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Authors

Connolly, Peter H

Caiozzo, Vincent J

Zaldivar, Frank

et al.

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Effects of exercise on gene expression in human peripheral blood mononuclear cells

Peter H. Connolly,[1,2] **Vincent J. Caiozzo,**[3,7] **Frank Zaldivar,**[1,2] **Dan Nemet,**[1,2] **Jennifer Larson,**[1,2] **She-pin Hung,**[4,6] **J. Denis Heck,**[5] **G. Wesley Hatfield,**[4,6] **and Dan M. Cooper**[1,2]

Departments of [2]*Pediatrics,* [3]*Orthopedics,* [4]*Microbiology and Molecular Genetics, and* [5]*Biological Chemistry,*
[1]*Center for the Study of Health Effects of Exercise in Children,* [7]*Department Physiology and Biophysics,*
[6]*Institute for Genomics and Bioinformatics, College of Medicine, University of California, Irvine, California 92697*

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Connolly, Peter H., Vincent J. Caiozzo, Frank Zaldivar, Dan Nemet, Jennifer Larson, She-pin Hung, J. Denis Heck, G. Wesley Hatfield, and Dan M. Cooper. Effects of exercise on gene expression in human peripheral blood mononuclear cells. *J Appl Physiol* 97: 1461–1469, 2004. First published June 11, 2004; 10.1152/japplphysiol. 00316.2004.—Exercise leads to increases in circulating levels of peripheral blood mononuclear cells (PBMCs) and to a simultaneous, seemingly paradoxical increase in both pro- and anti-inflammatory mediators. Whether this is paralleled by changes in gene expression within the circulating population of PBMCs is not fully understood. Fifteen healthy men (18–30 yr old) performed 30 min of constant work rate cycle ergometry ($\sim$80% peak O_2 uptake). Blood samples were obtained preexercise (Pre), end-exercise (End-Ex), and 60 min into recovery (Recovery), and gene expression was measured using microarray analysis (Affymetrix GeneChips). Significant differential gene expression was defined with a posterior probability of differential expression of 0.99 and a Bayesian *P* value of 0.005. Significant changes were observed from Pre to End-Ex in 311 genes, from End-Ex to Recovery in 552 genes, and from Pre to Recovery in 293 genes. Pre to End-Ex upregulation of PBMC genes related to stress and inflammation [e.g., heat shock protein 70 (3.70-fold) and dual-specificity phosphatase-1 (4.45-fold)] was followed by a return of these genes to baseline by Recovery. The gene for interleukin-1 receptor antagonist (an anti-inflammatory mediator) increased between End-Ex and Recovery (1.52-fold). Chemokine genes associated with inflammatory diseases [macrophage inflammatory protein-1α (1.84-fold) and -1β (2.88-fold), and regulation-on-activation, normal T cell expressed and secreted (1.34-fold)] were upregulated but returned to baseline by Recovery. Exercise also upregulated growth and repair genes such as epiregulin (3.50-fold), platelet-derived growth factor (1.55-fold), and hypoxia-inducible factor-I (2.40-fold). A single bout of heavy exercise substantially alters PBMC gene expression characterized in many cases by a brisk activation and deactivation of genes associated with stress, inflammation, and tissue repair.

microarray; inflammation; growth; immunity

IT IS WIDELY RECOGNIZED that relatively brief bouts of heavy exercise in humans lead to a robust increase in circulating peripheral blood mononuclear cells (PBMCs; lymphocytes, monocytes, and natural killer cells) and neutrophils (45, 57). PBMCs are among the essential cell mediators of stress and inflammation and produce cytokines, chemokines, and growth factors that can have powerful effects, beneficial or pathological, on tissues. In 1999, Ostrowski and coworkers (48) noted

that strenuous exercise led to increased circulating levels of proinflammatory mediators but that, simultaneously, ". . . cytokine inhibitors and anti-inflammatory cytokines restrict the magnitude and duration of the inflammatory response to exercise." Subsequent investigators have shown that other stressors, such as sepsis (51), also cause a seemingly paradoxical increase in the circulating levels of both pro- and anti-inflammatory cytokines. Finally, studies in adults and children now demonstrate that exercise stimulates growth and stress factors [such as growth hormone and interleukin (IL)-6] known specifically to antagonize one another (17, 18, 69).

The increasing understanding that exercise alters stress and inflammatory mediators combined with new knowledge that stress and inflammatory mediators play key roles in diseases ranging from arthritis to atherosclerosis (24, 38, 62) opens new areas of investigation into the mechanisms that link physical activity with health. However, levels of these mediators in the circulation do not necessarily reflect intracellular levels within the PBMCs and in target tissues (23), and it was not known whether exercise caused a similar dichotomous response of the cytokines, chemokines, and growth factors produced by the PBMCs themselves. Only a few investigators (21, 43, 63) have examined the effect of exercise on specific PBMC genes, with varying results showing no effect on inflammatory genes like IL-6 but increases in several of the heat shock proteins (HSPs).

Consequently, the purpose of this study was to characterize for the first time the genomic response of PBMCs obtained from healthy human subjects to an acute bout of exercise (30 min of strenuous cycling) with microarray technology. Our goal was to test two related hypotheses: *1*) genes that are altered by exercise in PBMCs reflect a dichotomy of pro- and anti-inflammatory, catabolic, and anabolic cytokines, chemokines, and growth factors; and *2*) genes that are altered by exercise in PBMCs are also associated with PBMC mediators known to be involved in specific metabolic and cardiovascular inflammatory diseases even in healthy human subjects.

