# Anabolic agents for improving muscle regeneration and function after injury

**Gordon S. Lynch, Jonathan D. Schertzer and James G. Ryall**

*Basic and Clinical Myology Laboratory, Department of Physiology,*
*The University of Melbourne, Victoria 3010, Australia*

## Summary

**1.** Muscle injury can result in a significant loss of function that can impact on quality of life. In this review we describe how muscles can be injured by external factors such as: contusion, laceration, or crush; by internal factors such as muscle strains during sudden and severe falls; or during the performance of some actions during sports. In addition, we describe the injury to a muscle that occurs when its blood supply is interrupted – an occurrence common in clinical settings. An overview of muscle regeneration is presented as well as a discussion of some of the potential complications that can compromise successful muscle repair and lead to impaired function and permanent disability.

**2.** Improving muscle regeneration is important for hastening muscle repair and restoring muscle function and this review describes ways in which this can be achieved. We describe recent advances in tissue engineering that offer considerable promise for treating muscle damage, but highlight the fact that these techniques require rigorous evaluation before they can become mainstream clinical treatments.

**3.** Growth promoting agents are purported to increase the size of existing and newly regenerating muscle fibres and therefore could be employed to improve muscle function if administered at appropriate times during the repair process. This review provides an update on the efficacy of some growth promoting agents, including anabolic steroids, insulin-like growth factor-I (IGF-I) and $\beta_2$-adrenoceptor agonists ($\beta_2$-agonists), to improve muscle function after injury. Although these approaches have clinical merit, a better understanding of the androgenic, IGF-I, and β-adrenergic signalling pathways in skeletal muscle is important if we are to devise safe and effective therapies to enhance muscle regeneration and function after injury.

## Introduction

Skeletal muscles can be injured by external factors such as: contusion, laceration, or crush[1-3] from road trauma, workplace accidents, or collisions on the sports field; or by internal factors such as strains, e.g. a hamstrings muscle tear when running or kicking;[4-6] or during surgery involving muscle laceration or during reconstructive or transplantation surgery, when muscles are excised by surgeons and transferred from one part of the body to another to provide supporting structures and help restore some level of function.[7,8] These transplantation procedures involve an unavoidable disruption (or interruption) to the muscle's normal blood supply (called 'ischaemia'). Subsequent return of the blood supply (reperfusion) is problematic in that a severe secondary injury can ensue mediated by production of damaging free radicals when blood flow is restored.[9-12] The same process occurs after revascularization of an amputated limb, compartment syndromes associated with vascular injury and following excessive tourniquet application.[13] Muscle injuries such as crush, ischaemia-reperfusion, and contraction-mediated damage involve injury to the muscle's support structures (including blood and nerve supply), such that functional repair is compromised.[14,15] All of these events can severely impair muscle structure and function, mobility and quality of life. Skeletal muscle injury is a significant health issue that costs billions in health care every year in most developed nations.

The cellular and molecular mechanisms of muscle regeneration after injury and degeneration have been described extensively.[16-20] Unfortunately, all evidence indicates that once muscles are damaged, the muscle repair/regeneration process is not always complete and can often be slow or complicated by fibrotic infiltration and scarring. Incomplete and slow repair can result in disability or handicap. Thus, developing therapeutic approaches to enhance the regeneration process and hasten restoration of muscle function is critical for improving the long-term physical outcome of patients and athletes suffering muscle injuries and for preventing or minimising functional disability after surgery.[5,21]

Muscle injury and repair involves a complex balance between local muscle fibre repair, regeneration, and scar-tissue formation.[22] A variety of methods have been examined for the purpose of hastening muscle regenerative processes in order to restore muscle function, by either enhancing muscle fibre growth and regeneration and/or promoting vascularity and nerve repair. Anti-inflammatory medications, corticosteroids, surgical methods, and exercise protocols have been studied.[21,22] Current research efforts are exploring closer interactions between developmental biology and tissue engineering in order to enhance existing tissue or develop new tissues to replace those that are damaged irreparably.[23,24] Regenerative medicine and tissue engineering provide novel therapeutic approaches to restore muscle structure and function to damaged skeletal muscles after injury or disease.[25-28] These approaches include the use of stem cells (including skeletal muscle-derived stem cells), bioinductive factors, and bioscaffolds to facilitate release of cells or biological growth factors to repair and/or

regenerate skeletal muscle.[28-31] While offering considerable promise for the treatment of muscle damage, realistically it will take many years before these emerging techniques are perfected and become mainstream clinical treatments.

To evaluate the current status of all the different approaches for treating muscle injury is beyond the scope of this brief review. Instead, we have focussed attention on therapies that have purported anabolic or growth promoting effects on skeletal muscle. The basic rationale is that growth promoting agents can hasten muscle regeneration by increasing the size of existing and newly regenerating muscle fibres and thereby improving muscle function. Muscle growth promoting agents include (but are not limited to) growth hormone, testosterone-derived or testosterone-like hormones such as anabolic steroids, insulin-like growth factor-I (IGF-I), and $\beta_2$-adrenoceptor agonists ($\beta_2$-agonists). We will provide a brief overview of the current state of knowledge regarding the efficacy of some of these growth promoting agents (anabolic steroids, IGF-I and β-agonists) to improve muscle function after injury.

