Insulin Attenuates Atrophy of Unweighted Soleus Muscle by Amplified Inhibition of Protein Degradation

Marc E. Tischler, Soisungwan Satarug, Anders Aannestad, Kathryn A. Munoz, and Erik J. Henriksen

Unweighting atrophy of immature soleus muscle occurs rapidly over the first several days, followed by slower atrophy coinciding with increased sensitivity to insulin of in vitro protein metabolism. This study determined whether this increased sensitivity might account for the diminution of atrophy after 3 days of tail-cast hindlimb suspension. The physiological significance of the increased response to insulin in unweighted muscle was evaluated by analyzing in vivo protein metabolism for day 3 (48 to 72 hours) and day 4 (72 to 96 hours) of unweighting in diabetic animals either injected with insulin or not treated. Soleus from nontreated diabetic animals showed a similar loss of protein during day 3 (−16.2%) and day 4 (−14.5%) of unweighting, whereas muscle from insulin-treated animals showed rapid atrophy (−14.5%) during day 3 only, declining to just −3.1% the next day. Since fractional protein synthesis was similar for both day 3 (6.8%/d) and day 4 (7.0%/d) of unweighting in insulin-treated animals, the reduction in protein loss must be accounted for by a slowing of protein degradation due to circulating insulin. Intramuscular (IM) injection of insulin (500 nmol/L) stimulated in situ protein synthesis similarly in 4-day unweighted (+56%) and weight-bearing (+90%) soleus, even though unweighted muscle showed a greater in situ response of 2-deoxy-[3H]glucose uptake to IM injection of either insulin (133 nmol/L) or insulin-like growth factor-I (IGF-I) (200 nmol/L) than control muscle. These findings suggest that unweighted muscle is selectively more responsive in vivo to insulin, and that the slower atrophy after 3 days of unweighting was due to an increased effect of insulin on inhibiting protein degradation.

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A TROPHY OF THE SOLEUS muscle by unweighting is associated with decreased protein synthesis and increased protein degradation as demonstrated both in vivo and in vitro. In adult animals, protein degradation increases after an initial rapid decrease in protein synthesis. This increase in proteolysis is transient, peaking after 2 weeks. Glucocorticoids seem to play a minimal role in the accelerated protein degradation of unweighting atrophy.

Some studies have examined specific contributions of myofibrillar proteins to unweighting atrophy. A relative loss of myofibrillar proteins has been observed over periods of unweighting ranging from several days to 8 weeks. Both myofibrillar and sarcoplasmic proteins show a decreased rate of synthesis, but only myofibrillar proteins undergo increased protein degradation. In contrast, sarcoplasmic proteins showed slower protein degradation to offset the decrease in their synthesis. In accordance with this observation, receptors for insulin and for β-adrenergic agonists, both components of the sarcoplasmic protein pool, are spared during unweighting atrophy of the soleus.

In a previous study, we evaluated the time course of changes in soleus muscle protein degradation in situ following hindlimb unweighting of juvenile rats. Whereas protein synthesis declined markedly within the first 24 hours, the increase in protein degradation was not appreciable until the second and third days of unweighting. Thereafter, protein degradation declined, but without any change in protein synthesis. Coincident with the decline in protein degradation in the soleus is the onset of increased binding and responsiveness to insulin. Prior results suggested that the decline in protein degradation after unweighting the hindlimbs of juvenile rats for 3 days was a consequence of an increased response to insulin related to the relative increase in insulin binding. In this study, we have considered this problem from a more physiological perspective by testing the effects of insulin in vivo. Diabetic animals in which insulin was either withdrawn or injected daily were used to reevaluate the role of circulating insulin in the decline of protein degradation during day 4 (72 to 96 hours) of unweighting compared with day 3 (48 to 72 hours). We hypothesized that unweighted, untreated diabetic animals should not exhibit a decline in soleus atrophy during day 4, largely because of preservation of elevated protein degradation, whereas insulin treatment should restore the decline in atrophy and protein degradation during day 4 normally characteristic of the unweighted juvenile soleus. The prior time-course study suggested that protein synthesis in the 4-day unweighted muscle may not show an increased response to insulin. Therefore, we compared the effect of intramuscularly (IM) injected insulin on in vivo protein synthesis in soleus muscles of normal and 4-day hindlimb-suspended animals.

