The Roles of IGF, IGF-Binding Proteins and IGF Receptors in Myogenesis

LeBris S. Quinn*
Richard A. Roeder

Introduction

The insulin-like growth factors (IGF-I and IGF-II) are important factors in skeletal muscle development, since both naturally-occurring and experimentally-induced perturbations of the IGF signalling system result in perturbations of skeletal muscle growth and development. However, little is known concerning the cellular and molecular mechanisms by which the IGFs affect muscle development. This review will summarize current knowledge concerning the steps in myogenic development which IGF can influence and the molecular pathways by which these effects are mediated. We will then attempt to suggest methods to manipulate muscle growth in developing food animals by means of perturbing one or more of these IGF-dependent pathways.

Effects of IGF on Skeletal Muscle Growth and Development

The IGFs produce a number of effects in skeletal myoblasts or muscle fibers; stimulation of glucose uptake, stimulation of amino acid uptake, inhibition of proteolysis, muscle fiber hypertrophy, stimulation of myoblast differentiation and stimulation of myoblast proliferation (reviewed in: Florini, 1987; Magri et al., 1991). The last two items on this list are particularly interesting, since stimulation of myoblast proliferation and stimulation of myoblast differentiation are usually considered contradictory processes (Olson, 1992). In fact, the observation that peptide mitogens such as basic fibroblast growth factor (bFGF) invariably inhibit or delay myoblast differentiation is supported by several decades of research conducted by many different groups (reviewed in Olson, 1992) and is now supported mechanistically by the findings that mitogens can inhibit the expression and action of the bHLH MRFs MyoD and myogenin by several redundant mechanisms (see preceding paper by D. Mulvaney for details). The IGFs are mitogenic for many types of cells, including myoblasts (Sara and Hall, 1990; Florini, et al., 1977; Ewton and Florini, 1980; Dodson et al., 1985; Allen and Boxhorn, 1989), and increased expression of IGF-II in particular is associated with neoplastic growth (Daughaday, 1990; Rogler et al., 1994). It is thus surprising that insulin and the IGFs have been repeatedly shown to stimulate myogenic differentiation (de la Haba, 1966; Ewton and Florini, 1981; Allen and Boxhorn, 1989). It is possible that the contradictory reports concerning the effects of the IGF family of molecules on myogenic cells could be due to the diversity of cell lines and primary myoblasts from several species which have been used in the experiments. However, simultaneous stimulation by IGF of both myoblast proliferation and of myoblast differentiation has been reported (Florini et al., 1986; Allen and Boxhorn, 1989; Johnson and Allen, 1990). Additionally, Florini et al. (1986) demonstrated that stimulation of differentiation by IGF in the L6 rat myoblast line exhibits a "biphasic" curve: low concentrations of IGF stimulate differentiation, while higher concentrations of IGF cause a progressive decrease in markers of differentiation. It is therefore likely that, in myoblasts, several signal transduction pathways, with different ligand concentration dependencies, are mediated by the IGFs.

One important factor in the complexity of the effects of IGF on skeletal myogenesis is the molecular complexity of the IGF signalling apparatus. Unlike simple signalling systems in which one ligand binds to one receptor, the IGF system includes at least three different ligands, at least three different species of receptors, six IGF-binding proteins (IGFBPs), and specific proteases which in turn can act on the IGFBPs (Figure 1). Developing muscle tissue either expresses or has access to almost all, if not all, of these molecules (Beguinot et al.,1985; Tollefsen et al.,1989a, b). Therefore, it is essential to determine which of these molecules is involved in each of the processes affected by the IGFs in myogenesis.

Molecules Comprising the IGF Signalling Pathway

Ligands

Three homologous ligands, insulin, IGF-I and IGF-II, comprise the IGF family of growth factors. All three are found in the circulation, but IGF-I or IGF-II are also produced locally in almost all tissues at some point in development or adult life (LeRoith and Roberts, 1991). In muscle tissue, myoblasts, muscle fibers and fibroblasts can all produce IGFs (e.g., Tollefsen et al., 1989a,b; Listrat et al., 1994). It is unclear whether circulating IGFs actually mediate induction of muscle growth via the classic "somatomedin hypothesis" of growth hormone (GH) action (Salmon and Daughaday, 1957), since infusion of IGF-I into the circulation fails to induce muscle growth in hypophysectomized animals (e.g., Glasscock et al. 1992). In contrast, in isolated hind limb preparations, GH-stimu-

*L.S. Quinn, Department of Biological Structure, SM-20 University of Washington School of Medicine, Seattle, WA 98195.

R.A. Roeder, Department of Animal and Veterinary Science, University of Idaho, Moscow, ID 83843.

Reciprocal Meat Conference Proceedings, Volume 47, 1994.

Figure 1
Molecular Components of the IGF Signal Pathway

LIGANDS:	Insulin	IGF-I	IGF-II	
RECEPTORS:	Insulin receptor	Type-1 IGF receptor	Type-2 IGF receptor	another IGF-II receptor?
bind:	Insulin (IGF-I) (IGF-II)	IGF-I IGF-II (insulin)	IGF-II (IGF-I)	IGF-II

IGF-BINDING PROTEINS (IGFBPs 1-6):
- six forms cloned so far
- not homologous to known insulin or IGF receptors
- soluble, present in serum and tissue fluid
- can be associated with extracellular matrix or cell membranes
- do not bind insulin
- can both positively and negatively modulate IGF action
- can act in a cell-type specific manner

IGFBP PROTEASES
- identified for IGFBPs -3, -4 and -5

Chart of molecular components of the IGF signalling pathway, including ligands, receptors, IGFBPs and IGFBP proteases. For receptors, high affinity ligands are identified without parentheses, while low affinity ligands are identified in parentheses. A fourth, putative IGF-II receptor is postulated on the basis of targeted gene mutation studies in mice (see text for details).

lated growth could be inhibited by anti-IGF antibodies (Schlecter et al., 1989). Also, transgenic animals which express high serum levels of IGF-I are not phenocopies of high GH transgenic animals (Quaife et al., 1989). GH may stimulate local tissue production of the IGFs which can act in an autocrine or paracrine fashion to promote muscle growth (Holly and Wass, 1989).

