Anabolic influences of insulin-like growth factor I and/or growth hormone on the diaphragm of young rats

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Lewis, Michael I., Thomas J. LoRusso, and Mario Fournier. Anabolic influences of insulin-like growth factor I and/or growth hormone on the diaphragm of young rats. J. Appl. Physiol. 82(6): 1972–1978, 1997.—It is controversial whether insulin-like growth factor I (IGF-I), growth hormone (GH), or their combination might enhance body growth and/or tissue anabolism in the well-fed animal with an intact somatotropic axis. To assess this further, we studied four groups of adolescent rats: 1) control (Ctr), 2) GH, 3) IGF-I, and 4) GH/IGF-I. IGF-I was given via an osmotic minipump, whereas GH was injected subcutaneously for a period of 72 h. Diaphragm (Dia) contractile and fatigue properties were determined in vitro. Quantitative histochemical and morphometric analyses were performed on Dia fibers. Total serum IGF-I levels were significantly increased in the groups receiving growth factors. Although body weight increased to a greater extent in the animals receiving growth factors, a further synergistic effect was noted in the GH/IGF-I animals compared with either GH or IGF-I groups. Costal Dia mass was greater in the groups receiving growth factors. The Dia of GH/IGF-I animals was more fatigue resistant than the Dia in Ctr. The cross-sectional area of types IIa and IIx fibers were increased to a similar extent in all groups receiving growth factors compared with Ctr. Succinate dehydrogenase activity of type IIa fibers was significantly greater in the GH/IGF-I animals compared with the other groups. We conclude that the short-term provision of growth factors to well-nourished, normally growing adolescent rats can accelerate body growth and promote selective hypertrophy of predominantly type II Dia fibers.

ACUTE NUTRITIONAL DEPRIVATION causes significant atrophy of diaphragm (Dia) muscle fibers in adolescent rats (21). We previously reported that insulin-like growth factor I (IGF-I) but not growth hormone (GH) diminished the reduction in cross-sectional areas (CSAs) of all Dia fibers in an adolescent rat model of acute nutritional deprivation for 72 h (20). It is controversial, however, whether IGF-I alone or in combination with GH can enhance body growth and/or tissue anabolism in the well-fed adolescent animal in which the somatotropic axis is intact. For example, Schalch and co-workers (29) reported a 41% increase in growth rate in malnourished young rats receiving an IGF-I infusion over 7 days but no change in growth rate in young rats fed ad libitum and given an identical regimen of IGF-I. Similar findings were noted in a follow-up study in which growth rate and nitrogen balance were unaffected by IGF-I infusion in well-fed young rats (34). In contrast, Hizuka and colleagues (14) reported significant increments in body weight gain, body length, tibial epiphyseal width, and organ weight in young rats administered IGF-I over a 7-day period. Similarly, Tomas et al. (33) reported a dose-dependent increase in body weight gain, nitrogen retention, and muscle protein synthesis in growing rats given IGF-I for 14 days. A number of well-described influences of IGF-I may underlie the apparent effect or lack of effect noted in some of these studies examining well-fed growing animals. For example, infusion of IGF-I reduces plasma levels of insulin and/or amino acids, thus diminishing overall anabolism (6, 15). In addition, in animal and human studies, IGF-I infusion has been reported to reduce the concentration of circulating GH, possibly by suppressing GH release (13, 27, 28). This may affect the direct effects and/or paracrine influences of IGF-I on target tissues. Reduced circulating levels of GH and insulin may alter serum levels of IGF binding proteins (IGFBP), because GH and insulin normally inhibit IGFBP1 and GH increases IGFBP3 (6). On the other hand, IGF-I may improve absorption and food efficiency by increasing the size of jejunal villi (27).

The aim of the present study was to evaluate the short-term effects of IGF-I alone or in combination with GH on Dia structure and function in a well-nourished adolescent rat model, by using a protocol similar to the one used in our acute nutrition-deprivation study (20). We specifically wished to address whether IGF-I or GH exerts an anabolic effect on Dia muscle fiber morphometry, and, if so, whether the effects of IGF-I and GH differ from each other. Furthermore, because GH may offset some of the influences of IGF-I treatment (for example, attenuating the decline in plasma insulin concentrations (17)), and the changes in IGFBPs, we wanted to assess whether the combination of GH and IGF-I exerts additive or synergistic effects.

