IGF-I and/or growth hormone preserve diaphragm fiber size with moderate malnutrition

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Lewis, Michael I., Andrea T. Feinberg, and Mario Fournier. IGF-I and/or growth hormone preserve diaphragm fiber size with moderate malnutrition. J. Appl. Physiol. 85(1): 189–197, 1998.—Resistance to the anabolic effects of growth hormone (GH) occurs with severe caloric deficit. This study examined whether moderate caloric deficit (50% of daily intake for 7 days) in the adolescent rat exceeds a critical threshold for GH action and whether a combination of GH and insulin-like growth factor I (IGF-I) would have enhanced anabolic effects on the diaphragm (Dia). Five groups of rats (4 wk old) were studied: 1) control (Ctl), 2) nutritionally deprived (ND), 3) ND + GH, 4) ND + IGF-I, and 5) ND + GH + IGF-I. IGF-I was given by continuous infusion (200 μg/day). GH was injected subcutaneously (250 μg every 12 h). Contractile and fatigue properties of the Dia were determined in vitro. Quantitative histochemical methods were used to determine Dia fiber type proportions, cross-sectional areas, and succinate dehydrogenase activities. The body weight of Ctl rats increased 46% compared with 7% in ND animals, whereas that of ND rats receiving growth factors was intermediate. Serum IGF-I levels were reduced 54% in ND animals and maintained with the provision of growth factors. Dia fatigue resistance was improved in ND animals receiving growth factors. There were no differences in Dia contractile properties, fiber type proportions, or succinate dehydrogenase activities across groups. ND resulted in atrophy/growth arrest of all Dia fibers (20–32%) compared with Ctl. Administration of IGF-I and/or GH completely prevented atrophy/growth arrest of all Dia fibers. No additive or synergistic effects were noted. We propose that these growth factors may provide useful short-term adjunctive nutritional support in circumstances in which the provision of optimal nutrition may be delayed or inadequate.

nourishment deprivation; diaphragm contractility and fatigue; fiber cross-sectional area; succinate dehydrogenase activity; growth factors

YOUNG GROWING ANIMALS exhibit significantly reduced nutritional reserve to unstressed starvation (fasting) or moderate degrees of malnutrition compared with adult animals. Goodman and colleagues (13) reported marked negative influences on protein turnover in limb muscles of young rats after short-term fasting, whereas no impact was observed in adult animals. In a recent study, Oster and co-workers (28) demonstrated greater depression of the insulin-like growth factor I (IGF-I) system to chronic energy restriction in 4-wk-old than in adult rats. We also showed significant atrophy of type I and II diaphragm muscle fibers in acutely malnourished adolescent rats but no effect on diaphragm fiber size in adult animals subjected to an identical nutritional insult (22, 23). We recently reported that the provision of IGF-I to acutely malnourished (fasted) adolescent rats significantly reduced the degree of fiber atrophy/growth arrest of type I, IIA, and IIX diaphragm fibers compared with untreated malnourished animals (20). By contrast, growth hormone (GH) had no impact on any diaphragm fibers, consistent with prior reports of peripheral resistance to the action of GH accompanying various degrees of energy and/or protein deprivation (20, 34, 35, 37).

We postulate that if sufficient calories are provided to exceed a “critical threshold,” GH would exert an anabolic influence on diaphragm fibers in the presence of moderate malnutrition. In 4-wk-old rats this critical threshold is likely >40% of caloric intake, inasmuch as this degree of energy deprivation was associated with reduced liver IGF-I mRNA levels, whereas 60% of the caloric intake was not (28). Furthermore, we postulate that the IGF-I and GH administered together would exhibit an additive or synergistic effect. This would be consistent with clinical studies in which enhanced nitrogen balance was reported with use of the combination of IGF-I and GH compared with IGF-I alone in subjects in whom moderate degrees of caloric restriction were imposed (18). Similarly, in an animal model of parenteral nutrition, the combination of both factors enhanced body weight gain to a greater extent than either factor alone (26). In well-nourished adolescent rats we recently reported a synergistic effect of combined IGF-I and GH on body weight compared with either agent alone (21). In 4-wk-old calorie-restricted rats, significant reductions in serum IGF-I, serum IGF-binding protein-3 (IGFBP-3), serum acid-labile subunit (ALS), serum GH-binding protein (GHBP), liver IGF-I mRNA levels, and serum insulin levels were reported (28). Inasmuch as IGF-I, IGFBP-3, ALS, and GHBP are GH-dependent products, the addition of GH to IGF-I may augment IGF-I levels, promote a more stable pool of circulating IGF-I (39), and prolong GH bioavailability (1). In addition, GH could blunt the decline in insulin levels induced by IGF-I and/or reduced caloric intake. IGF-I, on the other hand, may improve the absorption of macronutrients (35) and increase protein synthesis (11, 17).

