Mechanical Signals, IGF-I Gene Splicing, and Muscle Adaptation

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Muscle is a mechanical tissue, and when required it has the ability to increase power output by undergoing hypertrophy, which not only increases performance but reduces damage in muscles that would otherwise be “overstrained.” This is important because local damage occurs even in normal muscle, and since it is a postmitotic tissue there is no ongoing cell replacement; therefore, there must be an effective local cellular repair system. In this era of molecular biology, many developmental biologists tend to think of cellular phenotypes as being strictly programmed in the genome. However, mechanical signals are known to influence gene expression in muscle (10) and in other musculoskeletal cell types, including fibroblasts and osteoblasts. To physiologists, muscle is particularly interesting because it generates the forces to which it responds. It also provides an example of how a tissue mass is regulated by local as well as systemic factors, since when a muscle is exercised, it is that muscle that undergoes hypertrophy and not all of the muscles of the body.

Discovery of a Local Growth Factor that Initiates Muscle Hypertrophy

A little over 10 years ago, our group set about cloning the factor(s) that are involved in the autocrine regulation of muscle mass. For this purpose we needed to have an animal model in which we could make muscle grow rapidly. Previous work had shown that the tibialis anterior in the mature rabbit, when electrically stimulated while being held in the stretched position by plaster cast immobilization, increased in mass within one week (10). It was known that muscles rapidly adapt to a new functional length by adding sarcomeres in series at the ends of the existing myofibrils. However, if muscles are also subjected to electrical stimulation, they increase in girth and add more sarcomeres in parallel as well as in series. RNA was extracted while being held in the stretched position by plaster cast immobilization, increased in mass within one week (10). It was known that muscles rapidly adapt to a new functional length by adding sarcomeres in series at the ends of the existing myofibrils. However, if muscles are also subjected to electrical stimulation, they increase in girth and add more sarcomeres in parallel as well as in series. RNA was extracted from such muscles that were undergoing rapid growth, and by using differential display we detected an RNA transcript that is expressed in exercised but not in resting muscles (29). This mRNA was converted to cDNA and subcloned, and sequence analysis showed that this was a splice variant of the insulin-like growth factor-I (IGF-I) gene, although its 3’ exons were different from the liver or systemic type of IGF-I. However, it was noted that muscle also expresses the systemic type of IGF-I (IGF-IeA).

The terminology for the IGF-I variants is a problem when attempting to apply it to nonhepatic tissues and to different species. Also, we encountered a further problem with the hepatic IGF-I terminology, because the variant we discovered would be classified as IGF-IeB in the rat but IGF-IeC in the human (14). It became apparent that the muscle IGF-I isoforms, although they are derived from the same gene, have to be characterized separately. Therefore, we named this cryptic splice variant mechano growth factor (MGF), because it is expressed in response to mechanical stimulation and has a different carboxy peptide sequence from that of the liver type of IGF-I.
progenitor cells of the vasculature (26). It has been observed that MGF has a biological action, its cDNA was inserted into a plasmid vector with muscle-specific regulatory sequences. After a single intramuscular injection into the mouse, there was a 25% increase in mean muscle fiber cross-sectional area within 3 wk (9). Similar experiments have been carried out using the systemic or liver type of IGF-I using an adenoviral vector under the control of the myosin light chain (MLC) regulatory sequence that ensures muscle-specific expression (11). However, this took 4 months to produce a 15% increase and is probably due to the anabolic effect of IGF-I, which is common to all of the splice variants. Transgenic mice have been produced using IGF-IEa under the control of the chicken α-actin promoter (6) and later using the MLC I and III promoters (23), and perhaps it is not surprising that the expression of the introduced IGF-I is localized to muscle tissue. However, the structure of MGF and its high potency in initiating local hypertrophy indicated that it had a somewhat different function from that of other forms of IGF-I.

Replenishment of the Muscle Satellite (Stem) Cell Pool

Satellite cells in skeletal muscle were first described by Mauro (20), and it is now realized that these cells provide the extra nuclei for postnatal growth (22) and that they are also involved in repair and regeneration following local injury of muscle fibers (11). In normal adult undamaged tissue, the satellite cells are quiescent and usually detected just beneath the basal lamina. When activated, they commence to coexpress myogenic factors, including c-met, myoD, myf5, and, later, myogenin (26). Residual myoblasts are the main point of origin of satellite cells, although a small percentage may originate from pluripotent stem cells derived from progenitor cells of the vasculature (26). It has been established that even in normal muscle local injury does occur from time to time, but in certain diseases such as the muscular dystrophies, the muscle fibers are markedly more susceptible to damage, in particular to the membrane (5). The contractile system of muscle fibers also sustains damage during eccentric contractions (3). Therefore, there is an ongoing requirement in this postmitotic tissue for extra nuclei to be provided by satellite cells fusing with muscle fibers that are undergoing repair and/or adaptation.

