Overexpression of IGF-1 Exclusively in Skeletal Muscle Prevents Age-related Decline in the Number of Dihydropyridine Receptors*

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Excitation-contraction uncoupling has been identified as a mechanism underlying skeletal muscle weakness in aging mammals (sarcopenia). The basic mechanism for excitation-contraction uncoupling is a larger number of ryanodine receptors (RyR1) uncoupled to dihydropyridine receptors (DHPRs) (Delbono, O., O’Rourke, K. S., and Ettinger, W. H. (1995) J. Membr. Biol. 148, 211–222). In the present study, we used transgenic mice overexpressing human insulin-like growth factor-1 exclusively in skeletal muscle to test the hypothesis that a high concentration of IGF-1 prevents age-related decreases in DHPR number and in muscle force. Transgenic mice express 10–20-fold higher IGF-1 concentrations than nontransgenic mice at all ages (1–24 months). The number of DHPRs is 50–100% higher, and the DHPR/RyR1 ratio is 40% higher in transgenic soleus (predominantly type I fiber muscles), extensor digitorum longus (predominantly type II fiber muscles), and the pool of type I and type II fiber muscles than in nontransgenic young (6 months), adult (12 months), and old (24 months) mice. Furthermore, no age-related changes in DHPRs and the DHPR/RyR1 ratio were observed in transgenic muscles. The specific single twitch and tetanic muscle force in old transgenic soleus and extensor digitorum longus muscles are 50% higher than in old nontransgenic muscles. Taken together, these results support the concept that IGF-1-dependent prevention of age-related decline in DHPR expression is associated with stronger muscle contraction in older transgenic mice.

Insulin-like growth factor (IGF-1) is a trophic factor required for the proliferation of myoblasts, the proliferation of myogen differentiation, and subsequent growth and hypertrophy of myofibers (1). IGF-1 has been identified as a potent regulator of gene expression in skeletal muscle. Age-related decreases in plasma IGF-1 concentration is well established (2), which may contribute to the decrease in muscle size and strength in the elderly. In addition to effects on muscle development, IGF-1 facilitates skeletal muscle DHPR activity via tyrosine kinase-protein kinase C-dependent phosphorylation (3). Our laboratory has also shown that IGF-1-dependent DHPR modulation is impaired in aging skeletal muscles (4), which may explain, at least partially, the decline in muscle force with aging (5).

Specific muscle strength declines with aging in humans and in several animal models of aging (6–8). The impairment in sustaining the contraction tension during prolonged muscle activation seems to be a general phenomenon in aging mammals. It also becomes apparent from a series of studies on in vitro contractility that the decrease in muscle mass does not explain entirely the age-related decrease in skeletal muscle force (8, 9). Therefore, some other mechanisms are involved in age-dependent loss in muscle strength. Studies done in our laboratory in single human skeletal muscle fiber support the theory that sarcolemmal excitation-sarcoplasmic reticulum Ca²⁺ release uncoupling (EC uncoupling) is a basic mechanism underlying the reported age-related decline in specific skeletal muscle tension (10). EC uncoupling results from alterations in the number of dihydropyridine receptors (DHPRs) linked to the Ca²⁺ release channel/ryanodine receptor (RyR1). The DHPR, a voltage-gated L-type Ca²⁺ channel, expressed in the tubular sarcotrigem, and the RyR1, expressed in the terminal cisternae of the sarcoplasmic reticulum, are key molecules involved in skeletal muscle excitation-contraction coupling (11). The DHPR undergoes conformational changes that produce intramembrane charge movements. A hypothetical physical interaction between DHPR and RyR1 at the triadic junction leads to sarcoplasmic reticulum Ca²⁺ release into the myoplasm (12, 13). Ca²⁺ binding to contractile proteins initiates muscle contraction (14). We have reported that the molecular basis for EC uncoupling in Fisher 344 Brown Norway cross rats is a decrease in DHPR expression in fast- and slow-twitch muscles (15). The age-related decrease in DHPR expression leads to a significant impairment in the sarcolemmal action potential transduction into a Ca²⁺ signal and consequently in a diminished mechanical response.

