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Effect of single wrist exercise on fibroblast growth factor-2, insulin-like growth factor, and growth hormone

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Eliakim, Alon, Youngman Oh, and Dan Michael Cooper. Effect of single wrist exercise on fibroblast growth factor-2, insulin-like growth factor, and growth hormone. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R548–R553, 2000.—Anabolic effects of exercise are mediated, in part, by fibroblast growth factor-2 (FGF-2), insulin-like growth factor-I (IGF-I), and growth hormone (GH). To identify local vs. systemic modification of these mediators, 10 male subjects performed 10 min of unilateral wrist-flexion exercise. Blood was sampled from catheters placed in basilic veins of both arms. Lactate was significantly increased only in the exercising arm. FGF-2 decreased dramatically ($P < 0.01$) in both the resting (from 1.49 ± 0.32 to nadir at 0.11 ± 0.11 pg/ml) and exercising arm (1.80 ± 0.60 to 0.29 ± 0.14 pg/ml). Small but significant increases were found in both the resting and exercising arm for IGF-I and IGF binding protein-3 (IGFBP-3). GH was elevated in blood sampled from both the resting (from 1.04 ± 0.68 to a peak of 2.57 ± 0.53 ng/ml) and exercising arm (1.04 ± 0.66 to 2.43 ± 0.42 ng/ml, $P < 0.05$). Unilateral wrist exercise was not sufficiently intense to increase circulating lactate or heart rate, but it led to systemic changes in GH, IGF-I, IGFBP-3, and FGF-2. Low-intensity exercise involving small muscle groups can influence the circulating levels of growth factors.

growth factors; training; hypertrophy

IT IS BECOMING INCREASINGLY apparent that many of the health-promoting effects of exercise result from the interaction of specific hormones and growth factors such as insulin-like growth factor-I (IGF-I) and fibroblast growth factor-2 (FGF-2). These two mediators play important roles in muscle hypertrophy and angiogenesis, both of which characterize the anabolic adaptation to exercise. Little is known about the acute effects of exercise on circulating IGF-I or FGF-2, but such effects likely initiate the signaling pathways that eventually lead to tissue growth. Moreover, although IGF-I and FGF-2 may have endocrine and/or autocrine or paracrine functions, the role of local vs. systemic production and/or modification of these agents during exercise remains largely unexplored.

IGF-I is a major component of the growth hormone (GH)-IGF-I axis, a system of growth mediators, receptors, proteases, and binding proteins that control so-

matic and tissue growth in many species (24). IGF-I also plays a central role in exercise-associated muscle hypertrophy (1). Like IGF-I, FGF-2 is ubiquitously distributed. There is mounting evidence that FGF-2 also plays a role in exercise-associated muscle hypertrophy and/or angiogenesis (3, 6, 18, 25). Finally, recent work suggests the possible synergism of local tissue FGF-2 and systemic IGF-I in the regulation of muscle hypertrophy and growth (34).

The purpose of this study was to attempt to identify in human beings the local vs. systemic origins and/or modification of IGF-I and FGF-2 in response to exercise. A simple approach was used in which each subject performed a relatively low level of exercise consisting of unilateral, repeated flexion of the wrist against a relatively high resistance. To compare growth factor levels in the venous effluent of the exercising arm and in a site representing the central circulation, the basilic veins of both the exercising and resting arms were catheterized. We hypothesized that both FGF-2 and IGF-I would be increased only in the venous effluent of the exercising muscle.

It is now known that anabolic function of the GH-IGF-I axis cannot be determined simply by measurements of GH and IGF-I. IGF binding proteins (IGFBPs), binding protein proteolysis, and other carrier proteins play important regulatory roles in this system (26). Thus, to fully evaluate important functional constituents of the GH-IGF-I axis, we also measured serum levels of 1) GH; 2) IGFBP-3, the predominate binding protein of IGF-I in the circulating blood; 3) the degree of IGFBP-3 proteolysis; and 4) the acid-labile subunit (ALS), the third component of the circulating IGF-I-IGFBP-3 ternary complex that is known to be regulated by GH and may affect IGF bioactivity (15, 28).

