Effects of 30 min of aerobic exercise on gene expression in human neutrophils

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Radom-Aizik S, Zaldívar F Jr, Leu S-Y, Galassetti P, Cooper DM. Effects of 30 min of aerobic exercise on gene expression in human neutrophils. J Appl Physiol 104: 236–243, 2008. First published November 15, 2007; doi:10.1152/japplphysiol.00872.2007.—Relatively brief bouts of exercise alter gene expression in peripheral blood mononuclear cells (PBMCs), but whether exercise changes gene expression in circulating neutrophils (whose numbers, like PBMCs, increase) is not known. We hypothesized that exercise would activate neutrophil genes involved in apoptosis, inflammation, and cell growth and repair, since these functions in leukocytes are known to be influenced by exercise. Blood was sampled before and immediately after 30 min of constant, heavy (~80% peak O2 uptake) cycle ergometer exercise in 12 healthy men (19–29 yr old) of average fitness. Neutrophils were isolated using density gradients; RNA was hybridized to Affymetrix U133+2 Genechip arrays. With false discovery rate (FDR) <0.05 with 95% confidence, a total of 526 genes were differentially expressed between before and after exercise. Three hundred and sixteen genes had higher expression after exercise. The Jak/STAT pathway, known to inhibit apoptosis, was significantly activated (EASE score, P < 0.005), but 14 genes were altered in a way likely to accelerate apoptosis as well. Similarly, both proinflammatory (e.g., IL-32, TNFSF8, and CCR5) and anti-inflammatory (e.g., ANXA1) were affected. Growth and repair genes like AREG and FGFR2 receptor genes (involved in angiogenesis) were also activated. Finally, a number of neutrophil genes known to be involved in pathological conditions like asthma and arthritis were altered by exercise, suggesting novel links between physical activity and disease or its prevention. In summary, brief heavy exercise leads to a previously unknown substantial and significant alteration in neutrophil gene expression.

WE NOW KNOW from work done in this and other laboratories that exercise induces a robust increase in circulating peripheral blood mononuclear cells (PBMCs; lymphocytes, monocytes, and natural killer) and neutrophils (6, 27, 35) as well as changes in leukocyte function (28). The response of immune cells to physical activity is of growing interest because some of the key health effects of exercise, both beneficial (e.g., reduction of cardiovascular disease risk) and detrimental (e.g., exercise-induced asthma), may well be mediated through the activity of circulating leukocytes (11).

It is not surprising that exercise could influence immune cells so profoundly. The physiological perturbation induced by even brief bouts of physical activity includes thermodynamic (increased temperature (38)), physiochemical [lactic acidosis (23), hypoxia (34)], hormone [catecholamines (22)], cytokine (2), and physical [turbulence and dynamic shear forces (1)] factors, all of which can alter leukocyte function. In the case of PBMCs, it is now known that relatively brief exercise leads to substantial changes in cellular gene expression (9). Consequently, we hypothesized that exercise would also influence the gene expression profile in circulating neutrophils, a class of cells that until relatively recently had been (incorrectly) classified as terminally differentiated, incapable of altering gene regulation in response to environmental stresses (18).

Unlike the PBMCs that decrease rapidly from the circulation at the cessation of an exercise bout, neutrophils remain elevated or may even continue to increase during the postexercise recovery period (42). In humans, relatively brief episodes of exercise can activate neutrophils (25, 32, 40), but the ultimate impact of exercise on neutrophil function varies and depends on the duration and intensity of the exercise challenge (45–47). An intriguing emerging aspect of neutrophil biology is the mounting data that these cells play a role in tissue repair, remodeling, and growth (16, 43). Neutrophils act as an elegant paracrine type of hormonal system—the cells are attracted from the central circulation to a specific tissue (e.g., a wound or injured muscle) and then are induced to secrete mediators that impact tissue growth (16).

We reasoned that genes involved in cellular activation, inflammation, and growth and repair would likely be affected by a single bout of heavy exercise. Since these pathways involve a variety of genes, we used microarray technology along with verification by RT-PCR of selected genes. We chose to focus on neutrophil gene responses initially rather than on the complex ensuing physiological or functional changes. Conceptually, we wanted to test hypotheses on the mechanisms responsible for the early control of neutrophil function, and this would be best accomplished by examining gene regulation. By first identifying a discrete set of genes in circulating neutrophils that were altered by exercise, we could subsequently design more efficient experiments targeting specific proteins and physiological responses that were likely controlled by the identified genes.