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MATERIALS AND METHODS

Treatment of Animals

All procedures were approved by The University of Arizona Animal Care and Use Committee. Juvenile female rats (Sasco Sprague-Dawley, Omaha, NE) at body weights indicated in the figures were maintained on food and water ad libitum. Animals were killed by cervical dislocation after anesthesia by injection in a forelimb muscle with 0.1 mL/100 g body weight 10% (vol/vol) Innovar-vest (4 µg Sublimate and 200 µg Inapcine; Pitman-Moore, Washington Crossing, NJ). During casting or surgical procedures, animals were also anesthetized. For hindlimb unweighting, animals were tail-cast and suspended in a head-down position so that the hindlimbs were elevated above the floor of the cage for the period indicated.6 Casts consisted of Hexcelite orthopedic tape (Kirschner Medical, Timonium, MD) and 6382 RTS elastomer (Factor II, Lakeside, AZ).

Animals were made diabetic after an overnight fast by intraperitoneal (IP) injection of 8.5 mg streptozotocin/100 g body weight administered in 0.5 mL 0.9% NaCl solution. Animals were then refed. After 3 days, the animals were injected subcutaneously with 5 U protamine zinc insulin (Eli Lilly, Indianapolis, IN) in 0.2 mL 0.9% NaCl, and then maintained weight-bearing or were hindlimb-suspended the same day. Thereafter, animals continued to be injected daily either with insulin (diabetic insulin-treated) or with 0.9% saline (diabetic nontreated). Following insulin withdrawal, animals were confirmed to be diabetic as long as plasma glucose at the time of collection was at least 20 mmol/L.

Insulin-treated animals were similarly tested to verify the effectiveness of insulin treatment. After excision of the soleus, blood was drawn into a heparinized pipette tip from the severed hepatic artery, added to heparinized tubes, and then centrifuged (20 minutes at 2,000× g).

Plasma (0.2 mL) was deproteinized with 3.8 mL 79-mmol/L Ba(OH)2/79-mmol/L ZnSO4. Plasma glucose was determined spectrophotometrically.28

Protein Accretion or Loss

To study in vivo protein metabolism under conditions of altered weight-bearing and circulating insulin, we measured the protein content and protein synthesis of soleus muscles for weight-bearing diabetic animals without or with insulin treatment and for hindlimb-suspended animals that were normal or diabetic without or with insulin treatment. Protein content and protein synthesis were measured in contralateral muscles from the same animals. For each period studied (48 to 72 hours, 5 g or less on either side of the mean initial weight). The fractional rates (percent per day) of in vivo protein synthesis and the fractional rate of protein accretion for the 24-hour period, as described earlier. Animals were tranquilized as already described and then injected IP with 300 µCi and 40 µCi [1-14C]phenylalanine (ICN, Costa Mesa, CA) administered in 2 mL 1% saline solution/100 g body weight. Fifteen minutes later, one soleus muscle was removed and frozen in liquid nitrogen to terminate protein synthesis. After excision, the other soleus muscle was placed in 10% trichloroacetic acid to be processed for analysis of total protein as described earlier.

Specific activities of free intracellular (Sf) and protein-bound (Sp) phenylalanine were determined as described elsewhere,29 with modifications restricted to the volumes used.3 The fractional rate of synthesis was calculated as \( S_f/(0.9 \cdot S_f \cdot t) \), where \( t \) is the time in days between injection and muscle excision and 0.9 provides a correction factor for delay of label equilibration in the muscle.31

