Restoration of insulin-like growth factor I action in skeletal muscle of old mice

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Willis, P. E., S. G. Chadan, V. Baracos, and W. S. Parkhouse. Restoration of insulin-like growth factor I action in skeletal muscle of old mice. *Am. J. Physiol.* 275 (*Endocrinol. Metab.* 38): E525–E530, 1998.—This study examined the effects of long-term chronic voluntary wheel exercise on the ability of insulin-like growth factor I (IGF-I) to stimulate rates of protein synthesis in the soleus muscle of old C57Bl/6 mice. Factors contributing to any changes in hormone action were analyzed at the level of hormone receptor binding, protein content, and gene expression. Chronic exercise resulted in an increased skeletal muscle mass (10–22%) and a 56% increase in IGF-I-stimulated rates of protein synthesis ($P < 0.05$). IGF-I receptor mRNA was increased 46%, IGF-I receptor protein was increased 65%, and the binding capacity of the IGF-I high-affinity site was increased sixfold ($P < 0.05$) with chronic wheel exercise. Insulin receptor protein content was decreased 35% ($P < 0.05$), whereas GLUT-4 content was increased 47% with chronic exercise ($P < 0.05$). This study demonstrates that old animals retain a plasticity for IGF I receptor and glucose transporter expression that may have valuable physiological consequences.

protein synthesis; insulin-like growth factor I receptors; aging; insulin-like growth factor receptor mRNA

INSULIN-LIKE GROWTH FACTOR I (IGF-I) is an anabolic hormone that stimulates DNA synthesis, cell proliferation, protein synthesis, and glucose transport (26). The main role of IGF-I in skeletal muscle is in the promotion of protein synthesis, and decline in the circulating levels of this hormone with aging is thought to be a contributing factor in age-associated skeletal muscle atrophy (24). However, recent studies (6, 27, 28) have shown that IGF-I is unable to stimulate protein synthesis and amino acid transport in aged rat and mouse muscles. The aged rat skeletal muscle has also demonstrated resistance to insulin, suggesting that the resistance to the action of these two hormones may occur in parallel with aging.

Despite this observation, the degree of resistance to IGF-I or insulin varied depending on the metabolic process concerned in the aged rat skeletal muscle. Specifically, age-associated resistance to the stimulation of protein synthesis was greater for IGF-I than insulin, whereas the declines in IGF-I or insulin-mediated glucose transport were similar (6). The age-associated skeletal muscle resistance to the actions of IGF-I and insulin in rats was associated with large decreases in IGF-I receptor number, whereas insulin receptor levels were unchanged (6). Similarly, aged mouse muscle that also showed resistance to the action of IGF-I had a large decline in IGF-I receptor number (27). These findings suggest that the mechanisms accounting for the parallel resistance to IGF-I and insulin observed with aging may be different, despite the fact that these two hormones share common postreceptor events, including the phosphorylation of insulin receptor substrate 1 and activation of phosphatidylinositol 3-kinase (PI3-kinase).

We have recently shown a restoration in the ability of IGF-I to stimulate protein synthesis in IGF-I-resistant aged mouse skeletal muscle after a single acute bout of moderate intensity aerobic exercise (27, 28). The increased action of IGF-I was associated with increases in the IGF-I receptor binding capacity, affinity, and IGF-I receptor mRNA, with no significant increase in IGF-I receptor protein content (27). However, the effects of exercise training on IGF-I responsiveness in IGF-I-resistant tissues remains to be examined. In contrast to IGF-I, the effects of exercise training on insulin action in insulin-resistant tissues have been studied extensively. Exercise training has been shown to improve glucose tolerance in insulin-resistant tissues (4) and to improve the responsiveness of skeletal muscle to insulin (2, 11, 20). The improved insulin action is linked to concurrent training-induced elevations in GLUT-4 content, which are thought to parallel improvements in oxidative capacity (2, 19, 23). Whether exercise training similarly restores IGF-I action and the mechanisms for any alteration in the responsiveness to IGF-I remains to be examined.