We chose microarray approaches as an initial technique for a number of reasons. First, very little is known about the PBMC genomic response to exercise in adults. Only microarrays could definitively determine whether the leukocytosis of exercise was accompanied by a constrained or robust pattern of change in gene expression. Second, to test *hypotheses 1* and *2*, it would be necessary to have data on PBMC gene regulation from multiple systems. Only micro-

arrays provide the breadth and variety of information necessary to achieve this.

MATERIALS AND METHODS

Subjects. Fifteen healthy, adult men between the ages of 18 and 30 yr were recruited for this study. The decision to include only men in these initial studies was made because it is now known that a variety of metabolic and stress/inflammatory responses are gender dependent and likely to be influenced in women by the individual phase of the menstrual cycle (28, 30, 68), which could substantially complicate interpretation of the study results. Exclusion criteria included history of any chronic medical conditions, use of any medications, or training as a competitive athlete. Our Institutional Review Board approved this study, and written, informed consent was obtained by all participants. The studies were conducted at the University of California-Irvine General Clinical Research Center.

Height, weight, and body mass index. Standard calibrated scales and stadiometers were used to determine height, weight, and body mass index (weight/height^2).

Measurement of cardiorespiratory fitness and exercise intervention. Because many metabolic and immune responses to exercise are "dose" dependent (44), i.e., they are influenced by the relative magnitude and duration of the work input, care was taken to scale the exercise to the capability of each subject. We used an approach that has been successful in normalizing the exercise input in subjects with differing exercise capacities (7). We chose 30 min of heavy exercise (defined as work performed above the subject's anaerobic or lactate threshold) based on our laboratory's previous experience (13) with healthy nonathletes, because exercising above the lactate threshold leads to a robust metabolic and cardiorespiratory response.

Subjects participated in two phases. In *phase 1*, the volunteers performed a ramp-type progressive exercise test on an electronically braked, servo-controlled cycle ergometer to assess maximal exercise tolerance (Ergoline 800s SensorMedics, Yorba Linda, CA). Gas exchange was measured breath by breath, and the peak oxygen uptake ($\dot{V}O_2$) was determined by a ViaSys Vmax system (SensorMedics) as previously described (14). We included those subjects demonstrating a peak $\dot{V}O_2$ in the range of 35–45 ml·min^{-1}·kg^{-1}, a level consistent with a moderate level of fitness. In *phase 2* of the experiment, which was separated by at least 48 h from the completion of *phase 1*, subjects were required to exercise for 30 min at a work rate equal to ~80% of their peak $\dot{V}O_2$, as determined by subjects' lactate threshold and peak $\dot{V}O_2$.

Blood sampling. During *phase 2*, the blood was sampled from the antecubital vein and later separated into serum and PBMCs. A baseline sample was taken 30 min after the placement of the indwelling catheter and before the onset of exercise (Pre). We waited 30 min to ensure that measurable physiological parameters of stress (e.g., heart rate and blood pressure) were at baseline levels. Subjects then completed the 30-min exercise bout, and additional blood samples were obtained immediately after exercise (End-Ex) and after 60 min of postexercise recovery (Recovery). Figure 1 summarizes the experimental design and sampling points.

Serum lactate, circulating leukocytes, serum cytokines, and growth factors. Serum lactate was measured with a Yellow Springs Instruments lactate analyzer (YSI 1500, Yellow Springs, OH), with a sensitivity of 0.2 mg/dl. Complete blood counts were obtained by

standard methods from the clinical hematology laboratory at our institution. Serum IL-1 receptor antagonist (IL-1ra) and IL-6 levels were determined by commercially available ELISA kits for each subject at each of the sampling points (IL-1ra and IL-6; R&D Systems, Minneapolis, MN).

PBMC preparation. PBMCs were obtained from EDTA anti-coagulated peripheral blood with Ficoll-Hypaque density gradient centrifugation. Neutrophils were excluded from the genomic analysis because neutrophils account for upward of 60% of the population of circulating PBMCs. It was felt that their presence might obscure genomic changes in the more active populations of lymphocytes and monocytes. PBMCs were harvested and washed twice in ×1 HBSS. PBMC total RNA was stabilized with RNA*later* (Ambion). Standard and consistent practices were employed in an effort to minimize any potential changes in mRNA expression levels due to manipulation of PBMCs. The duration from blood draw to stabilization of RNA never exceeded 35 min.

RNA preparation and RNA pooling. Total RNA was isolated as recommended by Affymetrix (1). Total RNA was isolated from PBMCs using the RNeasy Midi columns method, according to the directions provided by the manufacturer (Qiagen, Valencia, CA). Eluted total RNAs were adjusted to a final concentration of 1 µg/µl. The quality of the total RNA was determined by electophoresis using a RNA Lab-On-A-Chip (Caliper Technologies, Mountain View, CA) that was read on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA). To reduce individual variation and other confounding events, such as spontaneous up- and downregulation of genes, the 15 subjects were pooled into 5 groups of 3 subjects each. The pools were not randomized; rather, we attempted to match the mean lymphocyte response to exercise across the five groups.