The aim of the present study was therefore to assess whether IGF-I and/or GH can partially or completely prevent diaphragm fiber atrophy/growth arrest in rapidly growing adolescent rats subjected to moderate levels of malnutrition. Furthermore, we wished to examine whether the combination of IGF-I and GH would exhibit additive or synergistic effects.
METHODS

Animals and Nutritional Protocol

Forty-two 4-wk-old male Sprague-Dawley rats were studied. The animals were divided into five groups: 1) control (Ctl) animals (n = 8); 2) nutritionally deprived (ND) animals (n = 9); 3) ND animals given IGF-I (ND-IGF-I, n = 8); 4) ND animals given GH (ND-GH, n = 9), and 5) ND animals given a combination of GH and IGF-I (ND-GH-IGF-I, n = 8). Ctl animals were fed ad libitum a purified powdered rodent diet (AIN 93G, Dyets) composed of a balanced mix of casein (200 g/kg), cornstarch (398 g/kg), sucrose (100 g/kg), soybean oil (70 g/kg), cellulose, t-butyldihydroquinone, salt mix, vitamin mix, L-cystine, and choline bitartrate. In all ND animals the diet was restricted to 50% of normal daily intake (i.e., 50% reduction in calorie and protein supply) over a 7-day period. Water was provided ad libitum to all groups. All animals were housed individually. The ambient temperature was maintained at 22°C, and the light-dark cycle was 12:12 h. The study was approved by the Animal Care and Use Committee of the Cedars-Sinai Medical Center Burns and Allen Research Institute.

Administration of IGF-I and GH

Recombinant human IGF-I was administered by continuous infusion via a subcutaneously implanted osmotic minipump (model 2001, Alzet). The daily dose was 200 µg. The infusion was given over a 7-day period. Recombinant human GH was administered by twice-daily subcutaneous injections. The dose of each injection was 250 µg. GH was administered over a 7-day period. To control the influences of surgical procedures and injections, Ctl and ND animals were subjected to identical surgeries, with creation of a subcutaneous pouch, and to saline infusions and injections. ND-GH animals were subjected to surgery and GH injections; the ND-IGF-I group received saline injections. The daily dosage regimens of IGF-I and GH were identical to the regimens that demonstrated anabolic effects in well-nourished and fasted adolescent rats (20, 21). In addition, the doses of growth factors used were known, from prior studies in our laboratory, to restore reduced levels of IGF-I due to malnutrition in the rodent model. The latter was believed to be a more useful guide to dosing than attempting to extrapolate clinical or experimental dosage regimens used in humans, which are commonly less on a per kilogram body weight basis.

In Vitro Assessment of Isometric Contractile and Fatigue Properties

The contractile and fatigue properties of the diaphragm in vitro were determined using methods identical to those described in detail in our earlier studies (23, 30). 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 cut from the right midcostal region, with care taken to maintain fiber attachments to the ribs and central tendon intact. The segment of diaphragm was vertically mounted in a tissue bath containing Krebs-Henseleit solution, which was maintained at 26°C and constantly aerated with 95% O2-5% CO2. A bath containing Krebs-Henseleit solution, which was main-

