The tubular (t-) system is a dynamic Ca^{2+}-buffer in human skeletal muscle fibres

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The tubular (t-) system of skeletal muscle fibres is an internalization of the plasma membrane that forms a junction with the sarcoplasmic reticulum (SR) in every sarcomere of the fibre. At these sites voltage-controlled Ca^{2+} release occurs to provide the change in cytoplasmic [Ca^{2+}] for the muscle to contract. This excitation-contraction coupling process can be compromised in a number of ways, including when [Ca^{2+}] becomes too high in the cytoplasm (Lamb *et al.*, 1995), a situation likely occurring during fatigue. The t-system is known to be able to vacuolate in response to osmotic stress, stretch and fatigue, and we hypothesized that this increase in volume of extracellular space in the fibre would provide a temporary storage compartment for Ca^{2+}. Such a mechanism could have important implications in exercise so we examined the Ca^{2+}-handling ability of vacuoles in the t-system of human muscle.

All procedures were approved by The Human Ethics Committee of The University of Queensland. Healthy males and females between the ages of 20-45 volunteered to give biopsies. Muscle biopsies were taken from the mid-portion of the *vastus lateralis* using a Bergstrom needle following an injection of local anaesthetic and a ~1.5cm incision through the skin and the fascia. Bundles of muscle fibres running 1-2 mm in length were isolated and exposed to a Physiological Solution containing 2.5 mM rhod-5N. A fibre was isolated from the bundle and mechanically skinned to trap rhod-5N in the t-system. Skinned fibres with t-system trapped rhod-5N were imaged by confocal microscopy while being bathed in internal bathing solutions that depleted the SR of Ca^{2+} or under otherwise resting conditions where the $[Ca^{2+}]_{cyto}$ was set between 28-1342 nM. All solutions contained 50mM EGTA. Experiments were performed at room temperature.

Imaging showed variation in numbers and volume of vacuoles, from none present to virtually totally obscuring the transverse tubules. SR Ca^{2+} release depleted transverse tubules *via* store-operated Ca^{2+} entry (SOCE) as previously reported (Launikonis *et al.*, 2003) but, when present, vacuoles took up Ca^{2+} and slowly released it. However, if vacuoles were exposed to high $[Ca^{2+}]_{cyto}$ or Ca^{2+} was released from a heavily Ca^{2+}-loaded SR, chronically activated SOCE did not deplete Ca^{2+} from vacuoles. Tens of minutes of bathing in a solution containing no Ca^{2+} was required for vacuoles to begin to decrease in size and therefore lower $[Ca^{2+}]_{t-sys}$. Upon application of 5mM Ca^{2+} and ionophore, vacuoles could show a many-fold increase in t-system volume and Ca^{2+}-holding capacity. Subsequent application of 0 Ca^{2+} caused t-system Ca^{2+} to deplete and vacuoles to deform in seconds. A further addition of 5 mM Ca^{2+} saw the transverse tubules reappear by filling with Ca^{2+}. We conclude that the t-system is a dynamic Ca^{2+}-buffer where vacuolation provides a store for significant Ca^{2+} levels when vacuoles are triggered to form. Vacuoles can deform as Ca^{2+} is lost.

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Launikonis BS, Barnes M, Stephenson DG (2003). Identification of the coupling between skeletal muscle store-operated Ca^{2+} entry and the inositol trisphosphate receptor. *Proc Natl Acad Sci USA* **100**, 2941-2944.