From targeted gene mutation studies in mice, it is clear that both IGF-I and IGF-II are important for fetal growth. Transgenic mice in which both copies of the IGF-I gene encoding have been inactivated weigh 60% of controls at birth (Liu et al, 1993; Powell-Braxton et al., 1993). The most obvious phenotypic deficit in these animals is a severe dysgenesis of skeletal muscle (Powell-Braxton et al., 1993). In animals and humans, the gene coding for IGF-II is paternally imprinted; that is, only the paternal allele is transcriptionally active in most tissues, and the maternal allele is generally transcriptionally inactive (reviewed in Nielsen, 1992). Mice in which the paternal gene encoding IGF-II is inactivated also weigh 60% of normal at birth (Liu et al., 1993). Mice in which both IGF-I and the paternal gene encoding IGF-II are inactivated display an even more severely dysgenic phenotype, resulting in only 30% of normal weight at birth (Liu et al., 1993).

As mentioned above, transgenic mice in which IGF-I is overexpressed by the liver, thereby inducing high serum (but not necessarily tissue) IGF levels, have been constructed (Quaife et al., 1989). Although larger than controls, these animals display considerably less growth induction than GH transgenic mice. In contrast, Coleman et al. (1994) have recently constructed transgenic mice in which expression of IGF-I is driven by a muscle-specific promotor, resulting in marked skeletal muscle fiber hypertrophy. These findings further underscore the importance of local tissue expression of IGF compared to serum IGF in muscle growth. Although growth-inducing treatments such as GH treatment raise serum IGF levels (Bates et al., 1993), this may be a correlative association only.

Overexpression of serum IGF-II likewise does not necessarily lead to increased growth or to a desirable phenotype. Rogler et al. (1994) constructed transgenic mice which expressed up to 30-fold higher serum levels of IGF-II post-natally. Both lean tissue mass and adipose mass of these animals were significantly reduced due to IGF-II-induced hypoglycemia; additionally, the animals were highly prone to development of diverse types of tumors. In humans, a condition known as Beckwith-Wiedemann syndrome is associated with elevated expression of IGF-II, due to duplication of the IGF-II gene, paternal isodisomy, or activation of the maternal IGF-II gene (Junien, 1992; Newsham and Cavenee, 1993; Weksberg et al., 1993). This condition results in increased bone and muscle growth, but is associated with a number of undesirable traits, including large tongues which must often be surgically resected, hypoglycemia-induced mental retardation, and susceptibility to several types of tumors, including tumors of skeletal muscle known as rhabdomyosarcomas (Newsham and Cavenee, 1993) .

Receptors

Three main types of receptors for the IGF family of ligands have been characterized: the insulin receptor; the type-1 IGF receptor; and the type-2 IGF receptor (for biochemical reviews, see Roth and Kiess, 1994). All three types of receptors are expressed by skeletal myoblasts and muscle fibers (Beguinøt et al., 1985; Tollefsen et al., 1989a,b). The insulin receptor binds insulin with high affinity, and also binds IGF-I and IGF-II. The type-1 IGF receptor (sometimes called the IGF-I receptor) binds both IGF-I and IGF-II with high affinity, although it binds IGF-I with higher affinity than it does IGF-II. The type-1 IGF receptor also binds insulin. Since the insulin receptor and the type-1 IGF receptor are both homologous, heterotetrameric molecules, hybrid receptors comprised of subunits from both types of receptors, can also form (Frattali et al., 1992; Oh et al., 1993). The functional significance of the hybrid receptors remains to be determined. In many cell types, the mitogenic actions of insulin and the IGFs are almost certainly mediated solely by the type-1 IGF receptor via its tyrosine kinase activity domain (Oh et al., 1993). The effects of insulin, IGF-I, and IGF-II on amino acid uptake, myoblast proliferation and myogenic differentiation are all apparently mediated via the type-1 IGF receptor (Kiess et al., 1987; Ewton et al., 1987; Magri et al., 1991; Quinn et al., 1994).

The type-2 IGF receptor binds IGF-II with high affinity, and binds IGF-with very low to no affinity (Roth and Kiess, 1994). This maternally-imprinted receptor apparently functions primarily to degrade IGF-II (Nielsen, 1992). Mice which lack a functional gene for the type-2 IGF receptor are viable and on average weigh 16% more than their normal littermates at birth (Forejt and Gregorova, 1992). A few reports which indicate signal transduction functions (as opposed to degradative func-

tions) of the type-2 IGF receptor have appeared (reviewed in Oh et al., 1993), but no overall consensus as to this potential additional aspect of type-2 IGF receptor physiology exists.

During differentiation of C2 skeletal myoblasts in culture, expression of the type-1 IGF receptor is increased only about 2-fold, whereas type-2 IGF receptor expression increases 6-fold (Tollefsen, 1989 a,b). Since expression of IGF-II increases more than 20-fold with differentiation (Tollefsen, 1989a), the type-2 IGF receptor may function to keep ambient levels of IGF-II within the range optimal for myogenic differentiation to occur (Quinn et al., 1993).

Evidence for an additional type of IGF receptor, important in fetal growth and utilized only by IGF-II, has been produced by comparing crosses of mice with targeted null mutations of the genes encoding the IGFs and the type-1 IGF receptor (Liu et al., 1993; Baker et al., 1993). Mice lacking the type-1 IGF receptor are phenotypically identical to mice lacking both the type-1 IGF receptor and IGF-I (45% normal birth weight), indicating that most, if not all, actions of IGF-I are mediated by the type-1 IGF receptor (Liu et al., 1993). However, deletions of both the type-1 IGF receptor and IGF-II result in an even more severely dysgenic phenotype (30% of normal birthweight), suggesting that IGF-II utilizes another receptor besides the type-1 IGF receptor (Liu et al., 1993). Further crosses indicated this additional receptor was not the type-2 IGF receptor (Baker et al., 1993). The molecular identity of this additional IGF-II receptor is unknown.

