Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders

MARIA A. FIATARONE SINGH,1–3 WENJING DING,1,4 THOMAS J. MANFREDI,5 GUIDO S. SOLARES,1 EVELYN F. O’NEILL,2 KAREN M. CLEMENTS,3 NANCY D. RYAN,2 J OSEPH J. KEHAYIAS,1 ROGER A. FIELDING,1,4 AND WILLIAM J. EVANS6

1Nutrition, Exercise Physiology, and Sarcopenia Laboratory, Jean Mayer United States Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston 02111; 2Hebrew Rehabilitation Center for Aged, Boston 02131; 3Department of Health Sciences, Sargent College of Health and Rehabilitation Sciences, Boston University, Boston, Massachusetts 02215; 4Exercise Science Laboratory, University of Rhode Island, Kingston, Rhode Island 02906; 6Nutrition, Exercise, and Metabolism Division, Donald W. Reynolds Department of Geriatrics and Geriatric Research, Education, and Clinical Center, University of Arkansas for Medical Sciences, Veterans Affairs Medical Center, Little Rock, Arkansas 72114; and 3School of Exercise and Sport Science, University of Sydney, Lidcombe, New South Wales 1825, Australia

Fiatarone Singh, Maria A., Wenjing Ding, Thomas J. Manfredi, Guido S. Solares, Evelyn F. O’Neill, Karen M. Clements, Nancy D. Ryan, Joseph J. Kehayias, Roger A. Fielding, and William J. Evans. Insulin-like growth factor I in skeletal muscle after weight-lifting exercise in frail elders. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E135–E143, 1999.—To assess muscle remodeling and functional adaptation to exercise and diet interventions, 26 men and women aged 72–98 yr underwent a vastus lateralis biopsy before and after placebo control condition, and progressive resistance training, multinutrient supplementation, or both. Type II atrophy, Z band, and myofibril damage were present at baseline. Combined weight lifting and nutritional supplementation increased strength by 257 ± 62% (P = 0.0001) and type II fiber area by 10.1 ± 9.0% (P = 0.033), with a similar trend for type I fiber area (+12.8 ± 22.2%). Exercise was associated with a 2.5-fold increase in neonatal myosin staining (P = 0.0009) and an increase of 491 ± 137% (P < 0.0001) in IGF-I staining. Ultrastructural damage increased by 141 ± 59% after exercise training (P = 0.034), strength increases were largest in those with the greatest increases in myosin, IGF-I, damage, and caloric intake during the trial. Age-related sarcopenia appears largely confined to type II muscle fibers. Frail elders respond robustly to resistance training with musculoskeletal remodeling, and significant increases in muscle area are possible with resistance training in combination with adequate energy intakes.

Methods
Subjects

A subset of 26 subjects (13 men and 13 women) who were eligible and willing to undergo the muscle biopsy procedures were recruited from among 100 subjects participating in a larger randomized controlled trial (the Boston FICSIT study; see Ref. 17) that was conducted at the Hebrew Rehabilitation Center for the Aged (HRCA) and the Jean Mayer Human Nutrition Research Center on Aging at Tufts University. Selection criteria for the subjects to undergo the muscle biopsy procedures additionally included the following: level of cognition was sufficient to consent to undergo a biopsy; no heparin or warfarin therapy; and no primary muscle disease or myopathy. The study was approved by the Human Investigation Review Committees at New England Medical Center and HRCA. Written informed consent was obtained from each subject.

Study Design

The randomization and study interventions have been described in detail previously (17). Briefly, 100 nursing home residents, 63 females and 37 males of mean age 87.0 ± 0.6 yr (range 72–98), were randomized in a factorial design into one of four treatment groups: a 10-wk course of either lower

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extremity resistance training or nutritional supplementation, both active interventions, or a placebo-controlled condition. Resistance training consisted of three sets of eight repetitions at 80% of the most recently determined one-repetition maximum (1-RM) for the hip and knee extensor muscles, 3 days/wk for 10 wk, using pneumatic resistance training equipment (Keiser Sports Health, Fresno, CA). The nutritional supplement (Exceed; Ross Laboratories, Columbus, OH) was a 240-mL liquid administered daily supplying 360 kcal in a 60% carbohydrate, 23% fat, 17% soy-based protein formula including one-third of the recommended daily allowance of essential vitamins and minerals. A nonnutritive placebo liquid (Crystal Light; Kraft General Foods, White Plains, NY) and nonresistive recreational activities were offered to the exercise and nutrition control subjects.

