Measurement and biochemical correlates of power fatigue resistance in transformed skeletal muscles

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Most people can define fatigue, but what is fatigue resistance? The question needs answering, for clinical as well as scientific reasons. Functional grafts of skeletal muscle are already being used to construct new sphincters and to provide assistance to a failing heart. These new surgical approaches depend on 'conditioning' the skeletal muscle with long-term electrical stimulation so that it does not fatigue when called upon to perform its task continuously. In these circumstances, it is important to define 'fatigue resistance' in relation to the work the muscle is required to sustain.

A muscle will be resistant to fatigue if it can supply, on a continuous basis, sufficient ATP to meet the prevailing energy costs of contraction. These costs derive mainly from the cyclic turnover of chemical bonds between actin and myosin and the transport of calcium between intracellular compartments. During the conditioning process, skeletal muscle is believed to acquire more favourable bioenergetics for sustained contraction as a result of changes both in the isoforms of myosin and in the kinetics of the release and uptake of calcium. At the same time, sustained production of ATP becomes possible through an increase in the capacity of oxidative pathways, particularly those involved in the breakdown of fatty acids. There is an associated increase in blood supply and mitochondrial volume. As a result, ATP production can match even extreme increases in ATP utilization (Clark et al. 1988; Mayne et al. 1991a).

Although the fatigue-resistant nature of conditioned muscle has been known for some years (Salmons & Sréter 1976; Hudlicka et al. 1977), the initial observations were made with fatigue tests based on isometric contractions. More recently, we have used a new ergometer apparatus (Jarvis & Salmons 1990) and a new experimental protocol to investigate the endurance of muscles under conditions in which they perform external work.

Physiological and biochemical measurement of power fatigue

Force-velocity curves were obtained from rabbit tibialis anterior muscles over the full physiological range of shortening velocity by the method of iso-velocity release. Measurements were digitized and processed, and the data plotted as force-velocity and power-velocity curves. For each muscle, single contractions were elicited at the velocity for maximum power (V_{opt}); activation was long enough to produce the full range of movement (19.5 mm). The work done by the muscles was then calculated as the area under the force-time curve (active–passive) multiplied by the velocity.

Since the work performed by the conditioned and control muscles in a single contraction was very different, the fatigue resistance of the two muscles was tested as follows. The muscle masses were estimated from our previous data. Repeated contractions were then set up at a frequency calculated to produce an initial power output, from each muscle, of 10 W/kg wet weight. The power output was monitored over a period of about 4 hours. At the end of the test the muscles were removed, weighed and snap-frozen for subsequent morphometric and biochemical analysis. The actual power output per kg used in the subsequent analyses was calculated using the measured muscle mass, rather than that estimated during the experiment.

After 2 weeks of continuous stimulation at 10 Hz, the conditioned muscle was able to maintain the initial power output over 5 hours, whereas the contralateral control muscle fatigued progressively. After 8 weeks, the instantaneous power output of the stimulated muscle was lower because of reductions in force and contractile speed. The initial working rate of 10 W/kg therefore represented 20% of the maximum instantaneous power output for the stimulated muscle, as against 2.5% for the control muscle. Nevertheless, the stimulated muscle could maintain this level of working better than the control muscle. After 12 weeks, 10 W/kg was close to the maximum

for the stimulated muscles: the duty cycle was about 50%, and the muscles were so slow that any further decrease in speed would not have allowed them to relax fully between contractions. These muscles had very high fatigue resistance—probably higher than that of naturally-occurring slow muscle. The homogeneity of muscles stimulated for these long periods is such that the whole-muscle properties probably reflect quite closely those of the constituent fibres.

We reasoned that it should be possible to measure fatigue resistance as the sustained working rate, in W/kg, at which ATP production is just able to keep pace with ATP consumption. To test this, we removed small samples from control muscles during a variety of stimulation régimes and analysed their metabolite composition by HPLC.

During the first 15 min of stimulation we observed the fall in ATP and PCr, and the corresponding rise in creatine and IMP, that are synonymous with fatigue. However, this was followed by spontaneous recovery of these metabolites to control levels, despite a continuing profound force fatigue (Mayne et al. 1991b). This phenomenon, which has been observed by others, may be due to a block in excitation-contraction coupling. Since force fatigue can occur in this way without exhaustion of intracellular ATP, metabolite levels do not provide an unambiguous indicator of fatigue.

Myosin isoforms and fatigue resistance

Recently, we have been comparing the effects of stimulation at 10 Hz and 2.5 Hz. Both the rate and extent of transformation were less when the muscles were stimulated at 2.5 Hz. Mechanical, biochemical and histochemical data were consistent with transformation of the 2B fibre type population of these muscles to the 2A type. Muscles stimulated at 2.5 Hz were significantly faster and more powerful than those stimulated at 10 Hz, yet they proved just as resistant to fatigue when tested under the conditions already described. Since there was no evidence that stimulation at 2.5 Hz for periods up to 12 weeks had induced synthesis of Type 1 myosin isoforms, we conclude that changes in the bioenergetics of contraction associated with myosin transitions are not a major factor in the development of fatigue resistance.

Conclusions

1. Fatigue resistance should be measured in terms of the sustainable rate of performing external work.
2. Fatigue can occur in the presence of normal levels of energy metabolites.
3. Changes in myosin isoforms appear to be much less important in relation to fatigue resistance than changes in blood flow, metabolism and, possibly, the energy costs of calcium transport.

References

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