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14. ABSTRACT

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ORIGINAL ARTICLE

Changes in transcriptional output of human peripheral blood mononuclear cells following resistance exercise

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Abstract Various types of exercise alter the population of circulating peripheral blood mononuclear cells (PBMCs) and change their transcriptional output. This work examines changes in PBMC populations and transcription in response to resistance exercise training (RET), and identify key transcriptional changes in PBMCs that may play a role in altering peripheral tissues in response to RET. Ten resistance-trained men (20–24 years), performed an acute bout of RET for ~30 min following a 12 h fast. Venous blood was sampled at rest, immediately following exercise, and at 2 h post-exercise and analyzed for total and differential leukocytes and global gene expression using Affymetrix Genechips. Results showed elevated leukocytes, monocytes, lymphocytes, and lactate values immediately post-exercise ($P < 0.05$) over baseline. At 2 h post-exercise, leukocytes, and granulocytes remained elevated ($P < 0.05$), whereas lymphocytes were lower than ($P < 0.05$) baseline values. Initial microarray results showed the greatest transcriptional changes in pathways related to immune response, inflammation, and cellular communication. The change in PBMC population (2 h time point) correlated with a dramatic decrease in the expression of CD160, and XCL1, markers of lymphocyte populations. At the 2 h recovery time point upregulation of matrix metalloproteinase 9, orosomucoid 1, dishevelled-associated activator of morphogenesis 2, and arginase 1 suggest an induction in muscle damage and repair during this time frame. These results demonstrate that an acute bout of RET disrupts cellular homeostasis, induces a transient redistribution of certain leukocytes, and results in transcriptional changes in PBMCs translating into systemic changes in response to RET.

Keywords Microarray · Gene expression · Immune response · Resistance exercise

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Introduction

Human skeletal muscle is a dynamic tissue that adapts to physical demands through constant regeneration and remodeling. Resistance exercise, especially eccentric exercise, results in muscle microtrauma followed by adaptation and repair in the muscle (Clarkson and Dedrick 1988; Clarkson and Tremblay 1988). It is well known that strenuous exercise of both types evokes increases in circulating levels of leukocytes that contribute to both changes in immune response (i.e., increased T-cells) and changes in transcriptional output (Radom-Aizik et al. 2007b; Connolly et al. 2004; Nieman et al. 1995c; Shinkai et al. 1992). Stereotypically following acute endurance and resistance

exercises, there is a biphasic alteration in circulating leukocytes (Shinkai et al. 1992), and a decrease in lymphocyte counts (Carlson et al. 2008; Koch et al. 2001), natural killer cell activity, and mitogen-induced lymphocyte proliferation (Nieman et al. 1995a; Shephard et al. 1994). To better understand the mechanism of change it is important to look at subsets of leukocyte populations to identify key cellular and transcriptional components contributing to changes in systemic physiology. This work will specifically examine changes in a subset of leukocytes, peripheral blood mononuclear cells (PBMCs), (Carlson et al. 2008; Clarkson and Tremblay 1988; Koch et al. 2001; Kraemer et al. 1996; Nieman 1995), in an attempt to correlate those changes with inflammation in the surrounding tissue and to muscle soreness and muscle damage, as a result of RET. These correlations are of extreme interest as the transcriptional regulation of the genes involve may provide insight into optimal training regimes and prescribed exercise.

The immune and inflammatory systems play key roles in health and disease processes. Therefore, determining the transcriptional changes in PBMCs that control these processes and how that changes the peripheral tissue will provide more knowledge with regards to exercise-induced stress response. Recent work by Connolly et al. (2004) and Radom-Aizik et al. (2007b) investigated the genomic response of PBMCs after an acute bout of strenuous cycling using microarray techniques and suggest that PBMCs mediate stress via production of cytokines, chemokines and growth factors (Radom-Aizik et al. 2007b). Following 30 min of cycling, both groups reported significant changes in the gene expression of circulating PBMCs involved in inflammation, and cell growth and repair. Many of the genes involved in aerobic metabolism and energy generation were also significantly upregulated suggesting and increase in energy demand in response to exercise. Identifying global alterations in genomic factors as a result of various exercise stimuli has the potential to afford a better understanding of adaptive responses to exercise.

There has been much investigation into changes in PMBC population and immunological changes in circulating cytokines in relation to acute endurance exercise (>75% VO_{2max}); however, less is known about genomic response to resistance exercise training (RET). The variations in leukocyte concentration during and after both endurance and resistance exercise may be the result of a transitory redistribution of immune cells between the peripheral lymphoid tissues and the circulation (Nieman 1997). RET programs can cause muscle damage and soreness because of the workload or resistances utilized; we hypothesize that the transcriptional response of PBMCs will differ when compared to the aforementioned work.

