Research article

Exercise training effects on skeletal muscle plasticity and IGF-1 receptors in frail elders

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Abstract

Age-related sarcopenia inhibits mobility, increasing the risk for developing many diseases, including diabetes, arthritis, osteoporosis, and heart disease. Tissue plasticity, or the ability to regenerate following stress, has been a subject of question in aging humans. We assessed the impact of 10-weeks of resistance training on markers of skeletal muscle plasticity and insulin growth factor-1 (IGF-1) receptor density in a sub sample of subjects who, in an earlier study, demonstrated enhanced immunohistochemical labeling of IGF following resistance training. Muscle biopsies from the vastus lateralis of five elderly men and women were taken prior to and following 10 weeks of resistance training ($N = 3$) or a control period ($N = 2$). Immunogold labeling and quantitative electron microscopy techniques were used to analyze markers of IGF-1 receptor density and tissue plasticity. The experimental subjects showed a $161 \pm 93.7\%$ increase in Z band damage following resistance training. Myofibrillar central nuclei increased $296 \pm 120\%$ ($P = 0.029$) in the experimental subjects. Changes in the percent of damaged Z bands were associated with alterations in the presence of central nuclei ($r = 0.668$; $P = 0.0347$). *Post hoc* analysis revealed that the relative pre/post percent changes in myofibrillar Z band damage and central nuclei were not statistically different between the control and exercise groups. Exercise training increased myofibrillar IGF-1 receptor densities in the exercise subjects ($P = 0.008$), with a non-significant increase in the control group. Labeling patterns suggested enhanced receptor density around the Z bands, sarcolemma, and mitochondrial and nuclear membranes. Findings from this study suggest that the age-related downregulation of the skeletal muscle IGF-1 system may be reversed to some extent with progressive resistance training. Furthermore, skeletal muscle tissue plasticity in the frail elderly is maintained at least to some extent as exemplified by the enhancement of IGF-1 receptor density and markers of tissue regeneration

Introduction

Production of insulin-like growth factor-1 (IGF-1) is stimulated by liver growth hormone receptors and released to the circulation. Localization of the growth hormone receptor in skeletal muscle is predominantly cytoplasmic (Lincoln et al. 1998), however, the exact location of the IGF-1 receptor within muscle is a topic with sparse documentation. Once the IGF-1 hormone binds to the receptor on muscle fiber sarcolemma, it signals a cascade of events in the muscle itself to initiate protein synthesis. Should the IGF-1 receptor become saturated, a typical situation when IGF-1 receptor number decreases, yet circulating IGF-1 hormone remains the same, the events downstream from the receptor will cease. This metabolic chain of events becomes more predominant with age and compromises protein synthesis.

Human (Castaneda et al. 2000; Marcell et al. 2001) and animal (Edwall et al. 1989; Ullman et al. 1990) studies suggest that IGF-1 is an important regulator of skeletal muscle growth. Human investigations report that serum (Rollero et al. 1998) and skeletal muscle (Welle et al. 2002) concentrations of IGF-1 are lower in older adults. The IGF-1 receptor plays a dominant role in age-related downregulation of the endogenous IGF-1 system (Lincoln et al. 1998; Marcell et al. 2001, Welle et al. 2002) that directly affects skeletal muscle strength and mass (Rudman et al. 1990; Oudin et al. 1998; Alexandrides et al. 1989; Adams 1998). In the sedentary older adult the ability to upregulate growth factors in response to overload such as exercise is especially compromised (Underwood and Van Wyk 1985). Many lifestyle factors, including exercise and nutrition influence the IGF-1 system's regulation of myogenesis and muscle metabolism (Willis and Parkhouse 1994; Frost and Lang 2003; Fiatarone et al. 1999; Poehlman and Copeland 1990; Willis et al. 1997; Nicklas et al. 1995). The consequences of physical inactivity are strength impairment and loss of muscle mass associated with a progressive decline in functional mobility and an elevated risk for metabolic-related diseases, including osteoporosis, arthritis, diabetes, and cardiovascular disease. Willis and Parkhouse (1994) measured protein turnover rates in in-vitro isolated soleus preparations in acutely exercised mice with and without IGF-1 presence. The authors reported that muscle protein synthesis was elevated with and without IGF-1 administration 12 h following an acute exercise bout.