METHODS

Subjects. Ten healthy adult male volunteers participated in the study. They ranged in age from 20 to 36 years old (mean 27.8 ± 1.4). Mean height was 178.1 ± 2.1 cm, and mean weight was 86.5 ± 10.8 kg. None of the subjects smoked, took long-term medications, or suffered from chronic

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disease. None were trained as competitive athletes. The study was approved by the University of Connecticut Health Center (UCHC) Institutional Human Subjects' Committee, and all volunteers granted informed consent.

Protocol. Subjects were admitted to the UCHC General Clinical Research Center, and an indwelling heparin lock catheter was inserted in the basilic vein of both arms. All tests began in the morning hours between 0800 and 1100. Subjects were postabsorptive, having been instructed only to drink water before the test. After a 30-min rest period, blood samples were drawn simultaneously from both arms before the onset of the exercise (7 ml per arm per sample), 5 min into the exercise burst, at the end of exercise, and every 10 min after exercise in the recovery period for 50 min. Blood samples were spun at 3,000 rpm at 4°C for 20 min. The serum was separated and stored at -20°C.

On the day of the exercise test, the subjects were instructed to limit their physical activity. We used the WedgeXE ergometer (Marcy Fitness Products; Evansville, IN), a commercially available, adjustable-resistance device designed to strengthen the wrist flexors (flexor carpi radialis and brachioradialis muscles). Unilateral wrist-flexion exercise was performed in the nondominant arm of each subject. Thus the exercising muscles were relatively unused muscles, and the potential confounding effects of training were diminished. Moreover, catheter insertion in the basilic vein is simple and safe, and the blood sampled from that site reflects, in large part, drainage from the wrist flexors.

At the beginning of the session, the subject flexed his wrist every 3 s against gradually increasing resistance until he could not tolerate any further increase. The range of motion was ~90°, and the exercise was primarily concentric. This process took ~1–2 min, then the subject continued exercising for a total of 10 min at the maximal tolerable resistance. A metronome was used to precisely pace the wrist flexion. Finally, heart rate (HR) was measured every 5 min during the exercise bout.

Serum measurements. To limit the total amount of blood sampled, we were unable to measure all variables at all time points. The sampling intervals for GH, IGF-I, and IGFBP-3 were selected on the basis of previous studies of a 10-min cycle ergometer exercise in which these substances were measured (7, 14, 30). Our previous studies indicated that IGF-I and IGFBP-3, even if elevated by the single wrist exercise protocol, would likely return to baseline well before the 50-min recovery period; consequently, we measured these substances during recovery at 10-, 20-, and 30-min intervals after exercise. Each sample consisted of 7 ml.

Serum lactate. Serum lactate was measured at rest (pre-exercise), at end exercise, and at 50 min into recovery. Lactate was measured spectrophotometrically with the use of the Behring Stat-pack rapid lactate test. The lactate intra-assay coefficient of variation (CV) was 2.8%, the interassay CV was 3.5%, and the sensitivity was 0.55 mmol/l.

Hematocrit. Hematocrit was measured at rest (preexercise), at end exercise, and at 50 min into recovery. A capillary tube sample of blood was spun at 3,000 rpm for 3 min, and the hematocrit was determined in conventional fashion.

FGF-2. FGF-2 serum concentrations were measured at rest, 5 min into exercise, end exercise, and at 10 and 50 min into recovery. FGF-2 serum concentrations were determined by ELISA with the use of the R&D System Quantikine High Sensitivity kit (R&D System; Minneapolis, MN). Interassay CV was 5.4–10.9%, and intra-assay CV was 5.0–10.0%. Assay sensitivity was 0.28 pg/ml. Undetectable levels of FGF-2 were arbitrarily assigned the value 0; however, the statistical analysis reported below was qualitatively the same when

we used 0.28 pg/ml for those FGF-2 measurements found to be below assay sensitivity.