Clinical Characteristics

Methods for assessment of health status, functional level, cognition, depressive symptoms, physical activity levels, and nutritional intake have been published previously (17). Three-day food weighing was used to analyze nutrient intakes. The average of 72-h counts of movement of three degrees or more in any direction were obtained from mercury accelerometers worn around both ankles during the period of dietary assessment (large scale integrated activity monitors).

Muscle Function

Muscle strength was measured in hip and knee extensors of both legs individually and was expressed in kilograms as the 1-RM for each muscle group (17). The right and left leg hip and knee extensor 1-RM measurements were added together to give a summary “strength” score for use in ANOVA and regression models. Lower extremity power was estimated from maximal chair-rise time (1).

Body Composition

Regional thigh muscle area was measured using blinded digital analysis of computerized tomography images using a Siemens DR3 (Somatom-Siemens, Erlangen, Germany) or Sytec 4000 (General Electric, Milwaukee, WI) scanner in the supine position at the nondominant midthigh. Total body water (liters) was estimated from bioelectric impedance (RJL Systems, Clinton, MI) measurements of resistance and reactance. Whole body potassium was measured as an index of body cell mass (7) in a K-40 counter calibrated daily. The coefficient of variation (CV) of weekly anthropomorphic phantom measurements was 5%.

Muscle Biopsy Procedure

A needle biopsy of the nondominant vastus lateralis was obtained at baseline and 4–6 days after the last exercise session under local anesthesia (1% xylocaine hydrochloride) using a 5-mm duchenne needle with applied suction (15). The sample obtained from each biopsy was divided into three pieces. The first piece was quick-frozen in liquid nitrogen. The second piece was oriented longitudinally, mounted in embedding medium (optimum cutting temperature compound [OCT]; Miles Laboratories, Naperville, IL), and frozen in isopentane cooled to the temperature of liquid nitrogen. Within 5 min after the biopsy material was obtained, the third piece was finely minced and fixed in 0.1 M cacodylate-buffered glutaraldehyde and paraformaldehyde for 3 h at 4°C, rinsed with 0.1 M sodium cacodylate buffer at least 3 × 5 min at room temperature (RT), and postfixed with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at 4°C.

After postfixation, the samples were rinsed with cacodylate buffer, dehydrated sequentially with methanol (30, 50, 70, 80, 90, and 100%), and infiltrated with propylene oxide. The samples were then embedded in LR white (medium). Semithin sections (1 μm) were cut using an Ultramicrotome (LKB, Bromma, Sweden) and stained with toluidine blue. Samples were dehydrated sequentially in graded methanol alcohol (30, 50, 70, 80, 90, and 100%) 15 min for each concentration at RT. Thin sections were cut and stained with uranyl acetate and lead citrate and examined with a Zeiss EM-10CA electron microscope (Carl Zeiss, Thornwood, NY). Stereological measurements of volume densities of sarcoplasmic space, Z bands, damaged Z bands, and myofibril damage were made with a 100-point isotropic semicircular test system, as described previously (18). Intersection counts were obtained with the test lines oriented at an angle of 19° to the direction of the longitudinal axis of the myofibrils. The ratio of damaged to total Z bands was determined for each time point along with the analysis of focal damage. Myofibril damage was defined as an area showing absent or disorganized myofilaments not associated with the Z band.

Muscle Histochemistry

The OCT-mounted samples were sectioned (8 μm) in a cryostat and stained for myofibrillar ATPase activity at a preincubation pH of 4.3 and 4.6 (31). Fiber type distribution and fiber areas were determined using a computer-operated image analysis system [image 1.39 from W. Rasband, National Institutes of Health (NIH)], as modified for our applications (Muscle Fiber Image from G. Solares), to threshold the image, trace the fiber boundaries, identify and count the light and dark fibers, and measure the cross-sectional areas of all the fibers. An average of 437 fibers per subject were measured for each time point. The mean CV for fiber measurements using this technique in our laboratory is 0.57% for type I fibers and 0.68% for type II fibers in elderly subjects.

