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Systemic vs. local cytokine and leukocyte responses to unilateral wrist flexion exercise

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Nemet, Dan, Suzi Hong, Paul J. Mills, Michael G. Ziegler, MaryAnn Hill, and Dan M. Cooper. Systemic vs. local cytokine and leukocyte responses to unilateral wrist flexion exercise. *J Appl Physiol* 93: 546–554, 2002. First published April 26, 2002; 10.1152/jappphysiol.00035.2002.—We hypothesized that brief exercise of a small muscle group would lead to local rather than systemic alterations in cytokines, peripheral blood mononuclear cells, and mediators of angiogenesis. Fifteen men and eight women (age range 22–36 yr old) performed 10 min of unilateral wrist flexion exercise. Blood was sampled from venous catheters in the resting and exercising arm at baseline, at the end of exercise, and at 10, 30, 60, and 120 min after exercise. Lactate was significantly elevated in the exercising arm ($+276 \pm 35\%$; $P < 0.0005$) with no change in the resting arm. In contrast, increases in both arms were observed for interleukin-6 ($+139 \pm 51\%$; $P < 0.0005$), growth hormone ($+1,104 \pm 284\%$; $P < 0.003$), natural killer cells ($+81 \pm 9\%$; $P < 0.0005$), and lymphocytes expressing CD62L, CD11a, and CD54. There were no significant differences in these increases between the resting and exercising arm. Catecholamines increased in both arms [epinephrine peak increase, $+226 \pm 36\%$ ($P < 0.001$); norepinephrine peak increase, $+90 \pm 15\%$ ($P < 0.01$)]. Fibroblast growth factor-2 initially decreased with exercise in both arms, and this was followed by a rebound increase. Vascular endothelial growth factor demonstrated a small but significant increase in both arms ($+124 \pm 31\%$; $P < 0.05$). Brief, low-intensity exercise leads to a systemic rather than local response of mediators that could be involved in inflammation, repair, or angiogenic adaptation to physical activity.

inflammation; single arm; white blood cells

SUBSTANTIAL RESEARCH INTEREST in recent years (24) has focused on the remarkable ability of physical exercise to modulate immune function by increasing circulating levels of proinflammatory cytokines like interleukin (IL)-1, IL-6, and tumor necrosis factor- α (TNF- α) and by altering circulating populations of peripheral blood mononuclear cells (PBMCs). However, the role of PBMCs in growth and repair of exercising tissue remains unclear. White blood cells can secrete growth modulating cytokines like IL-6 and other inflammatory

agents, some of which are now known to stimulate angiogenesis mediated by vascular growth factors [fibroblast growth factor-2 (FGF-2) (5) and vascular endothelial growth factor (VEGF) (12)].

A critical and as yet unresolved question is whether alteration in circulating white blood cells or elevation of inflammatory cytokines results from local mechanisms in the working muscle or, alternatively, by systemic effects of exercise such as stimulation of the autonomic nervous system. The purpose of this study was to attempt to differentiate, in human subjects, local from systemic mechanisms leading to alterations in PBMCs, cytokines, and related mediators.

Our laboratory recently demonstrated that unilateral wrist flexion exercise could be used to distinguish local from systemic effects of exercise (9). With this model, 10 min of exercise led to a fourfold increase in lactate levels in the venous effluent from the exercising arm with virtually no detectable systemic lactate increase or other manifestations of systemic responses such as increased heart rate. In the present study, we used this model to test the hypothesis that exercise-induced systemic alterations in PBMCs and cytokine responses originate predominately from the exercising tissue. As noted, controversy currently surrounds this question, with recent data from several laboratories suggesting that exercise can stimulate production of IL-6 mRNA by muscle tissue (18, 20, 32)

One confounding factor has been that virtually all previous studies have used exercise protocols of such great intensity that activation of autonomic nervous system and other systemic mechanisms clearly had occurred. The typical protocol for experiments focused on cytokine responses that have involved prolonged exercise (30 min to >2 h) at work rates well above 50% of peak values (e.g., Refs. 30, 31). Moreover, even studies focused on single-limb exercise have utilized protocols that are likely to be quite taxing; for example, Steensberg and co-workers (42) recently showed an increase in muscle-derived IL-6 in subjects who exercised the thigh muscles of a single limb for 5 consecu-

Address for reprint requests and other correspondence: D. M. Cooper, Univ. of California Irvine College of Medicine, Clinical Research Center, Bldg. 25, ZOT 4094-03, 101 The City Dr., Orange, CA 92868 (E-mail: dcooper@uci.edu).

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tive hours. In the present study, we chose a less intense and shorter protocol involving concentric exercise of a small muscle group, one less likely to cause systemic adrenergic and/or hormonal responses.

METHODS

Subjects

Twenty-three healthy adult volunteers (15 men, 8 women) participated in the study. They ranged in age from 22 to 35 yr old (mean 26.7 ± 0.8 yr). Mean height was 172.2 ± 1.9 cm (women 166.3 ± 3.7 cm, men 175.1 ± 1.9 cm) and mean weight was 77.8 ± 3.9 kg (women 66.4 ± 5.1 kg, men 83.9 ± 4.8 kg). None of the subjects smoked, took long-term medications, or suffered from chronic disease. None was trained as a competitive athlete or professional weightlifter. Subjects were instructed to limit their physical activity for 24 h before the study. The study was approved by the University of California, Irvine (UCI) Institutional Review Board, and informed consent was obtained.

