ACUTE NUTRITIONAL DEPRIVATION (ND) may be an important complicating factor in critical illness, enhancing both morbidity and mortality (2). We previously reported that acute ND (i.e., 90 h of complete food deprivation with water provided ad libitum) in adult rats produced no significant atrophy of either type I or type II diaphragm muscle fibers, despite a 20% reduction in body weight (18). By contrast, an identical experimental protocol of acute ND in rapidly growing adolescent rats reduced body weight by 32% and the cross-sectional areas (CSAs) of type I and type II diaphragm fibers by 22 and 40%, respectively (19). Thus young growing animals appear to have significantly reduced "nutritional reserve" and are unable to adapt biochemically to even short periods of unstressed starvation (13). For example, Goodman and co-workers (13) reported significant curtailment of protein synthesis, as well as enhanced protein degradation in limb muscles of 4-wk-old rats after acute ND for 2 days, whereas no impact on protein turnover was observed in 8-wk-old rats after an identical 2-day fast. In addition, Goldberg and Odessy (12) reported similar influences on protein turnover in diaphragms of young rats after 3 days of acute ND. The diaphragm, therefore, despite rhythmic activation of various motor units throughout life, does not appear to be protected from acute nutritional insults in young animals. As a result, a significant reduction in diaphragm mass (i.e., atrophy of muscle fibers) would be expected to reduce the force-generating capacity of this key inspiratory muscle, limiting its ability to sustain increased loads that could contribute to possible task/ventilatory failure and thus to enhanced morbidity and/or mortality. The rapidity with which the biochemical and morphometric changes occur in respiratory and limb muscles after acute ND in young animals provides a strong rationale for investigating whether the concomitant provision of growth factors with acute ND could offset the severe reductions in diaphragm fiber size. The rationale is further strengthened by the fact that, in the clinical context, numerous factors contribute to difficulties in providing optimal or, for that matter, any meaningful nutritional support acutely in critically ill children. In this context, the provision of growth factors may be considered as an important adjuvant measure to limit the adverse consequences of disordered protein turnover and negative nitrogen balance.

Insulin like growth factor I (IGF-I) is one of the principal polypeptide growth factors through which growth hormone (GH) is thought to exert its effects on protein metabolism, cartilage, and growth (29). IGF-I promotes anabolism by increasing protein synthesis and decreasing nitrogen excretion (7, 29). Serum IGF-I levels are significantly reduced after either acute ND (27) or in states of prolonged protein and/or calorie deprivation (26, 37). The reduction in IGF-I with ND may be age dependent, being greater in young animals (9).

Despite peripheral resistance to the action of GH accompanying various states of ND (33, 36), IGF-I administration was reported to reduce weight loss and/or nitrogen loss in mice (25) or rats (1) after acute ND. It is not known, however, whether the apparent anabolic effects of IGF-I in these circumstances would be sufficient to diminish the severe reductions in diaphragm fiber size noted with acute ND (19). Recent
studies in both animals (rats) (22) and humans (17), in whom caloric restriction was imposed, demonstrated that the addition of GH to IGF-I was significantly more anabolic than either agent alone. We questioned whether GH could indirectly add to the potential anabolic effects of IGF-I in the setting of acute ND.

The aim of the present study was, therefore, to assess in an adolescent-rat model of acute ND the anabolic effect of IGF-I, with or without added GH, on the CSAs of individual diaphragm muscle fibers. In addition, the impact of the growth factors on the contractile and fatigue properties of the ND diaphragm was assessed.

METHODS

Animal groups and nutritional protocol. Thirty-five adolescent Sprague-Dawley rats were studied 1 wk after weaning (i.e., at 4 wk of age, with initial body weights of ~85 g). The animals were divided into five groups: 1) control (Ctr; n = 8); 2) nutritionally deprived (ND; n = 7); 3) ND + administration of IGF-I (ND/IGF-I; n = 7); 4) ND + administration of GH (ND/GH; n = 7); and 5) ND rats given a combination of IGF-I and GH (ND/IGF-I/GH; n = 6).

