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Increase in muscle IGF-I protein but not IGF-I mRNA after 5 days of endurance training in young rats

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Eliakim, Alon, Mark Moromisato, David Moromisato, Jo Anne Brasel, Charles Roberts, Jr., and Dan M. Cooper. Increase in muscle IGF-I protein but not IGF-I mRNA after 5 days of endurance training in young rats. *Am. J. Physiol.* 273 (Regulatory Integrative Comp. Physiol. 42): R1557–R1561, 1997.—Five days of treadmill training in rats leads to increased muscle size and running time. This was used to examine the effect of exercise on circulating insulin-like growth factor I [IGF-I; radioimmunoassay (RIA)], local muscle (hindlimb) IGF-I (by RIA), and muscle IGF-I mRNA (by ribonuclease protection assay). Eight-week-old female Sprague-Dawley rats were divided into three groups: control ($n = 10$); single-exercise test ($n = 10$), untrained but with one maximal exercise test at the end of the study; and training ($n = 16$), trained for 5 days and one maximal exercise test on day 6. There were no differences among the groups with respect to circulating IGF-I. Muscle IGF-I protein in trained rats (4.2 ± 1.5 ng/g of muscle tissue) was significantly greater than both control (0.27 ± 0.1 ng/g) and single-exercise test (0.62 ± 0.19 ng/g, $P < 0.05$ by analysis of variance). There was no difference among the groups in IGF-I mRNA gene expression. These data suggest that there is an early, marked, local muscle increase in IGF-I protein in response to exercise. This increase, however, may not be related to increased muscle IGF-I gene expression. Moreover, the IGF-I response was probably local in nature since it was not matched by any increase in circulating IGF-I.

exercise; growth factor; gene expression

EXERCISE STIMULATES MUSCLE hypertrophy, and recent investigations suggest that insulin-like growth factor-I (IGF-I) might mediate this process. It was originally postulated that IGF-I production was stimulated predominantly by growth hormone (GH), but it now appears that IGF-I production can occur independently of GH in a number of situations including exercise-associated increases in muscle IGF-I. DeVol et al. (8) demonstrated a muscle-work-related increase in IGF-I mRNA in hypophysectomized rats, and Zanconato et al. (20) showed that 4 wk of treadmill training led to increased IGF-I mRNA and IGF-I protein levels in rats when GH secretion was blocked by inhibition of GH-releasing hormone (GHRH). These observations sug-

gested that local muscle IGF-I might play an initiating role in the muscle response to exercise training.

Although the rat is commonly used to gauge the metabolic, anatomic, and even molecular responses to exercise training, most studies involve relatively long durations of exercise. Even seemingly brief exercise inputs, e.g., 4 wk or so, span a proportionately longer "metabolic-developmental" stage in the rat compared with humans. In an attempt to gain insight into the early growth factor responses to training, we recently developed a 5-day treadmill training protocol that led to increased hindlimb muscle weight in young (late pubertal) female rats (11). Using this model, we hypothesized that the 5 days of training would be accompanied by significant increases in gene expression and protein levels of IGF-I in the trained hindlimb.

METHODS

Sample population. The study was approved by the institutional review board at Harbor-University of California, Los Angeles, Medical Center, and all national principles of laboratory animal care were adhered to. Thirty-six, 8-wk-old female Sprague-Dawley rats participated in the study. All rats were housed in steel cages and kept on a 12:12-h light-dark schedule. Food and water were provided to all rats ad libitum. Rats were brought to the laboratory 7 days before the protocol began so that they could become familiar with their surroundings (e.g., treadmill) and handlers. The rats were weighed at the beginning and at the end of the experimental period.

Rats were divided randomly into three groups: 1) control group ($n = 10$), these animals were never exposed to the treadmill; 2) single-exercise test group ($n = 10$), these rats did not train, but performed a maximal exercise test at the end of the study protocol; and 3) training group ($n = 16$), these rats trained for 5 days and performed a maximal exercise test on day 6. We chose an unequal number because we anticipated from our past experience that 4 or 5 of the rats in the training group would not be willing to complete the training and/or perform a maximal test.

