Skeletal muscle IGF-binding protein-3 and -5 expressions are age, muscle, and load dependent

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Spangenburg, Espen E., Tsghe Abraha, Tom E. Childs, J. Scott Pattison, and Frank W. Booth. Skeletal muscle IGF-binding protein-3 and -5 expressions are age, muscle, and load dependent. Am J Physiol Endocrinol Metab 284: E340–E350, 2003. First published October 22, 2002; 10.1152/ajpendo.00253.2002.—The purpose of the current study was to examine IGFBP-3, -4, and -5 mRNA and protein expression levels as a function of muscle type, age, and regrowth from an immobilization-induced atrophy in Fischer 344 × Brown Norway rats. IGFBP-3 mRNA expression in the 4-mo-old animals was significantly higher in the red and white portions of the gastrocnemius muscle compared with the soleus muscle. However, there were no significant differences in IGFBP-3 mRNA expression among any of the muscle groups in the 30-mo-old animals. There were no significant differences in IGFBP-5 mRNA expression in any of the muscle groups, whereas in the 30-mo-old animals there was significantly less IGFBP-5 mRNA expression in the white gastrocnemius compared with the red gastrocnemius muscles. Although IGFBP-3 and -5 proteins were detected in the type I soleus muscle with Western blot analyses, no detection was observed in the type II red and white portions of the gastrocnemius muscle. Aging from adult (18 mo) to old animals (30 mo) was associated with decreases in IGFBP-3 mRNA and protein and IGFBP-5 protein only in the soleus muscle. After 10 days of recovery from 10 days of hindlimb immobilization, IGFBP-3 mRNA and protein increased in soleus muscles from young (4-mo) rats; however, only IGFBP-3 protein increased in the old (30-mo) rats. Whereas there were no changes in IGFBP-5 mRNA expression during recovery, IGFBP-5 protein in the 10-day-recovery soleus muscle did increase in the young, but not in the old, rats. Because one of the functions of IGFBPs is to modulate IGF-I action on muscle size and phenotype, it is hypothesized that IGFBP-3 and -5 proteins may have potential modulatory roles in type I fiber-dominated muscles, aging, and regrowth from atrophy.

insulin-like growth factor-binding protein; insulin-like growth factor I; growth; hypertrophy; fiber type

SKELETAL MUSCLE IS A PLASTIC TISSUE that retains an intrinsic ability to alter its structural and functional properties to a given environment (12). For example, skeletal muscle atrophies under conditions of immobi-

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muscle is associated with a lowering of IGF-I mRNA (6). Furthermore, skeletal muscle IGF-I mRNA expression was decreased only during maturation, but not with aging (21). Therefore, on the basis of this evidence, it is likely that IGF-I has a significant role in the skeletal muscle growth process.

IGF-binding proteins (IGFBPs) are multifunctional proteins that transport IGFs in circulation, localize IGFs in specific cell types, and alter binding characteristics of IGFs to receptors (23). Six different IGFBP proteins have been cloned and sequenced (33). Initially, IGFBPs were thought to function as proteins that extended the half-life of IGFs in the circulation and also inhibited the binding of IGF to a specific receptor (33). The prevention of IGF-I from binding to a receptor was thought to be essential, since IGF-I can interact with the insulin receptor, and IGF-I concentrations in the serum are 1,000 times higher than those of insulin (30). Now it is known that IGFBPs can localize in the extracellular matrix, modulate IGFs’ interaction with a receptor, and/or act as a localized storage depot for IGFs (33). Thus IGFBPs likely modulate IGF-I’s effectiveness and exert IGF-I-independent effects on various tissues. Although the interactions of IGFBPs and IGF-I have been investigated very carefully, very little information is known about the changes in IGFBP protein expression in skeletal muscle during changes in loading patterns of the animals. Currently, there is less information available on whether IGFBP protein expression might be altered in old animals with age-induced muscle atrophy. Therefore, the purposes of the current report were to determine protein levels of IGFBP-3, -4, and -5 in slow and fast muscles and their response to increased loading of these muscles in young and old rats. The hypotheses of this study were that IGFBP-3, -4, and -5 protein concentrations would be the same in type I and II muscles, would be altered with aging, and would fail to respond during reloading of old muscles recovering from atrophy.