The Ctr animals were provided with food and water ad libitum (Purina rat chow: 56% carbohydrate, 23% protein, 4.5% fat, 6% fiber, and 10.5% ash minerals), whereas the ND animals were subjected to a 72-h period of complete food deprivation with water provided ad libitum. The animals were housed in individual cages. These studies were approved by the Cedars-Sinai Medical Center Burn BS and Allen Research Institute Animal Care and Use Committee.

Administration of growth factors. Recombinant human IGF-I and GH were utilized. IGF-I was administered by constant infusion, with the use of an implanted subcutaneous osmotic minipump (Alzet, model 2001). The minipump was implanted dorsally between the scapulae under sterile conditions and short-term general anesthesia (ketamine 100 mg/kg ip and xylazine 10 mg/kg ip). A 1-cm incision was closed with a surgical clip. IGF-I was delivered at a rate of 200 µg/day for the 72-h period of acute ND. GH was administered by subcutaneous injection twice daily (i.e., 250 µg every 12 h for 72 h). Sham surgery was performed in all animals not receiving IGF-I. Twice daily subcutaneous injections of saline were administered to all animals not receiving GH.

In vitro assessment of diaphragm contractile and fatigue properties. The methods used to determine the contractile and fatigue properties of the diaphragm in vitro have been described in detail in earlier studies (20, 31). Briefly, the entire diaphragm was rapidly excised after the induction of deep anesthesia (6 mg/100 g body wt ip pentobarbital sodium). A narrow 3- to 4-mm-wide strip of diaphragm was excised from the right midcostal region, maintaining fiber attachments to the ribs and central tendon intact. The segment of diaphragm was vertically mounted in a tissue bath containing Krebs-Henseleit solution that was maintained at a temperature of 26°C and constantly aerated with 95% O2-5% CO2. The costal margin clamp was attached to a calibrated force transducer (Grass FT10; Quincy, MA) and the central tendon clamp to a micromanipulator (Kopf; Topanga, CA). The diaphragm strip was directly stimulated by using 2-ms monophasic impulses at supramaximal intensity (Grass S88 stimulator). Neuromuscular transmission was blocked by the addition of d-tubocurare (12 µM) to the tissue bath. Muscle length was adjusted until maximum twitch force responses were obtained isometrically. Isometric contractile and fatigue properties were studied at this optimal length (L0), which was measured by using a digital caliper accurate to 1 µm (Mitutoyo, Japan).

Peak twitch force (P0), contraction time (time to P0), and one-half relaxation time (RT1/2; time for P0 to fall to half maximum) were determined from a series of single pulses. Force-frequency relationships were measured for a range of stimulus frequencies from 5 to 100 pulses/s (pps). The stimuli were presented in trains of 1-s duration, with an interval of at least 30 s intervening between each stimulus train. P0 and maximum tetanic force (Pt) were normalized for the estimated CSA of the muscle segment (CSA = muscle wt/1.056 × Lo, where 1.056 g/cm3 represents the density of muscle) and expressed in newtons per square centimeter. Pt was also normalized for the muscle strip weight and expressed in newtons per gram.

Fatigue resistance of the diaphragm muscle was determined by using a fatigue test, whereby repetitive stimuli were presented over a 2-min period (i.e., 40 pps in trains of 330 ms repeated each second). A fatigue index was calculated as the ratio of the force after 2 min of stimulation to the initial force.

Histochemical procedures: diaphragm fiber type proportions and CSA. After the physiological studies, the muscle segment and an adjacent separate strip of diaphragm were stretched to Lo and mounted on cork and then rapidly frozen in liquid nitrogen. Serial cross-sections of the diaphragm segments were cut at 10-µm thickness by using a cryostat (Reichert-Jung, model 2800E; Nussloch, Germany) kept at −20°C.

Diaphragm muscle fibers were classified based on difference in staining intensity for myofibrillar adenosine triphosphatase (mATPase) after alkaline (pH = 9.0) and acid (pH = 4.3 and 4.55) preincubations (5). One additional serial section was fixed in 2% paraformaldehyde at pH = 7.4 for 2 min at room temperature and then preincubated at pH = 10.4 (modification of method by Guth and Samaha; see Refs. 11, 15). These various staining procedures allow the classification of fibers into several types, i.e., types I, IIA, IIB, IIX, and IIC (11; see also Ref. 14). Fiber type proportions were determined from a sample of 200–300 fibers from each muscle. In previous studies, in both hamsters and rats, we verified that muscle fiber type immunohistochemically, with 95% or more correspondence between the mATPase-based classification and the major isoform of myosin heavy chain in single diaphragm fibers (11).

