Curve fitting model to quantify the rate and extent of force loss during different phases of fatigue in isolated skeletal muscle

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Fatigue induced with repeated tetani is often quantified using a fatigue index (Burke *et al.*, 1973), *i.e.*, the peak force at a defined stimulation time relative to that of the first tetanus. However, this approach fails to reveal detail about the time-course of fatigue. It is also established that several phases of fatigue exist that reflect different underlying mechanisms (Lännegren & Westerblad, 1991); hence there is a need to carefully quantify the rate and extent of each phase of fatigue. Therefore, the purpose of this study was to establish a curve-fitting methodology to provide the best quantitative description of fatigue profiles in isolated mammalian skeletal muscle.

Slow-twitch soleus or fast-twitch *extensor digitorum longus* (EDL) muscles were dissected from mice and bathed in control Krebs solution (4 mM K^+, 147 mM Na^+, 1.3 mM Ca^{2+}, 128 mM Cl^-) at 25°C. With altered K^+ solutions NaCl was exchanged with KCl. Isometric contractions were evoked by supramaximal electric field stimulation (parallel plate electrodes, 20V, 0.1ms pulses). Fatigue protocols involved repeated tetanic stimulation (30-125 Hz) for 500 ms, once every 1-3 s, for 100-300 s. The NLIN procedure available in the SAS statistical software package was used to fit the peak force data from either individual fatigue runs or pooled data from all fatigue experiments.

When fatigue was induced in soleus at 50 Hz for 500 ms, once every second, for 300 s, four distinct phases were apparent. The resulting fatigue profile was extremely well-fitted by a double sigmoid (negative) model with a mean square error of < 1%. The fitting efficacy for a double sigmoid function was better than for single sigmoid, or single and double exponential functions as revealed by the Akaiki Information Criterion. The second sigmoid (*i.e.*, late fatigue) depended on stimulation frequency between 30-125 Hz, and was abolished at the lower frequency of 30 Hz, over 100s. Fatigue induced at 125Hz for 500ms, once every second for 100 s, was well quantified by a double sigmoid model in both soleus and EDL. The first sigmoid (*i.e.*, early fatigue) involved ~10% reduction of peak force to an intermediate plateau in both muscle-types. With the second sigmoid, the maximum rate (slope) was 7-fold greater in EDL than soleus, and the extent of force loss was 83% initial in EDL and 63% initial in soleus. The double sigmoid model was also adequate to describe fatigue induced via nerve terminals, with longer inter-tetanus intervals, or with prolonged continuous tetanic stimulation. We have previously shown that the extent of fatigue is influenced by prior exposure to a physiological range of $[K^+]_o$, in soleus (Cairns, 2005), but there is now a contradictory finding (Zhang *et al.*, 2006). We subsequently utilised the double sigmoid methodology to further analyse our original fatigue data. Fitting parameters for the second sigmoid revealed that the maximum slope, time-constant (τ), and final plateau deteriorated with increasing $[K^+]_o$. For example, τ was 58.0 ± 4.3 s at 2 mM K^+, 52.8 ± 3.4 s at 4 mM K^+, and 43.6 ± 2.4 s at 7 mM K^+. This strengthens our claim that late fatigue is graded with the conditioning $[K^+]_o$.

In conclusion, a double sigmoid expression is a simple method to provide an accurate description of both the rate and extent of different phases of force loss in these models of fatigue.

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