] has indicated that a decrease in muscle carnosine content was not the cause of the decrease in $\beta m_{in\ vitro}$ following severe exercise training. It is also unlikely that the acute decrease in $\beta m_{in\ vitro}$ can be attributed to an acute change in muscle phosphate concentration following high-intensity exercise. It has previously been reported that there is no significant decrease in the total adenine nucleotide pool (ATP + ADP + adenosine monophosphate (AMP)) plus inosine 5′-monophosphate (IMP) following a 30-s maximal sprint [39], and no significant change in total muscle phosphate following exercise to exhaustion at 75% of VO_{2max} [37]. We therefore hypothesised that an acute decrease in $\beta m_{in\ vitro}$ following high-intensity exercise might be related to a decrease in protein buffering.

To date, no previous research has sought to quantify the relative contribution of both non-protein and protein buffering to the $\beta m_{in\ vitro}$ in humans or investigated possible changes in these components in response to different acute exercise stimuli. The current study therefore had four principal aims. The first was to verify if our previous results (i.e. an acute exercise-induced decrease in $\beta m_{in\ vitro}$ in women) would also be observed in men. The second aim was to determine if an acute decrease in $\beta m_{in\ vitro}$ is a common response to different types of high-intensity exercise (i.e. continuous and intermittent). The third aim was to determine if short-term endurance training can confer a protective effect on the acute, exercise-induced decrease in $\beta m_{in\ vitro}$. The final aim was to determine if acute changes in $\beta m_{in\ vitro}$ were attributable to acute changes in protein buffering. We hypothesised that decreases in $\beta m_{in\ vitro}$ would be the result of acute changes in protein buffering and would not be affected by exercise type or an interval-training program designed to increase $\beta m_{in\ vitro}$.

Materials and methods

Ethical approval

Subjects were informed of the study requirements, benefits and risks before giving written informed consent. The studies conformed to the latest revision of the *Declaration of Helsinki*, and approval for the study's procedures was granted by the Research Ethics Committee of the University of Western Australia.

Experimental overview

In order to perform this study, we recruited six well-trained endurance athletes (VO_{2max}=59.8±5.8 mL kg^{-1} min^{-1}; experiment A) and six well-trained, male, team-sport athletes (VO_{2max}=55.6±5.5 mL kg^{-1} min^{-1}; experiment B). All of the subjects first completed a familiarisation trial of the graded exercise test (GXT) and their respective high-intensity exercise test. During the subsequent main trials, each subject had a muscle biopsy from the vastus lateralis muscle at rest and immediately upon cessation of the high-intensity exercise test. Subjects in experiment B (who also performed 5 weeks of training) had their pre- and post-training tests conducted at the same time of day. Subjects were required to consume no food or beverages (other than water) 2 h prior to testing and were asked not to consume

alcohol or caffeine or to perform vigorous exercise in the 24 h prior to testing.

Graded exercise test

The GXTs were performed on an air-braked, track-cycle ergometer (Evolution Pty. Ltd., Adelaide, Australia; experiment A) or a motorised treadmill (Human Movement, Perth, Australia; experiment B) and consisted of graded exercise steps using an intermittent protocol (1-min break between stages). The tests commenced at 60 W or 8.5 $km \cdot h^{-1}$ (for experiments A and B, respectively) and thereafter, intensity was increased by 40 W or 1.5 $km \cdot h^{-1}$ (for experiments A and B, respectively) every 3 min until volitional exhaustion.

Gas analysis during the GXT

During the GXT, expired air was continuously analysed for O_2 and CO_2 concentrations using Ametek gas analysers (Applied Electrochemistry, SOV S-3A11 and COV CD-3A, Pittsburgh, PA, USA). Ventilation was recorded every 15 s using a turbine ventilometer (Morgan, 225A, Kent, England). The gas analysers were calibrated immediately before and verified after each test using three certified gravimetric gas mixtures (BOC Gases, Chatswood, Australia); the ventilometer was calibrated pre-exercise and verified post-exercise using a 1-l syringe in accordance with the manufacturer's instructions. The ventilometer and gas analysers were connected to an IBM PC which measured and displayed variables every 15 s. The sum of the two highest consecutive 15-s values was recorded as the subject's VO_{2max}.

