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The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy

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Adams, G. R., and F. Haddad. The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J. Appl. Physiol.* 81(6): 2509–2516, 1996.—Insulin-like growth factor-1 (IGF-1) is known to have anabolic effects on skeletal muscle cells. This study examined the time course of muscle hypertrophy and associated IGF-1 peptide and mRNA expression. Data were collected at 3, 7, 14, and 28 days after surgical removal of synergistic muscles of both normal and hypophysectomized (HX) animals. Overloading increased the plantaris (Plant) mass, myofiber size, and protein-to-body weight ratio in both groups (normal and HX; $P < 0.05$). Muscle IGF-1 peptide levels peaked at 3 (normal) and 7 (HX) days of overloading with maximum 4.1-fold (normal) and 6.2-fold (HX) increases. Increases in muscle IGF-1 preceded the hypertrophic response. Total DNA content of the overloaded Plant increased in both groups. There was a strong positive relationship between IGF-1 peptide and DNA content in the overloaded Plant from both groups. These results indicate that 1) the muscles from rats with both normal and severely depressed systemic levels of IGF-1 respond to functional overload with an increase in local IGF-1 expression and 2) this elevated IGF-1 may be contributing to the hypertrophy response, possibly via the mobilization of satellite cells to provide increases in muscle DNA.

growth factors; somatomedin; hypophysectomy; functional overload; ribonuclease protection assay

SKELETAL MUSCLES subjected to increased load bearing respond with a compensatory hypertrophy. This response includes quantitative and qualitative remodeling of muscle proteins leading to increases in mass and cross-sectional area (CSA) with corresponding changes in mechanical properties (3, 20, 27, 30). The mechanisms that link the mechanical stimulus resulting from increased loading to the hypertrophy response have not been clearly elucidated.

From the extensive literature on myogenic differentiation, it is clearly established that growth factors play crucial roles in the formation of skeletal muscles (17). Of the various growth factors, insulin-like growth factor 1 (IGF-1) alone appears to be involved with the expression of a complete spectrum of muscle-specific proteins (16, 17). IGF-1 is reported to have a variety of anabolic effects on skeletal muscle cells both in vivo and in tissue or cell culture (15, 17). Recently, there has been much interest in the role of IGF-1 in the adaptive growth response of mature skeletal muscle, especially with regard to possible autocrine and/or paracrine signaling (11, 12, 19, 22, 24, 25).

A potential confounding factor in studies of autocrine/paracrine IGF-1 pathways is the fact that growth hormone (GH)-stimulated hepatic production of IGF-1 maintains circulating IGF-1 concentrations that have been reported to range from 300 to 600 ng/ml (12, 14,

18) and may also stimulate IGF-1 production in other tissue such as skeletal muscle (7, 18, 31). GH-stimulated IGF-1 production might hamper in vivo investigations into the role of locally produced IGF-1. In an attempt to unmask the local production of IGF-1, researchers have used hypophysectomized (HX) animals to eliminate circulating GH and greatly reduce circulating IGF-1 (e.g., 10, 12). By using the HX model, Goldberg (19) reported that skeletal muscles exhibited work-induced hypertrophy. More recently, DeVol et al. (12) found that 8 days of functional overload increased IGF-1 mRNA eightfold in plantaris (Plant) muscles in HX rats. In transgenic mice, Coleman et al. (9) found that overexpression of IGF-1 (47-fold increase in IGF-1) in muscle lead to hypertrophy of muscles but not other tissues. IGF-1 has also been shown to stimulate myofiber hypertrophy in tissue culture (31). Taken together, these results suggest that IGF-1 may be involved in the hypertrophic adaptation of muscle in response to increased mechanical loading via a local feedback loop that operates independently of GH modulation.

In small animals such as rodents, surgical elimination of a targeted muscle's synergists has been used to increase the chronic mechanical activity imposed on specific skeletal muscles (see Refs. 27, 30). When a fast-twitch muscle such as the Plant is overloaded by removal of the soleus and gastrocnemius muscles, this muscle will increase mass due to an enlargement (cross section) of all the fibers comprising the muscle (3, 27). As noted above, several studies have reported that this hypertrophy response occurs in HX animals and thus appears to be independent of the GH axis of muscle growth control (12, 19).

There is a growing body of evidence that suggests that the compensatory hypertrophy response may require the stimulation of satellite cell proliferation and fusion with myofibers to maintain some finite ratio between muscle fiber size (e.g., cytoplasmic volume) and myonucleus number (1, 13, 26, 27). IGF-1 has been shown to stimulate satellite cell proliferation and fusion in primary rat satellite cell culture (2) and to increase myonuclei number and myofiber size in tissue culture (31). Collectively, these findings suggest that IGF-1 may be mediating the hypertrophy response via the stimulation of satellite cells.