Thus one of the goals of this study was to employ exercise wheels over an extended period to assess the effects of chronic activity on IGF-I action. The use of voluntary wheel exercise for aged animals has the additional advantage of modestly increasing the level of activity of senescent animals, a situation not too different from the aims of exercise programs geared for seniors. Specifically, we hypothesized that long-term chronic physical activity in old animals would restore the ability of IGF-I to stimulate skeletal muscle protein synthesis rates. We therefore analyzed the effects of long-term chronic voluntary wheel exercise on IGF-I action in skeletal muscle of aged mice. The factors contributing to any changes in IGF-I action were analyzed at the level of hormone receptor binding, protein content, and gene expression. Insulin receptor content was also assessed to identify any parallel changes in this protein with changes in IGF-I receptor

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protein. GLUT-4 content was assessed to determine whether long-term physical activity could overcome the aged skeletal muscles' apparent lack of plasticity associated with low training intensities.

METHODS

Chemicals. L-[2,6-^{3}H]phenylalanine, ^{125}I-labeled IGF-I, and [α-^{32}P]CTP were obtained from Amersham (UK). Plasmid vector pGEM3, *Eco*R I, and SP6 polymerase were obtained from BRL GIBCO (Burlington, ON, Canada). Recombinant IGF-I was obtained from Calbiochem (San Diego, CA). Rabbit polyclonal IgG specific for the β-chain of the IGF-I receptor, mouse monoclonal IgG specific for the insulin receptor β-chain, and goat polyclonal IgG specific for GLUT-4, and the secondary antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). All other reagents were from Sigma (St. Louis, MO).

Animals. Female C57Bl/6 mice were obtained at 12 mo of age from Charles River Breeding Laboratories (QC, Canada), housed under laminar airflow, maintained on a 12:12-h light-dark cycle (lights on 5:00 AM) with food (PMI Feeds Laboratory Rodent Diet 5001, St. Louis, MO) and water available ad libitum. Animals were maintained in our holding facilities for 3 mo after arrival to control for the stress of transport and differences in housing and dietary conditions. Animals were randomly assigned to either control or chronically exercised groups (n = 23–27 for each group). Chronically exercised animals were individually housed in cages with a freely rotating exercise wheel for up to 9 mo beginning at 15 mo of age. Animal activity patterns were continuously monitored as previously described (18). Exercise wheels were removed 48 h before the animals were killed. All animals were fasted for 12 h before being killed to avoid the influence of the last feeding on IGF-I action. All animals were killed at the same time of day. Citrate synthase, phosphofructokinase, and malate dehydrogenase activities were measured in control and chronically exercised soleus muscle homogenates according to the methods of Alp et al. (1). Animals were killed at 18, 21, and 24 mo of age, and the soleus, gastrocnemius, and extensor digitorum longus were carefully dissected to assess muscle mass.

Effects of IGF-I on soleus muscle rates of protein synthesis. Rates of protein synthesis were determined as described previously (27). Briefly, soleus muscles were preincubated in medium consisting of Krebs-Ringer bicarbonate (KRB) buffer [119 mM NaCl, 4.8 mM KCl, 1.25 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.24 mM NaH_2PO_4, and 1.0 mM $CaCl_2$ (pH 7.4)], 5 mM glucose, 0.05% BSA (99% fatty acid free; wt/vol), 2 mM HEPES-NaOH at pH 7.4, 0.3 μg/ml chloramphenicol, and 20 nM IGF-I. Muscles were then transferred into fresh medium of the same composition containing 0.1 μCi/ml L-[2,6-^{3}H]phenylalanine (55 Ci/mmol) and 1 mM unlabeled phenylalanine for 3 h. Contralateral muscles were incubated in the absence of IGF-I. Protein synthesis was measured by calculating the rate of incorporation of [^{3}H]phenylalanine into TCA-precipitable material, and intracellular phenylalanine was measured fluorometrically by HPLC analysis using methods of Jones and Gilligan (15). Muscle protein synthesis rates were calculated from protein-bound radioactivity and intracellular specific activity of phenylalanine for each muscle. Intracellular specific activity was not significantly different between groups and across treatments.