Immunohistochemistry

Embryonic (eMHC) and neonatal (nMHC) myosin heavy chain antibodies (Vector Laboratories, Burlingame, CA) were used to identify the presence of developmental myosin in myoblasts and mature muscle fibers. IGF-I antibodies (Chemicon International, Temecula, CA) were used to examine changes in the presence of this growth factor in response to the interventions. For immunohistochemistry, transverse sections (8 μm) were mounted on glass slides (Superfrost Plus) and incubated for 1 h at 37°C in primary antibody (IGF-I diluted 1:50 in PBS; eMHC diluted 1:20 in PBS; nMHC diluted 1:20 in PBS). Sections were subsequently rinsed in PBS (3 × 10 min) and incubated in secondary antibody. Sections were rinsed in PBS (3 × 10 min) and mounted on coverslips. The area positively stained by the above immunofluorescent technique for eMHC, nMHC, or IGF-I was quantified from digitized images of micrographs using NIH Image version 1.39 and was expressed as a percentage of the total cross-sectional fiber area examined.

Statistical Analysis

Data were analyzed using the SuperANOVA or Statview software packages (Abacus Concepts, Berkeley, CA). All values are reported as means ± SE. Baseline differences between groups were assessed by unpaired t-tests or chi square analysis as appropriate. Linear regression was used to determine the appropriateness of subsequent ANOVAs and analy-
Table 1. Baseline characteristics of subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exercise</th>
<th>Supplement</th>
<th>Exercise and Supplement</th>
<th>Control</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>83.5 ± 2.7</td>
<td>87.7 ± 1.8</td>
<td>84.0 ± 1.2</td>
<td>91.0 ± 1.8*</td>
<td>0.04</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>2/4</td>
<td>2/5</td>
<td>2/5</td>
<td>1/5</td>
<td>0.19</td>
</tr>
<tr>
<td>Functional status score (0–6)</td>
<td>1.3 ± 0.5</td>
<td>1.1 ± 0.3</td>
<td>2.4 ± 0.6</td>
<td>1.3 ± 0.2</td>
<td>0.18</td>
</tr>
<tr>
<td>Medical diagnoses</td>
<td>5.2 ± 0.9</td>
<td>5.0 ± 1.0</td>
<td>5.0 ± 0.6</td>
<td>3.3 ± 0.8</td>
<td>0.41</td>
</tr>
<tr>
<td>Depression score (0–30)</td>
<td>6.5 ± 2.3</td>
<td>12.1 ± 2.8</td>
<td>7.4 ± 2.4</td>
<td>9.8 ± 2.2</td>
<td>0.38</td>
</tr>
<tr>
<td>Habitual activity level, counts/day</td>
<td>23,988 ± 3,511</td>
<td>10,666 ± 2,686</td>
<td>17,424 ± 8,666</td>
<td>18,460 ± 2,701</td>
<td>0.33</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.9 ± 1.5</td>
<td>24.4 ± 1.2</td>
<td>26.4 ± 1.1</td>
<td>25.2 ± 1.5</td>
<td>0.74</td>
</tr>
<tr>
<td>Dietary energy intake, kcal/day</td>
<td>1,479 ± 69</td>
<td>1,646 ± 97</td>
<td>1,634 ± 102</td>
<td>1,449 ± 24</td>
<td>0.24</td>
</tr>
<tr>
<td>Dietary protein intake, g·kg⁻¹·day⁻¹</td>
<td>1.7 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.93</td>
</tr>
<tr>
<td>Lower body strength, kg</td>
<td>34.4 ± 4.5</td>
<td>30.5 ± 6.2</td>
<td>23.1 ± 3.4</td>
<td>30.5 ± 3.6</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. M, males; F, females. Functional status score determined using the Katz activities of daily living (ADL) index where 0 = independent in all ADLs and 6 = dependent in all ADLs (23). Depression score determined using the geriatric depression scale where 0 = no depressive symptoms and >9 is consistent with clinical diagnosis of depression (38). P value refers to baseline differences between groups in an ANOVA model in all cases. *Control group significantly different from the other 3 groups (P < 0.01).

Fig. 1. Strength changes in the four intervention groups. Strength was measured as the combined 1-repetition maximum (1-RM) of bilateral hip and knee extensor muscle groups after 10 wk of intervention. *Exercise group was significantly different from the exercise and supplement (Ex and Supp) group (P = 0.0091) and the supplement (Supp) group (P = 0.05). **Exercise and supplement group was significantly different from the exercise group (P = 0.0091), the supplement group (P = 0.0001), and the control group (P = 0.0002).
The observed changes in strength were greatest in those with the fewest depressive symptoms at baseline ($r = 0.511, P = 0.015$) and the greatest increases in energy intake ($r = 0.602, P = 0.003$) during the trial. The level of depression was unrelated to compliance with the exercise or placebo sessions. In a stepwise multiple-regression model including age, depression score, and changes in energy intake, 45% of the variance in strength gain was predicted by independent contributions of baseline depression and change in energy intake alone ($r = 0.671, P = 0.0034$).