METHODS

Animal groups. Adolescent Sprague-Dawley rats were studied 1 wk after weaning (i.e., 4 wk of age). Their initial body weight was 95 ± 7 g. Four groups of animals were studied: 1) control (Ctr; n = 11); 2) rats administered GH (GH; n = 8); 3) rats administered IGF-I (IGF-I; n = 8); and 4) rats administered both GH and IGF-I (GH/IGF-I; n = 8). All animals were provided with food and water ad libitum [Purina Rat Chow (in %): 56 carbohydrate, 23 protein, 4.5 fat, 6 fiber, and 10.5 ash minerals]. The animals were housed in individual cages. The research protocol was reviewed and approved by the Animal Care and Use Committee of Cedars-Sinai Medical Center and the Burns and Allen Research Institute.

Growth factor administration. Recombinant human IGF-I and GH were used in these experiments. IGF-I was administered by continuous infusion via an implanted subcutaneous
osmotic minipump (model 2001, Alzet) that delivered 200 µg/day for a period of 72 h. GH was given twice daily by subcutaneous injections of 250 µg each for 72 h. In animals not receiving IGF-I, sham surgery was performed, and subcutaneous saline injections were administered to animals not receiving GH. The primary rationale for the dosing regimens used in the present study was to provide identical doses to those doses showing an anabolic effect in our model of acute nutritional deprivation (20). In addition, the dose of the IGF-I infusion was chosen to avoid any hypoglycemia, even with concomitant nutritional deprivation (1, 20). Similarly, the dose of GH was selected based on prior studies showing no serious side effects in rats and no concomitant hyperglycemia (1). The dose of GH utilized was similar to that used by Lanz et al. (18) over prolonged periods but still substantially greater than conventional therapeutic or experimental dosage regimens used in humans (0.03–0.5 mg·kg

greater than conventional therapeutic or experimental dos-

0.5 mg·kg

ThedoseofGHutilizedwassimilartothatusedbyLanz

nutritional deprivation (20). In addition, the dose of the IGF-I used in the present study was to provide identical doses to those receiving GH. The primary rationale for the dosing regimens was to establish, it was measured, and the fresh adjacent strip was mounted on cork at that L₀ and rapidly frozen. Serial cross sections of the Dia segments were cut at 10-µm thickness with the use of a cryostat (model 2800 C, Reichert-Jung) kept at −20°C.

Dia muscle fibers were classified based on difference in staining intensity for myofibrillar adenosinetriphosphatase (mATPase) after alkaline (pH 9.0) and acid (pH 4.3 and 4.55) preincubations (12). 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 of Guth and Samaha (12); see also Ref. 9]. These various staining procedures allow the classification of fibers into several types, i.e., types I, IIa, IIb, IIx, and IIc (9, 11). Proportions of fiber types were determined from a sample of 200–300 fibers from each muscle. In previous studies in both hamsters and rats, we verified Dia muscle fiber type immunohistochemically, with 95% or more correspondence between the mATPase-based classification and the major isofrom of myosin heavy chain (MHC) in single Dia fibers (9).

In vitro assessment of isometric contractile and fatigue properties of the Dia. The contractile and fatigue properties of the Dia in vitro were determined by using methods identical to those described in detail in our earlier studies (22, 30). Briefly, the entire Dia was rapidly excised after the induction of deep anesthesia (pentobarbital sodium: 6 mg/100 g body wt ip). A narrow 3- to 4-mm-wide strip of Dia was cut from the right midcostal region, maintaining fiber attachments to the ribs and central tendon intact. The segment of Dia 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), and the central tendon clamp was attached to a micromanipulator (Kopf). The Dia strip was directly stimulated by using 2-ms monophasic impulses at supramaximal intensity (Grass S88 stimulator). d-Tubocurare (12 µm) was added to the tissue bath to block neuromuscular transmission. 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).

Peak twitch force (P₀), contraction time (CT; time to P₀) and half relaxation time (RT₀; 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 per second (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 (P₀) were normalized for the estimated CSA of the muscle segment (CSA = muscle wt/1.056 × L₀, where 1.056 g/cm² represents the density of muscle) and expressed in newtons per square centimeter.

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

Dia muscle fibers were classified based on difference in staining intensity for myofibrillar adenosinetriphosphatase (mATPase) after alkaline (pH 9.0) and acid (pH 4.3 and 4.55) preincubations (12). 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 of Guth and Samaha (12); see also Ref. 9]. These various staining procedures allow the classification of fibers into several types, i.e., types I, IIa, IIb, IIx, and IIc (9, 11). Proportions of fiber types were determined from a sample of 200–300 fibers from each muscle. In previous studies in both hamsters and rats, we verified Dia muscle fiber type immunohistochemically, with 95% or more correspondence between the mATPase-based classification and the major isofrom of myosin heavy chain (MHC) in single Dia fibers (9).