Total RNA to cRNA preparation. Total pooled RNA for each of the five groups consisted of 3.3 µg of total RNA from each individual (10 µg/group) for each of the corresponding time points. Double-stranded cDNA was synthesized from the mRNA present using the SuperScript double-stranded cDNA synthesis kit (Invitrogen, Carlsbad, CA). Double-stranded cDNA served as a template for the in vitro transcription reaction to generate biotinylated cRNA that included biotin-11-CTP and biotin-16-UTP [BioArray high-yield RNA transcript kabeling kit (T7), Enzo Diagnostics, Farmingdale, NY]. The 15 µg of the resulting biotin-tagged cRNA were fragmented into strands with an average of 110 bases in length following prescribed protocols (1).

Hybridization, staining, and scanning. Ten micrograms of fragmented target cRNA were hybridized for 16 h at 45°C (Affymetrix GeneChip hybridization oven 320) to probe sets present on the Affymetrix HU133A GeneChip. The GeneChip arrays were washed to remove any nonspecifically bound cRNA fragments and then stained (streptavidin-phycoerythrin) on an Affymetrix Fluidics Station 400, followed by scanning on a Hewlett-Packard GeneArray scanner.

Mircroarray data analysis. The statistical methods used for the analysis and interpretation of the data obtained from the DNA microarray experiments described in this paper are the same as those previously described by Hung et al. (29) and Baldi and Hatfield (5a). The DNA microarray data were background subtracted with the Affymetrix MAS 5.0 software package and normalized and modeled with the dChip program of Li and Wong (37). Statistical comparisons among the three time points were performed with the Cyber-T statistical program available online at www.igb.uci.edu. Only those

Fig. 1. Exercise protocol design and blood sampling points. The 30-min exercise consisted of constant work rate cycle ergometer pedaling at a work rate equivalent to 50% of the difference between the subject's lactate threshold and peak O$_2$ uptake ($\dot{V}O_2$) (see text). Pre, before exercise; End-Ex, immediately after exercise; Recovery, after 60 min of recovery.

Sample 1--PRE **Sample 2—END-EX** **Sample 3--RECOVERY**

30 min--exercise *60 min--recovery*
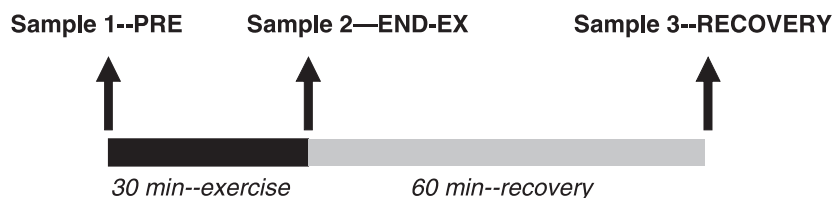
genes exhibiting an expression level of >0 in all replicates were used for statistical analysis. These gene expression level measurements were analyzed by a regularized t-test based on a Bayesian statistical framework implemented in the Cyber-T program (6, 39).

We ranked the mean gene expression levels of the replicate experiments in ascending order, used a sliding window of 101 genes, and assigned the average standard deviation of the 50 genes ranked below and above each gene as the Bayesian standard deviation for that gene. The global false-positive and -negative levels inherent in a DNA microarray experiment were computed using a mixture-model method described by Allison et al. (2). With this method, we can estimate an experimental wide, global false-positive level with a posterior probability of differential expression (PPDE) for each gene measurement (29, 39). For this study, we used a conservative PPDE value of $P \geq 0.99$ to define significant differential gene expression. This would be equivalent to a global false-positive level of $\leq 1\%$.

Statistical comparison of Pre and Recovery gene expression levels return PPDE values of zero. This results from the fact that, in the comparison of Pre and Recovery, an insufficient number of genes were differentially expressed, and it was not possible to model the data with a β distribution required for PPDE calculations. This does not mean, however, that there were, in truth, no differentially expressed genes. Therefore, to facilitate a comparison between gene expression in the Pre and Recovery sampling points, we conservatively used a Bayesian P value of <0.005.

Statistical analysis for leukocyte, lactate, and cytokine responses. We used a one-way analysis of variance with repeated measures to examine the effect of exercise on leukocyte numbers, lactate levels, and circulating cytokines. Values are expressed as means ± SE. Statistical significance was defined as a P value of ≤0.05.

RESULTS

Subjects. The mean ± SE for age, height, weight, and body mass index of the 15 subjects was 25 ± 1.2 yr, 178 ± 2.3 cm, 77 ± 3.4 kg, and 24 ± 0.9 kg/m^2, respectively. Peak $\dot{V}O_2$ was 37 ± 1.3 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$. The work rate performed in *phase 2* of the study corresponded to 80 ± 3.7% of peak $\dot{V}O_2$.

PBMC response to exercise. As shown in Table 1, the number of total WBCs, lymphocytes, and monocytes was found to be significantly elevated at End-Ex ($P < 0.001$), returning to near baseline levels [lymphocytes decreased significantly below baseline ($P < 0.001$) at Recovery]. The ratio of monocytes to lymphocytes was 24.0 ± 2.5% at Pre and decreased significantly to 15.6 ± 0.8% at End-Ex ($P < 0.003$). By Recovery, the ratio increased to 33.4 ± 3.1% ($P < 0.001$), which also differed significantly from the Pre value ($P < 0.02$). There were no changes in hematocrit during the course of exercise testing.

Serum lactate. The exercise bout caused a 10-fold increase ($P < 0.05$) in serum lactate levels [Pre (12.2 ± 1.25 mg/dl) vs. End-Ex (129.7 ± 10.1 mg/dl); $P < 0.05$] and approached baseline at Recovery (26.0 ± 3.0 mg/dl; $P < 0.05$).