Our investigation sought to examine the gene expression changes in PBMCs following an acute bout of resistance exercise using Affymetrix microarrays (Santa Clara, CA). Results of these microarray data will help discern whether immunological changes in response to RET coincide with the transcriptional changes in response to aerobic exercise or provide a host of unique transcriptional outputs in response to RET. We hypothesized that the RET insult would activate a transcriptional response involved in inflammation, cell growth, and tissue repair similar to that identified in sustained aerobic exercise and promote peripheral muscle remodeling in contrast to an increase metabolic pathways previously reported.

Methods

Participants

Ten moderately trained male college athletes ($n = 10$) gave their written informed consent to participate in this study, which was approved by the University's Institutional Review Board, and completed a medical-history questionnaire. All participants were members of NCAA Division III Ice Hockey team (post-season) and had been training for at least 5 years, with a minimum of 2 years of weight-lifting experience. Subjects' training throughout the year followed a typical periodization, which included both high volume and high intensity resistance training. Medical history results showed that no subjects had significant medical issues that would interfere with the study. Participant characteristics for experiments are presented in Table 1.

To minimize influence on each subject's immune system, participants were asked to adhere to specific instructions before exercise testing. This included abstinence from caffeine, alcohol, and anti-inflammatory medications for 24 h. Furthermore, participants agreed to abstain for 30 days from using large doses of vitamin/mineral supplements (>100% of recommended dietary allowances) until after the second exercise session. Participants were also instructed not to engage in exercise 24 h prior to each exercise testing session.

Table 1 Participant characteristics, mean ± SD

Variable	Mean/SD
Age (years)	22.3 ± 1.3
Height (cm)	181.1 ± 6.0
Body mass (kg)	90.0 ± 6.8
Body fat (%)	10.9 ± 3.9
1-RM back parallel squat (kg)	159.2 ± 18.0
1-RM leg press (kg)	410.0 ± 33.3
Years of training	5.0 ± 2.3

Participants were excluded from the study if they had an autoimmune disease (i.e., lupus, multiple sclerosis, rheumatoid arthritis, or insulin-dependent diabetes mellitus), tested positive for human immunodeficiency virus (HIV), or had been diagnosed with acquired immune deficiency syndrome (AIDS). Participants were also excluded if they were taking prescription medications, using steroids, using ergogenic supplements (e.g., creatine) within 30 days prior to testing, or had indicated that they experienced high psychological stress. Before each testing session, participants completed a second questionnaire to establish if they met the above pre-testing criteria and identify if they displayed any symptoms associated with upper respiratory tract infection (URTI) illness that would alter immune-cell parameters.

Procedures

Strength assessment and resistance-exercise protocol

One week before experimental testing, several baseline measurements were obtained which measured physical and strength parameters. This included measurements of baseline height, body weight, and body composition using the Jackson and Pollock's (1985) skinfold method, and strength assessment by performing one-repetition maximums (1-RMs) using the 1-RM testing protocol (Bachle et al. 2000) for both the leg press (Hoist Fitness Systems, San Diego, CA) and parallel back squat (Hoist, Fitness systems, San Diego, CA).

On the day of experimental testing, volunteers reported to the laboratory after 12 h of fasting. The temperature of the laboratory was ~21°C for all testing sessions and volunteers were tested at the same time of day as the strength assessment. Participants were required to complete an exercise-session checklist before participation to confirm adherence to pretesting instructions and absence of URTI symptoms. The resistance-exercise timeline (with differential gene expression data) used in this study is shown in Fig. 1. Briefly, blood was collected at three time points, pre-, post-, and 2 h following exercise. Prior to the first blood draw, each participant was required to rest quietly in a seated position for 10 min before sampling from an antecubital vein.

Exercise protocol

The resistance exercises employed were selected to recruit and activate a large amount of muscle tissue. Participants performed the parallel back squat and the seated leg press, both, which utilized major muscle groups in the lower extremities. The exercise protocol required each participant to complete six sets of the parallel back squat, followed by six sets of the seated leg press. Each exercise consisted of two warm-up sets of 10 repetitions at 45 and 55% of 1-RM and four sets of 10 repetitions at 65% of 1-RM. All repetitions were paced with the use of a metronome set at a 2:2 cadence, with a 2 min resting period between sets. The total time to complete the exercise protocol was approximately ~30 min. A second blood sample (post) was immediately collected after the completion of the exercise session. Participants were then instructed to rest quietly for 2 h. The final blood sample (2 h) was collected after the 2 h recovery period.

