J Musculoskel Neuron Interact 2004; 4(2):143-147

Perspective Article

Hylonome

Age-related loss of skeletal muscle function; impairment of gene expression

G. Goldspink

Royal Free and University College Medical School, Royal Free Campus, London, UK

Abstract

Mechano Growth Factor (MGF) is derived from the insulin-like growth factor (IGF-I) but its sequence differs from the systemic IGF-I produced by the liver. MGF is expressed by mechanically overloaded muscle and is involved in tissue repair and adaptation. It is expressed as a pulse following muscle damage and involved in the activation of muscle satellite (stem) cells. These donate nuclei to the muscle fibers that are required for repair and for the hypertrophy processes which may have similar regulatory mechanisms. Muscles in the elderly are unable to upregulate MGF in response to exercise. This is also true in certain diseases and this helps to explain muscle loss in those conditions. There is evidence that MGF is a local tissue repair factor as well as a growth factor and that it has an important role in damage limitation and inducing repair in other post-mitotic tissues. As there is no cell replacement in these tissues there has to be an effective local cellular repair mechanism. With advancing years this seems to become deficient and there is an increased chance that the damaged cells will undergo cell death leading to progressive loss of tissue function.

Keywords: Ageing, MGF, IGF-I Gene GH, Muscle Loss, Exercise

Introduction

Sarcopenia (age-related muscle loss) is a major care problem and is becoming more serious as average life expectancy increases. The most obvious reason is that when muscle function in the elderly declines they cannot generate enough power to correct their posture quickly enough and they tend to fall over and break bones that are brittle due to osteoporosis. Muscles generate the mechanical strain that is required to make the bones healthy. Therefore when muscle activity is reduced , this exacerbates the problem and a vicious circle is established. Another perhaps less obvious function of muscle is that it acts as a metabolic store and in times of emergency it produces the proteins and metabolites required for survival. Therefore the frail elderly person who has decreased muscle mass does not survive major surgery or a traumatic accident. The muscle cachexia associated with certain diseases e.g., cancer, HIV, makes this an even greater problem as medication might have time to work if the tissue loss could be prevented.

The work of my group over the last 10 years has been to identify the factors that are involved in inducing muscle hypertrophy and loss particularly during ageing and in certain neurological conditions. As well as marked atrophy there is a loss of muscle fibers. Damage to the innervation occurs throughout life but in old age the process of collateral sprouting of the neurons appears to become less effective in repairing innervation and muscle fibers are lost[1,2]. As the loss of muscle function is largely related to loss of muscle it was important to determine which growth/repair factors are involved in the molecular regulation of muscle mass and why their levels or effectiveness decrease.

Cloning of MGF and other IGF-I splice variants expressed by human muscle

About 12 years ago our group set about cloning these growth factor(s) and identifying the genes involved in regulation of muscle mass. For this purpose we needed to have

The author receives royalties relating to patents on MGF and research funding from UCL Biomedica.

Corresponding author: Prof. Geoffrey Goldspink, Professor of Anatomy and Developmental Biology, Division of Surgery and Reconstructive Medicine, Royal Free and University College Medical School, Royal Free Campus, University of London, Rowland Hill Street, London NW3 2PF, UK
E-mail: goldspink@rfc.ucl.ac.uk