Furthermore, the degree of net protein degradation was intensity related.

We recently explored the effects of a 10-week progressive resistance-training program on muscle plasticity in frail elders, aged 72–98 years (Fiatarone et al. 1999). Post muscle biopsy specimens showed an increased appearance of IGF-1 and regeneration potential from baseline atrophy. The 257% increase in strength following resistance training was associated with a 141% increase in ultrastructural damage and a 491% increase in IGF-I immunofluorescence staining. Since the IGF-1 receptor plays a dominant role in muscle IGF signaling, we speculated that an increase in IGF-1 receptor number in association with markers of muscle damage and regeneration can expand existing knowledge regarding the response of the IGF-1 receptor to exercise stress in older adults. One aim of this study was to identify, localize, and quantify the IGF-1 receptor in elderly human skeletal muscle in relation to regeneration markers (Fiatarone et al. 1999). Muscle biopsies taken from the vastus lateralis of five elderly (85–98 years) men ($N = 3$) and women ($N = 2$) prior to and following 10 weeks of resistance training were prepared for electron microscopic examination of immunogold labeling samples.

Materials and methods

Subjects

The tissue samples used in this study were taken from the vastus lateralis muscle from a subset of five subjects (three men and two women) who volunteered in a randomized control trial of progressive resistance training (Fiatarone et al. 1999). Two subjects were placed in a multi-nutrient supplementation (S) group and three experimental subjects underwent progressive resistance training (E, $N = 2$) or a combination of S plus E ($N = 1$). All statistical analyses of untrastructural and IGF-1 staining in the larger scale study (Fiatarone et al. 1999) showed that the nutritional supplement use had no independent or interactive effect on any of these variables, lending support for collapsing subjects into exercise or no exercise groups. This subset of subjects in this study comprised the exercise ($N = 3$: two men and one woman) and control ($N = 2$: one man and one

woman) groups. All subjects were explained the nature of the procedures, and informed consent was obtained following the explanation.

All activities were carried out in the Human Physiology laboratory of the Jean Mayer USDA Human Nutrition Research Center of Aging at Tufts University, and the Hebrew Rehabilitation Center for the Aged (HRCA). Inclusion criteria required that all participants were residents of HRCA, 70 years of age or older, willing to be randomized to training and/or dietary supplementation or control groups and have a muscle biopsy taken before and after training. All subjects were given an explanation of the nature of the procedures and informed consent was obtained following the explanation. The human Investigation Review Committees at the New England Medical Center and the Human Nutrition Research Center on Aging approved the study (Fiatarone et al. 1999).

The larger group of participants in the earlier study ($n = 26$) (Fiatarone et al. 1999) were randomized into one of four treatment groups: 1) a 10-week course of lower body resistance training, 2) nutritional supplementation, 3) a placebo-controlled condition or 4) both exercise and nutritional supplementation. The exercise group engaged in three sets of eight repetitions at 50% of a recently determined repetition maximum (1-RM) for the hip and knee extensor muscles, 3 days per week for 10 weeks, using pneumatic resistance training equipment (Keiser Sports Health, Fresno, CA). The subjects taking nutritional supplement (Exceed; Ross laboratories, Columbus, Ohio) were given a 240-ml liquid administered daily that supplied 360 kcal in a 60% carbohydrate, 23% fat, and 17% soy-based protein formula. The supplement provided one-third of the recommended daily allowance of essential vitamins and minerals. The control subjects were given a non-nutritive placebo liquid (Crystal Light; Kraft General Foods, White Plains, NY) and recreational activities that did not have a resistant exercise component.

Muscle biopsy

Needle biopsy specimens (5-mm Duchenne needle with applied suction) from the non-dominant vastus lateralis muscle were taken under local anesthesia (1% xylocaine hydrochloride), at baseline and 4 to 6 days after the last resistance training session.

