Effects of Growth Manipulation on IGF-I and IGF-II Expression in Skeletal Muscle

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Introduction

The insulin-like growth factors are a family of peptides which are fundamentally involved in growth regulation of mammalian and avian species. Insulin-like growth factor I (IGF-I) was first recognized by Salmon and Daughaday in 1957 and was originally termed "sulfation factor" (Salmon and Daughaday, 1957). IGF-I mediates many of the growth-promoting effects of growth hormone (GH), (Daughaday et al., 1972; Schoenle et al., 1985). Insulin-like growth factor II (IGF-II) appears to be a fetal counterpart to IGF-I (Moses et al., 1980; Daughaday et al., 1986; Adams et al., 1983). On the other hand, it is now apparent that certain adult tissues synthesize IGF-II (Lund et al., 1986; Rotwein et al., 1988; Turner et al., 1988), suggesting a possible role for IGF-II in post-natal growth and development.

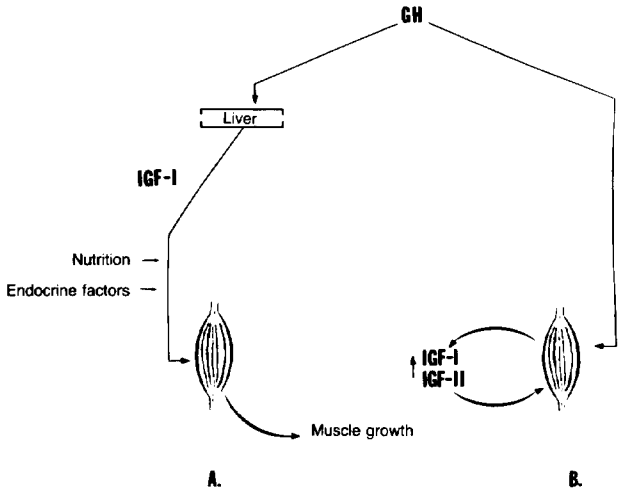
It was originally believed that the liver was the only source of IGF-I, and that liver-derived serum IGF-I (acting via the classic endocrine mechanism) was responsible for all of the biological actions of the IGFs during post-natal growth. In recent years, it has become apparent that many non-liver tissues also synthesize IGF-I and/or IGF-II (D'Ercole et al., 1984; Adams et al., 1984; Lund et al., 1986; Murphy et al., 1987a and 1987b; Shimatsu and Rotwein, 1987). Of interest to animal scientists are the findings that skeletal muscle cells (Hill et al., 1985a and 1985b; Jennische and Hansson, 1987) and muscle tissue (Turner et al., 1988) have been shown to produce IGF-I and IGF-II, and the synthesis of IGF-I and IGF-II mRNA in skeletal muscle is under the control of GH (Murphy et al., 1987a; Turner et al., 1988; Isgaard et al., 1989).

The production of IGFs by skeletal muscle raises the interesting possibility that these growth factors may regulate muscle growth in a localized, tissue-specific manner. This type of growth regulation could be either a result of an autocrine mechanism, whereby a specific cell type produces a growth factor which then stimulates growth of the same cell type, or a paracrine mechanism, where a cell type produces a growth factor which then stimulates growth of a different cell type(s) which is located nearby. These two types of regulatory mechanisms contrast with the classical endocrine mechanism, in which a regulatory molecule (either a protein or other type of hormone) is produced in one tissue or organ and then travels through the bloodstream and exerts its biological effect(s) on target organs at a distance. Figure 1 shows a schematic representation of the regulation of muscle growth by both liver-derived (endocrine) and muscle-derived (autocrine/paracrine) IGF-I.

A role for the IGFs in the autocrine/paracrine regulation of muscle growth is further supported by the well-documented effects of IGF-I and/or IGF-II on skeletal muscle cells and tissue. IGF-I and IGF-II stimulate growth of muscle cells and muscle tissue (Poggi et al., 1979; Ewton and Florini, 1980; Dodson et al., 1985; Ballard et al., 1986). In addition, both IGF-I and IGF-II receptors have been described in skeletal muscle (Poggi et al., 1979; Zorzano et al., 1988; Yu and Czech, 1984).

In this paper, we discuss a series of experiments to test the hypothesis that local production of IGF-I and/or IGF-II may regulate muscle growth in an autocrine/paracrine manner. In addition, the experiments described investigate the growth hormone dependence of IGF gene expression in skeletal muscle.

Figure 1



Schematic representation of growth hormone (GH) dependent muscle growth. A. Liver derived serum IGF-I may stimulate muscle growth via an endocrine mechanism. Liver production of IGF-I is under control of GH; however, other factors such as nutritional status and endocrine factors can affect serum IGF levels. B. Muscle derived IGF-I and IGF-II may stimulate muscle growth in an autocrine and/or paracrine manner. IGF-I and IGF-II mRNA levels in skeletal muscle are under GH control. In an autocrine or paracrine mechanism for muscle growth control, IGF which is made by skeletal muscle stimulates muscle growth.

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Reciprocal Meat Conference Proceedings, Volume 42, 1989.

Experimental Models and Methods

Compensatory hypertrophy of rat skeletal muscle was used as a model for muscle growth. When the distal tendon of the *gastrocnemius* muscle of the rat hindlimb is surgically cut, the *plantaris* and *soleus* muscles undergo rapid growth (Goldberg, 1967). This growth is termed "compensatory hypertrophy," as the *plantaris* and *soleus* grow to compensate for the loss of *gastrocnemius* function. This is a localized, tissue-specific growth response, suggesting that local regulatory mechanisms may be involved. Because the surgical procedure is performed on only one leg from each animal, the muscles of the contralateral leg serve as non-growing controls. Thus, the expression of the IGF genes can be evaluated in growing and non-growing muscles of the same animal. Any differences in the expression of the IGF genes observed between control and growing (hypertrophy) muscles must therefore be a result of local regulatory mechanisms.