Accepted 28 May 2004

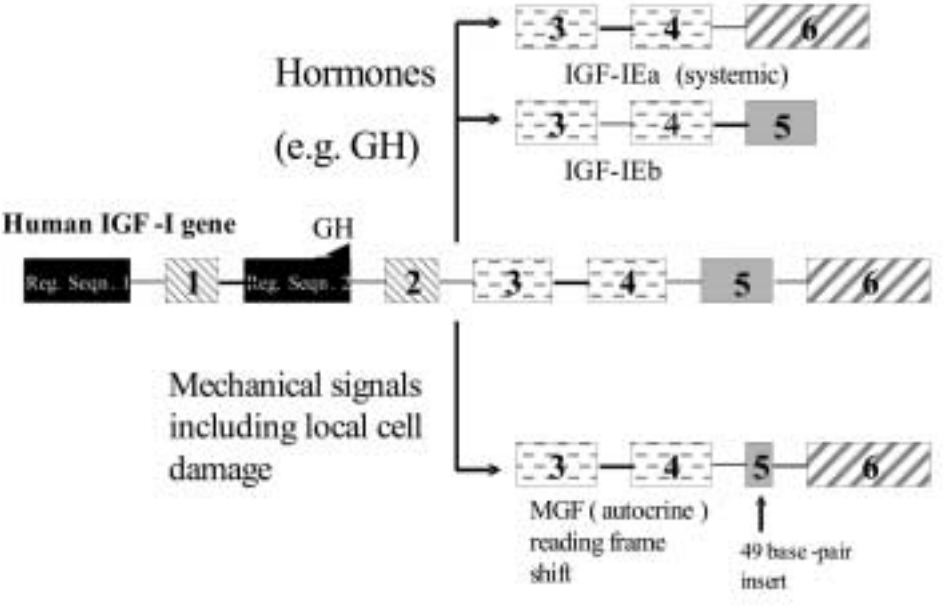

Figure. Splicing of the IGF-I gene as a result of exercise and/or muscle damage and hormones. In human muscle a 49 base insert changes the reading frame in MGF resulting in a different carboxy peptide. Hormones upregulate the expression of the IGF-I gene particularly the IGF-IEa but growth hormone (GH) apparently upregulates the primary RNA transcript of the IGF-I gene, and when in combination with exercise training, it results in more being spliced towards MGF. Human muscle also expresses an IGF-IEb form but its function is not known. The IGF-receptor domain encoded by exons 3 and 4, is responsible for the anabolic effect of IGF-I in all these splice variants.

an animal model in which we could make a muscle grow rapidly. Previous work had shown that if the tibialis anterior in the mature rabbit was electrically stimulated whilst held in the stretched position by plaster cast immobilisation it increased in mass by 35% within 7 days[3]. The mRNA in the stretch/stimulated muscles increased considerably but most of this was ribosomal RNA. Using the differential display with RT-PCR technique it was possible to detect an RNA transcript that was only expressed in the stretched/stimulated muscle and not in resting control muscles. This transcript was cloned and sequenced and when compared to sequences in the gene data bases it was seen to be derived from the insulin-like growth factor gene by alternative splicing. The terminology of the IGF-I splice variants is a problem when attempting to apply it to non hepatic tissues[4] and therefore we named this newly discovered splice variant "mechano growth factor" (MGF) as it is expressed in response to mechanical stimulation[5,6] and as it has a different downstream sequence to the liver type of IGF-I.

The sequence of the splice variant which we called mechano growth factor (MGF) proved to be extremely interesting not only because its expression was mechanosensitive but because it has a different carboxy peptide sequence. It was noted that its E domain has an insert in exon 5 that changes the reading frame. In the rat there is a 52 base insert and in the human the insert is 49 bases. Amino acids are coded for by triplets of bases and because this insert which is not a multiple of 3, is spliced into the MGF mRNA, then the down stream peptide sequence is different from that of the other IGF-I isoforms. This has important functional consequences as the different IGF-I carboxy peptide sequences are involved in the recognition of binding proteins. These stabilise and act as a time release for these growth factors. Also, in the case of MGF the carboxy peptide sequence (encoded in exons 5 and 6) acts as a different growth factor to the IGF-I receptor domain (exons 3 and 4). The E domain peptide has been shown to induce division of mononucleated myoblasts i.e. to activate the muscle satellite (stem) cells that are required for muscle hypertrophy and repair[7].

The systemic type of IGF-I (IGF-IEa) is expressed by many cell types including skeletal muscle. This or the mature IGF-I (IGF-I receptor domain) which is the commercially available recombinant IGF-I that has been used in a good number of *in vitro* and *in vivo* studies. The IGF-IEa cDNA has been used to produce transgenic mice[8] and also introduce into muscle by direct injection using a viral vector[9,10]. In these publications the expression has been described as muscle specific. This is misleading as muscle promoter/enhancer sequences were used in order to obtain muscle specific expression. This is compounded by the fact that when the full length cDNA of IGF-IEa was used this presumably included the carboxy peptide sequence and this recognises IGF-I

binding proteins. These include BP3 and BP5 which are expressed in muscle and they tend to retain the systemic type of IGF-I within the tissue. As far as can be discovered, the IGF-I cDNA used in these gene transfer experiments[10] was the systemic or liver type of IGF-I. Although this has a general anabolic effect, the induced hypertrophy takes months to develop in contrast to MGF where the same increase in muscle mass can be achieved in a few weeks. In the latter case the extra potency is apparently because MGF activates the muscle satellite (stem) cells and this "kick starts" the rebuilding process.