In Situ Insulin-Stimulated Protein Synthesis

To evaluate the effect of insulin on in situ protein synthesis and the linearity of insulin-stimulated protein synthesis, we measured fractional rates by a modification of the flooding-dose technique developed in our laboratory.3 The modification entailed IM rather than IP injection of a flooding dose of radiolabeled phenylalanine. We have previously used IM injections to successfully test in situ effects of protease inhibitors and hormones and to measure protein metabolism.5,10,27 To inject the soleus muscle, a 5-mm incision is made in the outer side of the shaved, ethanol-swabbed hindlimb of rats anesthetized as before. After the underlying fascia is cut, the soleus is gently exposed by hooking it with fine curved forceps. The belly of the muscle is injected using a 10-µL Hamilton syringe with a solution of 150 mmol/L NaCl containing 3H-phenylalanine (50 mmol/L; ~4 µCi) and 500 mmol/L porcine insulin (Eli Lilly) where indicated. The volume injected depended on muscle size: 4 µL/100 g body weight for control (weight-bearing) muscles or 3.2 µL/100 g body weight for 4-day unweighted muscles, based on prior studies of ratios of muscle to body weight. Based on previous results for distribution of injected radioactivity,3 only the middle approximately 50% of the muscle was excited. As described previously,3 the muscle was immediately washed twice for 10 minutes in 3 mL ice-cold 0.84% (wt/vol) NaCl solution containing 5 mmol/L cycloheximide to inhibit protein synthesis32 and 20 mmol/L cycloleucine to prevent leakage of intracellular phenylalanine by inhibiting its transport on the L-system.33 Muscles were then processed for analysis of fractional protein synthesis as described earlier, except that the initial step of freezing the muscles was skipped. Because the muscle was injected directly with radiolabeled phenylalanine, fractional rates of synthesis were calculated as \( S_p/(S_f \cdot t) \).

In Situ Measurement of 2-Deoxyglucose Uptake

In situ uptake of glucose was determined in soleus muscle of weight-bearing and 3- to 6-day hindlimb-suspended animals, as described previously.34 Soleus muscles were injected by the method described earlier, with 0.9% (wt/vol) NaCl solution containing 20 mmol/L [1,2-3H]deoxyglucose (300 µCi/mmol), 0.6 µCi/mL [1-14C]mannitol, and 0.1% (wt/vol) bovine serum albumin. Insulin (133 mmol/L) or IGF-I (200 mmol/L; generously supplied by Lilly Research Laboratories) were injected into the contralateral muscle as indicated, to measure insulin-sensitive transport. Twenty minutes after injection, the middle two thirds of each muscle was excised, blotted, frozen using champs dipped in liquid nitrogen, and weighed. Muscles were solubilized in 0.6 mL 0.5-mol/L NaOH. Then, 5 mL scintillant (Ecolume) was added for radioactive counting in the 3H and 14C channels. 14C radioactivity and 14C specific activity of the injected solution were used to determine the
extracellular volume. Net uptake of 2-deoxyglucose was calculated by subtracting 3H activity in the extracellular space from total 3H activity in each sample. The rate of uptake was then calculated using the specific activity of 2-[3H]deoxyglucose in the injected solution.

Statistical Analysis

Data are expressed as the mean ± SEM. The significance of differences between groups for measurements of in vivo muscle protein content and protein synthesis was analyzed using one-way ANOVA with Bonferroni correction. P values are given as the Bonferroni value, where P < .05 is significant, calculated using the Instat program (Graphpad Software, San Diego, CA). Statistical analyses of protein metabolism during the third day (48 to 72 hours) and fourth day (72 to 96 hours) are not possible, because synthesis is the average of means for the beginning and end of each 24-hour period. Protein accretion or loss is estimated as the difference between mean values for protein content, and degradation is calculated using both of these estimates.

RESULTS

Body Weight Changes

Changes in body weight were measured over a period of 4 days for each of eight groups. Percent changes (mean ± SEM) were compared using ANOVA. Normal weight-bearing animals gained 18% ± 3% in 4 days. Unweighting alone (13% ± 2%) did not significantly affect weight gain. When unweighted animals were made diabetic, both insulin (15% ± 3%) and saline (11% ± 2%)-injected animals gained significant weight similar to the controls. The value for diabetic saline-treated rats differed from the control value without Bonferroni correction. In contrast, both insulin (16% ± 4%) and saline (13% ± 2%)-treated diabetic, weight-bearing animals gained weight similar to the controls with or without Bonferroni correction.