METHODS

Materials. IGFBP-3, IGFBP-4, and IGFBP-5 antibodies were purchased from GroPep (Adelaide, BC, Australia). All remaining chemicals were purchased from Sigma Chemical (St. Louis, MO) or Fisher Scientific.

Animals. All animals were maintained in animal care facilities, kept on a 12:12-h light-dark cycle, and given rat chow and water ad libitum. Young (4-mo), adult (18-mo), and old (30-mo) male F1-generation Fischer 344 × Brown Norway rats were obtained from NIA (Harlan, Indianapolis, IN).

Hindlimb immobilization. The hindlimb immobilization of the rats was performed according to the previously described procedures of Booth (10). Rats were lightly anesthetized with an intraperitoneal injection of ketamine (50 mg/kg body wt) and acepromazine (5 mg/kg body wt) for attachment of the casting material. The hindlimbs of the animals were fixed in plantar flexion so that calf muscle was in a shortened position, as described previously (10). The animals were then checked daily for damage to casting material, which was subsequently repaired as necessary. After 10 days, the casting material was removed from anesthetized rats, and the animals were returned to their cages. The animals were allowed to recover for 10 days and then were killed. The muscles were removed and processed (see Muscle collection and preparation). There were multiple reasons for the use of this model. By utilizing a model of hindlimb immobilization, significant amounts of atrophy can be produced in both type I- and II-predominant muscles (~30–40% decrease in muscle wet weight and muscle protein content), and with the subsequent removal of the casting material, a reloaded muscle is induced to regrow through normal ambulation of the animal (10). In addition, after 30 days of recovery, whereas muscle in young animals regrows from limb immobilization (10), muscle in old animals does not regrow (14). Finally, we believe that this model mimics the response that would be exhibited by a human who is forced into extended periods of bed rest and then required to assume normal ambulation.

Muscle collection and preparation. At the specified time points, the animals were anesthetized (75 mg/kg ketamine, 3 mg/kg xylazine, and 5 mg/kg acepromazine), and the muscle was removed from the animals, weighed, then immediately frozen in liquid nitrogen, and finally stored at −80°C until further analysis. The muscle was later homogenized as 5% (wt/vol) in buffer containing 50 mM HEPES (pH 7.4), 0.1% Triton X-100, 4 mM EGTA, 10 mM EDTA, 15 Na3PO4·10 H2O, 100 mM β-glycerophosphate, 25 mM NaF, 5 mM NaVO4, 50 μg/ml leupeptin, 50 μg/ml pepstatin, and 33 μg/ml aprotinin. All homogenizations were performed on ice with a ground glass homogenizer by use of a variable speed motor. The homogenate was then centrifuged at 13,000 rpm for 10 min. Protein concentrations were determined on all samples through the Bradford protein assay (Bio-Rad, Hercules, CA).

Western blotting. Equal amounts of total protein were then resolved on SDS-PAGE gels and subsequently transferred to nitrocellulose membranes, as previously described (13). All blots were then incubated with Ponceau S (Sigma Chemical) to ensure equal loading (data not shown). The membranes were then blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline with 0.1% Tween (TBS-T) and 1 mM sodium orthovanadate. All membranes were then probed overnight with the appropriate antibody, which was diluted in 5% BSA-TBS. The antibodies were used at the following concentrations: IGFBP-3 (1:2,000), IGFBP-4 (1:10,000), IGFBP-5 (1:5,000). The membranes were washed in TBS-T and then incubated for 1 h with horseradish peroxidase (HRP)-conjugated rabbit, goat, or mouse secondary antibody (1:5,000 rabbit, 1:8,000 mouse; Amersham Pharmacia Biotech, Piscataway, NJ) (1:8,000 goat, Jackson Laboratories). Finally, the HRP activity was detected using enhanced chemiluminescence reagent (NECN Life Science Products, Boston, MA). The membrane was exposed to autoradiographic film (Kodak-XAR5), with the exposure time adjusted to keep the integrated optical densities within a linear and nonsaturated range. The bands were then scanned utilizing a densitometer (Molecular Dynamics) and quantified using ImageQuant software (Molecular Dynamics).