Protocol. The training protocol consisted of day 1, 21 m/min for 30 min; day 2, 21 m/min for 45 min; day 3, 24 m/min for 45 min; day 4, 24 m/min for 60 min; and day 5, 27 m/min for 60 min. The treadmill was maintained at 0% grade throughout the training protocol.

Maximal treadmill exercise test. As noted earlier, rats from the single-exercise test group and from the training group underwent a progressive treadmill exercise test to exhaustion at the end of the training period (*day 6*) to determine the functional effects of training. Because the single-exercise test group rats were unfamiliar with the treadmill, they were allowed 10-min periods of low-speed treadmill running on the last day of the experimental period. All animals were killed 4 h after the maximal exercise test. The whole hindlimb musculature (from its origin to its insertion) was carefully dissected from bone and other tissues and weighed.

Maximal tests were performed at 5% incline. A 4-min acclimation period was followed by an initial treadmill speed of 15 m/min for 4 min. Thereafter, treadmill speed was increased by 6 m/min every 4 min until the rats were unable to continue running. The 5 days of training led to an increased running time and decreased respiratory exchange ratio at maximal exercise, indicating that a functional improvement accompanied the increase in muscle weight. Although peak oxygen uptake tended to be greater in the trained rats, the increase did not achieve statistical significance (11).

Circulating GH and IGF-I. Radioimmunoassay (RIA) was used to measure rat serum GH using World Health Organization standard no. 66/217 polyclonal antisera generated in-house and human GH from National Institute of Diabetes and Digestive and Kidney Diseases for iodination purposes. The GH intra-assay variability was less than 10%, interassay variability was 12.6%, and the sensitivity was 0.5 $\mu\text{g/l}$. Double antibody RIA was performed to measure rat IGF-I serum concentrations, using the Diagnostic System Laboratories DBL 2900 kit. The intra-assay coefficient of variation was 3.3%, interassay coefficient was 5.4%, and sensitivity was 53 ng/ml. We used the acid-ethanol extraction method (6) to extract IGF-I from its binding proteins (BPs).

Solution hybridization ribonuclease protection assay. RNA extraction from skeletal muscles was performed according to the method of Chomczynski and Sacchi (4). RNA preparations were dissolved in sterile water and stored at -70°C . RNA was quantitated spectrophotometrically. The quantification and integrity of the RNA were then confirmed by visual inspection of the ethidium bromide-stained 28S and 18S rRNA in 10- μg aliquots of RNA that had been electrophoresed through agarose-2.2 M formaldehyde gels. Ribonuclease protection assays were performed as previously described in this and other laboratories (1, 20). Autoradiographs were scanned, and the intensity was digitized by computer. Typical gel results are shown in Fig. 1.

β -Actin. To determine the specificity of the training-induced changes in IGF-I gene expression, Northern blots of skeletal muscle mRNAs were hybridized to a rat β -actin cDNA probe that was labeled with [α - ^{32}P]dCTP by random priming.

Tissue IGF-I. IGF-I was extracted from the muscle according to the method described by D'Ercole et al. (7). Briefly, the whole right hindlimb musculature from each rat was carefully dissected and pulverized, and IGF-I was extracted by addition of 5 ml of 1 M acetic acid for each gram of tissue. After centrifugation, the supernatant was removed and subjected again to extraction with acetic acid. The two supernatants were then combined, frozen at -70°C , lyophilized, and reconstituted with 0.05 M tris(hydroxymethyl)aminomethane-HCl buffer (pH 7.8). The extract was clarified by centrifugation and frozen at -20°C . The RIA for IGF-I was carried out using the method described by Daughaday et al. (6) and using antibodies obtained from the National Institute of Diabetes and Digestive and Kidney Diseases National Hormone and Pituitary Program.

Statistical analysis. Analysis of variance (ANOVA) was performed on the results from the three groups. When the ANOVA was significant, modified post hoc *t*-tests were used to compare results among the groups. Data are presented as means \pm SE. $P < 0.05$ was considered statistically significant.