Diaphragm muscle fiber CSA was determined from microscopic images of digitized muscle sections by using a computer-based image-processing system. The latter is composed of a Leitz Laborlux microscope S (Leica; Deerfield, IL), charge-coupled device video camera system (model VI-470; Optronics Engineering; Goleta, CA), high-resolution Trinitron color video monitor (model PVM-1343MD; Sony, Ichiomiya, Japan), a 486 SX 50-MHz personal computer with a Targa+ imaging board (Truevision, Indianapolis, IN), and Mocha image-analysis software (version 1.20; Jandel, San Rafael, CA). A microscope stage micrometer was used to calibrate the imaging system for morphometry. The CSA of individual fibers was determined from the number of pixels within outlined fiber boundaries.

Biochemical analysis. Serum total IGF-I concentrations were determined at Genentech by radioimmunoassay (21), after precipitation of IGF binding proteins (IGFBP) by incubation in acid-ethanol (6). Serum glucose concentrations were measured with a Hitachi 736 autoanalyzer (Hitachi, Tokyo).

Statistical analysis. Statistical analysis was performed by using an analysis of variance, with the experimental factors being nutritional status, administration of IGF-I, and admini...
istration of GH. In comparing force-frequency relationships, analysis of variance with repeated measures was employed. Post hoc analysis (Newman-Keuls test) was used to compare differences in independent groups. An alpha level of 0.05 was used to compare differences in independent groups and to determine overall significance. All data are represented as means ± SD.

RESULTS

Body weights. The initial body weights of the animals were similar (85.4 ± 5.4 g). Whereas the body weights of Ctr animals increased by ~24% during the 72-h experimental period, the body weights of ND animals decreased ~32%. Thus, at the end of the 72-h period of acute ND, the body weights of ND animals were ~56% of those of Ctr animals (Fig. 1). The administration of individual growth factors to ND animals failed to prevent significant body weight loss. However, the degree of weight loss was less in the ND/IGF-I/GH animals (Fig. 1; P < 0.05).

Biochemistry. Serum glucose levels were similar in all groups, and hypoglycemia did not develop in either the ND animals or in ND animals receiving IGF-I (Table 1).

Total serum IGF-I concentrations were reduced ~51% in ND animals compared with Ctr (P < 0.01) (Table 1). Similarly, in ND animals receiving GH, IGF-I concentrations were reduced ~45% compared with Ctr (Table 1; P < 0.05). In animals receiving IGF-I infusions, the serum levels of IGF-I increased 2.2–3.6 times those of Ctr animals and 4.6–7.4 times those of ND animals (Table 1).

Diaphragm contractile and fatigue properties. Muscle L₀ was unaffected by ND or the provision of growth factors (Table 2). Analysis of twitch diaphragm characteristics revealed significant prolongation of RT₁/₂ in ND animals compared with Ctr (Table 2; P < 0.01). The provision of IGF-I and/or GH did not further alter the prolonged RT₁/₂ observed in ND animals (Table 2). P₁ and P₀ were unaffected by ND with or without the administration of growth factors (Table 2). The force-frequency relationships of the diaphragm were shifted up and to the left at frequencies of 30 pps or less in ND animals, compared with the Ctr group (Fig. 2; P < 0.01). No further alteration in force-frequency relationships was evident in the ND animals receiving IGF-I and/or GH (Fig. 2).

Whereas there was no significant difference in the fatigue indexes of the diaphragms of ND and Ctr animals, a trend toward improved fatigue resistance was noted in the ND animals (Table 2; P = 0.07). The fatigue index of ND animals receiving IGF-I and/or GH was not statistically different from that noted in Ctr animals (Table 2).