IGF-Binding Proteins

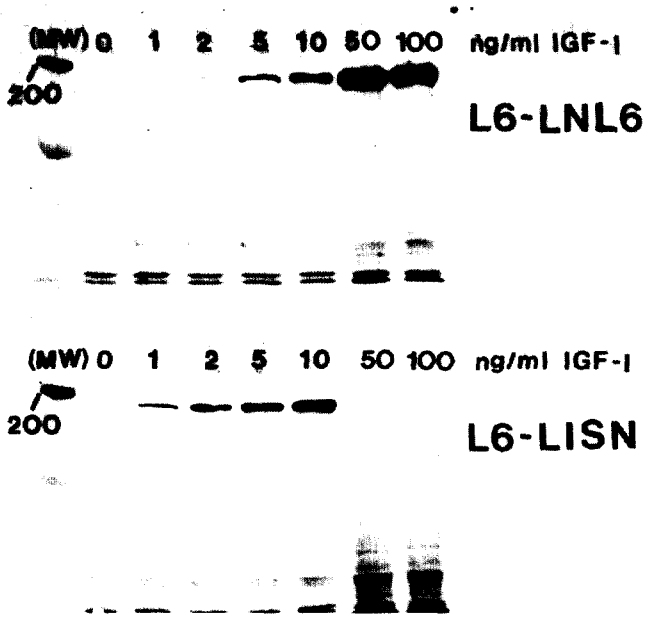
The IGFBPs are soluble molecules which can both positively and negatively modulate IGF action on cells (Shimasaki and Ling, 1991; Holly and Martin, 1994). Six forms have been identified (IGFBPs 1-6), and none are homologous to the IGF receptors (Shimasaki and Ling, 1991; Holly and Martin, 1994). IGFBPs are present in serum and tissue fluid, and are produced by cells in culture. IGFBPs 1 and 3 can bind to cell surfaces, and certain IGFBPs can be found in extracellular matrices (Shimasaki and Ling, 1991; Holly and Martin, 1994). Additionally, specific proteases for IGFBPs 3, 4 and 5 have been identified in serum and cell culture media (Holly and Martin, 1994), and may function to liberate IGF from IGFBP-IGF complexes (Blat et al., 1994). The precise roles of the IGFBPs in skeletal muscle development have not been characterized. However, since IGF is clearly important in myogenesis, these modulators of IGF activity will certainly be found to play major roles in regulating IGF action in muscle growth.

Studies on the production of IGFBPs by myoblasts and muscle fibers are conflicting. mRNA for IGF-BP1 is not detectable in fetal muscle tissue (Brinkman et al., 1988). Tollefson et al. (1989b) reported that mouse C2 myoblasts produced no detectable IGFBPs until the onset of terminal differentiation, when a 29 kDa IGFBP, later identified as IGFBP-5 by James et al., (1993), was induced. In contrast, Ernst. et al., (1992) detected production of a major 32 kDa form and minor 30 kDa and 24 kDa forms in C2C12 myoblasts, a subline of C2, and reported that IGFBP expression increased with proliferation and decreased with differentiation. Using rat L6 myoblasts, McCusker and Clemmons (1988) showed that L6 myoblasts produced two IGFBPs of 31 and 24 kDa. The 24 kDa form was associated with L6 myoblast proliferation and accumulated to very high levels in medium from differentiation-deficient L6 lines (McCusker et al., 1989). Using more recently available antibodies to the IGFBPs, McCusker and Clemmons (1994) showed the 24 kDa form was IGFBP-4 and also observed production of IGFBP-5 migrating at 31-32 kDa. It is important to emphasize that no functional roles, positive or negative, for any of the IGFBPs in myogenic proliferation or differentiation can be ascribed by these associations.

None of the myoblast lines produced significant quantities of IGFBP-3, so the primary nonvascular source of the IGFBP-3 in muscle tissue is probably fibroblasts (McCusker et al., 1989). In culture, fibroblasts from many species, including sheep and cattle, produce predominantly IGFBPs 3, 2 and 4 (e.g., Conover, 1990; Fowlkes and Freemark, 1992). Using IGF-II radioligand blots as well as IGF-I radioligand blots, Fowlkes and Freemark (1992) also detected additional (approximately 21 and 23 kDa) forms likely to be IGFBP-6 due to their markedly higher affinity for IGF-II (Shimasaki and Ling, 1991; Holly and Martin, 1994). Therefore, except for IGFBP-1, almost all the IGFBPs are potentially present in muscle tissue.

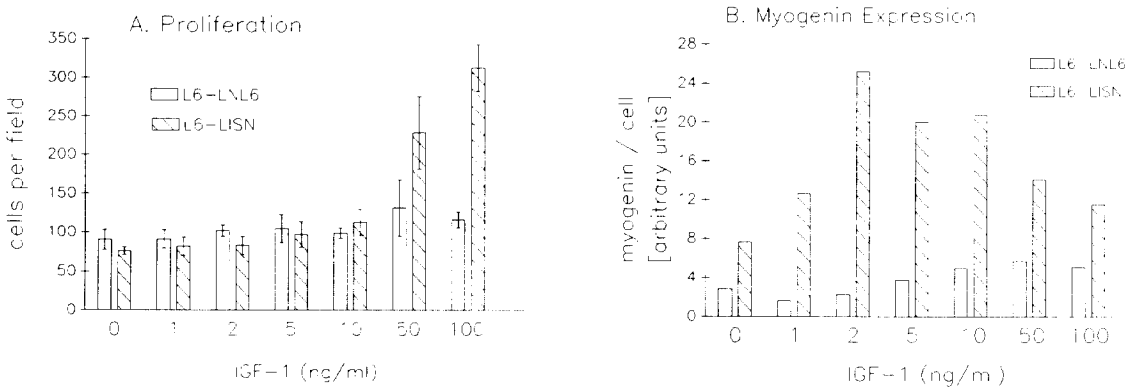
IGF-I strongly stimulates IGFBP-3 production by fibroblasts. Interestingly, however, this induction is not mediated by the type-1 IGF receptor nor by the other known receptors for IGF (Conover, 1991; Neely and Rosenfeld, 1992; Quinn et al., 1993).