The surgical procedure of Goldberg (1967; with minor modifications) was used to induce compensatory hypertrophy in the rat *soleus* and *plantaris*. Rats were allowed to undergo compensatory hypertrophy for 2, 4 or 8 days after surgery. Compensatory hypertrophy was induced in normal (pituitary-intact) and hypophysectomized (hypox) rats in order to investigate the GH dependence of the IGF response during compensatory hypertrophy.

The administration of rat growth hormone (rGH) to hypox rats was used as a model to determine the effects of GH replacement on the expression of the IGF genes in skeletal muscle. The surgery to induce compensatory hypertrophy was not performed on the rats receiving rGH injections. Comparison of the effects of compensatory hypertrophy and rGH administration of IGF gene expression will lend insight into whether there may be multiple mechanisms for controlling IGF gene expression.

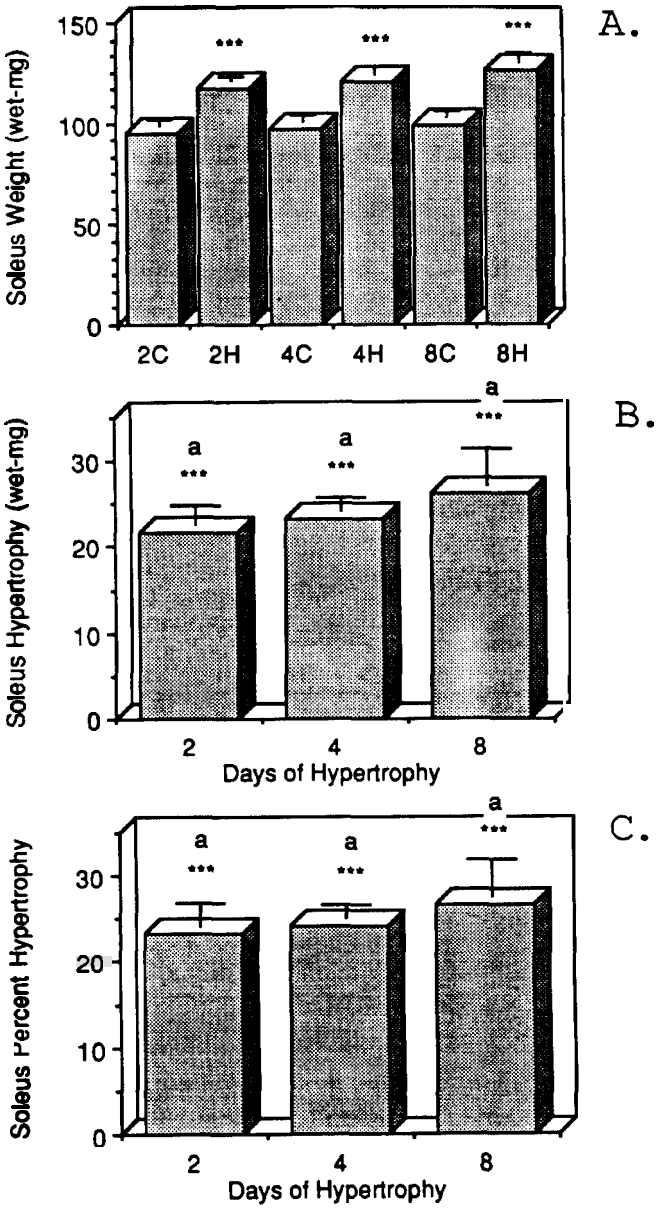
IGF-I and IGF-II mRNA levels in skeletal muscle were quantitated using a specific solution hybridization assay (Shimatsu and Rotwein, 1987). The IGF clones (used as probes to detect IGF-I and IGF-II mRNA) were kindly provided by the laboratory of P. Rotwein at Washington Unviersity Medical Center, St. Louis, Mo. Briefly, the procedure involves the annealing of ^{32}P-labelled IGF-I or IGF-II probe to RNA which has been isolated from the skeletal muscle of interest. The annealed RNA is subsequently digested with RNAses A and T1 and proteinase K, and electrophoresed on a polyacrylamide gel. The gel is then exposed to X-ray film for detection of the ^{32}P-labelled IGF-I or IGF-II mRNA. The procedure is quantitative and specific for IGF-I or IGF-II mRNA, depending on which probe is used.

Alll molecular biology techniques used (RNA isolation, detection of B-actin mRNA by northern transfer, synthesis and isolation of the IGF clones) were according to established and standard procedures (Birnboim and Doly, 1979; Towle et al., 1980; Maniatis et al., 1982; Thomas, 1983).