Blood collection, RNA extraction, and bioanalyzer analysis

Blood was sampled from the antecubital vein of each subject while seated at baseline, immediately post-exercise, and after 2 h of recovery. Vacutainers containing the anticoagulant (EDTA) were used for collection. Due to the delicacy of RNA, the duration from blood sampling to stabilization of RNA never exceeded 3 min. Blood was collected and immediately stored at −80°C at a ratio of four parts TriReagent BD to one part whole blood. Within 24 h, total RNA was extracted from the blood by adjusting the ratio of TriReagent to blood volume as described by the protocol, and further extracted using the standard blood protocol of TriReagent BD (Molecular Research Center, Cincinnati, OH). The resulting RNA was quantified on the

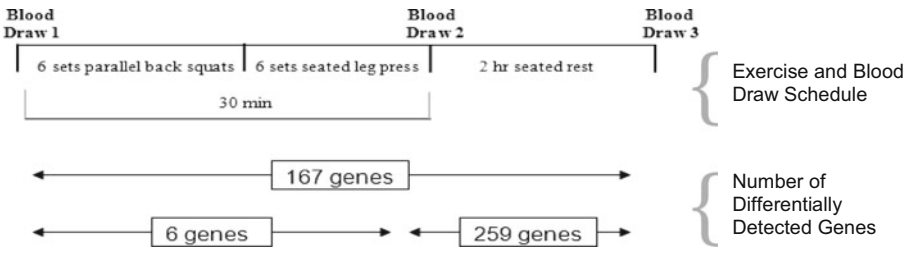

Fig. 1 Resistance exercise protocol timeline with corresponding gene response. Each exercise consisted of one set of 10 repetitions at 45% and one set at 55% of 1-RM and four sets of 10 repetitions at 65% of 1-RM. Repetitions were performed with a 2:2 cadence with 2 min rest between sets. Blood was immediately drawn from each participant and extracted for total RNA. Microarray analysis was performed and differentially expressed genes were calculated

NanoDrop ND1000 spectrophotometer and evaluated for integrity using Agilent 2100 Bioanalyzer. All RNA concentrations were between 13 and 77 ng/μL and had RIN values (RNA integrity numbers) greater than 7.0 indicating excellent quality RNA.

Microarray analysis

Microarray target preparation was performed using the NuGEN Ovation V2 and WB reagents (NuGEN Technologies, San Carlos, CA) as specified by the manufacturer with a total RNA input of 50 ng. The resulting cDNA was purified using the DNA clean and concentrator-25 (Zymo Research, Orange, CA), fragmented, biotin labeled, and prepared as a hybridization mixture as described in the methods of the NuGEN Encore labeling reagents. Microarray hybridizations were performed using the Affymetrix U133a 2.0 GeneChips for 16 h at 45°C at 60 rpm. GeneChips were subsequently stained using a double streptavidin phycoerythrin protocol on the FS450 fluidic station, scanned using the GS3000-7G scanner, and processed with the GeneChip Operating Software (Affymetrix Corp, Santa Clara, CA) (Affymetrix 2003). All gene chips passed standard Affymetrix guidelines for quality (Affymetrix 2003).

Microarray data analysis

Affymetrix GeneChip Operating Software (GCOS) was used to generate both image files (DAT and CEL) and probe intensity files (CHP). Data obtained from the GCOS CHP files includes quantitative gene signal information, which can be used in downstream complex data analysis. Quantitative data from each gene is determined by a set of DNA probes that interrogate at least 11 locations across each gene. Collectively, this "probe set" provides a single value summation of each gene response. Probe statistics are background corrected and normalized using the R Language and Environment for Statistical Computing with BioConductor tools (BioConductor: Open Source Software for Bioinformatics). Global expression statistics are calculated for each probe set from each sample using the robust multichip average (RMA) statistic of Speed et al. (Bolstad et al. 2003; Irizarry et al. 2003).

Immune cells and lactate

Whole blood containing EDTA was analyzed for complete blood counts and leukocyte subsets (Beckman Coulter AcT Diff2, Brea, CA). Total leukocytes and leukocyte subsets were corrected for changes in plasma volume via hematocrit and hemoglobin changes using the Dill and Costill (1974) method. Lactate determination at all three time periods was accomplished using fingersticks and analyzed using the Accutrend Lactate meter (Roche Diagnostics, Germany).