Table 1. *Anthropometric characteristics of the 15 subjects studied*

Age, yr	25.2±0.8
Height, cm	177.6±1.6
Mass, kg	76.9±2.3
BMI, kg/m^2	24.4±.6
$\dot{V}O_{2 \, peak}$, ml·min^{-1}·kg^{-1}	37.4±0.8
%$\dot{V}O_{2 \, peak}$ for constant WR exercise protocol	79.6±2.5

Values are means ± SE. BMI, body mass index; $\dot{V}O_{2 \, peak}$, peak oxygen consumption; WR, work rate.

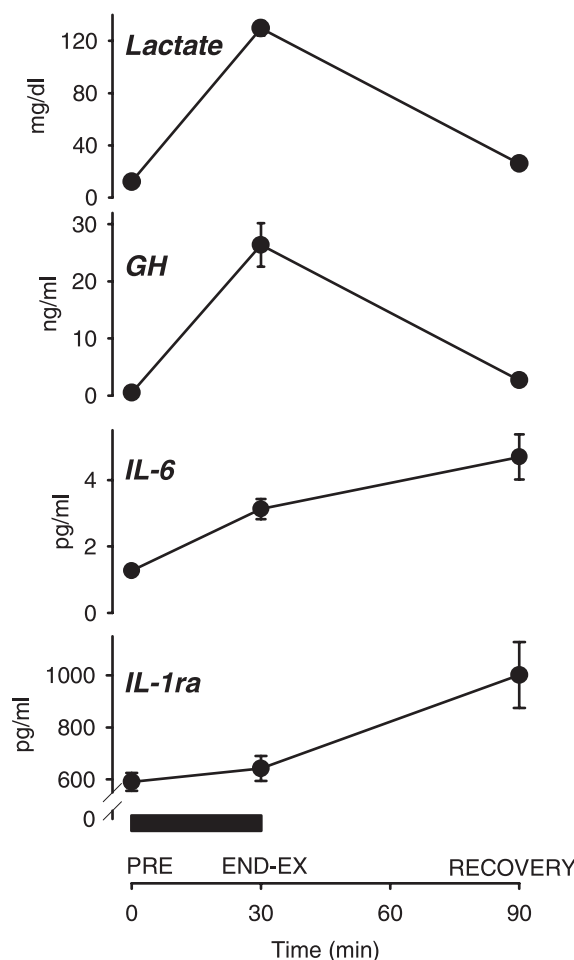
Fig. 2. Circulating factors reflecting stress, growth, and inflammatory responses to exercise. Data are means ± SE for lactate, growth hormone (GH), interleukin (IL)-6, and IL-1 receptor antagonist (IL-1ra). The 30-min exercise bout is designated by the filled bar above the x-axis. End-Ex lactate and GH were significantly ($P < 0.001$) greater than Pre and Recovery values. For IL-6, both End-Ex and Recovery were significantly greater than Pre ($P < 0.01$). For IL-1ra, Recovery was greater than both Pre and End-Ex ($P < 0.002$).

Serum cytokine response. IL-6 at Recovery was significantly greater than values obtained at Pre and End-Ex. IL-1ra at the Recovery time point was significantly greater than both Pre and End-Ex samples (Fig. 2).

Differential gene expression. A total of 311 genes were differentially regulated between Pre and End-Ex. As noted above, we used a PPDE of 0.99, and given this criterion the greatest Bayesian P value was <0.00008. A total of 552 genes was differentially regulated between End-Ex and Recovery. Again, with a PPDE of 0.99, the greatest Bayesian P value was 0.0001. In the comparison between Pre and Recovery, in which, as noted, PPDE could not be calculated, we found 292 genes demonstrating differential expression with a Bayesian P value of <0.005.

The overall pattern of gene up- and downregulation of the differentially expressed genes is shown in Fig. 3. Genes were categorized using the gene ontology database published by the Kyoto Encyclopedia of Genes and Genomes (www.kegg.org). As seen in Fig. 3, a pattern of upregulation predominated between Pre and End-Ex, and downregulation predominated between End-Ex and Recovery. With respect to Pre vs. Recov-
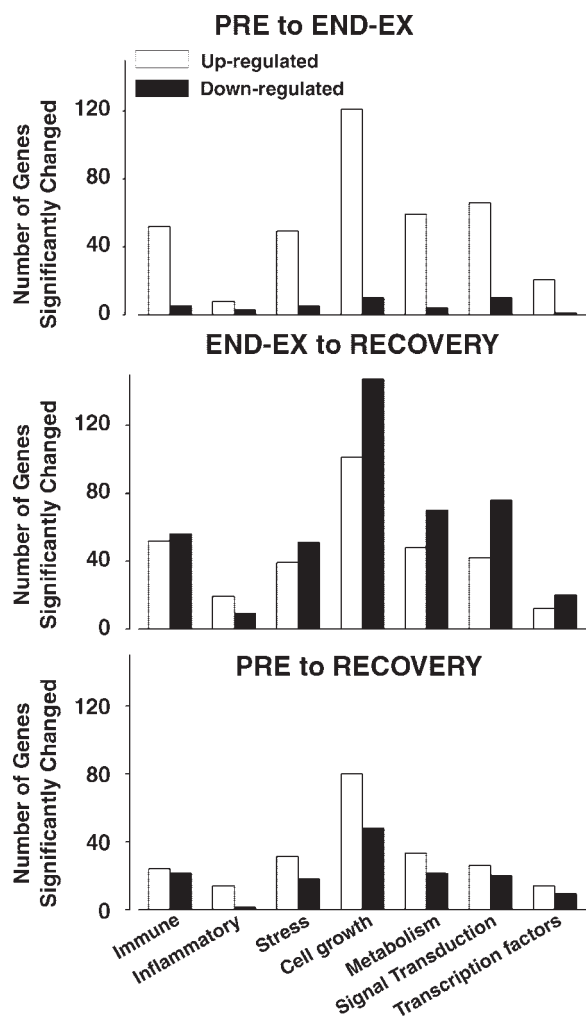
Fig. 3. Effect of 30 min of heavy exercise on the categories of peripheral blood mononuclear cell gene responses. The number of genes in each category (from the GO-gene ontology database) that were significantly up- or downregulated is shown. The majority of genes were upregulated from Pre to End-Ex, but expression of many of these genes returned to baseline by Recovery. Although exercise, as expected, influenced many genes classified in the immune, inflammatory, and stress categories, the exercise bout also affected a surprisingly large number of genes in the cell-growth category. (Some genes are classified in more than one category; consequently, adding the genes pictured in the graph will give a sum greater than the actual number of genes significantly altered by exercise.)