RNA extraction. Total RNA was extracted from preweighed frozen muscle samples using TRIzol reagent (Invitrogen Technologies, San Diego, CA) on the basis of previously described techniques (15). RNA concentrations were determined by measuring the optical density at 260 nm. The quality of the RNA was examined visually by ethidium bromide staining of 18S and 28S under ultraviolet light. The samples were subsequently stored at −80°C for subsequent analysis.
Two micromolars of total RNA were reverse transcribed for each sample with the SuperScript II RT (Invitrogen) mixed oligo(dT) (Invitrogen) and random decamers (500 µg/ml; Ambion, Austin, TX) in a 20-µl reaction volume at 42°C for 50 min. The reaction was inactivated by incubation at 70°C for 15 min. The samples were subsequently stored at 4°C for later analyses.

**PCR.** All methods were adapted according to the previously described methods (2). A semiquantitative RT-PCR was employed using 18S as an internal standard (Classic II 18S). The primer sequences utilized (which read in a 5′→3′ direction) were as follows: IGFBP-3 sense: 5′-CAGCAACCTGAGTGCTTACC-3′, IGFBP-3 antisense: 5′-CTGTCTCCGCCCTAGTACTG-3′ (product size 263 bp, GenBank M31837), IGFBP-4 sense: 5′-GTCGG- AGGGGTTGAAGCTGTTGTTGG-3′ (product size 214 bp, GenBank X76066), IGFBP-5 sense: 5′-CACGCTTCCGACGCAGTAACT-3′, and IGFBP-5 antisense: 5′-GTCCGG- GAATGGGGAGTGTCT-3′ (product size 214 bp, GenBank NM_012817). All primers were purchased from Invitrogen Technologies.

For all PCR reactions, the specific target and 18S were coamplified, therefore allowing the 18S signal to serve as an internal control and to further allow for differences in the total amount of RNA in each sample. The 18S primers were mixed with specific competitor primers (Ambion) at a ratio of 2:8 for all three IGFBP targets. Inclusion of the 18S competitors was necessary to ensure that the 18S signal was linearly amplified with the specific IGFBP target. For each primer set, the experimental conditions were tested to ensure that both the target mRNA and 18S signals were within a linear range of a semilog plot as a function of cycle number under our given conditions. In addition, for each sample and target, the identical solutions were used under the same conditions.

Two microliters of each reverse transcription reaction were mixed with 12.5 µl of AccuPrime Super Mix II (Invitrogen), 0.5 µM 18S primer/competimer mix, and 0.2 µM target primer mix in final 25-µl volume. Amplifications were performed in an Eppendorf Mastercycler with an initial denaturing step of 96°C for 3 min, followed by 25 cycles (IGFBP-5) or 30 cycles (IGFBP-3 or -4) of 1 min at 96°C, 1 min at 55°C, and 1 min at 72°C. The final cycle ended with 3 min at 72°C. The PCR products were then separated on a 2–5% agarose gel and stained with SYBR Gold Nucleic Acid Gel Stain (Molecular Probes, Eugene, OR). The gels were scanned, and signal quantification was performed with ImageQuant software. The signal determined for each target was subsequently normalized to the signal for the 18S target.

**Statistics.** All data were expressed as means ± SE. Statistical significance was determined using a one-way analysis of variance for multiple comparisons followed by a Tukey’s post hoc test. A P value of < 0.05 was considered significant.