Figure 2



Comparison of the effects of increasing doses of IGF-I on differentiation of L6-LNL6 (control) myoblasts and of L6-LISN myoblasts. L6-LISN myoblasts were genetically modified to produce 33 times higher levels of type-1 IGF receptors than the controls. Figure shows Western blot for muscle-specific myosin heavy chain expression, a marker of muscle differentiation, after seven days in serum-free culture with indicated concentrations of IGF-I. Note complete absence of muscle-specific myosin heavy chain expression by L6-LISN, but not L6-LNL6 myoblasts, at 50 and 100 ng/ml IGF-I.

Figure 3



Proliferation (A) and myogenin expression (B) by L6-LNL6 and L6-LISN myoblasts in response to increasing concentrations of IGF-I. Note increase in L6-LISN, but not L6-LNL6 myoblast cell number at 50 and 100 ng/ml IGF-I concomitant with inhibition of differentiation (see also Figures 2 and 4).

Overexpression of the Type-1 IGF Receptor in Skeletal Myoblasts

Since many of the important processes regulating skeletal myogenesis appear to be mediated by the type-1 IGF receptor, we wanted to determine if myoblasts exquisitely sensitive to IGF could be genetically engineered. We produced subvariants of L6 rat myoblasts (L6-LISN myoblasts) which expressed more than 30-fold higher levels of type-1 IGF receptors than controls (L6-LNL6 myoblasts) using the replication-deficient retroviral vector LISN (Kaleko et al., 1990; Quinn and Roh, 1993). L6-LISN myoblasts are slightly more sensitive to the differentiation-inducing effects of IGF-I at low concentrations of IGF (Figure 2). However, at very high IGF-I concentrations, differentiation of L6-LISN (but not control L6-LNL6) myoblasts is completely abolished (Figure 2). Concomitant with the inhibition of differentiation at high IGF-I concentrations, L6-LISN myoblast proliferation is massively induced (Figure 3A). L6-LISN myoblasts cultured with 50-100 ng/ml IGF-I no longer undergo contact inhibition and form several layers in culture (Figure 4). If the IGF concentration is reduced, these multilayered myoblasts can undergo differentiation (not shown). Thus, by manipulating IGF receptor levels and IGF concentrations, a vast increase in myoblast proliferation prior to differentiation can be induced in cell culture.

At all concentrations of IGF-I, even those which are not permissive for differentiation, expression of the bHLH MRF myogenin by L6-LISN myoblasts is higher than for L6-LNL6 myoblasts (Figure 3B). These findings confirm the hypothesis advanced by Florini (Florini et al., 1991) that the IGFs stimulate myoblast differentiation by induction of myogenin expression. At high IGF concentrations, myogenin activity may be inhibited by downstream products of mitogenic stimulation such as Id (Olson, 1992, and see review by D. Mulvaney, this volume.)

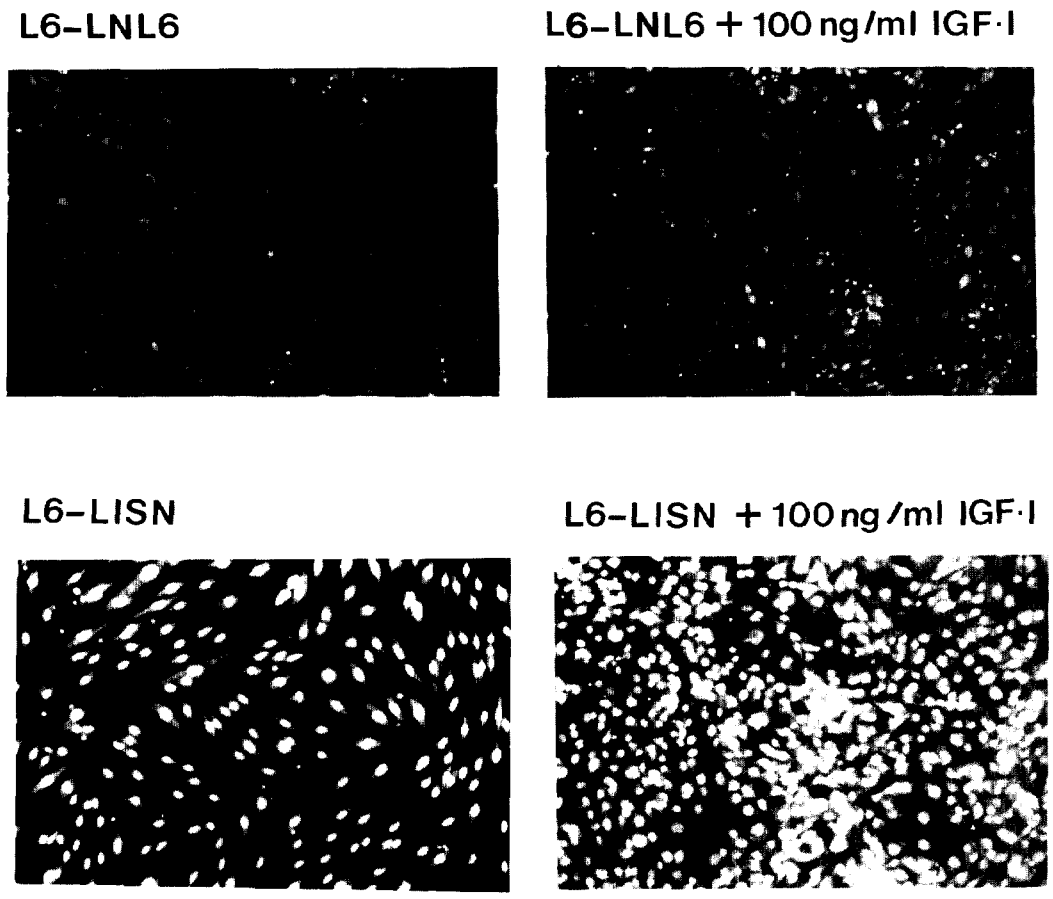
This work confirms the idea that both myoblast proliferation and myoblast differentiation are mediated by a single species of receptor, the type-1 IGF receptor. The two processes have different ligand concentration dependencies; the decision of whether a myoblast undergoes differentiation or another round of cell division seems to depend on the balance of intracellular signals for each of these processes (see also review by D. Mulvaney, this volume). Stimulation of differentiation may be mediated by induction of myogenin expression. Since myogenin is a transcription factor for many myofibrillar proteins, it is possible that this is also the mechanism whereby IGF induces skeletal muscle hypertrophy.