In Vitro Maximum Velocity of Unloaded Shortening

The protocol was similar to that originally described for the slack test by Edman (7). All experiments were performed on fresh costal diaphragm strips at 21°C. For these studies a computer-controlled ergometer (model 300B, Cambridge Technology, Watertown, MA) linked to a Keithley MetraByte/Asyst (Taunton, MA) DAS-1602 I/O interface board with customized data-acquisition and signal analysis software (MUSCLE, Integrated Scientific Resources, Santa Monica, CA) designed to control muscle force and length as well as pulse train was used. Data were sampled at 2 kHz. The muscle strip was maximally stimulated for 600 ms at 75 pps and allowed to reach its isometric plateau. Then changes in length steps of known distances (8–15% of L0) were rapidly made with a resultant drop in the force to zero. The duration of unloaded shortening for each quick release is a measure of the time between the release and the beginning of force redevelopment. The stimuli and length steps were presented with 1 min between each train. The slope of the line describing this distance-time relationship defines the maximum velocity of unloaded shortening, which was normalized for L0.

Histochemical Procedures

Muscle fiber type proportions and CSAs. The muscle segment used for physiological studies and an adjacent separate strip of diaphragm were stretched to L0 mounted on cork, and then rapidly frozen in isopentane that had been cooled to its melting point with liquid nitrogen. The unstimulated (fresh) segment of diaphragm adjacent to the segment used for muscle stimulation in vitro was used for all histochemical studies. Once L0 for the stimulated strip was established and measured, the fresh adjacent strip was mounted on cork at that L0, and rapidly frozen. Serial 10-µm-thick cross sections of the diaphragm segments were cut using a cryostat (model 2800E, Reichert-jung) kept at −20°C.

Diaphragm muscle fibers were classified on the basis of difference in staining intensity for myofibrillar ATPase (mATPase) after alkaline (pH 9.0) and acid (pH 4.3 and 4.55) preincubations (15). 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 (8) of method used by Guth and Samaha (15)]. These various staining procedures allow the classification of fibers into several types: I, IIa, IIb, IIx, and IIc (8, 14). Fiber type proportions were determined from a sample of 200–300 fibers.
The concentration of NBT diformazan (NBT-dfz) deposited within a muscle fiber was calculated using the Beer-Lambert equation

\[ [\text{NBT-dfz}] = \frac{\text{OD}}{K \times L} \]

where OD is the optical density of the muscle fiber measured at 570 nm (the peak absorbance wavelength for NBT-dfz), K is the molar extinction coefficient for NBT-dfz (26,478 mol/cm), and L is the path length (6-µm-thick section) for light absorbance (4).

The OD of muscle fibers was determined using a microdensitometric procedure implemented on the computer-based image-processing system. The video image was digitized (8-bit gray-level resolution) into a matrix of 1,024 pixels (picture elements). The gray levels of the video scanner were calibrated for photometry (OD units) by use of a series of neutral density filters (0.004–2.00 OD units, Melles Griot). We previously demonstrated that during the SDH reaction in the cat and rat the formation of NBT-dfz in diaphragm fibers increases linearly over a period of at least 9 min (4, 30, 32). In reactions in which succinate was absent from the reaction medium, there was measurable staining (i.e., reduction of NBT), but the OD did not change significantly across the same time periods. The tissue blank OD also corresponded to the OD measured at time 0 in reactions in which succinate was present in the medium. On the basis of these data, we justified the use of a single end-point measurement of OD, with a reaction time of 5 min. From these end-point measurements, a rate of SDH reaction was interpolated. Mean SDH activity of individual diaphragm muscle fibers was determined by averaging the OD of all pixels within outlined muscle fibers. To correct for the nonspecific formation of NBT-dfz, the tissue blank OD for each fiber was subtracted from the OD measured when substrate was added to the incubation medium. From the Beer-Lambert equation the mean SDH activity of each fiber was expressed as millimoles of fumarate per liter of tissue per minute (4, 30). Approximately 200–300 fibers were sampled from each diaphragm muscle.

### Biochemical Analysis

Serum total IGF-I concentrations were determined at Gentech by RIA (25). Before RIA, IGFBPs were precipitated by incubation in acid-ethanol (6). The maximum intra- and interassay coefficients of variation for total IGF-I measurement (extraction procedure and RIA) are 15 and 19%, respectively (25). Whole blood glucose concentrations were determined using a technique based on the glucose oxidase method.