Tissue preparation

The preparation and analysis of muscle biopsy specimens took place at the University of Rhode Island's Energy Metabolism Laboratory under the approval of the Institutional Review Board. The tissue samples were prepared for immunogold labeling of human skeletal muscle IGF-1 receptors following procedures previously described by Knibbs et al. (1990). The detailed immunogold procedures are in press (Urso et al., *Scanning Journal*).

Immunogold procedures

Quantitation and immunocytochemical localization of IGF-I receptors followed methods used by Riley et al. (1988). A Philips 300 transmission electron microscope equipped with an Advanced Microscopy Techniques imaging capturing system projected the images onto a Sony monitor. Once a clear picture was obtained on the monitor, images were captured using a Macintosh IICI computer equipped with a Data Translation image grabbing board and NIH 1.55 image analysis. Gold particles within a prescribed area were then counted. Ten myofibrillar regions for each sample were used for quantitation of IGF-1 receptors. Approximately 150 micrographs were taken and quantified for IGF-I receptor densities.

Ultrastructural qualitative analysis and quantitation of Z band and central nuclei

Routine qualitative ultrastructural analysis was performed to define markers typical of muscle atrophy and/or tissue turnover (Tomonaga 1977). Ultrastructural damage, including broadened and/or disrupted Z-lines, focal loss of myofibrillar architecture and broadened subsarcolemmal areas, was observed in all samples before and after training. Quantitation of Z band damage used stereological methods that employed point counting by placing a Weibel 100-point isotropic semicircular test system over electron micrographs of longitudinally oriented muscle fibers exposed at a final magnification of 13,500 $\times$. Z band damage was expressed as the percent of total volume density of Z bands that showed evidence of damage. Central nuclei quantitation was accomplished by observing thick sections using a light microscope and examining tissue sections from identical tissue blocks

Table 1. Physical characteristics of subjects.

Subject	Gender	Group	Age (years)	Height (cm)	Weight (kg)
1	Male	Control	85	165.5	66.3
2	Male	Exercise	81	163.5	66.3
3	Male	Exercise	91	159.5	59.4
4	Female	Control	87	143.5	47.0
5	Female	Exercise	85	137.0	36.7
Mean $\pm$ S.D.			85.8 $\pm$ 3.63	133.8 $\pm$ 12.8	55.1 $\pm$ 13.0

used for electron microscopic examination of thin sections. These procedures were used in several earlier studies (Fiatarone et al. 1999; Manfredi et al. 1996; Fielding et al. 1993, 2000; Dean et al. 2000).

Statistical analysis

Since the nutritional supplement use had no independent or interactive effect on all microscopic measurements in the larger scale study (Fiatarone et al. 1999), the data on our subset of five subjects were collapsed into control (no exercise training) and experimental (exercise training) groups. Data were analyzed using a SigmaStat 6.0 package. Subject descriptive statistics were expressed as mean $\pm$ standard error of the mean. The Wilcoxon signed

rank test was used to compare pre-to-post differences within the control and experimental groups. The Mann–Whitney Rank Sum Test was applied to compare the percent pre vs post changes between the control and experimental groups. Univariate associations between variables were examined using Pearson's correlation coefficients.

Results

Subject characteristics

Subject baseline characteristics are shown in Table 1. The mean $\pm$ SD age of the study group was 85.8 $\pm$ 3.63 years and was not different in age from the

Table 2. Ultrastructural and immunogold markers of muscle damage, regeneration, and plasticity in frail elders following resistance training.