High-intensity exercise

To determine if an acute decrease in $\beta m_{in\ vitro}$ is a common response to different types of high-intensity exercise, we performed two experiments—each with a different type of high-intensity exercise. These high-intensity exercise tests consisted of time to fatigue at 120% of the power output recorded at VO_{2max} (124±20 s; experiment A) or a repeated-sprint test (6× all-out 4-s sprints separated by 21 s of recovery; experiment B). All high-intensity exercise tests were performed on an air-braked, front-access cycle ergometer (Model Ex-10, Repco, Australia). Toe clips and heel straps were used to secure the feet to the pedals, and the test was performed in the seated position. Strong verbal encouragement was provided to each subject during the test.

Training intervention (experiment B)

Within 4–7 days of baseline testing, all subjects in experiment B started an interval-training program similar to that which had previously been reported to significantly increased $\beta m_{in\ vitro}$ [10, 11]. Subjects completed five to eight 2-min running intervals on an outdoor grass track at an initial intensity of ~100% of the velocity recorded at VO_{2max}. Each 2-min interval was interspersed with a 2-min passive recovery period. Three training sessions were performed per week (Monday, Wednesday, Friday) for five consecutive weeks, and training followed a periodised and progressive plan. All training was preceded by a 10-min standardised warm up involving general aerobic and dynamic stretching components.

Muscle sampling and analysis

On the day of the performance test, incisions were made under local anaesthesia (5 mL, 1% Xylocaine) into the vastus lateralis of each subject (mid-way between the anterior, superior iliac spine and the patella). The first muscle sample was taken (prior to warm-up) during supine rest. The second muscle sample was taken immediately (within 10 s) following the cessation of the respective performance test, while the subject remained on the cycle ergometer. The samples were then removed from the biopsy needle and immediately stored at −80°C until subsequent analysis.

Muscle buffering capacity; titration method ($\beta m_{in\ vitro}$) and [H^+]

Freeze-dried muscle samples (1.8–2.5 mg d.w.) were dissected free from visible blood, fat and connective tissue and then homogenised on ice for 2 min in a solution containing sodium fluoride (NaF; 10 mM) at a dilution of 30 mg dry muscle·mL^{-1} of homogenising solution [28]. Freeze-dried muscle samples (1.8–2.5 mg) were also deproteinised with the addition of 3% solid sulfosalicylic acid and centrifuged at 1,000×*g* for 10 min [31]. Deproteinisation does not alter the effectiveness of non-protein buffers in vitro [7]. Measurements of pH for both the whole-muscle and deproteinised muscle samples were then made at 37°C with a microelectrode (MI-415, Microelectrodes, Bedford, NH, USA) connected to a pH meter (SA 520, Orion Research, Cambridge, MA, USA). After initial pH measurement, muscle homogenates for both pre- and post-exercise samples (both the whole-muscle and deproteinised muscle samples) were adjusted to a pH of ~7.2 with a sodium hydroxide (NaOH) solution and then titrated to a pH of ~6.2 by the serial addition of 2 μL of hydrochloric acid (HCl; 10 mM). From the fitted titration trendline, the number of moles of H^+ (per kilogram of dry muscle) required to change the pH from 7.1 to 6.5 was interpolated (mmol $H^+ \cdot kg$ muscle $d.w.^{-1} \cdot 0.6\ pH^{-1}$). This value was also normalised to the whole pH unit for final

display as millimoles H^+ per kilogram dry muscle per unit pH (mmol $H^+ \cdot kg$ muscle d.w.$^{-1} \cdot pH^{-1}$) and determined as the subject's $\beta m_{in\ vitro}$. The protein buffer capacity was estimated by subtracting the deproteinised $\beta m_{in\ vitro}$ from the whole-muscle $\beta m_{in\ vitro}$.

Statistical analysis

All values are reported as mean ± SD. For experiment A, one-way analysis of variance (ANOVA) with repeated measures for time was used to test for main effects. For experiment B, two-way ANOVA with repeated measures for time was used. Least-squares linear regression analysis was used to calculate correlation coefficients between resting values and acute changes in $\beta m_{in\ vitro}$, using Pearson's product moment (r). Significance was accepted at $P<0.05$.