### Statistical Analysis

Before parametric analyses the distribution of all data was tested for normality. Statistical analysis was then performed (Crunch-3, Crunch Software, Oakland, CA) using an ANOVA, with the experimental factors being the administration of IGF-I or GH. ANOVA with repeated measures was employed to compare force-frequency relationships. If a significant interaction was found, post hoc analysis (Newman-Keuls test) was used to compare differences in independent groups. An α-level of 0.05 was used to compare differences in independent groups and to determine overall significance. Values are means ± SD.

### RESULTS

#### Animal Body Weights

The initial body weights of the 4-wk-old animals were similar across all groups. The mean initial body weight of all 42 animals was 98.7 ± 7.8 g. After the 7-day experimental protocol the body weight of Ctl animals increased by 46% compared with an increment of only 7% in the ND group (Fig. 1). Although a larger increment in body weight gain was observed in the ND groups receiving growth factors (12–20%), final body weights in these animals were still significantly lower than in Ctl animals (Fig. 1). Final body weights of ND-IGF-I and ND-IGF-I-GH groups were, however, significantly different from Ctl, P < 0.05. Although body weight of Ctl animals increased 46% over study period, provision of growth factors in ND groups had only a minimal, but significant, effect on body weight.

### Animal Body Weights

The initial and final body weights in control (Ctl) animals, nutritionally deprived (ND) animals, ND animals given insulin-like growth factor I (IGF-I; ND-IGF-I), ND animals given growth hormone (GH; ND-GH), and ND animals given IGF-I (ND-GH-IGF-I). Values are means ± SD. Although body weight of Ctl animals increased 46% over study period, provision of growth factors in ND groups had only a minimal, but significant, effect on body weight.

*Significantly different from Ctl, P < 0.05. †Significantly different from ND alone, P < 0.05.
significantly greater than that of the ND group (P < 0.05).

Serum IGF-I Assay and Blood Glucose Levels

Total serum IGF-I levels were reduced by 54% in ND animals compared with the Ctl group (Table 1; P < 0.05). The provision of growth factors to ND animals resulted in normalization of depressed IGF-I levels so that serum IGF-I levels in these groups were 1.35–2.42 times higher than in the ND group (Table 1). Hypoglycemia was not observed with the induction of ND alone or in ND animals given IGF-I and/or GH (Table 1).

In Vitro Diaphragm Isometric and Isotonic Contractile Properties

Diaphragm \( L_0 \) was similar across all five groups (Table 2). Similarly, \( P_t \) and twitch characteristics (i.e., CT and \( RT_{1/2} \)) were unaffected by ND or the provision of growth factors (Table 2). Force-frequency relationships are depicted in Fig. 2. The provision of growth factors had no effect on specific forces across all frequencies studied (Table 2, Fig. 2). In all ND groups receiving growth factors the fatigue index after the fatigue run was significantly greater, indicating enhanced fatigue resistance of the diaphragm muscle (Table 2, Fig. 3). No differences were observed across the groups in maximum velocity of unloaded shortening determined by means of the slack test (Table 2).

Diaphragm Fiber Proportions and CSA

Diaphragm fiber type proportions were similar among all groups (Fig. 4). Moderate ND resulted in significant atrophy/growth arrest of type I (reduced by 20%), IIa (reduced by 25%), and IIx (reduced by 32%) diaphragm fibers compared with Ctl animals (Fig. 5; P < 0.05).

The provision of IGF-I and/or GH to ND animals completely prevented any reduction in the size of type I, IIa, IIx, and IIc diaphragm fibers compared with Ctl (Fig. 5). The relative contribution of the different diaphragm fiber types to total costal diaphragm area was calculated from their relative proportions and CSAs. No differences were observed in the estimated relative contribution of type I, IIa, IIx, and IIc fibers after ND or ND with growth factors compared with Ctl (not shown).