**Anabolic steroids**

Androgenic-anabolic steroids (AAS) are synthetic derivatives of the male hormone testosterone capable of exerting strong effects on the human body that can benefit athletic performance.[32] Testosterone replacement therapy has been effectively used to counteract loss of lean body mass in hypogonadal men,[33,34] in older men with normal or low serum testosterone,[35,36] and HIV-infected men with low serum testosterone.[37] Similarly, muscle growth has been achieved in eugonadal states after supraphysiological administration to young, healthy men,[38,39] and HIV-infected men with normal testosterone levels.[40] Although some studies have demonstrated enhanced muscle strength following testosterone administration,[41] others have reported no effect of androgen therapy on muscle function despite increases in muscle size.[42] Although anabolic steroids have been used for the treatment of HIV- related wasting and other wasting conditions for many years, many questions remain unanswered, including those regarding appropriate and safe doses for long-term administration and the associated potential risks or side effects.[43,44]

There have been numerous studies that have investigated the effects of anabolic steroids on skeletal muscles that are simultaneously responding to other stimuli such as functional overload,[45] hindlimb suspension in rats[46] or heavy resistance training in humans.[47] However, few studies have examined the effect of anabolic steroids on skeletal muscle regeneration *per se*. One of the most important investigative techniques used in studying this process is to follow the muscle fibre degeneration and subsequent spontaneous fibre regeneration after an intramuscular injection of a myotoxin, such as snake venoms (e.g. notexin or cardiotoxin) or local anaesthetics such as bupivacaine hydrochloride.[48] Ferry and colleagues[49] examined whether treating rats with nandrolone deconoate improved regeneration of fast-twitch *extensor digitorum longus* (EDL) and slow-twitch *soleus* muscles after myotoxic injury caused by direct intramuscular injection of notexin. Nandrolone increased the mass of regenerating *soleus* muscles and decreased the relative amount of fast myosin heavy chain protein, but anabolic steroid treatment had no effect on regenerating EDL muscles.[49] In a follow-up study, the authors found that anabolic steroid treatment had no significant effect on the functional properties of regenerating EDL or *soleus* muscles at 21 days post notexin injury.[50] Beiner and colleagues[51] examined whether nandrolone deconoate could enhance the function of regenerating rat skeletal muscles following contusion injury. They found that at 7 days post-injury anabolic steroid treatment had no beneficial effect on the force producing capacity of *gastrocnemius* muscles *in situ* but by 14 days post-injury muscles from treated rats had improved twitch (but not tetanic) forces. Although interesting, this does not represent a definitive improvement in muscle strength since *in vivo*, all muscle actions result from graded tetanic (not twitch) contractions. However, the authors concluded that anabolic steroids could help the functional recovery of injured muscles and therefore "may have an ethical clinical application to aid healing in severe muscle contusion injury, and their use in the treatment of muscle injuries warrants further research".[51]

In a recent preliminary study, *tibialis anterior* (TA) muscles from castrated male mice were injured by intramuscular injection of the myotoxic agent, bupivacaine, and then treated with nandrolone decanoate to determine whether muscle regeneration could be enhanced.[52] Anabolic steroid treatment increased the incidence of small diameter fibres (as a proportion of the total number of fibres) at 14 days post-bupivacaine injury by 65% compared with injured muscles from untreated mice. At 28 days post-injury, there was no effect of treatment on the number of these smaller diameter fibres, but the incidence of large fibres (as a proportion of the total number of fibres) was two-fold greater in muscles from treated compared with untreated mice. It should be noted that the variable size of the regenerating muscle fibres could also indicate that bupivacaine injured some fibres but spared others. We have previously shown that the extent of muscle fibre injury in mice following an intramuscular injection of bupivacaine is significantly less than that after an intramuscular injection of a more powerful myotoxin such as notexin.[48] Regardless, the study showed that anabolic steroid treatment could improve myofibre growth during the later stages of muscle regeneration.[52]

Another preliminary study examined the effect of two doses of nandrolone deconoate on regeneration and satellite cells in mouse skeletal muscles following an intramuscular injection of venom from the jararacucu snake (*Bothrops jararacussu*) of South America.[53] At 6 mg/kg, the anabolic steroid increased the number of myotubes after 3 and 7 days post venom injection and the number of muscle fibres with normal morphology after 21 days. Muscle satellite cell proliferation at 7 and 21 days was also increased in mice that received this dose. However, regeneration was

not improved in the injured muscles of mice treated with nandrolone deconoate at a lower dose of 2 mg/kg. Thus, the higher dose (6 mg/kg) of the anabolic steroid was required in mice in order to produce a beneficial effect on muscle regeneration after severe myotoxic damage.[53]

Another important issue is whether anabolic steroids may have clinical application in treating the symptoms of skeletal muscle diseases especially where muscle repair mechanisms are defective and recurring episodes of fibre injury and inefficient and incomplete regeneration are a critical aspect of the pathophysiology, such as in Duchenne muscular dystrophy (DMD). In a study on dystrophic *mdx* mice, an animal model of DMD that also exhibits ongoing injury and regeneration in the limb muscles throughout the lifespan, treatment with anabolic steroids did not have a beneficial effect.[54] In fact, anabolic steroid treatment aggravated the dystrophic pathology in the EDL and *soleus* muscles, as evidenced from elevated creatine kinase activity and a doubling of the number of centrally nucleated muscle fibres (an index of accumulated injury and repair). Interestingly, the size of some fibre populations actually decreased in *mdx* mice after anabolic steroid treatment.[54]

**Insulin-like growth factor-I**

Regardless of the initial cause of muscle injury, effective fibre regeneration is dependent on the timed induction of myogenic regulatory factors and growth factors, including IGF-I.[3,20,55] IGF-I activates both myoblast proliferation and subsequently differentiation, crucial processes for successful muscle repair and regeneration.[56] The importance of IGF-I in muscle regeneration has been demonstrated in transgenic mice, where muscle-specific overexpression of IGF-I maintained regenerative capacity in aged mice[57] and reduced the skeletal muscle pathology in dystrophic *mdx* mice.[58,59] Exogenous administration of recombinant human IGF-I (rhIGF-I) increased the rate of functional recovery after myotoxic injury[60] and improved the dystrophic pathology in *mdx* mice.[61-63] Clearly, administration of IGF-I and other growth factors has the potential to accelerate healing processes and other tissues after trauma, but their use in sports medicine is restricted because of the potential for abuse as performance-enhancing agents.[64]