Results

Experiment A

Exercise to task failure at 120% of the power output recorded at VO_{2max} resulted in a significant decrease in muscle pH from 7.00±0.05 to 6.61±0.06. This was associated with a significant decrease in whole-muscle $\beta m_{in\ vitro}$ (146±9 to 138±6.8 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$, Fig. 1a) and no significant change in deproteinised $\beta m_{in\ vitro}$ (95 ±4 to 94±4 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$). Thus, it can be calculated that there was a significant decrease in protein $\beta m_{in\ vitro}$ (51±8 to 44±6 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$, Fig. 1b). Significant correlations were observed between initial $\beta m_{in\ vitro}$ and the decrease in $\beta m_{in\ vitro}$ following exercise for both whole-muscle ($r=0.75$, $P<0.05$, $n=6$) and protein ($r=0.93$, $P<0.05$, $n=6$) values.

Experiment B

The repeated-sprint task (6× all-out 4-s sprints separated by 21 s of recovery) resulted in a significant decrease in muscle pH from 6.98±0.05 to 6.79±0.07 pre-training and 6.99 ± 0.04 to 6.77 ± 0.08 post-training. This task was associated with a significant decrease in whole-muscle $\beta m_{in\ vitro}$ both before (139 ±9 to 131 ±7 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$, Fig. 2a) and after training (152±11 to 142±9 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$, Fig. 2b). The decrease in $\beta m_{in\ vitro}$ was almost completely attributed to a significant decrease in estimated protein buffering both before (52±8 to 45±7 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$) and after training (56±8 to 47±7 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$). A significant correlation was observed between initial protein $\beta m_{in\ vitro}$ and the decrease in protein $\beta m_{in\ vitro}$ following exercise ($r=0.79$, $P<0.05$, $n=12$, Fig. 3). There were significant increases in $\beta m_{in\ vitro}$, deproteinised $\beta m_{in\ vitro}$ (87±8 to 96±10 mmol $H^+ \cdot kg$ d.w.$^{-1} \cdot pH^{-1}$; $P<0.05$) and estimated protein $\beta m_{in\ vitro}$

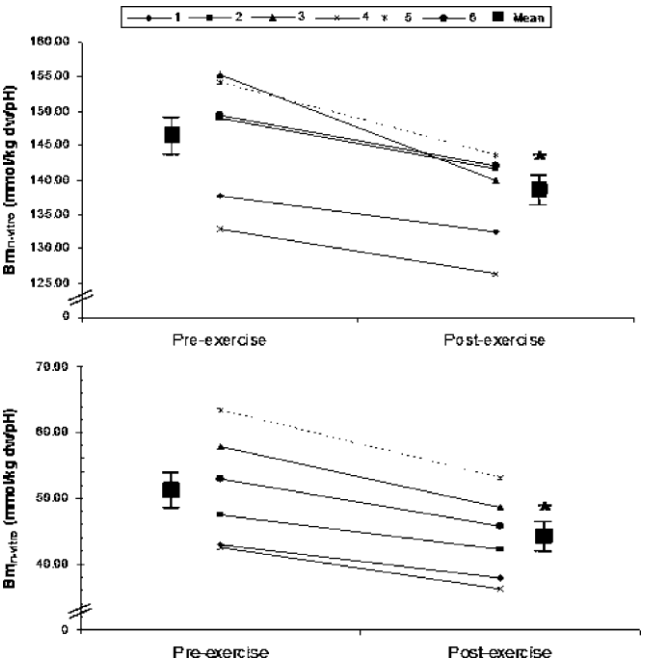

Fig. 1 Individual changes in titrated muscle buffer capacity ($\beta m_{in\ vitro}$; mmol $H^+ \cdot kg$ muscle d.w.$^{-1} \cdot pH^{-1}$) for whole muscle (a) and muscle protein (b) pre- and immediately post-exercise to fatigue at 120% of VO_{2max}. Also plotted are the mean values. *$P<0.05$, post-exercise mean significantly different from pre-exercise mean