Protocol

Subjects were admitted to the UCI General Clinical Research Center. All studies were performed in the afternoon at least 4 h after the subject's last meal. An indwelling heparin-lock catheter was inserted in the basilic vein of each arm. After 30 min of rest, blood samples were collected simultaneously from both arms before the onset of exercise. Moreover, catheter insertion in the basilic vein is simple and safe, and the blood sampled from that site reflects, in large part, the drainage from the wrist flexors.

Unilateral wrist flexion exercise was performed in the nondominant arm of each subject. We used WedgeXE ergometer (Marcy Fitness Products, Evansville, IN), a commercially available, adjustable, resistant device designed to strengthen the wrist flexors (flexor carpi radialis and brachioradialis muscles). All subjects were seated comfortably with both elbows resting on a table, allowing for easy access to the ergometer and for our staff to obtain blood samples from the indwelling catheters. The upper body was not fixed, but all subjects were asked to voluntarily keep both arms on the table throughout the protocol. At the beginning of the session, the subject flexed the wrist every 3 s (using a metronome to precisely pace the flexion) against gradually increasing resistance until he or she could not tolerate any further increase [maximal voluntary contraction (MVC)]. This process took 1–2 min, and then the subjects continued exercising at the same rate at the maximal tolerable resistance for a total of 10 min. The exercise was primarily concentric, and range of motion was $\sim 90^\circ$. Blood was drawn at baseline, at the end of exercise, and at 10, 30, 60, and 120 min after exercise. Finally, heart rate was measured every minute during the exercise bout with a monitor (Polar Accurex Plus, Polar Electro, Woodbury, NY).

Serum Measurements

Serum lactate. Serum lactate was measured spectrophotometrically (YSI 1500, Yellow Springs Instrument, Yellow Springs, OH). The intra-assay coefficient of variation (CV) was 2.8%. The interassay CV was 3.5%, and the sensitivity was 0.2 mg/dl.

Growth hormone. Growth hormone (GH) serum concentrations were determined by ELISA with the use of the DSL-10–1900 Active kit (Diagnostic System Laboratories, Web-

ster, TX). Intra-assay CV was 3.3–4.5%, interassay CV was 5.5–12.9%, and the sensitivity was 0.03 ng/ml.

FGF-2. FGF-2 serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit (R&D Systems, Minneapolis, MN). Intra-assay CV was 5.0–10.0%, interassay CV was 5.4–10.9%, and the sensitivity was 0.28 pg/ml. Undetectable levels of FGF-2 were arbitrarily assigned the value 0.

IL-6. IL-6 serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 3.8–11.1%, inter-assay CV was 7.1–29.5%, and the sensitivity was 0.0094 pg/ml.

TNF- α . TNF- α serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 8.7–14.8%, interassay CV was 16.1–22.6%, and the sensitivity was 0.18 pg/ml.

IL-1 β . IL-1 β serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 1.6–4.0%, interassay CV was 5.3–9.0%, and the sensitivity was 0.059 pg/ml.

IL-1 receptor antagonist. IL-1 receptor antagonist (IL-1ra) serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 3.1–6.2%, interassay CV was 4.4–6.7%, and the sensitivity was 22 pg/ml.

VEGF. VEGF serum levels were determined by ELISA with the use of the R&D Systems Quantikine High Sensitivity kit. Intra-assay CV was 5.1–6.7%, interassay CV was 7–8.8%, and the sensitivity was 5.0 pg/ml.

Flow cytometry. Blood was drawn in EDTA before, immediately after, and 10 and 30 min after each challenge and was maintained at room temperature (23°C). Complete blood count (CBC) analysis was performed by using a Cell-Dyn 4000 CBC counter (Abbott Diagnostics, Santa Clara, CA). Within up to 12 h of collection (due to transporting of samples to the flow cytometry laboratory), whole blood (100 μ l) was stained with various monoclonal antibodies (20 μ l) followed by 15-min incubation in the dark. Quantitation and phenotyping of blood samples for flow cytometric analyses were not affected by storage at room temperature up to 96 h (23). Monoclonal antibodies against phenotype (e.g., CD3, CD4, CD8, CD56CD16, etc.) and activation (e.g., CD62L) markers were conjugated to four-color fluorochromes: FITC, phycoerythrin (PE), peridinin chlorophyll protein, or allophycocyanin (Becton-Dickinson-PharMingen, San Diego, CA). After being stained with antibodies, erythrocytes were removed by using 2 ml of FACS lysing solution (Becton-Dickinson, San Jose, CA) followed by 10-min incubation in the dark. After centrifugation (5 min at 300 g), supernatant was aspirated, and cells were washed and resuspended in PBS with 5% formaldehyde.