MATERIALS AND METHODS

Participants

Twelve healthy men (19–29 yr old) participated in this study (see Table 1). The decision to include only men in these initial investigations was made because it is now known that a variety of metabolic and stress/inflammatory responses are sex dependent and likely to be influenced in women by the individual phase of the menstrual cycle...

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Our plan was to first determine whether key neutrophil genetic pathways were altered by exercise before determining the effect of sex on these putative responses. Individuals participating in competitive sports and with a history of any chronic medical conditions or use of any medications were excluded from participation. The Institutional Review Board at the University of California, Irvine, approved the study, and written informed consent was obtained from all participants upon enrollment.

**Anthropometric Measurements**

Standard, calibrated scales and stadiometers were used to determine height and body mass.

**Measurement of Fitness**

Each subject performed a ramp-type progressive cycle ergometer using the SensorMedics metabolic system (Ergoline 800S, Yorba Linda, CA). After resting on the cycle ergometer for 3 min and 1 min of unloaded pedaling, the work rate (WR) was incremented at a rate of 20–30 W/min to the limit of the subject’s tolerance. Subjects were vigorously encouraged during the high-intensity phases of the exercise protocol. Gas exchange was measured breath-by-breath and the anaerobic (lactate) threshold and peak VO₂ were calculated using standard methods (10).

**Exercise Protocol**

At least 48 h, but not exceeding 7 days, following the completion of the ramp test, each subject performed 30 min of constant work rate cycle ergometry at a work rate equivalent to ~50% of the difference between the lactate threshold and the peak VO₂. On average, this work represented ~80% of the participants’ peak VO₂.

**Blood Sampling and Analysis**

An indwelling catheter was inserted into the ante-cubital vein. A baseline sample was taken 30 min after the placement of the catheter and before the onset of exercise (Before). 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 (After). Complete blood counts (CBC) for white blood cell analysis were obtained by standard methods from the clinical hematology laboratory.

**Neutrophil Isolation**

Neutrophils were isolated from EDTA-treated peripheral blood using OptiPrep Density Gradient Medium (SIGMA). The duration from blood draw to stabilization of RNA never exceeded 60 min. Using hematoxylin staining, we determined that this approach to neutrophil isolation consistently yielded ≥98% purification.

**RNA Extraction**

Total RNA was extracted using TRIzol ( Gibco BRL Life Technologies, Rockville, MD) reagent and purified using the RNAeasy Midi columns method (Qiagen, Valencia, CA). RNA pellets were resuspended in diethyl pyrocarbonate-treated water. RNA integrity was assessed (prior to beginning target processing) by running out a small amount of each sample (typically 25–250 ng/well) onto a RNA Lab-On-A-Chip (Caliper Technologies, Mountain View, CA) that was evaluated on an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

**Preparation of Labeled cRNA**

The detailed protocol for preparation and microarray processing was performed as recommended by the manufacturer and is available in the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA). Briefly, 2 μg total RNA was used as a template for double-stranded cDNA synthesis. Single-stranded then double-stranded cDNA was synthesized from the poly A spike-in controls, and mRNA present in the isolated total RNA using the SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen) and a T7-oligo(dT) primer (Integrated DNA Technologies, Coralville, IA) that contains a T7 RNA polymerase promoter site added to its 3’ end. A portion of the resulting double-stranded cDNA was used as a template to generate biotin-tagged cRNA from an in vitro transcription reaction (IVT) using the Affymetrix GeneChip IVT Labeling Kit.

**Hybridization to Microarray**

A total of 15 μg of the resulting biotin-tagged cRNA was fragmented to an average strand length of 100 bases (range 35–200 bases) following prescribed protocols (Affymetrix GeneChip Expression Analysis Technical Manual). Subsequently, 10 μg of this fragmented target cRNA was hybridized at 45°C with rotation for 16 h (Affymetrix GeneChip Hybridization Oven 640) to probe sets present on an Affymetrix U133+2 arrays. The GeneChip arrays were washed and then stained (SAPE, streptavidin-phycocerythrin) on an Affymetrix Fluidics Station 450, followed by scanning on a GeneChip Scanner 3000.