The Expression of the IGF Genes During Compensatory Hypertrophy

When the distal portion of the *gastrocnemius* tendon is cut, the *soleus* and *plantaris* of the rat hindlimb undergo compensatory hypertrophy. The hypertrophy is a true hypertrophy, as significant increases in muscle weights are observed both on a wet and dry tissue basis. The dry tissue weight gain was highly correlated to an increase in protein content. The growth response which was observed after 2, 4 and 8 days of compensatory hypertropy in the *soleus* of normal (pituitary-intact) rats is shown in Figures 2 (wet tissue

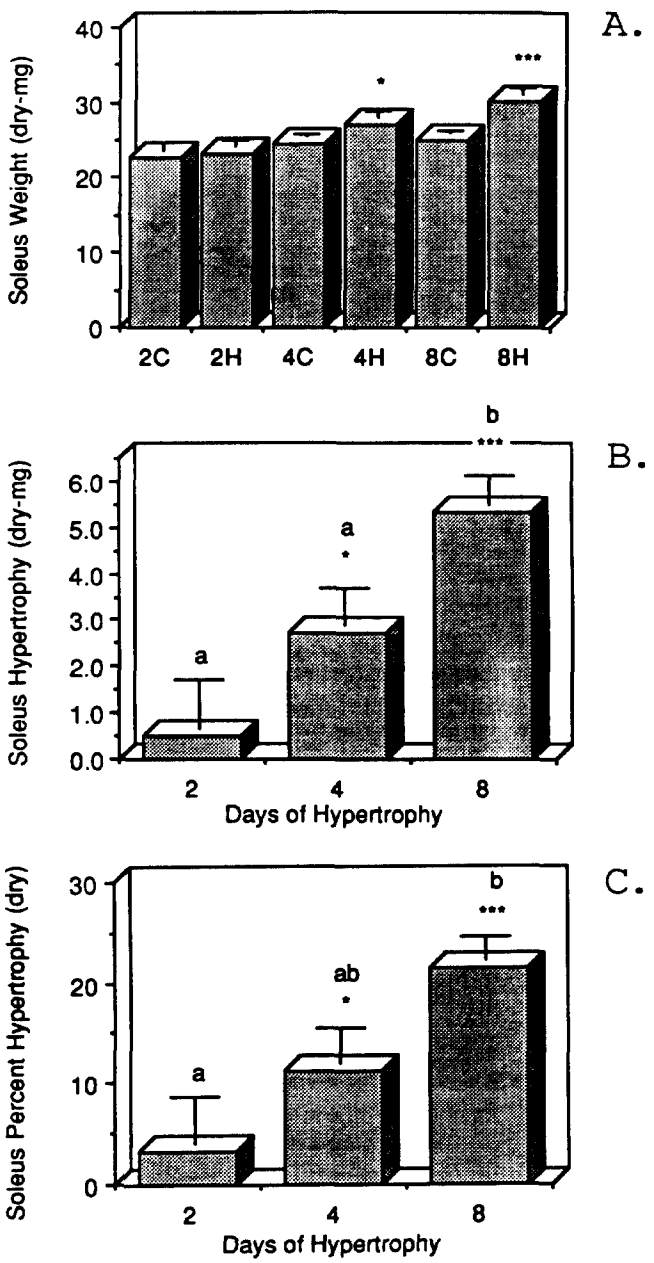
Figure 2



Compensatory hypertrophy of soleus muscle on a wet tissue basis. A. Soleus weight on a wet tissue basis. 2C, 4C, and 8C = 2, 4, and 8 day control (C) muscles. 2H, 4H, and 8H = 2, 4, and 8 day hypertrophy (H) muscles. C and H muscles are from the same rats. B. Soleus hypertrophy weight (hypertrophy-control muscle weight). C. Soleus percent hypertrophy (hypertrophy - control muscle weight)/control muscle weight *100). Values are means with standard error bars indicated. ***Significant difference between C and H muscles within days ($P<.001$; paired t-test). $^{a\text{-}c}$Bars with different letters above indicate a significant difference among days at the ($P<.05$) level (by analysis of variance).

weights) and 3 (dry tissue weights). Significant hypertrophy on a wet weight basis was observed in as little as 2 days, while significant hypertrophy on a dry weight basis was observed by 4 days of hypertrophy. In addition, the extent of dry weight hypertrophy increased significantly from 2 to 8 days of hypertrophy (Figures 3b and 3c). A similar growth response was observed in the *plantaris*, although to a slightly lesser extent.

Figure 3

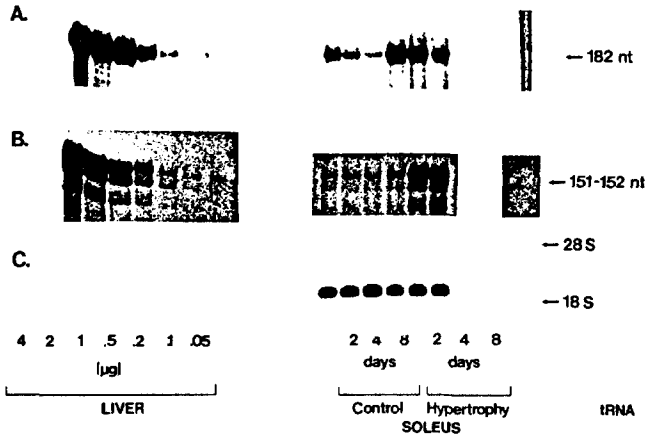


Compensatory hypertrophy of soleus muscle on a dry tissue basis. A. Soleus weight on a dry tissue basis. B. Soleus hypertrophy on a dry tissue basis. C. Soleus percent hypertrophy on a dry tissue basis. All axis labels and definitions are as described in Figure 2. *Significant difference between C and H muscles within days ($P<.05$). Other levels of significance are as indicated in Figure 2.

