APPS/PSNZ Meeting - Sydney 2003

Symposium 1: Stretch-induced muscle damage in sport and disease

Monday 29 September 2003

Chair: David Allen

Popping sarcomere hypothesis explains stretch induced muscle damage

D.L. Morgan and U. Proske, Monash University, Clayton, Vic 3800, Australia.

It has been known for over 100 years that active stretch of muscle, also known as eccentric or pliometric contraction, can lead to sore and stiff muscles, beginning the day after exercise and lasting up to a week. Mechanically eccentric contractions use muscles as brakes rather than motors, and occur in activities such as horse-riding, skiing and walking down hill. Histologically, such muscles show small areas of disrupted filament structure, confined to single fibres, and ranging in length from a single half sarcomere. Tension is also reduced more, and for longer, than after similar shortening contractions. Such exercise induces a rapid training effect, so that a second identical bout of exercise typically causes much reduced symptoms.

In 1990, it was suggested that the damage results from extremely non-uniform lengthening of sarcomeres, due to the instability of sarcomere lengths that results from the descending limb of the length-tension curve and the asymptote of the force-velocity curve (Morgan, 1990). Stretch of muscle beyond optimum length is concentrated in the sarcomere that has the lowest yield tension. This greater lengthening, on the descending limb of the length-tension curve, causes the isometric tension, and hence the yield point, to decrease. The assymptotic shape of the force velocity curve means that the sarcomere will be unable to support the existing tension at any velocity, and so will "pop", i.e. stretch rapidly and uncontrollably, limited only by passive viscosity and mass, until a length is reached where rising passive tension in that sarcomere increases to match the total tension being generated by the other un-lengthened sarcomeres. This will repeat with the next weakest sarcomere. The stretch then proceeds by popping sarcomeres in myofibrils, essentially one at a time in order from the weakest towards stronger. This explains why tension always rises during stretch, even beyond optimum length.

This hypothesis further postulated that the training effect consisted of growing extra sarcomeres in series to avoid stretch beyond optimum length. This was consistent with earlier observations that the number of sarcomeres in a fibre could change).

Since then, a number of results have supported this hypothesis. It has been shown in toad and rat muscle, that such stretch induced muscle damage is greater when the stretches are applied at longer length. It has been shown in rats and humans that training is accompanied by a shift in optimum length towards longer muscle lengths. In rats it has been confirmed that this is accompanied by an increase in the mean number of sarcomeres in the fibres of the muscle, and that the adaptation is ineffective if the stretches are moved to the same part of the length-tension curve rather than the same length.

Morgan, D.L. (1990) *Biophysical Journal*, **57**: 209-221.
Morgan, D.L. & Allen, D.G. (1999) *Journal of Applied Physiology*, **87**: 2007-2115.

Training with eccentric exercise to prevent hamstring strains

U. Proske[1], P. Percival[2] and D.L. Morgan[2], [1]Department of Physiology, and [2]Department of Electrical and Computer Systems Engineering, Monash University, Clayton, VIC 3800, Australia.

Eccentric exercise, where the contracting muscle is lengthened, is distinct from other forms of exercise because in someone unaccustomed to it, their muscles become stiff and sore next day. It is believed that the soreness is the result of microscopic damage to muscle fibres, leading to an inflammatory response and sensitisation of nociceptors. The soreness persists for about a week. A second period of eccentric exercise, a week after the first, is followed by much less soreness, the result of an adaptation process accompanying repair of the damage.

An indicator of muscle damage, present immediately after a period of eccentric exercise, is a shift of the muscle's length-tension relation in the direction of longer lengths (Jones *et al.*, 1997). It is believed that this is due to the presence of disrupted, non-functioning sarcomeres in series with still functional sarcomeres, and this produces an increase in whole-muscle series compliance. The shift reverses within 1–2 days. A second, sustained shift in the length-tension relation is apparent a week later. It persists for several weeks. This is the adaptation response of the muscle which is thought to involve the incorporation of additional sarcomeres into the repaired muscle fibres. As a result of this secondary shift, less of the muscle's working range lies on the descending limb of the length-tension relation, the region where disruption and damage is most likely to occur (Morgan, 1990).

Hamstring strains are the most important soft-tissue injury in the Australian Football League (AFL). There is evidence that hamstring strains occur while players are carrying out eccentric contractions during rapid knee extensions in sprinting and kicking a ball. We have recently proposed that the microscopic damage from eccentric contractions can, during repeated contractions, act as a point of weakness for development of a more major tear injury, involving many muscle fibres (Brockett *et al.*, 2001). The group at greatest risk of a hamstring strain are previously injured players. We have shown that optimum angles for torque in previously injured hamstrings were at shorter muscle lengths than for uninjured muscles, making them more susceptible to damage from eccentric exercise and therefore more prone to injury. This is because with a short optimum length more of the muscle's working range is on the descending limb of the length-tension curve, the potential region for damage. The reasons for the shorter optimum remain uncertain, but may include factors such as a player's natural predisposition, the development of scar tissue during healing and insufficient eccentric exercise during rehabilitation.