**Data Analysis**

**Microarray analysis.** The results were quantified and analyzed using GCOS 1.4 software (Affymetrix) using default values (Scaling Target Signal Intensity = 500). The microarray data were analyzed using ArrayAssist version 4.0.3 (STRATAGENE). We normalized the data using GC-RMA. Only probe sets that reached a signal value ≥20 in at least one array and a present call by MAS5 criteria in at least 18 arrays were selected for further analysis. Overall, 16,060 of 54,675 probe sets represented on the array met these criteria. The microarray cell files and GC-RMA normalized data have been deposited in the GEO database (http://www.ncbi.nlm.nih.gov/geo; series accession number = GSE8668). We further applied BRB-ArrayTools software Version 3.4.1 (http://linus.nci.nih.gov/~brb/ool.htm) to determine significantly changed probe sets from before to after exercise. Traditional Student’s paired t-test was first applied to each probe set and then significantly changed probe sets were decided with permutation tests (41). With 95% confidence, the final list of significantly changed probe sets has <5% FDR. The change from before to after exercise is presented with fold change, which is defined as the geometric mean of the ratio of after-exercise expression levels over before-exercise expression levels. [Note: The logarithm (base 2) of the geometric mean of X is equivalent to the arithmetic mean of log₂(X)].

**Gene annotation.** The final list of significantly changed probe sets was then additionally analyzed using the functional annotation tools provided by DAVID [Database for Annotation, Visualization and Integrated Discovery (http://david.abcc.ncifcrf.gov)] to classify the genes into functional groups and, when possible, into pathways using the KEGG database. Only functional groups with EASE score <0.05 are presented in this analysis. EASE score P value = 0 represents perfect enrichment. P value ≤0.05 is considered as gene enrichment in a specific annotation category (http://david.abcc.ncifcrf.gov/helps/functional_annotation.html#summary).

**Physiological data.** The physiological data were presented as mean and SE. The two-sided paired t-test was applied for testing changes from before to after the exercise and the significance level was set at 0.05. All analyses were done using SAS 9 (Cary, NC).

**Quantitative Real-Time Polymerase Chain Reaction**

For confirmation of microarray gene expression findings, quantitative real-time polymerase chain reaction (qPCR) was carried out on six genes that we felt were of particular physiological significance (five of which had higher expression after exercise). One microgram
of RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Reagents kit (Applied Biosystems) according to the manufacturer’s instructions, using random primers in a 100 µl reaction. The qPCR analysis was performed with the ABI PRISM 7000 Sequence Detection System (Applied Biosystems) by using TaqMan Universal PCR Master Mix and Assays-on-Demand Gene Expression probes (Applied Biosystems; HSPA1B: Assay ID: Hs00271244_s1, GZMB: Assay ID: Hs00188051_m1, STAT4: Assay ID: Hs00231372_m1, IL10RA: Assay ID: Hs00155485_m1, PRF1: Assay ID: Hs00169473_m1, EGR1: Assay ID: Hs00152928_m1). Actin beta was used as an endogenous control. We used Fisher Exact Test to evaluate the association between qPCR and microarray on their directional change of gene expression level due to exercise effect.

RESULTS

Anthropometric and Physiological Characteristics

The anthropometric and physiological characteristics of the 12 subjects appear in Table 1. The average BMI was within normal limits. The subjects were of relatively normal fitness (88.7 ± 3.7 of the subjects’ predicted VO$_{2}$max).

Serum Lactate

The exercise bout caused a mean increase of 9.0 ± 0.84 mM in serum lactate levels [Before (1.8 ± 0.07 mM) vs. After (11.0 ± 0.84 mM), P < 0.0001].

Leukocyte Response to Exercise

As shown in Table 2, the number of total white blood cells, lymphocytes, monocytes, and neutrophils was significantly elevated at peak exercise (P < 0.003).