The role of MGF in renewing the muscle satellite (stem) cell pool

Satellite cells in skeletal muscle were first described by Mauro[11] and it is now realized that these cells provide the extra nuclei for post-natal growth[12,13] and that they are also involved in repair and regeneration following local injury of muscle fibres[14]. In normal adult undamaged tissue, the satellite cells are quiescent and usually detected just beneath the basal lamina. When activated they commence to co-express myogenic factors including c-met, MyoD and myf5 and later myogenin[15,16,17]. Satellite cells are for the most part residual myoblasts although other pluripotent stem cells have been shown to fuse and adopt the muscle phenotype when introduced into dystrophic muscle and damaged muscle. It has been established that even in normal muscle, local injury does occur from time to time[18], but in certain diseases such as the muscular dystrophies, the muscle fibers are markedly more susceptible to damage, in particular to the membrane[19]. The contractile system of muscle fibers also sustains damage during eccentric contractions, that is to say, when the muscle is activated whilst being stretched. It is interesting to note that the forces generated by activation combined with stretch exceed even those of a maximal isometric contraction. In the muscle fibers involved, the sarcomeres may be pulled out to such a degree that there is no longer any overlap of the actin and myosin filaments, thus causing damage[20].

Two recent studies have indicated that one of the special functions of MGF is to induce the muscle satellite (stem) cells to divide. When IGF-I was added to C_2C_{12} cells in culture they increased in mass but they also fused to form myotubes. When the MGF E domain was added, then the mononucleated myoblasts increased in number but stayed as mononucleated cells. This effect, unlike the action of IGF-I, was not blocked by an antibody to the IGF-I receptor indicating that the MGF carboxy peptide is a growth/repair factor in its own right[7].

Using an *in vivo* approach, Hill and Goldspink[21] showed that following muscle damage MGF is produced as a pulse lasting a few days only. Although, it has been stated that IGF-I activates satellite cells it was not certain from these other studies whether this refers to inducing fusion of the satellite cells rather than proliferation or indeed what type of IGF-I was used. In our studies on local muscle damage, it was found that the expression of MGF preceded that of markers of satellite cell activation. On the other hand, IGF-IEa did not peak until 11 days after the damage, by which time satellite cell activation had virtually ceased.

Failure to maintain muscle loss during ageing and disease

Muscle loss (sarcopenia) is one of the most obvious effects of ageing. The work of Owino et al.[22] indicated that muscles in old rats, when surgically overloaded, were much less able to express MGF than those in younger animals. This was extrapolated to the human by Hameed et al.[23] who showed that the muscles in elderly male volunteers when exercised, were much less able to increase MGF levels as compared to muscles of young men. None of the other parameters measured showed a marked age-related decline although it has previously been known that circulating growth hormone levels in the over 70's are much lower than those in teenagers[24]. Whilst it was clear that mechanical activity plays a pivotal role in regulating local IGF-I expression in muscle, it was unclear as to whether there may be further regulation provided by hormones, notably growth hormone (GH). Some insight into this possibility was gained from the results of a recent longitudinal study where the relationship between exogenous GH administration and strength training exercise was studied in older people (age 74 ± 1 year). These were assigned to either resistance training with placebo, resistance training combined with GH administration or GH administration alone. GH administration without training did not change MGF mRNA levels when measured at 5 weeks, but significantly increased IGF-IEa levels (237%). In contrast, 5 weeks of resistance training significantly increased expression of MGF (163%) and to a lesser extent IGF-IEa (68%). However, when GH treatment was combined with exercise, MGF levels were dramatically increased (456%). These data suggest that exogenous GH administration causes an overall upregulation of transcription of the IGF-I gene prior to splicing, which results in more of the primary transcript of IGF-I. In the absence of strength training exercise, splicing is towards the IGF-IEa isoform, but when combined with the mechanical loading it splices towards the MGF isoform.

Muscle loss is a major problem in certain hereditary diseases. In dystrophic muscles it seems that there is an inability to produce MGF in response to mechanical overload[25]. The systemic type of IGF-IEa is also produced by muscle, including dystrophic muscle, but MGF is apparently required to ensure muscle fiber repair and survival. Therefore the role of the IGF-I splice variant MGF seems to be very relevant to understanding the etiology and the development of possible treatment in these muscle wasting conditions as well as sarcopenia.