Although rhIGF-I administration and transgenic IGF-I overexpression have beneficial effects on skeletal muscle, their mechanism of action differs considerably. Transgenic IGF-I overexpression in mice produced muscle hypertrophy[58] whereas rhIGF-I administration to mice did not.[61-63] We have speculated that these differential effects may be attributed to different interactions with IGF-binding proteins (IGFBPs) following systemic delivery of IGF-I to mice compared with muscle-specific overexpression of IGF-I in transgenic mice. Although the effects of rhIGF-I administration and IGF-I overexpression on skeletal muscle regeneration have been well characterised, the role of IGFBPs in skeletal muscle regeneration remains poorly understood. Recently, we examined whether inhibiting IGF-I interactions with IGFBPs influenced muscle regeneration after myotoxic injury using the aptamer NBI-31772 which binds all six IGFBPs with high affinity and releases "free" endogenous IGF-I. Continual release of NBI-31772 into the circulation of mice *via* a mini-osmotic pump increased the rate of functional recovery in mouse *tibialis anterior* muscles after notexin-mediated damage.[65] These results support the notion that abrogating IGFBP interactions with systemic IGF-I has therapeutic potential for enhancing muscle repair after muscle injury.

**$\beta_2$-adrenoceptor agonists**

Although $\beta_2$-adrenoceptor agonists ($\beta_2$-agonists) are traditionally prescribed for alleviating bronchospasm in the treatment of asthma because of their bronchodilatory effects on smooth muscle, some $\beta_2$-agonists actually have potent anabolic effects on skeletal muscle especially when administered systemically and at higher doses.[66-68] These muscle hypertrophic effects of $\beta_2$-agonists combined with their known lipolytic actions, have proved desirable for those working in the livestock industry trying to improve meat quality and yield.[69,70] Not surprisingly, $\beta_2$-agonists have also been used and abused by many athletes involved in competitive bodybuilding, strength- and power-related sports, and sports such as wrestling where athletes need to "make weight" in order to compete in specific weight classes.[71,72] However, because of their anabolic effects on skeletal muscle, $\beta_2$-agonists have significant clinical potential particularly for muscle wasting disorders including the muscular dystrophies.[72]

Skeletal muscle contains a significant proportion of β-adrenoceptors, mostly of the $\beta_2$-subtype, with approximately 7-10% $\beta_1$-adrenoceptors present and a sparse population of α-adrenoceptors, usually in higher proportions in slow-twitch muscles.[69,70,73,74] Slow-twitch muscles have also been shown to have a greater density of β-adrenoceptors than fast-twitch muscles.[70] Since β-adrenoceptors exist in the heart as well as skeletal muscle, any approach involving the systemic administration of exogenous β-agonists must take into account potential effects on tissues other than skeletal muscle, particularly the heart. Synthetic $\beta_2$-agonists promote skeletal muscle hypertrophy *via* activation of cAMP dependent mechanisms that increase protein synthesis and inhibit protein degradation pathways.[72,75] Recently, PI3K-Akt signalling, which is known to be implicated in skeletal muscle hypertrophy, has also been linked to $\beta_2$-adrenergic receptor signalling.[76]

We and others have also shown that systemic administration of β-agonists can promote regeneration of injured skeletal muscles, specifically to hasten the functional recovery of rat muscles after myotoxic injury with bupivacaine[77] or notexin.[48,78] Daily fenoterol administration to rats (1.4 mg/kg/day, *i.p.*) enhanced the force output of injured/regenerating rat EDL muscles by 19% at 14 days post injury, which was associated with increases in protein content and muscle fibre size.[77] Daily clenbuterol treatment to rats (2 mg/kg/day, by oral gavage) increased protein content in regenerating *soleus* muscles

and caused significant transitions from slow to fast fibres.[78] More recently, we have studied aspects of β-adrenoceptor signalling during early regeneration of rat EDL and *soleus* skeletal muscles after bupivacaine injury and found that despite β-agonist (fenoterol) treatment decreasing β-adrenoceptor density in regenerating rat EDL and *soleus* muscles, the cAMP response to β-adrenoceptor stimulation, relative to healthy (uninjured) muscles, remained elevated.[79]

The potential for β-agonists to improve the size and strength of muscles of human patients affected by neuromuscular diseases where muscle regenerative mechanisms are defective, has received relatively limited attention. Preliminary trials using the $\beta_2$-agonist, albuterol, to treat young boys with facioscapulohumeral dystrophy, found that year-long administration at doses of 16 and 32 mg/day had only limited beneficial effects on strength, and was associated with some adverse cardiovascular related events such as palpitations and in some cases, muscle tremor.[80] Fowler and colleagues[81] administered albuterol at a lower dose of 8 mg/day for 28 weeks to boys with DMD or BMD and found modest increases in strength with no side effects. Albuterol was well tolerated, but elicited only modest improvements in muscle mass and strength. It is our contention that one of the factors currently limiting the application of $\beta_2$-agonists for DMD and related disorders is that albuterol is simply not a powerful enough anabolic agent to counteract the severe muscle wasting and to stimulate muscle regenerative mechanisms sufficiently. We have shown unequivocally that newer generation $\beta_2$-agonists, such as formoterol, have powerful skeletal muscle anabolic effects (in mice and rats) even when administered in micromolar doses.[67,82] Most importantly formoterol is more selective for the $\beta_2$-adrenoceptor and its effects on the heart (comprising predominantly $\beta_1$-adrenoceptors) are much less than those of older generation $\beta_2$-agonists like albuterol or clenbuterol. Blocking stimulation of the $\beta_1$-adenoceptors is possible with highly selective $\beta_1$-adrenoceptor antagonists (such as CGP 20712A65) and the importance of blocking $\beta_1$-adrenoceptors in heart failure to abrogate cardiotoxic $\beta_1$-adrenoceptor-mediated effects is well known.[83,84]