ery, the response was mixed. Interestingly, the cell-growth category had the largest number of differentially regulated genes.

Table 3 is a subset of 59 genes selected from the complete set of all differentially regulated genes because of their potential role in pro- and anti-inflammatory, catabolic-anabolic physiological processes. The complete set of genes demonstrating significant responses is to be found Microarray Gene Expression Data Society. In general, with respect to inflammatory mediators, the majority of upregulated genes from Pre to End-Ex could best be classified as proinflammatory, but there were notable exceptions. For example, IL-1ra, an IL-1 and IL-6 inhibitor, was upregulated. Table 3 also highlights the large number of growth factors that were upregulated, such as hepatocyte growth factor and epiregulin.

DISCUSSION

Although it is clearly recognized that exercise can influence the number of circulating PBMCs, little is known about the effect of exercise on the regulation of physiological mediators produced by PBMCs. This is the first study to examine the effects of exercise on the global gene expression of PBMCs. Our findings are unique in several respects. First, we demonstrated that a relatively brief exercise regime significantly altered the expression of hundreds of genes. Second, we observed, immediately after heavy exercise, that a proinflammatory response in the PBMCs predominated, but that there were also increases in certain anti-inflammatory genes even in the acute phase. Third, the bulk of the proinflammatory gene responses were transient and returned to baseline, or even below baseline, by 1 h of recovery. Although exercise can quickly stimulate proinflammatory gene responses in PBMCs, anti-inflammatory signals must also be put into play that, in the healthy subjects of our study, rapidly quenched the development of a potentially deleterious chronic inflammatory state. Finally, whereas most investigators have focused on stress, immune, or inflammatory aspects of PBMC responses to exercise, the present data show that exercise influences genes known to be associated with other key physiological functions, such as growth and tissue repair.

Methodological considerations and limitations. The exercise was of sufficient intensity to cause a robust increase in circulating PBMCs, circulating cytokines, growth hormones, and levels of lactate, all indicating that the work done by the subjects was moderate to vigorous (Table 2, Fig. 2). As expected, this level of exercise led to significant increases in both IL-6 and growth hormone, which antagonize one another, and to increases in IL-1ra, which inhibits IL-6 and other proinflammatory cytokines. The exercise in this study consisted of a relatively brief interval (30 min), which was substantially less than many previous protocols in which the inflammatory response to exercise was examined after prolonged strenuous exercise, such as the running of a marathon (11, 58).

We cannot completely exclude the possibility that the changes in gene expression in the circulating blood after exercise resulted from the changing proportion of monocytes to lymphocytes in the circulation or from shifting cells with either higher or lower expression of a particular gene from the peripheral sites (lymph, lung, spleen, vascular margins) into the circulation. However, although the proportion of monocytes to lymphocytes did significantly decrease from Pre to End-Ex and then reached a value greater than Pre in Recovery, this shift alone could not explain the wide range of the magnitude in significant changes observed in gene expression (from a 6.48-fold increase to a -2.03-fold decrease in the Pre

Table 2. *Leukocyte response to exercise*

	Pre	End-Ex	Recovery
WBC	5.4 ± 0.21	$10.2\pm0.57^*$	7.0 ± 0.39
Lymphocyte	1.6 ± 0.10	$4.3\pm0.30^*$	1.2 ± 0.08
Monocyte	0.3 ± 0.03	$0.65\pm0.04^*$	0.37 ± 0.02

Values are means $\pm$ SE (in 10^3 cells/μl). WBC, white blood cell; Pre, before exercise; End-Ex, immediately after exercise; Recovery, after 60 min of recovery. *Significantly greater than Pre and Recovery ($P < 0.001$).

to End-Ex interval alone). Moreover, in Recovery, when the PBMC numbers had returned to baseline (with a small degree of lymphopenia), a number of genes remained significantly up- and downregulated (see Table 3). Finally, the rapidity of the genomic response accompanying exercise (within 30 min) may not indicate cellular migration over gene induction. Indeed, there are many reports of gene induction in a similar time frame (e.g., Refs. 16, 41), including studies of leukocytes in response to inflammatory stimuli (71). Whatever the mechanism, exercise changed not only the numbers but the functional capability of circulating PBMCs and their potential role in ensuing stress, inflammatory, growth, and immune processes.

