Growth Hormone Improves Body Mass Recovery with Refeeding after Chronic Undernutrition-Induced Muscle Atrophy in Aging Male Rats

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ABSTRACT The effects of growth hormone (GH) administration and refeeding after chronic undernutrition (UN) were investigated in Fischer 344 male rats aging into senescence (24.5 mo of age) during UN initiated at 12.5 mo of age that produced muscle atrophy and a 50% decrease in body mass. Muscle mass, protein, myosin heavy-chain (MHC) composition and circulating testosterone levels were measured and compared to controls with free access to food. Within 9 wk, refeeding + GH restored body mass to control levels, whereas it was still decreased with refeeding alone. By 24.5 mo of age, refeeding alone restored body mass, while addition of GH resulted in overshoot. UN uniformly decreased mass of the gastrocnemius, extensor digitorum longus, soleus and diaphragm muscles to 50–60% of controls. Refeeding and refeeding + GH restored these losses with some overshoot of gastrocnemius muscle suggesting hypertrophy. UN more than doubled slow Type I MHC composition and approximately halved fast Type IIB and IIX MHC in the deep gastrocnemius muscle while it increased Type IIA MHC in the diaphragm. Refeeding and refeeding + GH reversed these shifts. MHC shifts in the extensor digitorum longus and soleus muscles were not statistically significant, whereas UN increased fast Type IIA MHC followed by decrease with refeeding + GH. UN decreased testosterone levels to nearly zero followed by restoration with refeeding and refeeding + GH. We conclude that the phenotype of mixed-MHC muscles such as the gastrocnemius and diaphragm are most affected by chronic UN, which is reversible with refeeding and refeeding + GH. These alterations were associated with changes in circulating testosterone, which may be a key regulatory element in these processes.


KEY WORDS: • cachexia • Fischer 344 rats • senescence • undernutrition

Muscle atrophy, characterized by decreases of muscle girth and mass, occurs under many circumstances including limitation of nutrition (Ameredes et al. 1998a and 1999, McCarter et al. 1982, Prezant et al. 1994), muscle disease (Carmeli et al. 1993, Evans and Campbell 1993) and aging (Corpas et al. 1992, Evans and Campbell 1993, Larkin et al. 1998, Proctor et al. 1998). Muscle atrophy in elderly patients may be especially pronounced with certain diseases that are accompanied by cachexia (Donahoe 1997, Pape et al. 1991, Wolf et al. 1992), which is characterized as poor health accompanied by emaciation. The cachectic state is considered to be a function of which is characterized as poor health accompanied by emaciation. The cachectic state is considered to be a function of which is characterized as poor health accompanied by emaciation. The cachectic state is considered to be a function of

Chronically ill patients and elderly individuals in poor health are often undernourished due to reduced appetite and inability to feed properly (Donahoe 1997). In these instances, muscle atrophy can be due to sarcopenia or a loss of muscle mass associated with negative protein balance and persistence of a catabolic state (Clemmons and Underwood 1992, Donahoe 1997, Evans and Campbell 1993, Proctor et al. 1998). This situation is poorly responsive to nutritional repletion (Donahoe 1997). Thus, supplementation of nutritional repletion with anabolic hormones such as growth hormone (GH) has been considered (Clemmons and Underwood 1992, Donahoe 1997, Pape et al. 1991, Wolf et al. 1992), however, the specific effects of GH on muscle phenotype and function in the setting of aging and chronic undernutrition (UN) remain incompletely understood. A prior report indicated that GH administration in conjunction with nutritional repletion reversed some of the alterations produced by UN within the aged diaphragm muscle (Ameredes et al. 1999), therefore, GH effects on aged limb skeletal muscles would seem likely.

Moreover, the signals for these alterations in skeletal muscle phenotype are not known with certainty, particularly with UN in the context of aging. It is known that sarcopenia is associated with age-related decrements in function of the thyroid axis (Lamberts et al. 1997) and the pituitary-gonadal axis (Lamberts et al. 1997, Tenover 1997). Especially in males, decrements in circulating androgens such as testosterone and associated decreases in growth factors occur with UN (Lanz et
al. 1992) and aging (Everitt and Meites 1989), but have not been linked conclusively to the muscle atrophy process (Lamberts et al. 1997, Proctor et al. 1998, Tenover 1997). Interestingly, many of these same events occur with simple food restriction, which traditionally has been considered to retard or postpone the aging process (McCarter et al. 1982, Sojala and Weindruch 1996). This dilemma may have serious ramifications with regard to nutritional and hormonal treatment of aged cachectic patients who require these therapies to assist in the management of their disease. Thus, further understanding of these effects is desirable.

