

## Sustained Overexpression of IGF-1 Prevents Age-Dependent Decrease in Charge Movement and Intracellular $\text{Ca}^{2+}$ in Mouse Skeletal Muscle

Zhong-Min Wang,\* María Laura Messi,\* and Osvaldo Delbono\*†

From the Departments of \*Physiology and Pharmacology and †Internal Medicine, Gerontology, Wake Forest University School of Medicine, Winston-Salem, North Carolina 27157 USA

**ABSTRACT** In this work we tested the hypothesis that transgenic sustained overexpression of IGF-1 prevents age-dependent decreases in charge movement and intracellular  $\text{Ca}^{2+}$  in skeletal muscle fibers. To this end, short flexor digitorum brevis (FDB) muscle fibers from 5–7- and 21–24-month-old FVB (wild-type) and S1S2 (IGF-1 transgenic) mice were studied. Fibers were voltage-clamped in the whole-cell configuration of the patch-clamp technique according to described procedures (Wang, Z. M., M. L. Messi, and O. Delbono. 1999. *Biophys. J.* 77:2709–2716). Charge movement and intracellular  $\text{Ca}^{2+}$  concentration were recorded simultaneously. The maximum charge movement ( $Q_{\text{max}}$ ) recorded in young wild-type and transgenic mice was (mean  $\pm$  SEM, in nC  $\mu\text{F}^{-1}$ ):  $52 \pm 2.1$  ( $n = 46$ ) and  $54 \pm 1.9$  ( $n = 38$ ) (non-significant, ns), respectively, whereas in old wild-type and old transgenic mice the values were  $36 \pm 2.1$  ( $n = 32$ ) and  $49 \pm 2.3$  ( $n = 35$ ), respectively ( $p < 0.01$ ). The peak intracellular calcium  $[\text{Ca}^{2+}]_i$  recorded in young wild-type and transgenic mice was (in  $\mu\text{M}$ ):  $14.5 \pm 0.9$  and  $16 \pm 2.1$  (ns), whereas in old wild-type and transgenic mice the values were  $9.9 \pm 0.1$  and  $14 \pm 1.1$  ( $p < 0.01$ ), respectively. No significant changes in the voltage distribution or steepness of the  $Q$ - $V$  or  $[\text{Ca}^{2+}]$ - $V$  relationship were found. These data support the concept that overexpression of IGF-1 in skeletal muscle prevents age-dependent reduction in charge movement and peak  $[\text{Ca}^{2+}]_i$ .

### INTRODUCTION

Studies on muscle contractility in rodents and humans in vivo and in vitro have demonstrated that skeletal muscle contraction force declines with aging (Baumgartner et al., 1998; González and Delbono, 2000, 2001a,b; Roubenoff and Hughes, 2000). Several mechanisms have been postulated to explain age-related skeletal muscle weakness (for a review see Loeser and Delbono, 1999; Roubenoff and Hughes, 2000). It is evident that the loss of muscle mass does not entirely explain the decrease in contractile properties with aging (Delbono et al., 1997a; Moore, 1975). This means that the conservation of the muscle mass over age does not ensure a complete preservation of muscle tension. Studies on in vitro contractility showed that when the maximum isometric force for aged mice and rats is normalized to the smaller total muscle fiber cross-sectional area, a significant deficit in specific isometric force remains unexplained by atrophy (Brooks and Faulkner, 1988, 1994; González and Delbono, 2000; Renganathan et al., 1998). These data suggest that other factors in addition to reduction in contractile proteins contribute to muscle weakness in muscles from aged mammals. Previous work from our laboratory demonstrated that charge movement and peak intracellular  $\text{Ca}^{2+}$  recorded in old mice decrease significantly compared with middle-aged and young adult mice (Wang et

al., 2000). Therefore, we concluded that senescence, not maturation, accounts for excitation- $\text{Ca}^{2+}$  release uncoupling (Wang et al., 2000). Intramembrane charge movement and sarcoplasmic reticulum  $\text{Ca}^{2+}$  influx are part of a signaling cascade that determines the force of muscle contraction (Ashley et al., 1991; Melzer et al., 1995). The reduction in L-type  $\text{Ca}^{2+}$  channel expression in aging mice (Zheng et al., 2001) results in reduced peak cytosolic  $\text{Ca}^{2+}$ , with a subsequent decrease in skeletal muscle force. Based on the fine regulation exerted by  $\text{Ca}^{2+}$  on muscle force development (Ashley et al., 1991), prevention of the decline in intracellular  $\text{Ca}^{2+}$  associated with muscle weakness in senescent mammals is a major goal.