When the *soleus* and *plantaris* undergo rapid compensatory hypertrophy, both IGF-I and IGF-II mRNA levels increase within the muscles. Results from a representative solution hybridization assay to detect IGF-I and II mRNA in the *soleus* are presented in Figure 4. As can be seen, IGF-I mRNA levels increased by day 2 and IGF-II mRNA levels increased by day 4 of compensatory hypertrophy. When the results of several independent solution hybridization assays are averaged, it can be seen that IGF-I and IGF-II mRNA levels increased several fold in the hypertrophy *soleus* and *plantaris* muscles when compared to contralateral controls (Figure 5). The only control muscles which exhibited increased IGF mRNA levels were the 8 day control *plantaris* muscles, which exhibited a 4 fold increase in IGF-II mRNA levels compared to the 2 day control *plantaris* muscles. It is interesting to note that concomitant with this increase in control *plantaris* IGF-II message, there was a significant increase in 8 day control *plantaris* weights as compared to 2 and 4 day control *plantaris* weights (data not shown).

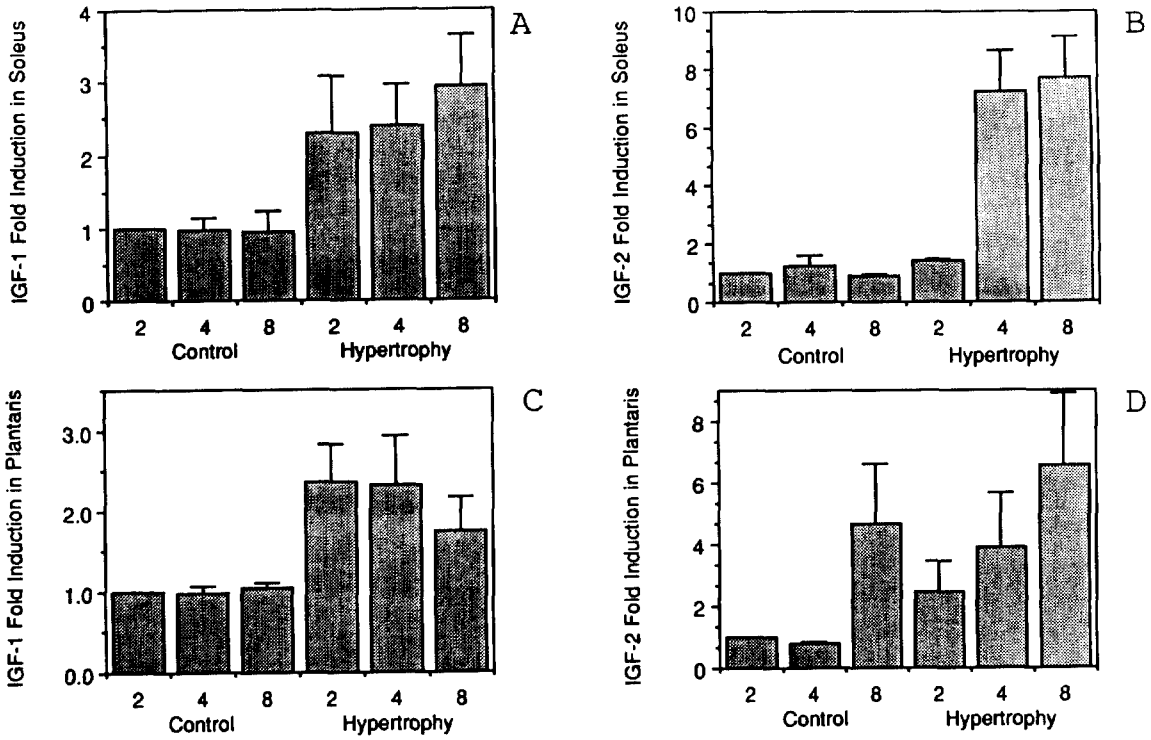
These results strongly suggest a role for the IGFs in the localized (autocrine/paracrine) regulation of skeletal muscle growth. The expression of the IGF-I and IGF-II genes increases in growing muscle, while contralateral control muscles (the same muscles in the opposite leg of the same animals) do not grow and show no increase in IGF mRNA

Figure 4



Changes in IGF-I and IGF-II mRNA levels in soleus muscle during compensatory hypertrophy. A. Modulation of IGF-1 mRNA during compensatory hypertrophy of the soleus. The left panel is a dilution series of adult liver RNA (which served as a positive control), the right panel is 8 µg of yeast tRNA (a negative control). In the experimental lanes, 8 µg of total RNA was loaded from each treatment. 2, 4, and 8 = days post-surgery. Hypertrophy = growing soleus from operated leg; Control = non-growing soleus from contralateral legs of the same rats. 182 nt indicates the position of the 182 base pair long portion of the IGF-I mRNA which is detected in this assay. B. Modulation of IGF-II mRNA during compensatory hypertrophy. The left panel is a dilution series of fetal liver RNA (a positive control), the right panel is 8 µg of yeast tRNA (a negative control). 8 µg of total RNA was loaded from each treatment. 151-152 nt indicates the position of the 151-152 base pair long portion of the IGF-II mRNA which is detected in this assay. C. Results of northern blot analysis to detect B-actin mRNA. Because B-actin mRNA is expressed as a constant proportion of the total RNA, it was used as a control to assure that similar amounts of RNA from each treatment were loaded onto gels. 28S and 18S indicate the positions of the 28S and 18S rRNA bands relative to the B-actin mRNA.