Similar experiments to those shown here have been conducted utilizing mouse C2 and mouse P2 myoblast lines, with the same conclusions (Quinn et al., 1993; Quinn et al., 1994). Importantly, by overexpressing the type-1 IGF receptor in myoblasts, it is possible to produce enhanced proliferation or differentiation responses which are ligand-dependent in myoblasts. This may be useful in producing controlled skeletal muscle hyperplasia and/or hypertrophy in food animals. However, a great deal of research must still be performed to accomplish practical applications.

Bovine Double-Muscling: A Natural Model of IGF Modulation?

"Double-muscling" (DM) is a hereditary skeletal muscle hyperplasia which occurs in several breeds of beef cattle (Nott and Rollins, 1979; Shahin and Berg, 1985), and is apparently caused by the inheritance of a single, autosomal co-dominant gene or allele (Rollins et al., 1972; Nott and Rollins, 1979). A specific molecular or endocrine basis for the phenotype has not been identified (Istasse et al., 1990; Arthur et al., 1990). Fetal, newborn and adult DM animals exhibit greatly increased muscle mass compared to genetically-related animals which do not carry the gene (Nott and Rollins, 1979; Shahin and Berg, 1985). Average muscle fiber diameters in DM and normal (NC) beef cattle are the same (Ashmore et al., 1974), while fiber numbers are increased (MacKeller, 1968), suggesting that skeletal myoblast hyperplasia during development is responsible for the extreme muscling which characterizes the DM phenotype. DM animals display other aberrations in the distribution of mesodermally-derived tissues, including a sub-

Figure 4



Morphological appearance of L6-/LNL6 and L6-LISN myoblasts cultured with and without 100 ng/ml IGF-I. At high concentrations of IGF-I, L6-LISN myoblasts do not differentiate, do not undergo contact inhibition and proliferate to form several layers of cells. Such multilayered L6-LISN myoblasts can subsequently undergo differentiation if placed in moderate concentrations of IGF-I (not shown).

stantial decrease in bone length and thickness (MacKellar, 1968), markedly decreased subcutaneous and intramuscular adiposity (Shahin and Berg, 1985; Istasse et al., 1990), and a decrease in intramuscular and intradermal collagen deposition (Bailey et al., 1982; Gerrard and Judge, 1993).

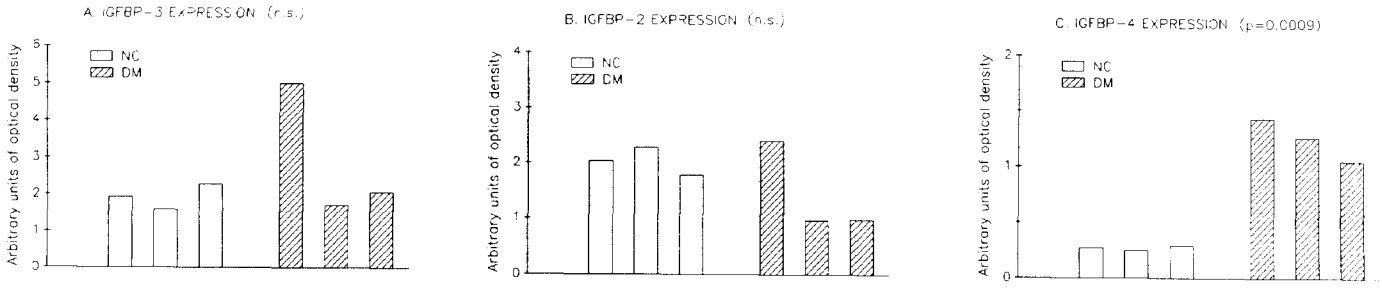
Studies by other groups have not identified consistent differences which might explain the DM phenotype in circulating concentrations of insulin, IGF-I, IGF-II, growth hormone, testosterone or thyroid hormone in developing DM animals (Istasse et al., 1990; Arthur et al., 1990). However, one study (Gerrard and Judge, 1993) has implicated unidentified fetal blood-borne growth factors for myoblasts in the development of the DM phenotype.

In a previous study, we described a cell culture model in which the myoblast hyperplasia characterizing the DM phenotype could be reproduced and studied (Quinn et al., 1990). Myogenic cultures from 90-day fetal DM muscle exhibited increased proliferative rates and a delay in the onset of differentiation compared to 90-day fetal NC myogenic cultures. Further investigation showed that DM fibroblasts produced a soluble factor (or factors) which could act mitogenically on myoblasts. Preliminary characterizations (Quinn et al., 1990) indicated that the myotrophic activity was not bFGF or one of the IGFs.

We examined IGFBP expression by muscle-derived fibroblasts from 3 NC and 3 DM 90-day fetuses (Figure 5). Both types of cells produced IGFBPs -3, -2 and -4. An average of 4.5-fold higher accumulation of IGFBP-4 by DM fibroblasts can be clearly observed ($p < 0.001$). Preliminary results suggest this difference is not regulated at the transcriptional level; it is possible that differences in expression or activity of proteases which degrade IGFBP-4 may play a role in the increased accumulation of IGFBP-4 in the DM cultures.

Differential expression of IGFBP-4 could affect the availability of IGF in developing muscle tissue. Of the five IGFBPs whose actions have been tested in cell culture systems, only IGFBP-4 has been found to be consistently inhibitory to IGF action on cells (Holly and Martin, 1994; McCusker and Clemmons, 1994). In contrast, IGFBPs 1, 2, 3 and 5 can either inhibit or potentiate IGF action, depending on the mode of administration and/or on the target cell type (Shimasaki and Ling, 1991; Holly and Martin, 1994; McCusker and Clemmons,

Figure 5



Expression of IGFBPs 3, 2 and 4 by cultured fibroblasts from 90-day fetal normal (NC) and (DM) muscle tissue. Samples were from 3 fetuses of each genotype; each bar within a plot represents one individual. Expression of IGFBP-3 and IGFBP-2 by NC and DM fibroblasts is not significantly different, but expression of IGFBP-4 by DM fibroblasts is significantly higher than that of NC fibroblasts (p = 0.0009).

