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Review

The Role of the Insulin-like Growth Factor 1 (IGF-1) in Skeletal Muscle Physiology

ANASTASSIOS PHILIPPOU[1,2], MARIA MARIDAKI[2], ANTONIS HALAPAS[1] and MICHAEL KOUTSILIERIS[1]

[1]*Department of Experimental Physiology, Medical School and*
[2]*Department of Sports Medicine and Biology of Physical Activity,*
Faculty of Physical Education and Sport Science, National and Kapodistrian University of Athens, Greece

Abstract. *The human insulin-like growth factor-1 (IGF-1) gene gives rise to multiple, heterogeneous mRNA transcripts through a combination of multiple transcription initiation sites, alternative splicing and different polyadenylation signals. These IGF-1 mRNA transcripts code different isoforms of the precursor peptide of IGF-1 (IGF-1Ea, IGF-1Eb and IGF-1Ec or MGF in human skeletal muscle), which also undergo post-translational modification. There is increasing interest in differential expression and implication of IGF-1 isoforms in the regulation of muscle fiber regeneration and hypertrophy following mechanical overloading and damage. The identification of a locally expressed, loading- or damage-sensitive IGF-1 isoform in skeletal muscle was one of the most attractive developments in the context of the autocrine/ paracrine actions of IGF-1. The concept that the competing processes of cellular proliferation and differentiation and the increased protein synthesis required for muscle repair or hypertrophic adaptation are regulated by a differential expression and by distinct roles of IGF-1 isoforms is discussed in the present review.*

There is a growing awareness that in certain cell types generally called mechanocytes, such as osteoblasts and skeletal muscle cells, gene expression is greatly influenced by mechanical signals (1, 2), although very little is known about the mechanisms linking the mechanical stimulus with gene expression. Similar to bone, skeletal muscle has the ability to adapt to an increased mechanical load by changing its mass and phenotypic expression *via* mechanisms that seem to be intrinsic to the muscle (3-5). Exercise is one of the most powerful stimuli for inducing structural, metabolic and functional re-organization of skeletal muscle cells. Research has mainly focused on the phenotypic nature of these adaptations and disproportionately fewer studies have systematically examined the role of specific gene expression or the expression profile of various genes in skeletal muscle adaptability (6-9) (see also review by Fluck and Hoppeler 10). It has been recognized (5, 11) that overexpression of certain growth factors largely mediate the intrinsic ability of skeletal muscle to develop hypertrophy in response to mechanical overload (12, 13) and to regenerate in response to metabolic or mechanical damage following unaccustomed or excessive exercise (5, 14, 15).

Cellular processes of myofiber regeneration and hypertrophy are enabled by the activation, proliferation and subsequent differentiation of quiescent mononuclear muscle stem cells (also called satellite cells or muscle precursor cells). These processes appear to be modulated by the autocrine (*i.e.* direct stimulation of protein synthesis) and/or paracrine (satellite cell proliferation, differentiation and fusion) activity of locally produced insulin-like growth factor 1 (IGF-1) (3, 11). In particular, there is an increasing interest in differential expression and implication of IGF-1 isoforms (IGF-1Ea, IGF-1Eb and IGF-1Ec) in the regulation of muscle fiber regeneration and hypertrophy following mechanical overloading and damage, however, this topic has received less notice in the literature.

In the present review, the focus has been directed on the specific context of such a potentially differential role of IGF-1 on skeletal muscle regeneration and adaptation.

IGF-1 Isoform Complexity

The human IGF-1 gene originally called somatomedin C spans a region of about 90 kb of genomic DNA (located on

Correspondence to: Dr. Michael Koutsilieris, MD, Ph.D, Department of Experimental Physiology, Medical School, National and Kapodistrian University of Athens, 75 Micras Asias St., Goudi-Athens, 115 27, Greece. Tel: +30210 7462597, Fax: +30210 7462571. e-mail: mkouts@medscape.com

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the long arm of chromosome 12) and contains six exons (16), which give rise to multiple, heterogeneous mRNA transcripts by a combination of: a) multiple transcription initiation sites (alternative promoter usage), b) alternative splicing and c) different polyadenylation signals (17, 18). These multiple IGF-1 mRNA transcripts code different IGF-1 isoforms of the precursor peptide of the IGF-1, which also undergo post-translational modification (19).

More specifically, the different transcription initiation sites lead to two different classes of IGF-1 isoforms: class 1 transcripts have their initiation site(s) on exon 1 (promoter 1), whereas class 2 uses exon 2 as leader exon (promoter 2). Exons 1 and 2 are differentially spliced to the common exon 3, producing class 1 (exon 1 to exon 3), or class 2 (exon 2 to the exon 3) mRNA transcripts. All possible combinations between promoter usage and terminal exon (5 or 6) can occur in different transcripts, *i.e.* a class 1 but also a class 2 IGF-1Ea and IGF-1Eb isoforms can be expressed (20, 21), as described in detail below. Transcripts initiating at promoter 1 are widely expressed in extra-hepatic tissues (local isoforms), whereas transcripts initiating at promoter 2 are common in the liver (circulating isoforms), which are thought to be more growth hormone-dependent (19, 22). However, it is possible that the two promoters are not mutually exclusive and the production of more primary transcripts by certain hormones could probably result in even higher expression of local isoforms (22, 23). It has been reported that the use of promoter 1-derived transcripts could be associated with the synthesis of paracrine IGF-1 and may promote the formation of the truncated IGF-1 peptide or influence interactions with IGF binding proteins (IGFBPs) (24).

Alternative splicing of the IGF-1 gene, whereby exons are spliced in different combinations from the primary RNA transcript, also results in different IGF-1 isoforms that contain either exon 5, generally classified as "IGF-1Eb", or exon 6 and are classified as "IGF-1Ea" (21, 24). Transcripts that containEb are thought to be more abundant in the liver, whereas transcripts contain either Ea are usually expressed in extra-hepatic tissues (25). Use of exon 5, *i.e.* the IGF-1Eb isoform, may be linked to an endocrine role of IGF-1, while the preferential use of exon 6, *i.e.* IGF-1Ea isoform, may be associated with local IGF-1 function (26).

In human skeletal muscle as well as in other tissues or cell lines, *e.g.* human liver, HepG2 cells (27), alternative splicing of the IGF-1 gene also generates a third isoform, the IGF-1 Ec, which corresponds to IGF-1Eb in rodents and contains both exon 5 and 6 (27). It results from an splice acceptor site in the intron preceding exon 6 and its cDNA differs structurally from its liver IGF-1Ea isoform by the presence of the first 49 base pairs from exon 5 (52 bp in the rat). It results in a different carboxy (C)-terminal peptide sequence due to a reading frame shift, which leads to a different mode of action compared with IGF-1Ea or IGF-1Eb isoform (23, 28, 29). This isoform named mechano growth factor (MGF) because it was found to be markedly up-regulated in exercised and damaged skeletal muscle (17, 22, 30, 31).