IGF-I receptor gene expression. Total RNA was extracted by the method of Chomczynski and Sacchi (5) and IGF-I receptor expression was determined as previously described (27). Briefly, a 265-bp rat IGF-I receptor cDNA containing bases complementary to 15 bases of the 5′-untranslated sequence as well as to the region encoding the signal peptide and the first 53 amino acids of the α-subunit (kindly provided by Dr. Werner, National Institutes of Health, Bethesda, MD) was subcloned into the plasmid vector pGEM3. The plasmid was linearized with *Eco*R I, purified, and used to generate [α-^{32}P]CTP-labeled antisense RNA with SP6 polymerase. Solution hybridization-RNase protection assays were carried out on 15 μg of total RNA. Protected hybrids were extracted with phenol-chloroform, ethanol precipitated, and separated electrophoretically on a 6% polyacrylamide-6 M urea denaturing gel. RNA isolated from *Caenorhabditis elegans* or tRNA acted as the negative control. Rat poly(A)$^+$ RNA acted as the positive control. Autoradiographic films were taken from each gel and quantified using scanning densitometry.

IGF-I receptor binding studies. A solubilized crude membrane fraction was isolated from mouse gastrocnemius muscle, and binding experiments were performed essentially as described in Willis et al. (27). Briefly, ^{125}I-IGF-I binding to 400–800 μg of the solubilized crude membrane fraction was carried out in a final volume of 200 μl of binding buffer [50 mM HEPES (pH 8.0), 150 mM NaCl, 0.3 g/l BSA, 100 U/ml bacitracin, and 0.25% Triton X-100] in the presence of 0.014 nM ^{125}I-IGF-I (2,000 Ci/mmol) and with increasing concentrations of unlabeled IGF-I (10^{-10} to 10^{-6} M). The bound receptors were precipitated, the supernatant was aspirated, and the radioactivity of the pellet was counted. Nonspecific binding (~10–20%) was estimated as that occurring in the presence of 10^{-6} M unlabeled IGF-I, and this was subtracted from the total activity. Displacement binding curves were generated, and the nonlinear curve-fitting LIGAND program (17) was used to obtain the equation of the line of best fit for the ligand binding experiments.

Immunoblotting. Crude membrane protein (200 μg) was mixed with 1× urea buffer [2% β-mercaptoethanol, 8 M urea, 0.054 M SDS, 0.1 M Tris (pH 6.8)], boiled for 5 min, and separated by SDS-PAGE on 10% polyacrylamide gels. Proteins were then transferred to polyvinylidene difluoride membrane (PVDF), visualized with Ponceau red (0.2% in 3% TCA, Sigma), washed in 50 mM Tris (pH 7.4)-150 mM NaCl-0.5% Triton X-100 (TTBS), and subsequently blocked in TTBS containing 3% BSA. After washing, membranes were incubated overnight with primary antibody (rabbit polyclonal IgG specific for β-chain of the IGF-I receptor, mouse monoclonal IgG specific for insulin receptor β-chain, goat polyclonal IgG specific for GLUT-4, or rabbit polyclonal IgG specific for p85α subunit of PI3-kinase; primaries 1:3,300 and secondaries 1:30,000) at 4°C. After three washes in TTBS, membranes were incubated at room temperature with the appropriate secondary antibody (anti-mouse, anti-goat, or anti-rabbit IgG-horseradish peroxidase) and subsequently washed. The proteins were visualized by the enhanced chemiluminescence and quantitated by laser densitometry (LKB Pharmacia).

Statistical analysis. For the determination of differences in rates of protein synthesis between contralateral muscles in the presence or absence of 20 nM IGF-I, a paired Student's t-test was used. Comparisons between control and chronically exercised groups were determined using an unpaired Student's t-test. $P \leq 0.05$ was considered statistically significant.