RESULTS

Circulating GH and IGF-I. At the end of the study period, there were no significant differences in circulating GH levels among the control (67.0 ± 29 ng/ml), single-exercise test (41.5 ± 9 ng/ml), and trained rats (65.3 ± 12 ng/ml). There were no significant differences in circulating IGF-I levels among the control (525.8 ± 32 ng/ml), single-exercise test (522.7 ± 23 ng/ml), and trained rats (540.0 ± 32 ng/ml).

Muscle IGF-I protein levels. ANOVA revealed a significant difference among the three groups. Post hoc analysis showed that muscle IGF-I was significantly greater ($P < 0.05$) in the trained rats compared with both the control and single-exercise test rats (Fig. 2). No significant difference was found in muscle IGF-I between the control and single-exercise test rats.

Muscle IGF-I mRNA. As previously described in this and other laboratories (1, 20) in liver, five major protected bands were observed with the IGF-I antisense probe. The smallest, at ~ 168 bp, represents exon 2 mRNAs. The remaining bands represent mRNAs derived from exon 1 and include a combination of mRNA initiated at *sites 1* and *2* (~ 530 bp), start *site 3* (~ 426 bp), alternatively spliced mRNA (~ 270 bp), and start *site 4* (~ 197 bp). In contrast, transcription initiation site usage differs in muscle compared with liver. In muscle, the predominant expression is found in exon 1

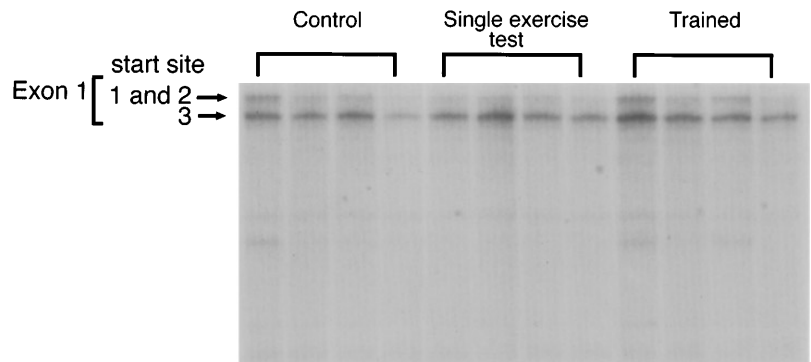


Fig. 1. Examples of solution hybridization-RNase protection assay using 20- μg aliquots of total RNA from skeletal muscles of individual rats. As previously described (see text), exon 1 start *sites 1-3* predominate in muscle. There were no differences in gene expression among control, single-exercise test, and trained groups.

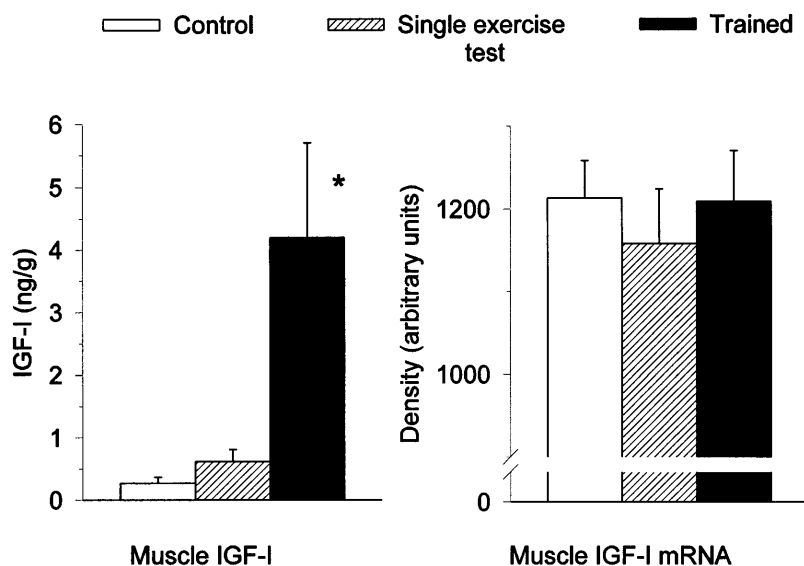


Fig. 2. Effect of a single maximal exercise test and 5 days of endurance-type treadmill training on muscle insulin-like growth factor I (IGF-I) levels and IGF-I gene expression. * $P < 0.05$, trained vs. control and/or single-exercise test groups.