1994). Thus, an increase in ambient levels of IGFBP-4 in developing muscle tissue could inhibit IGF-stimulated myoblast differentiation, keeping myoblasts in the proliferating compartment and thus playing a role in skeletal muscle hyperplasia. Alternatively, altered expression of IGFBP-4 could simply be a marker for some other physiologic or autocrine/paracrine alteration which is actually causal in development of the phenotype.

Our finding that NC and DM fibroblasts maintained differences in IGFBP-4 expression in isolated cell culture suggests these differences are tissue-intrinsic and not due to a systemic endocrine alteration, again emphasizing the importance of local tissue factors as determinants of skeletal muscle growth control.

Conclusions

A large body of both in-vitro and in-vivo experimental evidence indicates that the molecules which comprise the GH/IGF signalling system play major roles in controlling skeletal muscle growth and development. Local tissue production of the IGFs, as opposed to serum IGF, appears to be an important determinant of skeletal muscle growth. The IGFs can control several fundamental processes which determine muscle mass, such as myoblast proliferation and differentiation. Furthermore, in-vitro evidence suggests that molecular genetic manipulation of the components of the IGF signalling pathway, including various ligands, receptors, binding proteins and IGFBP proteases, could be a powerful method to modify skeletal muscle growth in developing agricultural animals to increase efficiency of food production. However, it is clear that the IGF signalling pathway is very complex. For example, a single receptor (the type-1 IGF receptor) can mediate two directly contradictory signal pathways in the same cells, myoblast differentiation and myoblast proliferation. Certain molecules whose expression is positively correlated with differentiation (e.g. the type-2 IGF receptor, certain IGFBPs) may actually function to degrade or sequester the molecules (IGF-II) which induce differentiation. Additionally, the IGFs can stimulate or inhibit differentiation at different concentrations, and most IGFBPs can enhance or inhibit IGF action on cells. Therefore, due to the molecular complexity of the IGF signalling pathway, it is unclear which of these molecules to up- or down-regulate (and when) in order to achieve a desired phenotype. Further research to untangle the roles of each of the molecules involved in IGF signalling and modulation of IGF action in myogenesis is needed.

ACKNOWLEDGMENTS

We thank Luan Ong, Jeffrey Roh and Brian Steinmetz for assistance with the experiments reported here. Dr. C.K. Smith provided the parental L6 myoblasts used in the studies described here. The LISN and LNL6 vectors were provided by Dr. Michael Kaleko. F5D hybridoma cells producing the anti-myogenin antibody were the gift of Dr. W. Wright. The MF-20 anti-muscle myosin heavy chain antibody (contributed by Dr. D. Fischman) was obtained from the Developmental Studies Hybridoma Bank maintained by the Dept. of Pharmacology and Molecular Science, Johns Hopkins University School of Medicine, Baltimore, MD, and the Dept. of Biology, University of Iowa, Iowa City, IA, under contract N01-HD-6-2915 from the NICHD. This work was supported by grants from the United States Department of Agriculture Cooperative State Research Service (Agreements 90-37265-5456 (LSQ and RAR) and 9303129 (LSQ), and by a grant from Eli Lilly and Company (LSQ).

References

Allen, R.E.; Boxhorn, L.K. 1989. Regulation of skeletal muscle satellite cell proliferation and differentiation by transforming growth factor-beta, insulin-like growth factor I, and fibroblast growth factor. J. Cell. Physiol. 138:311.

Arthur, P.F.; Makarechian, R.K.; Salmon, R.K.; Price, M.A. 1990. Plasma growth hormone and insulin concentrations in double-muscled and normal bull calves. J. Anim. Sci. 68:1609.

Ashmore, C.R.; Parker, W.; Stokes, H.; Doerr, L. 1974. Comparative aspects of muscle fiber types in fetuses of the normal and "double-muscled" cattle. Growth 38:501.

Bailey, A.F.; Enser, M.B.; Dransfield, E.; Restall, D.J.; Avery, N.C. 1982. Muscle and adipose tissue from normal and double muscled cattle: Collagen types, muscle fibre diameter, fat cell size, and fatty acid composition and organoleptic properties. In: J.W.B. King and F. Menissier (Ed.) Muscular Hypertrophy of Genetic Origin and Its Use to Improve Beef Production. Curr Top. Vet. Med. Anim. Sci. 16:178.

Baker, J.; Liu, J.-P.; Robertson, E.; Efstratiadis, A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. Cell. 75:73.

Bates, P.C.; Loughna, P.T.; Pell, J.M.; Schulster, D.; Millward, D.J. 1993. Interactions between growth hormone and nutrition in hypophysectomized rats: body composition and production of insulin-like growth factor-I. J. Endocrinol. 139:117.

Beguinot, F.; Kahn, C.R.; Moses, A.C.; Smith, R.J. (1985) Distinct biologically active receptors for insulin, insulin-like growth factor I, and insulin-like growth factor II in cultured skeletal muscle cells. J. Biol. Chem. 260:15892.

Blat, C.; Villaudy, J.; Binoux, M. 1994. In-vivo proteolysis of serum insulin-like growth factor (IGF) binding protein-3 results in increased availability of IGF to target cells. J. Clin. Invest. 93:2286.

Brinkman, A.; Groffen, C.; Kortleve, D.J.; Geurts van Kessel, A.; Drop, S.L.S. 1988. Isolation and characterization of a cDNA encoding the low molecular weight insulin-like growth factor binding protein (IBP-1). EMBO J. 7:2417.

Coleman, M.E.; Yin, K.C.; DeMayo, F.; Schwartz, R.J. 1994. Regulatory elements of the chicken -skeletal actin gene direct high level and tissue-specific expression of an hIGF-I cDNA in cultured myogenic cells and transgenic mice resulting in enhanced myogenesis and pronounced muscle hypertrophy. J. Cellular Biochem. Suppl. 18D:470.

Conover, C.A. 1990. Regulation of Insulin-like growth factor (IGF) binding protein synthesis by insulin and IGF-I in cultured bovine fibroblasts. Endocrinol. 126:3139.

Conover, C.A. 1991. A unique receptor-independent mechanism by which insulin-like growth factor I regulates the availability of insulin-like growth factor binding proteins in normal and transformed human fibroblasts. J. Clin. Invest. 88:1354.