Diaphragm muscle fiber type proportions and CSAs. Diaphragm fiber type proportions were similar between ND and Ctr groups. The provision of IGF-I and/or GH to ND animals did not alter fiber proportions (Table 3). Acute ND markedly reduced the CSA of diaphragm muscle fibers by 27–46% compared with Ctr (Fig 3). In ND animals receiving either IGF-I or IGF-I and GH, the reduction in CSA in type I fibers was not statistically different from that noted in Ctr animals (Table 2).

Table 1. Biochemistry

<table>
<thead>
<tr>
<th></th>
<th>Ctr</th>
<th>ND</th>
<th>ND/GH</th>
<th>ND/IGF-I</th>
<th>ND/IGF-I/GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum glucose, mg/dl</td>
<td>144 ± 24</td>
<td>113 ± 43</td>
<td>117 ± 47</td>
<td>117 ± 42</td>
<td>132 ± 40</td>
</tr>
<tr>
<td>Total IGF-I, ng/ml</td>
<td>264 ± 19</td>
<td>128 ± 91</td>
<td>*144 ± 48</td>
<td>*950 ± 972</td>
<td>*584 ± 389</td>
</tr>
</tbody>
</table>

Values are means ± SD. Ctr, control group; ND, nutritionally deprived group; ND/GH, ND animals given growth hormone (GH); ND/IGF-I, ND animals given insulin-like growth factor I (IGF-I); ND/IGF-I/GH, animals given combination of IGF-I and GH.*Significantly different from Ctr, P < 0.05; †significantly different from ND, P < 0.05.

![Fig. 1](http://jap.physiology.org/) Bar graphs depicting initial and final body weights in the 5 groups. Ctr, control; ND, nutritionally deprived; ND/GH, ND given growth hormone; ND/IGF-I, ND given insulin-like growth factor I; ND/IGF-I/GH, ND given a combination of IGF-I and GH. Values are means ± SD. *Significantly different from Ctr, P < 0.05; †significantly different from ND, P < 0.05.
DISCUSSION

This study demonstrates that the marked reductions in diaphragm fiber size observed after 72 h of acute ND in adolescent rats can be prevented to a significant degree by the concomitant administration of IGF-I. In contrast, GH had no significant effect on diaphragm fiber CSA after ND, indicating resistance to the action of GH in this model of acute ND. Furthermore, when GH was combined with IGF-I, no further increments in diaphragm fiber size were noted. Diaphragm contractile properties were not altered by the administration of either IGF-I and/or GH to the ND animals.

Diaphragm fiber proportions and morphometry: influence of growth factors. We have previously reported that diaphragm fiber proportions were unaffected by undernutrition in models of both acute (18, 19) and prolonged (20, 31) ND. Similarly, in the present study, no alterations in fiber proportions were observed after nutritional and/or hormonal manipulations over the short term. It is of interest that GH administered to rats over 2 wk to 6 mo had no impact on mATPase activity or fiber composition of limb muscles as well as the diaphragm (10). Thus lack of fiber conversion in the present study was not unexpected, especially in view of the short duration of ND.

The striking finding in this study was the positive impact of IGF-I on diaphragm fiber size with acute ND and the apparent resistance to the action of GH when administered alone or in combination with IGF-I. These findings are in keeping with the data of Asakawa and co-workers (1), who subjected rats to a 3.5-day fast using similar protocols of IGF-I or GH administration as used in the present study. Body and organ weights of IGF-I-treated animals were significantly greater than the weights in fasted controls, whereas GH animals exhibited no significant impact (1). In addition, IGF-I-treated animals demonstrated less urinary nitrogen excretion than fasted controls (1). Similarly, O’Sullivan and colleagues (25) also reported a reduction in body weight loss in mice after 36 h of starvation, compared with saline- or GH-treated animals subjected to an identical period of acute ND. No differences in the percentage of body water were noted between the IGF-I-, GH-, and saline-treated animals (25).