The biological significance of IGF-1 splice variants is unknown and the molecular and physiological mechanisms that regulate their expression are unclear, however, they probably reflect an underlying complexity of IGF-1 actions through its various isoforms (42). The IGF-1Ea isoform is similar to the main systemic (endocrine) IGF-1Ea produced in liver, with a similar exon sequence (32), while MGF seems to be designed for autocrine/paracrine mode of action (28), (discussed in detail below). The expression of IGF-1Eb isoform was first defined in the liver (33) and recently in the human skeletal muscle (23), however, its role in muscle is yet unknown.

The different IGF-1 mRNA transcripts encode several IGF-1 precursor proteins, which differ in the length of the amino-terminal (signal) peptide and the structure of the extension peptide (E-peptide) on the carboxy-terminal end (19).

IGF-1 polypeptides also contain a B amino-terminal domain, A and C domain and a D carboxy-terminal domain, sharing 62% homology with proinsulin which does not contain the D carboxy-terminal domain. The coding information for the mature IGF-1 peptide resides in the B, C, A and D domains (34, 35). The mature IGF-1 peptide is a 70-amino acid long single chain polypeptide with three intra-chain disulfide bridges and molecular weight 7,649 Da (19). It results from post-translational modification through protease cleavage of precursor polypeptides, by which the signal and the E-peptide are removed. It has been proposed that the E-peptide of the IGF-1 precursors may act as independent growth factor (36). The 5' end of exons 1, 2 and 3 encodes for the signal peptide of the prohormone (precursor IGF-1), since they all contain distinct translation initiation codons (AUG). In rodents and probably in humans, as the structure of the IGF-1 gene is highly conserved in mammals, exon 1 can also undergo alternatively undergo spicing (start sites 1 and 2), thereby further increasing the heterogeneity of IGF-1 mRNA transcripts (37). Exons 3 and 4 code the mature peptide. The first 16 amino acids of the E-peptide (*i.e.* the amino-terminal portion of the extension peptide) are coded by exon 4. Exons 5 and 6 encode, by alternative splicing, distinct portions of the E-peptide (called the E domain) with alternative carboxy-terminal sequences of the extension peptide and they also contain termination codons and 3'-untranslated regions. Exon 6 contains different polyadenylation sites and in humans, as opposed to rats, exon 5 also has a polyadenylation site (27). The presence of several polyadenylation sites in the 3'-untranslated sequence of the exon 5 and 6 introduces further post-transcriptional modification of the IGF-1 mRNA transcripts, which have different sizes. The length of the IGF-1 mRNAs appears to inversely influence their half-life (19, 24).

Post-translational cleavage of the IGF-1 precursor protein produces the signal, the mature and the E-peptide and two other protein products that have been identified in the human brain: a truncated IGF-1 isoform (-3N:IGF-1) that lacks the first tree amino acids of the NH2-terminal of the B domain probably due to alternate signal peptides, and the tripeptide GPE (glycyl-prolyl-glutamate) corresponding to the aminoterminal end of mature IGF-1 (38).

Four different transcription start sites are present in exon 1. Three of them have been studied (sites 2, 3 and 4) and their positions relative to the translational initiation codons (*i.e.* Met-48 and Met-25 located in exon 1, and Met-22 located in exon 3), can give rise to three distinct signal peptides from class 1 (exon 1 to exon 3) mRNA of the IGF-1 gene (see excellent review 19). Between two translation start sites contained in an mRNA, preference is given to the upstream site (39). Translation of mRNAs initiated at the three transcription start sites mentioned above produced IGF-1 precursor polypeptides with 48, 25 and 22 amino acids long signal sequences (19, 32, 39). From class 2 (exon 2 to exon 3) transcripts, three transcription start sites and their upstream position relative to the translational initiation codon Met-32 (which is located in exon 2) give rise to IGF-1 precursor polypeptides with 32 and 22 amino acid-long signal sequences (19, 39). It has been found, both *in vitro* and *in vivo*, that translational efficiency of IGF-1 mRNA and post-translational modification of the IGF-1 precursor peptide of rats are affected by the length and the structure of the 5'-untranslated regions: the smaller the length of the 5'-untranslated regions, the greater the translational efficiency, and the greater the number of the amino acids contained in the signal peptide the lesser is the extent of a glycosylation process (39, 40).

Alternatively, the splicing of exons at 3'-end of IGF-1 mRNA precursors increases the variety of IGF-1 transcripts and IGF-1 isoforms translated from these transcripts. Three mRNA variants, produced by alternative splicing of the 3'-end, have been identified in humans encoding three different E-peptides. Exon 4 to exon 6 splicing leads to an mRNA sequence that encodes the Ea-peptide, which contains 35 amino acids. The first 16 amino acids, which are common in all three different E-peptides, are encoded by the exon 4 and the remaining 19 are encoded by exon 6. The human Ea-peptide shares 91% homology with the mouse Ea-peptide (19, 32). Splice variant of exon 4 to exon 5, firstly identified in human liver (33) and more recently in skeletal muscle (23), yields the Eb-peptide (19). This extension peptide contains, apart from the 16 common amino acids encoded by the exon 4, 61 additional amino acids encoded by exon 5 resulting in the Eb-peptide, with a 77 amino-acid length. This isoform of the IGF-1 precursor appears to have a nuclear and nucleolar localization signal (reviewed in 19). The third mRNA splice variant is an exon 4-5-6 variant, also first identified in human liver (27) but in skeletal muscle as well (28). It contains exon 4, only 49 bp from exon 5 and then exon 6 and produces an E-peptide with a predicted length of 40 amino acids, *i.e.* 16 amino acids from the exon 4, 16 from the exon 5 and 8 amino acids from the exon 6, termed as Ec-peptide (19, 27). The human Ec extension peptide shares 73% homology with the rat Eb-peptide, which is its potential counterpart (27). It was thought to occur by use of a cryptic IGF_{633} donor splice site, located 49 bp downstream from the 5'-end of the exon 5 and deviates from the vertebrate 5'-donor splice site consensus. When this cryptic IGF_{633} donor splice site is not used, the alternative splicing of exon 4-5 occurs, *i.e.* the Eb extension peptide (27). The predicted molecular weights of the three different isoforms of the IGF-1 precursor peptide (based on the amino acid sequence derived from the three different IGF-1 mRNA transcripts) are: 17-17,3 kDa (153-156 aa) or 20,6 kDa (184 aa) for the pre-pro-IGF-1Ea, 21,8 kDa (195 aa) for IGF-1Eb pre-pro-peptide and 15,6 kDa (139 aa) for pre-pro-IGF-1Ec. Finally, the existence of an N-linked glycosylation site in the Ea-peptide which is absent in the Ec-peptide might reflect a differential biological action of the IGF-1Ea isoform mediated by this post-translational modification of the Ea-peptide (19, 34).