The purpose of the present study was to test the hypothesis that increases in muscle IGF-1 mRNA would result in increased muscle IGF-1 peptide levels and that the time course of these changes would be appropriate (i.e., concurrent with or preceding the hypertrophy) for the mediation of the hypertrophy response in overloaded skeletal muscles. A second hypothesis was that the mechanism of IGF-1 mediation of the hypertrophy response is the activation of satellite cells as reflected

by increases in absolute muscle DNA content and the maintenance of a constant DNA-to-muscle mass ratio.

This study employed the functional overload model to produce compensatory hypertrophy in rat Plant muscles of both intact and HX rats in an attempt to elucidate the role of locally produced IGF-1 in the hypertrophy process. The results of this study indicate that 1) the time course of increased IGF-1 expression is consistent with a role for this growth factor as a mediator of the hypertrophy response and 2) increased expression of IGF-1 in overloaded muscle appears to be stimulating an increase in the DNA content of the muscle (possibly via the mobilization of satellite cells), a process that may be necessary for the maintenance of some critical DNA-to-muscle protein ratio.

METHODS

One-hundred and twenty female Sprague-Dawley rats were purchased from Taconic Farms. The initial body weights of these animals were 205 ± 3 g. Sixty of these rats were surgically hypophysectomized by Taconic Farms. Intact and HX rats were randomly assigned to either a control group [normal control (NC) or HX control (HC)] or an overload group [normal overloaded (NOL) or HX overloaded (HOL)]. All groups consisted of a minimum of six animals. All animals were housed in standard vivarium cages and allowed food and water ad libitum. All treatment protocols were approved by the institutional animal research committee.

Overload

Animals from the overload groups were anesthetized ($80 \cdot 2$ mg⁻¹·kg⁻¹ ketamine/acepromazine), and the Plant muscles were overloaded via bilateral removal of the gastrocnemius and soleus muscles as described previously (3).

Tissue Collection

At 3, 7, 14, and 28 days postsurgery, groups of control and overloaded rats were killed via an overdose of pentobarbital sodium. Plant muscles from one leg were rapidly excised, cleaned of external connective tissue, weighed, freeze-clamped, and stored at -80°C . The Plant muscles from the second leg had transverse midbelly sections removed, mounted in Tissue-Tek (Miles Laboratories), and immersed in isopentane cooled in liquid N₂. Mounted muscles were stored at -70°C . In the 3- and 7-day groups, blood was withdrawn from the left ventricle and the plasma was collected and stored at -20°C for subsequent analysis.

Assessment of Muscle Hypertrophy

Muscle mass determination. At the time the animals were killed, left and right Plant muscles were quickly weighed on an electronic scale before further processing.

CSA determination. Mounted Plant muscles were sectioned with a cryostat at a thickness of 10 μm, mounted on glass slides, and air dried. Tissue sections were stained and classified as type 1, 2a, or 2b by using the myofibrillar ATPase technique described by Brooke and Kaiser (6). CSA of muscle fibers was measured on microscopic images by using an analysis system consisting of a charge-coupled device camera that transmitted the microscopic image to a frame-capture board in a personal computer. The Image software package (National Institutes of Health) was used to delineate fiber boundaries and to calculate CSA. An average of 360 ± 51 type

1, 271 ± 47 type 2a, and $1,910 \pm 274$ type 2b fibers were analyzed per muscle.

Muscle protein concentration determination. Muscle protein concentration was determined from whole muscle homogenate by using the biuret method (21). Total muscle protein was calculated from the product of the concentration and the wet weight of the muscle sample recorded at the time the animals were killed.

IGF-1 mRNA Analyses

RNA isolation. Total RNA was extracted from frozen muscles by using the RNAzol method (Tel-Test), which is based on the method described by Chomczynski (8). After extraction, the RNA pellet was suspended in 0.5% sodium dodecyl sulfate (SDS) and the RNA concentration was determined by optical density at 260 nm. Integrity of the RNA was checked by ethidium bromide staining of 1 μg RNA electrophoresed on 1% agarose, 0.5× TBE gel by using 0.5× TBE as the running buffer. For intact samples, both 28S and 18S (ribosomal RNA) bands can be visualized. Samples were stored at -20°C for subsequent analyses.

Probe synthesis. IGF-1 mRNA expression was determined by the RNase protection assay and was assessed relative to 28S ribosomal RNA (detailed below). Accordingly, two probes were synthesized. One probe is complementary to IGF-1 mRNA and protects 181 nucleotides (nt) in the RPA, and a second probe is complementary to 28S ribosomal RNA and protects a 115-nt fragment. The availability of these two probes allows the simultaneous detection of IGF-1 mRNA and the 28S rRNA. The IGF-1 probe was derived from a rat IGF-1 genomic clone containing parts of introns 3 (286 nts) and 4 (506 nts) and all of Exon 4 (181 nts) generously provided by Dr. P. Rotwein (Washington University School of Medicine) (4). The 28S ribosomal probe was derived from pTRI-RNA 28S antisense control template commercially available from Ambion. Probe transcription and labeling with biotin was achieved by using BrightStar BiotinStar nonisotopic in vitro transcription kit from Ambion. At the end of the reaction, the probe was purified by lithium chloride precipitation and suspended in 20 μl 0.5% SDS. Probe concentration was determined by ultraviolet (UV) absorption. Due to the abundance of the 28S ribosomal RNA, the probe concentration was adjusted to give a linear signal with increasing amount of input RNA. The specific activity of the biotin-labeled probe was lowered to generate a signal in the same range as IGF-1 signal. These adjustments were accomplished by mixing biotin-labeled and cold 28S RNA probe at a ratio of approximately 1:106. A MEGAscript RNA transcription kit from Ambion was used to synthesize large amounts of cold 28S RNA probe. One microgram of the 28S RNA probe mixture for each RNase protection reaction was sufficient to give a linear signal from 5 to 30 μg of total RNA input.