The satellite cell pool undergoes periods of replenishment lasting just a few days (4). Several studies have been carried out in which IGF-I has been claimed to activate satellite cells, but the problem is that there are two distinct processes involved: one is the replication of the mononucleated residual myoblasts, and the other is the fusion of these with the muscle fibers. Ground et al. (25) found that overexpression of the liver type of IGF-IEa (MLC/mGF-I transgenic mouse) did not increase the myoblast (satellite cell) proliferation during regeneration of whole tissue grafts. It would not make physiological sense for IGF-I (mature IGF-I or IGF-IE) to be involved in the process of replenishing the muscle stem cell pool, since IGF-IEa, unlike MGF, is produced constitutively and is detected at reasonably high levels in non-mechanically challenged muscle and indeed in virtually all cell types, as well the liver. IGF-IEa may activate satellite cells depending on their state of developmental commitment, but it enhances fusion with the muscle fibers, thus leaving no reserve pool of satellite cells. This seems to be the particular role of the unique carboxy-terminal peptide of MGF, i.e., to replenish this stem cell pool for repair and growth throughout life.

Two recent studies have indicated that one of the special functions of MGF is to activate the muscle satellite (stem) cells for division. When IGF-I was transferred into, or was added to, C2C12 muscle cells in culture, they increased in mass and fused to

**FIGURE 1.** The insulin-like growth factor gene is spliced in muscle as a result of exercise and/or muscle damage and hormones

In human muscle, a 49-base insert changes the reading frame in mechano growth factor (MGF), resulting in a different carboxy peptide. Hormones upregulate the expression of the insulin-like growth factor (IGF)-I gene, particularly IGF-IEa; e.g., growth hormone (GH) apparently upregulates the primary RNA transcript of the IGF-I gene, and when combined with exercise training, it results in increased splicing to MGF. Human muscle also expresses an IGF-IEb form, but the function is not known. In all of these splice variants, the IGF receptor domain encoded by exons 3 and 4 is responsible for the anabolic effect of IGF-I, but in MGF the downstream peptide sequence acts as a separate growth repair factor.
form myotubes. When the cells were transfected with the MGF cDNA or treated with its carboxy peptide, the mononucleated myoblasts increased in number but remained 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. The other study (16) showed that after damage MGF is produced as a pulse lasting only a few days. Although it has been stated that IGF-I activates satellite cells, it was not certain from these other studies whether fusion rather than replication of satellite cells was examined or indeed what type of IGF-I was used. In our in vitro studies, MGF was involved in the replication of mononucleated myoblasts (satellite cells). The in vivo studies showed that the expression of MGF preceded that of markers of satellite cell activation. IGF-I expression did not peak, however, until the expression of the satellite cell activation markers had peaked and declined back to baseline levels.

Interestingly, it seems that myostatin is a negative regulator of satellite cell activation (19) and that this puts these residual myoblasts into the quiescent state. On the other hand, MGF appears to be the positive regulator because it responds to mechanical stimuli and/or damage that not only activates the muscle satellite (stem) cell proliferation but, because it also has an IGF-I receptor domain, upregulates protein synthesis generally.

Recently, Bamman et al. (18) found that down-regulation of myostatins by resistance loading was not correlated with the upregulation of cyclins, but there was a correlation with increased MGF expression; therefore, it seems that the replenishment of the stem cell pool is via positive regulation by MGF.

Effects of Exercise on MGF and IGF-I/Ea Expression

It is clear that the type of exercise training in which the active muscles must overcome high loads is the type of exercise that results in muscle hypertrophy and that this is associated with an increase in IGF-I expression (7, 28, 29). However, these studies failed to distinguish between the different IGF-I splice variants. As mentioned above, the way MGF was discovered was through the study of the RNA transcripts of exercised and nonexercised muscle (29). Shortly after this, Adams et al. (12) showed that MGF is expressed earlier than IGF-I/Ea in response to exercise. We also found that, following mechanical strain and/or muscle damage, the IGF-I gene is first spliced toward MGF and then later toward IGF-I/Ea in rodent muscles (16). We measured the mRNA levels of MGF and IGF-I/Ea by using real-time quantitative PCR on muscle biopsy samples taken 2.5 h after a single bout of high-intensity knee extension (13). In young subjects, MGF mRNA levels were significantly increased as a result of resistance exercise, but no significant change was observed in older subjects. Furthermore, at this time point shortly after exercise, IGF-I/Ea mRNA levels were virtually unchanged in both groups. These observations were interesting because they were in agreement with animal experiments for which MGF levels were shown to increase before those of IGF-I/Ea, suggesting that the two isoforms were differentially regulated and that they have a different function.