In general, the complexity introduced by the transcriptional and splicing variants, post-transcriptional regulation and post-translational modifications of the IGF-1 gene (41), giving rise to various IGF-1 isoforms, probably indicates their different biological roles, particularly in human skeletal muscle following different stimuli or under various conditions.

One of the most attractive aspects recently developed is the potential differential role of the IGF-1 isoforms in muscle regeneration and adaptation processes following skeletal muscle overload and damage (3, 4, 42). There is evidence from animal studies that different modes of exercise (*i.e.,* lengthening, shortening or isometric contractions) result in differential expression responses of IGF-1 isoforms and in similar levels of compensatory hypertrophy (43). It will be of interest to determine if all isoforms of IGF-1 activate satellite cell proliferation, gene transcription, or protein translation, and if they act through the same or distinct signalling pathways. Therefore, there is a need for a more systemic approach to IGF-1 isoforms in the context of IGF-1 autocrine/paracrine and systemic action in skeletal muscle.

Autocrine/Paracrine Action of IGF-1 in Skeletal Muscle

Two major contexts have been developed in the literature concerning the role of IGF-1 in skeletal muscle: a) IGF-1 as a component of the growth hormone (GH)/IGF-1 axis in the regulation of muscle growth *via* metabolic and anabolic actions of IGF-1 and b) the role of IGF-1 in mitogenic and myogenic processes during muscle development, regeneration or hypertrophy (3, 5, 11, 19, 44-47).

With respect to the GH/IGF-1 axis, circulating or locally produced IGF-1 in target tissues mediates the muscle growth-promoting actions of GH (23, 48-51), although there is evidence that these GH actions on skeletal muscle occur at least partly independently of IGF-1 up-regulation (52). Circulating IGF-1 is mostly derived from the liver, but also from skeletal muscle and adipose tissue (53), and is thought to act in an endocrine manner, while circulating GH mainly controls its levels. However, circulating IGF-1 should be considered as a marker of GH action in the liver rather than a "second messenger" of GH action (54). Regulation of local production of IGF-1 may be tissue-specific concerning the molecular mechanisms by which its synthesis is modulated (18). The sustained local overexpression of IGF-1 was shown to promote myofiber regeneration and hypertrophy and to increase levels of myogenic regulatory factors and contractile protein mRNAs (46, 55, 56). On the other hand, the increase of serum IGF-1 with exogenous administration of GH or IGF-1 does not appear to stimulate myofiber hypertrophy in the absence of mechanical loading (57). During intensive exercise, most of the circulating IGF-1 is derived from the active muscles (4, 52), while following muscle damaging exercise, locally produced IGF-1 by the exercised muscles was found to increase with concominant increases (58), or no changes (59) in circulating IGF-1. Furthermore, the proportional expression of different IGF-1 isoforms may be regulated by the local concentrations of growth hormone (GH) and IGF-1 itself (23, 24).

An interesting aspect in the context of autocrine/ paracrine function of IGF-1 is whether the overexpressed IGF-1 protein by the muscle cells is available only to the cells from which it derives (autocrine action) or whether this protein acts on the adjacent cells (paracrine action), *e.g.* on the muscle satellite cells, as it was proposed (17, 60), or even enters the circulation acting in an endocrine manner (19). In the case of autocrine/paracrine action, IGF-1 should leave the muscle fiber to bind IGF-1 receptors on the external surface of the membrane of the same muscle cell or activate a signaling pathway *via* a different receptor, as it was proposed for MGF in satellite cells or myoblasts proliferation (17, 60). Moreover, it would be essential to know whether the IGF-1 isoforms expressed in human skeletal muscle are finally released in the circulation as different (IGF-1 Ea, Eb and Ec) peptides, or whether the final peptide that enters the circulation after an extracellular endoproteolysis of the IGF-1 prohormone (18), is only the mature IGF-1 peptide.

In general, use of exon 5 (IGF-1Eb isoform) may favor an endocrine role of IGF-1, while use of exon 6 (IGF-1Ea isoform) may be associated with local, autocrine/paracrine IGF-1 action (26). As mentioned above, the main systemic (endocrine) IGF-1 isoform is (class 2) IGF-1Ea, which is a predominant isoform expressed in liver and is similar to the predominant (class 1) IGF-1Ea isoform locally expressed in skeletal muscle and in other tissues (22, 46). Probably, the latter also has a systemic or endocrine mode of action (4). To distinguish this particular isoform expressed in muscle from the main systemic IGF-1 isoform, terms such "local muscle-specific" (mIGF-1) isoform (42, 46) or "muscle liver type" (L.IGF-1) isoform (22, 29) have also been adopted.

In contrast to the aspect of GH-dependent action of IGF-1, the context of GH-independent, autocrine/paracrine function of IGF-1 on skeletal muscle has recently been widened (3, 4, 61). One of the most attractive developments in the concept of the autocrine/paracrine signaling regulated by IGF-1 was the identification of a locally expressed IGF-1 isoform in skeletal muscle in response to mechanical damage or in response to changes in the loading state of muscle (*e.g.* mechanic overload), called the mechano growth factor (MGF) (22, 28, 29). This isoform is encoded by the exon 4-5-6 splice variant and, although its signal peptide has not been specified, it presumably corresponds to the human IGF-1 Ec isoform (19). It was hypothesized that MGF is probably the end product of mechanotransduction signaling pathways generated by and imposed upon skeletal muscle, however, questions about the specific exercise-induced stimuli that up-regulate MGF expression have not yet been answered, *e.g.* is muscle fiber membrane damage or mechanical overloading of muscle fiber responsible for the MGF production (30)? Nevertheless, it has been shown that skeletal muscle stretch is a main mechanical stimulus for changing gene transcription (62) and up-regulation of protein synthesis (63). This isoform was markedly up-regulated in response to both overload and damage and was thought to be the factor that controls local tissue repair, remodeling and maintenance (4, 30). However, expression of MGF has also been detected in resting skeletal muscles of humans (and rats), though at much lower levels than IGF-1Ea (mIGF-1) (22, 64), or following immobilization (30). It has also been reported that a single ramp stretch resulted in MGF expression, whereas a lower cyclical stretch regimen resulted in the expression of IGF-1 Ea but not of MGF (4). In general, the absolute mRNA levels of MGF in skeletal muscle appear to be much lower than those of IGF-1Ea (17, 22). Taking all these data into account, it could be suggested that the expression of MGF in muscle must be examined in more detail. Moreover, a third IGF-1 isoform, IGF-1Eb, was also found to be expressed in human skeletal muscle in response to mechanical overloading. IGF-1Eb mRNA encoded by the exon 4-5 splice variant was found to be up-regulated following resistance training (23). However, further studies are required in order to elucidate its specific biological role in skeletal muscle.