RNase protection assay. The nonradioactive RNase protection assay protocol employed provided simultaneous detection of protected bands from IGF-1 mRNA along with those from 28S ribosomal RNA. Approximately 20 μg total RNA sample were coprecipitated with 300 pg biotin-IGF-1 antisense riboprobe, ~1 μg biotin-28S antisense riboprobe, and 30 μg yeast transfer RNA. Hybridizations were performed in 10 μl Hybspeed hybridization buffer (Ambion) according to the Ambion protocol. The amount of RNase and digestion time were adjusted to account for the high 28S probe concentration. A blank, negative control, containing 50 μg yeast transfer RNA, was used along with the samples to check for complete digestion of the probes. The RNase digestion was stopped by addition of 0.75 ml RNA STAT-50 LS (Tel-Test) and

200 μ l chloroform. In this procedure, the protected RNA fragments were isolated in the aqueous phase and were precipitated by equal volume of isopropanol. Pellets containing the protected RNA fragments were washed once with 75% ethanol, dried, and suspended in a gel-loading buffer containing 80% deionized formamide and loaded on a 4% polyacrylamide 8 M urea denaturing gel. Electrophoresis was carried out at 30 V/cm in $1\times$ TBE until the bromophenol blue reached the bottom of the gel. The RNA was then electrophoretically transferred to a charged nylon membrane (NEN genescreen plus, DuPont) by using a mini-gel blotter apparatus (BioRad) and $0.2\times$ TBE as transfer buffer. Transfer was carried on for 2 h at 100 mA at the end of which the blots were washed for 5 min in $1\times$ TBE, UV irradiated to crosslink the RNA to the nylon, and stored dry until chemiluminescent detection. An Ambion BrightStar Biodetect detection kit was used for the chemiluminescent detection of the protected bands. In this assay, light emission reaches its peak in 2–4 h at which time it levels to a plateau that persists for several days. For the reported results, a 2-h exposure onto film (Kodak X-OMAT AR) was carried on the next day (Fig. 1). The integrated optical density for the detected bands was determined by laser scanning densitometry (Molecular Dynamics personnel densitometer). Each IGF-1 band signal was divided by its corresponding 28S signal.

Muscle IGF-1 Content

IGF-1 extraction. Muscle samples were pulverized under liquid nitrogen, and the resultant powder was transferred to a precooled, microcentrifuge tube (Kontes). After the transfer, the powdered muscle sample weights were recorded. Samples were then homogenized in acid/EtOH solution (12.5% 2N HCl-87.5% EtOH) at a concentration of 10 μ l per mg muscle by using a Teflon pellet dispersal pestle (Kontes). The acid/EtOH extraction is used to separate IGF-1 from IGF-1 binding proteins (5). The homogenate was then incubated for 1 h at 4°C followed by centrifugation at 3,000 g , 4°C for 30 min. The resultant supernatant was decanted and neutral-

ized with 0.855 M Tris base. The neutralized supernatant was incubated at -20°C for 60 min, then centrifuged at 3,000 g , 4°C for 30 min. This final supernatant was stored at -20°C for subsequent determination of IGF-1 by radioimmunoassay (RIA).

IGF-1 RIA. ^{125}I -IGF-1, rabbit anti-IGF-1 anti-serum, and Amerlex-M precipitating reagent were purchased from Amersham Life Science. IGF-1 standards and controls were purchased from Nichols Institute. One hundred microliters each of muscle extracts (or standards), IGF-1 anti-serum, and ^{125}I -IGF-1 were pipetted into duplicate RIA tubes. Tubes were vortexed and incubated with rotation at 4°C for 48 h. At the end of the incubation, 500 μ l of the Amerlex-M donkey anti-rabbit reagent was added to each tube followed by a 10-min incubation at room temperature. The tubes were then centrifuged at 2,500 g , 4°C for 10 min, to separate bound ^{125}I -IGF-1 from unbound. Immediately after centrifugation, the supernatant was removed by aspiration. All RIA tubes were then placed in a Beckman Gamma 4000 counter, and the bound ^{125}I -IGF-1 fraction radioactivity was counted for 2 min per tube. Nonspecific binding was assessed from tubes that contained assay buffer, tracer, and precipitant. Net counts per minute for standards, unknowns, and controls was determined by duplicate averaging and nonspecific binding subtraction. Linear regression analysis of the net counts per minute from five IGF-1 standards was used to calculate the unknown IGF-1 concentrations.