The Effects of Exercise on Neutrophil Gene Expression

Using FDR < 0.05 with 95% confidence, a total of 526 genes were differentially expressed before and after exercise. Three hundred and sixteen genes had higher expression after exercise and 210 genes had lower expression after exercise (see supplementary table contained in the online version of this article). Given this criterion, the paired test P value for these genes was <0.0005.

We attempted to characterize the genes that were affected by exercise in several ways. First we used the Gene Ontology (GO) database provided by DAVID that allows us to annotate genes and classify them into functional categories. Figure 1 presents six groups of genes that are particularly relevant to current knowledge of the ways in which the neutrophil impacts health. As can be seen, many genes that could be classified by GO are linked to regulation of cellular physiological process, immune response, defense response, response to stress, apoptosis, and signal transduction. In Table 3, we show genes associated with apoptosis, and in Table 4, we present genes associated with growth, tissue repair, and inflammation.

RT-PCR Verification of Specific Gene

We verified the change from before to after the exercise of 6 genes in 11 of 12 subjects using RT-PCR (note, one of the subjects did not have sufficient RNA for this verification). In Table 5, we show that the direction of exercise effects are highly associated (Fisher Exact Test P value <0.0001). In HSPA1B and IL10RA, the directional change was different between the two techniques in only one subject, respectively.

DISCUSSION

We found a substantial alteration of 526 genes in circulating neutrophils immediately following 30 min of heavy exercise (a work rate that elicited a large and significant increase in circulating lactate) in healthy young men with average fitness. We used stringent statistical approaches in analyzing the microarray data to limit the possibility of false-positive results. Moreover, we found that a different technique, RT-PCR in a small group of genes, corroborated the microarray data. Consistent with our hypothesis, many of the affected genes were linked to processes associated with neutrophil activation such as microbial killing (e.g., GZMA, GNLY, SPON2; see Table

Table 1. Anthropometric characteristics and exercise responses of the 12 subjects

<table>
<thead>
<tr>
<th>Characteristics/Responses</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Body mass, kg</th>
<th>BMI, kg/m$^2$</th>
<th>Peak VO$_2$, ml·kg$^{-1}$·min$^{-1}$</th>
<th>Average VO$_2$ during the constant work rate as percent of peak VO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23.3±1.0</td>
<td>178.2±3.2</td>
<td>76.5±3.9</td>
<td>24.0±0.8</td>
<td>39.1±1.6</td>
<td>76.5±2.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; peak VO$_2$, peak oxygen uptake.

Table 2. Leukocyte response to exercise

<table>
<thead>
<tr>
<th></th>
<th>Before Exercise</th>
<th>After Exercise</th>
<th>%Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>5,425.0±335.3</td>
<td>9,383.3±548.0*</td>
<td>78.6±13.3</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1,967.1±191.6</td>
<td>4,339.2±447.2*</td>
<td>144.3±28.0</td>
</tr>
<tr>
<td>Monocytes</td>
<td>412.8±42.9</td>
<td>710.1±78.4*</td>
<td>111.8±52.5</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2,871.6±210.0</td>
<td>4,001.9±231.1*</td>
<td>43.8±9.2</td>
</tr>
</tbody>
</table>

Values are means ± SE (cells/µl). WBC, white blood cell. *Significantly greater After Exercise than Before Exercise (P < 0.003).

