BEC preprints 2026.25
https://doi.org/10.26124/becprep2026-0025
Open Access

Effects of acidosis on mitochondrial respiratory sensitivity and capacity in human skeletal muscle

Baker Christopher J[1], Parry H[1], Peden D[2], Burnley M[1], Bailey S[1]

[1]School of Sport, Exercise & Health Sciences, Loughborough University; [2]David Greenfield Human Physiology Unit, School of Life Sciences, Nottingham NIHR Biomedical Research Centre, Univ Nottingham.
c.baker2@lboro.ac.uk



Contribution to MiP2026 – **Received** 2026-10-30 – **Online** 2026-11-02

Cite: Baker CJ, Parry H, Peden D, Burnley M, Bailey S (2026) Effects of acidosis on mitochondrial respiratory sensitivity and capacity in human skeletal muscle. BEC preprints 2026.25. https://doi.org/10.26124/becprep2026-0025

Keywords: skeletal muscle, acidosis, substrate sensitivity, mitochondria

Introduction

Resting human skeletal muscle pH is approximately 7.0-7.1 [1,2]. When mitochondrial ATP production is insufficient to meet cellular energy demands, glycolytic flux increases and cellular pH declines. Increased glycolytic flux and the resulting acidosis are consistently observed in skeletal muscle during high intensity exercise [1], and in pathologies where mitochondrial respiratory capacity is low such as MELAS syndrome. Given the association between exercise-induced acidosis and muscle fatigue, the influence of pH on skeletal muscle mitochondrial respiratory function has been addressed using *in vivo* techniques such as ^{31}P-MRS in humans [2,3] and with *ex vivo* techniques that compare respiratory capacity at a resting muscle pH against acidosis, e.g. 7.2 vs. 6.8 pH [4,5,6]. While some studies have reported acidosis to lower oxidative capacity [2,4], others have shown no differences between an acidic and neutral pH [3,5,6]. The evidence for how acidosis regulates mitochondrial respiratory sensitivity to substrates, such as ADP, is limited and available evidence suggests no decrease in sensitivity with acidosis [4]. The effects of an acidic pH on mitochondrial respiratory sensitivity to different substrates in human skeletal muscle has therefore yet to be determined. Accordingly, the purpose of the current study was to assess human skeletal muscle respiratory sensitivity to ADP, pyruvate and succinate as well as respiratory capacity through complex I and II, at a neutral (7.0) and acidic (6.5) pH.

Methods

Ten healthy young adults (n = 1 female; $\dot{V}O_2$peak = 52 ± 11 mL/min/kg) provided informed consent to participate in this ethically approved study. Participants underwent a muscle biopsy from the *m.vastus lateralis* under local anaesthesia using a Bergström muscle biopsy needle with suction. Fresh muscle samples were placed into ice-cold

BIOPS and separated on ice using fine-tip needles before being permeabilised in a BIOPS-saponin (50 µg/mL) solution for 30 minutes with gentle shaking. Fibre bundles were washed in MiR05 for 15 minutes, blotted dry and weighed for wet mass specific normalisation of respiratory flux. Permeabilised fibre respiration was measured in duplicate at 37°C in MiR05 supplemented with 20 mM creatine in either an acidic (6.5) or neutral (7.0) pH using the Oxygraph-O2k. Oxygen concentration was maintained between 200-450 µM. Two experiments were conducted per participant. The first experimental protocol was a modification of SUIT-008. Briefly, malate (2 mM) was injected into the O2k chambers followed by 25 µM ADP and subsequently a series of pyruvate titrations (in µM: 5, 15, 30, 50,100, 300, 1000, 3000, 5000 + 2000 µM) until saturation (a plateau in O_2 flux). ADP was then titrated until saturation (in µM: 25, 50, 100, 250, 1000, 4000, 7000) followed by addition of glutamate (10 mM), glycerophosphate (10 mM) and succinate (10 mM). Cytochrome c (10 µM) was injected into the chambers and an increase in respiratory rate of ≥10% was set as a threshold for exclusion of the sample from analysis. Serial titrations of carbonyl cyanide m-chlorophenyl hydrazone (0.5 µM) were made until a subsequent titration led to no further increase in O_2 flux and the experiment was concluded by injection of antimycin a (2.5 µM). In the second experiment, separated fibre bundles that had been stored in BIOPS were permeabilised in the same way as the first experiment and weighed before being added to chambers containing MiR05 with 20 mM creatine. Rotenone (0.5 µM) and ADP (10 mM) were added prior to a series of succinate titrations (in mM: 0.25, 0.5, 1, 2, 4, 8, 16, 24, 32) and finally cytochrome c (10 µM) and antimycin a (2.5 µM). Data were analysed using DatLab 7.4 and GraphPad Prism v11.0 was used to determine substrate sensitivity by application of the Michaelis-Menten model for enzyme kinetics. SPSS v31.0 was used for statistical analysis. All paired samples were tested for normality according to the Shapiro-Wilk test. Paired samples t-tests were conducted to assess between-condition differences with effect sizes reported as Hedges' g. Data are presented as mean ± SD with statistical significance accepted at $p<0.05$.