The role of MGF in other musculoskeletal tissues

Emerging evidence has indicated that MGF can be regarded as a cellular repair factor as well as a growth factor. Effective local tissue damage limitation and the initiation of local repair is particularly important in post-mitotic tissues such as muscle and neuronal tissue in which cell replacement is not a means of tissue repair. In these tissues, cell death must be prevented and repair initiated otherwise the ensuing cell death will result in a progressive loss of function. It was known that the myocardium expresses higher levels of IGF-I following ischemia or mechanical overload. This was shown using a general IGF-I antibody. However, recently we found that the type of IGF-I expressed in these circumstances is MGF. By transfecting cardiomyoctyes in culture using the Green Fluorescent Protein sequence attached to the 3' end of the cDNAs of MGF and IGF-IEa, that MGF becomes rapidly localized in the nucleus whilst the IGF-IEa peptide is localized through the cytoplasm. *In vivo* studies of ischemia and mechanical overload indicate that MGF reduces the size of the damaged area of the myocardium and this is in accord with the finding that MGF is expressed rapidly after tissue damage and prevents apoptosis. The other type of tissue in which we have found MGF is neuronal tissue. Recent work with a Polish group has shown that MGF ameliorates the effects of ischemia to the brain where we have shown it is expressed, particularly following damage. Neuronal tissue, as well as muscle, is post-mitotic tissue, and cell replacement is not a means of tissue repair. It appears that in the CNS, which is also a post mitotic tissue, MGF may have a similar role of ameliorating damage limitation and also possibly initiation of the repair processes. As MGF was involved in muscle repair, we thought that it may also be involved in maintaining the peripheral neurons particularly in re-innervation of muscle following damage. To investigate this we teamed up with Giorgio Terenghi's group at the University of Manchester, who has expertise in peripheral nerve repair. The approach involved cutting the sciatic nerve and putting the two cut ends in a conduit or nerve guide with a 2mm gap between the two stumps. The conduit was filled with a gel containing MGF, or IGF-IEa or the vector only. MGF was found to induce axonal growth over the gap and to restore muscle strength to 99% of normal muscle within 3 months following complete section of the nerve. The conduit with gel only controls was effective in promoting re-innervation as about 70% of the normal muscle function was restored. This demonstrates the need for bringing the two cut ends of the nerve into juxtaposition so the proximal axons can grow down the sheaths of the distal part. Further studies have shown that one of the ways in which MGF enhances re-innervation is by causing axonal branching; and this increases the chances of re-innervating more muscle fibres[26].

Benefits of exercise

As far as skeletal muscle is concerned, MGF is a splice variant of the IGF-I gene that is expressed early following muscle overload and/or damage. This results in the initial activation of the skeletal muscle satellite (stem) cells which fuse with the muscle fibres and donate nuclei. In this way MGF initiates the hypertrophy process via which they adapt to the type of physical activity they are required to perform. The second phase involves the splicing of the IGF-I transcript towards the systemic or liver type or IGF-IEa which provides more of the mature IGF-I. This produces the main anabolic response that upregulates gene expression in general. The evidence indicates that this is the mechanism via which muscle mass is locally regulated and the inability to express MGF in the elderly provides an explanation for the decline in muscle function during ageing.

Exercise is also known to elevate the serum levels of IGF-I[27,28]. Not only are the muscles producing more systemic IGF-I than the liver, they are also responsible for utilizing more during intensive exercise. As the IGF-IEa and its binding proteins 3 and 5 are expressed by muscle and upregulated by exercise it means that the IGF-IEa will bind to these binding proteins. Therefore, as this form of IGF-I is expected to have more effect on the muscles that produce it than on other muscles, its action can be regarded as paracrine as well as endocrine. The uptake from the serum seems to be a consequence of the greater abundance of the specific binding protein in trained muscle. The increase in circulating IGF-I is also believed to be one of the reasons why exercise is beneficial and that general tissue maintenance has improved. There is considerable evidence that IGF-I may be involved in the maintenance of the CNS and this may be the reason why regular exercise improves cognitive function.

Acknowledgments

This work was supported by funding from the Wellcome Trust, an EC grant (PENAM) and UCL Biomedica.

References

1. Campbell MJ, McComas AJ, Petito F. Physiological changes in ageing muscles. J Neurol Neurosurg Psychiatry 1973; 36:174-182.
2. Lexell J, Taylor CC, Sjostrom M. What is the cause of ageing atrophy? Total number, size and proportion of different fibre types studied in whole vastus lateralis muscle from 15-83 year old men. J Neurol Sci 1988; 84:275-294.
3. Goldspink G, Scutt A, Loughna P, Wells D, Jaenicke T, Gerlach G-F. Gene expression in skeletal muscle in response to mechanical signals. Am J Physiol 1992; 262:R326-R363.
4. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SDR. Clarification. J Physiol 2003; 549:553
5. Yang SY, Alnaqeeb M, Simpson H, Goldspink G. Cloning and characterisation of an IGF-I isoform expressed in skeletal muscle subjected to stretch. J Muscle Res Cell Motility 1996; 17:487-495.