start sites 1–3. Exon 2 expression is virtually insignificant in muscle (Fig. 1).

When calculated as the sum of IGF-I mRNA respective start site densities (all exon 1 start sites), no difference was found between the control, single-exercise test, and trained rats (Figs. 1 and 2).

β -Actin mRNA expression. There were no significant differences in muscle β -actin mRNA expression among the control, single-exercise test, and trained rats.

DISCUSSION

We had hypothesized that a relatively brief period of exercise training in young rats would lead to increases in both muscle IGF-I gene expression and protein. While we found remarkable increases in tissue IGF-I protein following 5 days of training, IGF-I mRNA levels were not affected either by 5 days of training or by a single maximal exercise bout (Figs. 1 and 2). Moreover, no changes in circulating IGF-I were observed after the training period. These results suggest that the early mechanisms of the training adaptation may involve translational or posttranslational increases in muscle tissue IGF-I. In addition, other nongenetic mechanisms could be considered, such as sequestration of circulating IGF-I in muscle tissue (perhaps by BPs) or by exercise-associated increases in muscle blood volume.

There is mounting evidence that the GH-IGF-I system plays a role in the adaptation to exercise (5). But it is also becoming clear that local IGF-I regulation (e.g., muscle tissue) may be dissociated from central control mechanisms (i.e., GH regulation of hepatic IGF-I production which is the predominant source of IGF-I in the circulation). The observation of marked increases in local IGF-I without changes in circulating levels of IGF-I supports the notion of relative independence of local and central regulation in the early phases of the adaptation to exercise. Consistent with this was our finding that GH levels did not differ among the groups, but the pulsatile nature of circulating GH limits the validity of single measurements to gauge GH secretory

patterns. Since we measured circulating growth factor levels only on days 1 and 5, we cannot rule out the possibility that transient increases in circulating IGF-I did indeed occur during exercise training.

Very few studies have examined the effect of brief exercise or training on muscle IGF-I levels. Interestingly, Yan et al. (17) demonstrated that an acute bout of eccentric exercise led to an increase in IGF-I immunoreactivity in rat type II muscle 4 days postexercise. The results of the present study suggest that exercise training initially leads to increases in muscle IGF-I but without changes in IGF-I gene expression. Moreover, there is evidence that early changes in other non-IGF components of the local adaptation to exercise training are mediated largely by posttranslational events (3). Town and Essig (16), for example, examined the effect of endurance training on mitochondrial enzymes and related proteins in the rat. They demonstrated increased aminolevulinate synthase activity after 3 days of training and elevated cytochrome oxidase activity after 7 days of training, but the investigators found no changes in the gene expression of these enzymes.

Longer periods of exercise training, however, do lead to stimulation of IGF-I gene expression both in the central neuroendocrine and local tissue components of the GH-IGF-I system. Zanconato et al. (20), for example, found that, by 4 wk of endurance training in young rats, hepatic IGF-I gene expression was increased. Yeh et al. (19) observed an increase in circulating IGF-I in rats after 9 wk of training. The data in the rats are corroborated by studies in humans showing that circulating IGF-I levels are correlated with fitness in large population, cross-sectional studies (10, 12, 15). Finally, Zanconato et al. (20) showed that 4 wk of endurance type training in the rat led to increases in exercising muscle IGF-I gene expression and protein.