Extra Benefits of Exercise

As mentioned above, IGF-I/Ea is also expressed in skeletal muscle as well as liver and several other nonhepatic tissues. It has the same sequence as the main hepatic IGF-I isoforms derived from the class 1 and class 2 primary transcripts in muscle and liver, which are produced in almost equal proportions, and therefore it is assumed that both types of IGF-I have systemic actions. Exercise is also known to elevate the serum levels of IGF-I (2, 15) and is believed to provide the main anabolic response for the body as a whole. Interestingly, during intensive exercise the muscles not only produce more systemic IGF-I than the liver, but they apparently use more of the circulating IGF-I. The uptake from the serum seems to be a consequence of the greater abundance of the specific IGF-I/Ea binding proteins in trained muscle. The increase in circulating IGF-I is also believed to be one of the reasons why exercise is beneficial in that general tissue maintenance is improved. There is considerable evidence that IGF-I is involved in the maintenance of the central nervous system, and this may be the reason why regular exercise is claimed to improve cognitive function.

Muscle Loss in Aging

Sarcopenia (age-related muscle loss) is a serious healthcare and socioeconomic problem. The most obvious aspect of this is that when muscle function in the elderly declines, they are less able to correct their posture quickly enough, and as they cannot generate enough power 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 osteoporosis problem and a vicious circle is established. Another, perhaps less obvious aspect is the function of muscle as a metabolic store. In times of emergency it produces the proteins and metabolites required for survival. Hence the frail elderly person who has decreased muscle mass does not survive major surgery or a traumatic accident. We therefore attempted to see if MGF was involved in the age-related loss of muscle tissue.

Members of our group have studied the ability of muscles of different ages to produce MGF as well as IGF-II and its IGF-I receptor. In young rats in which the muscle was surgically overloaded, there was a marked increase in expression of MGF (FIGURE 2). In middle-aged rats the increase in MGF expression was moderate, and in old rats it was very low and attenuated (24). This was extended to human studies in which young men (29.5 ± 1.5 yr) and elderly men (78.0 ± 1.0 yr) carried out a single acute bout of 10 repetitions of knee-extension exercise and then muscle biopsies were taken from the vastus lateralis 2.5 h after the cessation of this exercise. MGF expression was markedly increased in the muscles of the young subjects but not in those of the older men (13). This helps to explain the loss of muscle in the elderly. Older people can increase muscle mass by training; however, it apparently tends to take much longer than for teenagers, and the data is sometimes misleading when it is expressed as a percentage increase because the baseline for the elderly is much lower at the start of the training procedure.

One of the other marked growth factor changes associated with aging is the decline in circulating growth hormone (GH) levels, which in old age are only one third of that in our teenage years. Because GH is responsible for inducing IGF-I in the liver, we carried out some experiments (in cooperation with Kjaer et al.; Ref. 15) in which elderly males were given GH or a placebo (15). Some were subjected to a resistance exercise training regime for 5 or 12 wk, and in some groups this was combined with GH therapy. In the latter groups, MGF levels were markedly increased, as was muscle cross-sectional area. This indicates that the primary transcript of the IGF-I gene is probably upregulated by GH so that more is spliced toward MGF as a result of exercise. Interestingly, GH treatment alone resulted in upregulation of IGF-IIa, which is similar to what happens in the liver. GH alone did not produce a marked upregulation of MGF unless combined with exercise. This is in accord with the findings of Thorner et al. (17), who found that MGF is preferentially induced by GH in GH-deficient mice. It appears that to effectively prevent muscle loss in the elderly, the GH deficiency must also be treated (FIGURE 3). Alternatively, MGF could be administered instead of the GH, particularly for elderly people who are initially incapable of exercising. Muscle mass might then be maintained at a reasonable level once they become physically active again.

