Atrophy of the soleus muscle by hindlimb unweighting

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THOMASON, DONALD B., AND FRANK W. BOOTH. Atrophy of the soleus muscle by hindlimb unweighting. J. Appl. Physiol. 68(1): 1-12, 1990.—The unweighting model is a unique whole animal model that will permit the future delineation of the mechanism(s) by which gravity maintains contractile mass in postural (slow twitch) skeletal muscle. Since the origination of the model of rodent hindlimb unweighting almost one decade ago, about half of the 59 refereed articles in which this model was utilized have been published in the Journal of Applied Physiology. Thus the purpose of this review is to provide, for those researchers with an interest in the hindlimb unweighting model, a summation of the data derived from this model to date and hopefully to stimulate research interest in aspects of the model for which data are lacking. The stress response of the animal to hindlimb unweighting is transient, minimal in magnitude, and somewhat variable. After 1 wk of unweighting, the animal exhibits no chronic signs of stress. The atrophy of the soleus muscle, a predominantly slow-twitch muscle, is emphasized because unweighting preferentially affects it compared with other calf muscles, which are mainly fast-twitch muscles. The review considers the following information about the unweighted soleus muscle: electromyogram activity, amount and type of protein lost, capillarization, oxidative capacity, glycolytic enzyme activities, fiber cross section, contractile properties, glucose uptake, sensitivity to insulin, protein synthesis and degradation rates, glucocorticoid receptor numbers, responses of specific mRNAs, and changes in metabolite concentrations.

The unweighting model have appeared in The Physiologist and NASA Technical Reports but are not included in this review. In the decade elapsing since the pioneering efforts of Morey (48) and Musacchia et al. (50), at least 59 original publications employing hindlimb unweighting have appeared in refereed journals. Almost half of these papers have been published in the Journal of Applied Physiology. The purpose of this review is to summarize the findings in these papers, primarily with regard to the response of the soleus muscle. We occasionally point out inconsistent or limited information and speculate about the potential research that could resolve specific questions as investigators further explore the influence of gravitational forces on muscle gene expression.

Electromyogram Activity

One of the early assumptions of the unweighted hindlimb model was that it was a model for hypokinesia.
(reduced muscle contractile activity) and hypodynamia (reduced load-bearing or locomotor activity by muscles) (50). In 1987, Alford et al. (1) showed that hypokinesia does not occur except for the first few days in the tail-suspended unweighted hindlimbs. They recorded integrated electromyogram (EMG) activity in three hindlimb skeletal muscles for 28 days of unweighting, and a summary of their results is illustrated in Fig. 1.

In the soleus, a predominantly slow twitch postural muscle, hypokinesia (significantly reduced EMG activity from control) was observed only at the start of unweighting and not from 3 to 28 days of unweighting. In the medial gastrocnemius, a predominantly fast-twitch muscle, significant hypokinesia was shown at 0 and 3 days of unweighting but not from 7 to 28 days of unweighting. In the tibialis anterior muscle a significant increase in EMG activity was observed at 3 and 7 days of unweighting, and no significant difference was seen in EMG activity from control at other times of unweighting. Others (31, 46, 72) have previously noted that, during tail-suspended unweighting, the foot is plantar flexed so that the soleus and gastrocnemius muscles are shorter than normal while the tibialis anterior is stretched longer than normal. This accounts for the increased EMG activity in the tibialis anterior at 3 and 7 days of unweighting. Alford et al. (1) have indicated that the descriptive terms "hypokinesia" and "disuse" should not be applied to indicate the contractile status of muscle during hindlimb unweighting.

Whether there is a remodeling of the motoneural loops during prolonged hindlimb unweighting has not been fully explored. Observationally, there is an increase in "burst" EMG activity during unweighting (R. Roy, personal communication). A similar response occurs during limb immobilization (4). If indeed there is a similarity in motoneural response to these two atrophy models, further investigation of the hindlimb unweighting model may reveal motor end-plate degeneration, as has been observed during limb immobilization (53).

Thus the cause-and-effect relationship between motoneural activity and muscle atrophy during hindlimb unweighting has not been established. On one hand, integrated EMG activity returns to normal or remains elevated during atrophy of the soleus, medial gastrocnemius, and tibialis anterior muscles (1), implying that the presence of an active motoneural system does not prevent the atrophy. Therefore, hindlimb unweighting does not represent a functional denervation (2). However, some evidence exists, and hopefully more will be forthcoming, that the relationship between nerve and muscle during hindlimb unweighting cannot be simply represented by integrated EMG activity; questions remain concerning frequency of nervous discharge, end-plate morphology, afferent-efferent loops, and other motoneural phenomena.

**Indicators of Stress**

Hypophyseal release of adrenocorticotropicin (ACTH) is both an acute and chronic response to stress. Chronic ACTH release in stressed animals will produce adrenal hypertrophy, thymus involution, decreased food intake, and lower body weights relative to age-matched controls. All these responses have been monitored in the models of unweighting, and this information is discussed below.