Insulin-like growth factor 1 (IGF-1) is a peptide structurally related to proinsulin and has a primary role in promoting skeletal muscle differentiation and growth (Florini et al., 1996). IGF-1 regulates the ion permeation function of the dihydropyridine (DHP)-sensitive L-type  $\text{Ca}^{2+}$  channel (Delbono et al., 1997b; Renganathan et al., 1997c). However, it is unlikely that tyrosine-kinase/protein kinase C-dependent DHPR phosphorylation regulates excitation-contraction coupling. DHPR and ryanodine receptors (RyR1) and sarcoplasmic reticulum  $\text{Ca}^{2+}$  content are directly involved in regulating the amplitude of the muscle fiber  $\text{Ca}^{2+}$  influx (see Melzer et al., 1995). Prior studies from our laboratory have shown that age-related decrease in DHPR and RyR1 in skeletal muscle can be prevented by IGF-1 (Renganathan et al., 1997a,b). We have also shown that IGF-1 enhances skeletal muscle charge movement,  $[\text{Ca}^{2+}]_i$  binding sites, and DHPR $\alpha_{1S}$  expression in single muscle fibers from adult rats (Wang et al., 1999b). Whether the effects of IGF-1 on DHPR and RyR1 expression and function result in higher levels of intracellular

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Address reprint requests to Dr. Osvaldo Delbono, Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157. Tel.: 336-716-9802; Fax: 336-716-2273. E-mail: odelbono@wfubmc.edu

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$\text{Ca}^{2+}$  in response to sarcolemmal depolarization is not known.

In the present work we hypothesized that sustained overexpression of IGF-1 prevents age-related decline in charge movement and intracellular  $\text{Ca}^{2+}$ . To test this hypothesis sarcolemmal currents and intracellular  $\text{Ca}^{2+}$  have been recorded simultaneously in transgenic mice overexpressing IGF-1 in skeletal muscle (Coleman et al., 1995) and littermate wild-type FVB mice. Single fibers from flexor digitorum brevis muscle from young and old mice have been voltage-clamped in the whole-cell configuration of the patch-clamp technique (Wang et al., 1999a) and intracellular  $\text{Ca}^{2+}$  has been recorded using the low-affinity fluorescent indicator fluo-5N.

## METHODS

### Mouse skeletal muscle single fibers

Single skeletal muscle fibers from the flexor digitorum brevis (FDB) muscle were obtained from 5–7- (young adult group) or 21–24- (old)-month-old IGF-1 transgenic (S1S2) and wild-type (FVB) mice raised at the Animal Research Program of Wake Forest University School of Medicine (WFUSM). FVB (Taconic, Germantown, NY) is the background strain for S1S2 transgenic mice used in this work. Animal handling and procedures followed an approved protocol by the Animal Care and Use Committee of WFUSM. A total of 11, 12, 14, and 13 young wild-type, young transgenic, old wild-type, and old transgenic mice, respectively, have been used in the present study. FDB muscles were dissected in a solution containing 155 mM cesium aspartate, 5 mM magnesium aspartate<sub>2</sub>, and 10 mM HEPES (pH 7.4 with CsOH) (Beam and Franzini-Armstrong, 1997). Muscles were treated with 2 mg/ml collagenase (Sigma, St. Louis, MO) in a shaking bath at 37°C. After 3 h of enzymatic treatment, FDB muscles were dissociated into single fibers with Pasteur pipettes of different tip sizes.

### Charge movement recordings

Muscle fibers were voltage-clamped using an Axopatch-200B amplifier (Axon Instruments, Foster City, CA) in the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) according to procedures previously described (Wang et al., 1999a). Muscle fibers were transferred to a small flow-through Lucite chamber positioned on a microscope stage. Fibers were continuously perfused with the external solution (see below) using a push-pull syringe pump (WPI, Sarasota, FL). Only fibers exhibiting a clean surface and lack of evidence of contracture were used for electrophysiological recordings. Patch pipettes were pulled from borosilicate glass (Boralex) using a Flaming Brown micropipette puller (P97; Sutter Instrument Co., Novato, CA) and then fire-polished to obtain electrode resistance ranging from 450 to 650 k $\Omega$ . The pipette was filled with the following solution (in mM): 140 cesium aspartate; 2 magnesium aspartate<sub>2</sub>, 0.2 Cs<sub>2</sub> EGTA, and 10 HEPES, with pH adjusted to 7.4 with CsOH (Adams et al., 1990; Wang et al., 1999a). Membrane seal formation was attained in the following external solution containing (in mM): 150 TEA-CH<sub>3</sub>SO<sub>3</sub>, 2 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 10 Na-HEPES, and 0.001 tetrodotoxin (Delbono, 1992; Delbono et al., 1997b). Solution pH was adjusted to 7.4 with CsOH. Both the pipette and the bath solution were selected based on the ease of membrane seal formation and cell stability over time. For charge movement recording,  $\text{Ca}^{2+}$  current was blocked with the external solution containing 0.5 mM Cd<sup>2+</sup> and 0.3 mM La<sup>3+</sup> (Adams et al., 1990; Wang et al., 1999a).