Plasma 3,5,3'-L-Triiodothyromine (T_3) and L-Thyroxine (T_4) Analyses

T_3 and T_4 concentrations in plasma samples were measured by RIA by using a commercially available kit (Pantex).

DNA Determination

DNA concentration was measured in whole muscle homogenates by using a fluorometric assay for the DNA-binding fluorochrome bisbenzimidazole H-33258 (Calbiochem). Calf thymus DNA was used as a standard (23).

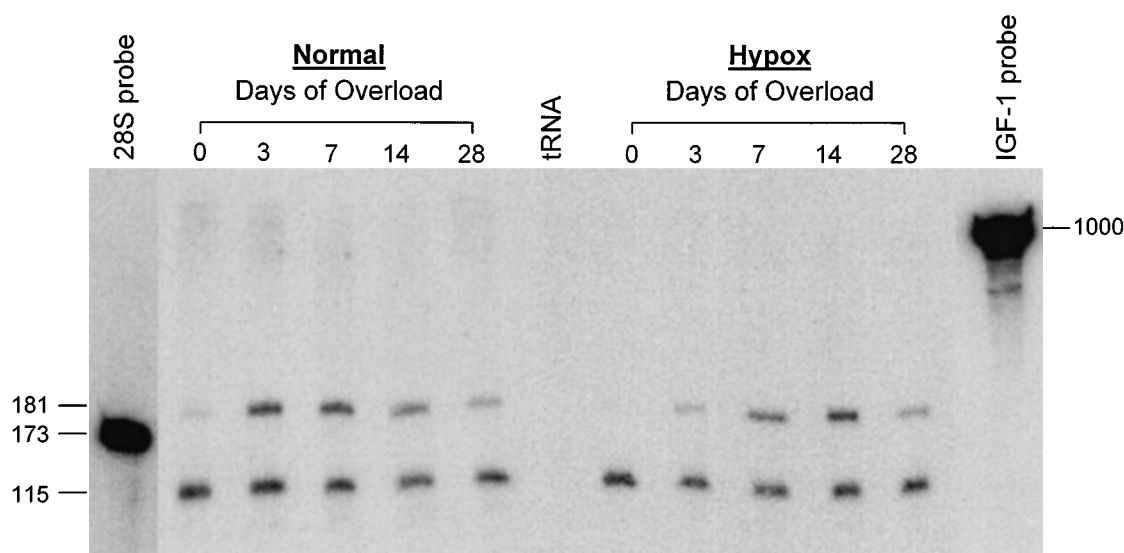


Fig. 1. Representative result of RNase protection assay used to determine levels of mRNA for insulin-like growth factor-1 (IGF-1) in total RNA from plantaris (Plant) muscle. Shown is simultaneous detection of IGF-1 mRNA and 28S ribosomal RNA in each sample. Assay was performed as described in METHODS. The 181-nucleotide (nt) protected band corresponds to IGF-1 mRNA; 115-nt protected band corresponds to 28S ribosomal RNA. Day 0 represents control groups, and days 3, 7, 14, and 28 represent overloaded Plant from hypophysectomized (HX) and intact (normal) rats.

Statistical Analysis

All values are reported as means \pm SE. For each time point, treatment effects for all four groups (NC, HC, NOL, HOL) were determined by analysis of variance with post hoc testing (Student-Newman-Keuls) using the Statmost software package (Data Most). Pearson's correlation analyses of relationships (comparing Plant DNA to mass and Plant DNA to IGF-1) were performed by using the Statmost package. For all statistical tests the 0.05 level of confidence was accepted for statistical significance.

RESULTS

Across the various parameters reported, the data obtained from control groups (NC and HC) at each time point were extremely consistent (note error bars at 0 points, see Figs. 3 and 4). Accordingly, these values have been grouped and presented on plots as the *day 0* time point to simplify the data presentation. However, as indicated in METHODS, all NC and HC group animals were started, maintained, and killed in parallel with the corresponding treatment groups, and statistical analyses were conducted by using the individual control groups appropriate for each time point.

Verification of Hypophysectomy

Over the 4-wk study period, the body weights of the HX rats remained unchanged, whereas those of the intact rats increased \sim 25% (Table 1). The plasma IGF-1 concentrations were fivefold greater in the NC group compared with the HX groups. Plasma IGF-1 concentrations did not change significantly as a result of treatments or elapsed time out to 7 days. The plasma concentrations of T_3 and T_4 indicated that the HX animals (HC and HOL) were significantly hypothyroid compared with the NC and NOL groups. The lack of growth and low circulating concentrations of IGF-1 and