## Conclusions: Overcoming safety concerns for anabolic treatments for muscle injury

It is clear that better understanding the androgenic, IGF-I, and β-adrenergic signalling pathways in skeletal muscle is important for devising and optimising safe therapies to enhance muscle regeneration and function following different types of muscle injury. Although many aspects of these signalling cascades have been described in detail elsewhere,[72,75] the complementary interactions between them especially in relation to the activation of pathways induced by anabolic agents specifically for enhancing muscle functional recovery after injury has not been described widely (see Figure 1). The extracellular and intracellular mechanisms of action of the three classes of anabolic agents discussed in this review: anabolic steroids, IGF-I and related therapeutics, and $\beta_2$-adrenoceptor agonists; exhibit significant "cross-talk" and converge on pathways responsible for protein synthesis. Extracellular cross-talk between these signals includes increased IGF-I levels and the modulation of IGFBPs due to $\beta_2$ agonist administration,[85] and increased levels of IGF-I as a consequence of anabolic steroid administration.[86,87] Intracellular cross-talk between these signals is extensive and includes activation of PI3K by the β/γ subunits of G-protein complex following andrenoceptor stimulation[72,75] and activation of PI3K and p70S6K by IGF-I and following AR stimulation.[88,89] Details regarding these signalling pathways and their interactions are incomplete and further delineation of novel signalling molecules will yield new therapeutic targets for enhancing skeletal muscle regeneration after injury (Figure 1).

For anabolic therapies, concerns regarding potential pharmaceutical toxicity and safety issues are often only related to high doses, so low-dose, short-term treatment strategies are likely to have less toxic effects and their clinical merit is worthy of testing. To this end, extensive preclinical and clinical studies are needed to determine the optimum doses and treatment regimens that will elicit significant improvements in muscle fibre size and strength without causing deleterious side effects such as cardiovascular complications or perhaps the formation of tumours if growth factors are administered systemically. Alternatively, intramuscular delivery and the use of emerging tissue engineering technologies that facilitate the timed and controlled release of growth factors, anabolic and/or antifibrotic agents, could help minimise potential side effects while exerting beneficial effects on regenerating muscle fibres to hasten restoration of muscle function after injury.

## Acknowledgements

Supported by research grant funding from the Australian Research Council Discovery-Project funding scheme (DP0665071, DP0772781), the National Health and Medical Research Council of Australia (350439, 454561, 509313), the Muscular Dystrophy Association (USA, 3595, 4167), and Pfizer Inc. (USA).

## References

1. Grounds MD, McGeachie JK. Skeletal muscle regeneration after crush injury in dystrophic *mdx* mice: an autoradiographic study. *Muscle Nerve* 1992; **15**: 580-6.
2. Menetrey J, Kasemkijwattana C, Day CS, Bosch P, Vogt M, Fu FH, Moreland MS, Huard J. Growth factors improve muscle healing. *J. Bone Joint Surg.* 2000; **82**: 131-7.
3. Huard J, Li Y, Fu FH. Muscle injuries and repair: current trends. *J. Bone Joint Surg.* 2002; **84**: 822-32.
4. Morgan DL, Allen DG. Early events in stretch-induced muscle damage. *J. Appl. Physiol.* 1999; **87**: 2007-15.
5. Brockett CL, Morgan DL, Proske U. Predicting hamstring strain injury in elite athletes. *Med. Sci.*

