# Using $^{31}$P-MRS to Explore the Effects of Iron Deficiency on Murine Skeletal Muscle Function and Metabolism During Exercise

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**Introduction:** Physical work or exercise capacity has been widely reported as being impaired by iron deficiency in both human and animal studies[1]. Recently, iron deficiency was shown to predict impaired exercise capacity in patients with chronic heart failure[2]. Moreover, chronic heart failure patients receiving ferric carboxymaltose demonstrated significant improvements in the distance achieved on the 6 minute walk test and quality of life assessments compared to placebo[3]. However, further investigation is needed to elucidate the mechanisms behind this effect. Previous, separate, studies using diet-induced iron deficient rats have shown decreased work rate capacity[4], diminished contractile force generation[5], and an impairment of energetics during exercise[6], when compared to rats on normal chow. The aim of this project was to develop a technique that allows for the simultaneous assessment of force production, fatigue resistance and metabolic function of murine gastrocnemius muscle during exercise. To address this, a method of *in vivo* gastrocnemius muscle stimulation was established, allowing the simultaneous measurement of energetics, by $^{31}$P magnetic resonance spectroscopy, and induced force production in a murine model of altered iron status.

**Methods:** *Animals* – Three week old C57BL/6 male mice were fed either an iron deficient diet (iron content 2-6 ppm) or a control diet (200 ppm) for 7-8 weeks before undergoing *in vivo* gastrocnemius muscle stimulation protocol.

*In vivo gastrocnemius muscle stimulation*- An anaesthetised mouse is placed in a bespoke Perspex cradle designed and manufactured for this protocol. The sciatic nerve is isolated surgically and electrodes are placed distal to the tibial nerve branch. The knee and ankle joints are immobilised, the calcaneal tendon is attached to a force transducer, via a suture thread, before a home-built $^{31}$P saddle-shaped RF surface coil is placed over the muscle. The cradle is positioned in the 7 T Agilent MR system and images of cross-sectional area (CSA) of the gastrocnemius muscle are obtained using the $^{1}$H volume coil. A stimulation protocol[7] consisting of a train of eight pulses of 100 μs at 30 Hz, followed by a rest period of 1.25 seconds, is repeated over a 10 minute period and induced force production is measured over this time using a PowerLab system. During the exercise period, and the subsequent 20 minute recovery period, a $^{31}$P spectrum is acquired every 100 seconds.

**Results:** Iron deficient mice have significantly decreased haemoglobin, haematocrit, serum iron and serum ferritin and significantly increased transferrin compared to controls ($p < 0.05$). Induced force production is significantly greater from 45 seconds of stimulation onwards in the iron deficient group compared to the controls (Figure 1, $p < 0.05$). However, there is no significant difference in the percent fatigue between the iron deficient and control groups, which are 74 % ± 0.98 and 76 % ± 2.36 respectively. Once reproducible fatigue of the muscle had been demonstrated, spectra were acquired successfully at rest, during exercise and recovery (Figure 2). Figure 2 shows the decrease in phosphocreatine (PCr) and increase in inorganic phosphate (Pi) expected during exercise and the loss of Pi and partial recovery of PCr at 10 minutes of recovery. The acquired spectra will be used to measure PCr, adenosine triphosphate (ATP), Pi and pH in iron replete versus iron deficient conditions in the future.

**Discussion:** A method of *in vivo* gastrocnemius muscle stimulation was successfully established. The protocol mimics the cyclical contraction pattern the gastrocnemius muscle would undergo during running type motion. Further experiments are needed to investigate why the iron deficient group generates significantly more force on average than the controls, which was not the expected result. Gastrocnemius muscles from both groups have been snap frozen for molecular analysis of gene and protein expression as well as enzyme activity to investigate why this is the case. However, the main aim of the project has been met, as a method has been established that facilitates the simultaneous assessment of skeletal muscle metabolism and function, including fatigue resistance. This can be used to explore the role of iron in work rate capacity in both diet-induced and genetic mouse models of iron deficiency.

**References:** [1] Haas, J.D. and Brownlie, T 4th. *J. Nutr.* 2001; 131: 676S-690S. [2] Jankowska, E.A., *et al. J. Cardiac Fail.* 2011; 17: 899-906. [3] Anker, S.D., *et al. N Eng J Med.* 2009; 361: 2436-48. [4] Finch, C.A., *et al. J Clin Invest.* 1976; 58(2): 447-53. [5] McLane, J.A., *et al. Am J Physiol.* 1981; 241: C47-C54. [6] Thompson, C.H., *et al. Acta Physiol Scand.* 1993; 147(1): 85-90. [7] Cole, M.A., *et al., Neruromuscl Disord.* 2002; 12(3): 247-57.

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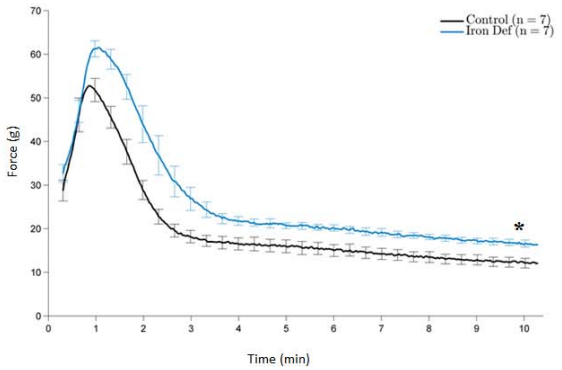


**Figure 1** - Maximum contractile force produced by the gastrocnemius muscle of control (black) and iron deficient (blue) mice over the ten minute stimulation period. Iron deficient mice generate significantly more force than the controls from 45 seconds onwards. Unpaired student two-tailed *t*-test. Mean ± SEM. * $p < 0.05$.

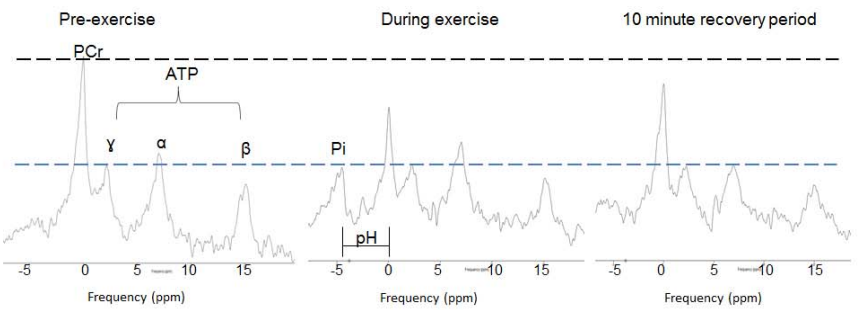


**Figure 2** – Representative $^{31}$P spectra acquired before exercise, during 10 minutes of exercise, and after 10 minutes of recovery, showing phosphocreatine (PCr), inorganic phosphate (Pi), pH and the γ, α, and β-ATP peaks. The black dashed line marks the height of the PCr peak at rest for comparison with during exercise and recovery. The spectra are scaled to the height of the γ-ATP peaks (blue dashed line).