Figure 5



Fold increase in IGF-I and IGF-II mRNA levels during compensatory hypertrophy of the soleus and plantaris in normal (pituitary-intact) rats. A. IGF-I mRNA fold induction in the soleus. B. IGF-II mRNA fold induction in the soleus. C. IGF-1 mRNA fold induction in the plantaris. D. IGF-II mRNA fold induction in the plantaris. All values are expressed relative to 2 day control values. The IGF mRNA levels of the different treatments were divided by the IGF mRNA levels of the 2 day control muscles, resulting in a fold increase or "fold induction" value relative to 2 day controls. The 2 day control muscles were therefore arbitrarily assigned a value of 1.0. 2, 4, and 8 = days post-surgery.

levels. The IGFs are known to stimulate the growth of muscle and muscle cells. In addition, the expression of IGF-I is controlled by altering mRNA levels (Bortz et al., 1988). It therefore seems likely that the increase in IGF mRNA levels plays a regulatory role in compensatory hypertrophy.

The tissue-specific and localized increase in IGF mRNA levels during compensatory hypertrophy also suggests a local mechanism for IGF gene regulation which is independent of the systemic (growth hormone) mechanism. Other researchers have reported increases in IGF-I and/or IGF-II mRNA levels in skeletal muscle in response to GH administration (Turner et al., 1988; Isgaard et al., 1989); however, muscles other than the *soleus* and *plantaris* were used in those experiments. In order to demonstrate that both local and systemic factors can regulate the expression of the IGF-I (and IGF-II) genes in the *plantaris* and *soleus,* the effects of rGH administration on IGF gene expression in the *plantaris* and *soleus* were investigated.

The Effects of Growth Hormone Administration on IGF Gene Expression

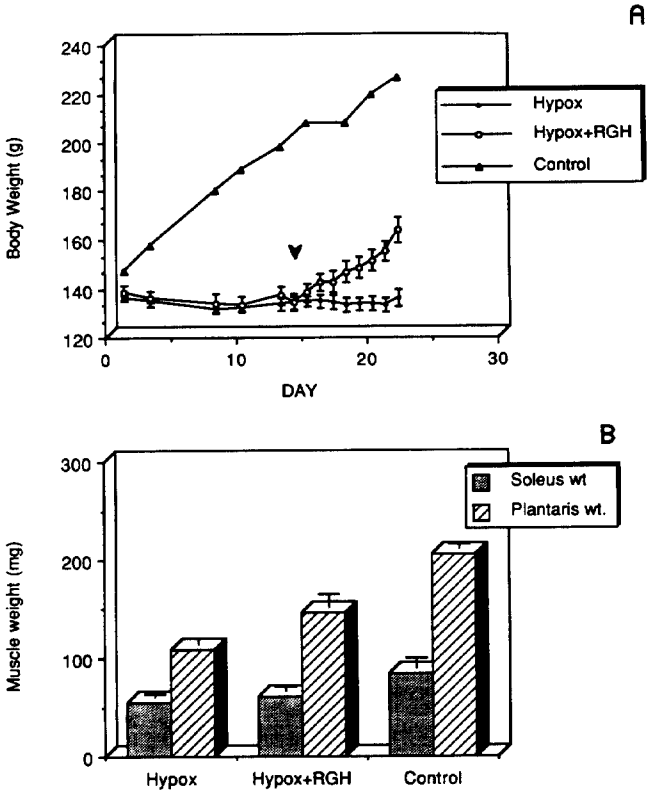
When hypox rats were injected with rGH, body weights increased 20% over 9 days, while hypox rats which did not receive rGH did not grow (Figure 6a). A growth response was also observed in the *soleus* and *plantaris* muscles (Figure 6b). The body and muscle weight increases demonstrate the efficacy of the rGH injections.

The replacement of rGH in hypox rats resulted in a 2 to 3 fold increase in IGF-I mRNA levels in both the *plantaris* and *soleus* muscles (Figure 7). These results are in agreement with the results of others who have reported increases in skeletal muscle IGF-I mRNA levels in response to GH administration (Murphy et al., 1987a; Turner et al., 1988; Isgaard et al., 1989).

IGF-II mRNA levels increased in response to rGH in the *soleus* but not in the *plantaris.* This was the only instance in which the *soleus* and *plantaris* differed in their response to any of the growth models investigated. It is possible that this difference reflects the different predominant fiber types present in these two muscles. It is interesting to note that the *soleus* exhibited a more dramatic response (fold increase in IGF-II mRNA) during compensatory hypertrophy than did the *plantaris.* Thus, the *soleus* may have more capacity to increase IGF-II mRNA levels than does the *plantaris.* This may partly reflect the observation that baseline (control) IGF-II mRNA levels were consistently higher in the *plantaris* than in the *soleus* (data not shown).