Dia muscle fiber CSA was determined from microscopic images of digitized muscle sections by using a computer-based imaging processing system. The latter is composed of a Leitz Laborlux S (Leica) microscope, charge-coupled device video camera system (model VI-470; Optonics Engineering, Goleta, CA), high resolution Trinitron color video monitor (model PVM-1343MD; Sony, Japan), 486 DX/50-MHz PC with a Targa+ imaging board (Truvision), 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.

Histochemical procedures: Dia fiber oxidative capacity. The methodology employed to quantify succinate dehydrogenase (SDH) activity of individual Dia muscle fibers has been described in detail in previous reports (2, 3, 31, 32). Briefly, in the histochemical reaction for SDH, the progressive reduction of nitroblue tetrazolium (NBT) to an insoluble colored compound [a deformazan (dfz)] is used as a reaction indicator. The reduction of NBT is mediated by H⁺ released in the conversion of succinate to fumarate. In a series of 6-µm sections, the incubation medium contained a large quantity of succinate (60 mM); thus the SDH reaction was not substrate limited (3). In other sections, succinate was absent from the incubation medium so that the reduction of NBT in these sections was nonspecific (3). These sections are referred to as tissue blanks.

The concentration of NBT-dfz deposited within a muscle fiber was calculated by using the Beer-Lambert equation

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

where OD is the optical density of the muscle fiber measured at 570 nm (the peak absorbance wave length for NBT-dfz), K is the molar extinction coefficient for NBT-dfz (26,478 mol/cm·L), and L is the path length (6-µm section thickness) for light absorbance (3).

The OD of muscle fibers was determined by using a microdensitometric procedure implemented on the computer-based image-processing system. The video image was then digitized (8-bit gray level resolution) into a matrix of 1,024 × 1,024 pixels (picture elements). The gray levels of the video
scanner were calibrated for photometry (OD units) by using a series of neutral-density filters (0.004–2.00 OD units, Melles Griot). We have previously demonstrated that during the SDH reaction in the cat and rat, the formation of NBT-dfz in Dia fibers increases linearly over a period of at least 9 min (3, 30, 32). In reactions where 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 where 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 Dia 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 fumarate per liter tissue per minute (3, 30). Approximately 200–300 fibers were sampled from each Dia muscle.

Biochemical analysis. Serum total IGF-I concentrations were determined at Genentech by radioimmunoassay (23). Before radioimmunoassay, IGFBP were precipitated by incubation in acid-ethanol (7). The maximum intra- and interassay coefficients of variation for total IGF-I measurement (extraction procedure and radioimmunoassay) are 15 and 19%, respectively (23).

Statistical analysis. Before parametric analyses, the distribution of all data was tested for normality. Statistical analysis was then performed (Crunch software, Crunch-3, Oakland, CA) by using an analysis of variance (ANOVA), with the experimental factors being the administration of either IGF-I or GH. ANOVA with repeated measures was employed in comparing force-frequency relationships. If a significant interaction was found, 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 and Dia weights. The initial body weights of the animals were similar, with the mean weight of the cohort being 95 ± 7 g (Ctr, 93.2 ± 4.8 g; GH, 96.5 ± 8.0 g; IGF-I, 96.8 ± 7.4 g; GH/IGF-I, 94.4 ± 8.6 g). During the experimental period, the body weight of Ctr animals increased 21.7%. The body weight increment of all groups receiving GH, IGF-I, or the combination of GH and IGF-I was significantly greater than Ctr (P < 0.05). In addition, the increment in body weight of the animals receiving the combination of GH and IGF-I was significantly greater than in the groups receiving either GH or IGF-I alone (Fig. 1; P < 0.01), indicating a synergistic effect.

The weight of the costal Dia was significantly greater in all the groups receiving growth factors, compared with Ctr (Fig 1; P < 0.05). However, the Dia weights were similar between the GH, IGF-I, and GH/IGF-I groups. The ratio of costal Dia weight (mg) to body weight (g) was similar between all the groups (Ctr, 1.9 ± 0.2; GH, 2.0 ± 0.2; IGF-I, 2.0 ± 0.2; GH/IGF-I, 1.9 ± 0.1). Although this similarity suggests that the increments in both body and Dia weights in the animals receiving growth factors were proportional, a confounding variable may be the response of individual tissues and organs to the growth factors, either alone or in combination.