**RESULTS**

**Body and muscle masses of 4-mo-, 18-mo-, and 30-mo-old animals.** There was a significant 40 and 48% increase in the body mass of the 18- and 30-mo-old animals compared with the 4-mo-old animals, respectively, in the aging study (Table 1). However, there was no significant difference in body mass between the 18- and 30-mo-old animals. In addition, there were no differences in absolute soleus muscle mass between any of the age groups, but there were significant reductions in the absolute gastrocnemius mass in the 30-mo-old animals by 17% compared with the 4- and 18-mo-old animals. However, the normalized soleus and gastrocnemius muscle masses were significantly less by 20 and 28% and by 28 and 44% in 18- and 30-mo-old animals compared with the 4-mo-old animals, respectively (Table 1). Only the normalized gastrocnemius muscle mass was significantly different between the 18- and 30-mo-old animals.

In the recovery study, the body, absolute soleus, and normalized soleus muscle masses from the 4-mo-old recovery animals were significantly less than those from the 4-mo-old control group by 15, 32, and 20%, respectively (Table 1). The body, absolute soleus, and normalized soleus masses from the 30-mo-old recovery animals were significantly less than the masses from the 30-mo-old control group by 19, 31, and 16%, respectively (Table 1).

Muscles composed of distinct fiber types express varying levels of IGFBP-3, IGFBP-4, and IGFBP-5 mRNA and protein in 4-, 18-, and 30-mo-old animals. IGFBP-3 mRNA expression was significantly higher by ~50% in the red and white gastrocnemius muscle compared with the soleus muscle in the 4-mo-old animals (Fig. 1A); however, there were no significant differences in IGFBP-3 mRNA expression between any of the different muscle types in the 30-mo-old animals (Fig. 1D).

Using an antibody specific for IGFBP-4, we were unable to detect IGFBP-4 expression at the protein level in skeletal muscle in any muscle or condition measured (data not shown). IGFBP-4 mRNA expres-

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**Table 1. Body and muscle masses from young and old animals 10 days after recovery from a 10-day bout of hindlimb immobilization**

<table>
<thead>
<tr>
<th>Age</th>
<th>Condition</th>
<th>BM, g</th>
<th>Muscle wet wt, g</th>
<th>Relative wt/BM, mg/g</th>
<th>Soleus</th>
<th>Gastrocnemius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Muscle wet wt, g</td>
<td>Relative wt/BM mg/g</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>364.34 ± 10.5</td>
<td>0.1664 ± 0.006</td>
<td>0.4568 ± 0.011</td>
<td>2.098 ± 0.081</td>
<td>5.7526 ± 0.07314</td>
</tr>
<tr>
<td>18</td>
<td>Control</td>
<td>509.18 ± 14.7</td>
<td>0.1852 ± 0.008</td>
<td>0.3648 ± 0.019*</td>
<td>2.1054 ± 0.058</td>
<td>4.1502 ± 0.17548*</td>
</tr>
<tr>
<td>30</td>
<td>Control</td>
<td>556.26 ± 9.82*</td>
<td>0.1766 ± 0.006</td>
<td>0.3182 ± 0.014*</td>
<td>1.7416 ± 0.053*†</td>
<td>3.2411 ± 0.14864*†</td>
</tr>
<tr>
<td>4</td>
<td>Regrowth</td>
<td>309.82 ± 2.42*</td>
<td>0.1132 ± 0.004*</td>
<td>0.3654 ± 0.014*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>30</td>
<td>Regrowth</td>
<td>452.36 ± 9.54‡</td>
<td>0.1210 ± 0.006§</td>
<td>0.2685 ± 0.015§</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Values are means ± SE per group of animals. BM, body mass. NA, not applicable. Significantly (P < 0.05) different from *4-mo controls; †18-mo controls; ‡30-mo controls.
Fig. 1. IGF-binding protein (IGFBP)-3 mRNA and protein expressions in various fiber type skeletal muscles isolated from 4-mo-old (A, mRNA; B, protein), 18-mo-old (C, protein), and 30-mo-old (D, mRNA; E, protein) animals ($n = 5$ for all groups). RT-PCR analyses were performed to determine IGFBP-3 expression for various fiber types by age group, and representative gels are depicted below graphs. Western analyses were performed for various fiber types by age group, and representative blots are depicted below graphs. *Statistically different from soleus muscle at $P < 0.05$ level.
sion was significantly lower in the red (~58%) and white gastrocnemius (~82%) muscles compared with the soleus muscle in the 4-mo-old animals (data not shown). There were no significant differences in IGFBP-4 mRNA expression between any of the muscle groups in the 30-mo-old animals (data not shown).