It should be emphasized that skeletal muscle produces both a generalized tissue-type IGF-1Ea isoform and the loading- or damage-sensitive MGF with differential regulation at the RNA level and different time courses, suggesting distinct roles of these isoforms (17, 64, 65). Unlike the IGF-1Ea isoform, MGF is not glycosylated, therefore it is smaller and probably has a

shorter half-life and seems to be designed for an autocrine/paracrine mode of action (4, 28). C-terminal MGF peptide lacks the domain present in the full-length peptide responsible for the binding of the IGF-1 receptor (66). It was found that the MGF E domain is probably mediated *via* a different receptor, since its action was not inhibited by blocking the IGF-1 receptor with a specific antibody (60). A synthetic peptide, which was generated from a unique region of MGF sequence, was used to raise a polyclonal antiMGF antibody in the rabbit. This antibody was found by immunoblotting to be specific only to MGF and not to IGF-1Ea (mIGF-1) (30). Furthermore, by using a proteomics approach, the specific E domain of MGF splice variant was found to result in binding to a different binding protein and so to a different mode of action compared to the IGF-1Ea isoform. The muscle-specific protein that binds MGF is expected to stabilize it and to localize its action within the muscle (since it would be unstable in the unbound form) and also to acts as a time-release mechanism (4, 30). In a bound state, MGF peptide was also detectable in resting muscles, probably as a residual MGF from an earlier muscle activity, reflecting the delayed release function of the MGF binding protein (4). Taking all these factors into account, it was argued that MGF acts as an independent autocrine factor (4, 60).

The fact that cells in the overloaded skeletal muscle apart from the IGF-1 also produce other factors of the IGF-1 regulatory system, such as IGFBPs, gives further support to the concept that an autocrine/paracrine IGF-1 system is active in skeletal muscle and is sensitive to the loading conditions of the muscle (11, 31, 64, 67). IGF-1 action is modulated by a family of seven IGFBPs (42). In overloaded or damaged skeletal muscle and in cultured myoblasts, IGFBB3 expression was associated with myoblast differentiation, IGFBP-4 with myoblast proliferation, IGFBP-5 with muscle differentiation and IGFBP-6 seems to play a role during the quiescence phase of myogenesis (42, 44, 59, 68). In general, IGFBPs increase the IGF-1 half-life in the circulation and would be expected to modulate and control the extent of IGF-dependent cellular effects *via* regulation of the IGF-1 free concentration and its local bioavailability in the muscle, probably *via* competition with type I IGF receptor (IGF-1.R for IGF-1 binding. They also provide tissue specificity for the local action of IGF-1 (5, 42, 59, 69, 70).

There is evidence that locally produced autocrine/paracrine IGF-1 may be important in the skeletal muscle regeneration process. The known effects of IGF-1 on skeletal muscle cells will be examined below in order to provide insight into the potential role(s) of this factor in muscle regeneration. Some of the processes that are known to be stimulated by IGF-1 during myofiber regeneration also promote muscle adaptation and hypertrophy following muscle overloading11, since it was observed that over-expression of IGF-1 causes hypertrophy (46, 62).

IGF-1 immunoreactivity was detected in satellite cell and in the cytoplasm of myoblasts and myotubes during skeletal muscle regeneration (71-73). IGF-1 gene expression is low in myoblasts and decreases slightly with their differentiation (74). It has been proposed that a slight decrease of IGF-1 gene expression in myoblasts during differentiation may be related to the reduced IGF-1.R gene expression. Thus, the mitogenic effect of IGF-1 may first delay the onset of myogenesis and subsequently stimulate myogenesis and promote differentiation (5, 74, 75). It appears that IGF-1 can stimulate myogenesis in the absence of proliferation (76). As mentioned above, in the case of exercise-induced adaptation and hypertrophy response, it seems to be a myogenic component, where myoblasts derived from satellite cells fuse with the existing myofibres, as they would with the damaged myofibres following injury (77-79). The requirement for additional nuclei to support both the regeneration and hypertrophy processes appears to be met *via* the proliferation, differentiation and fusion of satellite cells with the damaged, but still viable, myofibres or with the enlarging myofibres (80-82).

Among the well-characterized growth factors, only IGF-1 was consistently reported to promote each of these processes (11, 44). Increased mechanical loading or damage of skeletal muscle leads to satellite cell proliferation, differentiation and fusion and these myogenic processes in skeletal muscle are stimulated by IGF-1. It is postulated that IGF-1 isoforms are produced and released by myofibres in response to increased loading or muscle damage (3). It has also been shown that IGF-1 was produced by satellite cells in regenerating skeletal muscles (72). A relatively acute, but not chronic, overexpression of IGF-1 was shown to increase the number of times that satellite cells can proliferate *via* PI3K signal transduction pathway (83, 84).

IGF-1 gene and, more specifically, its isoform expression is seen as an early event in exercise-induced muscle overload or damage (17, 22, 23, 79) and IGF-1 mRNA and protein were detected in newly replicating rat skeletal muscle following injury (85). A general, unspecific effect of IGF-1 on muscle metabolism is the stimulation of protein synthesis, by acting rapidly on the rate of polypeptide chain initiation and by a slower action on mRNA synthesis (86). Furthermore, different roles for the MGF and the IGF-1Ea (mIGF-1) isoforms were suggested in the processes of myoblast proliferation and differentiation (60). To examine the biological activity and the potential local action of MGF, a plasmid gene construct containing MGF cDNA was transferred into myocytes in culture and also by intramuscular injection *in vivo*, under the control of muscle-specific regulatory sequence. It was found that, two weeks after direct injection, the mean fiber size of the injected muscle increased by 25% and its wet weight by approximately 20%

compared with the noninjected contralateral muscle or other controls (30, 87). In contrast, viral introduction of IGF-1Ea (mIGF-1) took 4 months to result in a 20% increase in muscle mass (88), indicating that MGF is more potent than IGF-1Ea in initiating and enhancing muscle hypertrophy (89). In culture experiments with C2C12 mouse myoblast cell line, a predicted synthetic MGF peptide was used and the muscle cell lines were transfected with either the MGF or IGF-1Ea (mature human IGF-1) peptide (60). It was found that MGF activated and proliferated mononucleated myoblasts 3 days post-transfection, but prevented their fusion to form myotubes. However, it should be noted that such a synthetic MGF peptide was reported to be rapidly degraded in serum or tissue fluids (66). To date, there is very little information or evidence regarding the presence of the MGF peptide *in vivo*, since there was only a mention of the detection of MGF peptide in a bound state with its bounding protein in muscles (4). On the other hand, it was found in the same study that IGF-1Ea increased cell proliferation, although at a lower rate than MGF and also promoted their terminal differentiation into myotubes (60). In other experiments, it was shown that mIGF-1 (IGF-1Ea) also enhance stem cell-mediated muscle regeneration (46, 90).