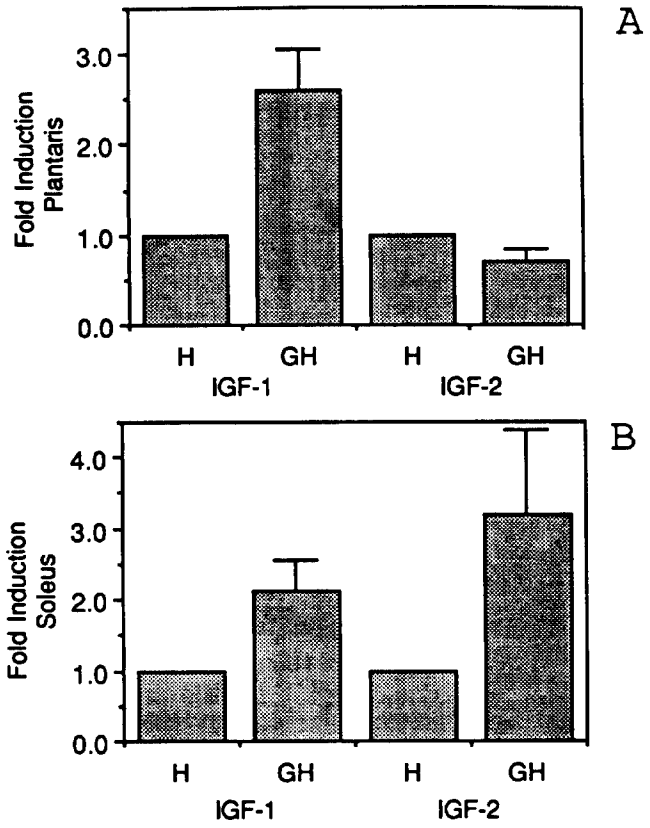
The observations that IGF-I mRNA levels are increased in rat *soleus* and *plantaris* during compensatory hypertrophy and as a result of GH administration strongly suggest (at least) two mechanisms for regulating IGF-I gene expression: a GH dependent (systemic) and a GH independent (local) mechanism.

Figure 6



A. Changes in body weights over time in non-hypophysectomized growing control (Control), hypophysectomized (Hypox), and hypophysectomized rats which received 100 μg rat growth hormone/day for 9 days (Hypox + RGH). Injections began on the day indicated by the arrow. B. Soleus and plantaris weights in Control, Hypox, and Hypox + RGH rats.

Figure 7



Fold increase in IGF-I and IGF-II mRNA levels in the soleus and plantaris following growth hormone administration to hypophysectomized rats. A. Fold induction of IGF-I and IGF-II mRNA in the plantaris. B. Fold induction of IGF-I and IGF-II mRNA in the soleus. H = control hypox rats; GH = hypox rats which were injected with 100μg of rat growth hormone/day for 9 days. Data is expressed relative to control hypox rat levels.

The increase in IGF-I mRNA levels observed during compensatory hypertrophy appears to be completely independent of GH. This is because any effects due to GH would be expected to affect both the hypertrophy muscle and the contralateral control, since they are from the same animal. However, from the above experiments it cannot be determined whether GH may be a "permissive" factor for IGF-I expression in skeletal muscle. Growth hormone may be necessary for the increase in IGF-I mRNA levels which occurs during compensatory hypertrophy, while not directly causing the increase in IGF-I. Alternatively, it cannot be ruled out that GH is directly involved in the increase in IGF-I mRNA levels in the hypertrophy muscles. The growing muscles may become more sensitive to GH than are the contralateral controls. This could occur by increasing GH receptor affinity and/or number. Therefore, while the above experiments strongly suggest GH independent expression of the IGF-I gene, they do not prove a GH independent mechanism.

The Expression of the IGF Genes During Compensatory Hypertrophy in the Absence of Growth Hormone

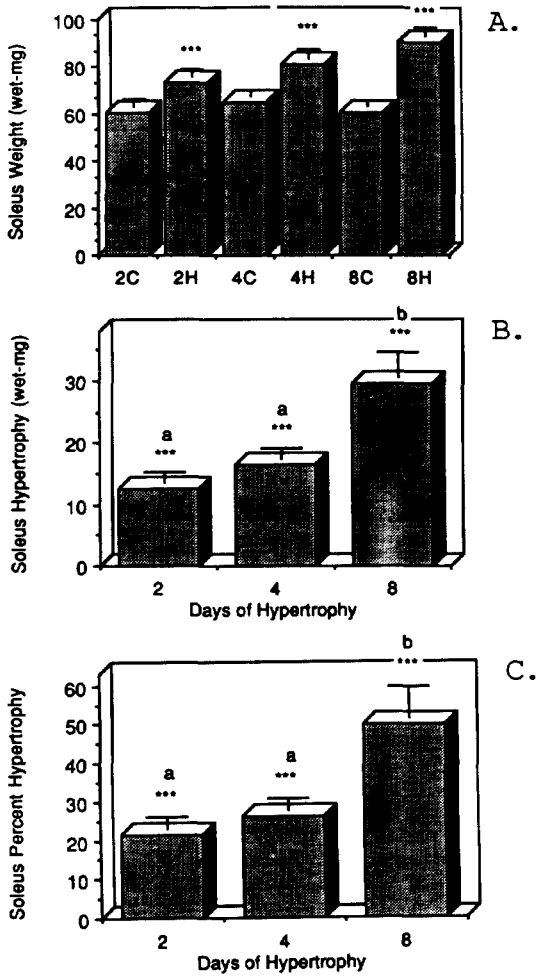
In order to positively demonstrate GH independent expression of the IGF-I and IGF-II genes, compensatory hypertrophy was induced in hypox rats. An even greater percent hypertrophy response was observed in the hypox rats than had been observed in normal (pituitary-intact) rats (Figure 8). The finding that hypox rats can undergo compensatory hypertrophy is in agreement with the results of Goldberg (1967), who first reported that both pituitary-intact and hypox rats can undergo compensatory hypertrophy. These results led Goldberg to postulate that there were two mechanisms for muscle growth, a growth hormone dependent and a growth hormone independent mechanism.

When compensatory hypertrophy was induced in hypox rats, there was an even greater increase in IGF-I mRNA levels than had occurred in normal rats. There was a 10 to 15 fold increase in IGF-I mRNA levels in the *soleus* by 8 days of hypertrophy, and a 5 to 8 fold increase by days 4 and 8 in the *plantaris* (Figure 9). The increase in IGF-II mRNA levels also occurred in hypox rats and were similar to the increases observed in normal (pituitary-intact) rats. These results clearly indicate that IGF-I and IGF-II gene expression can be regulated via a mechanism which is independent of GH.