Flow cytometry (FACSCalibur, Becton-Dickinson) equipped with CellQuest software (version 3.2, 1998) was used to quantify lymphocyte subpopulations and adhesion molecule expression. The fluorescence compensation was performed with CaliBRITE beads (Becton-Dickinson) and FACSCOMP software, and isotypic controls were used to determine non-specific antibody binding. A total of 8,000–15,000 events was analyzed per tube. Gating strategies were used as previously described (28). For example, T-cell population was identified with anti-CD3 antibody, and subsets were assessed with anti-CD4 and anti-CD8 under CD3⁺ gate. Leukocytes and adhesion molecule expression were obtained as percentage of total or gated cells and transferred into absolute numbers. For CD62L and CD11a density, flow cytometric estimation of antibodies bound per cell was performed with Quantibrite PE beads (Becton-Dickinson). Antibodies bound per cell, being

the number of antibodies that bind to the specific cell or microbead population, provides a good approximation of antigen density expressed on the cell. The Quantibrite PE beads were run at the same instrument settings as the assay, and the FL2 (PE) axis was converted into the number of PE molecules bound per cell.

Epinephrine and norepinephrine. Epinephrine and norepinephrine were measured by a radioenzymatic technique based on the conversion of the catecholamines to radiolabeled metanephrine and normetanephrine. One-milliliter plasma samples were extracted and then concentrated into a 0–1 ml volume before conversion into their radiolabeled metabolites. Sensitivity of the assay was 10 and 6 pg/ml for norepinephrine and epinephrine, respectively. The CV was 10 and 16%, respectively, for human plasma samples containing low catecholamine levels. Our laboratory has demonstrated that this technique is ~10 times more sensitive than the more commonly used assays and can thus reveal changes in venous epinephrine levels that often go undetected (21).

Statistical Analysis

All biochemical analyses were done in duplicate for each time point. Two-way (time \times 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 (i.e., exercising vs. resting arm of each subject), were accounted for in the covariance structure of the ANOVA models. For each outcome variable, *t*-test pairwise comparisons of interest (2-tailed) were made when main effects were found to be significant. Data are presented as means \pm SE. Statistical significance was set at $P < 0.05$.

RESULTS

Exercise Intensity

All subjects completed the 10-min protocol. The percent MVC averaged 40%, and this takes into account the fact that we reduced the resistance to some extent during the course of the exercise protocol to ensure that the subject would complete the required exercise. The average work per kilogram of body weight for the entire exercise period was 0.67 J/kg.

Heart Rate

The preexercise resting heart rate was 77 ± 2 beats/min, and immediately after exercise heart rate was 81 ± 2 ($P =$ not significant).

Lactate

The effect of a single-arm exercise on serum lactate is shown in Fig. 1. There was a significant increase in serum lactate immediately after exercise in the exercising arm only ($P < 0.0005$). This statistically significant difference between the exercising and resting arm was noted immediately after exercise and 10 min postexercise. Serum lactate returned to baseline levels by 60 min after exercise.

Hematocrit

Hematocrit (Hct) increased by 10 min of exercise in both the resting (from 43.7 to 44.8%) and exercising arm (from 43.7 to 45.7%); however, the increase in Hct

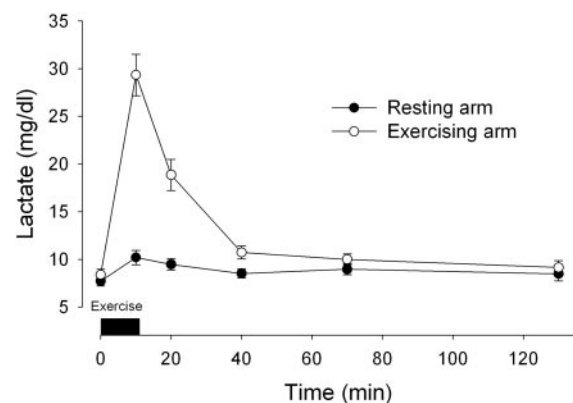


Fig. 1. Effect of unilateral wrist flexion exercise on venous blood lactate. A significant increase was noted only in the exercising arm ($P < 0.05$).

in the exercising arm was significantly greater ($P < 0.005$) than in the resting arm (data not included). This change is consistent with loss of water from the vascular space into the muscle interstitium [muscle water is known to increase after exercise (33)]. The overall change in Hct was roughly equivalent to only a 2–4.5% change in plasma water, and Hct levels returned to preexercise levels 10 min into recovery. The change in vascular water was lower in magnitude and different in timing from the significant changes we report in cytokines, growth factors, and PBMCs.

Circulating Growth Factors and Cytokines

GH. The effect of a unilateral wrist flexion exercise on serum GH in both arms is shown in Fig. 2. There was a significant increase (compared with preexercise levels) in serum GH, peaking at 20 min from the beginning of the exercise in both arms ($P < 0.003$). No difference was found between the resting and exercising arm.

Epinephrine and norepinephrine. Both epinephrine and norepinephrine increased markedly with exercise (Fig. 2). The increase in epinephrine was greater in the exercising compared with the resting arm ($P < 0.001$). In contrast, the increase in norepinephrine was less in the exercising compared with the resting arm ($P < 0.014$).

FGF-2. In agreement with our laboratory's previous study (9), FGF-2 decreased with exercise and reached a nadir by 70 min ($P < 0.03$; Fig. 3). However, in the present study, we extended the period of observation over that previously used and found that FGF-2 actually exceeded preexercise levels by the last sample obtained at 130 min. No difference was found between the arms.

VEGF. An increase in the levels of VEGF over time was noted (Fig. 3; $P < 0.05$). No difference was found between the arms.