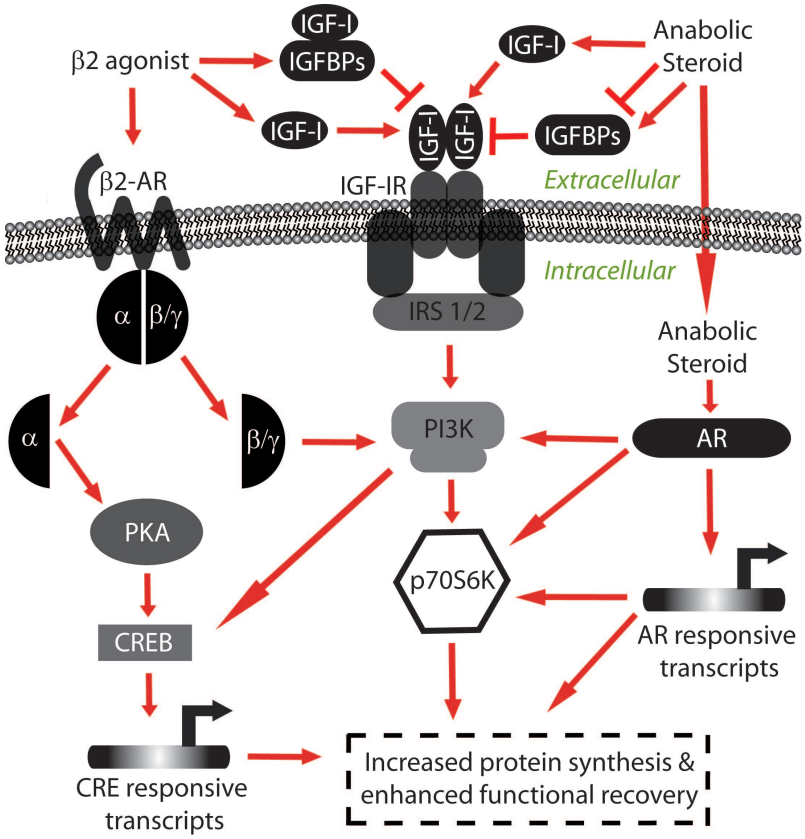


***Figure 1. Signalling cascades induced by anabolic agents that result in enhanced functional recovery after skeletal muscle injury.*** *The extracellular and intracellular mechanisms of action of anabolic steroids, IGF-I related therapeutics and $\beta_2$-adrenoceptor agonists, exhibit significant "cross-talk" and converge on protein synthetic pathways. Extracellular cross-talk between these signals includes increased IGF-I levels and modulation of IGFBPs (either increased or decreased levels of specific IGFBPs) following $\beta_2$ agonist administration,[85] and increased levels of IGF-I due to anabolic steroid administration.[86,87] Intracellular cross-talk between these signals is extensive and includes activation of PI3K by the β/γ subunits of G-protein complex following andrenoceptor stimulation (for review see Lynch & Ryall (2008)[72] and Lynch* et al. *(2007)[75]) and activation of PI3K and p70S6K by IGF-I and in response to AR stimulation.[88,89] These signalling pathways have not been characterised completely and further delineation of novel signaling molecules will yield new therapeutic targets for enhancing muscle regeneration. [α: alpha subunit of G-protein complex; β/γ: β and γ subunits of G-protein complex; AR: androgen receptor; B2-AR: $\beta_2$-adrenoceptor; CRE: 3′-5′-cyclic adenosine monophosphate (cAMP) response element; CREB: cAMP response element-binding protein; IGF-I: insulin-like growth factor-I; IGFBPs: insulin-like growth factor binding proteins; IGF-IR: insulin-like growth factor-I receptor; IRS ½: insulin receptor substrate 1/2; PI3K: phosphoinositide-3 kinase; p70S6K: 70 kDa ribosomal protein S6 kinase; PKA: protein kinase A].*

*Sports Exerc.* 2004; **36**: 379-87.

6. Proske U, Morgan DL, Brockett CL, Percival P. Identifying athletes at risk of hamstring strains and how to protect them. *Clin. Exp. Pharmacol. Physiol.* 2004; **31**: 546-50.
7. Kasemkijwattana C, Menetrey J, Bosch P, Somogyi G, Moreland MS, Fu FH, Buranapanitkit B, Watkins SS, Huard J. Use of growth factors to improve muscle healing after strain injury. *Clin. Orth. Rel. Res.* 2000; **370**: 272-85.
8. Chan YS, Li Y, Foster W, Fu FH, Huard J. The use of suramin, an antifibrotic agent, to improve muscle recovery after strain injury. *Am. J. Sports. Med.* 2005; **33**: 43-51.
9. Choudhury NA, Sakaguchi S, Koyano K, Matin AF, Muro H. Free radical injury in skeletal muscle ischemia and reperfusion. *J. Surg. Res.* 1991; **51**: 392-8.
10. Petrasek PF, Homer-Vanniasinkam S, Walker PM. Determinants of ischemic injury to skeletal muscle. *J. Vasc. Surg.* 1994; **19**: 623-31.
11. Lazarus B, Messina A, Barker JE, Hurley JV, Romeo R, Morrison WA, Knight KR. The role of mast cells in ischaemia-reperfusion injury in murine skeletal muscle. *J. Pathol.* 2000; **191**: 443-8.
12. Tupling R, Green H, Senisterra G, Lepock J, McKee N. Effects of 4-h ischemia and 1-h reperfusion on rat muscle sarcoplasmic reticulum function. *Am. J. Physiol. Endocrinol. Metab.* 2001; **281**: E867-77.
13. Blaisdell FW. The pathophysiology of skeletal muscle