In both our study and that of O’Sullivan et al. (25), total serum IGF-I concentrations were markedly reduced with acute ND, compared with free-eating Ctr animals. The anabolic effects observed thus appear to be related to augmented IGF-I levels with IGF-I administration, since in both studies GH administration failed to increase IGF concentrations above the levels observed in the fasted state. A concern with the induction of supranormal serum levels of IGF-I is the production of hypoglycemia (7). However, despite acute ND, the provision of significant doses of IGF-I did not produce a reduction in serum glucose levels in our model. Thus the mechanisms responsible for the acquired resistance to the action of GH in the setting of acute ND (28) were not shared by IGF-I (i.e., IGF-I appeared to “bypass” the cellular mechanisms precluding an anabolic action of GH in the setting of acute ND).

GH secretion is reduced in the rat model of acute ND, possibly related to a relative or an absolute increase in

![Image](image.png)

**Table 2. Diaphragm contractile and fatigue properties**

<table>
<thead>
<tr>
<th></th>
<th>L&lt;sub&gt;o&lt;/sub&gt;, mm</th>
<th>CT, ms</th>
<th>RT&lt;sub&gt;1/2&lt;/sub&gt;, ms</th>
<th>P&lt;sub&gt;t&lt;/sub&gt;, N/cm²</th>
<th>P&lt;sub&gt;o&lt;/sub&gt;, N/cm²</th>
<th>P&lt;sub&gt;o&lt;/sub&gt;, N/g</th>
<th>F&lt;sub&gt;t&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctr</td>
<td>13.4 ± 1.2</td>
<td>63 ± 8</td>
<td>71 ± 10</td>
<td>5.7 ± 0.1</td>
<td>16.8 ± 2.1</td>
<td>11.4 ± 1.6</td>
<td>49 ± 6</td>
</tr>
<tr>
<td>ND</td>
<td>12.8 ± 0.5</td>
<td>72 ± 8</td>
<td>120 ± 17*</td>
<td>6.8 ± 1.4</td>
<td>16.4 ± 2.6</td>
<td>12.1 ± 1.9</td>
<td>56 ± 8</td>
</tr>
<tr>
<td>ND/GH</td>
<td>13.3 ± 0.5</td>
<td>82 ± 15</td>
<td>122 ± 25*</td>
<td>5.6 ± 1.6</td>
<td>16.7 ± 3.8</td>
<td>11.1 ± 3.4</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>ND/IGF-I</td>
<td>13.4 ± 1.1</td>
<td>69 ± 7</td>
<td>104 ± 23*</td>
<td>6.2 ± 1.4</td>
<td>16.5 ± 3.2</td>
<td>10.8 ± 3.3</td>
<td>48 ± 5</td>
</tr>
<tr>
<td>ND/IGF-I/GH</td>
<td>13.4 ± 1.1</td>
<td>73 ± 2</td>
<td>99 ± 11*</td>
<td>6.9 ± 1.4</td>
<td>17.6 ± 2.2</td>
<td>12.4 ± 1.7</td>
<td>50 ± 5</td>
</tr>
</tbody>
</table>

*Significantly different from Ctr, P < 0.05.

**Table 3. Diaphragm fiber type proportions and relative contribution to total costal area**

<table>
<thead>
<tr>
<th></th>
<th>Ctr</th>
<th>ND</th>
<th>ND/GH</th>
<th>ND/IGF-I</th>
<th>ND/IGF-I/GH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber proportions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>29.2±2.4</td>
<td>33.8±4.0</td>
<td>31.4±3.3</td>
<td>30.7±1.9</td>
<td>32.8±3.6</td>
</tr>
<tr>
<td>Type IIa</td>
<td>33.2±4.3</td>
<td>30.6±4.0</td>
<td>26.7±2.2</td>
<td>27.9±4.0</td>
<td>28.8±7.6</td>
</tr>
<tr>
<td>Type IIx</td>
<td>29.2±2.2</td>
<td>27.7±3.6</td>
<td>30.6±1.0</td>
<td>36.4±6.0</td>
<td>34.7±7.1</td>
</tr>
<tr>
<td>Type IIc</td>
<td>8.4±3.3</td>
<td>7.9±1.9</td>
<td>11.3±2.8</td>
<td>5.0±2.4</td>
<td>5.0±2.3</td>
</tr>
<tr>
<td>Relative contribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>25.7±2.7</td>
<td>29.4±5.3</td>
<td>29.8±5.0</td>
<td>28.2±2.1</td>
<td>29.1±2.9</td>
</tr>
<tr>
<td>Type IIa</td>
<td>28.9±4.2</td>
<td>27.8±5.3</td>
<td>24.0±1.2</td>
<td>24.3±4.0</td>
<td>24.4±6.3</td>
</tr>
<tr>
<td>Type IIx</td>
<td>39.4±5.4</td>
<td>35.0±6.2</td>
<td>36.5±4.6</td>
<td>42.8±6.0</td>
<td>42.1±7.0</td>
</tr>
<tr>
<td>Type IIc</td>
<td>6.2±1.8</td>
<td>7.8±2.7</td>
<td>9.7±2.4</td>
<td>4.7±2.6</td>
<td>4.4±1.7</td>
</tr>
</tbody>
</table>