GH. GH serum concentrations were determined by ELISA with the use of the DSL-10-1900 Active kit (Diagnostic System Laboratories; Webster, TX). Interassay CV was 5.5–12.9%, and intra-assay CV was 3.3–4.3%. Assay sensitivity was 0.03 ng/ml.

IGF-I. IGF was extracted from IGFBPs with the use of the acid-ethanol extraction method (13). IGF-I serum concentrations were determined by a two-site Immunoradiometric Assay (IRMA) with the use of the DSL-5600 Active kit (Diagnostic System Laboratories). IGF-I interassay CV was 3.7–8.2%, and intra-assay CV was 1.5–3.4%. Assay sensitivity was 0.8 ng/ml.

IGFBP-3. IGFBP-3 was measured by IRMA with the use of the commercially available DSL-6600 Active kit (Diagnostic System Laboratories). IGFBP-3 interassay CV was 0.6–1.9%, and intra-assay CV was 1.8–3.9%. Assay sensitivity was 0.5 ng/ml.

IGFBP-3 proteolysis. Serum from four time points during each protocol (preexercise, end exercise, and 20 and 50 min into recovery) were assayed for the presence of IGFBP-3 protease activity, as described by Lamson et al. (23). Briefly, nonglycosylated *E. coli*-derived recombinant IGFBP-3 was iodinated by a modification of the chloramine T method to a specific activity of 150–300 mCi/μg. Two point five microliters of serum were mixed with 0.05 M Tris · HCl (pH 7.4), 0.5 mM CaCl₂, and ¹²⁵I-IGFBP-3 to a total volume of 25 μl and incubated for 5 h. At the completion of incubation, the samples were subjected to 12.5% SDS-PAGE overnight under nonreducing conditions. The gels were subsequently dried and exposed to X-ray film at -20°C for 18 h. The intensities of the autoradiographic bands were then determined by scanning photodensitometry. The amount of proteolysis was calculated as the percentage of the optical density of fragmented IGFBP-3 over the sum of all IGFBP-3-related optical densities.

ALS. Total and free ALS were measured with the use of the DSL-10-8200 and DSL-10-12100 ELISA kits, respectively. Measurements were made preexercise, immediately postexercise, and then at 10 and 20 min postexercise.

Statistical analysis. All biochemical analyses were done in duplicate for each time point. Two-way (time by arm) ANOVA for repeated measures was used to determine the effect of exercise on all variables. Repeated measures over time, as well as subjects' serving as their own controls (e.g., an exercising and nonexercising arm for each subject), were accounted for in the covariance structure of the ANOVA models. For each outcome variable, pairwise comparisons of interest (2 tailed) were made when main effects were found to be significant. Graphical data are presented as means ± SE. Statistical significance was set at $P < 0.05$.

RESULTS

HR. The preexercise resting HR was 68 ± 2 beats/min, and immediately postexercise HR was 76 ± 3 beats/min. This difference was not statistically significant.

Lactate. The effect of a single forearm exercise on serum lactate is shown in Fig. 1. Repeated-measures ANOVA revealed a significant effect of time ($F_{2,36} = 36.60$, $P < 0.01$), arm ($F_{1,18} = 14.12$, $P < 0.01$), and a time by arm interaction ($F_{2,36} = 20.06$, $P < 0.01$). There was a significant increase in serum lactate immediately after exercise in the exercising arm only

Fig. 1. A: effect of unilateral wrist-flexion exercise on venous blood lactate. A significant increase was observed only in the exercising arm (●). B: effect of unilateral wrist-flexion exercise on circulating fibroblast growth factor-2 (FGF-2). This growth factor decreased significantly in both the resting (○) and exercising arm. No differences between the arms were noted. *Statistical significance set at $P < 0.05$.

($t_{36} = 9.27$, $P < 0.01$). Serum lactate returned to baseline levels by 50 min after the exercise. A significant difference in serum lactate between the exercising and resting arm was noted immediately after exercise ($t_{36} = 7.22$, $P < 0.01$).