There has been a considerable interest in the use of exogenous IGF-1 to restore age-related losses in muscle mass and strength (16) because a number of studies in vitro have established that IGF-1 elicits pleiotropic effects on myogenic cells, including stimulation of myoblast replication and myogen differentiation (17). However, overexpression of IGF-1 in skeletal muscle with increased paracrine/autocrine secretion was more effective as a myogenic stimulus than exogenous IGF-1 (18). The expression of the α-skeletal actin/human IGF-1 hybrid gene induced overexpression of IGF-1 in FVB mice (18). In the present work, we used this animal model to test the hypothesis that high concentration of IGF-1 in skeletal muscle prevents age-related decreases in DHPRs and consequently in

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¶ The abbreviations used are: IGF, insulin-like growth factor; hIGF, human IGF; DHPR, dihydropyridine receptor; EC, excitation-contraction; EDL, extensor digitorum longus; Pipes, 1,4-piperazinediethanesulfonic acid.
muscle force. Human IGF-1 (hIGF-1) transgenic S1S2 mice showed a 10–20-fold increase in IGF-1 concentration and 50–100% increases in the number of DHPRs, whereas no significant change in RyR1 was detected. The prevention of age-related decline in DHPR expression in animals overexpressing IGF-1 was associated with a potentiation of muscle contraction in old mice. These results support the concept that the increase in autocrine/paracrine secretion of hIGF-1 exerts potent stimulatory effects on DHPR expression in adult skeletal muscle, improving its mechanical performance.

**EXPERIMENTAL PROCEDURES**

**S1S2 hIGF-1 Transgenic and FVB Nontransgenic Mice—** S1S2 transgenic mice overexpressing hIGF-1 exclusively in skeletal muscle and control wild-type FVB mice were used (18, 19). Dr. R. Schwartz (Department of Cell Biology, Baylor College of Medicine) provided the founder mice of the colony. Animals were housed in a pathogen-free area at Wake Forest University School of Medicine and fed ad libitum. Animal procedures followed an approved protocol by the Animal Care and Use Committee, Wake Forest University School of Medicine. FVB and S1S2 transgenic mice were killed by cervical dislocation.

**Determination of IGF-1 Concentration by Radioimmunoassay—** IGF-1 concentration from frozen quadriceps muscles of S1S2 transgenic and FVB nontransgenic mice was determined as described previously (19). Briefly, frozen quadriceps muscles were powdered under liquid N2 and then homogenized (1.5 v/v) in 0.1 M acetic acid using a Polytron tissue homogenizer. Homogenate was incubated for 2 h to separate the debris from the supernatant. Homogenate equivalent to 100 μg of protein was added to the reaction mixture containing 50 mM phosphate, pH 7.5, 50 mM NaCl, 10 mM EDTA, 3 mM sodium azide, and 0.25% bovine serum albumin. IGF-1 antiserum was a kind gift from NIDDK, National Institutes of Health. The concentration of antiserum required for the maximum detection of IGF-1 was determined from IGF-1-antiserum titration. IGF-1 antiserum was specifically withheld in some reaction tubes to determine nonspecific binding of radiolabeled IGF-1. The extent of hIGF-1 gene expression was determined using the lactoperoxidase and glucose oxidase procedure (20). The optimal counts needed for maximal IGF-1 detection were determined by titrating radiolabeled IGF-1 against known IGF-1 concentration. To initiate the reaction, 50,000 counts per tube were added and incubated overnight at room temperature. IGF-1-antiserum complex was precipitated by protein A for 30 min, and centrifuging at 3000 × g for 15 min pellets were precipitated. Radiolabeled IGF-1 in the pellet was estimated in a gamma counter. Data were expressed per mg of protein in relation to IGF-1 standards. The minimum and maximum detectable doses were 15 and 1000 pg of IGF-1.