Values are means ± SD.
circulating somatostatin concentrations in the fasted state (34). However, the lack of response to the administration of GH in the present study suggests, in addition, insensitivity or resistance to GH at a tissue level. The latter has been associated with a reduction in liver GH-binding sites with fasting (3, 27, 32) and/or a postreceptor response best characterized in protein-restricted rats (35, 36).

A variety of endocrine effects induced by ND may impact on the somatotropic axis. For example, dietary restriction or fasting is associated with reduced serum insulin levels, which are associated with reduced GH binding and low serum IGF-I concentrations. Reduced serum levels of IGF-I accompanying fasting appear to correlate with reduced levels of IGF-I mRNA in the liver and other tissues, including muscle, indicating reduced gene transcription (23, 37). In contrast, an increase in IGF-I receptor mRNA and increased binding of IGF-I in tissues has been reported with fasting in the rat (23). Thus the provision of exogenous IGF-I in the present study might be expected to promote an anabolic effect with acute ND by augmenting circulating ligand. Indeed, in fasted lambs, IGF-I infusion promoted protein conservation by reducing protein degradation as well as by augmenting protein synthesis in the liver, heart, and skeletal muscle, including the diaphragm (7). In addition, fasting may be associated with a reduction in 3,3',5-triiodo-L-thyronine (T₃) and increment in reverse T₃. A close relationship exists between reduced levels of IGF-I and T₃ after acute ND. Although thyroid hormone may exert some regulatory influences on the somatotropic axis (i.e., regulation of GH gene expression; blunted IGF-I response to GH with hypothyroidism), nutrient regulation is the more dominant factor (38).

The positive impact of IGF-I and IGF-I/GH on diaphragm fiber size in the present study was similar, whereas no significant influence was noted with the administration of GH alone. This suggests that the positive morphometric effects noted in the ND/IGF-I/GH group were solely due to the IGF-I component of the experimental regimen. We initially speculated that a combination of GH and IGF-I might exert a greater effect due to a number of mechanisms including augmentation of serum levels of the IGF-binding protein complex IGBP3/acid-labile subunit, which would prolong the half-life of IGF-I and promote a more stable pool of the growth factor (39), and possibly improve reduced insulin levels present in acute ND (24). IGF-I appeared to exert an anabolic effect on types I, IIa, and IIx diaphragm fiber types by attenuating growth arrest and/or atrophy of individual diaphragm fibers in the rapidly

Fig. 3. Bar graphs depicting cross-sectional areas (CSAs) for types I (A), IIa (B), IIx (C), and IIc (D) diaphragm muscle fibers. Note: a significant reduction in CSA of types I and II fibers is noted in ND animals compared with Ctr (P < 0.01). In ND animals receiving IGF-I (ND/IGF-I; ND/IGF-I/GH), CSA of types I, IIa, and IIx fibers was significantly greater than in ND (P < 0.01), whereas no impact on these fibers was observed in ND animals receiving GH alone. No added or synergistic effect was noted in group receiving combination of IGF-I and GH. Values are means ± SD. *Significantly different from Ctr, P < 0.05; 1significantly different from ND, P < 0.05.
growing adolescent animals. The extent of change appeared to be greater in diaphragm fibers more readily recruited (e.g., type I) than in fibers rarely recruited under normal eucaloric conditions (e.g., type IIx). For example, the CSA of type I fibers in ND/IGF-I and ND/IGF-I/GH groups was reduced 11.9 and 9.7%, respectively, relative to Ctr, compared with a 26.7 and 19.6% reduction in the CSA of type IIx fibers for these groups. Despite this, the estimated relative contribution of type I or II fibers to total costal diaphragm area was similar across the groups.

