## Iron overload in skeletal muscle; redox stress and exercise capacity

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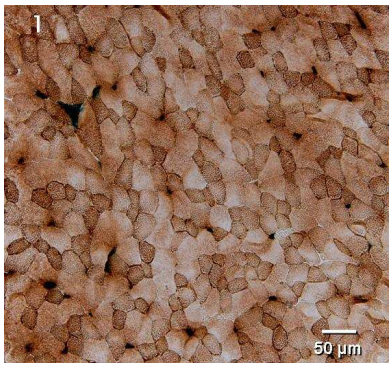
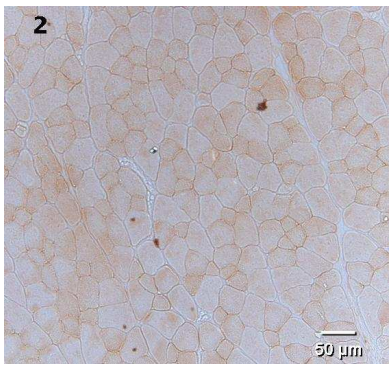
Iron accelerates the production of reactive oxygen species (ROS). Excessive levels of ROS are thought to accelerate skeletal muscle fatigue and contribute to the loss of skeletal muscle mass and function with age and disuse. Increased iron accumulation has been observed in these conditions and it has been proposed that iron may play a role (Jung *et al.*, 2008). Patients with an iron overload disorder frequently report symptoms of weakness and fatigue which is not entirely explained by reduced cardiac function (Davidsen *et al.*, 2007). The contribution of skeletal muscle to these symptoms is unknown.

Recent work from our laboratory has shown that iron accelerates skeletal muscle fatigue at 37°C (Reardon & Allen, 2007). Previous experiments under conditions in which there were trace amounts of iron in the perfusate, demonstrate that iron accelerates skeletal muscle fatigue at 37°C by reducing calcium sensitivity (Moopanar & Allen, 2005). Using a mouse model of iron overload lasting 30 days we determined the extent of iron accumulation in skeletal muscle and the change in the iron storage protein ferritin. The level of oxidative stress, changes in antioxidant enzymes and exercise performance were also assessed. The skeletal muscle analysed in this study was removed following cervical dislocation.

The iron content of the *tibialis anterior* muscle was assessed using inductively coupled plasma mass spectroscopy. Skeletal muscle iron was higher in the iron group (59.5 ± 3.5 μmoles/g dry weight) following the intervention compared to the control group (1 ± 0.1 μmoles/g dry weight) ($p < 0.001$; $n = 7$ per group). Importantly, the intracellular iron component (determined by a DAB enhanced Perls' stain) was also higher in the iron group (Figure 1) compared to the control group (Figure 2) ($p < 0.001$; $n = 6$ per group). The skeletal muscle content of the iron storage protein ferritin light chain was found to be 4 fold higher in the iron group ($p < 0.05$; $n = 5$ per group), indicating an increase in the iron storage capacity of skeletal muscle. The oxidative stress product malondialdehyde was also increased in the iron group compared to the control group ($p < 0.001$; $n = 5$ per group). The *extensor digitorum longus* muscle was used to measure the activity of the anti-oxidative enzymes glutathione reductase (GR) and glutathione peroxidase (GPx). GR activity increased in the iron group compared to the control group by 30% (3.7 ± 0.1 *vs.* 2.8 ± 0.1 nmol/mg/min respectively) ($p < 0.001$; $n = 7$ per group) and GPx activity increased in the iron group compared to the control group by 220% (1.5 ± 0.1 *vs.* 0.7 ± 0.1 nmol/mg/min respectively) ($p < 0.001$; $n = 7$ per group). The increased activity in the GR and GPx enzymes demonstrates that skeletal muscle has the ability to respond to the downstream oxidative stress of iron, but at the same time highlights the incomplete action of ferritin.




Exercise tests were performed before and after iron loading in both groups. Iron overload mice performed less work than the control group on a treadmill test designed to test endurance capacity (7 *vs.* 45 joules respectively) ($p < 0.001$; $n = 7$ per group). Iron overloaded mice produced less force than control mice on a maximal strength test ($p < 0.001$; $n = 7$ per group) and their performance over repeated trials deteriorated more rapidly compared to the control group ($p < 0.01$). Skeletal muscle weight was also lower in the iron group in absolute terms and relative to body weight following the intervention ($p < 0.001$; $n = 7$ per group).




In summary, iron accumulation in skeletal muscle may play a significant role in the reduced exercise capacity seen in iron overload disorders and in ageing, and may play an underlying role in skeletal muscle atrophy.

Davidsen ES, Liseth K, Omvik P, Hervig T, Gerdts E. (2007) *European Journal of Cardiovascular Prevention & Rehabilitation,* **14:** 470-5.

Jung SH, DeRuisseau LR, Kavazis AN, DeRuisseau KC. (2008) *Experimental Physiology,* **93:** 407-14.

Moopanar TR, Allen DG. (2005) *Journal of Physiology*, **564:** 189-99.

Reardon TF, Allen DG. (2007) *Proceedings of the Australian Physiological Society*, **38:** 170P.