**Muscle Homogenate and Radioligand Binding to DHPRs and RyR1s—** Extensor digitorum longus (EDL), soleus, and whole leg muscles were homogenized as described (19). Protein concentration was determined by Coomassie protein assay with bovine serum albumin as the protein calibration standard. DHPR and RyR1 concentrations were determined using the radioligands [3H]PN200–110 and [3H]ryanodine, respectively. Homogenates (1–2 mg/ml protein) were incubated either with 0.05–5 nM [3H]PN200–110 for 1 h at 23°C in 50 mM Tris-HCl, pH 7.5, 10 mM Ca2+, 1 mM diisopropyl fluorophosphate, and 5 μM leupeptin or 0.5–50 nM [3H]Ryanodine for 24–48 h at 10°C in 20 mM Pipes-NaOH, pH 7.0, 1 mM NaCl, 100 μM Ca2+, 5 mM AMP, 1 mM diisopropyl fluorophosphate, and 5 μM leupeptin. Membrane bound [3H]PN200–110 and [3H]Ryanodine were determined by filtration through Whatman GF/B filters using a Millipore unit (XX2702550, Millipore Corp., Bedford, MA). Filters were rinsed three times with 5 ml of ice-cold 200 mM choline chloride, 20 mM Tris-HCl, pH 7.5. Nonspecific [3H]PN200–110 and [3H]ryanodine binding was assessed in the presence of 10 μM unlabeled nifedipine (Sigma) or PN200–110 (Sandoz Pharmaceutical, East Hanover, NJ) and 10 μM unlabeled ryanodine (Calbiochem, San Diego, CA), respectively. Radioligand concentrations used resulted in occupancy of >95% of the high affinity binding sites (21). Linear regression and nonlinear least squares analysis were used to calculate nonspecific and total binding of the radioligands to the receptors. Specific binding of [3H]PN200–110 and [3H]ryanodine binding was assessed in the presence of 10 μM unlabeled nifedipine at each concentration was calculated by subtracting the nonspecific binding from the total binding obtained from the above analysis. The following equation,

\[ y = (ax + b)x + c \]

(Eq. 1)

where \( a \) = receptor number (\( B_{\text{max}} \)), \( b = K_d \) (dissociation constant), and \( c \) = the nonspecific binding or the low affinity site, was used to fit the binding isotherm. Data were also given in a graphical representation of the Scatchard plot. All values were analyzed for statistical significance using the unpaired Student's t test.

**Muscle Force Determination—** Small bundles of fibers from EDL and soleus muscles from 24–28-month-old transgenic and nontransgenic mice were dissected. The bundle of muscle fibers was equivalent to about 1/3 of the entire muscle. Whole muscle weight and bundle wet mass values are included in Table III. We used bundles of fibers to facilitate oxygenation of the core fibers and ameliorate pH and ionic gradients from the outer to the inner layers of the muscle (22, 23). All of the fibers were tested from a mouse a test visit within 4 h of muscle dissection. The bath buffered physiological solution used for contraction studies contained the following: NaCl, 121 mM; NaHCO3, 2.4 mM; glucose, 5.5 mM; KCl, 5 mM; CaCl2, 1.8 mM; MgCl2, 0.5 mM; NaH2PO4, 0.4 mM; and d-tubocurarine chloride, 0.025 mM (24). All of the experiments were carried out at room temperature (22–24°C). The solution pH was maintained at approximately 7.4 by buffering with a gas mixture of 95% O2 and 5% CO2. The fiber bundles were placed horizontally in a recording chamber, and one tendon was fastened to a micromanipulator that allowed bundle micropositioning in the three axes. The other tendon was tied to a Grass FT 03 force transducer (self-resonant frequency of 85 Hz). Analog signals were digitized using a 1200 Digidata (Axon Instruments, Foster City, CA). For data acquisition and analysis we used pClamp 6.3 software (Axon). Data were sampled at 2 kHz. IGF-1 antiserum was specifically withheld in some reaction tubes to determine nonspecific binding of radiolabeled IGF-1.

**RESULTS**

The aim of this study is to determine whether age-dependent decrease in DHPRs and the decline in muscle strength can be prevented or delayed by specific overexpression of IGF-1 in skeletal muscle. S1S2 transgenic mice overexpressing hIGF-1 exclusively in skeletal muscle and control FVB nontransgenic mice were used for that purpose (18). Age-related changes were determined using groups of 1-, 2-, 3-, 6-, 12-, and 24-month-old transgenic and nontransgenic mice. The extent of IGF-1 gene expression was determined by measuring IGF-1 concentration in individual quadriceps muscles. Skeletal muscles from S1S2 transgenic mice exhibited a 10–20-fold higher IGF-1 concentration compared with nontransgenic mice (Table I, P < 0.01). The increase in IGF-1 concentration in the transgenic mice used in the present study is similar to that reported previously (18, 19). The severalfold increases in IGF-1 concentration in skeletal muscle was sustained from 1- to 24-month-old mice (Table I). Therefore, the transgenic mice S1S2 is an appropriate model to determine whether age-dependent changes in skeletal muscle can be prevented by specific overexpression of IGF-1 in skeletal muscle.