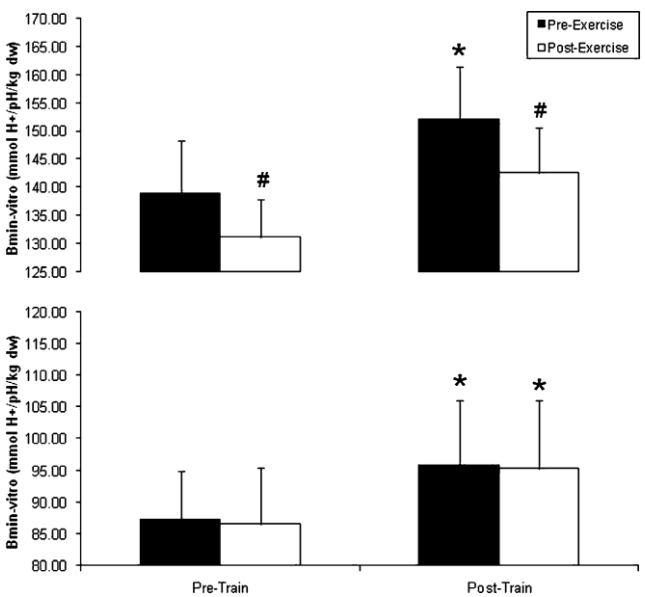

Fig. 2 Changes in titrated muscle buffer capacity ($\beta m_{in\ vitro}$; mmol $H^+ \cdot kg$ muscle d.w. $\cdot pH^{-1}$) for whole muscle (a) and deproteinised muscle (b) before and after 6 weeks of high-intensity interval training. *$P<0.05$, significant increase following training. # $P<0.05$, post-exercise mean significantly different from pre-exercise mean

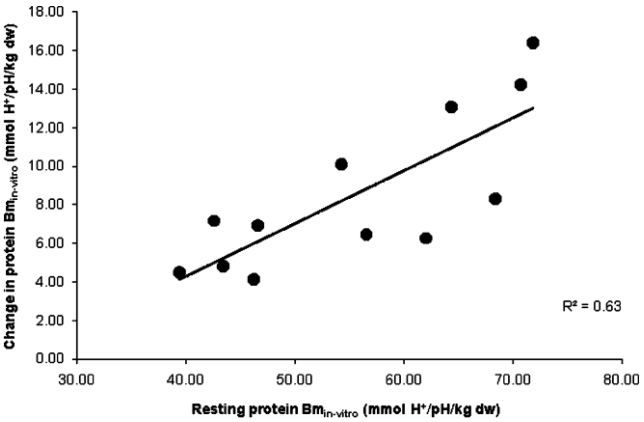

Fig. 3 Relationship between resting protein $\beta m_{in\ vitro}$ and change in protein $\beta m_{in\ vitro}$ following the repeated-sprint test in well-trained, team-sport athletes (experiment B). Data include the pre- and post-training data for six subjects (i.e. n=12)

(52±8 to 56±8 mmol H^+·kg d.w.$^{-1}$·pH^{-1}; P<0.05) following training.

Discussion

This is the first human study to report $\beta m_{in\ vitro}$ values for both whole and deproteinised human vastus lateralis muscle pre- and post-exercise. The main finding was that different types of high-intensity exercise were associated with a significant decrease in $\beta m_{in\ vitro}$ in all subjects tested (i.e. male endurance and team-sport athletes) and for both exercise tasks (i.e. continuous and repeated-sprint exercise). There were no acute changes in the non-protein buffering capacity which suggests that acute decreases in $\beta m_{in\ vitro}$ were due to an acute decrease in the buffering by proteins. There were strong negative correlations between the resting protein buffer capacity and the decrease in protein buffer capacity following high-intensity exercise (r=0.79–0.93; P<0.05). Consistent with this result, the significant increase in resting protein buffer capacity following interval training was associated with a trend for a greater acute decrease in protein buffer capacity post-training, but this did not reach statistical significance.

$\beta m_{in\ vitro}$

The titration method measures the contribution to physicochemical buffering by proteins, dipeptides and phosphates but excludes 'dynamic buffering' (e.g. the rephosphorylation of ADP by PCr) and the bicarbonate contribution (as CO_2 evaporates during the freeze-drying) [19]. It is also important to note that homogenisation, particularly on subsequent incubation at 37°C, increases the quantity of inorganic phosphate (P_i; and probably also hexose monophosphates; [29]) available to contribute to in vitro physicochemical buffering, with respect to in vivo. This is because the release of previously sequestered calcium triggers an acceleration of ATP turnover [35] which, coupled to the utilisation of anaerobic metabolism, results in almost complete (>95%) degradation of PCr and ATP [29]. Thus, muscle buffer capacity determined by the homogenate technique will differ slightly from intact preparations.