Hematocrit. Hematocrit changed over time ($F_{2,36} = 12.49$, $P < 0.01$) with significant increases in levels immediately after exercise in both the resting (from 43.2 ± 1.0 to $44.1 \pm 1.1\%$; $t_{36} = 2.28$, $P = 0.03$) and exercising arms (from 43.1 ± 1.0 to $44.8 \pm 1.1\%$; $t_{36} = 4.31$, $P < 0.01$). Hematocrit returned to baseline levels by 50 min after the exercise in both arms. These changes over time were not accompanied by a significant main effect of arm ($F_{1,18} = 0.03$, $P = 0.86$).

FGF-2. The effect of time ($F_{4,64} = 10.17$, $P < 0.01$) and arm ($F_{1,16} = 0.42$, $P = 0.53$) on serum FGF-2 are shown in Fig. 1. There was a significant drop in serum FGF-2 to almost nondetectable levels immediately after exercise in both arms (exercising arm: $t_{64} = 3.90$, $P < 0.01$; resting arm: $t_{64} = 3.78$, $P < 0.01$), and levels remained low for 50 min after the exercise (exercising arm: $t_{64} = 3.46$, $P < 0.01$; resting arm: $t_{64} = 2.66$, $P < 0.01$).

IGF-I. A significant effect of time ($F_{5,90} = 2.79$, $P = 0.02$), but not arm ($F_{1,18} = 0.01$, $P = 0.98$), on IGF-I was noted. IGF-I levels increased in the resting arm from 272 ± 25 to 293 ± 27 ng/ml at 10 min into

recovery and in the exercising arm from 277 ± 26 to 288 ± 26 ng/ml.

IGFBP-3. The effect of a single forearm exercise on serum IGFBP-3 is shown in Fig. 2. IGFBP-3 was significantly related to time ($F_{5,90} = 2.66$, $P = 0.03$), with significant, brief increases in levels in both arms immediately after exercise (exercising arm: $t_{90} = 2.35$, $P = 0.02$; resting arm: $t_{90} = 2.21$, $P = 0.03$). Serum IGFBP-3 returned to baseline levels after the exercise in the exercising arm, but IGFBP-3 at 40 min was still significantly higher than baseline in the resting arm ($t = 2.20$, $P = 0.03$). These changes over time were not accompanied by significant differences between arms ($F_{1,18} = 0.01$, $P = 0.99$).

IGFBP-3 proteolysis. In the resting arm, there was a small increase in IGFBP-3 proteolysis between preexercise (99.7 ± 9.0) and endexercise (105.6 ± 8.4 , $P < 0.05$). By 10 min postexercise, IGFBP-3 proteolysis was 103.9 ± 5.5 , and it virtually returned to preexercise values by 30 min postexercise (101.2 ± 8.5). A small increase in IGFBP-3 proteolysis was also observed in the exercising arm preexercise 94.4 ± 6.9 , endexercise 99.3 ± 4.9 , 10 min postexercise 108.6 ± 5.7 , and 30 min postexercise 110.6 ± 7.1 . Despite these small increases, time and arm did not significantly account for the variability in IGFBP-3 proteolysis ($F_{3,30} = 1.26$, $P = 0.31$; $F_{1,10} = 0.01$, $P = 0.94$, respectively).

Fig. 2. A: effect of unilateral wrist-flexion exercise on circulating growth hormone. Significant increases were observed in both the resting (○) and exercising (●) arm. B: effect of unilateral wrist-flexion exercise on circulating insulin-like growth factor binding protein-3 (IGFBP-3). Small but significant increases in IGFBP-3 were observed in both arms. No differences between the arms were noted. *Statistical significance set at $P < 0.05$.

GH. The effect of a unilateral wrist-flexion exercise on serum GH is shown in Fig. 2 (time: $F_{5,90} = 4.40$, $P < 0.01$; arm: $F_{1,18} = 0.02$, $P = 0.90$). There was a significant increase (as compared with preexercise levels) in serum GH, peaking at 20 min after exercise in both arms (exercising arm: $t_{90} = 2.57$, $P = 0.01$; resting arm: $t_{90} = 2.32$, $P = 0.02$).