**DHPR and RyR1 in Mixed Fiber-type Skeletal Muscles from S1S2 Transgenic and FVB Nontransgenic Mice—** The concentration of DHPR and RyR1 and their dissociation constant for high affinity radioligands were determined in a pool of muscles consisting of both fast- and slow-twitch muscle fibers. The DHPR concentration was assessed using the high affinity probe [3H]PN200–110. The sarcoplasmic reticulum RyR1 was quantitated by radioligand analysis using the neutral plant alkaloid [3H]Ryanodine as a high affinity probe in the same muscle homogenates. Fig. 1 shows [3H]PN200–110 (top) and [3H]ryanodine as high affinity probes in the same muscle homogenates.
2-month-old animals and the increase was sustained in increase at three months of age (and ryanodine receptors in FVB mice showed a significant groups and are given in Table I. The number of PN200–110 24-month (10 determinations in five different preparations) age 6-month (10 determinations in five different preparations), 12-month (8 determinations in four different preparations), and 24-month (6 determinations in three different preparations), and ryanodine receptors decreased 40% (P < 0.001), and ryanodine receptors decreased 12% (P < 0.05) compared with adult animals. These changes in maximum binding capacity cannot be explained by changes in the dissociation constant of both receptors for their ligand because no significant changes in

**Table I**

High affinity [3H]PN200–110 and [3H]ryanodine binding and their ratio to skeletal muscle isolated from S1S2 transgenic and nontransgenic mice

Values of B\(_{\text{max}}\), K\(_d\), and IGF-1 are mean ± S.E. and are expressed in pmol/mg of protein, nm, and pg/mg protein, respectively. Statistical significance (when observed) within the age group is given below the data, and difference between nontransgenic and transgenic is given next to the data.

| Age (months) | IGF-1 ([ng/mg]) | [3H]PN200–110 B\(_{\text{max}}\) (pmol/mg protein) | [3H]PN200–110 K\(_d\) (nM) | [3H]Ryanodine B\(_{\text{max}}\) (pmol/mg protein) | [3H]Ryanodine K\(_d\) (nM) | Ratio | P value |
|-------------|-----------------|---------------------------------|-----------------|---------------------------------|-----------------|------|---------|
| 1 Nontransgenic | 2032 ± 487 | 2.49 ± 0.08 | 1.66 ± 0.51 | 2.86 ± 0.22 | 12.8 ± 2.6 | 0.88 ± 0.04 | (P < 0.01) |
| 2 Nontransgenic | 2983 ± 466 | 2.28 ± 0.08 | 1.60 ± 0.19 | 2.33 ± 0.15 | 14.8 ± 0.2 | 0.98 ± 0.04 | (P < 0.01) |
| 3 Nontransgenic | 1215 ± 502 | 3.03 ± 0.03 | 1.77 ± 0.14 | 3.11 ± 0.08 | 8.4 ± 3.7 | 0.97 ± 0.02 | (P < 0.001) |
| 6 Nontransgenic | 2057 ± 652 | 2.77 ± 0.18 | 2.48 ± 0.84 | 3.06 ± 0.19 | 10.1 ± 2.3 | 0.91 ± 0.08 | (P < 0.001) |
| 12 Nontransgenic | 917 ± 120 | 2.79 ± 0.12 | 2.10 ± 0.29 | 2.94 ± 0.15 | 9.2 ± 0.9 | 0.99 ± 0.03 | (P < 0.05) |
| 24 Nontransgenic | 1536 ± 352 | 1.98 ± 0.08 | 1.48 ± 0.42 | 2.02 ± 0.09 | 19.5 ± 3.5 | 0.67 ± 0.03 | (P < 0.001) |
| 1 Transgenic | 2738 ± 5734 | 4.35 ± 0.27 (P < 0.001) | 1.39 ± 0.54 | 3.34 ± 0.17 | 23 ± 4 | 1.30 ± 0.03 (P < 0.01) | (P < 0.001) |
| 2 Transgenic | 25900 ± 8706 | 4.89 ± 0.38 (P < 0.001) | 3.01 ± 1.64 | 3.37 ± 0.45 (P < 0.05) | 22 ± 6 | 1.48 ± 0.14 (P < 0.02) | (P < 0.001) |
| 3 Transgenic | 15735 ± 6469 | 4.87 ± 0.21 (P < 0.002) | 2.07 ± 0.32 | 3.72 ± 0.19 | 13 ± 5 | 1.31 ± 0.03 (P < 0.01) | (P < 0.001) |
| 6 Transgenic | 25861 ± 7032 | 4.21 ± 0.10 (P < 0.001) | 2.05 ± 0.39 | 3.17 ± 0.13 | 16 ± 5 | 1.33 ± 0.02 (P < 0.01) | (P < 0.001) |
| 12 Transgenic | 22705 ± 7037 | 4.09 ± 0.13 (P < 0.001) | 1.24 ± 0.47 | 3.13 ± 0.14 | 9 ± 3 | 1.31 ± 0.03 (P < 0.01) | (P < 0.001) |
| 24 Transgenic | 16845 ± 7007 | 4.00 ± 0.09 (P < 0.001) | 2.58 ± 0.74 | 2.82 ± 0.07 (P < 0.01) | 15 ± 2 | 1.42 ± 0.04 (P < 0.01) | (P < 0.05) |