**Adrenal hypertrophy.** In a number of studies adrenal wet weights have been reported at various times of unweighting. These data, along with the duration of unweighting, are summarized in Table 1. The consensus of these data is that, after the first 2 wk following the start of unweighting, adrenal wet weights are not different from control. Thus the animal adapts to the unweighting model with a transient adrenal hypertrophy. A large variability exists in the degree of adrenal hypertrophy in the 1st wk of unweighting and could be caused by differences in animal handling and holding facilities among laboratories. Other authors have previously mentioned this possibility (17, 32). Indeed, a similar transient hypertrophy occurs when the animals are removed from non-weight bearing and allowed to recover (72), indicating that simply handling the animal and changing cages are sufficient to produce the observed changes. Other indirect indexes of stress (gastric ulcers and brain stem

![Fig. 1. Integrated EMG activity in 3 skeletal muscles as a function of duration of hindlimb unweighting. Control data are normalized to 100%, and unweighted data are expressed relative to control. [Data from Alford et al. (1).]](http://jap.physiology.org/)

TABLE 1. Adrenal wet weights after various times of hindlimb unweighting

<table>
<thead>
<tr>
<th>Days After Unweighting</th>
<th>% Change From Control</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
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<td>72</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>27, 32, 62</td>
</tr>
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<td>8</td>
<td>21-25</td>
<td>50, 64</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>64</td>
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<tr>
<td>16</td>
<td>24</td>
<td>72</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>23, 57, 72</td>
</tr>
<tr>
<td>35</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>56</td>
<td>0</td>
<td>72</td>
</tr>
<tr>
<td>206</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

FIG. 2. Plasma corticosterone as a function of duration of hindlimb unweighting in mice (Ref. 63) or rats (Ref. 37). Time (either 8 A.M. or 3 P.M.) of plasma collection is indicated on each curve.

phenylethanolamine N-methyltransferase activity) are at control values after 5 wk of unweighting (9), supporting the view that animals adapt to the unweighting.

Rats exhibit a normal diurnal variation in plasma corticosterone concentration, the lowest concentrations occurring at the onset of the light period and rising to a peak near the beginning of the dark period (11, 41, 42). Consistent with the initial stress of handling, 1-day unweighted rats (maintained on a diurnal cycle with lights on from 0500 to 1600 h) exhibited plasma corticosterone concentrations at least double those of control values at both 0800 and 1500 h (63), apparently abolishing the diurnal cycle (Fig. 2). By the 3rd day of unweighting, plasma corticosterone concentration was at the 1500-h control value at both 0800 and 1500 h, indicative of a lack of diurnal variation continuing into the 3rd day of unweighting (63). However, by the 7th day of unweighting, plasma corticosterone concentration had returned to the normal cycle observed for control animals (63). This is supported by an additional study in which plasma corticosterone levels reached a peak at the 3rd day of unweighting but then fell by the 6th day (Fig. 2) (37). These studies support the concept that stress is a transient event in unweighting and likely of most influence in the 1st wk. Furthermore, the transient peak in plasma glucocorticoids during hindlimb unweighting apparently precedes the transient increase in adrenal weight (Table 1). This suggests that the response to the stress of hindlimb unweighting is manifest during the first few days and that adrenal hypertrophy may not be an ideal indicator.

Thymus. Consistent with the rapid increase in circulating glucocorticoids at the onset of unweighting, thymus wet weight was 17, 37, and 50% lower after 1, 2, and 7 days, respectively, than in age-matched controls (62). These data support an early stress response to the unweighting.

Body weight and nutritional status. In many studies (5, 8, 9, 13, 18, 23, 24, 32, 40, 45, 46, 50, 51, 55, 59, 64, 72, 73, 75) a lower body weight is reported in unweighted groups than in age-matched controls. In some studies (24, 34, 37, 39, 57, 65, 66, 76) no effect of unweighting on body weight is reported for the duration of the experiment; in others (9, 50, 72) the effect of unweighting on body weight is observed during the first few days of unweighting where growth rates are depressed, but thereafter (days 3 or 4 onward) growth rates during unweighting are equal to those of age-matched controls. However, in another study (39) growth rates were similar to those of matched controls during the first 6 days of unweighting, but thereafter unweighted rats began to grow more slowly. The observation of differences among various laboratories in the response of body weight to unweighting supports the contention that variability in animal maintenance conditions among experiments could induce variable levels of stress at different times during unweighting (17, 32). The assumption underlying the previous conclusion is that a stressor reduces food intake, so weight gain is lessened. However, there is little evidence to suggest that the muscle atrophy induced by hindlimb unweighting has as its basis, even in part, malnutrition or changes in basal metabolism. In fact, animals undergoing some forms of rehabilitative exercise continue to exhibit decreased body weights, despite the fact that muscle atrophy is partially ameliorated (71). Nonetheless, tail-suspended rats or rats in weightlessness gain less weight per gram of food eaten than control rats (48). In addition, the absolute quantity of food eaten during the first 1–2 days of unweighting is reduced as measured by some (2, 8, 50) but not all laboratories (39).

A trophy of Unweighted Muscle

Effect of muscle type. A preferential atrophy of slow-twitch skeletal muscle in rats and mice during hindlimb unweighting has been reported by almost all laboratories (7, 9, 14, 18, 24, 32, 49, 51, 55, 58, 61, 64, 70, 71, 72, 75, 76).
77) (Fig. 3). Therefore, this review concentrates on the response of the soleus muscle, a slow-twitch ankle extensor that has been the focus of a large proportion of unweighting studies. Our discussion of the effects of unweighting on other muscles is limited to comparison with the soleus muscle.

Total protein loss. The decrease in protein content is greater in slow-twitch than in fast-twitch muscle during unweighting. For example, the total protein per soleus muscle was less than control by 26–34% at 3 days (46), 58% at 5 days (22), 26–32% at 7 days (2, 61), and 40% at 14 days (61) of unweighting. On the other hand, the protein content of gastrocnemius muscle was less than control by 31% at 5 days (21), 13–23% at 7 days (2, 61), and 14% at 14 days (61) of unweighting. Protein concentration of the unweighted soleus muscle is unchanged after 6–7 days of unweighting (2, 32, 40, 61), is either unchanged (61) or is decreased 30% (18) after 14 days of unweighting, and is increased 36% (75) after 6 wk of unweighting.