ischemia and the reperfusion syndrome: a review. *Cardiovasc. Surg*. 2002; **10**: 620-30.
14. Sato K, Li Y, Foster W, Fukushima K, Badlani N, Adachi N, Usas A, Fu FH, Huard J. Improvement of muscle healing through enhancement of regeneration and prevention of fibrosis. *Muscle Nerve* 2003; **28**: 365-72.
15. Li Y, Foster W, Deasy BM, Chan Y, Prisk V, Tang Y, Cummins J, Huard J. Transforming growth factor-β1 induces the differentiation of myogenic cells into fibrotic cells in injured skeletal muscle: a key event in muscle fibrogenesis. *Am. J. Pathol*. 2004; **164**: 1007-19.
16. Carlson BM, Faulkner JA. The regeneration of skeletal muscle fibers following injury: a review. *Med. Sci. Sports Exerc.* 1983; **15**: 187-98.
17. Grounds MD. Muscle regeneration: molecular aspects and therapeutic implications. *Curr. Opin. Neurol.* 1999; **12**: 535-43.
18. Grounds MD. Towards understanding muscle regeneration. *Pathol. Res. Pract.* 1991; **187**: 1-22.
19. Hawke TJ, Garry DJ. Myogenic satellite cells: physiology to molecular biology. *J. Appl. Physiol*. 2001; **91**: 534-51.
20. Chargé SP, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol. Rev.* 2004; **84**: 209-38.
21. Järvinen TA, Järvinen TL, Kääriäinen M, Aärimaa V, Vaittinen S, Kalimo H, Järvinen M. Muscle injuries: optimising recovery. *Best Pract. Res. Clin. Rheumatol.* 2007; **21**: 317-31.
22. Beiner JM, Jokl P. Muscle contusion injuries: current treatment options. *J. Am. Acad. Orthop. Surg*. 2001; **9**: 227-37.
23. Fodor WL. Tissue engineering and cell based therapies, from the bench to the clinic: the potential to replace, repair and regenerate. *Reprod. Biol. Endocrinol*. 2003; **13**: 1:102.
24. Corsi KA, Schwarz EM, Mooney DJ, Huard J. Regenerative medicine in orthopaedic surgery. *J. Orthop. Res*. 2007; 25: 1261-8.
25. Dennis RG, Kosnik PE 2nd. Excitability and isometric contractile properties of mammalian skeletal muscle constructs engineered in vitro. *In Vitro Cell Dev. Biol. Anim.* 2000; **36**: 327-35.
26. Bach AD, Beier JP, Stern-Staeter J, Horch RE. Skeletal muscle tissue engineering. *J. Cell. Mol. Med*. 2004;**8**: 413-22.
27. Stern-Straeter J, Bach AD, Stangenberg L, Foerster VT, Horch RE, Stark GB, Beier JP. Impact of electrical stimulation on three-dimensional myoblast cultures - a real-time RT-PCR study. *J. Cell. Mol. Med*. 2005; **9**: 883-92.
28. Bach AD, Arkudas A, Tjiawi J, Polykandriotis E, Kneser U, Horch RE, Beier JP. A new approach to tissue engineering of vascularized skeletal muscle. *J. Cell. Mol. Med*. 2006; **10**: 716-26.
29. Freed LE, Guilak F, Guo XE, Gray ML, Tranquillo R, Holmes JW, Radisic M, Sefton MV, Kaplan D, Vunjak-Novakovic G. Advanced tools for tissue engineering: scaffolds, bioreactors, and signaling. *Tissue Eng*. 2006; **12**: 3285-305.
30. Mikos AG, Herring SW, Ochareon P, Elisseeff J, Lu HH, Kandel R, Schoen FJ, Toner M, Mooney D, Atala A, Van Dyke ME, Kaplan D, Vunjak-Novakovic G. Engineering complex tissues. *Tissue Eng*. 2006; **12**: 3307-39.
31. Hutmacher DW, Cool S. Concepts of scaffold-based tissue engineering – the rationale to use solid free-form fabrication techniques. *J. Cell. Mol. Med*. 2007; **11**: 654-69.
32. Hartgens F, Kuipers H. Effects of androgenic-anabolic steroids in athletes. *Sports Med*. 2004; **34**: 513-54.
33. Bhasin S, Storer TW, Berman N, Yarasheski KE, Clevenger B, Phillips J, Lee WP, Bunnell TJ, Casaburi R. Testosterone replacement increases fat-free mass and muscle size in hypogonadal men. *J. Clin. Endocrinol. Metab.* 1997; **82**: 407-413.
34. Brodsky IG, Balagopal P, Nair KS. Effects of testosterone replacement on muscle mass and muscle protein synthesis in hypogonadal men – a clinical research center study. *J. Clin. Endocrinol. Metab.* 1996; **81**: 3469-75.
35. Snyder PJ, Peachey H, Hannoush P, Berlin JA, Loh L, Lenrow DA, Holmes JH, Dlewati A, Santanna J, Rosen CJ, Strom BL.. Effect of testosterone treatment on body composition and muscle strength in men over 65 years of age. *J. Clin. Endocrinol. Metab.* 1999; **84**: 2647-53.
36. Ferrando AA, Sheffield-Moore M, Yeckel CW, Gilkison C, Jiang J, Achacosa A, Lieberman SA, Tipton K, Wolfe RR, Urban RJ. Testosterone administration to older men improves muscle function: molecular and physiological mechanisms. *Am. J. Physiol. Endocrinol. Metab.* 2002; **282**: E601-7.
37. Bhasin S, Javanbakht M. Can androgen therapy replete lean body mass and improve muscle function in wasting associated with human immunodeficiency virus infection? *JPEN J. Parenter. Enteral Nutr*. 1999; **23**: S195-201.
38. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger B, Phillips J, Bunnell TJ, Tricker R, Shirazi A, Casaburi R. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. *N. Engl. J. Med.* 1996; **335**: 1-7.
39. Griggs RC., Pandya S, Florence JM, Brooke MH, Kingston W, Miller JP, Chutkow J, Herr BE, Moxley RT 3rd. Randomized controlled trial of testosterone in myotonic dystrophy. *Neurology* 1989; **39**: 219-22.
40. Fairfield WP, Treat M, Rosenthal DI, Frontera W, Stanley T, Corcoran C, Costello M, Parlman K, Schoenfeld D, Klibanski A, Grinspoon S. Effects of testosterone and exercise on muscle leanness in eugonadal men with AIDS wasting. *J. Appl. Physiol.* 2001; **90**: 2166- 71.
41. Schroeder ET, Terk M, Sattler FR. Androgen therapy