RESULTS

The activity patterns of the mice exposed to voluntary exercise wheels are shown in Table 1. Each value represents the average for at least 11 animals exercising over a 3-mo period. The number of runs per hour for each animal did not differ significantly from 15 to 24 mo of age. However, values for average run time (−27%),

Table 1. *Voluntary exercise data for the chronically exercised animals*

Age, mo	No. of Runs/h	Duration of Run, min	Velocity of Run, m/min	Distance Run, m/day
16–18	2.9 ± 0.2	9.7 ± 0.5	12.5 ± 0.5	4,220 ± 267
19–21	2.9 ± 0.2	8.6 ± 0.7*	11.0 ± 0.7*	3,292 ± 373*
22–24	2.6 ± 0.1	7.1 ± 0.4*	8.8 ± 0.5*	1,949 ± 263*

Values are means ± SE for 18-mo-old animals ($n = 31$), 21-mo-old animals ($n = 25$), and 24-mo-old animals ($n = 11$). Voluntary exercise data are for number of runs per hour, average run time of each run, average velocity of each run, and total distance run per day for mice aged 16–24 mo. Data are only tabulated for 12-h dark cycle. *Significantly different from 16- to 18-mo-old data, $P < 0.05$.

Table 2. *Effects of chronic exercise on animal and soleus muscle mass, PFK, CK, and CS activities in 24-mo-old mice*

	Control	Chronic Exercise
Animal wt, g	31.3 ± 0.9	31.2 ± 0.8
Soleus muscle wt, mg	7.9 ± 0.3	9.6 ± 0.4*
Activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$		
Soleus PFK	24.3 ± 1.4	29.0 ± 2.6
Soleus CK	364 ± 19	383 ± 33
Soleus CS	31.2 ± 1.1	35.4 ± 2.5

Values are means ± SE; $n = 6$ animals/group. PFK, phosphofructokinase; CK, creatine kinase; CS, citrate synthase. *Significantly different from control, $P < 0.05$.

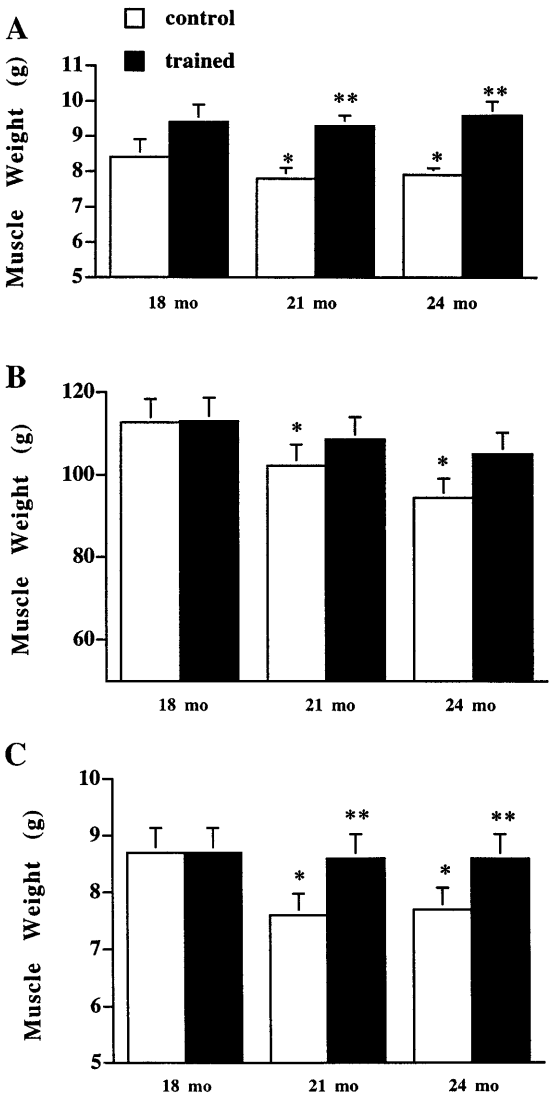

Fig. 1. Soleus (*A*), gastrocnemius (*B*), and extensor digitorum longus (*C*) muscle mass in control and chronically exercised animals as a function of age. Each value represents mean ± SE for $n = 6$ animals/group for 18-mo-old animals, $n = 12$ animals/group for 21-mo-old animals, and $n = 12$ for control and $n = 13$ for trained 24-mo-old animals. *Significantly different from control animals of same age, $P < 0.05$; **significantly different from age-matched control, $P < 0.05$.