Myofibrillar protein loss. Part of the loss of soleus muscle protein can be attributed to loss of myofibrillar protein. After 28 days of hindlimb unweighting, 80% of the myofibrillar protein in the soleus muscle of nongrowing adult female rats was lost (72), and 63% of the myofibrillar protein was lost from the soleus muscles of young female rats after 42 days of unweighting (75). In contrast, the unweighted plantaris muscle myofibrillar protein content decreases to a lesser extent, falling 29% after 42 days of hindlimb unweighting (75). Specific changes in myosin isoform content are discussed in detail later.

In addition to the larger magnitude of myofibrillar protein loss in the unweighted slow-twitch muscle relative to fast-twitch muscle, there is a preferential loss of myofibrillar protein relative to total protein (35, 40, 72, 75). For example, protein in the sarcoplasmic fraction of the soleus muscle of 160-g male rats was unchanged after 6 days of unweighting, whereas myofibrillar protein decreased 20–23% in the same muscles (37). After 28 days of unweighting, myofibrillar protein per gram of wet soleus weight was reduced ~50% in adult female rats (72). This preferential loss does not occur in the unweighted plantaris muscle (75). After 42 days of hindlimb unweighting of growing male rats there was 31% less myosin per plantaris muscle, 38% less muscle wet weight, and no change in myofibrillar protein concentration (mg protein/g wet wt) in the plantaris muscle (75).

Capillaries. Capillary density (capillaries/mm²) in the soleus muscle increases 34% after 5 wk of unweighting (9). However, because fiber area decreases to a greater extent (increasing the number of fibers per unit area, see below), the ratio of capillaries per fiber in the soleus decreases 46% (9). Since oxygen delivery is limited by capillary density, oxygen delivery to the unweighted soleus muscle should not be the limiting factor to muscle work.

Glycolytic enzyme changes. Changes in lactate dehydrogenase (LDH) activity of the unweighted soleus muscle are variable. In the whole soleus muscle, LDH activity has been reported to increase 12–20% after 2 or 5 wk of unweighting (9) and to decrease 36–37% after 3 wk of unweighting (9). Recently it has become possible to determine enzyme activity per muscle fiber and express the activity per fiber weight rather than per whole muscle weight. For example, when LDH activity is expressed per unit dry weight of single isolated fibers, it increased 56–72% in slow-twitch fibers of the soleus muscle but was unchanged in either the fast-twitch oxidative-glycolytic fibers or fast-twitch glycolytic fibers from the gastrocnemius muscle between 1 and 4 wk of hindlimb unweighting (16). The apparent increase in glycolytic potential of single soleus muscle slow-twitch fibers is confirmed in histochemical cross section by a 113% increase in α-glycerophosphate dehydrogenase (GPD) activity after 4 wk of unweighting (26). Increases in GPD activity of similar magnitude are observed in fast-twitch fibers of the soleus muscle and slow- and fast-twitch fibers of the deep region of the medial gastrocnemius muscle (26, 57), with no change in GPD activity in fast-twitch fibers in the superficial region of the medial gastrocnemius muscle (57). These data indicate an increase in the anaerobic capacity of individual slow-twitch fibers and fast-twitch oxidative-glycolytic fibers in the unweighted soleus and gastrocnemius muscles, whereas fast-twitch glycolytic fibers of the gastrocnemius muscle are little affected. The lack of a change in LDH activity of single fast-twitch oxidative-glycolytic fibers in the gastrocnemius muscle (16) may indicate that these fibers are able to rely more on oxidative pathways to provide reducing equivalents, in contrast to slow-twitch fibers, which must also rely on LDH.

The response of phosphofructokinase (PFK) activity to unweighting is also dependent on the mode of standardization. PFK activity in the unweighted soleus muscle increases relative to control values if it is normalized per muscle fiber but decreases when expressed per gram of whole soleus muscle. For example, PFK activity increased 71% per gram of single slow-twitch fiber in the soleus muscle at 4 wk of unweighting (16) but was
unchanged at 2 wk of unweighting. In contrast, PFK activity per gram of whole soleus muscle was reduced 38-48% after 3 wk of unweighting (59). However, the aforementioned increase of PFK activity per muscle fiber in the soleus muscle agrees with the suggestion that anaerobic capacity is increased in the unweighted soleus muscle. The reasons for the discrepancy between whole muscle and single fiber glycolytic enzyme activities may be due to a disproportionate increase in intracellular space relative to extracellular space (16).

**Oxidative enzyme changes.** Available evidence indicates that the respiratory capacity (aerobic potential) of individual slow-twitch fibers in the unweighted soleus muscle either does not change or increases. Succinate dehydrogenase (SDH) activity per gram of a single soleus muscle fiber is unchanged in slow-twitch fibers after 7 and 28 days of unweighting (23, 27) and is increased 27 and 40% in fast-twitch fibers of the soleus muscle after 7 and 28 days of unweighting, respectively (23, 27). Citrate synthase activity per gram of single slow-twitch fiber in the soleus muscle was increased at 1 wk (percentage not given) and increased 69% at 2 and 4 wk of unweighting (16). Thus, on a per gram basis, respiratory capacity of individual slow-twitch fibers in the soleus muscle increases during unweighting.

Earlier reports showed a decrease in respiratory capacity per gram of whole soleus muscle (9, 15, 18, 56, 59). These observations of different directional changes in respiratory capacity of the unweighted soleus muscle, depending on whether respiratory capacity is expressed per gram of single fiber (no change or increase) or per gram of whole soleus muscle (decrease), may indicate that the intercellular space is increasing relative to the intracellular space in the unweighted soleus muscle (16).