IGFBP-5 mRNA expression does not differ between the soleus and red or white gastrocnemius in the 4-mo-old animals (Fig. 2A). However, in the 30-mo-old animals there was significantly less IGFBP-5 mRNA (~30%) expression in the white gastrocnemius compared with the red gastrocnemius (Fig. 2D), whereas

Fig. 2. IGFBP-5 mRNA and protein expression in various fiber type skeletal muscles isolated from 4-mo-old (A, mRNA; B, protein), 18-mo-old (C, protein), and 30-mo-old (D, mRNA; E, protein) animals (n = 5 for all groups). RT-PCR analyses were performed to determine IGFBP-5 expression for various fiber types by age group, and representative gels are depicted below graphs. Western analyses were performed for various fiber types by age group, and representative blots are depicted below graphs. *Statistically different from soleus muscle; #statistically different from white gastrocnemius, both at P < 0.05 level.
there was no significant difference between the soleus and white gastrocnemius muscles \( (P = 0.13) \).

IGFBP-3 (Fig. 1, B, C, and E) and IGFBP-5 (Fig. 2, B, C, and E) protein expression levels were mainly restricted to the soleus muscle in all age groups. In addition, IGFBP-3 (Fig. 1, B, C, and E) and IGFBP-5 (Fig. 2, B, C, and E) protein expression levels were nearly undetectable in the red and white portions of the gastrocnemius muscle. Therefore, age comparisons were restricted to the soleus muscles.

**Age levels of IGFBP-3 and IGFBP-5 mRNA and protein in the soleus muscle.** IGFBP-3 mRNA expression was significantly higher in the soleus muscles from the 18-mo-old animals by 90 and 104\% compared with the 4- and 30-mo-old animals, respectively. However, there was no difference in IGFBP-3 mRNA expression in the soleus muscle between the 4- and 30-mo-old animals (Fig. 3A).

IGFBP-3 protein expression was significantly increased by 95\% in soleus muscle taken from 18-mo-old animals compared with that from 4- and 30-mo-old rats (Fig. 3B). There was no significant difference in IGFBP-3 protein expression in the soleus muscle between 4- and 30-mo-old rats.

There were no statistical differences in IGFBP-4 mRNA expression in the soleus muscles isolated from any age group (data not shown because its protein was not detectable).

IGFBP-5 mRNA expression was significantly higher by 70\% in the soleus muscle isolated from the 18-mo-old animals compared with that from the 4-mo-old animals (Fig. 4A), whereas there were no significant differences in IGFBP-5 mRNA expression in the soleus muscle isolated from the 4- and 30-mo-old animals (\( P = 0.069 \)).

IGFBP-5 protein expression was significantly reduced by 60\% in the soleus muscle taken from 30-mo-old animals compared with that taken from 4- and 18-mo-old animals (Fig. 4B). No significant differences were detected in IGFBP-5 protein expression between the 4- and 18-mo-old animals.

**Ten-day recovery levels of IGFBP-3 and IGFBP-5 mRNA and protein in the soleus muscle after a 10-day bout of hindlimb immobilization in 4- and 30-mo-old animals.** IGFBP-3 mRNA significantly increased after 10 days of recovery from a 10-day bout of immobilization by 49\% in the soleus isolated from 4-mo-old animals (Fig. 5A). However, there was no change (\( P = 0.16 \)) detected in IGFBP-3 mRNA expression in the soleus muscle after 10 days of recovery in the 30-mo-old animals (Fig. 5C).

IGFBP-3 protein expression was significantly elevated by 43 and 56\% in both the 4- and 30-mo-old 10-day-regrowth animals compared with the age-matched control animals (Fig. 5, B and D).