Therefore, the purpose of the following study was to determine the alterations in mammalian muscle phenotype under conditions of severe chronic UN, followed by nutritional repletion through refeeding (RF), and administration of GH. Physical characteristics and myosin heavy-chain (MHC) composition of selected prototypical fast (extensor digitorum longus, slow (soleus) and mixed (gastrocnemius) limb muscles were measured. Changes in circulating testosterone under these conditions were assayed as one possible signal in the trophic processes. The hypothesis tested was that GH, in combination with refeeding, would reverse the muscle atrophy and MHC shifts induced by chronic UN, and that these alterations would be associated with changes in circulating testosterone levels.

MATERIALS AND METHODS

General. The animal care and nutrition protocol was approved by the University of Pittsburgh Institutional Animal Care and Use Committee and conformed to the NIH guidelines (Guide for the Care and Use of Laboratory Animals, NIH Publication No. 86–23). Briefly, adult male Fischer 344 rats were given free access to a standard diet of Purina Rat Chow and weighed twice per week over the course of a 9-mo period from the delivery date. At this time they were sexed, and MHC isoforms were separated from myosin extracts by polyacrylamide gel electrophoresis (Fig. 1) as previously described (Ameredes et al. 1998a). Briefly, gels were prepared from a stock solution of 30% acrylamide containing 2.94 g/L of acrylamide and 0.06 g/L of bis (N,N'-methylene-bis-acrylamide). Electrophoresis was performed using a buffer composed of 20% acrylamide (Tm = 8% and stacking gel of Tm = 4%, Tm = total concentration of monomer (acrylamide + bis) at C = 2% (C = percentage of total monomer due to bis). Volumes of myosin extract (1–3 μL) containing 500-1000 ng of protein per well were loaded on the gels. Electrophoresis (275 V for 3.5 h then 178 V for 17.5 h) was performed using a vertical slab gel unit (SE600; Hoefer Scientific Instruments, San Francisco, CA) with Tris/glycine running buffer in a cold room maintained at 4°C. Separating gels were silver-stained. MHC gels were analyzed using a scanning densitometer (GS 300; Hoefer Scientific, San Francisco, CA) and densitometry software (GS 365; Hoefer Scientific) to quantify the area under individual isoform peaks. These data were used to determine the relative contributions of individual isoforms to their respective total MHC complements within the muscle. Total protein concentrations were measured on separate samples of muscle homogenates using the method of Lowry et al. (1951).

Testosterone analyses. Circulating testosterone levels were measured in blood samples taken from the abdominal aorta. A 25-gauge angiocatheter was inserted just above the renal artery bifurcation advanced 2–3 mm and tied in place with sutures previously placed around the vessel. One to two milliliters of blood were withdrawn, centrifuged (3000 × g) and separated. Serum was frozen at −80°C for later analysis. Duplicate serum samples for each rat were analyzed for total testosterone levels using an 125I radioimmunoassay kit (Coat-a-Count; Diagnostic Products Corp., Los Angeles, CA). The sensitivity of the kit was 0.14 nmol/L and the specificity for testosterone was 98%.

Statistics. Repeated-measures ANOVA (SigmaStat v.1.03; Jandel Corp., San Rafael, CA) was used to compare changes in body weight over the age interval studied with P < 0.05 considered significant. One-way ANOVA was used to compare muscle weight and MHC composition values within and between treatments at ages of 19.5 and 24.5 mo with P < 0.05 considered as significant. The general linear model of these ANOVA allowed compensation for unequal sample numbers between groups through sample number weighting of expected mean square values calculated for each group. Student-Newman-Keuls’ posthoc test was used to compare these variable values between discreet groups at a significance level of P < 0.05. Thus, statistical significance is reported as P values <0.05 for all tests. When a significant ANOVA F statistic was obtained, but an apparent discreet trend was nonsignificant by posthoc testing, the P value is reported as >0.05. When other trends appeared to be present, but the ANOVA F statistic was nonsignificant, it is given as the “ANOVA P.”