Whole-cell currents were acquired and filtered at 5 kHz with pCLAMP 6.04 software (Axon Instruments). A Digidata 1200 interface (Axon In-

struments) was used for A-D conversion. Membrane current during a voltage pulse,  $P$ , was initially corrected by analog subtraction of linear components. The remaining linear components were digitally subtracted on-line using hyperpolarizing control pulses of one-quarter test pulse amplitude (-P/4 procedure) as described for rat and mouse muscle fibers (Delbono, 1992; Delbono et al., 1997b). Four control pulses were applied before the test pulse. Potential voltage errors associated with whole-cell recording in large cells have been minimized by selecting small FDB fibers and by adequate compensation for whole-cell capacitance transients. The capacitance of the FDB fibers from young wild-type mice included in this study was (mean  $\pm$  SEM): 1560  $\pm$  145 pF (range: 812–2230 pF,  $n = 46$ ). These values were not significantly different from those recorded in young transgenic (1431  $\pm$  158,  $n = 38$ ), old wild-type (1515  $\pm$  201,  $n = 32$ ), and old transgenic (1581  $\pm$  223,  $n = 35$ ) mice.

Charge movements were evoked by 25-ms depolarizing pulses from the holding potential (-80 mV) to command potentials ranging from -70 to 70 mV with 10-mV intervals. Intramembrane charge movement was calculated as the integral of the current in response to depolarizing pulses (charge on,  $Q_{\text{on}}$ ) and is expressed per membrane capacitance (coulombs per farad). A linear  $Q_{\text{on}}-Q_{\text{off}}$  relationship confirmed the complete the inward  $\text{Ca}^{2+}$  current blockade (Wang et al., 1999a).

### Intracellular $\text{Ca}^{2+}$ transient recording

Intracellular  $\text{Ca}^{2+}$  transients were recorded simultaneously with sarcolemmal currents in voltage-clamped FDB muscle fibers. We used the low-affinity indicator fluo-5N AM (Molecular Probes, Eugene, OR) ( $K_{\text{Ca}} = 90 \mu\text{M}$ ) as the  $\text{Ca}^{2+}$  probe. The fluo-5N-AM was prepared as a 2 mM stock in DMSO and added to the recording solution at a final concentration of 5  $\mu\text{M}$ . The fibers were loaded with fluo-5N for 30 min. A group of fibers were incubated for 1–2 h to determine whether the dye is sequestered in the sarcoplasmic reticulum, as reported for amphibian muscle (Kabbara and Allen, 2001). We did not observe intraluminal  $\text{Ca}^{2+}$  transients or decreases in fluorescence, as the luminal counterpart of the  $\text{Ca}^{2+}$  release process, in any of the 10 cells studied (data not shown). In all of the cells studied, fiber stimulation resulted in elevations in fluorescence emission. The cells were perfused for 30 min with recording solution devoid of dye before recording. For fluorescent recordings the fiber was illuminated with an argon laser through a 20 $\times$  Fluor objective (Zeiss, Oberkochen, Germany). Images were acquired with a Noran O<sub>2</sub> confocal system (Noran, Middleton, WI) in the non-slit mode. Hardware control, image acquisition, and processing were done with Intervision software (Noran) run in a Silicon Graphics workstation (Mountain View, CA). Although the fluorescence was recorded from the whole cell, only a rectangular region of interest (ROI) of  $\sim 2000$ – $3000$  pixels near the patch pipette was analyzed. The patch pipette was not included in the ROI. Mean values of fluorescence changes corrected to basal fluorescence and transformed into  $\text{Ca}^{2+}$  concentration were plotted over time. Sequences of images for up to 2 s were acquired at 50 frames/s. Records were corrected for background fluorescence (optical pathway) and photobleaching. Fluorescent signals were transformed to  $\text{Ca}^{2+}$  concentration according to published methods (Tsien and Pozzan, 1989). All of the experiments were carried out at room temperature (22°C).

### Statistical analysis

Data are presented as means  $\pm$  standard error (SEM) with the number of muscle fibers examined as  $n$ . Experimental groups have been statistically analyzed using two-way analysis of variance (ANOVA) and Tukey-Kramer test.  $p < 0.05$  was considered significant.