average running velocity (−30%), and average total distance (−54%) that each animal ran per day were significantly less with increasing age. No significant differences in muscle mass were noted between groups at 18 mo of age (Fig. 1). By 21 mo of age, both the soleus (19%) and extensor digitorum longus (13%) muscles were significantly larger than their age-matched controls (Fig. 1). After the 9 mo of exposure to the exercise wheels, the soleus muscle mass was increased 22% and the extensor digitorum longus muscle mass increased 12% relative to controls ($P < 0.05$). Although the chronically exercised gastrocnemius muscle was larger than age-matched controls at both 21 (6%) and 24 mo of age (10%), these differences failed to reach significance (Fig. 1). Overall body mass was not altered by the chronic exercise (Table 2), whereas the soleus muscle mass was significantly increased in the chronically exercised animals compared with the control animals (Fig. 1). Despite the increase in soleus muscle mass, citrate synthase, phosphofructokinase, and creatine kinase activities were unaffected by the chronic physical activity (Table 2).

IGF-I was unable to stimulate rates of protein synthesis in the control animals (Fig. 2). In contrast, chroni-

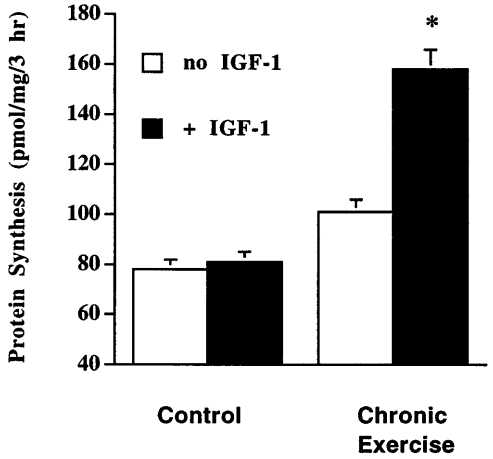

Fig. 2. Rates of protein synthesis in control (C) and chronically exercised (E) old animals determined in presence or absence of 20 nM insulin-like growth factor I (IGF-I). Measurements were made 48 h after cessation of chronic exercise. Each value represents mean ± SE for $n = 6$ animals for C and $n = 5$ animals for E. *Significantly different from C group (no IGF-I), $P < 0.05$.

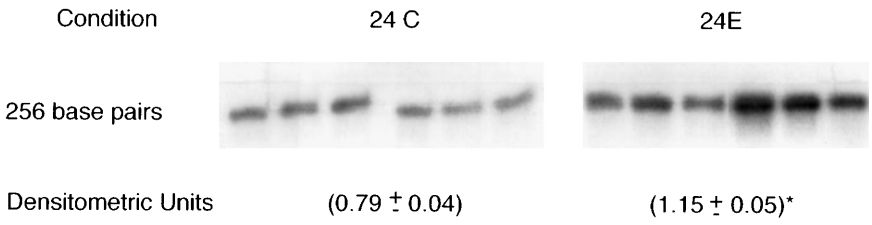

Fig. 3. IGF-I receptor expression by solution hybidization-RNase protection assay in gastrocnemius muscles of old C and E animals. Measurements were made 48 h after animals were removed from exercise wheels. Results are expressed in arbitrary units measured by scanning densitometry. Values represent means ± SE for $n = 6$ animals/group. *Significantly different from C values, $P < 0.05$.

cally exercised animals' rates of protein synthesis were significantly increased (58%) in the presence compared with the absence of IGF-I (Fig. 2). IGF-I receptor expression in the gastrocnemius muscles of old animals is illustrated in Fig. 3. The gastrocnemius muscles of the chronically exercised animals demonstrated a significant 46% increase in mRNA levels relative to the control gastrocnemius muscles, with no change in total RNA (Table 3). Scatchard analysis of the chronically exercised animals' IGF-I displacement curves revealed a significant approximately sixfold increase in binding capacity and a 75% decrease in affinity of the high-affinity site (Table 3). IGF-I receptor protein content was significantly increased (66%), whereas insulin receptor protein content was significantly decreased 35% (Fig. 4). GLUT-4 protein content was increased 47% in the exercised animals (Fig. 4).