Respiratory capacity per gram of single muscle fiber in fast-twitch muscle either does not change or decreases. SDH activity per gram of single fiber in the deep medial gastrocnemius muscle was unchanged in slow-twitch muscle fibers and decreased 24% in fast-twitch muscle fibers. In the superficial gastrocnemius muscle, SDH was decreased 37% in fast-twitch fibers after 28 days of hindlimb unweighting (57).

**Fiber dimensions.** The gross atrophy of the soleus muscle during unweighting is caused by a decrease in fiber size with no change in fiber number (68). Compared with age-matched controls, the decrease in cross-sectional area of type I (slow-twitch) fibers in the unweighted soleus is rapid, occurring during the first 2 wk of unweighting (Table 2). The decrease in cross-sectional area of type II (fast-twitch) fibers in the unweighted soleus muscle is similar to the slow-twitch fibers in that most of the atrophy occurs in the first 2 wk of hindlimb unweighting (Table 2).

**Contractile Properties**

**Fiber type percentages.** In most studies, a decrease in the percentage of slow-twitch fibers is reported in the unweighted soleus muscle (Table 3). In contrast, in some studies no change in the percentage of slow-twitch fibers was found in the unweighted soleus muscle after 21 (60) or 28 days (55). However, the consensus is that the percentage of fibers staining as slow-twitch fibers decreases in the unweighted soleus muscle. The combination of fiber atrophy and the decreased percentage of slow-twitch fibers necessarily changes the contractile properties of the unweighted soleus muscle.

**Myosin isoforms.** The relative losses of myosin protein in the unweighted soleus muscle exceed the percentage decline of mixed soleus proteins. These findings are consistent with the preferential loss of myofibrillar protein in the unweighted soleus muscle mentioned earlier. The concentration of myosin per milligram of soleus wet weight decreases from 22 (control) to 9 µg/mg in the 2-wk unweighted soleus muscles (65). By 8 wk of unweighting, 77% of the myosin protein has been lost (72). Superimposed on this high preferential loss of myosin protein in the unweighted soleus muscle are shifts in the relative amounts of fast and slow myosin isoforms. After 3-4 wk of unweighting, slow myosin decreased from 68 (control) to 62% of the total myosin in the soleus muscle (65). By 8 wk of unweighting, 64% of the soleus myosin was the slow form compared with 96% in control (72). Because the latter study showed no further loss in slow

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**TABLE 2. Soleus muscle fiber cross-sectional areas during hindlimb unweighting**

<table>
<thead>
<tr>
<th>Days After Unweighting</th>
<th>%Decrease Relative to Control</th>
<th>Type I</th>
<th>Type II</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>36</td>
<td>35</td>
<td>Rat</td>
<td>27</td>
<td></td>
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<tr>
<td>14</td>
<td>37</td>
<td>34</td>
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<td>9</td>
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<tr>
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<td>45-60</td>
<td>38-51</td>
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</tr>
<tr>
<td>28</td>
<td>40</td>
<td>40</td>
<td>Rat</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>63</td>
<td>63</td>
<td>Rat</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>27</td>
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<td>Dystrophic hamster</td>
<td>12</td>
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</tr>
<tr>
<td>206</td>
<td>58</td>
<td>50</td>
<td>Rat</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3. Soleus muscle type I fiber expression during hindlimb unweighting**

<table>
<thead>
<tr>
<th>Days After Unweighting</th>
<th>% of Fibers</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Unweighted</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>Rat</td>
</tr>
<tr>
<td>28</td>
<td>74</td>
<td>66</td>
<td>Rat</td>
</tr>
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<td>55</td>
<td>44</td>
<td>Hamster</td>
</tr>
<tr>
<td>150</td>
<td>63</td>
<td>37</td>
<td>Dystrophic hamster</td>
</tr>
<tr>
<td>206</td>
<td>90</td>
<td>62</td>
<td>Rat</td>
</tr>
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</table>
myosin isoform of the soleus muscle beyond 4 wk of unweighting, these data imply that most, if not all, of the loss of slow myosin is complete by the 4th wk of unweighting. In addition, the relative percentage of fast myosin increases in the unweighted soleus muscle (55, 72), as well as the absolute quantity of fast myosin isoforms (72). However, there may be considerable plasticity in myosin isoform quaternary structure, such that the myosin isoforms expressed during unweighting are not necessarily those observed in rodent skeletal muscle during weight bearing (70, 74). For example, after 2 wk of unweighting, a myosin isoform appears in the soleus muscle that comigrates with a fast myosin isoform but contains only slow light chains (17). Furthermore, an intermediate band of myosin, which migrates during electrophoresis between the slow myosin isoform and the fast myosin isoforms, increases from 21 to 59% of total in the 2-wk unweighted soleus muscle (68). There is little change in myosin light chain isoform composition in the suspended soleus muscle (17, 55, 72).

Myofibrillar adenosinetriphosphatase (ATPase) activity. The activity of myofibrillar ATPase expressed per microgram of myofibril protein is unaltered in the soleus muscle by 56 days of unweighting (72) and increased 18% by 36 days of unweighting in another study. The reason for the lack of a major change, despite a shift in myosin isoform profile to those of greater ATPase activity (69), is not known.

Mechanical properties. Mechanical measurements of contractile speed show that the slow-twitch soleus muscle acquires faster contractile properties during its unweighting (Table 4).