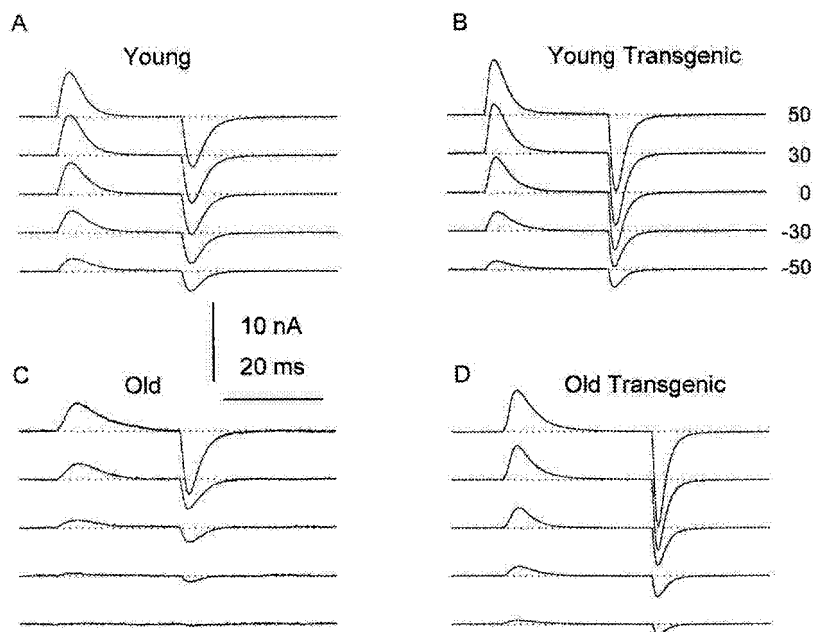


FIGURE 1 Charge movement recorded in an FDB muscle fiber from 7- (*A*), 6- (*B*), and 24-month-old (*C* and *D*) mice. Traces in *A* and *C* correspond to wild-type and *B* and *D* to IGF-1 transgenic mice, respectively. Charge movement was evoked by 25-ms depolarizing voltage steps from the holding potential ( $-80$  mV) to the command potentials ranging from  $-50$  to  $50$  mV. The dotted line represents the baseline after subtracting the linear components (see Methods).

## RESULTS

### Charge movement in skeletal muscle fibers from young adult and old wild-type and IGF-1 transgenic mice

Intramembrane charge movement was recorded after blocking the inward  $\text{Ca}^{2+}$  current (see Methods). Fig. 1 shows a group of charge movement traces recorded in muscle fibers from young adult wild-type (*A*, 7 months old), young adult IGF-1 transgenic (*B*, 6 months old), old wild-type (*C*, 24 months old), and old transgenic (*D*, 24 months old) mice. Charge movements have been evoked by applying 25-ms depolarizing voltage steps from the holding potential ( $-80$  mV) to the command potentials ranging from  $-70$  to  $70$  mV. Only charge movement recorded at  $-50$ ,  $-30$ ,  $0$ ,  $30$ , and  $50$  mV is illustrated. The current recorded after blocking the  $\text{Ca}^{2+}$  current is the intramembrane charge movement because it shows saturation at both extremes of the voltage range, and the amount of charge moved during depolarization ( $Q_{\text{on}}$ ) is equal to the charge that returns during the repolarization ( $Q_{\text{off}}$ ). This has been demonstrated previously for adult skeletal muscle fibers voltage clamped in the whole-cell configuration of the patch-clamp technique (Wang et al., 2000). It is apparent that charge movement does not differ in wild-type and IGF-1 transgenic young adult mice (Fig. 1, *A* and *B*), whereas there is a significant decrease in fibers from old wild-type mice (Fig. 1 *C*). It is also apparent that the charge moved by fibers from old IGF-1 transgenic mice (*D*) differs from that re-

corded in old wild-type, but it is similar to that recorded in young mice (wild-type or transgenic). For the analysis of the voltage-dependence of the charge, data points were fitted to a Boltzmann equation of the form:

$$Q_{\text{on}} = Q_{\text{max}} / \{1 + \exp[z_Q F (V_{1/2Q} - V_m) / RT]\}, \quad (1)$$

where  $Q_{\text{max}}$  is the maximum charge,  $V_m$  is the membrane potential,  $V_{1/2Q}$  is the charge movement half-activation potential,  $z_Q$  is the effective valence, and  $F$ ,  $R$ , and  $T$  have their usual thermodynamic meanings. Fig. 2 shows the voltage distribution of the charge for young adult (*A*) and old (*B*) mice. The best-fitting parameters for  $Q_{\text{max}}$ ,  $V_{1/2Q}$ , and  $z_Q$  recorded in muscle fibers from young adult and old mice are included in Table 1. From these measurements we conclude that the pronounced age-related decline in maximum charge movement is prevented by chronic overexpression of IGF-1 in skeletal muscle.