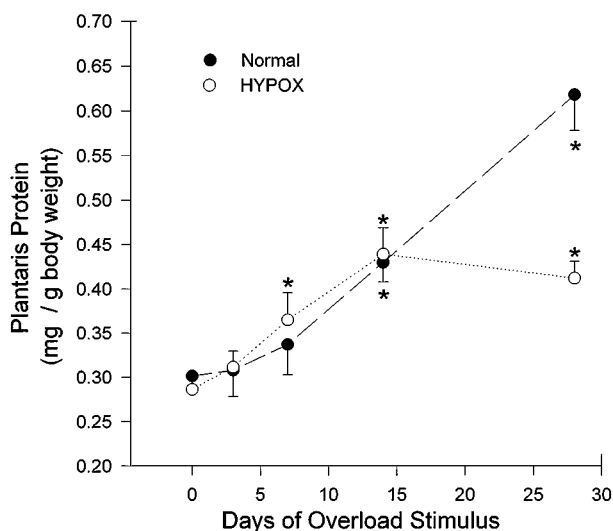


Fig. 2. Total protein from control and overloaded Plant muscles expressed as milligram protein per muscle per gram body weight. Values from normal (NC) and HX (HC) control animals from each time point were grouped and plotted at *day 0*. HYPOX, HX animals. *Significantly different compared with corresponding control values, $P < 0.05$.

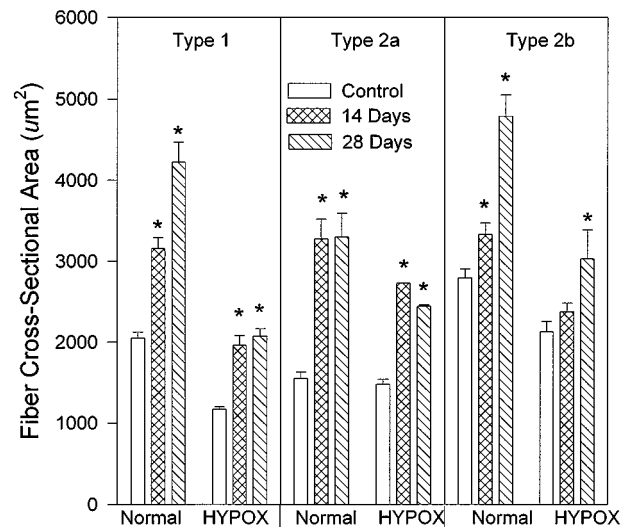


Fig. 3. Plant muscle fiber cross-sectional areas by fiber type from normal and HX rats. Control (NC and HC) and overloaded [normal overloaded (NOL) and HX overloaded (HOL)] Plant muscles at 14 and 28 days of overloading. Control values from both time points were grouped for plotting. *Significantly different compared with corresponding control values, $P < 0.05$.

T_3/T_4 of the HX animals indicated that the hypophysectomies were successful (12).

Compensatory Hypertrophy Response

Muscle mass. Normal rats continued to grow during the course of the experiments; for this reason, we are presenting data that have been normalized to body weights for both groups to factor out any differences due to differential development. When normalized to body weight, Plant muscle weights were significantly increased at 14 and 28 days in both the NOL and HOL groups (Table 2). The relative Plant muscle weights of the NOL groups increased between 14 and 28 days of overload, whereas the HOL relative weights did not increase from 14 to 28 days.

Muscle protein content. Plant muscle protein content is reported as $\text{mg} \cdot \text{muscle}^{-1} \cdot \text{g body wt}^{-1}$. The normalization to body weight was included to account for the

Table 1. Body weights, plasma IGF-1, and plasma T_3 and T_4 values

	NC	NOL	HC	HOL
Body wt at <i>day 0</i> , g	220 \pm 5	213 \pm 3	197 \pm 5	196 \pm 2
Body wt at <i>day 28</i> , g	270 \pm 4 [†]	267 \pm 4 [†]	199 \pm 4	193 \pm 4
Plasma IGF-1 at <i>day 3</i> , ng/ml	487 \pm 22	485 \pm 5	95 \pm 3*	103 \pm 9*
Plasma IGF-1 at <i>day 7</i> , ng/ml	549 \pm 66	524 \pm 65	99 \pm 1*	91 \pm 1*
Plasma T_3 at <i>day 3</i> , ng/dl	62 \pm 3.3	43 \pm 3.0*	10 \pm 2.7*	10 \pm 1.1*
Plasma T_4 at <i>day 3</i> , $\mu\text{g/dl}$	6.0 \pm 0.1	5.6 \pm 0.3	1.6 \pm 0.2*	1.6 \pm 0.1*

Values are means \pm SE. T_3 , 3,5,3'-triiodothyronine; T_4 , thyroxine; NC, normal control; NOL, normal overloaded; HC, hypophysectomized control; HOL, hypophysectomized overloaded; IGF-1, insulin-like growth factor-1. Significantly different ($P < 0.05$) compared with: *NC; [†] *day 0*.

difference in growth between the normal and HX groups. By using this measure, there was significant hypertrophy (27%) of the Plant in the HOL group at 7 days of overloading. Plant protein content was increased 43 and 53% compared with controls in the NOL and HOL groups, respectively, at 14 days. The hypertrophy response continued to increase out to 28 days in the NOL group, whereas it plateaued in the HOL group (Fig. 2). Subjective observations of normal and HX rats suggest that the HX animals are less active. The continued hypertrophy response observed in the NOL group may be a result of differences in activity levels between the two groups.