IL-6. There was a significant increase in IL-6 after exercise ($P < 0.0005$; Fig. 4), and there was no difference between the exercising and resting arm.

IL-1ra. There was a significant increase in IL-1ra after exercise (Fig. 4) with a pattern of change similar

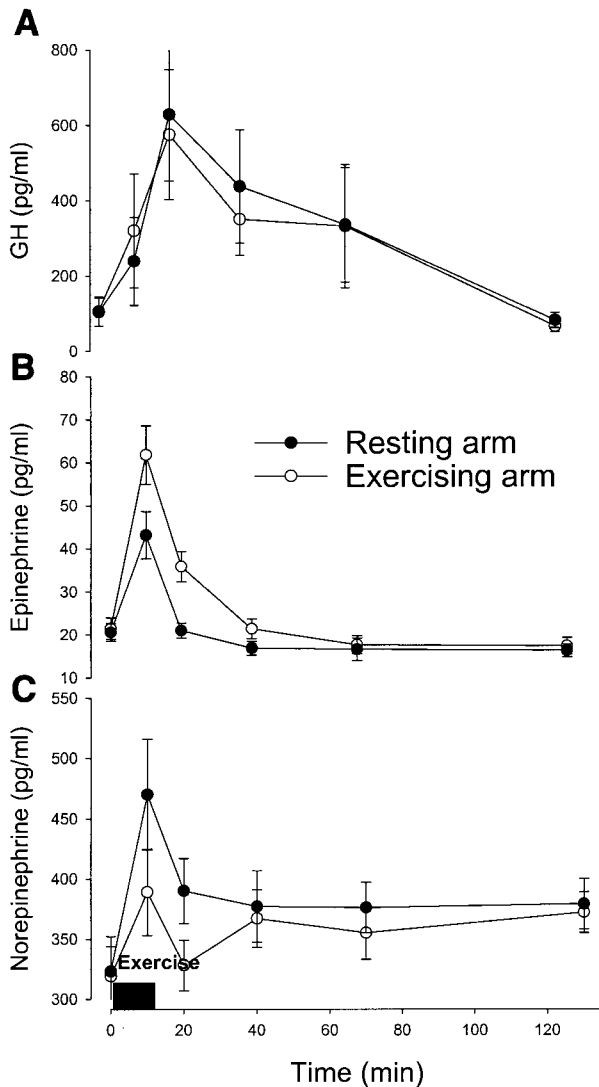


Fig. 2. *A*: effect of unilateral wrist flexion exercise on venous blood growth hormone (GH). Significant increases were observed in both the exercising and resting arm ($P < 0.003$); no difference was found between the exercising and resting arm. *B*: effect of unilateral wrist flexion exercise on venous blood epinephrine. Significant increases were observed in both the exercising and resting arm ($P < 0.005$). The increase in epinephrine was greater in the exercising compared with the resting arm ($P < 0.001$). *C*: effect of unilateral wrist flexion exercise on venous blood norepinephrine. Significant increases were observed in both the exercising ($P < 0.009$) and resting arm ($P < 0.005$). The increase in norepinephrine was less in the exercising compared with the resting arm ($P < 0.014$).

to that observed for IL-6. There was a significant change in IL-1ra over time ($P < 0.018$). No difference was found between the arms.

TNF- α and IL-1 β . No effect of exercise was observed in either the resting or exercising arm.

PBMCs and adhesion molecules We found substantial exercise-associated increases in total PBMCs that were largely explained by the increase in natural killer (NK) cell number (Fig. 5). Moreover, there were no differences between the resting and exercising arm. The exercise led to a significant increase in the number of circulating white blood cells ($P < 0.001$), including

granulocytes ($P < 0.008$), unseparated lymphocytes ($P < 0.0005$), CD19⁺ B cells ($P < 0.001$), CD3⁺ T cells ($P < 0.002$), CD3⁺CD8⁺ T-cytotoxic cells ($P < 0.0005$), and NK cells ($P < 0.0005$). The number of CD3⁺CD8⁺ T-cytotoxic cells expressing CD62L (CD8⁺CD62L⁺) increased significantly ($P < 0.02$), as did the number of T-cytotoxic cells not expressing CD62L (CD8⁺CD62L⁻; $P < 0.0005$; Fig. 6). The number of NK cells expressing CD62L (CD3⁻CD16⁺CD56⁺CD62L⁺; $P < 0.0005$) increased significantly, as did the number NK cells not expressing CD62L (CD3⁻CD16⁺CD56⁺CD62L⁻; $P < 0.0005$). In both the NK and T-cytotoxic cells, a preferential increase in CD62L⁻ cells was observed. Both CD54⁺ and CD54⁻ unseparated lymphocytes increased in circulation ($P < 0.0005$). The density of CD62L on unseparated lymphocytes decreased after the exercise ($P < 0.0005$; Fig. 6), whereas the density of CD11a on unseparated lymphocytes increased (from $20,227 \pm 1,056$ to $22,644 \pm 1,200$; $P < 0.0005$). No significant change was found in the number of monocytes, CD19 B cells, CD3⁺CD4⁺ T-helper cells, and CD4 cells expressing CD62L.