### Intracellular $\text{Ca}^{2+}$ recording

Intracellular  $\text{Ca}^{2+}$  has been recorded with the low-affinity  $\text{Ca}^{2+}$  fluorescent indicator fluo-5N-AM (see Methods). The advantage of using fluo-5N for intracellular  $\text{Ca}^{2+}$  recordings is twofold. The dye's low affinity for  $\text{Ca}^{2+}$  enables reliable measurement of peak  $\text{Ca}^{2+}$  concentration without saturation, and its high quantum yield allows for recordings of changes in the dye/ $\text{Ca}^{2+}$  complex with the photodetector used in the present work at a higher sampling rate. There-

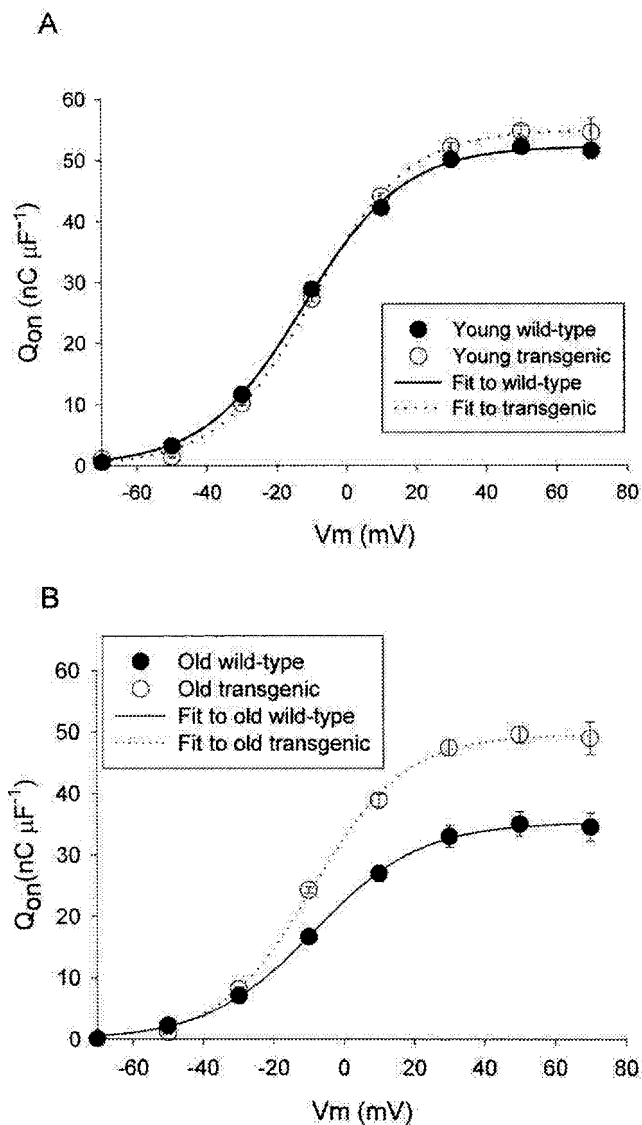


FIGURE 2 Charge movement ( $Q_{on}$ )- $V_m$  relationship for muscle fibers from young wild-type and IGF-1 transgenic mice (A) and old wild-type and IGF-1 transgenic mice (B). The data points were fitted to a Boltzmann equation (Eq. 1).

fore, saturation of the fluo-5N with  $\text{Ca}^{2+}$  is not expected based on the peak  $\text{Ca}^{2+}$  transients reported in the literature for adult muscle fibers (Delbono and Meissner, 1996; Delbono and Stefani, 1993; Garcia and Schneider, 1993; Wang et al., 2000). Fig. 3 shows representative traces of intracellular  $\text{Ca}^{2+}$  transients recorded in fibers from young adult wild-type and transgenic (A and B) and from old wild-type and IGF-1 transgenic (C and D) mice, respectively. It is apparent that the peak intracellular  $\text{Ca}^{2+}$  recorded in the fiber from the old mouse (C) is significantly smaller than that recorded in fibers from young mice, either wild-type (A) or IGF-1 transgenic (B), respectively. It is also evident that sustained overexpression of IGF-1 prevents the age-

related decline in the peak intracellular  $\text{Ca}^{2+}$  concentration (D). Fig. 4 shows the voltage-dependence of the peak  $\text{Ca}^{2+}$  transient recorded in muscle fibers from young adult (A) and old mice (B), respectively, from -50 to 50 mV. The data points were fitted to Eq. 1 and the best-fitting parameters are included in Table 1. No significant differences in maximum  $\text{Ca}^{2+}$  concentration,  $\text{Ca}^{2+}$  transient half-activation potential ( $V_{1/2}$ ), and  $z$  between muscle fibers from young and young IGF-1 transgenic mice were found. However, the peak  $\text{Ca}^{2+}$  concentration in fibers from old mice was significantly reduced compared to fibers from young adult wild-type and transgenic mice. No significant changes in the  $V_{1/2Q}$  and  $z$  parameters were recorded in fibers from these two groups (Table 1). In summary, sustained overexpression of IGF-1 in skeletal muscle resulted in maintained peak intracellular  $\text{Ca}^{2+}$  concentration across ages.