**Disease-Related Muscle Loss**

Muscle loss is one of the major causes of death in patients with certain neurological diseases. In some cases, the muscle loss can be linked to the inability to express MGF (9). We found that muscles of the mdx dystrophic mouse, which is a model for human Duchenne muscular dystrophy, are unable to respond to mechanical stimuli by splicing the IGF-I gene toward MGF. Measurements in human
Cardiomyocytes transfected with adenoviral vector

A IGF-IEa cDNA  
B MGF cDNA

MGF peptide expression using immunostaining in levator ani muscle
C After easy delivery  
D After difficult delivery

patients with autosomal dystrophies also showed that they have a problem upregulating MGF following exercise. Indeed, the levels in their muscles were not significantly different from zero and are below those of very elderly men, who are also losing muscle. The production of MGF must presumably, directly or indirectly, involve some type of mechanotransduction mechanism. The reason this is defective in dystrophy is not known, although it may be associated with the defective cytoskeletal complex. In the case of Duchenne muscular dystrophy, the most severe form of muscular dystrophy, the large dystrophin protein is missing from this complex. In the autosomal dystrophies, other proteins are missing, and in some cases these anchor the dystrophin complex to the extracellular matrix of the β-actin and myofibrils. Therefore it is possible that this cytoskeletal complex acts as a mechanotransduction system, as it seems to be too elaborate a structure just for stiffening the membrane. Support of this mechanotransduction function was recently provided by Layten et al. (8). They found that when mesenchymal stem cells were introduced into dystrophic muscles of the mdx mouse, this restored the sarcolemmal expression of dystrophin and reinstated the expression of MGF. Because of the absence of dystrophin, the muscle fibers are more susceptible to damage than normal muscle. When damage occurs in normal muscle, repair is apparently initiated by MGF, but this is not so in dystrophic muscle; hence, there is presumably an inability to renew the satellite cell pool, resulting in a progressive loss of muscle fibers.

Regulation of MGF in Skeletal and Cardiac Muscle

It was known that the myocardium expresses higher levels of IGF-I following ischemia or mechanical overload, as shown using a general IGF-I antibody. However, we recently found that the type of IGF-I that is upregulated following a major infarct is MGF. In vivo studies of ischemia and mechanical overload indicate that MGF increases the percentage of the viable tissues of the myocardium. This is in accordance with the finding that MGF is expressed rapidly after tissue damage and that it prevents apoptosis of cardiomyocytes. Investigation if its role in remodeling the surviving myocardium tissues following a major infarct is now underway as part of a study concerning the effects of MGF on cardiac muscle. Modus Operandi of MGF

By transfected cardiomyocytes in culture using the green fluorescent protein sequence attached to the 3’ end of the cDNAs of MGF and IGF-IEa, it was found that the MGF peptide, which may be just the carboxy-terminal peptide, becomes rapidly localized in the cytoplasm, whereas the IGF-IEa peptide is localized throughout the cytoplasm (FIGURE 4, TOP). This is in accord with Chew et al. (27), who located a nuclear signal in exon 5 of the IGF-I gene. The IGF-I receptor domain appears to have signaling sequences in exons 1 and 2 (21, 27), but these are not nuclear signals and thus IGF-IEa remains in the extracellular circulation where it acts on the IGF-I receptors in the plasma membrane of muscle and other tissue. Our recent work has shown that the carboxy-terminal peptide of MGF not only has a different signaling pathway from that of the IGF-I receptor domain, but it also has a short half-life in the extracellular compartment, presumably due to proteolysis. Also, by using Western blotting we
found that MGF does not bind to the known IGF-I binding proteins that stabilize IGF-I in muscle as well as in the blood. Therefore, because it is produced as a pulse after exercise/damage and does not survive in the extracellular compartment for any appreciable length of time, it has thus to be regarded as an autocrine growth factor. The particular morphological niche of the satellite cells, i.e., under the basal lamina, probably means that they are close enough to be activated by MGF:

A monoclonal as well as a polyclonal antibody has been made to the carboxy-terminal peptide of MGF and its association with mechanical stress, including damage to the pelvic floor musculature following childbirth, has been confirmed in several studies. The immunostaining of lavator ani muscle biopsies from women following difficult labor were found to have markedly upregulated MGF (FIGURE 4, BOTTOM). These muscle tissues showed an increase in MGF mRNA expression following delivery that was two orders of magnitude higher than that in the lavator ani muscle of nonpregnant women who acted as controls.

The human genome has just been sequenced, and somewhat to our surprise there are only about 35,000 genes required to code for a human being. Yet there are many more different proteins. This greater number of proteins is because a good number of genes are spliced to give rise to several separate messages, which are in turn translated into different proteins. By combining physiological studies with molecular biology techniques, we are beginning to understand the signals, including mechanical signals, that are involved in not only switching on or switching off certain genes but also in directing the splicing to give one or more different gene products. One example of this gene splicing is MGF: a growth/repair factor that apparently “kick starts” muscle hypertrophy of skeletal muscle and is involved in the local cellular repair process. It is also expressed in other postmitotic tissues and is neuroprotective as well as cardioprotective. Its expression has been found to be much reduced in certain diseases and during aging. Hence MGF may have considerable therapeutic potential, and therefore it is important to understand the mechanical signaling system involved and its physiological actions.

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References