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** Photograph of SDS-PAGE gel showing typical gastrocnemius muscle myosin heavy-chain isoform band separation. Shown are myosin heavy-chain bands for Type I (slow), IIA, IIB and IIX isoforms from muscles at 24.5 mo of age.
initiation (20.75 mo of age). Body mass in the RF group was greater than the UN group by the second week of their initiation of the study (24.5 mo of age; Fig. 2). Average body mass of both RF and RF + GH groups were significantly greater than the UN group by the second week of their initiation (20.75 mo of age). Body mass in the RF + GH group was significantly greater than RF beginning at 21.75 mo of age, becoming not different than controls by 22.25 mo of age. At that same time, RF alone was still significantly lower than controls and remained lower through the end of the study.

RESULTS

The body mass decrement produced by UN was significantly different from freely-eating controls by the second week of food restriction (12.75 mo of age; Fig. 2). Average body mass of both RF and RF + GH groups were significantly greater than the UN group by the second week of their initiation (20.75 mo of age). Body mass in the RF + GH group was significantly greater than RF beginning at 21.75 mo of age, becoming not different than controls by 22.25 mo of age. At this time, body mass with RF alone was still significantly lower than controls and remained lower through the duration of the study.

Body mass and masses of all muscles sampled at the termination of the study (24.5 mo of age; Fig. 3) were significantly lower in UN rats (P < 0.05). Both RF and RF + GH produced significant increments, restoring mass toward that of control, however, RF + GH increased both the body mass and the gastrocnemius muscle to values above that of the RF group (P < 0.05). This trend also was suggested in the DIAlm (nonsignificant).

Total protein was decreased (nonsignificant) with UN in the deep GSTRCm at 24.5 mo of age (Table 1) with RF and RF + GH resulting in significantly greater amounts of protein in this muscle. The superficial GSTRCm showed similar trends which did not reach statistical significance (ANOVA, P = 0.80). No demonstrable alterations were observed for the EDLm and SOLm.

No statistically significant changes in MHC composition occurred as a result of these treatments in the EDLm and SOLm at 24.5 mo of age, although there was some suggestion of shifts toward increased Type I and IIA MHC in the EDLm (Table 2). At 19.5 and 24.5 mo of age, Type IIA MHC of the hemi-diaphragm was increased while type IIX was decreased with UN at 19.5 mo of age (Table 3). These trends were suggested at 24.5 mo of age but did not reach statistical significance (Type IIA: ANOVA P = 0.09, Type IIX ANOVA P = 0.053). RF + GH significantly decreased Type I and IIA MHC as compared to UN (P < 0.05). A significant increase in Type IIX MHC was observed with RF alone, and a similar increase was suggested with RF + GH, which did not reach statistical significance (ANOVA P = 0.053).

At 24.5 mo of age with UN, type I MHC of the deep gastrocnemius was significantly increased (Fig. 4), while type IIB and IIX MHC were significantly decreased (P < 0.05). RF and RF + GH reversed these shifts to levels not different from controls. At this same age, percentages of type I (P > 0.05), IIA (P < 0.05), and IIX (P < 0.05) MHC were less, while type IIB MHC was significantly greater in the superficial gastrocnemius with UN. RF and RF + GH typically reversed these shifts, again restoring respective MHC isoform levels toward those of the controls. An effect of aging on MHC composition was observed between 19.5 and 24.5 mo of age in the control superficial gastrocnemius with a significant increase in type I and IIA MHC and a decrease in type IIB MHC over time (P < 0.05; main factor: time).

A significant decrease in serum testosterone was observed with aging in the control animals (Fig. 5). UN at 19.5 mo of age depressed testosterone levels to nearly zero. A similar depression was observed with UN at 24.5 mo of age, however, it was not statistically different from the respective age-matched controls (P > 0.05). Both RF and RF + GH resulted in significantly greater levels of circulating testosterone as compared to that in the UN rats.