Results

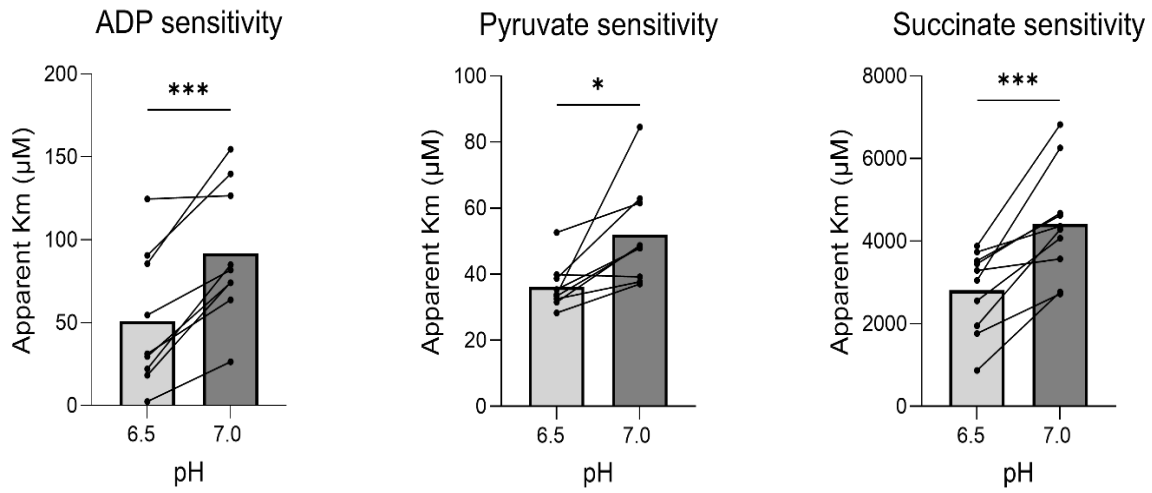


Figure. 1. Effects of acidosis on mitochondrial substrate sensitivity in human skeletal muscle. * = p < 0.05; *** = p < 0.001. n = 9-10

The apparent K_m was lower at pH 6.5 vs. 7.0 for ADP (51 ± 41 μM vs. 89 ± 40 μM; p < 0.001; *g* = -1.73), pyruvate (36 ± 7 μM vs. 51 ± 15 μM; p = 0.013; *g* = -0.95) and succinate (2807 ± 994 μM vs. 4417 ± 1321 μM; p < 0.001; *g* = -1.51). There was no difference in wet mass-normalised ADP-stimulated capacity for complex I (CI; 52 ± 20 pmol/s/mg vs. 55 ± 15 pmol/s/mg; p = 0.532; *g* = -0.21) or the glycerophosphate dehydrogenase complex (CGPDH; 9 ± 9 pmol/s/mg vs. 8 ± 8 pmol/s/mg; p = 0.956; *g* = 0.02). ADP-stimulated capacity for complex II (CII; 77 ± 27 pmol/s/mg vs. 87 ± 16 pmol/s/mg; p = 0.122, *g* = -0.52) and CI + CGPDH + CII (85 ± 14 pmol/s/mg vs. 97 ± 11 pmol/s/mg; p = 0.087, *g* = -0.73) were not different between conditions. Uncoupled respiratory capacity through CI + CGPDH + CII was not different between conditions (105 ± 19 pmol/s/mg vs. 119 ± 13 pmol/s/mg; p = 0.159; *g* = -0.57) nor was the ratio between the maximum theoretically coupled to uncoupled respiratory capacity (P/E; 0.81 ± 0.11 vs. 0.81 ± 0.04; p = 0.919; *g* = 0.09).

Discussion

The principal novel findings from the current study were that, compared to a neutral pH, skeletal muscle mitochondrial respiratory sensitivity to ADP, pyruvate and succinate were greater (apparent K_m was lower) in an acidic medium (Fig. 1). Also, while complex-specific ADP-stimulated capacity was not lower at an acidic pH, the differences in combined CI + CGPDH + CII ADP-stimulated capacity approached statistical significance (p=0.09).

Mitochondrial respiratory sensitivity to ADP has been shown to be comparable between resting muscle and acidic pH (7.2 vs. 6.8) in a murine model [4]. Consistent with our protocol, mitochondrial respiratory sensitivity to ADP was measured in the

presence of pyruvate and malate which might suggest that the observed differences with our results are explained by different forms of mitochondrial respiratory control in mouse and human skeletal muscle or by the extent of the decrease in pH (6.5 in our study vs. 6.8). They also demonstrated that pyruvate-supported respiration was lower with maximal pyruvate concentrations at 6.8 pH but not different at submaximal levels of pyruvate, possibly indicating enhanced respiratory sensitivity to pyruvate although this was not reported.