Both *in vivo* and *in vitro* studies revealed that MGF was rapidly activated and subsequently depressed in damaged or overloaded muscles, indicating that this IGF isoform, and more specifically its E domain, probably act as a separate growth factor (60). It was suggested that it is responsible for satellite cell activation (17, 65), for the prolongation of myogenic cell proliferation and for depression of their terminal differentiation into myotubes (22, 60). In contrast, IGF-1Ea appeared to have a more delayed expression profile, to increase the mitotic index, to enhance terminal differentiation and to promote fusion of the myogenic cells (17, 60). It has been shown that post-mitotic expression of IGF-1 induced dramatic morphological changes in hypertrophic myofibres, with accumulation of actin and formation of nuclear rings within the body of fibres (76). Both, the MGF and IGF-1Ea isoform, appear to up-regulate protein synthesis since they both share the same mature peptide (exon 3 and 4) which promotes protein synthesis. This role is mediated predominantly by IGF-1Ea, since most of the mature IGF-1 peptide is derived from IGF-1Ea, whereas the E domain of the MGF peptide is thought to be involved in the activation of satellite cells (17, 22). It was suggested that MGF is much more potent than IGF-1Ea in inducing rapid hypertrophy (17).

It was found in animal studies, that these two IGF-1 isoforms not only act as different growth factors, with apparently different function, but that they also have different expression kinetics (17, 22, 64). The MGF transcript showed a relatively rapid increase and peaked 24 h, probably even earlier, following mechanical (or chemical, by bupivacaine injection) muscle damage (17) or mechanical overloading of muscle (64) and it declined within a few days, whereas IGF-1Ea expression peaked 7 and 2 days following muscle damage (17) and mechanical overloading (64), respectively. The initial pulse of MGF expression following muscle damage was thought to activate the satellite cells, providing the extra nuclei required for the repair process, while an up-regulation of protein synthesis also is promoted. The later expression of IGF-1Ea further up-regulates protein synthesis to complete the repair and/or the hypertrophy process (17, 91). Moreover, it was suggested that the impaired regenerative ability of old skeletal muscle is probably a result of the down-regulation of IGF-1 system and particularly the decreased MGF mRNA responses to muscle overloading and the lower levels of IGF-1.R compared to young muscles (65). However, IGF-1.R number, affinity and binding capacity were found to increase in skeletal muscle of old mice following exercise (92).

The imposition of a second bout of resistance exercise on skeletal muscle was shown to result in sustaining increases of MGF and IGF-1Ea transcripts. These increases were more pronounced where the rest intervals between the two exercise bouts were longer (*i.e.* 48 h *versus* 24 h or 8 h rest) (64). These results indicated a likely summation of IGF-1 responses to the repeated stimuli, with a potential optimum time-point to perform a second bout so as to facilitate greater responses (64). It was hypothesized that signaling mechanisms may enter a refractory period after an exercise bout that blunts the response to a shortly repeated second bout, whereas an optimum rest interval between the exercise bouts protects muscle (64). It was reported that long-term (12 weeks) voluntary exercise did not affect the expression levels of IGF-1 but decreased its circulating levels in rats (93). These findings were attributed to a marginal role of IGF-1 in the adaptation process of rat skeletal muscle during chronic exercise (93). In another study, using gene-array technology for simultaneous analysis of the expression of 184 different genes in human skeletal muscle following cycling exercise, it was found that more than 85% of the analyzed genes reducted in abundance. It was suggested that an inhibition of the transcription process or a degradation of mRNA might occur during or after the exercise, before initiation of the selective transcription initiates (94).

It is important to point out that, current studies provide information on IGF-1 isoforms mainly at the mRNA level, since the available techniques restrict or do not allow the identification and differential quantitation of the different IGF-1 isoforms at the protein level. The development of epitope-specific antibodies for distinguishing the different IGF-1 peptides and both their production and distribution is

needed for a more definitive analysis of IGF-1 expression in skeletal muscle following different exercise stimuli (19).

In humans, there is little information about the expression kinetics of IGF-1 or its different isoforms following exercise-induced mechanical overloading or damage of skeletal muscle, since the potential to observe the responses over time requires a number of biopsy samples from the same (exercised) muscle. Various responses in IGF-1 transcriptional levels have been reported following resistance exercise. An early decrease in IGF-1 mRNA levels was found 1 to 12 h following isometric or dynamic (eccentric-concentric) contractions (67, 95), whereas an increase in IGF-1 mRNA levels was reported 48 h following eccentric (lengthening) contractions (59). No changes in IGF-1 mRNA levels were found 24 h after electromyostimulation resistance exercise (31). More specifically, regarding the particular expression of IGF-1 isoforms in skeletal muscle, it was observed that MGF increased 2.5 h after resistance exercise (22) and 24 h after electromyostimulation resistance exercise in spinal cord injury patients, but not in controls (31, 67). IGF-1Ea isoform at the mRNA level seemed to remain unchanged 2.5 h after resistance exercise (22), or to be down-regulated during the initial part of recovery from resistance exercise (1-48 h post exercise) (95). In addition, all IGF-1 isoforms expressed in human skeletal muscle (IGF-1 Ea, b and c) were found to be increased 24 h after the completion of a resistance training program (23), while IGF-1Ebc (IGF-1Eb and IGF-1Ec isoforms) mRNA levels were unaffected 1-48 h following resistance exercise (95). Finally, in contrast to the results from animal studies (64), the imposition of a second bout of resistance exercise 24 or 72 h after the second bout resulted in an increase in MGF mRNA levels only in spinal cord injury patients (31, 67).

Conclusion

Different IGF-1 isoforms coded by multiple IGF-1 splice variants, which also undergo post-translational modification, appear to be sensitive to muscle loading or damage conditions. However, the precise biological and functional characterization of IGF-1 isoforms in skeletal muscle are particularly important, in terms of elucidating the specific signaling pathways that promote both the competing processes of cellular proliferation and differentiation in muscle regeneration and hypertrophy. Moreover, it is possible that regulation of local production of IGF-1 isoforms may be tissue-specific concerning the molecular mechanisms by which synthesis is modulated. It remains a challenge to elucidate and identify the specific stimuli and mechanotransduction signaling mechanisms by which IGF-1 isoform synthesis is modulated at the skeletal muscle cellular level.

References

1 Goldspink G: Changes in muscle mass and phenotype and the expression of autocrine and systemic growth factors by muscle in response to stretch and overload. J Anat *194*: 323-334, 1999.