*Statistically significant difference was seen between nontransgenic and transgenic mice (P < 0.01).*
IGF-1 Overexpression and EC Coupling in Skeletal Muscle

TABLE II

High affinity [3H]PN200–110 and [3H]ryanodine binding to soleus and EDL muscles of S1S2 transgenic and nontransgenic mice

Values of Bmax are mean ± S.E. and are expressed in pmol/mg of protein. Statistical significance (when observed) within the age group is given below the data, and the difference between nontransgenic and transgenic is given next to the data.

| Age | [3H]PN200–110 | [3H]Ryanodine | Ratio | [3H]PN200–110 | [3H]Ryanodine | Ratio |
|-----|--------------|---------------|-------|--------------|---------------|-------|
|     | Soleus muscle |               |       | EDL muscle    |               |       |
| months |              |               |       |              |               |       |
| Nontransgenic |             |               |       |              |               |       |
| 1    | 3.54 ± 0.44  | 6.21 ± 0.43   | 0.57 ± 0.06 | 6.10 ± 1.56  | 6.40 ± 1.39   | 0.91 ± 0.07 |
| 2    | 3.12 ± 0.47  | 4.96 ± 0.52   | 0.63 ± 0.07 | 6.05 ± 0.41  | 6.85 ± 0.99   | 0.90 ± 0.07 |
| 3    | 2.31 ± 0.36  | 5.22 ± 1.08   | 0.45 ± 0.02 | 5.95 ± 1.00  | 6.20 ± 0.92   | 0.96 ± 0.02 |
| 6    | 3.45 ± 0.50  | 6.50 ± 0.52   | 0.53 ± 0.07 | 6.20 ± 0.1   | 6.46 ± 0.23   | 0.96 ± 0.02 |
| 12   | 2.54 ± 0.23  | 6.44 ± 0.46   | 0.39 ± 0.02 | 6.74 ± 0.91  | 6.70 ± 0.6    | 1.00 ± 0.05 |
| 24   | 1.93 ± 0.21  | 5.74 ± 0.48   | 0.33 ± 0.02 | 4.69 ± 0.4   | 6.60 ± 0.25   | 0.71 ± 0.06 |

Transgenic |             |               |       |              |               |       |
| 1    | 6.84 ± 0.9 (P < 0.05) | 7.57 ± 1.19 | 0.91 ± 0.02 (P < 0.001) | 12.22 ± 1.12 (P < 0.02) | 10.04 ± 0.97 | 1.22 ± 0.04 (P < 0.01) |
| 2    | 6.55 ± 1.22 (P < 0.05) | 7.71 ± 1.49 | 0.85 ± 0.04 (P < 0.03) | 11.15 ± 2.21 (P < 0.05) | 7.27 ± 1.31 | 1.54 ± 0.10 (P < 0.01) |
| 3    | 4.6 ± 0.41 (P < 0.05) | 6.21 ± 0.65 | 0.74 ± 0.04 (P < 0.01) | 8.95 ± 0.45 (P < 0.03) | 7.04 ± 0.43 | 1.27 ± 0.03 (P < 0.01) |
| 6    | 4.62 ± 0.32 (P < 0.05) | 5.25 ± 0.23 | 0.88 ± 0.04 (P < 0.01) | 7.85 ± 0.72 (P < 0.03) | 5.93 ± 0.53 | 1.32 ± 0.02 (P < 0.01) |
| 12   | 5.47 ± 0.1 (P < 0.001) | 6.97 ± 0.69 | 0.81 ± 0.07 (P < 0.001) | 10.45 ± 1.12 (P < 0.05) | 8.37 ± 0.89 | 1.26 ± 0.08 (P < 0.05) |
| 24   | 4.81 ± 1 (P < 0.001) | 7.44 ± 1.42 | 0.66 ± 0.09 (P < 0.05) | 10.14 ± 0.97 (P < 0.01) | 8.38 ± 0.89 | 1.22 ± 0.07 (P < 0.01) |