In Vivo Protein Metabolism in Response to Circulating Insulin

In a previous study, we showed that after an initial period (3 days) of significant atrophy in the unweighted juvenile muscle, there followed a marked slowing in the extent of loss of total proteins accounted for by a slower degradation of myofibrillar proteins. We proposed that an increased response of protein breakdown to insulin might account for the decreased loss of myofibrillar proteins. To test this idea, we conducted a time-course study for 2 to 4 days using diabetic animals that were either weight-bearing or hindlimb-unweighted and either treated or not treated with insulin. Measurements of muscle mass, protein content, and fractional protein synthesis were made at each time point (Table 1). At the 48-hour time point, the normalized (to initial body weight) protein content was similar in all groups. Diabetic weight-bearing animals, from which insulin was withdrawn at the beginning of the first day and was not replaced thereafter, showed a significant (P < .05) decline (11%) in muscle protein content during the third and fourth days, which was prevented by administration of insulin. Insulin also prevented the increase in blood glucose (data not shown).

There was a tendency for protein content to increase (≈5% to 6%) in the soleus of insulin-treated animals, but this change was not statistically significant. Administration of insulin to these animals was effective, as illustrated by the restoration of protein synthesis to approximately normal values; in a previous study, the fractional rate of total protein synthesis was 18.4%/d for normal animals.

When nontreated diabetic animals were unweighted, after 48 hours muscle protein synthesis was significantly (P < .05) slower (51%) compared with nondiabetic unweighted animals. After 96 hours, there was marginally (P < .1) less protein and slower protein synthesis in the muscles of nontreated animals compared with either nontreated or insulin-treated animals. Without insulin, unweighted animals tended to show slower protein synthesis at all time points compared with the other unweighted groups (Table 1).

In insulin-treated diabetic animals, protein content was decreased (29% to 34%) significantly by unweighting after 72 and 96 hours, and protein synthesis was significantly slower (51% to 62%) at all time points. Even when insulin was not administered, unweighting tended to produce a lower protein content and slower protein synthesis compared with that in the weight-bearing muscle, especially after 96 hours (P < .1).

When hindlimbs of nondiabetic animals were unweighted, there was a significant loss of protein during the third day but not during the fourth day, consistent with the prior observation of slower muscle atrophy after 3 days. Fractional protein synthesis in these muscles did not differ significantly after 3 or 4 days of unweighting compared with 2 days of unweighting. When diabetic animals were unweighted but not treated with insulin, protein content declined steadily during both the third and fourth days of unweighting, even though protein synthesis remained constant. When diabetic unweighted animals were treated with insulin, protein content declined markedly during the third day but showed only a small change during the fourth day, and protein synthesis did not differ at any time point. These results suggest that the presence of circulating insulin could

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Weight-Bearing Diabetic</th>
<th>Unweighted Diabetic</th>
<th>Fractional Protein Synthesis (%/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>48</td>
<td>8.2 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>6.7 ± 1.1*</td>
</tr>
<tr>
<td>72</td>
<td>7.3 ± 0.3*</td>
<td>9.1 ± 0.2</td>
<td>6.9 ± 1.1*</td>
</tr>
<tr>
<td>96</td>
<td>6.5 ± 0.4*</td>
<td>8.6 ± 0.4</td>
<td>6.9 ± 1.8</td>
</tr>
</tbody>
</table>

NOTE. Values are the mean ± SE for soleus muscles from 10 animals (100 to 115 g initial body weight) subjected to each condition for the duration indicated. In separate experiments at the end of each period, either muscle protein content or in vivo fractional protein synthesis by an IP injection technique were measured.