Whitney et al. (67) suggested that excessive in vitro handling of leukocytes leads to a gene expression ''signature'' of cell stress, including upregulation of the dual-specificity phosphatase (DUSP) genes. However, despite the fact that the cells were handled in an identical manner at all three time points in the present study, the gene expression of DUSP2, for example, was upregulated only at End-Ex (Table 3), negating the idea that in vitro manipulation of the cells was responsible. Finally, although we cannot completely exclude the possibility that spontaneous, temporal variations in PBMC gene expression not related to exercise might have influenced our results, the use of 15 subjects and pooling of samples would mitigate against the likelihood that idiosyncratic gene expression changes in a particular individual would predominate.

Although neutrophil concentrations in the circulation are markedly influenced by exercise, they were excluded from the present analysis only because we wanted to focus on those cells, lymphocytes, and monocytes that are more likely to respond to perturbation with robust genomic regulation. Even with our focus on the PBMCs without neutrophils, we recognize that we may have obscured or masked substantial genomic changes in specific cell types (e.g., one of the lymphocyte subtypes alone or monocytes alone). Because a global study of this nature had yet to be performed, our goal for this initial effort was to determine the range and magnitude of the genomic regulatory response to exercise in a large subpopulation of circulating leukocytes.

As noted in METHODS, the PPDE provides a measure of the global false-positive and false-negative rates. One of the unique aspects of this study is that we employed a highly conservative PPDE value of 0.99 to define differential gene expression. Although there are, as yet, no universally accepted approaches as to which PPDE should be used, we chose to use a rigorous PPDE of 0.99 because it provides a very high degree of assurance that the genes identified in Table 3 and Fig. 3 were indeed significantly up- or downregulated. It should be noted, however, that had we employed a less conservative but, arguably, quite reasonable PPDE value of 0.95, then the number of differentially regulated genes would have been substantially larger. For example, with the use of the 0.95 criteria, 531 genes changed from Pre to End-Ex and 870 genes changed from End-Ex to Recovery. Among them were vascular endothelial growth factor (VEGF), which increased by 1.36-fold from Pre to End-Ex (Bayesian P value of <0.001). VEGF is critical in the process of angiogenesis that can accompany exercise training (10, 35).

Does exercise produce a paradoxical stress/inflammatory PBMC gene response? In their original paradigm of the paradoxical pro- and anti-inflammatory response to exercise, Os-

trowski and coworkers (48) focused on the circulating levels of the proinflammatory cytokines TNF-α, IL-1β, and the "inflammation responsive cytokine IL-6," each of which had been previously reported to be increased in response to exercise. Furthermore, Ostrowski and coworkers focused on cytokine inhibitors IL-1ra, the soluble TNF receptors-1 and -2, and the anti-inflammatory cytokine IL-10, each of which had been reported in various studies to be increased by exercise and to balance the inflammatory effects. Since 1999, the majority of studies (56) have confirmed the large exercise increase in circulating IL-6 and IL-1ra, but there is great variability in the responses reported for other stress and inflammatory mediators. The source of many of these cytokines was initially hypothesized to be the immune system. However, recent studies demonstrate that IL-6, at least in strenuous exercise, appears to emanate from the exercising muscle as demonstrated by direct studies of muscle tissue and the findings that neither IL-6 protein nor IL-6 mRNA were increased in PBMCs after exercise (31, 49, 59).

The present study confirms the previous observation regarding IL-6 in that we found no global increase in the PBMC expression of IL-6 mRNA after exercise. Nor did we find global increases in the PBMC gene expression of IL-10 or soluble TNF receptors. We did, however, note a significant increase in the PBMC gene expression of the cytokine inhibitor IL-1ra between End-Ex and Recovery. IL-1ra is stimulated by the presence of inflammatory mediators such as IL-6 and IL-1 (4). Intriguingly, the timing of the gene expression of IL-1ra in PBMCs paralleled the increase in circulating levels of IL-1ra (Fig. 2). There has been much recent focus on IL-1ra as a potential effective therapeutic agent for inflammatory diseases, much in the same way that research on the soluble TNF receptors have led to improvements in the treatment of rheumatoid arthritis (4, 55).

Since the compelling paradigm proposed by Ostrowski and coworkers in 1999 (48), there have been major shifts in the current understanding of how stress, inflammatory, and immune processes interact in disease. Indeed, the classification of what constitutes pro- or anti-inflammatory responses has changed in the intervening years. Our data on global gene expression in PBMCs show the extent to which these processes are activated and regulated in healthy people performing activities routinely done by humans. For example, prostaglandin D2 synthase was increased from Pre to End-Ex, suggesting a role for the lipocalins, a group of mediators involved in the regulation of immune and inflammatory responses in many tissues (64, 65). We found, as well, a number of upregulated genes of more newly discovered functional pairs of pro- and anti-inflammatory mediators. Both cystatin and cathepsin were upregulated. Cystatin is an immunomodulatory mediator that inhibits the protease activity of cathepsin (66). There is growing interest in developing pharmacological inhibition of cathepsin and other cysteine proteases as a means of treating a variety of inflammatory diseases (26).