ALS. Neither free nor total ALS were influenced by exercise in either the exercising or control arm (free ALS-time: $F_{3,24} = 0.63$, $P = 0.60$; free ALS-arm: $F_{1,8} = 0.01$, $P = 0.99$; total ALS-time: $F_{3,24} = 0.75$, $P = 0.53$; total ALS-arm: $F_{1,8} = 0.02$, $P = 0.90$). In the control arm, total ALS at preexercise, postexercise, and 10 and 30 min into recovery were 17.6 ± 1.5 , 18.2 ± 2.1 , 17.0 ± 1.9 , and 16.8 ± 1.50 , respectively. In the exercising arm, corresponding total ALS values were 17.7 ± 1.8 , 17.8 ± 2.3 , 16.9 ± 2.3 , and 17.6 ± 1.5 . In the control arm, free ALS values were 1.7 ± 0.4 , 1.8 ± 0.5 , 1.2 ± 0.3 , and 1.8 ± 0.4 . In the exercising arm, free ALS values were 1.5 ± 0.5 , 1.2 ± 0.4 , 1.7 ± 0.4 , and 1.9 ± 0.6 .

DISCUSSION

The experiment was designed so that it would be possible to distinguish between systemic and local effects of exercise performed by a relatively small muscle group. This goal was achieved in large measure. As seen in Fig. 1, lactate concentrations increased significantly in the venous blood obtained from the exercising, but not the resting, arm.

The most remarkable finding in the present study was the virtual disappearance of FGF-2 from the circulation (Fig. 1). This occurred in venous blood from both resting and exercising arms, and it persisted virtually throughout the entire recovery period. Much work has been done in the last few years on FGF-2 as a potential therapeutic vascular growth factor in cardiac and other tissues (5, 35). Far less is known about the role of FGF-2 naturally found in the circulation.

Normally, concentrations of FGF-2 are low in the circulation, with several notable exceptions such as Duchenne muscular dystrophy (12), certain neoplasms (31), and ischemic cardiovascular disease (19). Hill and co-workers (21) found elevated circulating levels of FGF-2 in pregnant diabetic women with retinopathy in the second and early third trimesters. These authors speculated that the high FGF-2 levels resulted from excessive production in the uteroplacental compartment and might be causally related to the development of diabetic retinopathy. Rohovsky and co-workers (27) found that circulating levels of FGF-2 were markedly elevated in patients with vascular insufficiency and suggested that the high circulating levels reflected a physiological response to limb ischemia.

Our finding is consistent with preliminary data (in abstract form) of Scheinowitz and co-workers (29), who showed a similar reduction in circulating FGF-2 levels in healthy subjects after only 30 s of very heavy cycle-ergometer exercise. But the exercise-associated reduction in systemic FGF-2 observed here in *in vivo* studies contrasts with results obtained from *in vitro* studies of

cell systems. For example, Clarke and co-workers (11) found a clear linear relationship among the amount of mechanical load placed on skeletal muscle, the amount of myofiber wounding, and the amount of FGF-2 released from the cytoplasm of the wounded muscle cells. Moreover, Clarke and Feedback (10) showed that blockade of FGF-2 actually inhibited the usual mechanical load-induced growth response in differentiated skeletal muscle cultures.

The mechanism of the marked reduction in circulating FGF-2 after unilateral wrist-flexion exercise is not readily apparent. One possibility is that exercise somehow enhances local accumulation of FGF-2 in the muscle (e.g., through increased tissue receptor-binding affinity). In this model, the bulk of circulating FGF-2 is then bound to receptors on local endothelial and/or muscle tissues. This FGF-2 "captured" from the circulation may play a role in subsequent muscle hypertrophy and/or angiogenesis.