Fig. 1. Groups of genes that were altered by exercise [classified by Gene Ontology (EASE score <0.0004)].
of the expression level before the exercise. A fold change of 1.3 indicates the expression level after the exercise is in peripheral blood leukocytes in several subjects in whom example, Campbell and coworkers (8) studied gene expression over intervals of time in the range of 30 min. For gene expression over short intervals, the existing literature rest. Although not much data exist on changes in neutrophil changes in leukocyte gene expression over a 30-min period of in which one arm of the study would consist of observing overlap in gene response suggests that neutrophils respond differently to exercise than do PBMCs. Our own experience having extracted RNA from both neutrophils and PBMCs in many subjects), and, as a consequence the contribution of putative changes in gene expression among the various leukocytes is virtually impossible to determine unless the cells are isolated. In addition, the type of exercise and its duration differs greatly among the previous studies. We did not design this investigation as a crossover protocol. To the best of our knowledge, this is the first report demonstrating an acute effect of exercise on gene expression in circulating neutrophils. Other investigators have studied the effects of exercise on gene expression in whole blood (e.g., Refs. 7, 29, 50). Comparing the results of these earlier studies with those of the present are, however, problematic. First, because it has been known since the 1980s that neutrophils produce low amounts of RNA (Ref. 4; this is corroborated in our own experience having extracted RNA from both neutrophils and PBMCs in many subjects), and, as a consequence the contribution of putative changes in gene expression among the various leukocytes is virtually impossible to determine unless the cells are isolated. In addition, the type of exercise and its duration differs greatly among the previous studies. We showed earlier that the same exercise protocol used in the present study in a different group of participants altered PBMC gene expression in 311 genes (9). Interestingly, only 16% of the exercise-altered genes in neutrophils were similarly affected by exercise in the PBMCs. Among these similar genes, KSP37, GADD45B; see Table 4).

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Table 3. Representative genes known to regulate apoptosis

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Fold Change</th>
<th>Effect of Gene Change on Apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL2-associated transcription factor 1</td>
<td>BCLAF1</td>
<td>0.6</td>
<td>↑</td>
</tr>
<tr>
<td>Growth arrest and DNA-damage-inducible, beta</td>
<td>GADD45B</td>
<td>0.7</td>
<td>↑</td>
</tr>
<tr>
<td>BCL2-associated athanogene 5</td>
<td>BAG5</td>
<td>0.8</td>
<td>↑</td>
</tr>
<tr>
<td>Tumor necrosis factor (ligand) superfamily, member 8</td>
<td>TNFSF8</td>
<td>1.3</td>
<td>↑</td>
</tr>
<tr>
<td>Peptidyl-tRNA hydrolase 2</td>
<td>PTHR2</td>
<td>1.3</td>
<td>↑</td>
</tr>
<tr>
<td>Ras homolog gene family, member B</td>
<td>RHOB</td>
<td>1.5</td>
<td>↑</td>
</tr>
<tr>
<td>Annexin A1</td>
<td>ANXA1</td>
<td>1.5</td>
<td>↑</td>
</tr>
<tr>
<td>Fem-1 homolog b (C. elegans)</td>
<td>FEM1B</td>
<td>1.5</td>
<td>↑</td>
</tr>
<tr>
<td>PreB-cell colony enhancing factor 1</td>
<td>PBEF1</td>
<td>0.7</td>
<td>↑</td>
</tr>
<tr>
<td>Phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)</td>
<td>PDE4B</td>
<td>1.5</td>
<td>↑</td>
</tr>
<tr>
<td>Granzyme a (granzyme 1, cytotoxic t-lymphocyte-associated serine esterase 3)</td>
<td>GZMA</td>
<td>2.1</td>
<td>↑</td>
</tr>
<tr>
<td>Granzyme h (cathepsin g-like 2, protein h-ccpx)</td>
<td>GZMH</td>
<td>2.8</td>
<td>↑</td>
</tr>
<tr>
<td>Perforin 1 (poreforming protein)</td>
<td>PRF1</td>
<td>3.1</td>
<td>↑</td>
</tr>
<tr>
<td>Granzyme b</td>
<td>GZMB</td>
<td>3.9</td>
<td>↓</td>
</tr>
<tr>
<td>CD27-binding (Siva) protein</td>
<td>SIVA</td>
<td>0.6</td>
<td>↑</td>
</tr>
<tr>
<td>Signal transducer and activator of transcription 1, 91 kDa</td>
<td>STAT1</td>
<td>0.6</td>
<td>↓</td>
</tr>
<tr>
<td>Interferon induced with helicase C domain 1</td>
<td>IFIH1</td>
<td>0.7</td>
<td>↓</td>
</tr>
<tr>
<td>B-cell CLL/lymphoma 10</td>
<td>BCL10</td>
<td>0.7</td>
<td>↓</td>
</tr>
<tr>
<td>Purinergic receptor P2X, ligand-gated ion channel, 1</td>
<td>P2RX1</td>
<td>0.7</td>
<td>↓</td>
</tr>
<tr>
<td>Tumor necrosis factor (ligand) superfamily, member 10</td>
<td>TNFSF10</td>
<td>0.8</td>
<td>↓</td>
</tr>
<tr>
<td>Heat shock 70 kDa protein 1B</td>
<td>HSPA1B</td>
<td>1.9</td>
<td>↓</td>
</tr>
</tbody>
</table>