IGF-I levels. Total serum IGF-I levels were significantly increased in the groups receiving growth factors, compared with Ctr (P < 0.05; Table 1). As noted in Table 1, serum IGF-I levels in experimental groups ranged from 1.5 times Ctr level (i.e., GH group) to 2.5 times Ctr level (i.e., GH/IGF-I group).

In vitro Dia contractile and fatigue properties. Dia L_o was unaffected by GH, IGF-I, or a combination of GH and IGF-I (Table 2). Twitch characteristics (CT; RT_1/2) were similar across the groups (Table 2). P_1 and P_o were
Similarly increased in all groups receiving growth either IGF-I or GH alone. Costal Dia weight was unaffected by the provision of growth factors (Table 2), and the force-frequency relationships of the Dia (normalized for maximum force) were similar across all groups (Fig. 2). The fatigue index (FI) of the Dia was significantly increased in the GH/IGF group, compared with Ctr animals, indicating improved fatigue resistance of the Dia (Table 2; P < 0.05). However, no significant differences in FI were observed between the groups receiving growth factors (Table 2).

Histochemistry: fiber proportions and CSA. Dia fiber type proportions were similar among all the groups (Table 3). No significant differences among the groups were observed in the CSA of type I fibers (Fig. 3). However, the provision of IGF-I, GH, or the combination of IGF-I and GH resulted in a significant increment in the CSA of type IIa fibers, compared with Ctr (P < 0.05; CTA ~27-32% larger than Ctr; Fig. 3). In addition, the CSA of type IIx fibers in the IGF and GH/IGF groups were significantly greater than in Ctr animals (P < 0.05; CTA ~26% larger than Ctr; Fig. 3). There was also a tendency for type IIx fibers in the GH group to be larger than Ctr (P < 0.1; CTA ~19% larger than Ctr; Fig. 3). Thus the provision of growth factors had a significant impact on type II Dia fibers.

Histochemistry: fiber SDH activity. SDH activity was significantly greater in type IIa fibers of animals receiving the combination of GH and IGF-I compared with Ctr (P < 0.05; Fig. 4). No other differences were observed for SDH activity in the other fiber types among the various groups (Fig. 4).

**DISCUSSION**

This study demonstrated a significant increment in body weight in well-fed adolescent rats after short-term administration of IGF-I, GH, or the combination of IGF-I and GH, with a greater effect observed in the group receiving the two growth factors combined than either IGF-I or GH alone. Costal Dia weight was similarly increased in all groups receiving growth factors. Improved Dia fatigue resistance and increased SDH activities of type IIa fibers were observed in the GH/IGF-I group. Moreover, increased CSA of type II fibers (IIa; IIx) was found after growth factor treatment.

Body weights. Controversy exists concerning whether well-fed animals with an intact somatotropic axis exhibit an anabolic response to growth factors (e.g., IGF-I). For example, it has been argued, in studies showing a minimal or absent somatogenic response to IGF-I (6, 29, 34), that suppression of serum insulin and amino acid levels may occur with the use of IGF-I, which may enhance proteolysis and limit substrate uptake (6, 15, 26). Furthermore, IGF-I suppresses plasma levels of GH, decreases the GH response to growth hormone-releasing factor challenge, and down-regulates hepatic GH receptors (13, 27, 28). IGF-I may also modulate levels of IGFBP. For example, IGF-I infusion for 3 days resulted in increased levels of IGFBP1, an effect likely related to suppressed serum insulin levels (6, 19). IGFBP1 has the potential to inhibit the action of administered IGF-I (16).

In the present study, a positive impact on body weight was noted with the administration of growth factors, with greatest effect observed after administering the combination of GH and IGF-I. In keeping with our findings, recent studies both in animals (24) and in humans (17), with imposition of modest caloric restriction, reported that the addition of GH to IGF-I was significantly more anabolic than either agent alone. Possible mechanisms for this observation include blunt-

**Table 1. Serum IGF-I concentrations**

<table>
<thead>
<tr>
<th>Group</th>
<th>IGF-I, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>248 ± 128</td>
</tr>
<tr>
<td>GH</td>
<td>375 ± 42*</td>
</tr>
<tr>
<td>IGF-I</td>
<td>535 ± 67††</td>
</tr>
<tr>
<td>GH/IGF-I</td>
<td>627 ± 109††</td>
</tr>
</tbody>
</table>

Values are means ± SD. GH, growth hormone; IGF-I, insulin-related growth factor I. *Significantly different from control; †significantly different from GH. P value <0.05 was regarded as significant.