Table 1. Serum IGF-I and blood glucose concentrations

<table>
<thead>
<tr>
<th>Group</th>
<th>IGF-I, ng/ml</th>
<th>Glucose, mg/dl</th>
</tr>
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<tbody>
<tr>
<td>Ctl</td>
<td>279 ± 64</td>
<td>143 ± 28</td>
</tr>
<tr>
<td>ND</td>
<td>129 ± 73*</td>
<td>112 ± 24*</td>
</tr>
<tr>
<td>ND-IGF-I</td>
<td>275 ± 90*</td>
<td>114 ± 21*</td>
</tr>
<tr>
<td>ND-GH</td>
<td>174 ± 58</td>
<td>110 ± 23*</td>
</tr>
<tr>
<td>ND-GH-IGF-I</td>
<td>311 ± 85*</td>
<td>106 ± 26*</td>
</tr>
</tbody>
</table>

Values are means ± SD. Ctl, control; GH, growth hormone; IGF-I, insulin-like growth factor I; ND, nutritionally deprived. *Significantly different from Ctl (P < 0.05); †significantly different from ND (P < 0.05).

Diaphragm Fiber SDH Activity

Neither ND nor the administration of growth factors had any impact on SDH activity in type I, IIa, IIx, and IIc diaphragm fibers compared with Ctl (Fig. 6).

DISCUSSION

After 7 days of a moderate degree of protein/calorie malnutrition, diaphragm fiber CSAs were reduced by 20–32%. With restoration of reduced serum IGF-I levels by growth factor administration to ND animals, no atrophy/growth arrest of diaphragm fibers was observed. The growth factor regimens produced similar effects on the diaphragm of ND animals, and no additive or synergistic effects were observed with a combina-

Table 2. Diaphragm contractile and fatigue properties

<table>
<thead>
<tr>
<th></th>
<th>( L_0 ), mm</th>
<th>CT, ms</th>
<th>( RT_{1/2} ), ms</th>
<th>( P_t ), N/cm²</th>
<th>( P_o ), N/cm²</th>
<th>( V_o ), L/sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctl</td>
<td>13.7 ± 1.4</td>
<td>39 ± 10</td>
<td>41 ± 13</td>
<td>4.7 ± 0.7</td>
<td>16.1 ± 1.2</td>
<td>7.8 ± 1.2</td>
</tr>
<tr>
<td>ND</td>
<td>13.1 ± 0.7</td>
<td>44 ± 8</td>
<td>45 ± 12</td>
<td>5.5 ± 1.1</td>
<td>17.4 ± 1.7</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>ND-GH</td>
<td>13.5 ± 0.6</td>
<td>44 ± 9</td>
<td>44 ± 8</td>
<td>5.9 ± 1.4</td>
<td>17.3 ± 3.4</td>
<td>6.8 ± 1.8</td>
</tr>
<tr>
<td>ND-IGF-I</td>
<td>13.0 ± 0.5</td>
<td>47 ± 7</td>
<td>49 ± 8</td>
<td>5.8 ± 1.4</td>
<td>16.1 ± 2.0</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>ND-GH-IGF-I</td>
<td>13.6 ± 0.4</td>
<td>40 ± 7</td>
<td>46 ± 7</td>
<td>5.6 ± 0.9</td>
<td>16.6 ± 2.5</td>
<td>6.6 ± 0.9</td>
</tr>
</tbody>
</table>

Values are means ± SD. \( L_0 \), optimal length; CT, contraction time; \( RT_{1/2} \), half-relaxation time; \( P_t \), peak twitch force; \( P_o \), maximum tetanic force; \( V_o \), maximum velocity of unloaded shortening.
tion of IGF-I and GH compared with either agent alone. Isometric and isotonic contractile properties of the diaphragm were unaffected by the provision of growth factors. Similarly, oxidative capacity of diaphragm fibers was unaffected by ND with or without the provision of growth factors.

Nutrient Effects on the IGF-I System

Malnutrition may exert significant adverse effects on the IGF-I system. In the present study, serum levels of IGF-I were reduced by ∼54% in ND animals consuming 50% of their usual intake of a balanced purified powdered diet. In rats of similar age, Oster et al. (28) reported similar reductions in serum IGF-I levels when caloric intake was reduced to 40 or 60% that of control rats. Furthermore, significant reductions in serum IGFBP-3, ALS, GHBP, and insulin levels were observed (28). The deficits were greater with the degree and duration of caloric restriction (28). In addition, reduced IGF-I mRNA in the liver and other tissues, as well as muscle, has been reported after acute ND as well as protein restriction in young growing rats (27, 28, 38). Such reductions are accompanied by increased IGF-I receptor mRNA and increased IGF-I binding in tissues (27). Thus it seems logical to assume that systemic and/or local augmentation of IGF-I levels by administration of IGF-I and/or GH might exert an anabolic effect in states of moderate malnutrition, if sufficient calories and/or protein are provided to offset the peripheral insensitivity/resistance to the action of GH noted with states of severe malnutrition or fasting (2, 20, 29, 33, 36, 37).