It is possible to provide protection against the damage from eccentric exercise by means of a controlled program of eccentric training. Such a program would be designed to keep all damage at the microscopic level, yet produce an adequate shift of the optimum angle, so that less of the muscle's working range included the descending limb of the length-tension relation. We are therefore proposing a strategy of regular testing of optimum angles together with a program of mild, targetted eccentric exercise as a means of reducing the incidence of hamstring strains, indeed, of strain injuries in all susceptible muscles.

Brockett, C.L., Morgan, D.L. & Proske, U. (2001) *Medicine and Science in Sports and Exercise,* 33:783-790.

Jones, C., Allen, T., Talbot, J., Morgan, D.L. & Proske, U. (1997) *European Journal of Applied Physiology and Occupational Physiology,* 76:21-31.

Morgan, D.L. (1990) *Biophysical Journal*, 57:209-221.

Stretch-activated channels in stretch-induced muscle damage - role in muscular dystrophy

E.W. Yeung[1] and D.G. Allen[2], [1]Department of Rehabilitation Sciences, Hong Kong Polytechnic University, Hong Kong, and [2]Institute for Biomedical Research and Department of Physiology, University of Sydney, NSW 2006, Australia.

Unaccustomed eccentric contractions result in damage to skeletal muscles which can last for up to one week. In normal individuals, this muscle damage represents a transient weakness and discomfort after unaccustomed exercise. However in muscular dystrophy repetitive damage cannot be adequately repaired and contributes to progressive weakness and muscle degeneration.

We have studied the causes of stretch-induced muscle damage in single mouse muscle fibres which were stretched by 40% of optimal length (L_o) during 10 maximal tetani (Balnave & Allen, 1995). As a consequence of eccentric contractions, the recognised features of damage included: (i) reduced maximal force; (ii) greater reduction of force at low stimulation frequencies; and (iii) a shift in L_o to a longer muscle length, which is characteristic of sarcomere disorganisation. Isometric tetani or stretches of resting fibres produced none of these features.

The cause of the reduced force and muscle damage are not established but one theory is that tears in the muscle membrane allow influx of ions such as Na^+ and Ca^{2+} and the efflux of proteins such as creatine kinase. To investigate this mechanism we measured intracellular sodium concentration ($[Na^+]_i$) after both isometric or eccentric tetani. $[Na^+]_i$ was unaffected by isometric tetani but increased after eccentric contractions from the resting level of 7.2 ± 0.5 mM to 16.3 ± 1.6 mM over 1-2 min and the increase persisted for more than 30 min. There was no evidence of localised elevations of $[Na^+]_i$ which might result from membrane tears but, instead, the rise could be prevented by gadolinium (Gd^{3+}), a blocker of stretch-activated channels (Yeung *et al.*, 2003). These results suggest that a stretch-activated Na^+ permeable channel is opened following eccentric contractions and causes the increased $[Na^+]_i$. Since Gd^{3+} reduced Na^+ influx we tested whether it could prevent muscle damage as measured by the force production 10 min after eccentric contractions. When Gd^{3+} was applied over the period in which $[Na^+]_i$ rises (i.e. for the first 10 min after the eccentric contractions), it increased the force from 36 ± 5 to 49 ± 4%.

Given that Gd^{3+} prevented Na^+ entry and minimised force reduction following eccentric contractions in wild-type fibres, we examined the same phenomena in *mdx* muscles. We establish that *mdx* fibres have a higher than normal resting $[Na^+]_i$ and show that single fibres from *mdx* muscle are more susceptible to eccentric damage. The rise in $[Na^+]$ following eccentric contraction was greater in *mdx* compared to wild-type mice. This rise in $[Na^+]_i$ could be reduced by Gd^{3+} and, as with wild-type fibres, the force after eccentric contractions was increased when Gd^{3+} was applied over the period in which $[Na^+]_i$ rose.

Stretch-activated channels are also permeable to Ca^{2+}, so they could provide a leak pathway for Ca^{2+} to enter the cell causing cellular damage. Investigations in Ca^{2+} handling as a result of activity of the stretch-sensitive channels after eccentric contractions should enhance our understanding of muscle damage in muscular dystrophy

Balnave, C.D. & Allen, D.G. (1995) *Journal of Physiology*, 488, 25-36.
Yeung, E.W., Ballard, H.J., Bourreau, J-P. & Allen, D.G. (2003) *Journal of Applied Physiology*, 94, 2475-2482.

Mechanisms of muscle damage in muscular dystrophy

G.S. Lynch, Department of Physiology, The University of Melbourne, Victoria 3010, Australia.

Muscular dystrophies are a group of neuromuscular disorders characterised by progressive and extensive muscle wasting and weakness. Patients with Duchenne muscular dystrophy (DMD) have mutations in the gene for the subsarcolemmal protein dystrophin. The muscles of the *mdx* mouse, an animal model for DMD, also lack dystrophin. Although *mdx* mice exhibit a relatively benign phenotype, the lack of dystrophin renders their limb muscles more susceptible to contraction-induced injury (Brooks 1998; DelloRusso *et al.*, 2002). Due to its role in linking the cytoskeleton to the extracellular matrix, dystrophin is postulated to have a mechanical function, namely the stabilisation of the muscle fibre membrane integrity in both quiescent and contracting muscles (Lynch *et al.*, 2000). Support for this hypothesis has been demonstrated by the sarcolemmal fragility of fibres from *mdx* mice which have a greater susceptibility to rupture following osmotic shock and active muscle lengthening, although the findings remain controversial (Brooks, 1998). In many cases, the severity of the contraction protocols used, make it difficult to discern genuine differences between the injury susceptibility of normal and dystrophin-deficient skeletal muscle.