Age-related Changes in the Number of PN200–110 and Ryanodine Receptors in soleus and EDL Muscles of S1S2 Transgenic and Nontransgenic Mice—To determine whether the prevention of the age-related decline in the number of PN200–110 and the PN200–110/ryanodine ratio in transgenic skeletal muscle was due to an effect on fast- and/or slow-twitch muscle fibers, binding assays on fast- or slow-twitch muscles were performed. For this purpose, transgenic and nontransgenic 1-, 2-, 3-, 6-, 12-, and 24-month-old mice were studied. The Bmax values of PN200–110 and ryanodine in soleus and EDL muscles of nontransgenic mice were determined by a single saturating concentration of [3H]PN200–110 and [3H]ryanodine. Table II shows [3H]PN200–110 and [3H]ryanodine binding to soleus and EDL muscle homogenates from 1-, 2-, 3-, 6-, 12-, and 24-month-old nontransgenic mice. The decrease in PN200–110/ryanodine ratio in aged EDL and soleus muscles is mainly due to the decrease in the number of PN200–110 receptors in soleus muscle sites as seen in muscle pool. In FVB nontransgenic mice, the number of PN200–110-uncoupled ryanodine receptors in soleus is larger than EDL muscles due to lower number of PN200–110 receptors in soleus muscle (25). Consequently, the PN200–110/ryanodine binding ratio is less in soleus muscle than in EDL muscle. These values are similar to those reported by us earlier in rat (15) and mouse (19).

The Age-related Changes in the Number of DHPRs and the DHPR/RyR1 Ratio Depend on IGF-1 Concentration—A two-factor analysis of variance was used to see whether the number of PN200–110 receptors and the PN200–110/ryanodine ratio are affected by interaction of the two different factors namely aging and IGF-1 concentration (transgenic IGF-1 expression). The effect of aging on DHPRs and the DHPR/RyR1 ratio depends on what level of IGF-1 is present in skeletal muscle. There is a statistically significant interaction between age and status of IGF-1 expression in the number of DHPRs (P = 0.005) and the DHPR/RyR1 ratio (P < 0.001). The age-related decrease in the number of DHPRs depends on whether the animals are nontransgenic or transgenic. In aged nontransgenic animals, the number of DHPRs and the DHPR/RyR1 ratio decrease, whereas in transgenic animals, the specific overexpression of IGF-1 in skeletal muscle prevents age-related decrease in the number of DHPRs and the DHPR/RyR1 ratio. No statistically significant interaction between age and status of IGF-1 expression was observed in the case of RyR1.

In aged animals, the PN200–110/ryanodine ratio is 0.67 due to the decrease in the number of PN200–110 receptors. These results indicate that in aged skeletal muscles, ½ of the available RyR1s are coupled to DHPRs, and the other ½ of RyR1s are uncoupled due to the age-related decrease in the number of DHPRs. The PN200–110/ryanodine ratio of 0.67 in aged muscle is similar to that reported for aging F344BNF1 rat muscle (15).

S1S2 transgenic mice show a 50–100% increase in the number of PN200–110 receptors compared with nontransgenic mice at different ages. These results indicate that specific overexpression of IGF-1 in skeletal muscle results in an increased expression of DHPR. The difference in the number of RyR1 between transgenic and nontransgenic muscles is not statistically significant. Also, in aged transgenic mice, the number of RyR1 depicts a significant age-related decrease, as observed in control mice (P < 0.05). This suggests that IGF-1 does not modulate ryanodine receptor expression. Consequently, the PN200–110/ryanodine ratio in transgenic animals is about 30% higher in transgenic animals compared with nontransgenic animals (P < 0.01), and the increase is similar to results reported earlier for adult transgenic mice (19). The age-related decrease in the number of PN200–110 receptors and PN200–110/ryanodine ratio seen in control mice is absent in transgenic animals. These results demonstrate that the increased availability of IGF-1 in skeletal muscle by exclusive overexpression of hIGF-1 gene prevents age-related decrease in the number of DHPRs and the DHPR/RyR1 ratio.
skeletal muscle modulates PN200–110 receptors and PN200–110/ryanodine ratios equally in muscles consisting almost exclusively of slow- or fast-twitch muscle fibers. Two-way analysis of variance was done to determine whether the number of PN200–110 receptors and the ratio of PN200–110/ryanodine are affected by interaction of the two different factors namely aging and IGF-1 concentration in EDL and soleus muscles. The effect of aging on the number of PN200–110 receptors and the ratio of PN200–110/ryanodine ratio does not depend on what level of IGF-1 is available in EDL and soleus muscles. There was no significant interaction between age and IGF-1 concentration in EDL and soleus muscles. The levels of PN200–110 receptors and the ratio of PN200–110/ryanodine ratio does not depend on what level of IGF-1 is available in EDL and soleus muscles. There was no significant interaction between age and IGF-1 concentration in EDL and soleus muscles. The levels of PN200–110 receptors and the ratio of PN200–110/ryanodine ratio does not depend on what level of IGF-1 is available in EDL and soleus muscles. There was no significant interaction between age and IGF-1 concentration in EDL and soleus muscles.