In the present study the total caloric and protein intake was reduced by 50%. Given the fact that a clear anabolic effect was observed after the administration of GH to the ND animals, we may assume that the total energy provided to the malnourished rats was in excess of a “critical threshold energy,” below which increments in protein intake fail to augment serum IGF-I levels in response to GH administration (16). It would appear that the total amount of calories consumed is a far more important factor in the nutrient regulation of IGF-I than the degree of protein restriction, provided this “caloric threshold” is exceeded (16).

Diaphragm Fiber Size and Biochemistry

In the present study the provision of IGF-I and/or GH completely prevented any atrophy/growth arrest in diaphragm fibers of malnourished young rats. IGF-I likely plays an integral role in the maintenance of fiber size.
size and appropriate growth hypertrophy in the adolescent rat. This is supported by recent studies performed in our laboratory in which we evaluated the impact of IGF-I gene deletion in a mouse knockout model. In the diaphragm and limb muscles of 2-mo-old IGF-I knockout mice, significant reductions in muscle fiber size and number were observed, highlighting the importance of IGF-I on cell proliferation during embryogenesis (i.e., hypoplasia) as well as the maintenance of growth during postnatal development into adulthood (9, 10).

Fig. 5. Cross-sectional areas (CSA) of type I (A), IIa (B), IIx (C), and IIc (D) diaphragm fibers. Values are means ± SD. *Significantly different from Ctl, \((P < 0.05)\). †Significantly different from ND alone (i.e., restoring CSA to Ctl values), \(P < 0.05\).

Fig. 6. Succinate dehydrogenase (SDH) activities for type I (A), IIa (B), IIx (C), and IIc (D) fibers of costal diaphragm. Values are means ± SD. No differences were observed between groups.
IGF-I and GH produce anabolic effects by influencing protein turnover. GH promotes an increase in protein synthesis (12), whereas IGF-I, like insulin, prevents protein breakdown (19). Recently, it has been suggested that the availability of IGF-I to promote protein synthesis in the rat is attenuated or offset by the concomitant reduction in plasma amino acids and insulin induced by IGF-I (17). Although the latter may impact on the hormonal actions of IGF-I, they should not influence the local autocrine/paracrine actions of the peptide in muscle.

Despite the very positive impact of IGF-I or GH on diaphragm muscle in malnourished adolescent rats, we failed to observe an additive or synergistic effect with the combination of the two growth factors. Theoretically, the addition of GH to IGF-I would be expected to counteract the insulin-suppressive effects of IGF-I, enhance total serum IGF-I levels, promote protein synthesis, stabilize and increase the half-life of the IGF pool by increasing IGFBP-3 and ALS, and promote autocrine/paracrine actions of IGF-I in target tissues, e.g., muscle. Indeed, the combination of IGF-I and GH has been reported to produce additive anabolic effects in human and animal models of caloric restriction (18, 26). Possible reasons for the lack of an additive effect on diaphragm fiber size with the combination of IGF-I and GH include 1) maintenance of the CSAs of all diaphragm fiber types with the use of either growth factor with no “overshoot” above that expected for animals of this age, 2) suppressive effects of IGF-I on endogenous insulin levels, plasma amino acids, and GH that were greater than the positive impact provided by the administered GH, and 3) failure of the current dosage regimen to augment total serum IGF-I levels sufficiently. It is possible that the administration of higher doses of GH and/or IGF-I would have increased IGF-I levels sufficiently to exert an additive or synergistic effect on muscle fiber size. In the present study we did not evaluate whether the combined dosage regimen exhibited an additive effect with respect to measures of nitrogen balance or on other target organs. Indeed, differing organ selectivity, including responses in skeletal muscle, has been reported with the use of IGF-I or GH (26).