Daughaday, W.H. 1990. The possible autocrine/paracrine and endocrine roles of insulin-like growth factors of human tumors. Endocrinology 127:1.

de la Haba, G.; Cooper, G.W.; Elting, V. 1966. Hormonal requirements for myogenesis in vitro: Insulin and somatotropin. Proc. Natl. Acad. Sci. USA. 56:1719.

Dodson, M.V.; Allen, R.E.; Hossner, K.L. 1985. Ovine somatomedin, multiplication stimulating activity and insulin promote skeletal muscle satellite cell proliferation in vitro. Endocrinol. 117:2357.

Ernst, C.W.; McCusker, R.H.; White, M.E. 1992. Gene expression and secretion of insulin-like growth factor-binding proteins during myoblast differentiation. Endocrinol. 130:607.

Ewton, D.Z.; Florini, J.R. 1980. Relative effects of the somatomedins, multiplication-stimulating activity, and growth hormone on myoblasts and myotubes in culture. Endocrinol. 106:577.

Ewton, D.Z.; Florini, J.R. 1981. Effects of the somatomedins and insulin on myoblast differentiation in vitro. Devel. Biol. 86:31.

Ewton, D.Z.; Falen, S.L.; Florini, J.R. 1987 The type II IGF receptor has low affinity for IGF-I analogs: pleiotropic actions of IGFs on myoblasts are apparently mediated by the type I receptor. Endocrinol. 120:115.

Florini, J.R. 1987. Hormonal control of muscle growth. Muscle & Nerve 10:577.

Florini, J.R.; Nicholson, M.L.; Dulak, N.C. 1977. Effects of peptide anabolic hormones on growth of myoblasts in culture. Endocrinol. 101:32.

Florini, J.R.; Ewton, D.Z.; Falen, S.L.; Van Wyk, J.J. 1986. Biphasic concentration dependency of stimulation of myoblast differentiation by somatomedins. Am. J. Physiol. 250:C771.

Florini, J.R.; Ewton., D.Z.; Roof, S.L. 1991. Insulin-like growth factor-I stimulates terminal myogenic differentiation by induction of myogenin gene expression. Molec. Endocrinol. 5:718.

Forejt, J; Gregorova, S. 1992. Genetic analysis of genomic imprinting: An Imprintor-1 gene controls inactivation of the mouse Tme locus. Cell. 70:443.

Fowlkes, J.; Freemark, M. 1992. Developmental regulation of insulin-like growth factor binding protein production: Studies in fetal, postnatal, and pregnant sheep. J. Cell. Physiol. 152:19.

Frattali, A.L.; Treadway, J.L.; Pessin, J.E. 1992. Insulin/IGF-1 hybrid receptors: Implications for the dominant-negative phenotype in syndromes of insulin resistance. J. Cell. Biochem. 48:43.

Gerrard, D.E.; Judge, M.D. 1993. Induction of myoblast proliferation in L6 myoblast cultures by fetal serum of double-muscled and normal cattle. J. Anim. Sci. 71:1464.

Glasscock, G.F.; Hein, A.N.; Miller, J.A.; Hintz, R.L.; Rosenfeld, R.G. 1992. Effects of continuous infusion of insulin-like growth factor I and II, alone and in combination with thyroxine or growth hormone, on the neonatal hypophysectomized rat. Endocrinol. 130:203.

Holly, J.M.P.; Wass, J.A.H. 1989. Insulin-like growth factors; autocrine, paracrine, or endocrine? New perspectives of the somatomedin hypothesis in the light of recent developments. J. Endocrinol. 122:611.

Holly, J.M.P.; Martin, J.L. 1994. Insulin-like growth factor binding proteins: A review of Methodological Aspects of their purification, analysis and regulation. Growth Regul. 4 (suppl. 1):20.

Istasse, L.C.; Van Eenaeme, P.; Evrard, A.; Gabriel, P.; Baldwin, G.; Maghuin-Rogister, J.M.; Bienfait. 1990. Animal performance, plasma hormones and metabolites in Holstein and Belgian Blue growing-fattening bulls. J. Anim. Sci. 68:2666.

James, P.L.; Jones, S.B.; Busby, W.H.; Clemmons, D.R; Rotwein, P. 1993. A highly conserved insulin-like growth factor binding protein (IGF-BP5) is expressed during myoblast differentiation. J. Biol. Chem. 268:22305.

Johnson, S.E.; Allen, R.E. 1990. The effects of bFGF, IGF-I, and TGF- on RMo skeletal muscle cell proliferation and differentiation. Exp. Cell Res. 187:250.

Junien, C. 1992. Beckwith-Wiedemann syndrome, tumourigenesis, and imprinting. Curr. Opin. Genet. Devel. 2:431.

Kaleko, M.; Rutter, W.J.; Miller, A.D. 1990. Overexpression of the human insulin-like growth factor I receptor promotes ligand-dependent neoplastic transformation. Molec. Cell. Biol. 10:464.

Kiess, W.; Haskell, J.F.; Lee, L.; Greenstein, L.A.; Miller, B.E.; Aaron, A.L.; Rechler, M.; Nissley, S.P. 1987. An antibody that blocks insulin-like growth factor (IGF) binding to the type II receptor is neither an agonist nor an inhibitor of IGF-stimulated biologic responses in L6 myoblasts. J. Biol. Chem. 262:12745.

LeRoith, D.; Roberts, C.T. 1991. Insulin-like growth factor I (IGF-I): a molecular basis for endocrine versus local action? Molec. Cell. Endocrinol. 77:C57.

Listrat, A.; Gerrard, D.E.; Boulle, N.; Groyer, A.; Robelin, J. 1994. In situ localization of muscle insulin-like growth factor-II mRNA in developing bovine fetuses. J. Endocrinol. 140:179.

Liu, J.-P.; Baker, J.; Perkins, A.S.; Robertson, E.J.; Efstratiadis, A. 1993. Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (Igf1r). Cell. 65:59.

MacKellar, J.C. 1968. Muscular hypertrophy in South Devon cattle. Fellowship thesis, Royal College of Veterinary Surgeons, London. In: Structure and Development of Meat Animals. Prentice-Hall, Englewood Cliffs, N.J.