The Plant muscle protein-to-body weight ratio remained constant in both control groups (NC and HC; pooled as *day 0* data points in Fig. 4) and in the vastus lateralis muscles of all 4 groups (data not shown).

Myofiber CSA. At 14 and 28 days the mean CSAs of muscle fibers from both overload groups were significantly increased compared with the appropriate controls (Fig. 3). At the 14-day time point, the magnitude of the increase in muscle fiber CSA was similar within fiber type for both groups (e.g., for type 2a CSA, NOL increased 2.1-fold, HOL increased 1.9-fold). However, CSA of type 1 and 2b fibers continued to increase in the NOL group, whereas fiber CSAs in the HOL group were similar at 14 and 28 days.

Hypertrophy data presentation. Muscle mass and in some cases CSAs tended to be elevated at time points earlier than 14 days; however, this could have been the result of edema. For this reason, we have focused on the 14- and 28-day time points when there is clear evidence of hypertrophy in both groups using the criterion of muscle protein content (Fig. 2).

IGF-1 mRNA

At peak response, Plant IGF-1 mRNA levels increased more than sixfold during overloading in both the NOL and HOL groups (Fig. 4A). IGF-1 mRNA levels peaked at 3 days in the NOL rats and at 7 days in the HOL animals. From their peak increase, IGF-1 mRNA levels gradually declined but remained significantly elevated at all time points out to 28 days.

IGF-1 Peptide Concentration

Plant IGF-1 peptide concentrations at 3 days were 34 ± 2.3 and 11 ± 0.7 ng/g in the NC and HC groups, respectively. As indicated above, muscle mass values might not provide reliable representation of muscle size

Table 2. *Plantaris muscle wet weight normalized to body weight*

NC	1.04 ± 0.02	HC	1.05 ± 0.03
NOL <i>day 14</i>	1.66 ± 0.10*	HOL <i>day 14</i>	1.80 ± 0.13*
NOL <i>day 28</i>	2.15 ± 0.04*	HOL <i>day 28</i>	1.76 ± 0.06*

Values are means ± SE in milligrams muscle weight per gram body weight. *Significantly different compared with corresponding control values, $P < 0.05$.

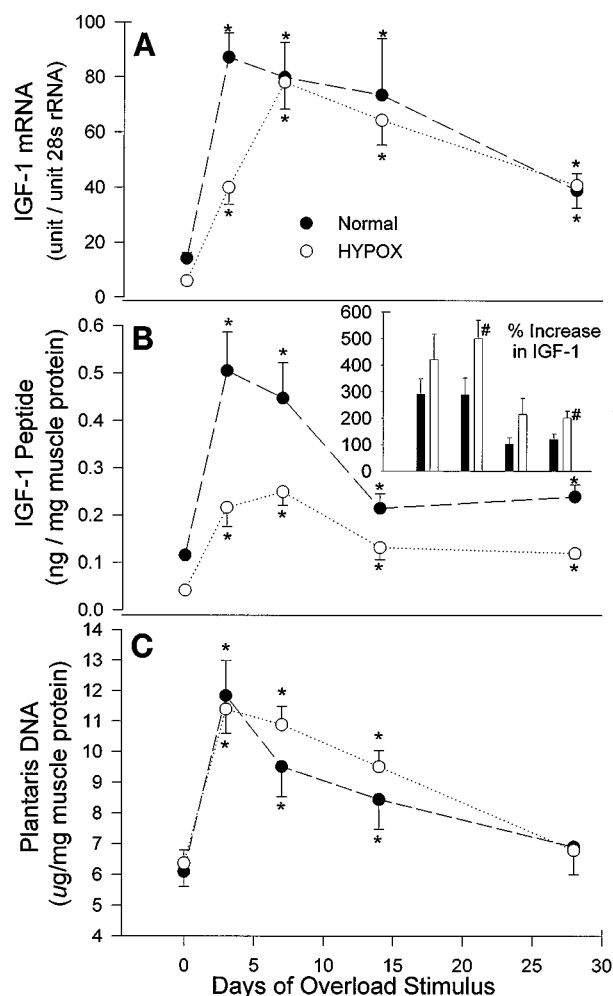


Fig. 4. Time course of changes in Plant IGF-1, IGF-1 mRNA, and DNA with overloading. In normal and HX rats, control (NC and HC) = *day 0*, overloaded (NOL and HOL) = *days 3, 7, 14, and 28*. A: IGF-1 mRNA determined from RNase protection assay. B: IGF-1 peptide concentration. Inset: percent change from corresponding control in IGF-1 peptide concentration. Solid bars, normal; open bars, HX. #Significantly different compared with NOL, $P < 0.05$. C: Plant total DNA content. *Significantly different compared with corresponding control values, $P < 0.05$.

during the adaptation to overload in this model. For this reason, all subsequent IGF-1 data pertaining to the overload protocol have been expressed per milligram muscle protein.