In contrast, DeVol et al. (8) showed that a significant increase in IGF-I mRNA accompanied compensatory hypertrophy of the plantaris and soleus muscle only 2 days after unilateral excision of the gastrocnemius

tendon (levels of IGF-I protein were not measured in these studies). The apparent discrepancy between the results of the present study and those of DeVol et al. (8) suggests that different types and amount of muscular work could lead to a different time course and pattern of IGF-I increase. Interestingly, inhibition of GH [by hypophysectomy in the study by DeVol et al. (8) and by GHRH antibody in the study by Zanconato et al. (20)] actually enhanced the local IGF-I response to increased muscular work.

The mechanisms responsible for our observed increase in muscle IGF-I without changes in either local gene expression or circulating levels of IGF-I are not readily apparent. One possibility is that increases in local IGF-I gene expression and circulating IGF-I actually did occur, but were transient. Had we sampled at more frequent time points (i.e., between *days 1* and *5*), we might have observed these changes.

Alternatively, regulation of IGF-I is complex both from a biosynthetic and physiological perspective (13), and there is evidence to support a variety of mechanisms whereby IGF-I protein could increase without changes in IGF-I mRNA. For example, Yang et al. (18) showed that IGF-I mRNAs potentially encode multiple forms of preproIGF and that specific differences in their 5'-untranslated regions provide a molecular basis for translational control of IGF-I biosynthesis.

Alternatively, local tissue sequestration of IGF-I (perhaps, from the circulation) is a possible explanation for the increased tissue IGF-I after 5 days of training. Along these lines, Phillip et al. (14) examined the mechanisms responsible for the rapid (24–48 h) accumulation of IGF-I in the experimental (streptozotocin-induced) nephropathic kidney. They concluded that the increase in IGF-I was not due to an increase in local synthesis of IGF-I, but rather to an increase in IGF-I uptake from the circulation due to nonmembrane-associated IGFBP-1. One could envision a number of mechanisms related to exercise in which IGF-I might be sequestered in muscle tissue. Exercise-associated changes in pH and/or intracellular Ca^{2+} could affect the circulating ternary complex of IGF-I (IGF-I, IGFBP-3, and the acid labile subunit), and/or endurance training could lead to changes in local IGFIBPs and/or receptors. Finally, exercise training is known to increase muscle capillary density. As a consequence, local capillary volume and, consequently, the associated amount of IGF-I, would be greater.

In summary, we found marked increases in local skeletal muscle IGF-I production following only 5 days of training in the rat. These changes were not observed 4 h after a single bout of strenuous exercise, nor does it appear that the local increases were the result of increased muscle IGF-I gene expression or that they were accompanied by increases in circulating IGF-I. A number of mechanisms might explain these results, including translational-mediated or posttranslational-mediated increase in IGF-I concentration. Alternatively, sequestering of circulating IGF-I could occur following exercise training by increased muscle IGFIBPs or by a larger capillary blood volume in exercis-

ing muscle. The increased IGF-I in the trained muscle might serve a role in muscle hypertrophy seen as a consequence of exercise training.

Perspectives

The mechanisms that link the act of exercise or physical activity to the profound structural, biochemical, and functional aspects of the training response are under intense scrutiny in many laboratories. IGF-I, which is known to stimulate satellite cell proliferation (2, 9), seems to be a likely candidate for regulating in part, at least, exercise-induced growth of muscle. Our data suggest the possibility that initial local IGF-I responses to exercise may not necessarily result from increased gene expression. Indeed, IGF-I hindlimb muscle levels were high after only 5 days of training without concomitant increases in gene expression. Although it is certainly possible that IGF-I mRNA had transiently increased between *days 1* and *5*, it is known that IGF-I mRNA in the muscle increases following exercise training sessions lasting weeks, rather than days. Our finding of an apparent dissociation between IGF-I mRNA levels and IGF-I tissue levels after only 5 days of training in the rat provides an experimental model that may be useful in determining the translational, posttranslational, physiological (e.g., changes in local muscle blood volume), or endocrinological (e.g., local changes in IGFIBPs) mechanisms for IGF-I regulation in the muscle after exercise.

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