More recently, contraction protocols have been devised that might more accurately test the hypothesis that dystrophin deficiency increases the likelihood of contraction-mediated damage. These protocols are important for testing whether muscles from transgenic *mdx* mice, expressing different truncated dystrophins are protected against damage caused by muscle activity. In fact, injection of adeno-associated viruses carrying micro-dystrophins into dystrophic muscles of immunocompetent *mdx* mice results in a significant reversal of the histopathological features of the disease, and protection from contraction injury, highlighting the clinical potential of these therapeutic approaches (Harper *et al.*, 2002).

It is generally accepted that damage to membranes in dystrophic muscle represents a component of the initial mechanism of injury that does not occur in normal muscles. Membrane disruption could allow influx of calcium that triggers the cellular pathways of destruction, leading to necrosis. However, the lack of dystrophin may not be the sole reason for the greater susceptibility of dystrophic muscles to contraction-mediated damage. Other studies have suggested that the appearance of significant numbers of abnormally branched fibres in dystrophic muscles might also contribute to the aetiology of damage. Branched fibres and their specific branching points may render them inherently weaker than non-branched fibres and this may help explain why regeneration ultimately fails (Schmalbruch, 1984).

Traditionally, it was thought that larger calibre fibres were more susceptible to contraction-mediated damage than small calibre muscle fibres, and that increasing the size of dystrophic muscle fibres following treatment with anabolic agents may actually increase injury susceptibility. Instead, recent work by Bogdanovich and colleagues (2002) suggests that making muscle fibres larger may ameliorate the symptoms of the disease, as advocated previously (Lynch, 2001). Although these results are encouraging from a clinical perspective, it is still possible that these hypertrophied dystrophic muscles remain vulnerable to extreme stress (Zammit & Partridge, 2002).

Bogdanovich, S., Krag, T.O.B., Barton, E.R., Morris, L.D., Whittemore, L-A., Ahima, R.S. & Khurana, T.S. (2002) Nature 420, 418-421.

Brooks, S.V. (1998) Journal of Muscle Research and Cell Motility 19, 179-187.

DelloRusso, C., Crawford, RW, Chamberlain, J.S. & Brooks, S.V. (2002) Journal of Muscle Research and Cell Motility 22, 467-475.

Harper, S.Q., Hauser, M.A., DelloRusso, C., Duan, D., Crawford, R.W., Phelps, S.F., Harper, H.A., Robinson, A.S., Engelhardt, J.F., Brooks, S.V. & Chamberlain, J.S. (2002) Nature Medicine 8, 253-261.

Lynch, G.S., Rafael, J.A., Chamberlain, J.S. & Faulkner, J.A. (2000) American Journal of Physiology (Cell Physiology) 279, C1290-C1294.

Lynch, G.S. (2001) Expert Opinion on Therapeutic Patents 11, 587-601.

Schmalbruch, H. (1984) Neurology 34, 60-65.

Zammit, P.S. & Partridge, T.A. (2002) Nature Medicine 8, 1355-1356.

Supported by research grants from the Muscular Dystrophy Association (USA)

The role of Dystrophin in muscle maintenance within the zebrafish embryo and the identification of zebrafish models of human muscular dystrophy

P.D. Currie, Victor Chang Cardiac Research Institute, 384 Victoria Street, Darlinghurst NSW 2010, Australia. (Introduced by David Allen)

Large-scale mutatgenic screens of the zebrafi sh genome have identifi ed numerous mutations that disrupt differentiation and maintenance of skeletal muscle within the zebrafi sh embryo. Mutants possess phenotypes that range from a failure of myoblasts to elongate and fuse into a mulinucleate muscle fi bres to those that exhibit muscle degeneration reminiscent of human muscular dystrophies. Homozygous mutants of this latter class form myofi brils normally but are lost focally or globally, depending on the loci involved, during early larval life. Here we present data specifi cally on one member of the zebrafi sh dystrophic mutant class and reveal that its phenotype results from mutations within the zebrafi sh Dystrophin orthologue. We will present a detailed characterisation of the phenotype that arises as a consequence of the loss of Dystrophin expression within the embryonic and larval myotomes of zebrafi sh. This analysis points to the critical and novels roles that the Dystrophin and its associated-glycoprotein complex plays in the ontogeny of zebrafi sh muscle. We will compare and contrast the function of Dystrophin in teleost and mammalian muscle fi bre biogenesis. This analysis represents the fi rst identifi cation of a zebrafi sh model of a human muscular dystrophy and we will discuss the possible application of zebrafi sh genetic methodologies to the study of the human dystrophic condition.