Determination of heat production in human skeletal muscle from measurements of basal Ca^{2+} movements

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Skeletal muscle is essential for posture and movement in almost all animals. In mammals it also performs another *vital* role. Skeletal muscle in mammals generates heat that is used to maintain body temperature independently of the ambient temperature. The evolutionary achievement of utilizing skeletal muscle as a heat generator has seen the spread of mammals to all parts of the globe (Rowland *et al.*, 2015). Muscle must be a heat generator in mammals when the muscles are resting. These animals stay warm even when they are lying still (Rolfe & Brown, 1997). To understand this, one must understand the processes inside resting muscle fibres that are responsible for heat generation. These processes must also be regulated, as the demand for heat generation can be transient. The generation of heat in resting muscle is largely attributable to the ATP splitting activity of the sarcoplasmic reticulum (SR) Ca^{2+} pump (Bal *et al.*, 2012) but the mechanisms are not understood. This enzyme pumps Ca^{2+} back into the SR following Ca^{2+} release that is essential for contraction and also pumps Ca^{2+} back into SR at rest, as the SR constantly leaks Ca^{2+} through the ryanodine receptor (RyR) (Cully *et al.*, 2018). The muscle spends most of its time at rest, so harvesting heat from the muscle in this state provides an effectively constant generation of heat.

Recently the Ca^{2+} leak of the SR through the RyR has been shown in resting human muscle fibres using a novel confocal imaging technique utilizing a Ca^{2+}-sensitive dye trapped in the sealed tubular (t-) system of skinned fibre prepared from Bergstrom needle biopsies (Cully *et al.*, 2018). These measurements exploited the fact that the “junctional space” sandwiched between the SR terminal cisternae and t-system have a $[Ca^{2+}]$ that is dependent on local RyR Ca^{2+} leak.

Assessing RyR Ca^{2+} leak *via* measurements of Ca^{2+} uptake into the sealed t-system is the net outcome of a complex set of events. To understand these events to allow the prediction of heat generated by the muscle a model was devised that divided the system into 7 discrete spaces across the t-system, SR, cytoplasm and junctional space. Ca^{2+} movements between these spaces were linked by the RyR, PMCA, NCX and SERCA. Within the junctional space and cytoplasm Ca^{2+} was buffered by EGTA and inside the SR Ca^{2+} was buffered by calsequestrin. A system of 7 inter-related differential equations using published rate constants and mechanistic descriptions of PMCA, SERCA and NCX were solved using Maple software. The initial model was refined using parameter sweeps to determine influential parameters and optimise the model. The factors determined to influence t-system Ca^{2+} uptake were PMCA density, PMCA Ca^{2+} affinity, pH and t-system leak rate.

The model was then used to quantify the SR leak rate in human muscle. A leak rate between 0.01 and 0.02 s^{-1} was consistent with experimental measurements (Cully *et al.*, 2018). The model was then used to quantify Ca^{2+} efflux from SR to estimate heat production associated with Ca^{2+} cycling between SR and cytoplasm of ~0.4 W kg^{-1}, which is about half of the likely heat production of quiescent (or resting) human skeletal muscle.

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