*P < .01 v weight-bearing diabetic + insulin by ANOVA.
1P < .05 v preceding time point by ANOVA.
Table 2. Effects of Unweighting and Insulin on Protein Metabolism

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Weight-Bearing</th>
<th>Diabetic</th>
<th>Unweighted</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein accretion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48-72</td>
<td>-11.0</td>
<td>5.8</td>
<td>-13.5</td>
<td>-16.2</td>
</tr>
<tr>
<td>72-96</td>
<td>-11.0</td>
<td>5.5</td>
<td>-1.6</td>
<td>-14.5</td>
</tr>
<tr>
<td></td>
<td>Protein synthesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48-72</td>
<td>6.8</td>
<td>17.2</td>
<td>8.0</td>
<td>4.3</td>
</tr>
<tr>
<td>72-96</td>
<td>6.9</td>
<td>16.3</td>
<td>6.8</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Protein degradation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48-72</td>
<td>17.8</td>
<td>11.4</td>
<td>21.5</td>
<td>20.5</td>
</tr>
<tr>
<td>72-96</td>
<td>17.9</td>
<td>10.8</td>
<td>8.4</td>
<td>19.5</td>
</tr>
</tbody>
</table>

NOTE. Fractional protein accretion (negative = loss) and protein synthesis were calculated from the data in Table 2. Protein accretion (loss) is calculated as the percent change in protein content between 48 and 72 hours or 72 and 96 hours for the third and fourth days of treatment, respectively. Protein synthesis during the third or fourth day is the mean of values at the beginning and end of each day (ie, the value for the third day, 48 to 72 hours, is the average from 48 and 72 hours of duration). Fractional protein degradation was calculated as protein synthesis minus protein accretion. Statistical analysis is not possible, as these are calculations from mean rather than individual values.

Table 3. Linear Insulin-Stimulated Protein Synthesis Using IM Injection

<table>
<thead>
<tr>
<th>Time After Injection (min)</th>
<th>Fractional Rate (%/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>35 ± 6</td>
</tr>
<tr>
<td>20</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>30</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>40</td>
<td>36 ± 3</td>
</tr>
</tbody>
</table>

NOTE. Values are the mean ± SE for soleus muscles from 7 animals (105 to 120 g) injected with 500 nmol/L insulin and 3H-phenylalanine for the duration indicated. Fractional protein synthesis using the IM injection technique was determined.
INSULIN LESSENS ATROPHY OF UNWEIGHTED MUSCLE

Table 4. Effect of IM Insulin Injection on Protein Synthesis

<table>
<thead>
<tr>
<th>Fractional Rate (%/d)</th>
<th>Inulin</th>
<th>Insulin</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight-bearing muscle</td>
<td>20 ± 3</td>
<td>38 ± 6</td>
<td>90 ± 21*</td>
</tr>
<tr>
<td>Unweighted muscle</td>
<td>9 ± 2</td>
<td>14 ± 3</td>
<td>56 ± 12*</td>
</tr>
<tr>
<td>Difference (%)</td>
<td>-55 ± 18</td>
<td>-54 ± 20</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Values are the mean ± SE for contralateral soleus muscles of 12 weight-bearing or 4-day unweighted animals (105 to 120 g) injected IM for 20 minutes with radiolabeled phenylalanine in the absence or presence of 500 nmol/L insulin.

*P < .005 v unweighted by paired Student's t test.
**P < .001 v weight-bearing by ANOVA.

Since it was possible that an enhanced effect of insulin on muscle processes due to unweighting might not be measurable in situ, we tested this possibility by measuring insulin-stimulated uptake of 2-deoxyglucose at 3 and 6 days after unweighting (Fig 1). In control muscle, insulin increased the uptake of 2-deoxyglucose nearly threefold, whereas in soleus of unweighted animals, the effect of insulin was even greater (fourfold to fivefold stimulation) after 3 or 6 days. Stimulation with IGF-I produced comparable results: 2.5-fold increase in control compared with 3.5- to 4.5-fold in 3- or 6-day unweighted muscle. Therefore, the failure of insulin to increase protein synthesis to a greater extent in 4-day unweighted versus weight-bearing muscle could not have been due to an inability to detect an increased response to insulin in situ.