2 Jones DB, Nolte H, Scholubbers JG, Turner E and Veltel D: Biochemical signal transduction of mechanical strain in osteoblast-like cells. Biomaterials *12*: 101-108, 1991.

3 Adams GR: Autocrine and/or paracrine insulin-like growth factor-I activity in skeletal muscle. Clin Orthop Relat R *403S*: S188-S196, 2002b.

4 Goldspink G, Williams P and Simpson H: Gene expression in response to muscle stretch. Clin Orthop Relat R *403S*: S146-S152, 2002.

5 Husmann I, Soulet L, Gautron J, Martelly I and Barritault D: Growth factors in skeletal muscle regeneration. Cytokine Growth F R *7*: 249-258, 1996.

6 Carson JA, Nettleton D and Reecy JM: Differential gene expression in the rat soleus muscle during early work overload-induced hypertrophy. FASEB J *16*: 207-209, 2002.

7 Chen YW, Nader GA, Baar KR, Fedele MJ, Hoffman EP and Esser KA: Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling. J Physiol *545*: 27-41, 2002.

8 Chen YW, Hubal MJ, Hoffman EP, Thompson PD and Clarkson PM: Molecular responses of human muscle to eccentric exercise. J Appl Physiol *95*: 2485-2494, 2003.

9 Barash IA, Mathew L, Ryan AF, Chen J and Lieber RL: Rapid muscle-specific gene expression changes after a single bout of eccentric contractions in the mouse. Am J Physiol Cell Physiol *286*: C355-C364, 2004.

10 Fluck M and Hoppeler H: Molecular basis of skeletal muscle plasticity from gene to form and function. Rev Physiol Biochem Pharmacol *146*: 159-216, 2003.

11 Adams GR: Invited Review: Autocrine/paracrine IGF-I and skeletal muscle adaptation. J Appl Physiol *93*: 1159-1167, 2002a.

12 Watt PW, Goldspink G and Ward PS: Changes in fiber type composition in growing muscle as a result of dynamic exercise and static overload. Muscle Nerve *7*: 50-53, 1984.

13 Harridge SD, Kryger A and Stensgaard A: Knee extensor strength, activation, and size in very elderly people following strength training. Muscle Nerve *22*: 831-839, 1999.

14 Carlson BM and Faulkner JA: The regeneration of skeletal muscle fibers following injury: a review. Med Sci Sports Exerc *15*: 187-198, 1983.

15 McArdle A, Vasilaki A and Jackson M: Exercise and skeletal muscle ageing: cellular and molecular mechanisms. Ageing Res Rev *1*: 79-93, 2002.

16 Kim SW, Lajara R and Rotwein P: Structure and function of a human insulin-like growth factor-I gene promoter. Endocrinology *5*: 1964-1972, 1991.

17 Hill M and Goldspink G: Expression and splicing of the insulin-like growth factor-I gene in rodent muscle is associated with muscle satellite (stem) cell activation following local tissue damage. J Physiol *549*: 409-418, 2003.

18 Tang LL, Wang YL and Sun CX: The stress reaction and its molecular events: splicing variants. Biochemical and Biophysical Research Communications *320*: 287-29, 2004.

19 Shavlakadze T, Winn N, Rosenthal N and Grounds MD: Reconciling data from transgenic mice that overexpress IGF-I specifically in skeletal muscle. Growth Horm IGF Res *15*: 4-18, 2005.

20 Jansen E, Steenbergh PH, LeRoith D, Roberts CT and Sussenbach JS: Identification of multiple transcription start sites in the human insulinlike growth factor-I gene. Mol Cell Endocrinol *78*: 115-125, 1991.

21 Okazaki R, Durham SK, Riggs BL and Conover CA: Transforming growth factor-beta and forskolin increase all classes of insulin-like growth factor-I transcripts in normal human osteoblast-like cells. Biochem Bioph Res Co *207*: 963-970, 1995.

22 Hameed M, Orrell RW, Cobbold M, Goldspink G and Harridge SD: Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. J Physiol *547*: 247-254, 2003.

23 Hameed M, Lange KH, Andersen JL, Schjerling P, Kjaer M, Harridge SD and 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 *555*: 231-240, 2004.

24 Bloor CA, Knight RA, Kedia RK, Spiteri MA and Allen JT: Differential mRNA expression of insulin-like growth factor-1 splice variants in patients with idiopathic pulmonary fibrosis and pulmonary sarcoidosis. Am J Respir Crit Care Med *164*: 265-272, 2001.

25 Stewart CE and Rotwein P: Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. Physiol Rev *76*: 1005-1026, 1996.

26 Gilmour RS: The implications of insulin-like growth factor mRNA heterogeneity. J Endocrinol *140*: 1-3, 1994.

27 Chew SL, Lavender P, Clark AJL and Ross RJM: An alternatively spliced human insulin-like growth factor-I transcript with hepatic tissue expression that diverts away from the mitogenic IBE l peptide. Endocrinology *136*: 1939-1944, 1995.

28 Yang S, Alnaqeeb M, Simpson H and Goldspink G: Cloning and characterization of an IGF-I isoform expressed in skeletal muscle subjected to stretch. J Muscle Res Cell Motil *17*: 487-495, 1996.

29 McKoy G, Ashley W, Mander J, Yang SY, Norman W, Russell B and Goldspink G: Expression of insulin growth factor-1 splice variants and structural genes in rabbit skeletal muscle induced by stretch and stimulation. J Physiol *516*: 583-592, 1999.

30 Goldspink G: Gene expression in skeletal muscle. Biochem Soc T *30*: 285-290, 2002.

31 Bickel CS, Slade JM, Haddad F, Adams GR and Dudley GA: Acute molecular responses of skeletal muscle to resistance exercise in ablebodied and spinal cord-injured subjects. J Appl Physiol *94*: 2255-2262, 2003.

32 Jansen M, van Schaik FM, Ricker AT, Bullock B, Woods DE, Gabbay KH, Nussbaum AL, Sussenbach JS and Van den Brande JL: Sequence of cDNA encoding human insulin-like growth factor I precursor. Nature *306*: 609-611, 1983.

33 Rotwein P: Two insulin-like growth factor I messenger RNAs are expressed in human liver. Proc Natl Acad Sci *83*: 77-81, 1986.

34 Duguay SJ, Lai-Zhang J and Steiner DF: Mutational analysis of the insulin-like growth factor I prohormone processing site. J Biol Chem *270*: 17566-17574, 1995.

35 Denley A, Cosgrove LJ, Booker GW, Wallace JC and Forbes BE: Molecular interactions of the IGF system. Cytokine Growth F R *16*: 421-439, 2005.