6. McKoy G, Ashley W, Mander J, Yang SY, Williams N, Russell B, Goldspink G. Expression of insulin-like growth factor-I splice variant and structural genes in rabbit skeletal muscle induced by stretch and stimulation. J Physiol 1999; 516:583-592.
7. Yang SY, Goldspink G. Different roles of the IGF-IEc peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. FEBS Lett 2002; 522:156-160.
8. Mathews LS, Hammer RE, Behringer RR, D'Ercole AJ, Bell CL, Brinster RL, Palmiter RD. Growth enhancement of transgenic mice expressing human insulin-like growth factor I. Endocrinology 1988; 123:2827-2833.
9. Barton-Davis E, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor-I blocks the aging-related loss of skeletal muscle function. Proc Natl Acad Sci USA 1998; 95:15603-15607.
10. Musaro A, McCullagh K, Paul A, Houghton I, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, Rosenthal N. Localized IGF-I transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet 2001; 27:195-200.
11. Mauro A. Satellite cells of skeletal muscle fibres. J Biophys Biochem Cytol 1961; 9:493-495.
12. Moss FP, Leblond CP. Satellite cells are the source of nuclei in muscles of growing rats. Anat Rec 1970; 170:421-435.
13. Schulz E. Satellite cell proliferative compartments in growing skeletal muscle. Dev Biol 1996; 175:84-94.
14. Grounds MD. Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. Ann N Y Acad Sci 1998; 854:78-91.
15. Cornelison DD, Wold BJ. Single-cell analysis of regulatory gene expression in quiescent and activated mouse skeletal muscle satellite cells. Dev Biol 1997; 191:270-283.
16. Beauchamp JR, Heslop L, Yu DS, Tajbakhsh S, Kelly RG, Wernig A, Buckingham ME, Partridge TA, Zammit PS. Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol 2000; 151:1221-1234.
17. Qu-Petersen Z, Deasy B, Jankowski R, Ikezawa M, Cummins J, Pruchnic R, Mytinger J, Cao B, Gates C, Wernig A, Huard J. Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration. J Cell Biol 2002; 175:851-864.
18. Wernig A, Irintchev A, Weisshaupt P. Muscle injury, cross-sectional area and fibre type distribution in mouse soleus after intermittent wheel-running. J Physiol 1990; 428:639-652.
19. Cohn RD, Campbell KP. Molecular basis of muscular dystrophies. Muscle Nerve 2000; 23:1456-1471.
20. Lieber RL, Friden J. Mechanisms of muscle injury after eccentric contraction. J Sci Med Sport 1999; 2:253-265.
21. Hill M, Goldspink G. Expression and splicing of the insulin-like growth factor gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. J Physiol 2003; 549:409-418.
22. Owino V, Yang SY, Golsdpink G. Age-related loss of skeletal muscle function and the inability to express the autocrine form of insulin-like growth factor-1 (MGF) in response to mechanical overload. FEBS Lett 2001; 506:259-263.
23. Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SDR. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol 2003; 547:247-254.
24. Rudman D, Kutner MH, Rogers CM, Lubin MF, Fleming GA, Bain RP. Impaired growth hormone secretion in the adult population: relation to age and adiposity. J Clin Invest 1981; 67:1361-1369.
25 Goldspink G, Yang SY, Skarli M, Vrbova G. Local growth regulation is associated with an isoform of IGF-I that is expressed in normal muscles but not in dystrophic muscles. J Physiol 1996; 495P:162-163.
26. Yang SY, Tohill M, Goldspink P, Dijk S, Ashe A, Terenghi G, Goldspink G. Functional muscle re-innervation following MGF-enhanced axonal regeneration. 2004; (to be submitted for publication).
27. Brahm H, Piehl-Aulin K, Saltin B, Ljunghall A. Net fluxes over working thigh of hormones, growth factors and biomarkers of bone metabolism during lasting dynamic exercise. Calcif Tissue Int 1997; 60:175-180.
28. Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD, Goldspink G. The effect of recombinant human growth hormone and resistance training on IGF-I mRNA expression in the muscles of elderly men. J Physiol 2004; 555:231-240.