Oxidative capacity determined by phosphocreatine recovery kinetics in human skeletal muscle has been compared between resting and acidic muscle pH. One study that predicted V_{max} of the medial gastrocnemius after 9 vs. 30 seconds of rapid voluntary plantar flexion at ~30% of maximal voluntary contraction found no change in oxidative flux despite a large difference in intracellular pH (7.04 vs 6.45) [3]. In another human study, participants performed high force contractions of the muscles of the anterior leg and hand for 24 seconds at a high frequency and in a separate bout performed 7 minutes of repeated contractions at a constant peak force with lower frequency. The short-lived exercise induced a greater rise in ADP but no difference in oxidative flux during recovery was detected between the two exercise protocols which the authors attributed to the development of mild acidosis in the short duration exercise condition (~6.90 pH) [2]. Contrasting results with ^{31}P-MRS could be explained by differences in exercise protocol, oxygen availability and equations used to predict oxidative flux. *Ex vivo* assessments of mitochondrial respiratory capacity in rat and mouse skeletal muscle similarly conflict in reporting either inhibition or no effect of acidosis on respiratory capacity [4,5,6] which could be explained by species differences and experimental protocol [4].

While the mechanisms of the changes in respiratory substrate sensitivity were not investigated in this study, the apparent K_m for ADP is much lower (higher sensitivity) when measured in isolated mitochondria versus permeabilised slow twitch skeletal muscle fibres. Proteases that putatively degrade cytoskeletal proteins that interact with the mitochondrial outer membrane to regulate outer membrane transport of ADP have been shown to drastically decrease the apparent K_m for ADP in permeabilised fibres to a level close to isolated mitochondria [7]. Given that the apparent K_m for ADP in this study at 7.0 pH (89 μM) is considerably higher than in isolated mitochondria (~20 μM), a change in the interaction between cytoskeletal proteins and the mitochondrial outer membrane could explain the effect of pH on respiratory sensitivity to ADP. It is also possible that changes in membrane potential and protonation of respiratory complexes and transporters explain the observed effect.

In conclusion, the results of the current study suggest that lowering muscle pH to a level that can be achieved during exhaustive high intensity exercise can acutely enhance mitochondrial respiratory sensitivity to ADP, pyruvate and succinate in healthy young adult humans. These findings might have implications for metabolic

responses observed in exercising humans and in mitochondrial myopathies where respiratory capacity is limited.

References

[1] Sahlin K, Harris RC, Nylind B, Hultman E (1976) Lactate content and pH in muscle samples obtained after dynamic exercise. Pflugers Arch. 367(2), 143–149. https://doi.org/10.1007/BF00585150

[2] Jubrias SA, Crowther GJ, Shankland EG, Gronka RK, Conley KE (2003) Acidosis inhibits oxidative phosphorylation in contracting human skeletal muscle in vivo. J Physiol. 553(2), 589–599. https://doi.org/10.1113/jphysiol.2003.045872

[3] Walter G, Vandenborne K, McCully KK, Leigh JS (1997) Noninvasive measurement of phosphocreatine recovery kinetics in single human muscles. Am J Physiol Cell Physiol. 272(2), C525–C534. https://doi.org/10.1152/ajpcell.1997.272.2.C525

[4] Frangos SM, DesOrmeaux GJ, Holloway GP (2023) Acidosis attenuates CPT-I-supported bioenergetics as a potential mechanism limiting lipid oxidation. J Biol Chem. 299(9), 105079. https://doi.org/10.1016/j.jbc.2023.105079

[5] Willis WT, Jackman MR (1994) Mitochondrial function during heavy exercise. Med Sci Sports Exerc. 26(11), 1347–1353. http://www.ncbi.nlm.nih.gov/pubmed/7837955

[6] Hedges CP, Bishop DJ, Hickey AJR (2019) Voluntary wheel running prevents the acidosis-induced decrease in skeletal muscle mitochondrial reactive oxygen species emission. FASEB J. 33(4), 4996–5004. https://doi.org/10.1096/fj.201801870R

[7] Kuznetsov AV, Tiivel T, Sikk P, Kaambre T, Kay L, Daneshrad Z, Rossi A, Kadaja L, Peet N, Seppet E, Saks VA (1996) Striking differences between the kinetics of regulation of respiration by ADP in slow-twitch and fast-twitch muscles in vivo. Eur J Biochem. 241(3), 909–915. https://doi.org/10.1111/j.1432-1033.1996.00909.x

Author contributions

All authors designed the study. CB performed the experiments. CB wrote the manuscript and prepared the figure. SB reviewed the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

Copyright ©2026 The authors. This is an Open Access preprint (not peer-reviewed) distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. © The authors, who have granted BEC preprints an Open Access preprint license in perpetuity