DISCUSSION

Overview

The primary goals of this report were to demonstrate that an increased inhibitory response to insulin of protein degradation accounted for the slowing of muscle atrophy subsequent to 3 days of unweighting, and that protein synthesis in vivo was immune to enhanced sensitivity. Diabetic animals were used to address these problems, and these data permitted comparisons of in vivo insulin responsiveness of the synthesis and degradation of proteins within this study and relative to other studies.

Protein Metabolism in Weight-Bearing Muscle of Diabetic Animals

Just 48 hours after insulin withdrawal, the protein content of the soleus remained similar to that of insulin-treated animals despite much greater protein synthesis following insulin treatment (Table 1). This result is consistent with a previous study showing that following insulin withdrawal growth of the gastrocnemius continued during the first 24 hours, followed by growth cessation during day 2, and finally a marked reduction in protein mass thereafter. The delay in protein loss by the gastrocnemius was attributed to protein breakdown not exceeding protein synthesis until after day 2, even though there was an appreciable decrease in synthesis after 2 days, just as in our study. Consistent with this conclusion, the results here show considerable protein loss during days 3 and 4 because of slower protein synthesis and faster protein degradation. Other studies have yielded similar results. The effect of diabetes on protein degradation has been controversial. One study reported slower proteolysis; however, a later study using 1-day diabetic rats showed increased myofibrillar protein catabolism that was reversed by treatment with insulin. Such discrepancies may be a consequence of differences in the severity, mode of induction, or duration of diabetes.

In our study, insulin treatment had a larger effect on synthesis than on degradation of protein (Table 2). Similarly, daily insulin treatment had a larger effect on protein synthesis in the gastrocnemius of diabetic rats. In contrast, hyperinsulinemia in humans showed degradation to be more responsive than synthesis to insulin in vivo. When the effect of insulin on human muscle was examined in vitro, the results were more consistent with those herein in that synthesis was more responsive to insulin. A possible explanation for this discrepancy is that in studying hyperinsulinemia, basal insulin levels may have already elicited a near-maximal effect on synthesis but not on degradation; thus, degradation appeared more responsive. In contrast, when the basal condition is nontreated diabetes or incubation without insulin, protein synthesis may show more responsiveness because it is sensitive to lower amounts of insulin than protein degradation.

Protein Metabolism in Unweighted Muscle of Diabetic Animals

Unweighting the soleus in nontreated diabetic animals increased the rate of protein loss by 32% to 47% (Table 2). This greater atrophy was accounted for by contributions from both decreased synthesis and increased degradation of protein, with the absolute changes in rates similar for the two processes. Therefore, the lack of circulating insulin did not alter the typical responses of protein synthesis and degradation to unweighting. Similarly, in insulin-treated diabetic animals, the marked atrophy caused by unweighting was a consequence of both a decrease in synthesis and an increase in degradation of total proteins.

The primary purpose for using diabetic rats was to demonstrate in vivo whether increased responsiveness of protein metabolism to insulin might account for the slowing of muscle protein loss. Day 4 of unweighting was characterized by a
marked decline in protein loss in unweighted muscle of nondiabetic or insulin-treated animals but not nontreated diabetic animals. This protein loss occurred solely because protein degradation declined by 52% to 61% when circulating insulin was present. Consequently, during day 4, degradation was considerably more rapid (93% to 132%) in the absence of circulating insulin. Therefore, diminished protein loss after 3 days of unweighting must be a consequence of a reduction in muscle protein degradation owing to an increased ability of circulating insulin to retard this process.

Unlike degradation, synthesis rates were unchanged during day 4 of unweighting (Table 2), even though in vitro data suggested that insulin sensitivity might affect protein synthesis as it does protein degradation. These findings suggested that protein synthesis in vivo may resist the predicted increase in sensitivity. In accordance with this conclusion, comparison of the effects of IM injected insulin on protein synthesis showed a smaller rather than a greater response in unweighted muscle (Table 4), even though under similar conditions the uptake of 2-[1H]deoxyglucose in unweighted soleus showed an increased response to both insulin and IGF-1 (Fig. 1). This discrepancy prior in vitro findings and the current in vivo results emphasizes the potential problems associated with drawing firm conclusions from in vitro studies and the need to follow-up such studies in vivo.