36 Siegfried JM, Kasprzyk PG, Treston AM, Mulshine JL, Quinn KA and Cuttitta F: A mitogenic peptide amide encoded within the E peptide domain of the insulin-like growth factor IB prohormone. Proc Natl Acad Sci *89*: 8107-8111, 1992.

37 Adamo ML, Ben-Hur H, Roberts Jr CT and LeRoith D: Regulation of start site usage in the leader exons of the rat insulin-like growth factor-I gene by development, fasting, and diabetes. Mol Endocrinol *5*: 1677-1686, 1991.

38 Sara VR, Carlsson-Skwirut C, Drakenberg K, Giacobini MB, Hakansson L, Mirmiran M, Nordberg A, Olson L, Reinecke M and Stahlbom PA: The biological role of truncated insulin-like growth factor-I and the tripeptide GPE in the central nervous system. Ann N Y Acad Sci *692*: 183-191, 1993.

39 Yang H, Adamo ML, Koval AP, McGuinness MC, Ben-Hur H, Yang Y, LeRoith D and Roberts CT Jr: Alternative leader sequences in insulin-like growth factor I mRNAs modulate translational efficiency and encode multiple signal peptides. Mol Endocrinol *9*: 1380-1395, 1995.

40 Simons G, van den Heuvel W, Reynen T, Frijters A, Rutten G, Slangen CJ, Groenen M, de Vos WM and Siezen RJ: Overproduction of bovine beta-casein in *Escherichia coli* and engineering of its main chymosin cleavage site. Protein Eng *6*: 763-770, 1993.

41 Zhang J, Whitehead RE and Underwood LE: Effect of fasting on insulin-like growth factor (IGF)-IA and IGF-IB messenger ribonucleic acids and prehormones in rat liver. Endocrinology *138*: 3112-3118, 1997.

42 Mourkioti F and Rosenthal N: IGF-I, inflammation and stem cells: interactions during muscle regeneration. Trends Immunol *26*: 535-542, 2005.

43 Adams GR, Cheng DC, Haddad F and Baldwin KM: Skeletal muscle hypertrophy in response to isometric, lengthening, and shortening training bouts of equivalent duration. J Appl Physiol *96*: 1613-1618, 2004.

44 Florini JR, Ewton DZ and Coolican SA: Growth hormone and insulin like growth factor system in myogenesis. Endocrine Rev *17*: 481-517, 1996.

45 Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B and LeRoith D: Normal growth and development in the absence of hepatic insulin-like growth factor I. Proc Natl Acad Sci USA *96*: 7324-7329, 1999.

46 Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL and Rosenthal N: Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. Nat Genet *27*: 195-200, 2001.

47 Sheffield-Moore M and Urban RJ: An overview of the endocrinology of skeletal muscle. TRENDS Endocrin Met *15*: 110-115, 2004.

48 Hayes VY, Urban RJ, Jiang J, Marcell TJ, Helgeson K and Mauras N: Recombinant human growth hormone and recombinant human insulin-like growth factor I diminish the catabolic effects of hypogonadism in man: metabolic and molecular effects. J Clin Endocr Metab *86*: 2211-2219, 2001.

49 Brill KT, Weltman AL, Gentili A, Patrie JT, Fryburg DA, Hanks JB, Urban RJ and Veldhuis JD: Single and combined effects of growth hormone and testosterone administration on measures of body composition, physical performance, mood, sexual function, bone turnover, and muscle gene expression in healthy older men. J Clin Endocr Metab *87*: 5649-5657, 2002.

50 Goldspink G and Harridge SDR: Growth factors and muscle ageing. Exp Gerontol *39*: 1433-1438, 2004.

51 Bjarnason R, Wickelgren R, Hermansson M, Hammarovist F, Carlsson B and Carlsson LMS: Growth hormone treatment prevents the decrease in insulin-like growth factor I gene expression in patients undergoing sbdominal surgery. J Clin Endocr Metab *83*: 1566-1572, 1998.

52 Sotiropoulos A, Ohanna M, Kedzia C, Menon RK, Kopchick JJ, Kelly PA and Pende M: Growth hormone promotes skeletal muscle cell fusion independent of insulin-like growth factor 1 up-regulation. Proc Natl Acad Sci USA *103*: 7315-7320, 2006.

53 Naranjo WM, Yakar S, Sanchez-Gomez M, Perez AU, Setser J and LeRoith D: Protein calorie restriction affects nonhepatic IGF-I production and the lymphoid system: Studies using the liver-specific IGF-I gene-deleted mouse model. Endocrinology *143*: 2233-2241, 2002.

54 Sonksen PH: Insulin, growth hormone and sport. J Endocrinol *170*: 13-25, 2001.

55 Coleman ME, DeMayo F, Yin KC, Lee HM, Geske R, Montgomery C and Schwartz RJ: Myogenic vector expression of insulin-like growth factor I stimulates muscle cell differentiation and myofiber hypertrophy in transgenic mice. J Biol Chem *270*: 12109-12116, 1995.

56 Fiatarone Singh MA, Ding W, Manfredi TJ, Solares GS, O'Neill EF, Clements KM, Ryan ND, Kehayias JJ, Fielding RA and Evans WJ: Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am J Physiol *277*: E135-E143, 1999.

57 Bamman MM, Clarke MS, Feeback DL, Talmadge RJ, Stevens BR, Lieberman SA and Greenisen MC: Impact of resistance exercise during bed rest on skeletal muscle sarcopenia and myosin isoform distribution. J Appl Physiol *84*: 157-163, 1998.

58 Brahm H, Piehl-Aulin K, Saltin B and Ljunghall S: Net fluxes over working thigh of hormones, growth factors and biomarkers of bone metabolism during short lasting dynamic exercise. Calcif Tissue Int *60*: 175-180, 1997.

59 Bamman MM, Shipp JR, Jiang J, Gower BA, Hunter GR, Goodman A, McLafferty CL Jr and Urban RJ: Mechanical load increases muscle IGF-I and androgen receptor mRNA concentrations in humans. Am J Physiol Endoc M *280*: E383-390, 2001.

60 Yang SY and Goldspink G: Different roles of the IGF-I Ec peptide (MGF) and mature IGF-I in myoblast proliferation and differentiation. FEBS Lett *522*: 156-160, 2002.

61 Hameed M, Harridge SDR and Goldspink G: Sarcopenia and hypertrophy: a role for insulin-like growth factor-1 in aged muscle? Exerc Sport Sci Rev *30*: 15-19, 2002.

62 Yang H, Alnaqeeb M, Simpson H and Goldspink G: Changes in muscle fibre type, muscle mass and IGF-I gene expression in rabbit skeletal muscle subjected to stretch. J Anat *190*: 613-622, 1997.

63 Williams PE and Goldspink G: The effect of immobilization on the longitudinal growth of striated muscle fibres. J Anat *116*: 45-55, 1973.