Effect of Gender

A post hoc analysis was performed to determine whether or not gender differences were observed in the

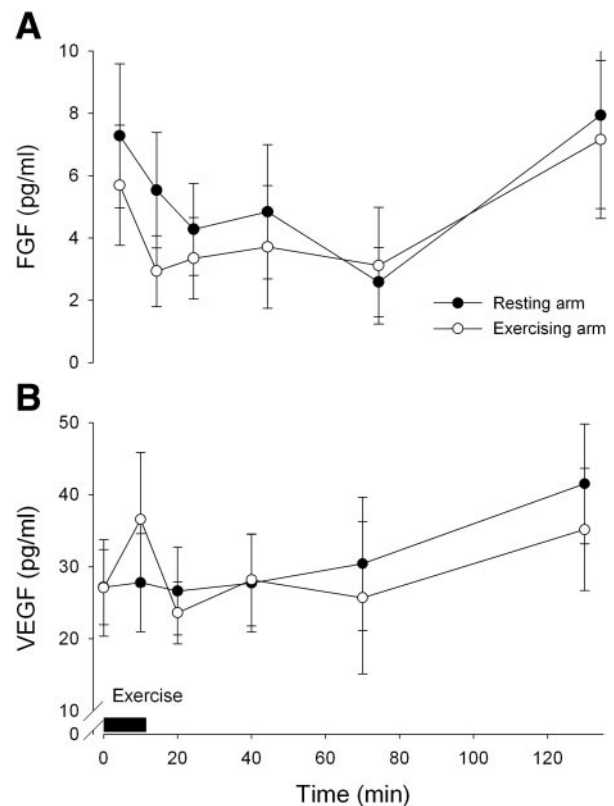


Fig. 3. *A*: effect of unilateral wrist flexion exercise on venous fibroblast growth factor-2 (FGF). This angiogenic factor showed a pattern of an initial significant decrease ($P < 0.03$) followed by a rebound increase in both the resting and exercising arm. *B*: effect of unilateral wrist flexion exercise on venous blood vascular endothelial growth factor (VEGF). An increase in the levels of VEGF over time was noted ($P < 0.05$). No difference was found between the arms.

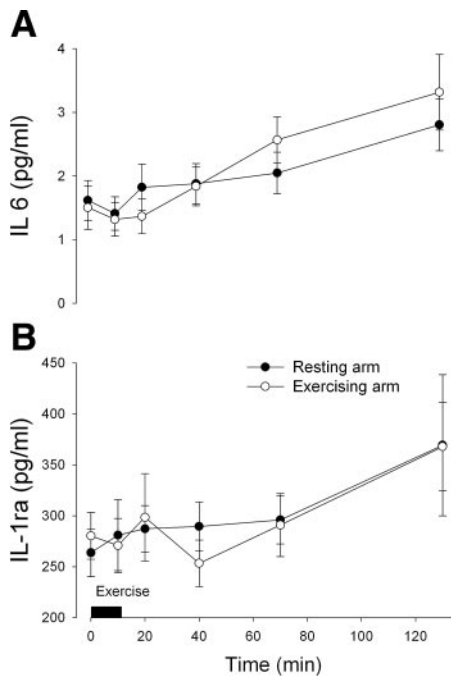


Fig. 4. Effect of unilateral wrist flexion exercise on venous blood interleukin-6 (IL-6; A) and interleukin-1 receptor antagonist (IL-1ra; B). There was a significant increase in IL-6 after exercise ($P < 0.0005$), with no difference between the exercising and resting arm. A similar pattern of change similar was observed for IL-1ra, demonstrating an increase in IL-1ra over time ($P < 0.018$). No difference was found between the arms.

responses to unilateral wrist flexion exercise. No significant differences in any of the variables described above were noted.

DISCUSSION

In this study, we made novel observations regarding the ability of small-muscle-mass exercise to stimulate growth factors and alter leukocyte trafficking systemically in human subjects. We had hypothesized that brief, unilateral wrist flexion exercise would alter inflammatory mediators and PBMCs only in the exercising arm. Indeed, we found, as in our laboratory's previous study (9), that lactate increased significantly only in the venous blood of the exercising arm, as would be expected when metabolites of exercise are produced primarily in the working muscle tissue (Fig. 1). Although we found significant exercise effects on IL-6, IL-1ra, GH, FGF-2, VEGF, and PBMCs, in none of these variables did we detect a different response between the resting and exercising arm. Thus relatively brief, low-level, unilateral exercise can increase the concentration of inflammatory mediators, growth factors, and PBMCs throughout the circulation. In contrast to our hypothesis, this increase does not seem to originate from the working muscles.

There are strengths and weaknesses to the present experimental model. Physiologically, this approach is a well-described, two-compartment, venous-venous model (35). Moreover, the lactate data (Fig. 1) are very consistent with a model of this nature in which sampling

occurs at two sites: the first is adjacent to the site of metabolite production (in this case lactate produced by the exercising muscle), and the second is a site reflecting central venous (systemic) blood (in our case the contralateral arm). As shown in Fig. 1, lactate is quite high in blood sampled from the exercising arm, and lactate levels are virtually unchanged when drawn simultaneously from the systemic sampling site.