Discussion

The observation that IGF-I mRNA levels increase dramati-

Figure 8

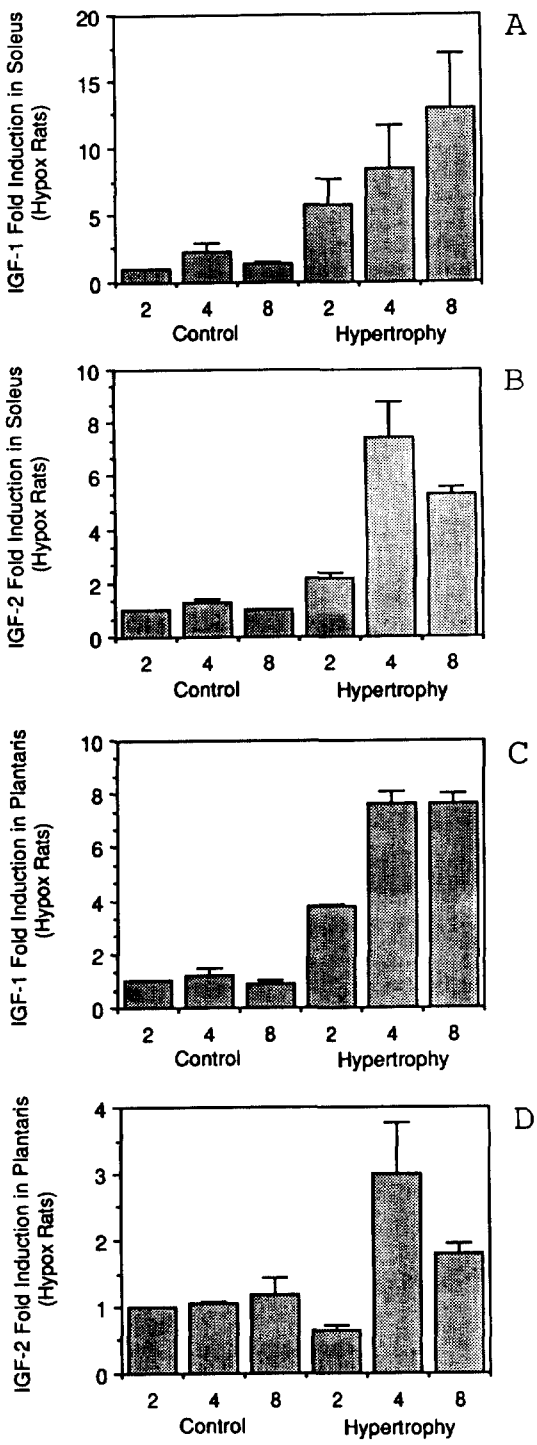


Soleus compensatory hypertrophy (wet tissue basis) in hypophysectomized rats. A. Soleus weight during compensatory hypertrophy in hypophysectomized rats. B. Soleus actual hypertrophy in hypophysectomized rats. C. Soleus percent hypertrophy in hypophysectomized rats. Values are means with standard error bars indicated. *All axis labels and definitions are as described in Figure 2.*

cally during compensatory hypertrophy in the absence of GH clearly indicates that IGF-I mRNA levels can be regulated in a GH independent manner. When taken together, the above series of experiments demonstrate two mechanisms for IGF gene expression: a GH dependent (systemic) mechanism and a GH independent (local) mechanism. Thus, while GH dependent and GH independent muscle growth can occur, the increased expression of the IGF genes may be the underlying mechanism for both types of muscle growth.

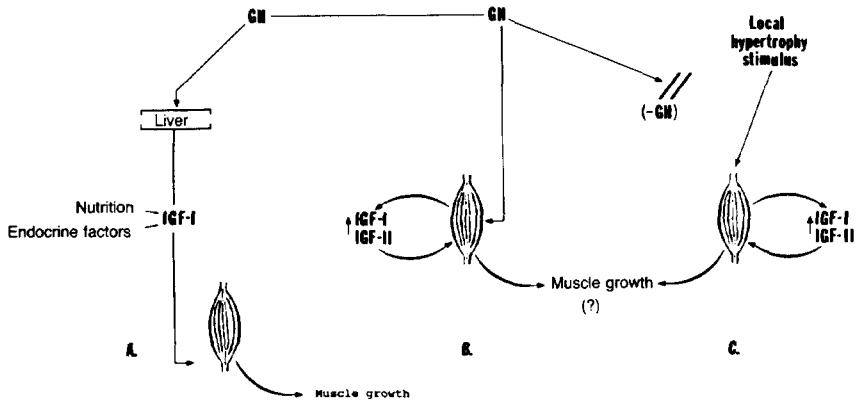
The results also strongly suggest that the IGFs may regulate muscle growth via an autocrine and/or paracrine mechanism. Although direct proof of a cause and effect relationship between the increase in IGF expression and compensatory hypertrophy may be very difficult to obtain, several indirect lines of evidence support this hypothesis. First, a greater compensatory hypertrophy response was observed in hypox rats than in normal rats, and associated with this was a greater fold increase in IGF-I mRNA levels. Second, a greater hypertrophy response was observed in