The present data demonstrate that relatively brief exercise acutely alters circulating FGF-2. To our knowledge, the only study that has focused on the effect of more chronic levels of activity on circulating FGF-2 was the recent work of Clarke and co-workers (9). Circulating FGF-2 was not changed in three groups of young men after 14 days of 1) bed rest, 2) a combined program of bed rest and resistive exercise, or 3) a resistive exercise program in normally active subjects (note: circulating FGF-1 was decreased by bed rest alone, but it was increased by bed rest in combination with resistive exercise). Whether different types of training (e.g., aerobic vs. resistive) might influence circulating levels of FGF-2, or, alternatively, whether differing levels of fitness could affect the acute circulating FGF-2 response to exercise is simply not known. However, our data do clearly indicate that bouts of physical activity occurring immediately before blood sampling must be taken into account when interpreting circulating FGF-2 levels in humans.

Unlike FGF-2, circulating IGF-I increased in response to exercise, and the magnitude of the changes were relatively small. But similar to FGF-2, unilateral wrist-flexion exercise led to changes in IGF-I in both the resting and exercising arms. Small increases in circulating IGF-I have been previously observed in this and other laboratories in response to heavy cycle-ergometer exercise (4, 7), but the mechanism of this response has not been fully elucidated.

The bilateral, simultaneous increase in IGF-I in response to unilateral exercise that we observed in the present study diminishes the probability that the local exercising muscle was the source of the IGF-I. Loss of water from the vascular space to the exercising tissues could lead to increased concentration of all polypeptides remaining within the vascular space. Indeed, magnetic resonance imaging studies with the use of secondary signal decay time-weighted signal enhancement demonstrate large exercise-associated shifts of water into the muscle (2). We did find an increase in hematocrit in both the resting and exercising arms, indicating a loss of vascular water possibly equivalent

to 2–4%, but this estimated decrease in vascular water was less than the roughly 7–8% increase observed in IGF-I concentrations with exercise.

As noted, physiological function of circulating IGF-I is influenced by a variety of binding proteins like ALS and IGFBP-3 (the predominant circulating IGF-I binding protein). ALS was not influenced by exercise in this study, but IGFBP-3 increased in both the resting and exercising arms (Fig. 2). IGFBP-3 is the predominant circulating IGF binding protein, and it often parallels changes in IGF-I itself. The mechanism for the observed systemic increase in IGFBP-3 is not readily apparent. IGFBP-3 proteolysis can lead to increased IGFBP-3 concentrations (16) and is known to accompany pregnancy (20), some catabolic states (26), and heavy cycle-ergometer exercise (30). But, in the present study, although IGFBP-3 proteolysis seemed to increase with exercise, the change did not achieve statistical significance.

We were surprised to find significant GH increases in both the exercising and resting arms. Many previous studies indicated that exercise does not stimulate GH release unless the exercise input is sufficient to cause a sizeable metabolic and/or neuroendocrine systemic response (14, 22, 32, 33). But, in the present study, there was a small but significant GH response despite the lack of systemic effects of the limited exercise (no increase in HR or circulating lactate). We speculate that factors such as perceived exertion with its attendant psychological stress [a known stimulator of GH (8)] led to activation of the hypothalamic-pituitary axis (HPA) and GH release. Alternatively, there is new evidence to suggest that certain forms of GH may be released during exercise via the actions of afferent nerve fibers from fast skeletal muscles (17). Direct neural links between activated muscle and the HPA remain an intriguing but relatively unexplored mechanism for the GH increase.

Perspectives

In this study, brief, unilateral exercise of small muscle groups significantly altered circulating levels of a number of growth mediators. The virtual disappearance of FGF-2 from the circulation suggests that exercise promotes redistribution of FGF-2 into some as yet unidentified tissue sink. IGF-I and IGFBP-3 were each elevated, indicating as yet unidentified systemic growth factor mediation by local exercise. Finally, GH was also elevated by an exercise input that had no effect on HR or circulating lactate levels. The data show that mechanisms must exist in which even low-intensity exercise involving small muscle groups can influence the circulating levels of biologically potent growth factors.

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