Statistical analyses

One-way analysis of variance (ANOVA) was used to determine whether there were significant changes in the dependent variables over time. A Tukey post hoc analysis was used to isolate differences across time. Based on our previous work (Carlson et al. 2008), a change of 25–50% with this type of training intensity. Based on this observation, we assumed that a similar change could be expected in the current study and would be considered meaningful thus we estimated that 6–12 participants would provide sufficient statistical power ($\beta = 0.20$) and an alpha of 0.05 to detect a difference in immune responses (Tran et al. 1997).

Linear modeling was performed on the microarray data using the Bioconductor limma package, which implements the method of Smythe (2005). Smythe's (2005) method borrows information across genes to improve inference based on small sample sizes. It provides, for each contrast, a moderated t statistic, P value, and P value adjusted (Klipper-Aurbach et al. 1995) for the purpose of controlling the false discovery rate (FDR). Differentially expressed probes were loaded into the ingenuity pathways analysis (Ingenuity® Systems, http://www.ingenuity.com) database for biological pathway analysis. Thresholds used for pathway analysis were $P = 0.01$ or less, and an expression fold change of at least 1.7× or greater.

Results

Lactate and immune response

A significant effect ($P < 0.05$) for time was observed for lactate. The resistance exercise induced a ~sevenfold increase ($P < 0.05$) from baseline to post-exercise while after 2 h post-exercise, the lactate levels subsided to approximately twofold over baseline (Fig. 2). A significant main effect ($P < 0.05$) for time was observed for leukocytes, monocytes, granulocytes, and lymphocytes. Tukey post hoc analysis revealed that leukocytes, monocytes, and lymphocytes were elevated immediately post-exercise ($P < 0.05$) over baseline values. At 2 h post-exercise, leukocytes and granulocytes remained elevated ($P < 0.05$), whereas lymphocytes were lower than ($P < 0.05$) baseline values (Fig. 2).

Gene expression and statistical analysis

Using a conservative FDR of $P < 0.05$ and a twofold change of or higher threshold, 167 genes were

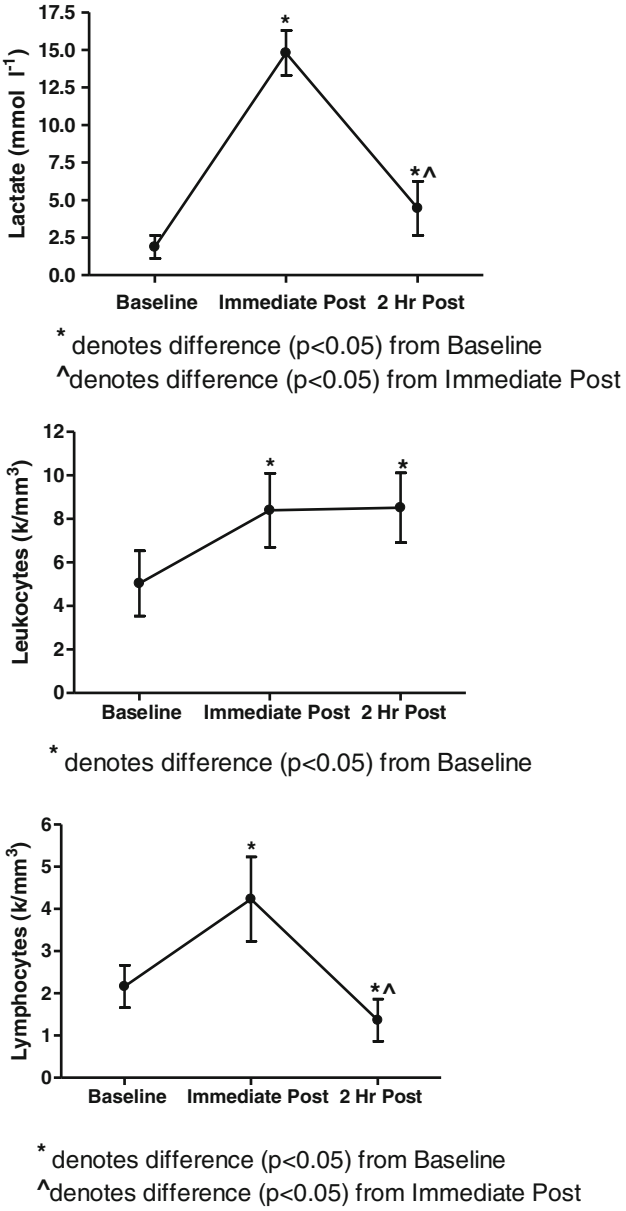

Fig. 2 Lactate, leukocyte, and lymphocyte data show changes in response to exercise time points