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

<table>
<thead>
<tr>
<th>Group</th>
<th>L₀, mm</th>
<th>CT, ms</th>
<th>RT₀, ms</th>
<th>P₀, N/cm²</th>
<th>Pₜ, N/cm²</th>
<th>FI, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14.7 ± 0.9</td>
<td>65 ± 8</td>
<td>87 ± 17</td>
<td>5.6 ± 0.7</td>
<td>15.8 ± 1.7</td>
<td>47 ± 6</td>
</tr>
<tr>
<td>GH</td>
<td>14.6 ± 1.2</td>
<td>75 ± 13</td>
<td>97 ± 21</td>
<td>5.1 ± 1.5</td>
<td>15.6 ± 2.5</td>
<td>50 ± 4</td>
</tr>
<tr>
<td>IGF-I</td>
<td>14.5 ± 1.3</td>
<td>64 ± 10</td>
<td>95 ± 25</td>
<td>6.0 ± 1.1</td>
<td>15.6 ± 2.5</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>GH/IGF-I</td>
<td>14.9 ± 0.9</td>
<td>65 ± 8</td>
<td>83 ± 19</td>
<td>6.1 ± 1.2</td>
<td>17.2 ± 2.4</td>
<td>54 ± 4*</td>
</tr>
</tbody>
</table>

Values are means ± SD. L₀, optimal length; CT, contraction time; RT₀, half relaxation time; P₀, peak twitch force; Pₜ, maximum tetanic force; FI, fatigue index. *Significantly different from control. P value <0.05 was regarded as significant.

**Table 3. Diaphragm fiber type proportions**

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Control</th>
<th>GH</th>
<th>IGF-I</th>
<th>GH/IGF-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>30.6 ± 1.7</td>
<td>32.6 ± 2.9</td>
<td>32.4 ± 4.4</td>
<td>30.9 ± 3.1</td>
</tr>
<tr>
<td>Type IIa</td>
<td>31.9 ± 2.6</td>
<td>30.3 ± 3.2</td>
<td>30.6 ± 4.3</td>
<td>30.4 ± 3.6</td>
</tr>
<tr>
<td>Type IIx</td>
<td>31.7 ± 3.1</td>
<td>33.8 ± 4.6</td>
<td>34.3 ± 4.2</td>
<td>35.2 ± 4.3</td>
</tr>
<tr>
<td>Type IIc</td>
<td>5.8 ± 3.8</td>
<td>4.1 ± 2.2</td>
<td>2.7 ± 0.7</td>
<td>3.5 ± 2.2</td>
</tr>
</tbody>
</table>

Values are means ± SD in percent.
Fig. 3. Cross-sectional areas (CSAs) of types I (A), IIa (B), IIx (C), and IIc (D) Dia muscle fibers. CSA of type IIa fibers was significantly greater in groups receiving growth factors compared with Ctr. In animals receiving IGF-I and GH/IGF-I, CSA of type IIx Dia fibers was significantly greater than in Ctr group. A similar tendency was also noted in GH group (P < 0.1) compared with Ctr. Values are means ± SD. *Significantly different from Ctr. P value < 0.05 was regarded as significant.

Fig. 4. Succinate dehydrogenase (SDH) activities of types I (A), IIa (B), IIx (C), and IIc (D) Dia muscle fibers. Values are means ± SD. *Significantly higher SDH activity in type IIa fibers of GH/IGF-I animals compared with Ctr. P value < 0.05 was regarded as significant.
ing the decline in serum insulin levels induced by IGF-I, with the coadministration of GH, and increasing the serum levels of IGFBP-3 and its acid-labile subunit (ALS). Both IGFBP3 and ALS are GH responsive and could promote a more stable pool of circulating IGF-I (35). In addition, GH itself could promote protein synthesis (25) systemically or locally by enhancing autocrine/paracrine effects on target extrahepatic tissues. IGF-I, on the other hand, may also improve the absorptive capacity of the jejunum, by increasing the size of jejunal villi and crypts (5), and increase protein synthesis (10, 15), thus contributing to positive protein turnover in this model.