Subject	Percent of Z bands damaged			Central nuclei			IGFI receptor density		
	Pre	Post	Percent change (%)	Pre	Post	Percent change (%)	Pre	Post	Percent change (%)
Control A	25.4	18.5	−37.3	8.4	10.2	27.4	27.2	37.8	38.9
Control B	25.7	17.8	−44.3	2.76	4.56	65.2	40.3	70.6	75.2
Mean	25.6	18.2	−40.8	5.58	7.38	46.3	33.8	54.2	57.1
SEM	0.15	0.35	3.5	2.82	2.82	18.9	6.55	16.4	18.2
<i>P</i> value ¹			0.043			0.001			0.286
Exercise A	17.7	40.4	128	8.17	20.7	153	17.3	55.3	219
Exercise B	36.7	43.6	18.8	3.77	11.4	202	35.4	74.5	110
Exercise C	13.0	57.0	338	2.68	17.0	534			
Mean	22.5	47.0	161	4.87	16.4	296	26.4	65.0	165
SEM	7.25	5.09	93.7	1.68	2.70	120	9.05	10.7	54.5
<i>P</i> value ²			0.150			0.029			0.008
<i>P</i> value for [Percent change (%)] differences between groups			0.193			0.205			0.333

Data were collapsed into control and exercise groups since supplement used had no independent or interactive effect on all variables tested in the larger sample (Fiatarone et al., 1999, Table 4). *P* value¹ and *P* value² are *P* values for pre–post differences regarding percent (%) change for the control and exercise groups, respectively. *P* values for percent (%) change differences between groups refers to main effects of exercise on percent (%) change scores in all cases. A Wilcoxin Signed Rank Test was applied to percent (%) change within groups and Mann Whitney Rank Sum Test was applied to percent (%) change differences between groups.

subjects used in the larger scale training study ($15; 86.5 \pm 1.1$ years). The clinical characteristics of the sub-group (Table 1) were also similar to volunteers in our earlier study (Fiatarone et al. 1999).

Qualitative analysis

Light and electron microscopic analysis of skeletal muscle offered information regarding structural and ultrastructural features of frail skeletal muscle before and following 10 weeks of resistance training. The qualitative evaluation of frail subjects used in this study was compared to biopsy samples taken from younger healthy subjects in our laboratory, ranging in age from 20 to 50 years (Manfredi et al. 1991). Light microscopic examination of all subjects at baseline showed evidence of muscle fiber atrophy, central nuclei and nuclear chains, lipofuscin, and inflammatory cell infiltration. At the light and electron microscopic levels, various degrees of myofibrillar damage and sarcolemmal waviness suggested homeostatic disruption.

Quantitation of Z band damage and central nuclei

The control subjects who did not exercise for 10 weeks showed a significant decline ($P = 0.043$) in the percent of Z bands damaged (Table 2; mean $\pm$ SEM = $-40.8 \pm 3.5\%$). The three trained subjects who engaged in the exercise program showed remarkable

but non-significant increases in Z band damage of 128%, 18.8%, and 338% (Table 2; $161\% \pm 93.7\%$).

Central nuclei appeared in tissue samples of all subjects prior to the 10 weeks period of exercise Vs control (Table 2). The intervention period resulted in significant increases in the presence of central nuclei in the control ($46.3\% \pm 18.9\%$; $P = 0.001$) and exercise groups ($296\% \pm 119.7\%$; $P = 0.029$). However, *post hoc* analysis revealed that the pre/post percent changes in myofibrillar Z band damage and central nuclei were not statistically different between the control and exercise groups. The changes in Z band damage were associated with changes in central nuclei ($r = 0.668$, $P = 0.0347$).

IGF-I receptor localization

To aid in understanding the role that the IGF-1 receptor plays in skeletal muscle remodeling and maintenance, careful attention was paid to the localization of the particles. Within the cytoplasm, the greatest concentrations of IGF-1 receptors appeared in regions adjacent to the Z band (Figure 1). Gold labels for IGF-1 receptors were also seen along the sarcolemma, and mitochondrial (Figure 1) and nuclear membranes. The labeling of the gold particles appeared to be greater in all regions of the experimental subjects following resistance training. Because of the lack of uniformity of IGF-1 receptor labeling throughout the mitochondrial and cell border regions,

Figure 1. Electron micrograph of one subject taken at $91,000\times$ before and after 10 weeks of resistance training. Gold particles labeling IGF-1 receptors (arrows) are more sparse adjacent to the Z band (Z) prior to resistance training. Gold particles are also identified along the periphery of the mitochondria (M). The myofibrillar receptor density appears to be greater following training.

quantitation of the IGF-1 receptor is presented only in the myofiber region.