Figure 9



Fold induction of IGF-I and II in the soleus and plantaris muscles of hypophysectomized rats during compensatory hypertrophy. A. IGF-I mRNA fold induction during compensatory hypertrophy of the soleus in hypophysectomized rats. B. IGF-II mRNA fold induction in the soleus during compensatory hypertrophy in hypophysectomized rats. C. IGF-I mRNA fold induction during compensatory hypertrophy of the plantaris in hypophysectomized rats. D. IGF-II mRNA fold induction during compensatory hypertrophy of the plantaris in hypophysectomized rats. All values are expressed relative to 2 day control values (see Figure 5 for explanation). 2, 4, and 8 = *days post-surgery.*

Figure 10



A proposed model for growth hormone (GH) dependent and GH independent regulation of muscle growth by the insulin-like growth factors (IGF-I and IGF-II). Goldberg (1967) first suggested GH dependent and GH independent muscle growth. The results of our studies suggest that the IGFs can be regulated in skeletal muscle in a GH dependent and GH independent manner. A. Liver derived serum IGF-I, which is GH dependent, may stimulate muscle growth. B. Production of IGF-I and IGF-II by skeletal muscle is stimulated by GH. Locally produced IGFs, under the influence of systemic GH, may stimulate muscle growth in an autocrine and/or paracrine manner. C. IGF-I and IGF-II mRNA levels increase in skeletal muscle during compensatory hypertrophy, even in the absence of systemic GH (– GH). Localized muscle hypertrophy may therefore be under the control of locally produced IGFs which are expressed independently of GH control. The results demonstrate two mechanisms for control of IGF gene expression in skeletal muscle: a GH dependent and a GH independent mechanism.

the *soleus* than in the *plantaris* of hypox rats, and this was correlated with a greater fold increase in IGF-I and IGF-II mRNA in the *soleus* muscle. Third, the only control muscles which demonstrated an increase in IGF message were the 8 day control *plantaris* muscles from normal (pituitary-intact) rats, which showed elevated IGF-II mRNA levels when compared to other control muscles. Coupled with the elevated IGF-II mRNA levels was a significant increase in weight of these muscles. Finally, the known growth-promoting effects of the IGFs on skeletal muscle and the existence of receptors for IGF-I and IGF-II in skeletal muscle are consistent with a mechanism whereby skeletal muscle can be regulated by the IGFs in an autocrine and/or paracrine manner.

A revised model for the mechanisms by which the IGFs may influence muscle growth, taking into account the results of the above studies, is presented in Figure 10. There now appear to be two mechanisms for regulating IGF gene expression in skeletal muscle: a GH dependent and a GH independent mechanism. The finding that skeletal muscle responds to two different types of growth stimuli (GH and compensatory hypertrophy) by increasing IGF-I and IGF-II mRNA levels suggests an important role for locally produced (autocrine and/or paracrine) IGFs in skeletal muscle growth.

As shown in Figure 10, both serum and locally derived IGFs may exert effects on skeletal muscle. The relative importance of locally produced IGFs versus serum IGFs in regulating muscle growth is not known. Riss et al. (1986) reported that the effects of GH and compensatory hypertrophy were additive in causing muscle growth. Their results suggested that GH and compensatory hypertrophy stimulate muscle growth through different mechanisms; however, it is possible that neither GH administration nor compensatory hypertrophy alone caused a maximal IGF response in skeletal muscle. Therefore, the effects of these two stimuli could be additive by increasing IGF expression to levels above that which either stimulus alone can cause.

There is increasing evidence that autocrine and paracrine mechanisms are important regulators of growth. As biotechnology tools and techniques continue to advance and our understanding of local mechanisms of growth control continues to improve, it may be possible to target growth factors and other key regulatory molecules to specific tissues. The desired effect could be either stimulatory or inhibitory, for example to promote muscle growth or inhibit adipose tissue formation. For animal and meat scientists, this could eventually allow the "fine tuning" of animal growth and composition.

Summary

We have shown that there must be (at least) two mechanisms for controlling IGF gene expression in skeletal muscle: a GH dependent and a GH independent mechanism. Thus, it would appear as if both local and systemic factors can control the expression of the IGF genes. Two independent means of inducing muscle growth, GH administration and compensatory hypertrophy, both increase IGF mRNA levels in skeletal muscle. The results suggest that muscle-derived IGF-I and IGF-II may play a fundamental role in regulation of muscle growth via an autocrine/paracrine mechanism.

ACKNOWLEDGEMENTS

The work discussed in this paper was done while the author was a Ph.D. student at the University of Illinois, under the direction of Dr. Peter Bechtel. The author gratefully acknowledges his guidance and support. Dr. Peter Rotwein of Washington University in St. Louis provided the IGF clones and assistance with the IGF solution hybridization methodology. David DeVol was a recipient of a Jonathan Baldwin Turner Graduate Fellowship at the University of Illinois, and wishes to acknowledge the support of this fellowship. Finally, the author wishes to thank the AMSA, Dr. Don Beermann, and the committee of the growth/gene expression session for the opportunity to present this research.

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Discussion

S. Mills: Relating to Terry's talk, we all recognize the effects of growth hormone (GH) on adipose tissue metabolism, specifically as it relates to the inhibition of lipogenic activity and the effect of the treatment to prevent adipose tissue accretion. Terry, I think you present some very compelling evidence that there are some direct actions, but I think that there is probably just as equally strong evidence to suggest (perhaps) the opposite. There is a model which suggests that if you feed animals inappropriately, (i.e., not enough protein of energy) that you may not get the same prevention of lipid accretion in the pST-treated animal. So my question obviously is, is the direct effect of pST on adipose tissue sufficient to explain the decrease in adipose accretion? Perhaps to carry it further and more importantly, if you