Although the lactate data serve to validate the model, albeit in an indirect manner, this approach lacks an intramuscular probe. Thus we are unable to

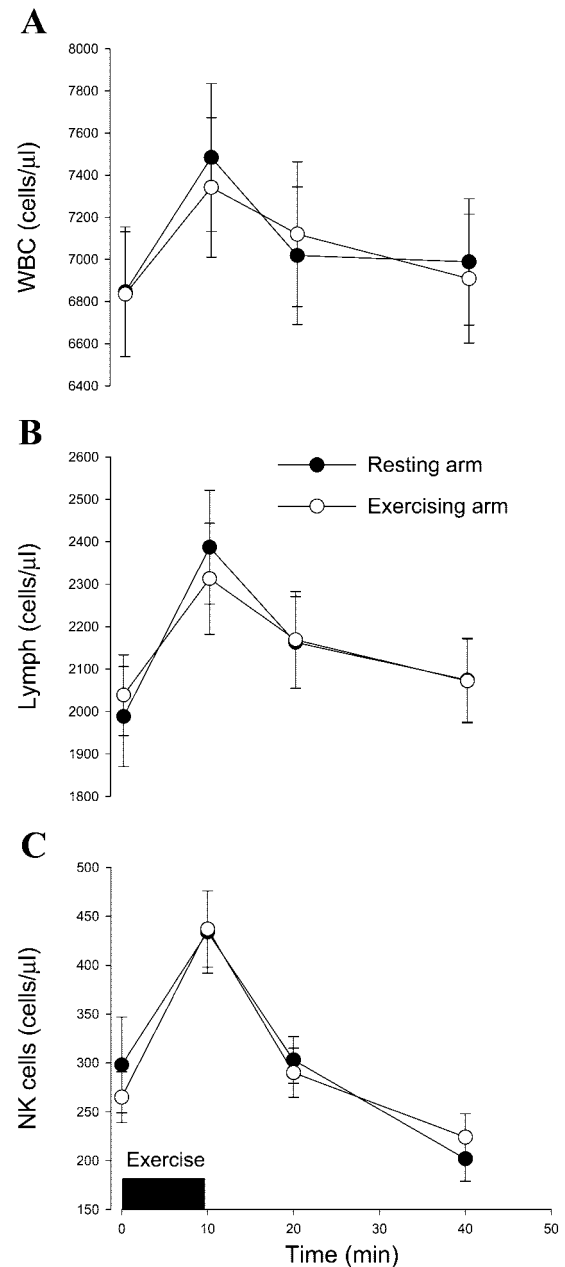


Fig. 5. Effect of unilateral wrist flexion exercise on peripheral blood mononuclear cells. The exercise led to a significant increase in the number of circulating white blood cells (WBC; A; $P < 0.001$), unseparated lymphocytes (B; $P < 0.0005$), and natural killer (NK) cells (C; $P < 0.0005$) in both the exercising and resting arms.

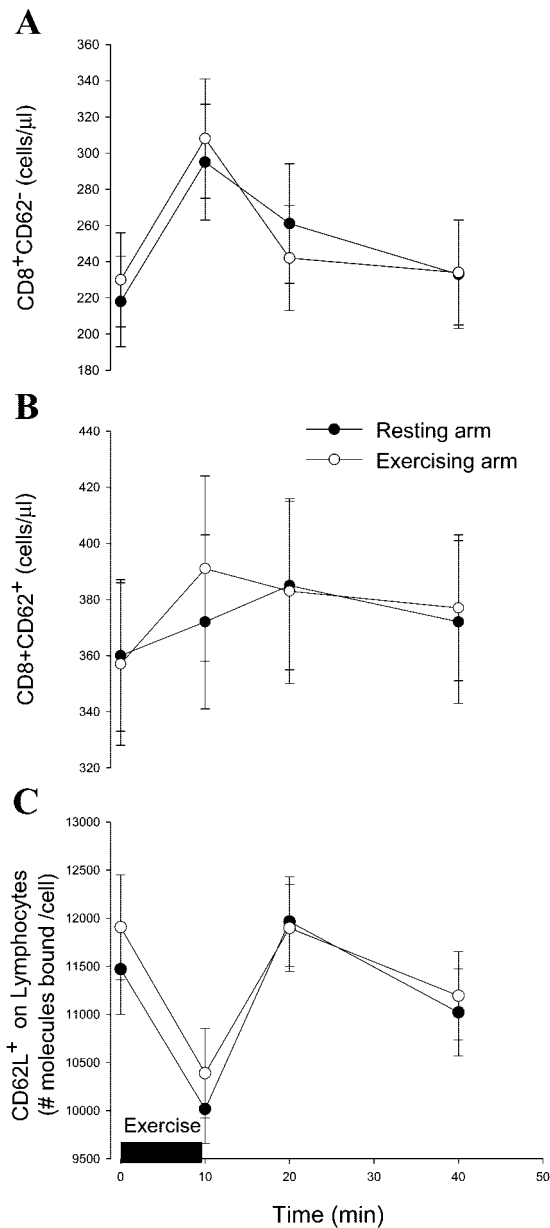


Fig. 6. Effect of unilateral wrist flexion exercise on CD62L (L-selectin). CD62L⁻ (A; $P < 0.0005$) and CD62L⁺ (B; $P < 0.02$) T-cell subsets increased significantly in both the exercising and resting arm. There was a preferential increase of CD62L⁻. The number of CD62L⁺ molecules on circulating lymphocytes decreased significantly (C; $P < 0.0005$).

address the issue of degree of damage or injury actually caused by the exercise protocol. Similarly, we are unable to determine the effect of local sequestering of hormonal mediators or leukocytes that could occur in response to exercise in muscle tissue.