IGF-I receptor quantitation

As shown in Table 2, there was a significant effect of resistance training on skeletal muscle myofibrillar IGF-1 receptor density ($P = 0.008$). The muscle fiber IGF-1 receptor densities of the exercise trained adults increased $165\% \pm 54.5\%$ compared to a $57.1\% \pm 18.2\%$ in the control subjects (Table 2).

Discussion

Local overexpression of IGF-1 in exercised or injured muscle is indicative of rescue from age-related atrophy (Musaro et al. 2001) and compensatory growth and proliferation (Edwall et al. 1989; McKoy et al. 1999; Hill and Goldspink 2003; Barton et al. 2002; Musaro et al. 2004). Elevated levels of muscle IGF-1 protein in overexpressed myosin light chain/muscle IGF-1 (MLC/mIGF-1) transgenic mice are believed to remain in the muscle bed without entering the circulation (Musaro et al. 2001; Barton et al. 2002), although other isoforms may enter the systemic circulation. There is now increasing interest in the various isoforms of IGF-1 with some detailed reviews available (e.g. Spangenburg 2003; Shavlakadze et al. 2004, 2005; Winn et al. 2002). In a study using older mice, Musaro et al. (2001) found that IGF-1 transgenic expression sustained muscle hypertrophy and regeneration. The authors based their findings in part from the presence of central nuclei in muscle fibers and expression of myosin heavy chain (MHC) in the transgenic mice and not in the controls. These findings support the Fiatarone Singh et al. (Fiatarone et al. 1999) report of a 2.5-fold increase ($P = 0.0009$) in neonatal MHC and a $491\% \pm 137\%$ ($P = 0.0001$) increase in IGF staining in elderly adults following 10 weeks of resistance training. Ultrastructural Z band damage increased $141\% + 59\%$ ($P = 0.034$). The present study used a sub-sample from the Fiatarone Singh et al. study (15) and found a dramatic increase in Z band damage in the exercise group and a $40.8 \pm 3.5\%$ decline in Z band damage in the control subjects. The observed increase in muscle damage following resistance training was statistically associated with a greater occurrence of muscle fiber central nuclei. Several recent studies have reported the pres-

ence of central nuclei in aging muscle, attributing their presence with myofiber regeneration (McGeachie et al. 1993) and denervation (Larsson 1995) associated with compromised re-innervation (Carlson et al. 2001). The increased presence of central nuclei in concert with greater damage to the Z bands following resistance training suggests cellular events associated with injury and repair processes in response to the overload of resistance training.

Skeletal muscle IGF-1 availability may not play as major a role in regenerating muscle as does the IGF-1 receptor presence (Grounds and McGeachie 1999), especially in aging muscle where IGF-1 system downregulation has been reported in animals (Owino et al. 2001) and humans (Hameed et al. 2003). Dardevet et al. (1994) reported an 80% decrease in IGF-1 receptors in rat epitrochlearis muscle with aging with no associated decline in IGF-1 mRNA, suggesting receptor degradation with age. Martineau et al. (1999) observed an age-related downregulation of the IGF-1 system at the IGF-1 receptor site. Clearly, the resistance to IGF-1 in aged rodents is associated with a dramatic decline in receptor numbers (Willis et al. 1997; Dardevet et al. 1994). It is speculated that the preservation of healthy muscle phenotype with aging plays a vital role during regeneration in old MLC/mIGF-1 mice (Musaro et al. 2001), and that early muscle regeneration is regulated by local and not systemic events (Martineau et al. 1999). The maintenance of muscle mass with age preserves healthy muscle phenotype and minimizes downregulation of the IGF-1 system, in particular the IGF-1 receptor's role in hypertrophy (Grounds 2002; Barton-Davis et al. 1998). Progressive resistance training can effectively prevent myofiber atrophy and preserve fast-twitch fibers and motor units in adults well into the sixth decade (Fiatarone et al. 1999; Klitgaard et al. 1990; Fiatarone and Evans 1993; Fiatarone et al. 1994). Klitgaard et al. (1990) examined vastus lateralis muscle biopsies from young and elderly swimmers, runners, strength-trained, and sedentary subjects and reported a higher content of myosin heavy chain type -1 and slow myosin heavy chain type -2 in sedentary elderly adults compared to young adults. Their findings that older adults possessed larger mean slow twitch fiber area in association with selective atrophy of fast twitch fibers suggest possible cessation of activities that stress the fast twitch motor units. Most striking was the finding that the older strength trained