As noted, the DUSP family was upregulated by exercise. These agents are responsible for dephosphorylation of the critical phosphothreonine and phosphotyrosine residues within the mitogen-activated protein kinases (MAPK), thereby deactivating MAPKs (12). MAPKs play a role in stimulating the proinflammatory factor IL-1β (33). DUSPs are involved in the deactivation of the stress-activated protein kinase pathways as

Table 3. *Effect of 30 min of heavy exercise on selected stress, inflammatory, growth, and transcription genes in healthy young men*

Gene Symbol	Description	Fold Change		
		Pre → End-Ex	End-Ex → Recovery	Pre → Recovery
Stress response				
DUSP1	Dual-specificity phosphatase 1	5.69	NC	2.98
DUSP2	Dual-specificity phosphatase 2	4.45	−5.19	NC
DUSP5	Dual-specificity phosphatase 5	1.46	−1.55	NC
HIF1A	Hypoxia-inducible factor 1, alpha subunit	NC	NC	1.26
HSPB1	Heat shock 27-kDa protein 1	NC	NC	1.73
DNAJB1	DnaJ (HSP40) homolog, subfamily B, member 1	2	−2.03	NC
HSPA1A	Heat shock 70-kDa protein 1A	1.96	NC	2.43
HSPA1B	Heat shock 70-kDa protein 1B	3.8	−3.1	NC
HSPCA	Heat shock 90-kDa protein 1, alpha	1.49	NC	1.43
HSP105	Heat shock 105-kDa protein	NC	NC	2.2
RTP801	HIF-1 responsive RTP801	2.41	−1.89	NC
SPON2	Spondin 2, extracellular matrix protein	2.92	−4.63	−1.58
Inflammatory response				
CCL3	Chemokine (C-C motif) ligand 3 (MIP-1α)	1.84	−1.72	NC
CCL4	Chemokine (C-C motif) ligand 4 (MIP-1β)	2.88	−3.42	NC
CCL5	Chemokine (C-C motif) ligand 5 (RANTES)	1.34	−1.44	NC
CD14	CD14 antigen	NC	1.84	1.36
CD22	CD22 antigen	−1.76	NC	−1.51
CD69	CD69 antigen (p60, early T-cell activation antigen)	2.89	−3.01	NC
CSF3R	Colony-stimulating factor 3 receptor (granulocyte)	NC	1.81	1.33
CST7	Cystatin F (leukocystatin)	2.57	−3.05	NC
CTSW	Cathepsin W (lymphopain)	2.22	−2.88	−1.3
GNLY	Granulysin	2.35	−3.18	−1.35
GZMA	Granzyme A	2.0	−2.97	−1.49
GZMB	Granzyme B	2.88	−2.96	NC
IL-18RAP	Interleukin 18 receptor accessory protein	2.17	−3.05	−1.41
IL-1R2	Interleukin 1 receptor, type II	NC	NC	1.63
IL-1RN	Interleukin 1 receptor antagonist	NC	1.52	NC
IL-2RB	Interleukin 2 receptor, beta	1.88	−2.54	−1.35
IL-6R	Interleukin 6 receptor	NC	1.52	NC
NCR1	Lymphocyte antigen 94, natural cytotoxicity receptor 1	2.42	−3.46	−1.43
NCR3	Lymphocyte antigen 117	2.0	−2.73	−1.37
PRF1	Perforin 1 (pore-forming protein)	2.01	−2.57	−1.28
PTGDR	Prostaglandin D2 receptor (DP)	2.09	−3.02	−1.44
PTGDS	Prostaglandin D2 synthase 21 kDa (brain)	3.13	−4.54	−1.45
XCL1	Chemokine (C motif) ligand 1 (lymphotactin)	2.66	−3.24	NC
XCL2	Chemokine (C motif) ligand 2	3.28	−4.2	NC
Growth factors and transcription				
AKR1C3	Aldo-keto reductase family 1, member C3	3.49	−5.12	−1.47
CYP1B1	Cytochrome *P*-450, subfamily I, polypeptide 1	−2.06	3.14	1.53
CYR61	Cysteine-rich, angiogenic inducer, 61	NC	NC	1.74
ECGF1	Endothelial cell growth factor 1 (platelet-derived)	NC	1.5	NC
EGR1	Early growth response-1	NC	1.9	1.57
EREG	Epiregulin	3.5	−3.22	NC
GRN	Granulin	NC	1.56	NC
IGFBP7	Insulin-like growth factor binding protein 7	2.16	−2.41	NC
NCAM1	Neural cell adhesion molecule 1	2.51	−2.76	NC
PDGFRB	Platelet-derived growth factor receptor, β polypeptide	1.55	−1.69	NC
STAT4	Signal transducer and activator of transcription 4	1.45	−1.6	NC
AHR	aryl hydrocarbon receptor	NC	1.88	1.47
CREM	cAMP responsive element modulator	3.09	−1.61	1.92
FOS	v-*fos*	NC	NC	1.86
JUN	v-*jun*	NC	NC	−1.82
MYC	v-*myc*	−1.73	2.03	NC
NR4A2	Nuclear receptor subfamily 4, group A, member 2	6.48	−4.99	NC
RGS1	Regulator of G-protein signaling 1	5.14	−4.65	NC
TCF8	Transcription factor 8 (represses IL-2 expression)	2.25	−2.38	NC
CLIC3	Chloride intracellular channel 3	3.77	−5.33	−1.41
GPR56	G protein-coupled receptor 56	2.53	−3.87	−1.53
GPR66	G protein-coupled receptor 66	NC	−4.08	−2.05
GPR86	G protein-coupled receptor 86	−1.61	2.19	1.36

For the comparison of gene expression between Pre to End-Ex (Pre → End-Ex), sampling interval and for the End-Ex to Recovery (End-Ex → Recovery) sampling interval, statistical significance was achieved if the posterior probability of differential expression (PPDE) was greater than 0.99 (see text). For the Pre to Recovery (Pre → Recovery) interval, PPDE could not be calculated, and we used a Bayesian P value of <0.0005. NC, no significant change in gene expression. The complete set of results of differential gene expression that met these statistical standards can be found online in supplemental data (see http://jap.physiology.org/cgi/content/full/00316.2004/DC1).

well. Stress-activated protein kinase pathways play a key role in the cellular response to proinflammatory cytokines (60). Thus activation of the DUSP genes in PBMCs by exercise may indicate an early response to perturbation in homeostasis in which activated proinflammatory pathways are attenuated.