DISCUSSION

Body and muscle mass alterations. The body mass of rats was dramatically lower with UN, attaining a value of one-half that of freely-eating controls. The effect was manifest early (within 2 wk), reaching a sustained plateau within about 5 mo. Initiation of RF and RF + GH resulted in rapid replenishment of body mass with the RF + GH group demonstrating a greater
and sustained increment (Fig. 3). Moreover, the RF + GH group attained the control body mass value by 21.25 mo of age, which was 2.5 mo more rapid than RF alone. As shown in Table 4, limb and respiratory muscle masses at 24.5 mo of age with UN were approximately one-half that of respective controls. These data indicated that the UN/control ratios of both body and muscle mass attained values similar to the ratio of UN/control food provision (~0.50). Subsequently, the overshoot of body and GSTRCm mass, along with the trend in the DIAm, were suggestive of a hypertrophic effect of GH when administered with RF. Thus, the relative masses of these two muscles followed changes in the body mass under these conditions. These findings demonstrate the ability of this nutritional/hormonal paradigm to produce significant muscle atrophy and hypertrophy over a time span that encompasses nearly the whole adult life of the rat.

**Muscle total protein changes.** The significant decreases in protein concentration with UN and the subsequent increases with RF and RF + GH in the deep GSTRCm suggest that it was most affected by the experimental regime in this study. While the protein values suggested increase with RF + GH vs. RF alone, this increment was not statistically significant. Interestingly, the superficial GSTRCm, comprised of mostly fast Type IIB and IIX MHC, was not significantly affected, similar to the results seen with the EDLm, which is a prototypical fast-fibered muscle. These results suggest that protein turnover rates may have been most affected in largely mixed-MHC muscles, i.e., those in which one MHC type does not dominate the total MHC composition. Furthermore, this notion is consistent with a prior study (Kelly et al. 1984) reporting protein turnover rate to be slowest in muscles with predominantly fast-twitch fibers and higher in muscles such as the medial gastrocnemius that have substantial mixed fast and slow fiber composition. In the present study, the profound muscle atrophy with UN and subsequent hypertrophy with RF + GH observed in the GSTRCm (Fig. 2) are likewise consistent with these ideas.

**MHC shifts.** MHC shifts were most pronounced in the GSTRCm. As with the protein concentrations, the alterations in MHC composition with RF + GH vs. RF alone were not significant. In the deep GSTRCm, a ≥2× increase of Type I MHC and approximately the same reduction in Type IIB and IIX MHC occurred with UN into senescence. These changes were reversed with RF + GH alone. In the superficial GSTRCm, an effect of aging into senescence was noted in controls with >2× increases in Types I and IIA, and a >2× decrease in Type IIB, MHC. These changes were strikingly reversed back to either zero or the prior control value by UN. A trend for increased Type IIX MHC into senescence also was suggested followed by a significant halving with UN. All of these findings are consistent with the previous findings of Inoki et al. (1995) reporting that muscle fast fiber composition was most affected in the GSTRCm and DIAm that have substantial mixed fast and slow MHC. These changes were consistent with these ideas.

### Table 1

Protein concentrations of muscle homogenates from rats at 19.5 and 24.5 mo of age with free access to food (control, C), chronic undernutrition (UN), refeeding (RF), and refeeding + growth hormone (RF + GH)

<table>
<thead>
<tr>
<th>Age</th>
<th>C 19.5 mo</th>
<th>UN 19.5 mo</th>
<th>RF 24.5 mo</th>
<th>RF + GH 24.5 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg protein/mg muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDLm</td>
<td>—</td>
<td>—</td>
<td>264 ± 22</td>
<td>236 ± 33</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td>(5)</td>
<td>(7)</td>
</tr>
<tr>
<td>SOLm</td>
<td>—</td>
<td>—</td>
<td>251 ± 9</td>
<td>283 ± 35</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(6)</td>
<td>(5)</td>
<td>(7)</td>
</tr>
<tr>
<td>GSTRCm Deep</td>
<td>220 ± 31</td>
<td>236 ± 18</td>
<td>290 ± 35</td>
<td>203 ± 23†</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(10)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
<tr>
<td>GSTRCm Superficial</td>
<td>167 ± 11</td>
<td>190 ± 13</td>
<td>185 ± 45</td>
<td>158 ± 26</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(10)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n as shown in parentheses beneath each mean; †P = 0.053 as compared to Control group (C) at same age, §P < 0.05 compared to UN group at same age; SOLm = soleus muscle, EDLm = extensor digitorum longus muscle, GSTRCm = gastrocnemius muscle.