Plant IGF-1 peptide concentrations were significantly elevated at all time points in both the NOL and HOL groups (Fig. 4B). At peak response, IGF-1 concentrations were increased 4.1-fold in the NOL group and 6.2-fold in the HOL group. The peak occurred earlier in the NOL (3 days) group compared with the HOL (7 days) group. The magnitude of the increase in IGF-1 of the HOL group tended to be greater than that seen in the NOL group (Fig. 4B, inset). It is important to note that the reported IGF-1 concentrations have been normalized to muscle protein; thus any increases observed were not a result of general upregulation of protein production in overloaded muscles.

Plant DNA Concentration

Functional overload resulted in significant increases in the DNA concentration ($\mu\text{g}/\text{mg}$ muscle protein) in the Plant muscles of both the NOL and HOL groups at 3, 7, and 14 days (Fig. 4C).

IGF-1/DNA Relationship

Comparison of the IGF-1 and DNA time courses plotted in Fig. 4 suggests that these changes were following a similar pattern of increase and decline. When total DNA content (mg/muscle) is plotted as a function of Plant muscle weight, a significant correlation is found for both the NOL and the HOL groups (Fig. 5). Analyses of the relationship between Plant DNA and IGF-1 concentrations also result in a significant correlation for both normal ($r = 0.53$, $P = 0.0001$) and HX rats ($r = 0.73$, $P > 0.0001$) (data not shown). When the DNA/IGF-1 relationship is plotted with the data grouped by time point, the similarity of the pattern of response for the two groups becomes evident (Fig. 6).

DISCUSSION

The primary aim of this study was to elucidate the role of locally produced (i.e., within the target muscle) IGF-1 in the hypertrophy that results from overloading induced by the removal of synergistic muscles. Although it may be less physiologically representative than intact animal models, the use of HX rats may be more illustrative of the capacity of muscle to autoregulate the interaction between loading state and myofiber size. This model essentially eliminates GH-stimulated IGF-1 production by the liver, greatly reducing circulating IGF-1 levels. In addition, HX eliminates the variable of GH-stimulated IGF-1 production in muscle. Despite the substantial differences in hormonal state and somatic growth, the general pattern of response was similar for NOL and HOL rats for each measured parameter, excluding body weight, up to 14 days of overloading. The IGF-1 response to overloading was very similar between the NOL and HOL rats; however, presumably due to the low baseline, the magnitude of

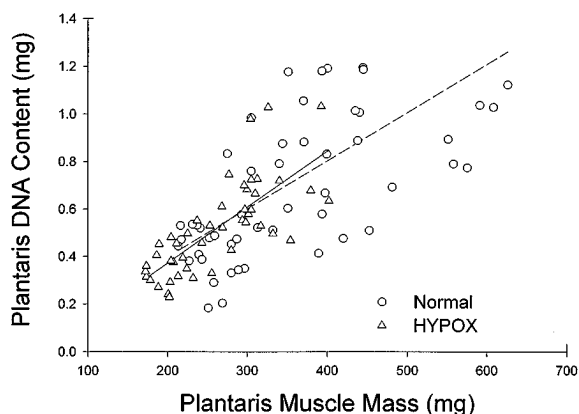


Fig. 5. Plant DNA content (mg/muscle) as function of Plant muscle mass. $r = 0.67$ for normal (solid line) and $r = 0.75$ for HX rats (dashed line). Values are significantly different at $P < 0.05$.

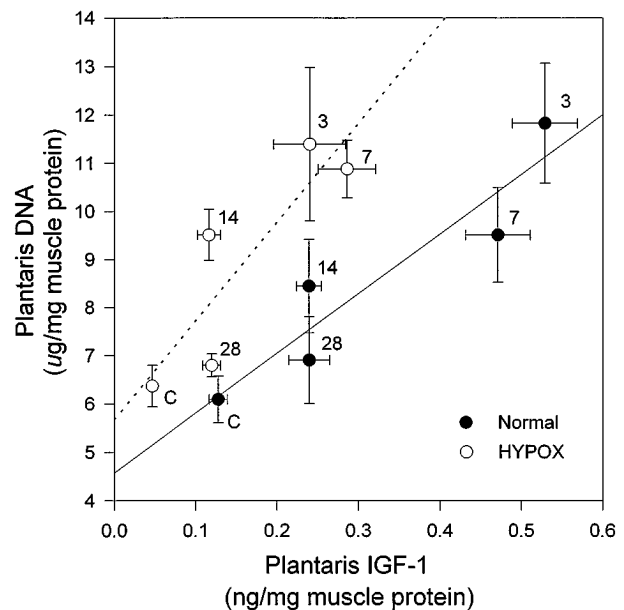


Fig. 6. Plant DNA concentration as function of IGF-1 concentration by time point, using data from Fig. 4, B and C. C, pooled control group values. Numbers, time points in days. Lines represent best fit for the pooled control values and overloaded group values for each time point. Analyses of individual datum points indicated significant correlations (see RESULTS).

the change (i.e., relative change) tended to be much greater in the HOL group.