differentially detected between baseline and 2 h post-exercise, 259 genes between 2 h post-exercise and immediately post-exercise, and six genes between immediately post-exercise and baseline indicating the greatest gene response was seen at the 2 h post-exercise (Fig. 1). Further analysis of these differentially detected genes by plotting using similarity overlapping in a Venn diagram, indicate that although cellular populations and lactate levels are changing, that the majority of differentially expressed genes remain the same as indicated by the 139 genes in the overlap region. Conversely, the non-overlapping genes may be related to cellular population, lactate, or other changes (Fig. 3). The Venn diagram also displays 10 of the highest and lowest differentially expressed genes (by gene symbol) for the corresponding region. Complete data sets are available in the NCBI's Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/gds).

Principal component analysis (PCA, Ringner 2008), as well as sample clustering (heatmap and sample dendrogram, not shown) indicated clear separation between the 2 h post-exercise samples and all other samples as well as the expected inherent variation between participants reflected on the axis of PC 2 (Fig. 4).

The top canonical pathways identified by Ingenuity Pathways Analysis (Ingenuity® Systems, http://www.ingenuity.com) from the genes differentially expressed between the 2 h post-exercise and the pre-exercise blood samples are presented in Table 2. The percentage of these genes that are differentially regulated in the 2 h post-exercise samples is indicated. All of the pathways were statistically significant at $P < 0.05$, suggesting that the differentially expressed genes identified between the samples are most likely involved in these particular pathways due to the biology of the system.

Discussion

This study focused on identifying significant transcriptional and immunological changes in human PBMCs, in response to resistance exercise. This is one of the first studies to examine the changes in PBMC population and gene transcription in response to RET. While increases in immune cell population in response to both endurance and resistance exercises have been well documented, the transcriptional changes of the genes involved are not well understood. Using a microarray approach, this study identified differential gene expression in PBMCs (167 genes at $P < 0.01$) between pre- and 2 h post-exercise and grouped genomic changes into relevant key signaling pathways. The data presented here clearly demonstrates that a brief bout of RET stimulated PBMC both leukocyte and leukocyte-subset mobilization and a unique set of genomic changes. We also identified several specific genes from resistance exercise, as having potential roles into the mechanism of both skeletal and cardiac muscle recovery, remodeling and immune cell mobilization.

PBMC transcriptional response

Microarray data suggest that the genetic response 2 h post-exercise is significant, and presents a strong signature of immunological stress (Table 2). This was verified using a transcriptional principal components analysis (PCA), which graphically reveals total sample variation from microarray data into components that capture most of that