Fold change is defined as the geometric mean of expression levels of After/Before. A fold change of 0.6 indicates the expression level after exercise is ~60% of the expression level before the exercise. A fold change of 1.3 indicates the expression level after the exercise is ~130% of the expression level before the exercise.

4), viability (e.g., HSPA1B, BCLAF1, ANXA1, STAT1; see Table 3), and the regulation of growth mediators (e.g., EGR1, KSP37, GADD45B; see Table 4).

The impact of exercise on neutrophil apoptosis has yet to be completely elucidated. In rats, Lagranha et al. (19) demonstrated increased apoptotic markers (like annexin V) in neutrophils immediately following a 1-h bout of strenuous exercise. By contrast, in humans, Hsu et al. (14) showed no immediate effect of 30 min of heavy exercise on neutrophil apoptosis (using mitochondrial membrane potential), but did show accelerated neutrophil apoptosis the day after the exer-
The effect of exercise was proinflammatory, for example, when the exercise was performed a day after neutrophils were isolated from the blood. We also observed changes in gene expression of the neutrophil, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated 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performance that occurs in exercise-associated angiogenesis, one of the known mechanisms of improved physical activity performance that occurs in exercise-associated angiogenesis, one of the know...
expression responses following exercise to responses caused by other stimuli, we compared the changes in neutrophil gene expression from the present study with those observed by Zhang et al. (49) using an in vitro stimulation of neutrophils with live, opsonized *Escherichia coli* or LPS or chemotactant formyl-methionyl-leucylphenylalanine (Table 6). We did find a set of common genes in neutrophils that responded to both the in vivo exercise and in vitro immunologic stimuli. However, as shown in Table 6, the response pattern in this common set of genes, i.e., whether the genes were up- or downregulated, differed substantially.

As noted above, even brief exercise is accompanied by substantial perturbations in temperature, pH, oxygen availability, and cytokines, each of which is known to influence neutrophil function (3, 23, 24, 39, 44). Remarkably, we found changes in neutrophil gene expression of proteins that could provide mechanistic insight, in part, to each of these previously observed changes in neutrophil function (e.g., HSPA8, HSPA1B). Collectively, these observations suggest that the neutrophil gene expression accompanying exercise is more than just a stereotypic stress response and likely represents an integrated or summed response to the various physiological perturbations associated with physical activity. Exactly how these changes in gene expression translate into actual changes in protein production with physiological consequences has yet to be determined.

Two alternative mechanisms might explain the robust effect of exercise on neutrophil gene pathways: the first, a direct effect of exercise on gene expression within the population of circulating neutrophils, and the second, an indirect effect, the mobilization into the circulation of neutrophils that were expressing genes differently in their marginal pools (medullary or extramedullary) into the circulation of neutrophils that were expressing genes, i.e., whether the genes were up- or downregulated, differed substantially.

Consider first, for example, the gene with the highest increase in expression-endothelial differentiation, sphingolipid G protein-coupled receptor 8 (EDG80). The 4.2-fold increase that we observed for this gene could occur, however, only if marginal neutrophils that entered the circulation during exercise (note, we observed a 44% increase) were expressing EDG80 at levels ~11-fold greater than the circulating neutrophils. The decrease in expression for another gene, EGR-1, which had a fold change of 0.2, is even harder to model. If, in the extreme case, the marginal neutrophils had no detectable expression of this gene, the lowest possible postexercise ratio would be 0.69. Thus it is reasonable to speculate from the current database that exercise has some effect on gene expression even in the circulating population of neutrophils.