**Functional Significance**—We examined the functional significance of the exclusive overexpression of IGF-1 in skeletal muscle by measuring the mechanical output of small bundles of EDL and soleus muscle fibers. Because a decrease of 30–40% in muscle strength has been recorded in aging humans and rodents (6, 8), we focused this part of the work on the comparison of the mechanical performance of hIGF-1 transgenic and nontransgenic old mice (24–26 months of). Eight EDL and 8 soleus muscles from hIGF-1 transgenic and FVB muscles were studied. A set of 29–31 bundles was examined for each group. Fig. 2 shows muscle bundle contractions in response to supramaximal single pulses of 50-ms duration or a series of 10-ms pulses at various stimulation rates. Fig. 2, A and B, shows twitch and tetanus contractions in a control EDL bundle from FVB mouse. The mechanical force developed in response to a single pulse (Fig. 2C) or a train of pulses (Fig. 2D) was potentiated in transgenic EDL. In EDL muscles from both FVB and S1S2 mice, the stimulation rates needed to reach the peak tetanic response were 70 ± 3 and 68 ± 4 Hz, respectively. This difference was not statistically significant. Although the dissection of bundles of fibers partially cancelled differences in muscle sectional area between control and transgenic mice (18), muscle force was systematically normalized to the cross-sectional area to determine whether overexpression of IGF-1 in skeletal muscle results in significant differences in specific muscle force. Fig. 2 shows twitches and tetanic contractions in soleus bundles from control (E–G) and S1S2 (F–H) mice, respectively. It is apparent that the twitch tension developed in soleus muscle bundles is lower than in EDL. Also, that the peak tetanic tension is similar in both soleus and EDL muscle (see Table III). The frequency needed to attain a maximum tetanic contraction in soleus muscle bundles was 40 ± 4 and 38 ± 5 Hz in control and transgenic mice, respectively. This difference was not statistically significant. As for EDL muscle, S1S2 soleus muscle bundles developed more specific tension than control (see Fig. 2 and Table III). The tetanus/twitch (P_{t}/P_{T}) ratio for EDL and soleus muscle bundles was not significantly different for FVB and S1S2 mice. This supports the concept that the mechanical output is enhanced in partially and fully activated hIGF-1 transgenic mice compared with control FVB mice. This is in agreement with the lack of significant difference in the stimulation rates needed to obtain maximal muscle activation in FVB and S1S2 mice. These results rule out changes in the frequency-force relationship as the underlying mechanism for twitch and tetanus potentiation in muscles from S1S2 mice (see under “Discussion”).