SDH activities in individual diaphragm muscle fibers were unaffected by ND with or without the provision of GH or IGF-I. We previously reported preservation of SDH in the diaphragm of malnourished rats, despite reduced activity in the medial gastrocnemius of these animals (30). Our present results are consistent with these observations (30). The effect of GH on oxidative capacity of limb muscles in well-nourished rats has been reported to be variable. For example, citrate synthase activity was reduced in the plantaris muscles of rats given GH, although activity was increased in the soleus muscle of these animals (5). In the present study, SDH activities were not affected by GH or IGF-I, despite the anabolic effects observed in diaphragm fibers of these ND animals. Clearly, the complex factors controlling the myofibrillar pool are separate from those affecting enzymatic activity within diaphragm muscle fibers.

Diaphragm Contractile Properties

Malnutrition had no impact on the isometric and isotonic contractile properties of the diaphragm. Similarly, the administration of growth factors had no further influence. In part, these observations are consistent with preservation of the relative contribution of the different fiber types to total costal diaphragm area in the Ctl and experimental groups. Similarly, maintenance of fiber type proportions infers no major shift in the expression of myosin heavy chain isoforms, which could impact on the velocity of shortening of the diaphragm muscle. In ND animals there was a tendency for fatigue resistance of the diaphragm to be increased, whereas significant increments in fatigue resistance were observed in the ND animals receiving GH and/or IGF-I. Improved fatigue resistance of the diaphragm has been well documented in the states of undernutrition (24, 30). The mechanism(s) accounting for the improved fatigue indexes in the present study is unclear. No changes were observed in SDH activity in animals receiving growth factors. Although a general correlation exists between fatigue resistance and oxidative capacity, this relationship is not necessarily tightly linked, nor is the relationship linear. In addition, substrate utilization may differ in the in vitro preparation and be less reliant on oxidative pathways, particularly with repeated stimulation at 40 pps. Although no significant changes in fiber composition were observed among the groups, a small increase in the relative proportion and contribution of type I fibers to total diaphragm area in animals receiving growth factors may have contributed in part to the small changes in fatigue resistance. This could also reflect a slight change in the rate of ATP utilization. Finally, it is of interest to speculate whether the growth factors altered substrate transport systems in muscle, such as those described for glucose (e.g., GLUT-4).

Clinical Implications

As highlighted in the introduction, young growing animals exhibit significantly reduced nutritional reserve compared with adults. Indeed, distinct developmental differences in the response of the IGF-I system, protein turnover, and respiratory muscle and organ effects to severe and chronic caloric malnutrition have been highlighted by Oster and colleagues (28), Goodman et al. (13), and Lewis and Sleck (22, 23).

Significant atrophy/growth arrest of diaphragm muscle fibers after moderate degrees of malnutrition would be expected to significantly curtail the total force-generating capacity of the entire costal diaphragm as well as its functional force reserve. Conversely, the preservation of diaphragm fiber CSAs and L0 in ND animals receiving growth factors would be expected to preserve the total force-generating capacity, especially in view of its maintained specific force. This may have important clinical implications for chil-
seriously ill children in whom "poor nutritional reserve" may thus be a useful adjunctive nutritional measure in the short term. Fatigue and task failure under conditions of increased load. The provision of growth factors in the short term serve to offset incipient or overt diaphragm muscle fiber size in adolescent rats over a relatively short period. The administration of IGF-I and/or GH did not exert significant adverse effects on the maintenance of diaphragm muscle fiber size in old male Fischer 344/Brown Norway rats. 

In summary, moderate degrees of malnutrition may exert significant adverse effects on the maintenance of diaphragm fiber size in adolescent rats over a relatively short period. The administration of IGF-I and/or GH completely prevented diaphragm muscle fiber atrophy/growth arrest in malnourished young rats. No additive or synergistic effects were observed with the combination of IGF-I and GH. We postulate that the preserved total diaphragm force and functional reserve would serve to offset incipient or overt diaphragm muscle fatigue and task failure under conditions of increased load. The provision of growth factors in the short term may thus be a useful adjunctive nutritional measure in seriously ill children in whom "poor nutritional reserve" exists and in whom clinical circumstances may prevent or delay the institution of optimal nutritional support. 

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