IGFBP-4 mRNA expression significantly decreased at 10 days of recovery in the soleus muscle of the 4-mo-old animals (data not shown), but there was no change after 10 days of recovery in the 30-mo-old animals (data not shown as its protein was not detectable).

IGFBP-5 mRNA expression did not change after 10 days of recovery in the soleus muscle of the 4-mo-old (Fig. 6A) or the 30-mo-old (Fig. 6C; \( P = 0.061 \)) animals. IGFBP-5 protein expression was significantly elevated by 29\% in regrowing soleus muscle compared with soleus muscle isolated from 4-mo-old control animals (Fig. 6B). However, there was no change in IGFBP-5 protein expression in regrowing 30-mo-old soleus muscle compared with that in its age-matched control (Fig. 6D).

**DISCUSSION**

Although IGFBP-3 and -5 proteins were found in the type I muscle fiber-dominated (soleus) muscle, their absence, at least at the sensitivity of Western analysis,
Translated mechanisms exist for the regulation of IGFBP-3, -4, and -5 expression in type II fiber-dominated rat muscle (Table 2).

Previous work on the expression of IGFBP proteins in skeletal muscle fiber types had been restricted to tissue culture. Conditioned media containing rabbit adult myoblasts obtained from type I muscle exhibited the presence of a 30- to 31-kDa band with ligand blotting that was interpreted by Barjot et al. (7) to correspond to IGFBP-5. However, IGFBP-5 protein was not detected in the conditioned media from adult myoblasts isolated from a rabbit muscle consisting of nearly 100% type II fibers (7). The current observed association (Fig. 3) for a differential expression of IGFBP-5 proteins between types I and IIb muscles extends the aforementioned developmental relationship and allows formulation of a hypothesis that IGFBP-5 either has a causal effect on fiber type phenotype or is a secondary consequence of muscle phenotype. The role of IGFBP-5 in the modulation of the IGF system between types I and II muscles in mature and aged animals remains to be defined.

Barjot et al. (7) found no differences in the amount of IGFBP-3 protein secreted into the media between types I and II myoblasts. In contrast, whereas IGFBP-3 protein was found in the type I soleus muscle, it was undetectable with Western analysis in whole muscle homogenates of the red and white gastrocnemius (Fig. 1). Because previous reports and current data detected IGFBP-3 mRNA in type II muscles (5, 7), IGFBP-3 protein expression may be regulated at the translational and/or posttranslational level in muscles made up of mainly type II fibers.

Because multiple publications report that excess IGF preferentially increases the proportion of type II fibers while decreasing that of type I fiber, support then exists for a hypothesis that the IGF-I system could play a role in fiber type determination in adult skeletal muscles. Lynch et al. (25) reported that IGF-I treatment increased the proportion of type Ia and type IIb fibers and decreased the proportion of type I fibers in the extensor digitorum longus (EDL) muscles of dystrophic mice. Hennessy et al. (22) found that administration of recombinant growth hormone for 6 mo induced significant increases in IGF-I expression, which were associated with significant increases in muscle strength and in the proportion of type II fibers. Specific overexpression of IGF-I in striated muscles of transgenic mice was associated with a shift toward more oxidative fiber types [likely types Ia and IIX (16)]. In addition, this finding was extended to a second IGF-I transgenic mouse line that specifically overexpressed IGF-I in skeletal muscle, by report of an increase in type IIb fibers and a decrease in type Iia fibers in the EDL muscle compared with wild-type mice (27). Therefore, because several studies have reported fiber type shifts due to excess IGF-I, it is possible to suggest that high levels of IGFBP-3 and -5 protein expression in slow fibers could attenuate and/or prevent IGF-I from modulating the fiber type expression in the soleus muscle. However, this suggestion remains to be tested.