### Table 2

Myosin heavy chain composition of prototypical fast (EDLm) and slow (SOLm) limb muscles in rats at 24.5 mo of age with free access to food (control, C), chronic undernutrition (UN), refeeding (RF), and refeeding + growth hormone (RF + GH)

<table>
<thead>
<tr>
<th>MHC2</th>
<th>SOLm</th>
<th>EDLm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>UN</td>
</tr>
<tr>
<td>Type I, %</td>
<td>1 ± 1</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Type IIA, %</td>
<td>12 ± 4</td>
<td>27 ± 5</td>
</tr>
<tr>
<td>Type IIB, %</td>
<td>54 ± 8</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>Type IIX, %</td>
<td>33 ± 4</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n as shown; EDLm = extensor digitorum longus muscle, SOLm = soleus muscle.
2 MHC = myosin heavy chain.
these UN-induced changes in the superficial GSTRCm were reversed with RF and RF + GH. Numerically, average Type IIA MHC composition of the prototypical fast EDLm was more than doubled with UN (ANOVA $P = 0.15$) (Table 2), whereas Type IIA MHC of the prototypical slow SOLm was one-third less (ANOVA $P = 0.18$). These differences displayed a trend in the appropriate paradoxical directions suggestive of hormonal regulation of Type IIA MHC expression in these specific prototypical muscles (Schiaffino and Reggiani 1994). A slightly greater sample number in each group would likely show these differences to be statistically significant.

Finally, the mixed-MHC DIAm did show some UN- and GH-dependent shifts, in agreement with our prior results (Ameredes et al. 1998a). Taken together, these data suggest:

1. chronic UN may drive some muscles toward expression of certain predominant MHC isoforms,
2. RF and RF + GH can reverse these adaptations, and
3. these effects are pronounced in muscles with largely mixed-MHC composition.

Thus, these results are consistent with the idea that largely mixed-MHC muscles such as the deep GSTRCm and DIAm may have greater adaptive flexibility than those with more homogeneous MHC composition, such as the SOLm and EDLm.

Our methods allowed quantification of the relative MHC composition of the muscles, but not assessment of the actual amount of MHC within the muscles. Therefore, we cannot say conclusively whether these MHC shifts occurred due to either increases in the amounts of MHC, decreases in the amounts of MHC, or a combination of both.

### Table 3

<table>
<thead>
<tr>
<th>MHC</th>
<th>19.5 mo C</th>
<th>19.5 mo UN</th>
<th>24.5 mo C</th>
<th>24.5 mo UN</th>
<th>24.5 mo RF</th>
<th>24.5 mo RF + GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I (%)</td>
<td>31 ± 4</td>
<td>33 ± 1</td>
<td>31 ± 2</td>
<td>34 ± 2</td>
<td>30 ± 1</td>
<td>28 ± 1†</td>
</tr>
<tr>
<td>Type IIA (%)</td>
<td>23 ± 1</td>
<td>29 ± 1*</td>
<td>21 ± 2</td>
<td>24 ± 1</td>
<td>20 ± 2</td>
<td>19 ± 1†</td>
</tr>
<tr>
<td>Type IIB (%)</td>
<td>14 ± 3</td>
<td>11 ± 2</td>
<td>17 ± 2</td>
<td>14 ± 3</td>
<td>12 ± 3</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>Type IIX (%)</td>
<td>33 ± 2</td>
<td>26 ± 1*</td>
<td>32 ± 3</td>
<td>28 ± 3</td>
<td>38 ± 2†</td>
<td>36 ± 2</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, $n$ as shown; treatment group designations as in Table 1; *$P < 0.05$ UN vs. C at same age; †$P < 0.05$ RF or RF + GH vs. UN at same age.
2 MHC = myosin heavy-chain.