improves muscle mass and strength but not muscle quality: results from two studies. *Am. J. Physiol. Endocrinol. Metab.* 2003; **285**: E16-24.
42. Wang C, Cunningham G, Dobs A, Iranmanesh A, Matsumoto AM, Snyder PJ, Weber T, Berman N, Hul, L, Swerdloff RS. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J. Clin. Endocrinol. Metab.* 2004; **89**: 2085-98.
43. Abrams D. Use of androgens in patients who have HIV/AIDS: what we know about the effect of androgens on wasting and lipodystrophy. *AIDS Read*. 2001; **11**: 149-56.
44. Gold J, Batterham MJ, Rekers H, Harms MK, Geurts TB, Helmyr PM, Silva de Mendonça J, Falleiros Carvalho LH, Panos G, Pinchera A, Aiuti F, Lee C, Horban A, Gatell J, Phanuphak P, Prasithsirikul W, Gazzard B, Bloch M, Danner SA; E-1696 Study Investigators. Effects of nandrolone decanoate compared with placebo or testosterone on HIV-associated wasting. *HIV Med*. 2006; **7**: 146-55.
45. McClung JM, Lee WJ, Thompson RW, Lowe LL, Carson JA. RhoA induction by functional overload and nandrolone decanoate administration in rat skeletal muscle. *Pflügers Arch*. 2003; **447**: 345-55.
46. Bricout VA, Serrurier BD, Bigard AX, Guezennec CY. Effects of hindlimb suspension and androgen treatment on testosterone receptors in rat skeletal muscles. *Eur. J. Appl. Physiol. Occup. Physiol*. 1999; **79**: 443-8.
47. Eriksson A, Lindström M, Carlsson L, Thornell LE. Hypertrophic muscle fibers with fissures in power-lifters; fiber splitting or defect regeneration? *Histochem. Cell Biol*. 2006; **126**: 409-17.
48. Plant DR, Colarossi FE, Lynch GS. Notexin causes greater myotoxic damage and slower functional repair in mouse skeletal muscles than bupivacaine. *Muscle Nerve* 2006; **34**: 577-85.
49. Ferry A, Noirez P, Page CL, Salah IB, Daegelen D, Rieu M. Effects of anabolic/androgenic steroids on regenerating skeletal muscles in the rat. *Acta Physiol. Scand*. 1999;**166**: 105-10.
50. Ferry A, Vignaud A, Noirez P, Bertucci W. Respective effects of anabolic/androgenic steroids and physical exercise on isometric contractile properties of regenerating skeletal muscles in the rat. *Arch. Physiol. Biochem*. 2000; **108**: 257-61.
51. Beiner JM, Jokl P, Cholewicki J, Panjabi MM. The effect of anabolic steroids and corticosteroids on healing of muscle contusion injury. *Am. J. Sports Med*. 1999; **27**: 2-9.
52. Carson JA, White JP, Baltgalvis KA, Washington TA, Jepson MJ, Thompson RW. Nandrolone decanoate administration and skeletal muscle regeneration. *FASEB J*. 2007; **21**: 769.26 (Abstract).
53. Souza R, Goncalves W, Dal Pai-Silva M, Gallacci M. Influence of anabolic steroid treatment upon muscle regeneration and satellite cells of mice following envenoming by *Bothrops jararacussu* snake venom. *Toxicol. Lett*. 2007; **172**: S237 (Abstract).
54. Krahn MJ, Anderson JE. Anabolic steroid treatment increases myofiber damage in *mdx* mouse muscular dystrophy. *J. Neurol. Sci*. 1994; **125**: 138-46.
55. Philippou A, Halapas A, Maridaki M, Koutsilieris M. Type I insulin-like growth factor receptor signaling in skeletal muscle regeneration and hypertrophy. *J. Musculoskelet. Neuronal. Interact*. 2007; **7**: 208-18.
56. Engert JC, Berglund EB, Rosenthal N. Proliferation precedes differentiation in IGF-I-stimulated myogenesis. *J. Cell Biol*. 1996; **135**: 431-40.
57. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, Rosenthal N: Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat. Genet*. 2001; **27**: 195-200.
58. Barton ER, Morris L, Musaro A, Rosenthal N, Sweeney HL: Muscle specific expression of insulin-like growth factor I counters muscle decline in *mdx* mice. *J. Cell Biol.* 2002; 157: 137-48.
59. Shavlakadze T, White J, Hoh JF, Rosenthal N, Grounds MD. Targeted expression of insulin-like growth factor-I reduces early myofiber necrosis in dystrophic *mdx* mice. *Mol. Ther*. 2004; **10**: 829-43.
60. Schertzer JD, Lynch GS. Comparative evaluation of IGF-I gene transfer and IGF-I protein administration for enhancing skeletal muscle regeneration after injury. *Gene Ther*. 2006; **13**: 1657-64.
61. Gregorevic P, Plant DR, Leeding KS, Bach LA, Lynch GS: Improved contractile function of the *mdx* dystrophic mouse diaphragm muscle after insulin-like growth factor-I administration. *Am. J. Pathol*. 2002; **161**: 2263-72.
62. Gregorevic P, Plant DR, Lynch GS. Administration of insulin-like growth factor-I improves fatigue resistance of skeletal muscles from dystrophic *mdx* mice. *Muscle Nerve* 2004; **30**: 295-304.
63. Schertzer JD, Ryall JG, Lynch GS: Systemic administration of IGF-I enhances oxidative status and reduces contraction-induced injury in skeletal muscles of *mdx* dystrophic mice. *Am. J. Physiol. Endocrinol. Metab*. 2006; **291**: E499-505.
64. Creaney L, Hamilton B. Growth factor delivery methods in the management of sports injuries: the state of play. *Br. J. Sports Med*. 2008; (in press, doi:10.1136/bjsm.2007.040071 Nov 5, 2007).
65. Schertzer JD, Gehrig SM, Ryall JG, Lynch GS. Modulation of insulin-like growth factor (IGF)-I and IGF-binding protein interactions enhances skeletal muscle regeneration and ameliorates the dystrophic pathology in *mdx* mice. *Am. J. Pathol*. 2007; **171**: 1180-8.
66. Ryall JG, Plant DR, Gregorevic P, Sillence MN, Lynch GS. $\beta_2$-Agonist administration reverses muscle wasting and improves muscle function in aged rats. *J. Physiol*. 2004; **555**: 175-88.
67. Ryall JG, Sillence MN, Lynch GS. Systemic

administration of $\beta_2$-adrenoceptor agonists, formoterol and salmeterol, elicit skeletal muscle hypertrophy in rats at micromolar doses. *Br. J. Pharmacol*. 2006; **147**; 587-95.