Fig. 4. IGFBP-5 mRNA (A) and protein (B) expression of the soleus muscle isolated from 4-, 18-, and 30-mo-old animals (n = 5 for all group A). RT-PCR analyses were performed to determine IGFBP-5 expression by age group and representative gels are depicted below graph (A). *mRNA statistically different from that of 4-mo-old animals at P < 0.05 level. Western blot analyses were performed by age groups, and a representative blot is depicted below each graph bar (B). #Protein statistically different from that of 4- and 18-mo-old animals at P < 0.05 level.

In type IIb (white gastrocnemius) muscle fiber-dominated muscles was unexpected, because two studies have reported the existence of mRNAs for IGFBP-3 and -5 in type II muscles (5, 7), and their mRNA presence in type II muscles was verified in the current study (Figs. 1 and 2). The rat soleus muscle is composed of 86% type I muscle fibers, whereas the red and white portions of the gastrocnemius muscles of 5-mo-old rats had ~20% type I myosin heavy-chain expression (20) and no type I muscle fibers, respectively (18). Thus a novel suggestion of the aforementioned observations may be that important translational and post-

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Previous publications have indicated that IGFBP mRNA transcript concentrations differ among muscle types (6, 8). For example, IGFBP-5 mRNA levels are lower in the soleus muscle (predominantly type I fibers) compared with the gastrocnemius and tibialis anterior muscles (predominantly type II fibers) of 200-g male Wistar rats (8). However, in the current study, IGFBP-5 mRNA showed no difference in muscle type specificity in the young animals, whereas the IGFBP-5 protein shows the opposite fiber type predominance by being higher in the soleus muscle of 4-mo-old rats (Fig. 2; Table 2). In addition, the current study found that IGFBP-3 mRNA expression is higher in the red and white portions of the gastrocnemius muscles compared with the soleus muscle of 4-mo-old rats. This again differs from the findings of Bayol et al. (8), who found no differences in IGFBP-3 mRNA expression between muscle types, and who did not report IGFBP protein levels. The difference in the above mRNA findings could be partially explained by the different strain, as well as the different age, of the rats. More specifically, our 4-mo-old rats were F1-generation Fischer 344 × Brown Norway, whereas Bayol et al. utilized ~2-mo-old Wistar rats. Thus our data suggest that IGFBP-3, -4, and -5 protein expression appears to be regulated at the posttranscriptional level in the red and white portions of the gastrocnemius muscle.

Also, previous publications have indicated that IGFBP mRNA expression differs depending on the load of the muscle (6). IGFBP-5 mRNA decreased at the 2nd and 8th days of compensatory overload of the soleus muscle in 2-mo-old mice (6); yet on the 10th day of regrowth from atrophy in the current study, IGFBP-5 mRNA did not change, whereas IGFBP-5 protein increased in the soleus muscles of 4-mo-old rats (Fig. 6; Table 2). This suggests that a posttranscriptional mechanism may be regulating the increase in IGFBP-5 protein expression in the soleus muscle with increased load. Intriguingly, the hypothesized posttranscriptional mechanism might be dysfunctional in the 30-mo-old rats, in that there was no increase in IGFBP-5 protein expression with increased load.
Owino et al. (28) concluded that the downregulation of the IGF-I system, particularly the attenuated increase in the autocrine skeletal muscle isoform of IGF-I mRNA (designated as “mechano growth factor” mRNA) levels, as well as IGF-I receptor mRNA levels in response to overload, is likely to have an important role in the impaired growth potential of old skeletal muscle. The current observation that IGFBP-5 protein