Muscle Fiber Type Distribution and Areas

Baseline. There were no significant differences between groups in fiber type ratio or areas. Type II (fast-twitch) fibers comprised $57.0 \pm 3.7\%$ of the fibers counted in these subjects, and abnormal fiber type grouping (areas predominated by one fiber type rather than the normal variegated pattern) and small, angular fibers were often observed in specimens. The type II-to-type I ratio was greater with higher baseline energy intake ($r = 0.519, P = 0.0189$).

Mean type I fiber cross-sectional area was $3,603 \pm 244\, \mu m^2$, comparable to five young controls (mean age $27.0 \pm 2.1\, yr$) whose mean type I fiber area was $3,379 \pm 462\, (P = 0.61)$. Type II fibers were often small and irregular in appearance under light microscopy, with a mean fiber area of $2,229 \pm 146\, \mu m^2$, which is $\sim 60\%$ of the area of young controls ($3,857 \pm 565\, (P = 0.0003$).

Clinical features related to baseline fiber areas are shown in Table 2. In a model including gender, age, leg power, and activity level, age was the only independent predictor of type II fiber area ($P = 0.0189$).

Regional and whole body measures of lean tissue [midthigh muscle area by computed tomography (CT) scan, whole body potassium, and total body water] were all highly correlated with type II fiber area but unrelated to type I fiber area, as seen in Fig. 2, A and B, and Table 2.

Intervention effects. In response to the intervention, type II fiber area increased significantly in the combined exercise-supplement group (10.1 \pm 9.0\%), with no change or decreases in the other treatment groups, as shown in Table 3 ($P = 0.033$). This increase in type II fiber area was associated with higher baseline energy intake ($r = 0.642, P = 0.0055$). No significant effect of the intervention was seen on type I fiber area, although similar trends as with type II fibers were seen, and the changes in type I and II fiber area were directly correlated ($r = 0.555, P = 0.0208$).

Muscle Damage

Baseline. At baseline, there was extensive evidence of Z band and myofibril disruption (see Table 4). Approxi-
and the baseline value of the relevant variable. All analyses were done as ANCOVA models of exercise and supplement main effects and interaction terms, adjusted for age, gender, for clarity of data presentation and effect size, as the nutritional supplement use had no independent or interactive effect on any of the above variables. All analyses were done as ANCOVA models of exercise and supplement main effects and interaction terms, adjusted for age, gender, and the baseline value of the relevant variable. P value refers to the main effect of exercise on the adjusted percent change scores in all cases.

IGF-I. BASELINE. At baseline, 1–17% of the analyzed muscle fiber area was positively stained for IGF-I, averaging 6.7 ± 1.3%, as shown in Table 4. Higher baseline presence of IGF-I was predictive of increases...
INTERVENTION EFFECTS. Exercise training resulted in a 500% increase in IGF-I staining, as shown in Table 4 and Fig. 5, with no significant effects of the nutritional supplement. Positive IGF-I staining was observed in mature muscle fibers and smaller fibers consistent with myogenic precursor cells. IGF-I staining after strength training was localized to both pericellular and intracellular regions of transverse sections (see Fig. 6, A and B). The increase in IGF-I was greatest in subjects with fewer depressive symptoms at baseline ($r = -0.708, P = 0.0217$). IGF-I increases closely paralleled increases in both damage ($r = 0.855, P = 0.0301$) and developmental myosin ($r = 0.901, P = 0.0009$).

DISCUSSION

This study provides the first immunohistochemical and ultrastructural evidence of skeletal muscle remodeling in response to resistance training in frail individuals of extreme old age. We have demonstrated that, in individuals with a high burden of chronic disease, skeletal muscle is characterized by a preservation of type I fiber area, severe selective type II fiber atrophy, widened sarcoplasmic spaces, and Z band and myofibrillar disruption. In addition, we find that female gender, low physical activity level, undernutrition, and depression are linked to sarcopenia, extending findings that have been seen in other studies (16). This would suggest that interventions aimed specifically at stimulating type II fibers (e.g., contractions demanding high force output) may be most effective in the prevention and treatment of sarcopenia. Although hypertrophy of similar magnitude was seen in type I fibers in the combined exercise and supplement group, the response was more variable and not statistically significant in this sample.