The increased responsiveness to insulin seems to be due to the increased binding of insulin by the soleus muscle after at least 3 days of unweighting. Other observations support the idea that this is not a specific effect on the insulin receptor, but rather a generalized sparing of membrane components. Ultrastructural studies showed that membranes of fibers of soleus muscle unweighted for 7 days have a wavier appearance. This adaptation created a 29% increase in the ratio of cell membrane to muscle volume, thus representing a relative conservation of the cell membrane. Accordingly, we have shown a general sparing of sarcoplasmic proteins, which include those in the membrane. Furthermore, the binding capacity of the β-adrenergic receptor is also increased, as is the responsiveness of the unweighted soleus to β-adrenergic agonists. Since epinephrine appears to slow muscle proteolysis, it is possible that increased responsiveness to catecholamines may also retard muscle protein loss with chronic unweighting.

Despite the expectation that all insulin responses should be enhanced in conjunction with increased insulin binding capacity, protein synthesis in vivo and amino acid uptake in vitro contradict this supposition. It is noteworthy that these are both processes potentially linked to mitogenic responses in the muscle. Most likely due to altered signal transduction, partial "resistance" of these processes to insulin negates the increased sensitivity associated with greater insulin binding capacity. Resistance of protein synthesis and amino acid transport alone implies that just a portion of the insulin signal transduction pathway was affected by muscle unweighting. The observation of such differential responses is indicative of the multiple signal transduction pathways linked to insulin action. The plausibility of specific resistance of protein synthesis and amino acid transport via a branch of the insulin signal transduction pathway is supported by a recent study in which insulin control of metabolic and mitogenic processes was compared. Analogs of insulin with a broad range of $K_d$ values were used to assess the relative metabolic (3-O-[3H]methylglucose uptake) and mitogenic ([3H]thymidine incorporation) responses following analog treatment. Analogs with an increased half-life of the receptor-ligand complex produced a much greater mitogenic response. This was associated with sustained phosphorylation of the S6c protein but not of insulin receptor substrate-1 (IRS-1). These findings lead to the conclusion that phosphorylation of S6c may be associated with mitogenic responses, whereas metabolic responses are linked to phosphorylation of IRS-1. This leads us to speculate that perhaps the responses in unweighted muscle that show increased sensitivity (eg, glucose uptake and protein degradation) are mediated through IRS-1, whereas the attenuated responses of protein synthesis and amino acid uptake may use phosphorylated S6c protein.

The effect of unweighting on the insulin receptor was more pronounced in young than in adult rats. Accordingly, the increased responsiveness of glucose uptake and metabolism was less pronounced in adult muscle. One may then predict that insulin would be less effective in slowing protein loss during chronic unweighting of adult muscle. Such a result might explain the different time course of protein metabolism associated with unweighting of juvenile versus adult muscle. In adult muscle, proteolysis reaches a peak after 2 weeks of unweighting, compared with just 3 days in juvenile muscle. Still, in endeavoring to develop countermeasures to atrophy caused by chronic unweighting of adult muscle, researchers should not overlook taking advantage of even moderate increases in the binding capacity of insulin and β-adrenergic receptors.

In conclusion, some, but not all, processes show an increased in vivo response to insulin when its binding capacity is enhanced in unweighted muscle. This finding has important implications for the long-term rate of atrophy in unweighting compared with other perturbations leading to muscle atrophy. Thus, the selective sparing of certain proteins in unweighting atrophy culminates in ultimately slowing the loss of myofibrillar proteins.

REFERENCES

5. Munoz KA, Satarug S, Tischler ME: Time course of the response of myofibrillar and sarcoplasmic protein metabolism to unweighting of the soleus muscle. Metabolism 42:1006-1012, 1993
INSULIN LESSENS ATROPHY OF UNWEIGHTED MUSCLE