Table 2. Comparison of published directional changes in selected IGFBP mRNA and protein expression in skeletal muscles under various conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>IGFBP-3 mRNA</th>
<th>Protein</th>
<th>IGFBP-4 mRNA</th>
<th>Protein</th>
<th>IGFBP-5 mRNA</th>
<th>Protein</th>
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</thead>
<tbody>
<tr>
<td>Slow vs. fast muscle</td>
<td>↓ (Fig.1)</td>
<td>↑ (Fig.1)</td>
<td>↑ DNS ↔ (8)</td>
<td>N/D</td>
<td>↓ (6), ↔ (Fig.2)</td>
<td>↑ (Fig.2)</td>
</tr>
<tr>
<td>Young/adult vs. old</td>
<td>↔ (31)</td>
<td>↔ (Fig.3)</td>
<td>↔ DNS</td>
<td>N/D</td>
<td>↔ (19), (Fig.4)</td>
<td>↓ (Fig.4)</td>
</tr>
<tr>
<td>Regrowth from atrophy</td>
<td>↑ 4 mo ↔ 30 mo (Fig.5)</td>
<td>↑ (Fig.5)</td>
<td>↓ 4 mo ↔ 30 mo; DNS</td>
<td>N/D</td>
<td>↔ (Fig.6)</td>
<td>↑ 4 mo ↔ 30 mo (Fig.6)</td>
</tr>
<tr>
<td>Overload hypertrophy</td>
<td>↑ (23a)</td>
<td></td>
<td>↑ (6)</td>
<td></td>
<td>↑ (23a)</td>
<td></td>
</tr>
<tr>
<td>Anabolic hypertrophy</td>
<td>↑ (23a)</td>
<td></td>
<td>↑ (6)</td>
<td></td>
<td>↑ (6)</td>
<td></td>
</tr>
<tr>
<td>Clenbuterol hypertrophy</td>
<td>↑ (6a)</td>
<td></td>
<td>↑ (6a)</td>
<td></td>
<td>↑ (6a)</td>
<td></td>
</tr>
<tr>
<td>Suspension atrophy</td>
<td>↔ (6)</td>
<td></td>
<td>↔ (6)</td>
<td></td>
<td>↔ (6)</td>
<td></td>
</tr>
<tr>
<td>Resistance exercise</td>
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IGFBP, IGF-binding protein. DNS, data not shown. N/D, nondetectable expression levels. Up arrow, increase; down arrow, decrease; horizontal arrow, no change. Nos. in parentheses indicate appropriate figure within this study or published reference.
increased in the soleus muscle only of 4-mo-old, but not of 30-mo-old, rats during muscle regrowth (Fig. 6) extends the observations of Owino et al. (28) to the protein level and to another component of the IGF-I system. However, not all components of the IGF-I system are altered with aging, as similar significant increases in IGF-IEa mRNA (28) and in IGFBP-3 protein (Fig. 5) occurred in the young and old overloaded and atrophied-regrowing muscles, respectively.

Some of the changes in IGFBP mRNAs and proteins are not in parallel with aging. At the mRNA and protein levels, IGFBP-3 protein expression in the soleus muscle was not different between the 4- and 30-mo-old rats (Fig. 3), confirming previous gastrocnemius data (31). However, IGFBP-3 mRNA and protein and normalized muscle mass were significantly elevated in the soleus muscle of 18-mo-old animals compared with 4-mo-old rats in the current study. Although absolute and normalized soleus muscle mass did not change from 18 to 30 mo of age, soleus muscles in similarly aged rats of the same strain are atrophied in another report (5), so it is noteworthy that both IGFBP-3 and -5 proteins are decreased before the expected mass loss in the 30-mo-old soleus muscles in the current study. IGFBP-5 mRNA expression remained elevated at 18 and 30 mo of age in the soleus muscle (Fig. 4), extending similar results for the gastrocnemius muscle (21). Again, results of the present study, combined with other data (21), suggest that regulation at the level of protein translation or posttranslation could determine the expression levels of IGFBP-5 protein in skeletal muscle during aging.

The results of the current study demonstrate that IGFBP-3 and -5 protein expression in skeletal muscles differs between types I and II fiber-dominated muscles, mature and aging animals, and young and old rats during regrowth from limb immobilization. Through the examination of our data and data published by other groups, it appears that IGFBP-3 and IGFBP-5 protein expression in skeletal muscle could be regulated at the level of protein translation/posttranslaction, whereas soleus muscle IGFBP-3 expression could be partially regulated at the transcriptional level. Clearly, additional experiments are necessary to determine how IGFBP-3 and -5 modulate IGF-I function in skeletal muscle.

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