### Figure 4

Myosin heavy-chain (MHC) percentage composition of the superficial and deep gastrocnemius muscle in rats at 19.5 and 24.5 mo of age with free access to food (control), chronic undernutrition (UN), refeeding (RF), and refeeding + growth hormone (RF + GH). Shown are averages of Type I (slow), IIA, IIB and IIX MHC isoforms, in control, UN, RF and RF + GH. Zeros indicate MHC percentage composition was 0% Type I for the superficial gastrocnemius in control and UN groups at 19.5 mo of age, and in UN group at 24.5 mo of age. Animal $n$’s for 19.5 mo and 24.5 mo of age for control were 5; $n$’s for 19.5 mo and 24.5 mo of age for control were 5; $n$ for 19.5 mo RF + GH were 8. Error bars are SEM; *$P < 0.05$ as compared to control group; †$P < 0.05$ as compared to UN group.

### Figure 5

Serum testosterone concentrations in rats at 19.5 and 24.5 mo of age, with free access to food (control), chronic undernutrition (UN), refeeding (RF) and refeeding + growth hormone (RF + GH). Shown are control; UN; RF; and RF + GH groups. Error bars are SEM; *$P < 0.05$ UN as compared to control group, †$P < 0.05$ control 19.5 vs. control 24.5 mo of age, ‡$P < 0.05$ as compared to UN group.
TABLE 4

Body mass and muscle mass ratios in rats at 24.5 mo of age with free access to food (control), chronic undernutrition (UN), refeeding (RF), and refeeding + growth hormone (RF + GH)*

<table>
<thead>
<tr>
<th>Mass ratio</th>
<th>UN/C</th>
<th>RF/C</th>
<th>RF + GH/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>0.49</td>
<td>0.95</td>
<td>1.13</td>
</tr>
<tr>
<td>GSTRCm</td>
<td>0.57</td>
<td>0.97</td>
<td>1.12</td>
</tr>
<tr>
<td>Hemi-diaphragm</td>
<td>0.55</td>
<td>1.04</td>
<td>1.14</td>
</tr>
<tr>
<td>SOLm</td>
<td>0.66</td>
<td>0.92</td>
<td>0.99</td>
</tr>
<tr>
<td>EDLm</td>
<td>0.58</td>
<td>0.93</td>
<td>0.88</td>
</tr>
</tbody>
</table>

* Values computed as ratio of means for each group, respective group n’s shown below ratio in parentheses; C = control, GSTRCm = gastrocnemius muscle, SOLm = soleus muscle, EDLm = extensor digitorum longus muscle.

MHC, or “switching” of isoforms (Izumo et al. 1986). For instance, in the deep GSTRCm decreased amounts of type IIB and IIX MHC are a possibility, perhaps due to protein catabolism that occurs with chronic food restriction (McGilvery 1979). However, switching of MHC isoforms also is likely, perhaps due to hormone-driven shifts toward slower MHC isoform expression (Izumo et al. 1986). We suggested previously (Ameredes et al. 1998a, and 1999) that this will remain unknown until reliable methods are developed to determine the absolute amount of type-specific MHC within muscles.

Potential significance of MHC shifts. It may be that the UN-driven increases in type I and IIA MHC composition of the GSTRCm may be beneficial because it is characteristic of slower muscle fibers that utilize more efficient oxidative processes for the energy provision (Siek 1988). Thus, in the face of limited energy substrates, a shift toward predominance of MHC isoforms and fibers associated with efficient energetic processes would seem to be an advantageous adaptation to chronic UN. This would allow efficient use of the limited energy substrates, however it could significantly compromise the force, velocity, and power capacity of the muscles (Ameredes et al. 1999, Schiaffino and Raggani 1994), making response to a physical challenge difficult. This issue may be of critical importance when therapy for replenishment of muscle bulk, strength and physical capacity of the aged cachectic individual is considered.

Possible role of GH and testosterone. GH is considered to be one of the most important protein anabolic agents in the body and is essential for protein synthesis throughout life (Everitt and Mettes 1989). It has been shown to be decreased with aging (Corpas et al. 1992) and to reverse catabolic states (Clemmons and Underwood 1992). Testosterone levels also have been shown to fall with food restriction and aging in adult male mammals (Corpas et al. 1992, Howland 1975, Lamberts et al. 1997). Because the anabolic actions of these hormonal factors are important in maintenance of mammalian male muscle mass and function throughout life (Everitt and Mettes 1989, Lamberts et al. 1997, Proctor et al. 1998, Wu 1997), it would be logical that decreases might significantly influence muscle phenotype. Consequently, it might also be expected that GH administration would reverse some of these changes. As indicated above, trends in MHC alterations in the SOLm and EDLm were suggestive of a UN and subsequent GH effect. We did, however, observe significant RF and RF + GH-driven MHC shifts, protein elevations and mass increments in the GSTRCm concomitant with increased circulating testosterone levels (Fig. 5). These results are consistent with the possibility that GH is one of the critical factors regulating atrophy, hypertrophy and MHC shifts in the present model.