## DISCUSSION

In the present study we investigated the role of sustained overexpression of IGF-1 in the decrease in charge movement and intracellular peak  $\text{Ca}^{2+}$  concentration in skeletal muscle from aging mice. We have shown that localized overexpression of IGF-1 prevents age-dependent decrease in intracellular  $\text{Ca}^{2+}$  in skeletal muscle fibers that could result in a maintained specific muscle force at older ages. Intracellular  $\text{Ca}^{2+}$  transients evoked by sarcolemmal depolarization under voltage-clamp conditions in muscle fibers from old mice are significantly smaller than those recorded in fibers from young adult mice. A publication from our laboratory demonstrated that senescence induces a substantial reduction in intracellular  $\text{Ca}^{2+}$  concentration upon fiber activation (Wang et al., 2000). The sarcoplasmic reticulum  $\text{Ca}^{2+}$  supply to contractile proteins is a crucial step in sarcolemmal excitation-contraction coupling. Also, free cytosolic  $\text{Ca}^{2+}$  concentration regulates muscle force (Ashley et al., 1991). In the present study we have demonstrated that the reported age-dependent reduction in charge movement and peak intracellular  $\text{Ca}^{2+}$  concentration can be prevented by sustained IGF-1 overexpression in skeletal muscle. The significant reduction in free  $\text{Ca}^{2+}$  accounts, at least partially, for the reported decline in specific muscle tension (tension normalized to cross-sectional area) not explained by atrophy in skeletal muscle fibers from aging mammals (González and Delbono, 2000, 2001a,b).

Absolute reductions in the number and/or function of the DHPR and/or RyR1 are potential explanations for the age-related impairment in intracellular  $\text{Ca}^{2+}$  mobilization in skeletal muscle from aging mammals (Delbono et al., 1997a; Renganathan et al., 1997a). In the present work we measured charge movement as an indication of the level of expression of DHPR based on the fact that this channel contributes principally to the total charge movement recorded (Adams et al., 1990). The percent of decline in



**TABLE 1** Best-fitting parameters describing the voltage-dependence of charge movement and intracellular  $\text{Ca}^{2+}$  simultaneously recorded in FDB muscle fibers

Best-fitting parameters		Young wild-type mice ( <i>n</i> = 46)	Young IGF-1 mice ( <i>n</i> = 38)	Old wild-type mice ( <i>n</i> = 32)	Old IGF-1 transgenic mice ( <i>n</i> = 35)
Charge movement	$Q_{\max}$ (nC $\mu\text{F}^{-1}$ )	$52 \pm 2.1$	$54 \pm 1.9$	$36 \pm 2.1$	$49 \pm 2.3$
	$V_{Q1/2}$ (mV)	$-12 \pm 1.6$	$-9.5 \pm 0.8$	$-8.5 \pm 1.5$	$-8.8 \pm 1.4$
		ns*	(ns) <sup>†</sup>	(ns) <sup>‡</sup>	
	<i>z</i>	$1.7 \pm 0.19$	$1.9 \pm 0.21$	$2.1 \pm 0.17$	$1.6 \pm 0.23$
Intracellular			ns*	(ns) <sup>†</sup>	(ns) <sup>‡</sup>
	Maximum [ $\text{Ca}^{2+}$ ] ( $\mu\text{M}$ )	$14.5 \pm 0.9$	$16 \pm 2.1$	$9.9 \pm 0.1$	$14 \pm 1.1$
			ns*	( $P < 0.01$ ) <sup>†</sup>	( $P < 0.01$ ) <sup>‡</sup>
	$V_{1/2}$ (mV)	$4.1 \pm 0.08$	$3.8 \pm 0.04$	$3.1 \pm 0.07$	$2.9 \pm 0.09$
			ns*	(ns) <sup>†</sup>	(ns) <sup>‡</sup>
	<i>z</i>	$2.2 \pm 0.18$	$2.2 \pm 0.18$	$2.1 \pm 0.17$	$1.8 \pm 0.15$
			ns*	(ns) <sup>†</sup>	(ns) <sup>‡</sup>

*n*, number of muscle fibers; ns, nonsignificant.

\*Comparison between young transgenic and young wild-type mice.

<sup>†</sup>Comparison between old wild-type and young wild-type or young IGF-1 transgenic mice.

<sup>‡</sup>Comparison between old transgenic and old wild-type mice.

$Q_{\max}$  is the maximum charge,  $V_{1/2Q}$  is the charge movement or calcium concentration half-activation potential, and *z* is the effective valence.

myoplasmic  $\text{Ca}^{2+}$  concentration mentioned above is similar to the magnitude of the decrease in total charge movement.