It is not entirely clear why some studies (14, 33), including the present study, demonstrated a positive anabolic impact on body weight after growth factor administration, whereas other studies did not (6, 27, 29, 34). Differences in species, age, gender, size, and growth profile of the animals tested, as well as the duration of administration, may explain some of the variances. In rat studies, the dose of IGF-I also varied with regard to both daily dose (120–278 µg/day sc by infusion) and total dose administered (600–3,892 µg). No clear trend could be discerned among the variables listed above. In studies in rats, positive responses were noted in young growing animals (14), and a dose response was noted in female rats (33), but strong inferences regarding these observations cannot be made.

Dia morphometry and biochemistry. In the present study, a significant increment in the size of type IIa Dia fibers was observed after IGF-I, GH, or the combination IGF-I and GH treatment, with a similar impact on type IIx Dia fibers in the IGF-I and GH/IGF-I animals, whereas no effect was noted in type I fibers. The mechanisms underlying this selective effect are not presently known. Whether differences exist in receptor density, sensitivity to ligand binding, or levels of IGFBP within muscle fibers [e.g., IGFBP4, which may modulate the effects of IGF-I within muscle fibers (16, 35)] is speculative and needs to be explored further. It is of interest, however, that Lanz and co-workers (18) also reported an apparent preferential effect of GH on Dia myofibers containing fast MHC isoforms in a refeeding model in the adult rat. In their study, after a period of malnutrition, the CSA of Dia fibers containing MHC 2B and MHC 2X isoforms were still reduced compared with Ctr animals, despite either 5 or 9 wk of refeeding (18). The provision of GH, in addition to food ad libitum, for 5 wk resulted in a return of the fast fiber CSA to Ctr values (18).

Despite our finding of a significant effect of either GH or IGF-I alone on both costal Dia weight and type II Dia muscle fiber CSA, we did not observe a synergistic impact on either Dia weight or Dia muscle CSA with the combination of GH and IGF-I, as was observed with the change in body weight over the experimental period in the GH/IGF-I animals. As body weight encompasses both carcass and organ weights, it is possible that a greater effect was observed in some organs with the combination of GH and IGF-I than with either agent alone. This may be accounted for in part by the fact that both IGF-I and GH exhibit some degree of organ selectivity, for example, greater splenic sensitivity with IGF-I (6). It is unclear, however, why a synergistic effect was not observed in the Dia of GH/IGF-I animals. Perhaps dosage regimens producing higher serum IGF-I levels or a longer duration of administration would have yielded a greater effect with the combination of growth factors. Biochemically, however, improved oxidative capacity of type IIa Dia fibers was observed in the GH/IGF-I animals but not in the animals receiving GH or IGF-I alone.

Dia contractile and fatigue properties. The use of GH, IGF-I, or the combination of GH and IGF-I had no impact on L_c, twitch characteristics, specific force, or force-frequency relationships of the Dia. The preservation of specific force can in part be explained by no alteration in the estimated relative contribution of the various fiber types to total costal Dia area in the groups receiving growth factors, compared with the Ctr animals. However, because of a greater myofibrillar mass with preserved specific force, the total force-generating capacity of the costal Dia would be expected to increase in the animals receiving growth factors.

Dia fatigue resistance was improved in the group receiving the combination of GH and IGF-I. This may in part be related to the increase in SDH activity in type IIa Dia fibers. Although a significant correlation exists between fatigue resistance and SDH activity in motor unit studies (8), the contribution of SDH activity to fatigue resistance in whole muscle preparations cannot be quantified, as a variety of factors may interact to influence muscle fatigability. These include alterations in the relative contribution of various fiber types to total muscle CSA, reduced creatine phosphate, changes in calcium flux, mATPase activity, capillarity, substrate and electrolyte content, and alterations in redox state. The improved Dia fatigue resistance, together with the likely increment in the total Dia force-generating capacity in the GH/IGF-I animals, would be expected to enhance the endurance capacity of the Dia.

In summary, these studies demonstrate that GH and IGF-I may exhibit an anabolic effect in well-nourished adolescent rats. A synergistic effect on body weight gain was observed with the combination of GH and IGF-I compared with the administration of either agent alone. GH, IGF-I, or the combination of GH and IGF-I had a positive but similar impact on both Dia weight and the CSA of predominantly type II Dia fibers (IIa; IIx). No synergistic effect was noted with the combined use of GH and IGF-I, apart from higher SDH activity in type IIa fibers. Although the results of this study are somewhat provocative, the potential implications for use of these growth factors, either alone or in combination, in conditions of short stature or growth retardation secondary to chronic disease in children will require further investigation in both animal and human studies (4).

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