Skeletal muscle is a heterogeneous tissue (e.g., satellite cells, fibroblasts) and thus gross measurements of total muscle IGF-1 mRNA and peptide might not reflect expression in myofibers. However, IGF-1 mRNA expression in muscles has been localized specifically to the myofibers (7). Furthermore, Yan et al. (32) have reported that eccentric contractions resulted in increased IGF-1 immunoreactivity in a large number of muscle fibers. These results suggest that myofibers have the capacity to express IGF-1 at both the message and peptide levels. The clear coincidence in time course of increased IGF-1 mRNA and peptide levels in the present study strongly suggests that the increased IGF-1 is being produced by myofibers in response to the overload stimulus. Alternatively, it would be necessary to postulate that 1) overloading stimulates an increased uptake and accumulation of IGF-1 by muscles from the circulation to explain these results and 2) the increased IGF-1 mRNA present in overloaded muscles is not being translated.

DeVol et al. (12) have reported that IGF-1 mRNA was significantly increased in overloaded muscles. Both the magnitude and direction of the changes seen herein are in close accord with those reported by DeVol et al. For example, both studies report a 13-fold increase in IGF-1 mRNA in the HOL rats at ~ 1 wk of overloading. The present study further elucidates this response detailing the increase and subsequent decline in IGF-1 mRNA and the concurrent expression of IGF-1 peptide.

In the present study, increases in IGF-1 peptide expression preceded the observed hypertrophy of the overloaded Plant muscles (see Figs. 2 and 4). This

suggests that the time course of increased muscle IGF-1 expression is consistent with a role for this growth factor in the mediation of muscle protein accretion in response to overload.

It is apparent from examination of the time course data presented in Fig. 4 that the changes in IGF-1 peptide and mRNA followed a similar pattern to changes in DNA. Analysis of the relationship between the concentration of Plant IGF-1 and DNA indicated that there was a significant positive correlation between these two variables that was stronger in the HX group. When the data points from Fig. 4, *B* and *C*, were used to plot this relationship (DNA/IGF-1), the similarity in response becomes evident (Fig. 6). At 3 and 7 days of overloading, IGF-1 and DNA are greatly elevated then gradually decline toward control at later time points.

As has been suggested by others (13), a correlation between DNA and muscle mass (Fig. 5) indicates that there may be some critical or optimal myonuclear number-to-fiber size ratio that needs to be maintained in muscle fibers as they compensate for increased loading. Because mature myofiber nuclei are thought to be mitotically inactive (29), increased DNA content in skeletal muscle cells suggests activation of satellite cells. Other studies indicate that satellite cell proliferation may be required for compensatory hypertrophy to proceed (1, 13, 26, 28).

IGF-1 has been shown to stimulate satellite cell proliferation and fusion in primary rat satellite cell culture (2) and to increase myonuclei number and myofiber size in tissue culture (31). Recently, Coleman et al. (9) reported that overexpression of IGF-1 in muscle (47-fold increase in IGF-1) lead to hypertrophy of muscles but not other tissues of transgenic mice. Although these cell culture and transgenic paradigms may be to varying extents developmental models, the parallelism of result with the present study strongly suggests that the IGF-1 system, and specifically locally produced IGF-1, may be mediating muscle hypertrophy at least in part via the activation and incorporation of satellite cells into existing myofibers in the muscles of adult rats. In a preliminary study, we found that 3 days of overloading in both HX and normal rats resulted in an approximately fivefold increase in nuclei that incorporated bromo-deoxyuridine (BRdU) after a bolus injection 1 h before the animals were killed; when overloading was conducted for 1 wk with constant BRdU infusion via osmotic pump, the ratio of BRdU positive nuclei to muscle fiber counted increased from 0.08 ± 0.01 in NC to 0.83 ± 0.12 in NOL rats, an approximately 11-fold increase. (G. R. Adams, unpublished observations). These preliminary findings suggest that the increases in DNA reported in this study most probably reflect satellite cell proliferation.

Various developmental studies have resulted in data that indicate that IGF-1 may be working via the induction of members of the MyoD family gene products (17). This mechanism would be consistent with the activation and differentiation events necessary for satellite cells to contribute to the hypertrophy process. However, it should be noted that none of the data

presented in this study precludes some direct effect of IGF-1 on myofibers and their existing nuclei.

In summary, a comprehensive analysis of overloaded muscles indicates that 1) the muscles of animals with both normal or low circulating IGF-1 will upregulate their expression of IGF-1 peptide in response to overloading leading to compensatory hypertrophy and 2) IGF-1 appears to be mediating some aspect of the hypertrophy response via an increase in DNA that may be maintaining some critical DNA-to-protein ratio, possibly via the activation and incorporation of satellite cells.

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