Although the decrease in charge movement and myoplasmic  $\text{Ca}^{2+}$  concentration at older ages is similar, it may be possible that the smaller peak  $\text{Ca}^{2+}$  transient in fibers from older animals does not result entirely from the deficit in charge movement. Previous studies from our laboratory demonstrated that the number of DHPR and RyR1 expressed in mouse EDL muscles decrease with aging (Renganathan et al., 1997b). The age-dependent decrease in RyR1 occurs at later ages in rat than in mouse EDL muscle (Renganathan et al., 1997a). In these studies, no significant changes in the dissociation constant of the DHPR and RyR1 for [ $^3\text{H}$ ]PN200–110 and [ $^3\text{H}$ ]ryanodine was detected despite the significant decrease in the maximal binding capacity in aging rodents. The reduction in the number of DHPR measured by radioligand binding assay is consistent with the decrease in charge movement reported here. The ratio between the number of DHPR and RyR1 in adult EDL muscle showed a mean value of 0.92. This ratio suggests that every fourth RyR1 is linked to a group of four DHPR (Delbono and Meissner, 1996). The reduction in the number of DHPR and charge movement indicates that every sixth to eighth RyR1 is linked to a group of four DHPR in muscles from aging mice. The lack of changes in the receptor affinity for the ligand does not completely rule out functional alterations in the DHPR in muscle fibers from aging mice. To completely ascertain this point, single DHPR function needs to be recorded in muscle fibers. However, the DHPR is not accessible to patch pipettes due to its location in the sarcolemmal infoldings. Similar considerations can be applied to the RyR1 expressed in the sarcoplasmic reticulum. Activity of single RyR1 recordings in living muscle fibers have not been reported yet due to technical difficulties in gaining access to an intracellular

organelle. Another potential explanation for the lower peak myoplasmic  $\text{Ca}^{2+}$  transient in muscle fibers from old mice is a sarcoplasmic reticulum  $\text{Ca}^{2+}$  depletion faster than in fibers from young mice. Shorter depolarizations could deplete SR luminal  $\text{Ca}^{2+}$  in fibers from older mammals. A series of experiments argue against this possibility. We have found that there is residual free luminal  $\text{Ca}^{2+}$  in fibers from older humans at the end of prolonged depolarization to very positive potentials (Delbono et al., 1995). Also, caffeine can elicit further increases in myoplasmic  $\text{Ca}^{2+}$  concentration after a maximal activation (Delbono et al., 1995). To explore this issue more in depth, direct recordings of sarcoplasmic reticulum luminal  $\text{Ca}^{2+}$  in muscle fibers from animals of different ages are needed. For this application, low-affinity fluorescent  $\text{Ca}^{2+}$  indicators exclusively sequestered in the sarcoplasmic reticulum of mammalian species are required. In this study we explored whether fluo-5N can be used as reported for frog muscle (Kabbara and Allen, 2001). No significant sequestration of fluo-5N was found in the sarcoplasmic reticulum monitored with confocal microscopy in any of the cells studied (see Methods).

This work shows that IGF-1 enhances charge movements significantly in skeletal muscle from aging mice. Prior studies from our laboratory demonstrated that IGF-1 induces  $\alpha_{1s}$  gene expression in vitro (Wang et al., 1999b) and in vivo (Zheng et al., 2001). However, the mechanism by which the trophic factor activates DNA transcription remains unknown. It has been shown that IGF-1 regulates the transcription of a number of genes encoding proteins involved in growth and metabolism (Florini et al., 1993, 1996). Immediate early genes such as *c-fos* and *c-jun* associated with muscle cell proliferation are activated by IGF-1 (Angel et al., 1988). These may be the early events leading to products of Fos and Jun protein dimerization to bind the DNA consensus sequence known as TPA response element

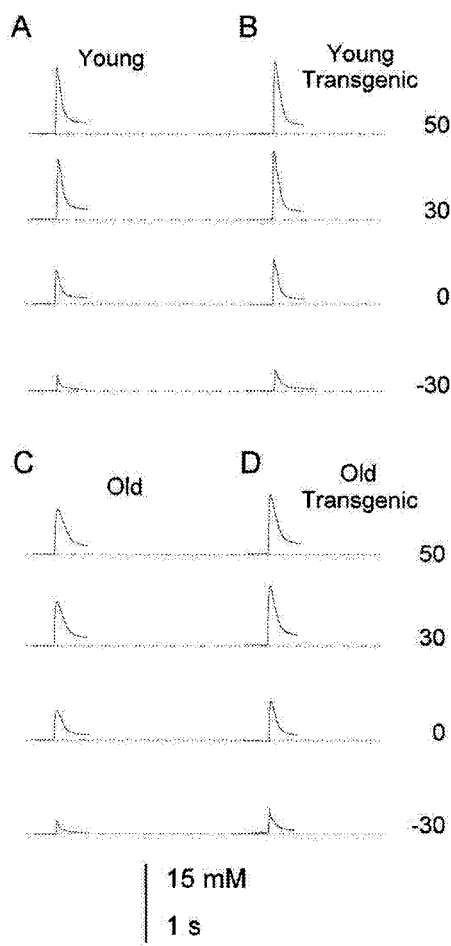


FIGURE 3 Intracellular  $\text{Ca}^{2+}$  transients recorded in the muscle fibers illustrated in Fig. 1 from  $-30$  to  $50$  mV. Intracellular fluorescence was recorded with fluo-5N AM as a  $\text{Ca}^{2+}$  probe. The dotted line represents the basal fluorescence after subtracting the background fluorescence.