Surprisingly, no previous research has sought to quantify the relative contribution of both non-protein and protein buffering to the $\beta m_{in\ vitro}$ in humans. However, our non-protein $\beta m_{in\ vitro}$ values (87–95 mmol H^+·kg muscle d.w.$^{-1}$·pH^{-1} or 60–65% of the whole-muscle $\beta m_{in\ vitro}$) are very similar to the results reported for marathon runners (~90 mmol H^+·kg muscle d.w.$^{-1}$·pH^{-1}; [31]). Similarly, another study has estimated that 60–70% of non-bicarbonate buffering in mammalian skeletal muscle is due to non-protein substances [1]. From our results, we can calculate that the protein buffering capacity was ~52 mmol H^+·kg muscle d.w.$^{-1}$·pH^{-1} (30–35%) for both male triathletes and male team-sport athletes in the present study. This value closely approximates the value of 50 mmol H^+·kg muscle d.w.$^{-1}$·pH^{-1} that can be calculated from the reported histidine concentration in human muscle protein (2.7 g/100 g of protein; [16]), the protein content in muscle tissue (~170 g·kg w.w.$^{-1}$; [17]) and the pKa value of the imidazole ring in protein-bound histidine residues (6.25; [42]). These calculations however, must be treated with caution as theoretical calculations of the buffering power of protein-bound histidine residues are complicated by uncertainty regarding the pKa value of histidine (5.97 at 37°C) when incorporated into proteins [6]. Nonetheless, our results are consistent with values that can be estimated from previous research and confirm that proteins are an important buffer within human skeletal muscle.

Acute decreases in $\beta m_{in\ vitro}$

Consistent with previous research [3, 20], we observed a significant decrease in $\beta m_{in\ vitro}$ following an acute bout of high-intensity exercise. As previously reported in women, this acute decrease in $\beta m_{in\ vitro}$ was observed in all subjects (Fig. 1). Furthermore, this acute decrease occurred following both constant-intensity and repeated-sprint exercise. Interestingly, this acute decrease was positively related to the resting $\beta m_{in\ vitro}$ value. Thus, the resting $\beta m_{in\ vitro}$ value is a more important determinant of the subsequent decrease in $\beta m_{in\ vitro}$ than the type of high-intensity exercise performed.

Although an acute decrease in $\beta m_{in\ vitro}$ has previously been observed [3], we report for the first time that this appears to be predominately due to a decrease in protein buffering. In both experiments A and B, acute, high-intensity exercise resulted in no significant change in the non-protein buffer capacity (i.e. the buffering by intramus-

cular phosphates and carnosine). This is consistent with previous research which has not observed changes in the total adenine nucleotide pool (ATP+ ADP +AMP+ IMP) [39] or total muscle phosphate [37] following high-intensity exercise. While one study has reported that acute, intense exercise results in a 10% loss of carnosine from the muscle [9], using a pKa of 6.83 [26] and a typical resting muscle carnosine concentration of 20 mmol kg muscle d.w.$^{-1}$ [19, 27, 31], it can be calculated that this would at most contribute to a decrease in $\beta m_{in\ vitro}$ of 1 mmol $H^+ \cdot$kg muscle d.w.$^{-1} \cdot pH^{-1}$. The unchanged non-protein buffer capacity also argues against the possibility that the decrease in $\beta m_{in\ vitro}$ is an artefact caused by a different chemical composition of the pre- and post-exercise muscle samples (e.g., differences in the quantity of P_i and hexose monophosphates will affect the non-protein and whole-muscle buffer capacity due to differences in their respective pKa). Thus, these previous findings are consistent with our observation that a decrease in protein buffering is predominately responsible for the acute decreases in $\beta m_{in\ vitro}$ observed following high-intensity exercise.

