# High frequency doublet stimulation enhances the rate of force development in fast-twitch skeletal muscle by increasing early binding of $Ca^{2+}$ to the second binding site of troponin C

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**Introduction:** Fast-twitch skeletal muscle fibres are often exposed to high frequency motorneuron double discharges which are reported to increase both the rate of contraction and the magnitude of the resulting force responses. Previous findings have suggested that an initial 200 Hz doublet action potential at the start of tetanic stimulation enhances force production by significantly increasing the amplitude of initial sarcoplasmic reticulum (SR) $Ca^{2+}$ release compared to controls (no doublet, Cheng *et al.*, 2013). However, limitations in previous tracking of cytosolic $Ca^{2+}$ ($[Ca^{2+}]_c$) due to the use of a high-affinity $Ca^{2+}$ indicator and insufficient temporal resolution (500 Hz) have led to a distorted view of the tetanic $Ca^{2+}$ response in previous reports (Cheng *et al.*, 2013). Consequently, the relationship between doublet stimulation, the resulting changes in $[Ca^{2+}]_c$ and rates of force development are unlikely to have been accurately determined. The aims of this study were to accurately track $[Ca^{2+}]_c$ during doublet stimulation in isolated fast–twitch fibres and to use this data to model the effects of doublet-induced SR $Ca^{2+}$ release on intracellular $Ca^{2+}$ binding and force enhancement in fast twitch fibres

**Methods:** Mice were euthanased and the interosseous muscles removed. Single interosseous fibres (isolated *via* collagenase digestion) were loaded with Mag-Fluo-4 (5μM), and maintained in Kreb's Ringer, containing the myosin inhibitor BTS (100 μM). Fibres were activated by 10 action potentials at 120 Hz with or without (control) an initial 200 Hz doublet action potential. $Ca^{2+}$ fluorescence was captured at 9 kHz using a Zeiss 5 Live confocal microscope in line-scan mode. Modelling of the effects of doublet stimulation on binding of $Ca^{2+}$ to cytosolic buffers and force production was undertaken using a similar approach to that of Baylor & Hollingworth (2003).

**Results:** In this study, 200 Hz doublet stimulation did not significantly alter the amplitudes of the $Ca^{2+}$ responses. However, doublet stimulation did increase the minimum fluorescence value between $Ca^{2+}$ transient spikes (MFBCS) by approximately 200% compared to controls (control initial MFBCS: 61.06 ± 2.82% of 6th $Ca^{2+}$ response; doublet: 111.27 ± 13.19% of 6th response, $P<0.05$). Modelling of the changes in $Ca^{2+}$ binding to the main intracellular $Ca^{2+}$ buffers of troponin, parvalbumin and the SR $Ca^{2+}$ pump during tetanic $Ca^{2+}$ release showed that the main effect of the second response in the doublet is to more rapidly increase the occupation of the second $Ca^{2+}$ binding site on troponin C ($TnC_2$), resulting in earlier activation of force.

**Conclusion:** Doublet stimulation maintains high $Ca^{2+}$ levels for longer in the early phase of the $Ca^{2+}$ response, resulting in earlier saturation of $TnC_2$ with $Ca^{2+}$, faster initiation of cross-bridge cycling, and a more rapid onset of force development.

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