DISCUSSION

The results of the present study have demonstrated that low-intensity exercise can alter IGF-I action, IGF-I, and insulin receptor content as well as GLUT-4 content in aged skeletal muscle. These current data are the first to show that chronic voluntary wheel exercise can restore IGF-I action in old mouse muscle that before exercise was unable to respond to the anabolic actions of IGF-I. IGF-I receptor mRNA was elevated with chronic activity and was associated with an increase in IGF-I binding capacity and IGF-I receptor protein. The restoration of IGF-I action may have contributed to the increased muscle mass observed in the chronically exercised mice.

Changes in IGF-I receptor binding could be contributing to the enhanced IGF-I action after chronic exercise in skeletal muscles of the old animals. Steady-state binding to the membrane-enriched fraction with IGF-I exhibited curvilinear Scatchard plots. Although this could be explained by negative cooperativity (7), high-affinity IGF-I receptors have been successfully separated from the low-affinity binding sites (25). Chronic wheel exercise resulted in a sixfold increase in high-affinity site binding capacity. This finding of a large increase in high-affinity binding site number is consistent with the acute exercise-induced 10-fold increase in binding capacity of the high-affinity site previously demonstrated in old mice (27). The large increase in high-affinity site binding capacity in this study occurred despite the observation that IGF-I receptor mRNA and IGF-I receptor protein were only increased 46 and 65%, respectively. This finding may be attributed to the 100–1,000 times more IGF-I low-affinity than high-affinity sites (25). Thus any selective increase in the capacity of the high-affinity site would have minimal effect on IGF-I receptor protein as determined by immunoblots.

Our results provide evidence that the increases in IGF-I receptor expression as a consequence of long-term exercise result from regulation at the level of the IGF-I receptor gene as well as possible regulation at the mRNA and/or protein turnover levels. Because IGF-I receptor mRNA per unit total cellular total RNA was

Table 3. *Receptor binding and gene expression in control and chronically exercised 24-mo-old mice*

	Control	Chronic Exercise
Crude membrane protein, mg/g tissue	54.0 ± 3.2	55.9 ± 2.7
IGF-I HA binding capacity		
nmol/mg membrane protein	0.052 ± 0.010	0.300 ± 0.128*
$nM^{-1} \cdot (10^9\ M^{-1})$	27.4 ± 5.2	6.9 ± 2.9*
Total RNA, μg/g tissue	595 ± 46	624 ± 27
IGF-I receptor mRNA		
AU/15 μg total RNA	100 ± 5	146 ± 7*
AU/g tissue	3,967 ± 198	6,060 ± 242*

Values are means ± SE; $n = 6$ animals/analysis. Separate animals were used for the binding and mRNA analyses. IGF-I, insulin-like growth factor I; HA, high affinity; AU, arbitrary units. *Significantly different from control, $P < 0.05$.