(Rosenzweig et al., 1994). Further studies on the activation of sarcolemma-nucleus signaling mediated by IGF-1 might help to clarify the mechanism(s) by which this growth factor increases L-type  $\text{Ca}^{2+}$  channel  $\alpha_1$ -subunit expression.

The effects of IGF-1 on DHPR and RyR1 expression can account for the increase in the peak  $\text{Ca}^{2+}$  transient in old transgenic compared with old wild-type mice. However, the possibility that sustained high concentrations of IGF-1 in skeletal muscle modulates the expression of sarcoplasmic reticulum proteins such as calsequestrin and  $\text{Ca}^{2+}$ -ATPase, and subsequently sarcoplasmic reticulum  $\text{Ca}^{2+}$  storage and release, needs further investigation.

The concentration of IGF-1 in muscle and the magnitude of DHPR overexpression have important physiological implications. We have reported that a 12-fold increase in muscle IGF-1 concentration in young and old transgenic mice resulted in  $\sim 100\%$  increase in the number of DHPR. More recently, we found that normal plasma concentrations of IGF-1 (20 ng/ml) enhance DHPR  $\alpha_1$ -subunit expression

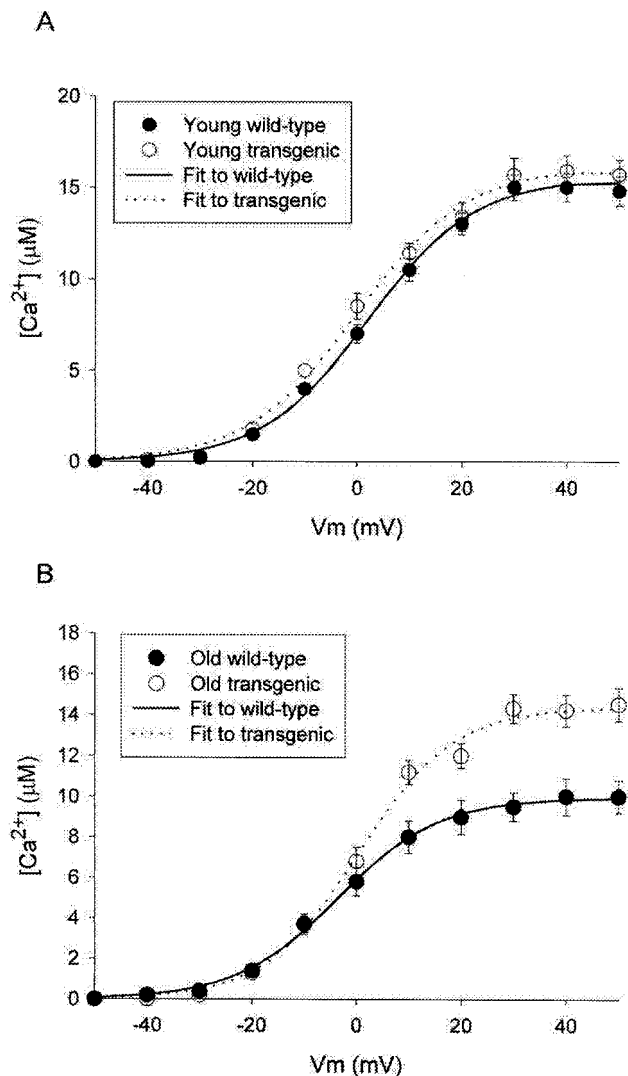


FIGURE 4 Intracellular  $\text{Ca}^{2+}$  concentration-membrane voltage relationship recorded in FDB muscle fibers from young wild-type and IGF-1 transgenic (A) and old wild-type and IGF-1 transgenic (B) mice. The data points were fitted to a Boltzmann equation (Eq. 1).

in differentiated myotubes (Wang et al., 1999b) to an extent similar to that reported in transgenic mice. The comparison between these two models is not simple because the availability of IGF-1 to interact with the IGF-1R through an autocrine/paracrine mechanism in the transgenic model is not known.

In summary, the age-dependent reduction in charge movement and peak myoplasmic  $\text{Ca}^{2+}$  concentration recorded in fibers from the FDB muscle is associated with a decreased DHPR $\alpha_{1S}$  and RyR1 gene expression (Zheng et al., 2001), phenomena that can be prevented by skeletal muscle IGF-1 overexpression. The impact of these events on single muscle fiber contractility needs to be addressed by measuring specific contraction force and peak intracellular

## Ca<sup>2+</sup> simultaneously in single muscle fibers from wild-type and IGF-1 transgenic mice.

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