Although brief exercise is usually tolerated well, exercise can elicit an immunologic “danger” type of stress and inflammatory response that, on occasion, becomes dysregulated and detrimental to health (11). In many cases, such as exercise-induced asthma, anaphylaxis, and chronic diseases like arthritis, there is mounting evidence that neutrophils play a pathologic role. Consequently, we found in our data a subset of neutrophil genes that were influenced by brief exercise and that also have been identified by other investigators to be influenced by asthma (Ref. 31; Table 7) or arthritis (15) (Table 8). Several

**Table 7.** Five genes whose expression in neutrophils was influenced by 30 min of exercise in healthy adults (current study) and were also linked to asthma [data from Ober and Hoffjan et al. (31)].

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Fold Change</th>
<th>P Value (Paired t-Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signal transducer and activator of transcription 4</td>
<td>STAT4</td>
<td>2.6</td>
<td>7.6E-06</td>
</tr>
<tr>
<td>T-box 21</td>
<td>TBX21</td>
<td>2.5</td>
<td>6.0E-07</td>
</tr>
<tr>
<td>GATA binding protein 3</td>
<td>GATA3</td>
<td>1.7</td>
<td>2.3E-05</td>
</tr>
<tr>
<td>Chemokine (C-C motif) receptor 5</td>
<td>CCR5</td>
<td>1.6</td>
<td>4.2E-05</td>
</tr>
<tr>
<td>Toll-like receptor 10</td>
<td>TLR10</td>
<td>0.7</td>
<td>4.5E-04</td>
</tr>
</tbody>
</table>

Fold change is defined as the geometric mean of expression levels of After/Before. A fold change of 0.7 indicates the expression level after exercise is ~70% of the expression level before the exercise. A fold change of 1.7 indicates the expression level after the exercise is ~170% of the expression level before the exercise.

**Table 8.** Five genes whose expression in neutrophils was influenced by 30-min of exercise in healthy adults (current study) and were also found to be downregulated in neutrophils in arthritis patients [data from Jarvis et al. (15)].

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Fold Change</th>
<th>P Value (Paired t-Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Granulysin</td>
<td>GNLY</td>
<td>2.5</td>
<td>9.8E-06</td>
</tr>
<tr>
<td>Dual specificity phosphatase 2</td>
<td>DUSP2</td>
<td>2.2</td>
<td>4.5E-05</td>
</tr>
<tr>
<td>Granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)</td>
<td>GZMA</td>
<td>2.1</td>
<td>7.5E-06</td>
</tr>
<tr>
<td>Suppressor of cytokine signaling 1, SSF-1</td>
<td>SOCS1</td>
<td>1.6</td>
<td>1.2E-04</td>
</tr>
<tr>
<td>Growth arrest and DNA-damage-inducible, beta</td>
<td>GADD45B</td>
<td>0.7</td>
<td>1.8E-05</td>
</tr>
</tbody>
</table>

Fold change is defined as the geometric mean of expression levels of After/Before. A fold change of 0.7 indicates the expression level after exercise is ~70% of the expression level before the exercise. A fold change of 1.6 indicates the expression level after the exercise is ~160% of the expression level before the exercise.
studies (2–5) had shown that TBX21 and Gata 3 have been associated with an asthma or atopy phenotype. This subset of neutrophil genes that are both influenced by exercise and altered in disease states may prove as useful targets for further investigations into how physical activity acts to prevent or exacerbate disease.

In summary, relatively brief but strenuous exercise caused a substantial, specific, and seemingly paradoxical, change in neutrophil genomic response is dynamic not only under in vitro conditions but also under physiological conditions in vivo, suggesting that the genomic response in neutrophil immediately following the perturbation of exercise might be characterized as a cellular “wake-up” call. It is then the influence of subsequent physiological stimuli that ultimately determines which cellular pathway is actually activated or inhibited leading, in some cases, to change in neutrophil function. It well may be that the balanced response observed with this protocol could be upset when exercise is of longer duration and/or greater intensity. Nor did our data permit us to determine the impact of exercise on neutrophil gene expression beyond the end-exercise (30 min) time point that we chose to sample. Our study demonstrated that the neutrophil genomic response is dynamic not only under in vitro conditions shown in previous studies in which cultured cells are stimulated by a variety of factors ranging from cytokines to LPS (30, 49), but also under physiological conditions in vivo, like exercise, that occur frequently in the daily lives of human beings.

GRANTS

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