**DISCUSSION**

**IGF-1 Overexpression Prevents Age-related Decline in DHPRs and the DHPR/RyR1 Ratio**—The present study reports that specific overexpression of hIGF-1 in skeletal muscle prevents age-related decline in the number of DHPRs and the DHPR/RyR1 ratio. The quantitative comparison between DHPR and RyR1 was done in parallel determinations of [3H]PN200–110 (DHPR) and [3H]ryanodine (RyR1) binding to the same muscles. Transgenic mice express 10–20-fold higher concentrations in IGF-1 concentration than nontransgenic mice in all ages tested. Furthermore, in aged transgenic mice, the increase in IGF-1 concentration is sustained, suggesting that the transgenic mouse is an ideal animal model to test whether increased availability of IGF-1 in skeletal muscle prevents age-related decline in the number of DHPRs and the DHPR/RyR1 ratio. When skeletal muscles from S1S2 transgenic mice were compared with FVB nontransgenic mice, a significant increase in the number of DHPRs was evident in young, adult, and old mice. The levels of RyR1, which functions as the Ca^{2+} release channel in EC coupling (12, 13), did not change significantly in transgenic compared with nontransgenic muscles. Consequently, young, adult, and old mice showed a 30–40% increase in the DHPR/RyR1 ratio in transgenic muscles compared with nontransgenic muscles. The DHPR is a voltage-gated Ca^{2+} channel, and its activation by t-tubule membrane depolarization evokes Ca^{2+} release from sarcoplasmic reticulum through RyR1 into the myoplasm (13). Contractile proteins on binding Ca^{2+} initiate muscle contraction and force development. Hence, DHPR and RyR1 play a central role in the mechanism of excitation-contraction coupling in skeletal muscle. The age-related reduction in the number of DHPRs and the DHPR/RyR1 ratio alters the electrome-
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Role of hIGF-1 Overexpression in Muscle Force Potentiation—DHPR and RyR1 are key molecules for skeletal muscle force development. Therefore, we tested the hypothesis that IGF-1-mediated prevention of the age-related decline in DHPR and RyR1 expression results in higher muscle strength in transgenic than in nontransgenic muscles. Based on the radio-ligand binding assays, a significant effect of IGF-1 overexpression on receptor expression in slow- and fast-twitch fiber subtypes was apparent. Thus, we investigated whether overexpression of IGF-1 results in more powerful contractions in bundle of muscle fibers consisting almost exclusively of slow-twitch (soleus muscle) or fast-twitch (EDL) cells. Regardless of the muscle fiber subtype, overexpression of IGF-1 exclusively in skeletal muscle resulted in a significant potentiation of the specific contraction force. The stimulation rate needed to obtain a maximal fused mechanical response was similar for transgenic and nontransgenic mice and in both soleus and EDL muscle bundles. A plausible explanation for these results is that the increase in DHPR density precludes the reported age-dependent EC uncoupling (5). It is known from previous studies that the acute exposure to IGF-1 does not alter the voltage dependence of charge movements in muscle fibers (3). Because the voltage dependence of charge movements is not altered by IGF-1, changes in the voltage dependence of sarcoplasmic reticulum calcium release are also unlikely. These findings support the concept that the increase in receptor density is directly linked to muscle force potentiation in hIGF-1 transgenic muscles. An increase in contractile proteins in SIS2 content has been suggested by the larger muscle size in transgenic mice than in control FVB mice (18). In the present work, the muscle force was normalized to a bundle cross-sectional area, providing direct information about muscle-specific force. Therefore, an increase in contractile protein content in transgenic muscles does not account for the reported significant difference in tension. The expression of DHPR has been associated with skeletal muscle activity (30); it should be mentioned that transgenic and nontransgenic mice were housed in similar cages and exhibited similar physical activity. Although acute exposure to IGF-1 enhances calcium influx through DHPR (3), it is unlikely that potentiation of calcium influx plays a significant role during brief contractions of mouse skeletal muscle. Calcium influx through DHPR has been suggested to play a role in sustained tetanic tension (31–33). However, intracellular calcium concentration measured before and after blocking calcium influx through DHPR does not change significantly in mature single muscle fibers and in rat myotube.2 Modulatory effects of IGF-1 on protein kinases through a paracrine mechanism have been reported in acute experiment (3). This has been the mechanism by which IGF-1 regulates calcium influx through DHPR. Tyrosine kinase and protein kinase C activation in response to IGF-1/IGF-1R interaction results in facilitation of calcium influx through DHPR or a shift in the current activation curve toward more negative potentials. It is obvious from the arguments discussed above that this mechanism cannot account for the results reported here. In addition to a direct regulation of the DHPR-RyR1 coupling, other potential mechanisms may be involved in the IGF-1-dependent modulation of skeletal muscle force. The higher DHPR/RyR1 ratio is a crucial phenomenon that leads to stronger contractions in hIGF1-transgenic skeletal muscle. However, this may not be the exclusive mechanism that may ac-
count for the results of the contraction tests reported in this work. IGF-1-evoked modulation of the expression of other proteins involved in excitation-contraction coupling such as FKBP12 (34) and/or changes in the DHPR and RyR1 spatial arrangement are some of the mechanisms that need to be explored (35).

In summary, the magnitude of EDL and soleus muscle force enhancement is proportional to the difference in DHPR/RyR1 between hIGF-1 transgenic and nontransgenic aging mice. Exclusive overexpression of IGF-1 in skeletal muscle prevents age-related decline in DHPR and DHPR/RyR1 ratio, a phenomenon that is clearly associated with the prevention of the reported decrease of 30–40% in EDL and soleus muscle-specific force.

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