Magri, K.A.; Ewton, D.A.; Florini, J.R. 1991. The role of the IGFs in myogenic differentiation. p. 57 In: Molecular Biology and Physiology of Insulin and Insulin-like growth factors. M.K. Raizada and D. LeRoith, Plenum Press, New York.

McCusker, R.H.; Clemmons, D.R. 1988. Insulin-like growth factor binding protein secretion by muscle cells: Effect of cellular differentiation and proliferation. J. Cell. Physiol. 137:505.
McCusker, R.H.; Clemmons, D.R. 1994. Effects of cytokines on insulin-like growth factor binding protein secretion by muscle cells in vitro. Endocrinol. 134:2095.
McCusker, R.H.; Camacho-Hubner, C.; Clemmons, D.R. 1989. Identification of the types of insulin-like growth factor-binding proteins that are secreted by muscle cells in vitro. J. Biol. Chem. 264:1989.
Neely, E.K.; Rosenfeld, R.G. 1992. Insulin-like growth factors (IGFs) reduce IGF-binding protein-4 (IGFB-4) concentrations and stimulate IGFBP3 independently of IGF receptors in human fibroblasts and epidermal cells. Endocrinol. 130:985.
Newsham, I.; Cavenee, W. 1993. Tumors and developmental abnormalities associated with Wilms tumor. Med. Ped. Oncol. 21:199.
Nielsen, C.F. 1992. The molecular and cellular biology of insulin-like growth factor II. Prog. Growth Factor Res. 4:257.
Nott, C.F.G.; Rollins, W.C. 1979. Effects of the m gene for muscular hypertrophy on birth weight and growth to one year of age in beef cattle. Growth 43:221.
Oh, Y.; Muller, H.L.; Neely, E.K.; Lamson, G.; Rosenfeld, R.G. 1993. New concepts in insulin-like growth factor receptor physiology. Growth Regul. 3:113.
Olson, E.N. 1992. Interplay between proliferation and differentiation within the myogenic lineage. Devel. Biol. 154:261.
Powell-Braxton, L.; Hollingshead, P.; Warburton, C.; Dowd, M.; Pitts-Meek, S.; Dalton, D.; Gillett, N.; Stewart, T.A. 1993. IGF-I is required for normal embryonic growth in mice. Genes & Devel. 7:2609.
Quaife, C.J.; Mathews, L.S.; Pinkert, C.A.; Hammer, R.E.; Brinster, R.L.; Palmiter, R.D. 1989. Histopathology associated with elevated levels of growth hormone and insulin-like growth factor I in transgenic mice. Endocrinol. 124:40.
Quinn, L.S.; Roh, J.S. 1993. Overexpression of the human type-1 insulin-like growth factor receptor in rat L6 myoblasts induces ligand-dependent cell proliferation and inhibition of differentiation. Exp. Cell Res. 208:504.
Quinn, L.S.; Ong, L.D.; Roeder, R.A. 1990. Paracrine control of myoblast proliferation and differentiation by fibroblasts. Devel. Biol. 140:8.
Quinn, L.S.; Ehsan, M.; Steinmetz, B.; Kaleko, M. 1993. Ligand-dependent inhibition of myoblast differentiation by overexpression of the type-1 insulin-like growth factor receptor. J. Cell. Physiol. 156:453.
Quinn, L.S.; Steinmetz, B.; Maas, A.; Ong, L.; Kaleko, M. 1994. Type-1 insulin-like growth factor receptor overexpression produces dual effects on myoblast proliferation and differentiation. J. Cell. Physiol. 159:387.
Rogler, C.E.; Yang, D.; Rossetti, L.; Donohoe, J. ; Alt, E.; Chang, C.J.; Rosenfeld, R.; Neely, K.; Hintz, R. 1994. Altered body composition and increased frequency of diverse malignancies in insulin-like growth factor-II transgenic mice. J. Biol. Chem. 269:13779.
Rollins, W.C.; Tanaka, M.; Nott, C.F.G.; Thiessen, R.B. 1972. On the mode of inheritance of double-muscled conformation in bovines. Hilgardia 41:433.
Roth, R.A.; Kiess, W. 1994. Insulin-like growth factor receptors: Recent developments and new methodologies. Growth Regul. 4 (suppl. 1):31.
Salmon, W.D.; Daughaday, W.H. 1957. A hormonally controlled serum factor which stimulates sulfate incorporation by cartilage in vitro. J. Lab. Clin. Metab. 49:825.
Sara, V.R.; Hall, K. 1990. Insulin-like growth factors and their binding proteins. Physiol. Review 70:591.
Schlechter, N.L.; Russell, S.M.; Spencer, E.M.; Nicoll, C.S. 1986. Evidence to suggest that the direct growth promoting effects of growth hormone on cartilage in vivo are mediated by local production of somatomedin. Proc. Natl. Acad. Sci. USA. 83:7932.
Shahin, K.A.; Berg, R.T. 1985. Growth patterns of muscle, fat, and bone, and carcass composition of double-muscled and normal cattle. Canad. J. Anim. Sci. 65:279.
Shimasaki, S; Ling, N. 1991. Identification and molecular characterization of insulin-like growth factor binding proteins (IGFBP-1, -2, -3, -4, -5, and -6). Prog. Growth Factor Res. 3:243.
Tollefsen, S.E.; Sadow, J.L.; Rotwein, P. 1989a. Coordinate expression of insulin-like growth factor II and its receptor during muscle differentiation. Proc. Natl. Acad. Sci. USA 86:1543.
Tollefsen, S.E.; Lajara, R.; McCusker, R.H.; Clemmons, D.R.; Rotwein, P. 1989b. Insulin-like growth factors (IGF) in muscle development. Expression of IGF-I, the IGF-I receptor, and an IGF binding protein during myoblast differentiation. J. Biol. Chem. 264:13810.
Weksberg, R.; Shen, D.R.; Fei, Y.L.; Song, Q.L.; Squire, J. 1993. Disruption of insulin-like growth factor 2 imprinting in Beckwith-Wiedemann syndrome. Nature-Genetics. 5:143.