subjects had maximal isometric torques, speed of movements, cross-sectional areas, and myosin and tropomyosin isoform content identical to their younger counterparts and in contrast to the old swimmers, runners, and sedentary adults.

Although the sample size employed in this study was limited, the use of immunogold to label ultrastructures is a powerful tool that provides valuable information about the IGF-1 receptor in human skeletal muscle. The use of this method for IGF-1 receptors has not been reported in the literature to date. An increase in IGF-1 receptor density accompanied by exercise induced muscle damage and the increased presence of central nuclei also supports earlier suggestions that the IGF-1 receptor plays a role in muscle regeneration (Musaro et al. 2004; Grounds and McGeachie 1999; Martineau et al. 1999). The evidence that exercise increases IGF-1 receptor numbers supports a central role for IGF-1 in myofiber hypertrophy (Adams 1998; Musaro et al. 2001) and also suggests a central role of IGF-1 in the upregulation of endogenous myosin isoforms. The upregulation of myosin isoforms appears to be a dominant factor regarding the metabolic response to overload in animal (Owino et al. 2001) and human studies (Fiatarone et al. 1999; Hameed et al. 2003; Dardevet et al. 1994).

Muscle biopsy samples of frail elders with compromised mobility show ultrastructural evidence of selective atrophy of slow-twitch fibers, widened sarcoplasmic spaces, low actin-myosin filament density, and elevated Z band damage when compared young healthy adults (Fiatarone et al. 1999; Manfredi et al. 1991). These anatomical features of tissue damage in association with a lower muscle mass to body weight ratio in the elderly may contribute to an elevated inflammatory response to eccentric induced muscle damage compared to their younger counterparts (Manfredi et al. 1991; Hameed et al. 2003). Early adaptation to resistance training includes muscle damage, central nucleation, the presence of developmental myosin isoforms (Fiatarone et al. 1999), and enhance IGF-1 receptor numbers as part of the remodeling process that leads to muscle regeneration. Thus, age-related sarcopenia associated with inactivity, nutritional status, and advanced age (Fiatarone and Evans 1993; Fiatarone et al. 1994, 1999) can be modified when progressive resistance training is used as rehabilitative or preventive measure. The biological events associated with muscle regeneration and

strength and mobility gains in active elders suggest that muscle plasticity is maintained to some degree in senile muscle.

This study reported a significant association between Z band damage and central nuclei ($r = 0.668$, $P = 0.0347$) and is the first to associate markers of muscle damage with increases in IGF-1 receptor density in skeletal muscle of frail elders. The localization of the IGF-1 receptor in the mitochondrial and nuclear membranes and the sarcolemma, with the greatest densities located around the myofibrillar Z and I bands, added specificity to earlier reports of IGF-1 receptors localization.

Findings from this study imply that the age-related downregulation of the skeletal muscle IGF-1 system may be reversed to some extent with progressive resistance training. The exercise stress placed on the elderly muscle resulted in enhanced tissue damage and a greater presence of central nuclei and myofibrillar IGF-1 receptors, suggesting that frail elders maintain some degree of tissue plasticity and regenerative capability. Future research efforts that focus on possible associations between human growth hormone, the IGF-1 receptor, and muscle protein metabolism (Nindl et al. 2003) in elders involved in properly prescribed exercise regimes (Frontera et al. 1988) can provide valuable information about the intriguing question of muscle plasticity in older adults.

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