HSP genes in the PBMCs were upregulated by exercise (e.g., HSP70 increased by 3.8-fold from Pre to End-Ex). These studies are consistent with observations made by Niess and coworkers (46, 47) and Fehrenbach and coworkers (20). The latter group examined HSP27 and HSP70 in circulating leukocytes (neutrophils, lymphocytes, and monocytes) using RT-PCR in 12 athletes who completed a half-marathon (~3 h of running). Levels of extracellular HSP72 also increase during exercise and are now known to be signals activating innate immunity (22). Recent work (40) demonstrates a relationship between HSP and nuclear factor-κB (NF-κB). NF-κB is a transcription factor that regulates the expression of many genes involved in immunity and inflammation. NF-κB clearly plays a role in a variety of human diseases, like atherosclerosis, in which chronic, low-level inflammation contributes to the pathophysiology (70). Some HSPs inhibit NF-κB, and this may explain the HSP70 cardioprotective effect that has been noted previously (32, 52).

Effect of exercise on gene expression of growth mediators. A remarkable observation from the present study was the extent to which exercise acutely altered genes from systems not typically considered to be part of stress, inflammatory, or immune functions. We found that a brief bout of exercise upregulated PBMC growth factor genes such as epiregulin, which is effective in healing wounds and can stimulate growth of hepatic cells (19, 34). Platelet-derived growth factor was also upregulated, and it too is involved in widespread anabolic functions such as angiogenesis and wound healing (15, 27).

A related finding was the upregulation of hypoxia-inducible factor-1 (8) and early growth response-1. Both of these agents mediate the response to hypoxia in endothelial cells (61). Activated under hypoxic conditions that can occur during exercise, hypoxia-inducible factor-1 potentiates protective cellular function by inducing transcription of target genes involved in oxygen delivery to hypoxic tissue as well as genes that compensate for metabolic function altered by hypoxia. Hypoxia-inducible factor-1-dependent target genes include the gene for VEGF. As noted above, using a "relaxed" PPDE of 0.95, VEGF increased from Pre to End-Ex. VEGF is upregulated in muscle tissue after exercise (9).

Indeed, there is evidence to support the idea that PBMCs play a role not only in the "classical" immune system activity of eliminating invading organisms but also in the context of adaptation to exercise as agents of tissue growth and repair. A number of new studies on angio- and arteriogenesis, arguably, one of the most functionally important adaptive responses to exercise in mammals (53), suggest that there exist subpopulations of monocytes/macrophages that act as endothelial progenitor cells and secrete angiogenic growth factors like VEGF (54). Moreover, Heil et al. (25) have demonstrated that blood monocyte concentration is critical for enhancement of collateral artery growth. PBMCs and their mediators influence growth in other tissues as well (42).

Are the PBMC genes altered by exercise associated with specific inflammatory disease states? Many of the PBMC genes that were altered by exercise are known to play a role in disease processes. This was particularly evident in the chemotactic cytokine (chemokine) classification of genes. These low-molecular weight proteins represent a large family of mediators of inflammation and immunity with functional similarities to cytokines (36). Upregulation between Pre and End-Ex in the inflammatory chemokines genes, CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5-regulation-on-activation, normal T cell expressed and secreted is associated with multiple sclerosis, rheumatoid arthritis, asthma, transplant rejection, and nephritis (5). Both MIP-1α and MIP-1β are involved in the activation of neutrophils, eosinophils, and basophils and appear to be involved in acute neutrophilic inflammation (3). They also induce the synthesis of other proinflammatory cytokines such as IL-1 and TNF-α in fibroblasts and macrophages. Regulation-on-activation, normal T cell expressed and secreted is chemotactic for T cells, human eosinophils, and basophils and plays an active role in recruiting leukocytes into inflammatory sites. In addition, we also observed a significant increase in the homing chemokine XCL1-lymphotactin, which may be involved in the pathogenesis of human nephropathy (50).

Summary. We found a significant acute effect of exercise on PBMC gene expression. A predominately proinflammatory gene response was noted immediately after exercise, but this rapidly subsided. The mechanisms of the anti-inflammatory component of the response are not entirely clear but likely are related to exercise-associated stimulation of IL-1ra, HSP, DUSP, and other mediators that may act by deactivating MAPK or stress-activated protein kinase pathways to prevent the development of chronic inflammation. In addition to the stress/inflammatory responses, exercise led to the activation of a number of growth factor-related genes in the PBMCs that might be important for physiological responses to exercise, such as angiogenesis.

The recent study from Whitney et al. (67) was among the first to establish the feasibility and utility of microarray analysis of whole blood and PBMCs in adult humans. They noted that "Peripheral blood is an accessible source of cells with which to investigate these questions. Moreover, circulating leukocytes can be viewed as scouts, continuously maintaining a vigilant and comprehensive surveillance of the body for signs of infection or other threats." Our data additionally suggest that the PBMC genomic response to exercise may prove a useful area of investigation in attempting to determine the mechanisms that link physical activity with health.

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