Consistent with the finding of decreased contractile and half-relaxation times are the reports of increased maximal velocity \(V_{\text{max}}\) during contraction of the unweighted soleus muscle. In the whole soleus muscle \(V_{\text{max}}\) is increased 13 and 46% at 1 wk of unweighting (17, 32), 124% at 2 wk (17), and 0% at 4 wk (77). The \(V_{\text{max}}\) of individual fibers from the soleus muscle is also increased 29 and 31% at 2 and 4 wk of unweighting, respectively (20, 55). In one of these studies (20) two subpopulations of slow-twitch fibers were found in the 2-wk unweighted soleus muscle. In one population no increase in \(V_{\text{max}}\) was shown, whereas in a second subpopulation of individual slow-twitch fibers a doubling in \(V_{\text{max}}\) was demonstrated. At 2 wk of unweighting the highest value for \(V_{\text{max}}\) of shortening by individual slow-twitch fibers from the soleus muscle was still much less than the mean \(V_{\text{max}}\) for fast-twitch fibers from control gastrocnemius muscle (20), indicative of hybrid fibers containing physiologically significant proportions of both fast and slow myosin isoforms.

As might be expected from the preferential loss of contractile protein in the unweighted soleus muscle, absolute tetanic tension (units of grams weight or Newtons) and stress (units of force per unit muscle cross-sectional area) are reduced (Table 5).

Similar directional changes occur in single slow-twitch fibers, where stress is decreased 27-29 and 12% at 2 and 4 wk of unweighting, respectively (20, 55). These findings support the hypothesis that the functional changes that occur in the soleus muscle as a result of unweighting have as their basis fundamental contractile deficiencies at the single fiber level. Furthermore, the fact that the contractile changes in single fibers are, in part, due to a downregulation of contractile protein expression (as inferred from the preferential loss of myofibril protein in the whole soleus muscle) is supported by the "moth-eaten" appearance of the fibers when visualized by light microscopy (65).

Calcium activation properties. Two weeks of unweighting also decreases the negative log of the \(\text{Ca}^{2+}\) concentration producing 50% of maximal tension \((pC_{\text{so}})\) of single slow-twitch fibers of the soleus muscle (20). Furthermore, the calcium activation curve for the individual fibers becomes steeper (20). These authors interpret their data as a greater cooperativity in \(\text{Ca}^{2+}\) activation of contraction in the unweighted soleus muscle (20), consistent with the soleus muscle fibers taking on fast-twitch properties.

Fatigue properties. The fatigability of the soleus muscle does not change after 1 (14, 32), 2 (24), or 4 wk (7, 77) of unweighting. This phenomenon is probably a result of the sustenance of mitochondrial respiratory density in the unweighted soleus muscle (16, 23, 27). In contrast, for unweighted fast-twitch muscle some investigators

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**TABLE 4. Soleus muscle twitch temporal properties during hindlimb unweighting**

<table>
<thead>
<tr>
<th>Days After Unweighting</th>
<th>% Decrease Relative to Control</th>
<th>Animal</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Contraction time</td>
<td>Half-relaxation time</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>-12</td>
<td>0</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>-14</td>
<td>0</td>
<td>Rat</td>
</tr>
<tr>
<td>14</td>
<td>-30</td>
<td>-70</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>-39</td>
<td>0</td>
<td>Rat</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>-25</td>
<td>Rat</td>
</tr>
<tr>
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<td>Mouse</td>
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<td>Dystrophic hamster</td>
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<tr>
<td>206</td>
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<td>-43</td>
<td>Rat</td>
</tr>
</tbody>
</table>
report an increase in fatigability (14, 77), whereas others observe no change in its fatigability (7, 32).

**Energy Stores**

Glucose and glycogen. The basal rate of uptake of 2-deoxy-D-glucose in the absence of insulin decreased by 19 and 26% in the soleus muscle at the 4th and 24th h of hindlimb unweighting, respectively (30). Basal rates of glucose oxidation and of glycogen synthesis were decreased by 34 and 44%, respectively, in the 24-h unweighted soleus muscle (30). These changes were likely due to local effects in the unweighted soleus muscle, because the extensor digitorum longus muscle did not demonstrate the changes (30). By the 3rd day of unweighting, basal rates of 2-deoxy-D-glucose were not different from control (29).

Whereas the sensitivity to insulin for the uptake of 2-deoxy-D-glucose into the unweighted soleus muscle was unchanged at 1 day of unweighting (29), the sensitivity was increased after 3 (29), 6 (31), and 28 days (5) of unweighting. These findings suggest that the increased sensitivity to insulin reflected a concentration of insulin receptors due to loss of muscle mass (29). The return of soleus EMG activity (Fig. 1) may also have a role in this process. Insulin caused a greater release of lactate and pyruvate and increased glucose oxidation in 3- (29), 6- (31), and 28-day (5) unweighted soleus muscle compared with control muscle. Insulin sensitivity for the processes of glycogen synthesis is increased in the 6- (31) and 28-day (5) unweighted soleus muscles of growing rats, but not in the 28-day unweighted soleus muscle from adult rats (5). Insulin binding per unit of wet weight for the unweighted soleus muscle is increased at 6 days (31) and 28 days (5).