Diaphragm contractile properties and functional implications. The administration of GF had no significant impact on isometric contractile and fatigue properties in ND animals. This may in part be explained by the similar estimated relative contributions of type I and II fibers to total costal diaphragm area across the various groups. Nevertheless, the influence of acute ND on total diaphragm force production would be markedly curtailed. Eddinger and Moss (8) reported that the specific force (i.e., force/unit CSA) of type II diaphragm fibers was 1.5 times that of type I fibers. Taking into account the proportions of diaphragm fibers, the mean CSA of type I and II, and the relative differences in specific force for type I and II fibers, we estimated that total force of the costal diaphragm would be reduced ~45% in the ND group. We further estimated that total force production by the costal diaphragm would be much less impacted in the ND/IGF and ND/IGF-I/GH groups (i.e., reduced ~21 and 17%, respectively). Similar estimates of total diaphragm force reduction in ND animals and attenuation of force reduction in groups receiving IGF-I were also made based on similar yet valid assumptions. Because L₀ of diaphragm muscle fibers was not changed in any of the experimental groups, any changes in muscle CSA would be expected to reflect the relative diaphragm mass available for force generation. Total diaphragm area in ND animals was estimated to be 55.9% that in Ctr animals, whereas in the groups receiving IGF-I total CSA was estimated to be 79.6% (ND/IGF-I) and 84.3% (ND/IGF-I/GH) of Ctr values. As diaphragm force corrected for muscle strip weight was similar among all groups, reduction in total CSA of the diaphragm should be a valid representation of the relative loss in total force-generating capacity. Whereas the reduction in total diaphragm force production in ND animals is unlikely to impact on resting ventilation, as eucaloric efforts require only ~10–15% of the total force-generating capacity of the diaphragm (30), the functional reserve of the diaphragm would be very limited. With loaded efforts, the critical ratio of force to maximum force may easily be exceeded with ensuing task (ventilatory) failure (4). We speculate that the improved total CSA (i.e., contractile mass) of the diaphragm in ND animals responding to IGF-I would almost certainly improve the reserve capacity of the muscle and its ability to meet added loads.

In summary, adolescent animals subjected to acute ND exhibit reduced nutritional reserve, resulting in significant atrophy of all fiber types. The administration of IGF-I diminishes the reduction in diaphragm fiber size noted with acute ND. In contrast, GH failed to augment IGF-I levels or improve diaphragm fiber CSA in ND animals, indicating resistance to the action of GH in this ND model. We speculate that if sufficient calories are provided to offset the peripheral tissue resistance to GH action (i.e., exceed a critical energy threshold) that the combination of GH and IGF-I may indeed have an added anabolic effect on diaphragm fiber morphometry (16, 17, 22). This hypothesis is supported by the studies of Kupfer et al. (17), who demonstrated significantly enhanced nitrogen balance using a combination of GH and IGF-I compared with IGF-I alone in subjects in whom moderate caloric restriction was imposed and by the work of Lo et al. (22), who reported enhanced body weight gain in rats receiving the combination of GH and IGF-I than either agent alone in a surgical stress-total parenteral nutrition model. The choice of an adjunctive GF regimen and the anticipated outcome on respiratory muscle structure and function may, thus, depend on the severity of the nutritional insult.

The authors gratefully acknowledge the superb assistance of Ling Tang and Darlene Ford in these studies as well as Drs. Shirono Melamed and Ross Clark for their encouragement and advice and Dr. S. Melmed for his insightful review of the manuscript. This study was supported by National Heart, Lung, and Blood Institute Grants HL-01907 and HL-47537 and by a generous gift (GH and IGF-I) from Genentech, Inc.

Address for reprint requests: M. I. Lewis, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Rm. 6732, Los Angeles, CA 90048.

Received 23 May 1996; accepted in final form 2 November 1996.

REFERENCES

Growth factors effect on diaphragm of malnourished rats