The fact that muscle hypertrophy was linked to higher caloric intake at baseline and was only significant in those who received nutritional supplementation and exercise suggests that adequate energy balance is critical to treatment of sarcopenia with exercise in frail elders. It is notable in this regard that, in results previously published from this trial (17), we showed
that subjects who received supplementation without exercise suppressed their habitual dietary intake so that, despite compliance with the study supplement, they had no significant net gain in calorie intake. Thus nutritional status appears to be important for muscle hypertrophy, but altering it in frail elders may require attention to physical activity levels, not simply access to additional food.

Adaptation at both the physiological and functional levels was blunted in individuals with lower calorie intakes before and during the trial, as well as in those with more depressive symptoms at baseline. These effects were independent of each other and require further investigation. These clinical factors, in addition to age, may contribute to the heterogeneous hypertrophic response to resistance training seen in previous trials in elderly populations. Most of the previous studies of resistance training in the elderly (3, 5, 19, 25, 33) demonstrate muscle fiber hypertrophy in response to exercise, although the responses are variable (27), and two report no significant hypertrophy (1, 4). When these studies are examined in relation to subject age, there is a significant inverse relationship between type II fiber hypertrophy within the vastus lateralis and mean age of the study group, as shown in Fig. 7, suggesting age and/or age-related disease may moderate the degree of cellular adaptation to loading.

The baseline atrophy of type II fibers in our study was accompanied by levels of ultrastructural damage that have not been seen in healthy subjects at younger ages (12). The etiology of this damage is unknown, but alterations such as the age-related impairment in the ATP-dependent ubiquitin pathway regulating protein degradation in muscle may contribute (8). Our resistance training regimen, which included eccentric contractions as the weight was lowered, markedly increased the baseline damage. Whereas acute experimental damage is accompanied by edema, neutrophil and cytokine localization in muscle tissue (18), and has been shown to be associated with delayed-onset muscle soreness and reduced force-generating capacity (29, 30), the long-term adaptation to the milder eccentric component during resistance training in this study was, by contrast, characterized by damage associated with large gains in strength. This suggests that not all damage is alike and, when accompanied by regenerative processes, such as shown here, may in fact be a positive adaptation to stress. Whether such damage is essential to strength gains or whether it occurs in concentric-only weight lifting is unknown at this time.

Our biopsy results suggest that the early adaptation to progressive resistance training includes such muscle damage as a step in a remodeling process that ultimately leads to muscle regeneration. We have demon-
activation, increased protein synthesis, decreased pro-
ing IGF-I, are known to be mediators of satellite cell
regeneration after injury (22). Growth factors, includ-
not in mature muscle cells unless they were undergoing
activity has been seen in myogenic precursor cells but
muscle growth (26). In animal models, IGF-I immunore-
xists as an autocrine growth factor regulating skeletal
skeletal muscle tissue after progressive resistance train-
a substantial increase in the presence of IGF-I in
"injury" during resistive exercise may be the stimulus
for regeneration of myofibrils, via liberation of satellite
cells from between the basal lamina and the sarco-
lemma, which could serve as the source of developmen-
tal myosin. The fact that strength gains were highest in
those with the greatest damage and regenerative adap-
tations suggests that these adaptations are in fact part of
the cellular mechanism underlying improvements in
muscle function.

In conclusion, we have shown for the first time in
human skeletal muscle simultaneous appearance of
ultrastructural damage and developmental myosin and
IGF-I immunoreactivity in response to resistance train-
ing. Compared with some previous training studies, the
extent of hypertrophy induced may have been attenu-
ated by the presence of factors that we have now
identified to be linked to muscle fiber atrophy in this
population (very advanced age, inactivity, depressive
symptoms, and nutritional status), but functional gains
were nonetheless substantial. Age-related sarcopenia
clearly has modifiable contributants, and skeletal
muscle retains remarkable plasticity in individuals as
old as 98 yr of age. Future preventive and rehabilitative
strategies for age-related sarcopenia should be de-
signed to maximally exploit this potential.

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Address for reprint requests and other correspondence: M. A.
Fiatarone Singh, Nutrition, Exercise Physiology and Sarcopenia
Laboratory, Jean Mayer USDA Human Nutrition Research Center on
E143

IGF-I and DEVELOPMENTAL MYOSIN IN SKELETAL MUSCLE

Aging, Tufts University, 711 Washington St., Boston, MA 02111
(E-mail: m.singh@chhs.syd.edu.au)

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