Whereas in the previous study (31) an increase in glucose uptake was observed in the unweighted soleus muscle at a given concentration of insulin, in a second study glucose uptake was found to be depressed as a result of unweighting (15). Experimental conditions varied between these two studies. An increased glucose uptake in response to insulin was noted in tail-suspended rats in which glucose uptake was determined in vitro (31), whereas the decreased glucose uptake into the rat hindquarter in response to similar doses of insulin was found in whole hindlimb-suspended rats in which glucose uptake was measured in a perfused hindquarter preparation (15). In addition, there may be a difference due to the sex of the animals. Estradiol-17β increases insulin-stimulated glucose uptake in mouse soleus muscle (54) and could contribute to the increased insulin sensitivity (31). An additional explanation of this discrepancy is that the soleus muscle represents only ~1% of the muscle mass of the hindquarter muscles and may contribute little to the total glucose uptake of the mostly fast-twitch muscle of the rat hindquarter. It is possible that insulin resistance in the fast-twitch muscle of the hindquarter would mask an increased insulin sensitivity in slow-twitch soleus muscle.

Soleus muscle glycogen concentrations are increased by 22% after 1 day of unweighting (29), by 38% after 6 days of unweighting (31), and by 69% after 7 days of unweighting (15). The increased glycogen concentration in the 1-day unweighted muscle can be explained by its decreased degradation rate because of decreased muscle use (29). Thereafter the increased glycogen concentration in the unweighted soleus muscle likely is the factor for the observed decrease in the activity ratio of glycogen synthase (29). Although the decrease in the percentage of glycogen synthase in the glucose 6-phosphate-independent form in the 3- and the 6-day unweighted soleus muscles is 70 (28) and 78% (31), respectively, the activity of the glucose 6-phosphate dependent form of glycogen synthase increases (31). In response to insulin, the rate of soleus muscle glycogen synthesis increased more in the 1- (29) and 3-day (29) unweighted muscle than in the control muscle. The 6-day response was likely due to a 50% increase in concentration of insulin receptors in the unweighted soleus muscle (no change in the absolute number of receptors per muscle) (31).

High-energy phosphate levels. Unweighting of the soleus muscle for 6 days resulted in a 38% greater ATP concentration (35), but this apparent increase in energy charge had dissipated by 5 wk of unweighting (9). No changes in soleus muscle ADP and AMP concentrations were noted at either 6 days (35) or 5 wk (9) of unweighting. Creatine phosphate concentration in the soleus muscle was also unaltered by 5 wk of unweighting (9).

**Protein Expression**

DNA and RNA concentrations. Unweighting of the soleus muscle for 3–14 days did not alter the amount of DNA per whole muscle in three studies (2, 46, 61) and decreased the DNA content only 14% in a fourth report (22). In contrast, the quantity of RNA per whole unweighted soleus muscle was markedly decreased: 23% at 3 days (46), 58% at 5 days (22), 43 and 44% at 7 days (2, 61), and 50% at 14 days (61). Similar percentage losses in protein per whole soleus muscle during unweighting have also been noted (2, 22, 46, 61). Thus, hindlimb unweighting apparently has little effect on soleus muscle nuclei but profound effects on the expression of the protein synthetic machinery.

Protein synthesis. When considering the role of protein synthesis in hindlimb unweighting-induced atrophy of soleus muscle, one must consider the possible effects that muscle growth may exert on the atrophy process. For example, protein synthesis rates seem to be depressed less in unweighted soleus muscles taken from young rats than in those from mature rats. In rapidly growing 100-g male rats, mixed protein synthesis rates in the unweighted soleus muscle in vivo are decreased 21% at 3 days (46), 58% at 5 days (22), 43 and 44% at 7 days (2, 61), and 50% at 14 days (61). Similar percentage losses in protein per whole soleus muscle during unweighting have also been noted (2, 22, 46, 61). Thus, hindlimb unweighting apparently has little effect on soleus muscle nuclei but profound effects on the expression of the protein synthetic machinery.
due to the absence of growth (or changes in growth) superimposed on the atrophy response.

**Protein degradation.** It is extremely difficult to obtain valid estimates of the true rates of protein degradation in the unweighted soleus muscle. One approach is to estimate protein degradation rate in the excised soleus muscle in vitro by measuring amino acid release into the bathing medium. Using this approach, a 43% (35) and a 55% increase (34) in tyrosine release by the 6-day unweighted soleus muscle was observed during a 2-h incubation. The shortcoming with such estimates is that protein degradation rates in soleus muscles from 100-g rats are much higher in vitro than in vivo (19). A second approach has been to calculate the fractional rates of protein degradation by subtracting the mean growth rate over a multimodality from the mean fractional rate protein synthesis in vivo (21, 47). By use of this approach, protein degradation rates were estimated to be increased 108% at 3 days (46), 55% at 4-6 days (34), and 243% at 5 days of unweighting (22). The shortcoming of these estimates is that growing rats were used. In the reports giving increases of 108% (46) and 243% (22) in protein degradation rates of the unweighted soleus muscle, increases of 153 (46) and 148% (22) were given for the unweighted extensor digitorum longus muscle, which atrophied. On the other hand, in the study reporting the smallest increase in protein degradation rate in the unweighted soleus muscle (34) there was no change in either the protein degradation rate or the wet weight of the unweighted extensor digitorum longus muscle (34). It is possible that less stress during unweighting could spare the extensor digitorum longus muscle from atrophy during unweighting.