An approximate doubling of IL-6 occurred in venous blood from both arms simultaneously (a pattern quite distinct from the lactate results), suggesting a central systemic mechanism for the increased IL-6. This finding of a systemic rather than local IL-6 response is somewhat surprising in light of a number of recent studies that have identified the exercising muscle itself

as the source of increased IL-6. For example, Ostrowski and co-workers (32) reported that IL-6 mRNA was elevated in muscle but not in blood mononuclear cells, a well-described source of elevated systemic cytokines production in sepsis (10). Similarly, Jonsdottir and co-workers (19) showed, in a model using single-leg exercise in the rat, that exercise resulted in elevated IL-6 mRNA in the exercising but not the resting muscle. Qualitatively similar results were found recently in exercise protocols in humans (20). Thus it is possible that the inflammatory response to exercise has several mechanisms that vary with exercise type, intensity, and duration. Finally, it is also possible that some cytokine and leukocyte stimulation occurred as a result of the perception of stress, because most of the subjects perceived the exercise to be strenuous, particularly by the end of the session. Psychosocial stress is known to stimulate the hypothalamic-pituitary-adrenal (HPA) axis and increase inflammatory cytokines (14, 15).

We did not find increases in IL-1 β or TNF- α . The preponderance of recent data suggest that these particular proinflammatory cytokines may require quite intense exercise for long periods of time before changes in the circulation occur (32, 36, 45), and in the present protocol, exercise was brief with no increase in circulating lactate in the systemic circulation. IL-1ra, which did increase in parallel with IL-6, is an anti-inflammatory cytokine. A major stimulator of IL-1ra is IL-6 (3); thus the similar and roughly simultaneous elevation of both of these mediators would be expected.

It is currently held that increased circulating IL-6 (such as accompanies systemic viral illnesses) is derived from IL-6 production in PBMCs (1), and the increased IL-6 levels parallel increased numbers of specific cell types. Consistent with this, we found marked changes in PBMCs and adhesion molecules (Figs. 5 and 6) with the unilateral wrist flexion exercise. Like the IL-6 response, these changes did not appear to result from alterations in PBMC trafficking in the exercising muscles per se; rather, they were systemic in nature.

The decreased density of CD62L and the increased density of CD11a on circulating lymphocytes are consistent with prior exercise studies (14). There was also a preferential increase in CD62L⁻ vs. CD62L⁺ T-cell subsets (Fig. 6). The relative increase in circulating CD62L⁻ lymphocytes is believed to result from a preferential release into the circulation of CD62L⁻ cells from the marginal pool rather than an actual down-regulation of L-selectin in response to exercise (22, 27). Because L-selectin is typically shed from T lymphocytes as the cell transitions from a naive to a post-antigen-presented memory T cell (4), these findings suggest that unilateral wrist flexion exercise leads to recruitment of memory T cells into the peripheral circulation. These memory T cells (marked also as CD45RO) are known to be associated with increased production of inflammatory cytokines such as IL-6 and TNF- α (6, 18). Lymphocytes expressing CD54 (known also as intercellular adhesion molecule-1) also increased in response to exercise in both arms, a re-

sponse similar to what has been observed previously in heavier exercise (34). CD54 is constitutively expressed on the surface of some lymphocytes and is upregulated in response to a variety of inflammatory mediators, including proinflammatory cytokines, certain hormones, cellular stresses, and virus infection (37).

What then might have been responsible for the systemic, rather than local, effect of exercise on PBMCs, IL-6, and IL-1ra? It is known that IL-6 secretion from PBMCs rapidly follows stimulation by epinephrine (41). Indeed, the magnitude and timing of the cytokine and PBMC response observed in our subjects were remarkably similar to the changes in these variables recently observed in a series of studies in resting, healthy adult human subjects after a supraphysiological intravenous injection of epinephrine (40).

Consistent with the idea that some part of the exercise-associated increase in IL-6 and IL-1ra may be mediated by epinephrine was our finding that the unilateral wrist flexion exercise was associated with increases in epinephrine and norepinephrine in blood sampled from both the resting and the exercising arm (Fig. 2). We did note differences in catecholamines between the resting and exercising arms; namely, epinephrine was higher and norepinephrine lower in the exercising arm than in the resting arm. Venous catecholamine concentration results from catecholamine levels in the arterial blood, clearance at the capillary level and the lung, release of catecholamines by nerve endings and adrenal glands, and the dynamics of blood flow from the arteries to the veins across the exercising muscle (38). Epinephrine is released into the circulation from the adrenal gland and is then partially cleared from the arterial blood by uptake into the sympathetic nerves and endothelium of the arterioles and capillaries. Consequently, arterial levels of epinephrine are higher than venous. When blood flow increases in response to exercise in the working muscle (functional sympatholysis), clearance is less efficient, the venous blood is "arterialized," and epinephrine levels increase in the venous blood draining the exercising muscle, as we observed in the present study.

