# Effects of carnosine on sarcoplasmic reticulum $Ca^{2+}$-handling and contractile properties in human *vastus lateralis* muscle fibres

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Carnosine, an endogenous cytoplasmic dipeptide (ß-alanyl-*l*-histidine), has been shown to be important in a myriad of cellular processes such as pH buffering, membrane stabilization, acting as an osmotic shock protector, anti-oxidant and anti-aging agent (see reviews Derave *et al*., 2010; Sale *et al*., 2010). However, its effects on excitation-contraction (EC) coupling are not well defined. There is considerable interest into the potential ergogenic and therapeutic effects of carnosine supplementation (Kendrick *et al*., 2009; Baguet *et al*., 2010a & b; Derave *et al*., 2010; Sale *et al*., 2010). Consequently, we sought to characterize what effect carnosine, at levels attained by supplementation, has on human muscle function, using a skinned fibre preparation in which all key EC coupling proteins are in their *in situ* positions.

All protocols and procedures were approved by the Human Research Ethics Committee at Victoria University. A muscle needle biopsy was taken from the middle third of the *vastus lateralis* muscle from four healthy subjects who had given written informed consent. After injection of a local anaesthetic into the skin and fascia (1% lidocaine (Xylocaine)), a small incision was made and a muscle sample taken (~150 mg) using a Bergstrom biopsy needle. Individual fibre segments, obtained from the biopsy, were mechanically skinned and their sarcoplasmic reticulum (SR)-$Ca^{2+}$-handling and contractile apparatus properties were characterized. Thereafter, western blotting was performed on the same fibre segments to precisely determine their fibre-type. All solutions (including carnosine-containing solutions) used in this study are identical to those described in Dutka & Lamb (2004). A carnosine stock was made similar to the standard K-HDTA solution but with 80mM carnosine and reduced [HEPES]. When used at the final [carnosine] changes to osmolality and ionic strength were minor.

The effects of carnosine on the properties of the contractile apparatus were determined by exposing each fibre segment to a series of heavily $Ca^{2+}$-buffered solutions containing progressively higher free [$Ca^{2+}$] until maximum $Ca^{2+}$-activated force was produced. Hill curves were fitted and the mean change (Δ) in $pCa_{50}$ (where $pCa50 = -\log_{10}[Ca^{2+}]$) determined. Compared to control levels, the $Ca^{2+}$-sensitivity of the contractile apparatus was significantly increased by the presence of 8 and 16 mM carnosine (Δ $pCa_{50}$ for six type I fibres: 0.073±0.007 and 0.116±0.006 pCa units for 8 and 16 mM respectively, and 0.063±0.018 and 0.103±0.013 pCa units for 8 and 16 mM respectively in five type II fibres). This equates to an increase in absolute force of ~20% (*e.g.* 50% force would be 70% in the presence of carnosine). Caffeine (8 mM)-induced responses were potentiated by 8 mM carnosine in both type I and II fibres, with the level of potentiation in type II fibres being entirely explicable by the increase in $Ca^{2+}$-sensitivity of the contractile apparatus caused by carnosine. However, the potentiation of caffeine-induced responses caused by carnosine in type I fibres was beyond that expected from the associated increase in $Ca^{2+}$-sensitivity of the contractile apparatus and suggestive that carnosine potentiated $Ca^{2+}$-induced $Ca^{2+}$-release.

These findings suggest that increasing muscle carnosine content *via* supplementation or by dietary means could confer benefits on muscle performance in both type I and type II fibres based on the increase in $Ca^{2+}$-sensitivity of the contractile apparatus. However, the potentiation of caffeine-induced SR $Ca^{2+}$ release caused by carnosine in type I fibres may not be manifested *in vivo* when $Ca^{2+}$ release is strictly controlled by dihydropyridine receptors (Lamb *et al*., 2003). Increasing $Ca^{2+}$-sensitivity of the contractile apparatus and potentiating $Ca^{2+}$ release might help to lessen the decline in force output observed during muscle fatigue.

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