The third approach, which has been used to estimate protein degradation rates in the unweighted soleus muscle of relatively nongrowing rats, modifies the second approach by employing computer modeling (70). For example, a Lagrangian interpolation of data points for myofibrillar protein content provides an estimate of myofibrillar protein content at all time points during unweighting. From this continuous function the rate of change of myofibrillar protein can be calculated. Also the best-fit function for an exponential decay in myofibrillar protein synthesis, as determined from existing measurements (70), provides an estimate of myofibrillar protein synthesis at all time points during unweighting of the soleus muscle. Thus the difference between the rate of protein synthesis and the rate of change of protein yields the myofibrillar protein degradation rate at all time points during soleus muscle unweighting (Ref. 70, Fig. 4). The estimates derived by this method are consistent with the first two approaches; i.e., an increased fractional rate of myofibrillar protein degradation occurs as the duration of unweighting-induced soleus muscle atrophy proceeds. However, as mentioned previously, differences between growing male rats and slowly growing female rats must be considered. For example, in the 6-day unweighted soleus muscle of young male rats, mixed protein degradation rate was estimated to increase 46% in the first approach (35), whereas myofibrillar protein degradation rate was estimated to be only 7% greater in the 6-day unweighted soleus muscle of relatively nongrowing female rats by use of the third approach (70). The shortcoming of the third approach is that protein degradation rates were derived rather than measured.

The consensus of these reports is that protein degradation rates increase more slowly than the decrease in synthesis rate in the soleus muscle during the first few days of its unweighting. Furthermore, the data from the third approach provide a testable hypothesis that predicts maximal increases in the fractional rates of myofibrillar protein at the 15th day of unweighting in the soleus muscle of adult female rats (70).

**Factors Altering Protein Turnover in Unweighted Soleus Muscle**

Many of the mechanisms that have been considered as possibly influential in controlling soleus muscle atrophy during unweighting are consistent with the rapid phases of atrophy (acute response) but fail to explain the chronic maintenance of atrophy. The maintenance of atrophy is not explained by the factors considered to date because the potential mechanisms eventually accommodate during the unweighting.

**Mechanical initiator.** In Fig. 4, measurements of soleus muscle EMG activity during hindlimb unweighting (1) have been superimposed on the computer-generated lines for myofibrillar protein turnover (70). Comparisons of the directional changes in these values predict that there is no correlation and, thus, no cause-and-effect relationship between EMG activities and protein synthesis in the unweighted soleus muscle. Although EMG activity and myofibrillar protein synthesis rate both decrease markedly during the first few hours of unweighting of the soleus muscle, protein synthesis rate remains depressed when EMG activity returns to control values at the 7th day of unweighting. Furthermore, although EMG activity and myofibrillar protein degradation rates increase in parallel from the 1st to the 14th day of soleus muscle unweighting, if the return of EMG activity to normal were sufficient to maintain muscle mass, increasing EMG activity should prevent the increase in protein
degradation rate in the unweighted soleus muscle. A speculative hypothesis is that the increase in myofibrillar degradation rate may be related to the unloaded contraction of the soleus muscle. Nonetheless, unweighted contractions by the soleus muscle are insufficient to return rates of both myofibrillar protein synthesis and degradation back toward control values. These and other comparisons suggest that the major mechanical factor for maintaining myofibrillar protein in slow twitch muscle over long periods of time appears to be "weighted" contraction.

**Glucocorticoids.** The transient rise in plasma corticosterone appears to coincide with the early decrease in myofibrillar protein synthesis rate in the unweighted soleus muscle. Plasma corticosterone peaks after 1 day of unweighting (69) (Fig. 2). The major decrease in protein synthesis rate occurs by the end of the 1st day of unweighting (Fig. 4). However, at the 7th day of unweighting, when plasma corticosterone has returned to control values (63), protein synthesis remains depressed in the unweighted soleus muscle (Figs. 2 and 4). Thus the plasma level of glucocorticoids alone does not explain the lower rates of protein synthesis from the 1st wk of soleus muscle unweighting. In support of this interpretation is the report that adrenalectomy did not abolish atrophy of the unweighted soleus muscle (36, 40).

Some of the more acute effects of increased plasma corticosterone concentration during the 1st wk of hindlimb unweighting may be amplified by an apparent increase in sensitivity of the soleus muscle to cortisol, as measured by branched-chain amino acid flux. Breakdown of these amino acids by the soleus muscle is increased after 6 days of unweighting (36). This increase is abolished by adrenalectomy and is reestablished with cortisol treatment. These authors suggest that an increase in glucocorticoid receptor number in the unweighted soleus muscle could have some role in the increased leucine flux. Indeed, binding of \[^{3}H\]dexamethasone (an index of available glucocorticoid-binding receptors) to unweighted soleus muscle cytosolic binding sites shows a transient increase. After 7 days of unweighting, specific binding of \[^{3}H\]dexamethasone increases \(-140\%\) in soleus muscle extracts (63). However, the \(63\%\) increase in binding after 14 days of unweighting is less than at 7 days (63). We speculate that the transient increase in glucocorticoid receptor number in the unweighted soleus muscle could have some role in the transient rise in protein degradation rate (see Fig. 4). Supporting such a speculation is the similarity in the time course of the rise in both glucocorticoid receptors and protein degradation. However, more time points are needed to test this correlation. We do not believe that increased glucocorticoid receptor density in the unweighted soleus muscle has a role in the rapid decrease in protein synthesis rate for the following reason. No change in glucocorticoid receptor number was noted in the gastrocnemius muscle after the first 5 h of hindlimb immobilization in the shortened position (59), even though the gastrocnemius muscle protein synthesis rate is decreased at this early time point (6). Measurements of glucocorticoid receptor in the soleus muscle at early time points of hindlimb unweighting are required to test whether there is a similarity with the limb immobilization findings.

**Prostaglandins.** There is a decrease in phosphatidylethanolamine (PE) concentration (nmol/mg tissue) in the soleus muscle after 2 wk of unweighting that is uniform with respect to fatty acid composition (67), including linoleic and arachadonic acids. After 2 wk of unweighting and 1 wk of recovery, soleus muscle PE pools did not increase (67). The administration of indomethacin during the recovery repleted the PE pools (67). Thus, soleus muscle PE turnover apparently increases during recovery from unweighting. Whether this represents a cause-and-effect relationship is not known.