In contrast, norepinephrine in the bloodstream is primarily derived from sympathetic nerves, and about half of the norepinephrine in blood is cleared during passage through the lungs. As a result, venous levels of norepinephrine are higher than arterial levels. Again, during exercise, blood flow increases in the working muscle and the "arterialization" of the venous blood leads to lower venous norepinephrine levels in the exercising arm.

In most previous studies of the catecholamine response to low levels of exercise, metabolic rates were at least 20% of peak O₂ uptake and heart rate was substantially increased. Isometric handgrip exercise, in which subjects continuously squeeze a device (such as a sponge) or attempt to turn an unmovable device for varying durations, are arguably most similar to the unilateral wrist flexion exercise of the present study. Handgrip does increase sympathetic nervous system tone and leads to increases in circulating levels of

catecholamines (46). But previous handgrip studies are not entirely comparable to unilateral wrist flexion exercise of the present study in that heart rate in handgrip studies is substantially increased (14). Whether the systemic sympathetic stimulation that occurs with handgrip translates into elevation of IL-6 or IL-1ra has not, to our knowledge, been studied. What is clear is that the present study corroborates our laboratory's previous observation (9) that low-level exercise can lead to systemic responses such as an increase in GH and shows that stimulation of the adrenals occurs as well.

These results support the speculations by Mostoufi-Moab et al. (29) that muscle afferents are engaged early in exercise at relatively low tension development and, through central mechanisms, enhance the synchronization of motor and autonomic responses to contraction.

As reported in our laboratory's previous study (9), an unexpected increase in GH from what appears to be a low level of exercise was observed. Many previous studies [including studies from our own laboratory (11)] tend to indicate that exercise does not stimulate GH release unless the exercise input is sufficient to cause a sizeable metabolic and/or neuroendocrine systemic response. 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 heart rate or circulating lactate). We speculate that factors such as perceived exertion with its attendant psychological stress [a known stimulator of the HPA (14)] led to activation of the 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 (16). Direct neural links between activated muscle and the HPA remain an intriguing but relatively unexplored mechanism for the GH increase.

As noted, IL-6 and GH can stimulate the production of mediators of angiogenesis like VEGF and FGF-2. Our laboratory had previously observed that unilateral wrist flexion exercise led to a remarkable systemic reduction in circulating FGF-2 (9). In the present study, this observation was corroborated in a new group of subjects, but, in addition, we noted a rebound in FGF-2 that began at ~60 min after the 10-min exercise bout and continued through the end of the observation period (~2 h after exercise) (Fig. 3). As in our laboratory's previous study, no differences in the FGF-2 response were noted between the two arms (9). Our laboratory had speculated previously that FGF-2 might be sequestered in the exercising muscle, accounting for its apparent loss from the circulation (9). The present study suggests that this process is relatively short lived (9). No obvious relationship between exercise-associated IL-6 and FGF-2 was observed in the present study, and the dynamic response of circulating FGF-2 to unilateral exercise remains an enigma.

Previous investigators have noted from *in vitro* studies that adrenergic activation can stimulate VEGF mRNA (44); moreover, exercise stimulates VEGF

mRNA and protein in skeletal muscle (2, 7). To our knowledge, the acute effects of exercise on circulating VEGF have not been studied. We observed a small but significant progressive increase in VEGF in both the resting and exercise arm. Because circulating mononuclear cells and platelets are a major source of circulating VEGF (25), it is likely that the increase in VEGF in the present study represents a systemic effect of low-intensity exercise. We speculate that the increase in catecholamines and IL-6 contributed to the small but significant elevation in circulating VEGF [because each of these factors is known to independently stimulate VEGF (39, 43)]. VEGF levels in the circulation are known to be elevated during conditions of increased angiogenesis, such as certain malignancies, and increased inflammation (26); however, the physiological significance of increasing concentrations of VEGF in the circulation are, as yet, unknown.

Gender-related differences in the metabolic response to exercise have been observed, particularly with references to neuroendocrine control of substrate utilization (8). Accordingly, as noted, a post hoc analysis to determine gender differences in the exercise did not reveal any significant differences between male and female subjects. (However, note that we did not time the study so that all the women would be at the same point in the menstrual cycle.) There were no significant gender differences at baseline, with almost identical responses to the exercise protocol. The lack of gender differences in our study is probably explained by the fact that the exercise in terms of duration and intensity was relatively low; this in contrast to previous studies showing gender differences when longer and more strenuous exercise protocols were used.

In summary, brief, unilateral exercise of a small muscle group led to small but significant increased levels of the inflammatory cytokine IL-6 and the anti-inflammatory cytokine IL-1ra along with substantial changes in PBMCs and adhesion molecule expression. The data suggest a systemic origin for these changes and that the changes likely resulted from exercise-associated catecholamine stimulation of white blood cells rather than from the exercising muscle itself. Despite the low level of exercise and the apparent lack of a detectable increase in heart rate, systemic manifestations of low-level exercise include increased epinephrine, norepinephrine, and GH. These latter agents may work together to stimulate VEGF secretion from platelets or white blood cells. Finally, we corroborated the previous finding of a disappearance of FGF-2 from the circulation after brief exercise (9) and showed a rebound in these levels by ~2 h into recovery. Brief, local muscle group, low-intensity exercise is capable of stimulating systemic mediators that could be involved in inflammation, repair, or angiogenic adaptation to physical activity.

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