68. Ryall JG, Schertzer JD, Lynch GS. Attenuation of age-related muscle wasting and weakness in rats after formoterol treatment: therapeutic implications for sarcopenia. *J. Gerontol. A. Biol. Sci. Med. Sci.* 2007;**62**: 813-23.
69. Sillence MN, Matthews ML. Classical and atypical binding sites for beta-adrenoceptor ligands and activation of adenylyl cyclase in bovine skeletal muscle and adipose tissue membranes. *Br. J. Pharmacol*. 1994; **111**: 866-72.
70. Sillence, MN, Matthews ML, Moore NG, Reich MM. Effects of BRL-47672 on growth, $\beta_2$-adrenoceptors, and adenylyl cyclase activation in female rats. *Am. J. Physiol*. 1995; **268**: E159-67.
71. Delbeke FT, Desmet N, and Debackere M. The abuse of doping agents in competing body builders in Flanders (1988-1993). *Int. J. Sports Med.* 1995; **16**: 66-70, 1995.
72. Lynch GS, Ryall JG. Role of β-adrenergic signaling in skeletal muscle: implications for muscle wasting and disease. *Physiol. Rev*. 2008; (in press, doi:10.1152/physrev.00028.2007).
73. Kim YS, Sainz RD, Molenaar P, Summers RJ. Characterisation of $\beta_1$- and $\beta_2$-adrenoceptors in rat skeletal muscles. *Biochem. Pharmacol*. 1991; **42**: 1783-9.
74. Rattigan S, Appleby GJ, Edwards SJ, McKinstry WJ, Colquhoun EQ, Clark MG, Richter EA. α-adrenergic receptors in rat skeletal muscle. *Biochem. Biophys. Res. Commun*. 1986; **136**: 1071-7.
75. Lynch GS, Schertzer JD, Ryall JG. Therapeutic approaches for muscle wasting disorders. *Pharmacol. Ther*. 2007; **113**: 461-87.
76. Kline WO, Panaro FJ, Yang H, Bodine SC. Rapamycin inhibits the growth and muscle-sparing effects of clenbuterol. *J. Appl. Physiol*. 2007; **102**: 740-7.
77. Beitzel F, Gregorevic P, Ryall JG, Plant DR, Sillence MN, Lynch GS. $\beta_2$-Adrenoceptor agonist fenoterol enhances functional repair of regenerating rat skeletal muscle after injury. *J. Appl. Physiol*. 2004; **96**: 1385-92.
78. Bricout VA, Serrurier BD, Bigard AX. Clenbuterol treatment affects myosin heavy chain isoforms and MyoD content similarly in intact and regenerated soleus muscles. *Acta Physiol. Scand*. 2004; **180**: 271-80.
79. Beitzel F, Sillence MN, Lynch GS. β-Adrenoceptor signaling in regenerating skeletal muscle after β-agonist administration. *Am. J. Physiol. Endocrinol. Metab*. 2007; **293**: E932-40.
80. Kissel JT, McDermott MP, Mendell JR, King WM, Pandya S, Griggs RC, Tawil R. Randomized, double-blind, placebo-controlled trial of albuterol in facioscapulohumeral dystrophy. *Neurology* 2001; **57**: 1434-40.
81. Fowler EC, Graves MC, Wetzel GT, Spencer MJ. Pilot trial of albuterol in Duchenne muscular dystrophy. *Neurology* 2004; **62**: 1006-8.
82. Harcourt LJ, Schertzer JD, Ryall JG, Lynch GS. Low dose formoterol administration improves muscle function in dystrophic *mdx* mice without increasing fatigue. *Neuromuscul. Disord*. 2007; **17**: 47-55.
83. Molenaar P, Parsonage WA. Fundamental considerations of β-adrenoceptor subtypes in human heart failure. *Trends Pharmacol. Sci*. 2005; **26**: 368-75.
84. Molenaar P, Chen L, Parsonage WA. Cardiac implications for the use of $\beta_2$-adrenoceptor agonists for the management of muscle wasting. *Br. J. Pharmacol*. 2006; **147**: 583-6.
85. Awede BL, Thissen JP, Lebacq J. Role of IGF-I and IGFBPs in the changes of mass and phenotype induced in rat soleus muscle by clenbuterol. *Am. J. Physiol. Endocrinol. Metab*. 2002; **282**: E31-7.
86. Lewis MI, Horvitz GD, Clemmons DR, Fournier M. Role of IGF-I and IGF-binding proteins within diaphragm muscle in modulating the effects of nandrolone. *Am. J. Physiol. Endocrinol. Metab*. 2002; **282**: E483-90.
87. Hobbs CJ, Plymate SR, Rosen CJ, Adler RA. Testosterone administration increases insulin-like growth factor-I levels in normal men. *J. Clin. Endocrinol. Metab*. 1993; **77**: 776-9.
88. Rommel C, Bodine SC, Clarke BA, Rossman R, Nunez L, Stitt TN, Yancopoulos GD, Glass DJ. Mediation of IGF-1-induced skeletal myotube hypertrophy by PI3K/Akt/mTOR and PI3K/Akt/GSK3 pathways. *Nat. Cell. Biol*. 2001; **3**: 1009-13.
89. Xu T, Shen Y, Pink H, Triantafillou J, Stimpson SA, Turnbull P, Han B. Phosphorylation of p70s6 kinase is implicated in androgen-induced levator ani muscle anabolism in castrated rats. *J. Steroid Biochem. Mol. Biol*. 2004; **92**: 447-54.

---

Received 28 January 2008, in revised form 17 February 2008. Accepted 17 February 2008.


*Author for correspondence:*

Gordon S. Lynch, Ph.D.
Department of Physiology
University of Melbourne
Victoria 3010 Australia

Tel: +61 3 8344 0065
Fax: +61 3 8344 5818
E-mail: gsl@unimelb.edu.au