**Messenger RNAs (mRNAs).** Although decreases in specific mRNAs do occur in unweighted soleus muscle of relatively nongrowing adult rats, these declines occur after the decrease in protein synthesis rate in soleus muscle of adult rats. For example, there is no decrease in either \(\alpha\)-actin mRNA concentration at 1 day of unweighting (33) or in \(\beta\)-myosin heavy chain mRNA concentration at 7 days of unweighting (69), even though myofibrillar protein synthesis rates are decreased in the 1- and 7-day unweighted soleus muscle (70). In addition, the \(29\%\) decrease in \(\alpha\)-actin mRNA concentration of the 7-day unweighted soleus muscle (2) is less than the \(19\%\) decrease in myofibrillar protein synthesis rate in the 7 day unweighted soleus muscle (70). Although clenbuterol administration during unweighting prevented most of the decrease in \(\alpha\)-actin mRNA in the soleus muscle, loss of protein by the unweighting was not abated (3). We interpret the above information to suggest that both translational and transcriptional downregulation occur in the unweighted soleus muscle of adult rats. Nevertheless, the acute decrease in protein synthesis rates in the unweighted soleus muscle of the adult female rat likely occurs because of an early downregulation of translational processes.

In young growing rats an early decrease in \(\alpha\)-actin mRNA concentration likely has a role in growth inhibition of the unweighted soleus muscle. \(\alpha\)-Actin mRNA and cytoplasmic \(\beta\)-actin mRNA concentrations are decreased in the 1-day unweighted soleus muscle of growing rats (21, 33). The different responses of \(\alpha\)-actin mRNA concentrations in the soleus muscle at 1 day of unweighting in young rats (where it decreased) and in mature nongrowing rats (where it was unchanged) suggest that caution should be exercised in extrapolating responses of mRNAs in unweighted soleus muscles from rapidly growing muscles to expected responses of mRNAs in adult human skeletal muscles during weightlessness.

However, despite the decrease in some mRNAs, unweighting increases the soleus muscle poly A+ mRNA-directed in vitro synthesis of specific proteins corresponding to 16-, 17.4-, and 23-kDa proteins (33). The identity of these proteins is unknown, but the result clearly demonstrates that the unweighting-induced atrophy displays some measure of control and selectivity with respect to the expression of mRNA.

**Glutamine.** Glutamine concentration decreases \(41\%\) in the 6-day unweighted soleus muscle (38). In a later experiment (34), glutamine concentration was un-
changed in the 6-day unweighted soleus muscle and no correlation was observed between glutamine concentration and size of the soleus muscle.

Because unweighting increases glutamine synthase in the soleus muscle (38), Jaspers et al. propose that decreased ammonia availability in the unweighted soleus muscle likely accounts for the decreased glutamine production and, thus, concentration. They speculate that a decreased flux through the purine nucleotide cycle, as a result of decreased energy demand in the unweighted soleus muscle, could account for the decreased amount of ammonia availability (38).

Proteases. Some lysosomal enzymes show increased activity in the soleus muscle homogenates after unweighting. After 4 days, acid protease activity was increased 32% (65); after 5 days, cathepsin D activity was increased 42%, but cathepsin B was unchanged (22); and after 14 days, acid protease activity was increased 35% (66). The relationship between these increases in soleus muscle homogenate protease activity and soleus muscle protein degradation in vivo needs to be explored further.

Nitrogen losses in urine. Neither 3-methylhistidine nor urea excretion is increased after 1 day of unweighting (50, 64). Theretofore their excretion is elevated (50, 51, 64), although in one study no increased urinary urea excretion was found at the 12th day of unweighting (39). In contrast to the unchanged 3-methylhistidine and urea excretion after 1 day of unweighting, ammonia excretion is doubled within the first 24 h (50) and remains elevated during unweighting (50, 51, 64), except in a single study in which no increase in ammonia excretion was reported at 12 days of unweighting (39).

Because the soleus muscle comprises <1% of the mass of skeletal muscle in the rat hindlimb, it is difficult to extrapolate from urinary excretions to this small muscle mass. In addition, 3-methylhistidine is released mainly from fast-twitch, rather than slow-twitch muscle, (25, 43, 44), so it is also difficult to extrapolate from an assay of this product to protein degradation rate in the slow-twitch soleus muscle.

Conclusion

We have outlined the changes that occur in the soleus muscle during hindlimb unweighting. The hindlimb unweighting model was first devised to mimic the lack of weight bearing that occurs in a weightless environment. However, hindlimb unweighting at 1-G is undoubtedly slightly different from weightlessness. Nevertheless the data obtained from animal models of unweighting should prove to be very valuable for at least two reasons: 1) because of the limited scope of data on humans in weightlessness the unweighting model has provided information that would not otherwise be available, and 2) because of the likelihood of limited future data on humans in weightlessness, the unweighting model should make the planning of human research more meaningful.

Each section of our discussion outlined specific data. Some areas have numerous individual reports, whereas in other areas the data are sparse and contradictory, especially with regard to the effect of age on the atrophy process. Although we have considered possible mechanisms for soleus muscle atrophy, no single biochemical signal has been identified as the trigger for the atrophy, and probably a single signal does not exist. This leaves considerable room for further experiments and reinterpretation of the data presented here. As a popular animal model, hindlimb unweighting will undoubtedly continue to yield fruitful information.

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