As histidine is the only amino acid able to exert a buffering function in the intracellular pH range [21], our results therefore suggest that there was an acute, exercise-induced decrease in the buffering by protein-bound histidine. Such changes could potentially be brought about by a loss of protein from the muscle, the release of histidine bound to protein and/or chemical changes in the imidazole ring belonging to histidine (and subsequent decreases in the apparent pKa). Using the Hendersen–Hasselbach equation, it can be calculated that the decrease in protein buffering observed in the present study (~7–9 mmol $H^+ \cdot$kg muscle d.w.$^{-1} \cdot pH^{-1}$) would require the loss of ~15% of intracellular protein. While exhaustive endurance exercise in rats has been reported to be associated with a 13% loss of protein from muscle [8], it seems unlikely that such changes would have occurred within the time frame observed in our study (<3 min). Nonetheless, it is possible that smaller losses of protein did contribute to our observed acute decrease in protein buffering, and studies investigating the acute loss of protein during brief, high-intensity exercise (>100% VO_{2max}) are warranted.

While we are unaware of any previous research that has investigated changes in muscle histidine following brief, intense exercise, it also seems unlikely that the release of histidine bound to protein can completely account for our findings. Only slight increases in histidine concentration (5–10%) have been reported following 1 h of exercise at approximately 70% of VO_{2max} [5]. Furthermore, much larger increases following brief, high-intensity exercise are improbable as reported changes in other muscle amino acids (e.g., alanine) are similar following either 4 min of exercise at 100% of VO_{2max} [25] or 1 h of exercise at 70% of VO_{2max} [5]. It should be noted however, that the release of histidine bound to protein is not necessary to explain our observations and that exercise-induced changes in the imidazole ring belonging to histidine (and a subsequent decrease in the apparent pKa) may also contribute to the acute decrease in protein buffering observed in this study. While we are unaware of research investigating exercise-induced changes in the apparent pKa of histidine, only a very small decrease in pKa (<0.15) would be required to explain our results. Furthermore, rather than being attributable to one cause, acute changes in protein buffer capacity may be due to small contributions from all three of the above hypothesised mechanisms (i.e. the loss of protein from the muscle, the release of histidine bound to protein and/or chemical changes in the imidazole ring belonging to histidine).

Training and $\beta m_{in\ vitro}$

The significant increase in $\beta m_{in\ vitro}$ following training in experiment B is consistent with the majority of training studies that have used high-intensity intervals interspersed with relatively short rest periods (work to rest ratio ≥1:1) [2, 10, 11]. While the changes observed in this study are not as large as those reported in some studies, this can probably be attributed to the relatively high starting levels of our subjects and the previous observation that there is a negative relationship between starting values and training-induced changes in $\beta m_{in\ vitro}$ [10]. A novel observation of the present study, however, is that the increase in $\beta m_{in\ vitro}$ was due to an increase in both protein and non-protein buffering. As intracellular phosphate concentration has not typically been reported to change in response to training [18], an increase in non-protein buffering is likely to be explained by the previous observation that high-intensity training is associated with an increase in carnosine [41]. While it has been suggested that increased $\beta m_{in\ vitro}$ following training can largely be attributed to changes in carnosine concentration [30], our results suggest that changes in protein are also important. This is consistent with previous reports of increases in protein concentration with training [33] and the observation that protein concentration is higher in trained sportsmen when compared with sedentary controls [36].

A final observation in the present study was that training did not significantly alter the acute decrease in $\beta m_{in\ vitro}$ following high-intensity exercise (experiment B), although there was a trend for a greater decrease following training. This can probably be related to the finding that the decrease in protein buffer capacity during intense exercise was correlated with the resting protein buffer capacity (Fig. 3). Interestingly, training has also been reported not to protect against the acute decrease in lactate transport induced by exhaustive exercise [14].

Summary

In conclusion, the results of this study confirm that proteins are an important buffer within skeletal muscle. More importantly, it appears that it is a decrease in this protein buffering that is responsible for the acute decrease in $\beta m_{in\ vitro}$ in response to high-intensity exercise. Further research is required to determine the relative contributions of a loss of protein from the muscle, the release of histidine bound to protein and/or chemical changes in the imidazole ring belonging to histidine to this acute decrease in protein buffering capacity. These findings have important implications for study design when examining training-induced changes in $\beta m_{in\ vitro}$. Resting and post-exercise muscle samples cannot be used interchangeably to determine $\beta m_{in\ vitro}$, and researchers must try to exclude the possibility that reported training-induced changes in $\beta m_{in\ vitro}$ are not influenced by the acute effects of the last training bout. Future studies should verify these findings and also examine the time course of the acute changes that we have reported.

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