64 Haddad F and Adams GR: Selected contribution: Acute cellular and molecular responses to resistance exercise. J Appl Physiol *93*: 394-403, 2002.

65 Owino V, Yang SY and Goldspink 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 *505*: 259-263, 2001.

66 Dluzniewska J, Sarnowska A, Beresewicz M, Johnson I, Srai SK, Ramesh B, Goldspink G, Gorecki DC and Zablocka B: A strong neuroprotective effect of the autonomous C-terminal peptide of IGF-I Ec (MGF) in brain ischemia. FASEB J *19*: 1896-1898, 2005.

67 Bickel CS, Slade J, Mahoney E, Haddad F, Dudley GA and Adams GR: Time course of molecular responses of human skeletal muscle to acute bouts of resistance exercise. J Appl Physiol *98*: 482-488, 2005.

68 Awede B, Thissen J, Gailly P and Lebacq J: Regulation of IGF-I, IGFBP-4 and IGFBP-5 gene expression by loading in mouse skeletal muscle. FEBS Lett *461*: 263-267, 1999.

69 Baxter RC and Martin LJ: Binding proteins for the insulin like growth factors: structure, regulation and function. Prog Growth Factor Res *1*: 49-68, 1989.

70 Baxter RC: Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. Am J Physiol Endoc M *278*: E967-E976, 2000.

71 Jennische E and Hansson HA: Regenerating skeletal muscle cells express insulin-like growth factor 1. Acta Physiol Scand *130*: 327-332, 1987.

72 Jennische E, Skottner A and Hansson HA: Satellite cells express the trophic factor IGF-I in regenerating skeletal muscle. Acta Physiol Scand *129*: 9-15, 1987.

73 Tollefsen SE, JL Sadow and Rotwein PS: Coordinate expression of insulin-like growth factor-II and its receptor during muscle differentiation. Proc Natl Acad Sci USA *86*: 1543-1547, 1989.

74 Rosenthal SM, Brunetti A, Brown EJ, Manuela PW and Goldfine JD: Regulation of insulin-like growth factor (IGF) I receptor expression during muscle cell differentiation. *In*: Potential Autocrine Role of IGF-II. J Clin Invest 87: 1212-1219, 1991.

75 Ewton DZ, SL Roof, KA Magri, FJ McWade and Florini JR: IGF-II is more active than IGF-I in stimulating L6A1 myogenesis: greater mitogenic actions of IGF-I delay differentiation. J Cell Physiol *16*: 277-284, 1994.

76 Musaro A and Rosenthal N: The role of local insulin-like growth factor-1 isoforms in the pathophysiology of skeletal muscle. Curr Genom *3*: 149-162, 2002.

77 Salleo A, LaSpada G, Falzea G, Denaro MG and Cicciarello R: Response of satellite cells and muscle fibers to long-term compensatory hypertrophy. J Submicrosc Cytol Pathol *15*: 929-940, 1983.

78 Garry D, Meeson A, Elterman J, Zhao Y, Yang P and Bassel-Duby R: Myogenic stem cell function is impaired in mice lacking the forkhead/winged helix protein MNF. Proc Natl Acad Sci USA *97*: 5416-5421, 2000.

79 Hawke TJ and Garry DJ: Myogenic satellite cells, physiology to molecular biology. J Appl Physiol *91*: 534-551, 2001.

80 Rosenblatt JD, Yong D and Parry DJ: Satellite cell activity is required for hypertrophy of overloaded adult rat skeletal muscle. Muscle Nerve *17*: 608-613, 1994.

81 Allen DL, Monke SR, Talmadge RJ, Roy RR and Edgerton VR: Plasticity of myonuclear number in hypertrophied and atrophied mammalian skeletal muscle fibers. J Appl Physiol *78*: 1969-1976, 1995.

82 McCall GE, Allen DL, Linderman JK, Grindeland RE, Roy RR, Mukku VR and Edgerton VR: Maintenance of myonuclear domain size in rat soleus after overload and growth hormone/IGF-I treatment. J Appl Physiol *84*: 1407-1412, 1998.

83 Delaughter MC, Taffet GE, Fiorotto ML, Entman ML and Schwartz RJ: Local insulin-like growth factor I expression induces physiologic, then pathologic, cardiac hypertrophy in transgenic mice. FASEB J *13*: 1923-1929, 1999.

84 Chakravarthy MV, Abraha TW, Schwartz RJ, Fiorotto ML and Booth FW: Insulin-like growth factor-I extends *in vitro* replicative life span of skeletal muscle satellite cells by enhancing G1/S cell cycle progression *via* the activation of phosphatidylinositol 3'-kinase/Akt signaling pathway. J Biol Chem *275*: 35942-35952, 2000.

85 Caroni P and Schneider C: Signaling by insulin-like growth factors in paralyzed skeletal muscle: rapid induction of IGF1 expression in muscle fibers and prevention of interstitial cell proliferation by IGF-BP5 and IGF-BP4. J Neurosci *4*: 3378-3388, 1994.

86 Monier S and Marchand-Brustel YL: Effects of insulin and IGF-I on RNA synthesis in isolated soleus muscle. Mol Cell Endocrinol *37*: 109-114, 1984.

87 Goldspink G: Method of treating muscular disorders. United States patent *6(221)*: 842B1, 2001.

88 Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N and 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 *95*: 15603-15607, 1998.

89 Goldspink G: Skeletal muscle as an artificial endocrine tissue. Best Pract Res Cl En M *17*: 211-222, 2003.

90 Musaro A, Giacinti C, Borsellino G, Dobrowolny G, Pelosi L, Cairns L, Ottolenghi S, Cossu G, Bernardi G, Battistini L, Molinaro M and Rosenthal N: Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1. PNAS *101*: 1206-1210, 2004.

91 Hill M, Wernig A and Goldspink G: Muscle satellite (stem) cell activation during local tissue injury and repair J Anat *203*: 89-99, 2003.

92 Willis PE, Chadan SG, Baracos V and Parkhouse WS: Restoration of insulin-like growth factor I action in skeletal muscle of old mice. Am J Physiol *275*: E525-530, 1998.

93 Matsakas A, Nikolaidis MG, Kokalas N, Mougios V and Diel P: Effect of voluntary exercise on the expression of IGF-I and androgen receptor in three rat skeletal muscles and on serum IGF-I and testosterone levels. Int J Sports Med *25*: 502-508, 2004.

94 Cameron-Smith D: Exercise and skeletal muscle gene expression. Clin Exp Pharmacol P *29*: 209-213, 2002.

95 Psilander N, Damsgaard R and Pilegaard H: Resistance exercise alters MRF and IGF-I mRNA content in human skeletal muscle. J Appl Physiol *95*: 1038-1044, 2003.

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