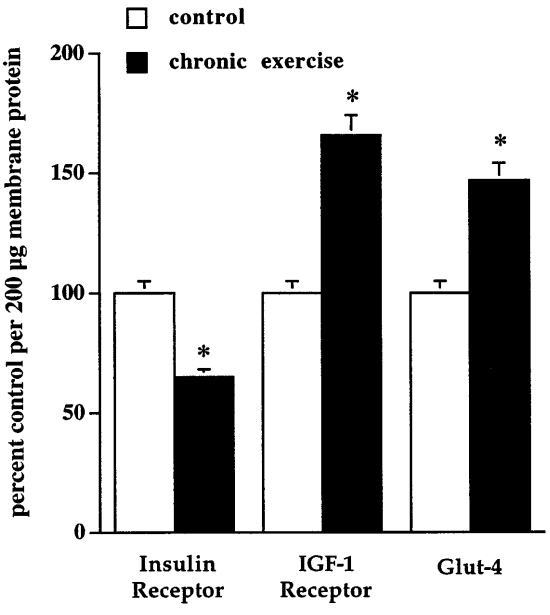

Fig. 4. Insulin receptor, IGF-I receptor, and GLUT-4 protein content as assessed by immunoblots of C and E animals. Results are expressed in arbitrary units measured by scanning desitometry. Values represent means ± SE for $n = 6$ animals/group. *Significantly different from C values, $P < 0.05$.

elevated, the increase in IGF-I receptor content with exercise is likely to be due to an increase in gene transcription. However, we cannot rule out the possibility that exercise resulted in changes in IGF-I receptor protein stability and/or IGF-I receptor mRNA turnover. The chronic exercise also induced a small decline in binding affinity (75%) of the high-affinity site. This finding is contrasted by our acute exercise study in which we observed a large increase (14-fold) in affinity (27). It thus appears that the acute and chronic exercise elicited different responses in terms of their effects on IGF-I affinity.

In this study, levels of receptor protein for insulin and IGF-I do not respond similarly to the chronic exercise. Insulin receptor levels were decreased by the chronic activity, whereas IGF-I receptor protein content was increased. The decrease in insulin receptor protein content is in contrast to other studies reporting either no change (12) or an approximate doubling of insulin receptor number (8) as assessed by ligand binding after chronic exercise in young rats. These differential effects of exercise on insulin receptor protein content may be attributed either to the higher relative intensity exercise bouts used for younger animals or, alternatively, to an age-associated difference in the ability of skeletal muscle to upregulate insulin receptor levels. The necessity of increasing IGF-I receptor protein content may be linked to its age-associated declines (27) and its more important role of stimulating protein synthesis as opposed to its role in increasing glucose transport (21). In contrast, impaired insulin action with aging in skeletal muscle has been associated with decreases in insulin-stimulated tyrosine kinase activity, insulin receptor autophosphorylation, decreased GLUT-4 levels, and decreased glucose transporter translocation to the plasma membrane (3). The increased GLUT-4 content may compensate for any age-associated decreases in insulin receptor function, since increases in insulin responsiveness have been associated with increases in GLUT-4 transporter content (2, 11, 20).

The exercise-induced increase in GLUT-4 content observed in this study is similar in magnitude to other exercise studies employing adult (9–11) and old rats (29). However, other investigators failed to demonstrate a significant increase in GLUT-4 in aged rats with exercise (13, 16). The finding of a significant increase in our study may be attributed to the threefold longer exercise program employed and the finding that GLUT-4 decreases with age in the rat (13, 16). Thus the increases that we observed in GLUT-4 may be due not only to the chronic activity but also to an attenuation of this age-associated decline in GLUT-4 content.

The increase in skeletal muscle mass as a result of the long-term activity may have been due to the nearly twofold increase in the action of IGF-I. However, other factors, such as a decrease in the rates of protein degradation, may also have contributed to the increased muscle mass. This increase in muscle mass occurred without a substantial increase in citrate synthase activity. This finding is in agreement with the observations of Gulve et al. (13) and implies that only a minimal intensity level of exercise is necessary to prevent age-associated skeletal muscle atrophy.

In summary, chronic activity increased muscle mass and restored the ability of old skeletal muscle to respond to IGF-I. This indicates that exercise may be involved in the prevention of IGF-I insensivity in aged animals. This restoration of IGF-I action was associated with increases in IGF-I binding, IGF-I receptor mRNA, and IGF-I receptor protein. In contrast, insulin receptor protein content was decreased after training, implying potentially different mechanisms for the parallel insulin and IGF-I resistance frequently observed in aged skeletal muscle. This study demonstrates that old animals retain a plasticity for IGF-I receptor and glucose transporter expression that may have valuable physiological consequences.

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