Another potentially important factor in the hormonal regulation of muscle phenotype is insulin-like growth factor I (IGF-I). IGF-I is the active anabolic agent derived from GH and therefore is the critical mediator of anabolic effects typically attributed to GH (Daughaday and Rotwein 1989). IGF-I also is known to produce significant hypertrophy and increased MHC content in skeletal myofibers (Vandenburgh et al. 1991). Similar to testosterone, IGF-I levels also decline with aging (Corpas et al. 1992) and UN (Lanz et al. 1992, Thissen et al. 1994) and have been implicated as a possible signal responsible for decrements in protein synthesis capacity (Clemmons and Underwood 1992, Richardson 1981). Given that IGF-I was previously shown to be decreased with chronic UN in this same rat sex and strain (Lanz et al. 1992), and in other hypocaloric models (Thissen et al. 1994) the changes in muscle and body mass that we observed with chronic UN suggest a possible role for IGF-I. Moreover, GH is a potent stimulus for IGF-I production (Thissen et al. 1994). Thus, an increase in circulating IGF-I with RF + GH (Lanz et al. 1992) could be a likely factor promoting the reversal of UN-induced changes we observed.

The exact nature of the interaction between testosterone, GH and IGF-I in our experiments is unknown. Possibly, chronic UN resulted in decreased activity of the thyroid resulting in a decrement in IGF-I production (Keis et al. 1993, McGilvery 1979, Thissen et al. 1994). The levels of triiodothyronine with chronic UN as compared to controls (UN = 560 vs. C = 890 ng/L) were reported elsewhere (Ameredes et al. 1998a) and are consistent with this possibility. Unfortunately, simple linkage of decreased thyroid hormone production to rat skeletal and respiratory muscle MHC profile has been nonuniform (Florini 1989, Gosselin et al. 1996, Izumo et al. 1986) and possibly dependent on the methods utilized to induce hypothyroidism. Further study is needed to better establish the relationships between these critical anabolic factors in long-term models of aging and undernutrition.

Conclusion and critique. In conclusion, we found that GH in conjunction with RF resulted in greater and more rapid increments in body mass replenishment after chronic UN in aging rats. This effect was reflected as a trend toward mass overshoot with GH treatment in the whole gastrocnemius muscle. We also found that mixed-fiber type muscles such as the gastrocnemius and the diaphragm displayed the greatest MHC shifts in response to RF and RF + GH. These changes were associated with alterations in circulating testosterone levels, which may be a key factor in these processes. Although we did not measure IGF-I, the known property of GH as a precursor for IGF-I (Daughaday and Rotwein 1989) and data from a prior study in a similar model (Lanz et al. 1992) suggest that it may have played a role as well.

It should be noted that, while the final changes observed in muscle mass, MHC shifts, protein concentrations and testosterone at senescence were not significantly different between the treatments of RF + GH and RF alone. This may have been due to the design of our study. The design ended the study with achievement of the control body mass by the group treated with RF alone as opposed attainment of that body mass by the RF + GH group. The fact that the RF + GH group
attained this endpoint significantly (2.5 mo) sooner and remained greater than the RF group suggests that muscle sampling at, or just after, 21.75 mo of age could have resulted in significant differences in the muscle characteristics between the treatments of RF + GH and RF alone. Moreover, this time point also qualifies as senescence for this species (Yu et al. 1985) and therefore indicates that GH treatment was effective in enhancing the effects of RF into senescence. These findings suggest that GH administration in conjunction with RF can reverse atrophy and reestablish body mass significantly more rapidly than RF alone, and therefore should be further considered in treatment of aged chronically undernourished individuals.

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LITERATURE CITED

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