The impact of high dose vitamin C supplementation on skeletal muscle of healthy humans

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Background: Vitamin C is an antioxidant and is the most common vitamin supplement taken by adult Australians. Its antioxidant effects in humans are unclear and it's not known how effectively vitamin C can be taken up by important metabolic tissues such as skeletal muscle. Furthermore, there is evidence to suggest that increasing the exogenous levels of antioxidants in metabolic tissues like skeletal muscle could actually reduce the levels of endogenous antioxidant enzymes. Indeed, we have recently shown that long-term antioxidant vitamin E supplementation reduces antioxidant enzyme levels in the skeletal muscle of rats (Strobel *et al.*, 2011). Furthermore, long-term, high-dose vitamin C supplementation significantly reduces antioxidant enzyme levels in the liver of rodents (Selman *et al.*, 2006). Therefore, the aims of this study were to determine how quickly high-dose vitamin C supplementation can increase the levels of vitamin C in human skeletal muscle and whether this reduced skeletal muscle levels of antioxidant enzymes.

Methods: Five healthy males (mean ± SEM, 24.6 ± 1.7 years, 80.2 ± 3.9 kg, BMI 25.3 ± 1.6 kg/m^2) underwent placebo and vitamin C supplementation (0.5g twice daily) in a double-blind, randomized, counter-balanced, cross-over manner. The placebo and vitamin C treatments each lasted 6 weeks with a 4 week washout between treatments. Blood and *vastus lateralis* muscle samples were collected at baseline and following 1, 7 and 42 days of treatment. Vitamin C levels in the plasma and skeletal muscle were measured using reverse-phase high performance liquid chromatography using a C18 column with UV detection. The protein abundance of antioxidant enzymes such as catalase and superoxide dismutase (SOD)-1 and 2 in skeletal muscle was determined via western blotting using commercially available antibodies.

Results: After 1 day of supplementation, plasma levels of vitamin C had increased by 65% (P<0.05), although vitamin C levels in skeletal muscle were not significantly altered (P>0.05). Seven days of supplementation significantly (P<0.05) increased vitamin C levels by 84% in plasma and by 46% in skeletal muscle. There were no further increases in vitamin C levels in muscle and plasma during the remaining 42 days of treatment. The protein abundance of catalase, SOD1 and SOD2 were not significantly altered by vitamin C supplementation throughout the 42 day trial. Placebo treatment did not alter skeletal muscle antioxidant enzyme levels or vitamin C levels in skeletal muscle or plasma throughout the 42 day trial (P>0.05).

Conclusions: Several days of high-dose supplementation are required to significantly increase skeletal muscle vitamin C levels in humans. High-dose vitamin C supplementation for six weeks does not alter the levels of major antioxidant enzymes in the human skeletal muscle of healthy individuals.

Selman C, McLaren JS, Meyer C, Duncan JS, Redman P, Collins AR, Duthie GG & Speakman JR. (2006). Life-long vitamin C supplementation in combination with cold exposure does not affect oxidative damage or lifespan in mice, but decreases expression of antioxidant protection genes. *Mechanisms of Ageing and Development* **127**, 897-904.

Strobel NA, Peake JM, Matsumoto A, Marsh SA, Coombes JS & Wadley GD. (2011). Antioxidant supplementation reduces skeletal muscle mitochondrial biogenesis. *Medicine and Science in Sports and Exercise* **43**, 1017-1024.