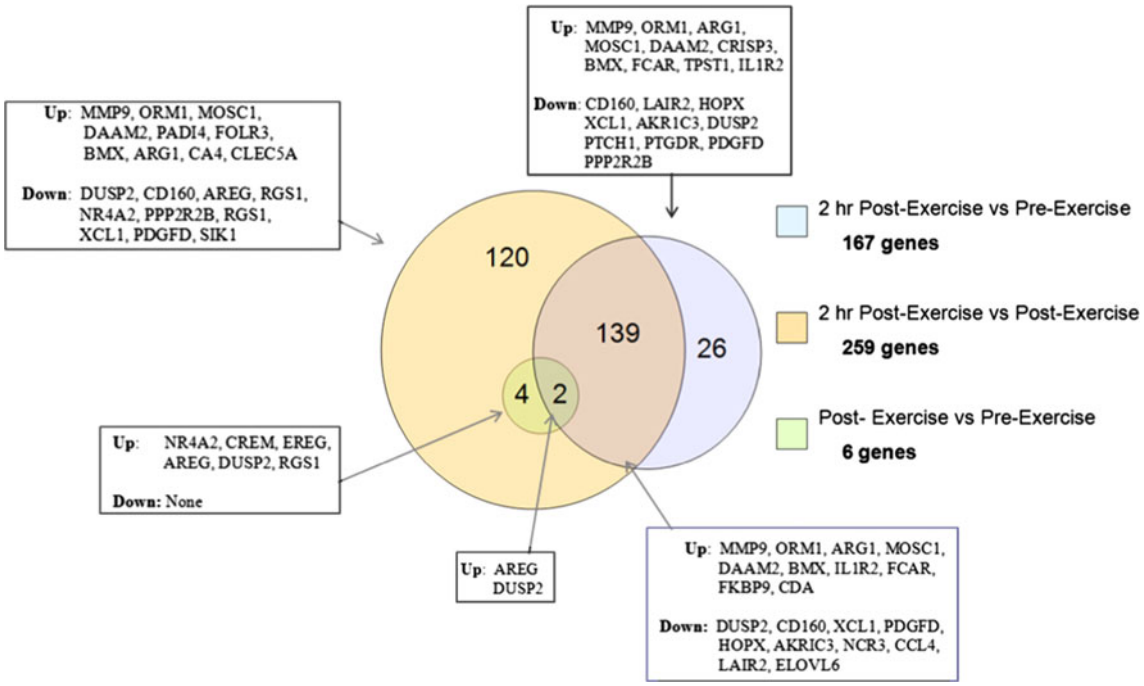

Fig. 3 Venn diagram showing the number and similarity of differentially detected genes from each comparison. These data indicate that 139 of the167 genes differentially detected between the 2 h after exercise and pre-exercise were the same genes as those detected between 2 h after exercise versus immediate post-exercise. All six genes detected for the immediate post-exercise data versus pre-exercise were also seen in the 2 h after exercise versus immediate post-exercise

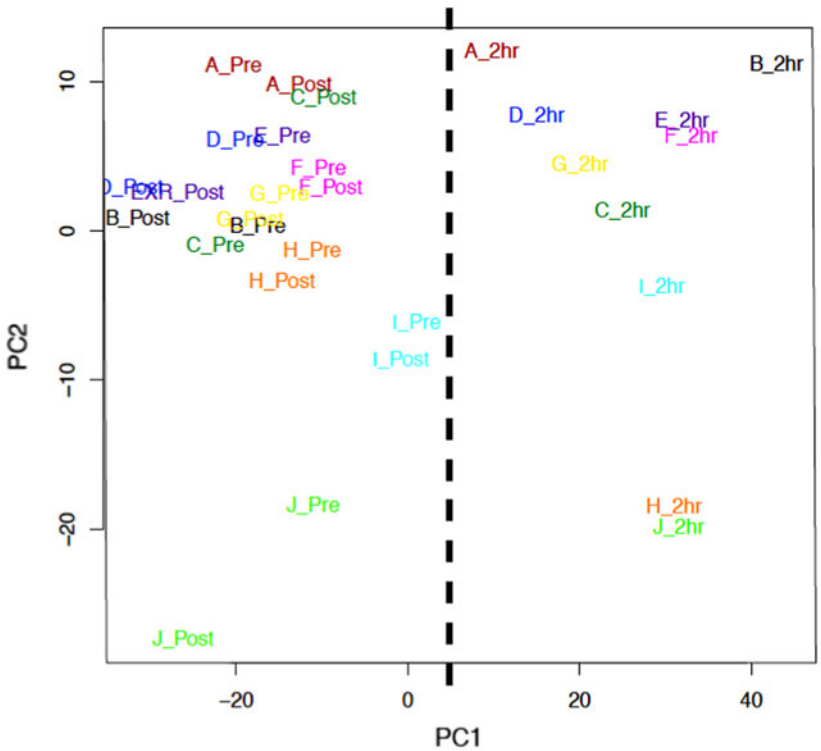

Fig. 4 The principle component analysis (PCA) plot of the microarray data indicates that the primary source of variation among samples correlates with the sample time points, distinguishing the 2 h post-exercise samples from the pre- and immediate post-exercise samples (PC1, *dashed line*). The second principal component (PC2) largely captures differences in expression between participants. Samples are labeled by participant (*A*–*J*) and sample time point (*pre* pre-RE, *post* immediate post-exercise, and *2 h* 2 h post-exercise), and color-coded by participant

Table 2 Top pathways, functions, and networks identified by ingenuity pathways analysis of the genes differentially expressed (DE) between 2 h post-exercise and immediate post-exercise blood samples ($P \leq 0.01$, fold change of 1.7× or higher)

Canonical pathways	*P* value	Number of differentially expressed molecules to total in pathway (%)
Natural killer cell signaling	1.6×10^{-7}	20/115 (17.4)
NF-κB signaling	8.9×10^{-5}	17/149 (11.4)
Cytotoxic T lymphocyte-mediated apoptosis of target cells	3.7×10^{-4}	7/31 (22.6)
T cell receptor signaling	9.9×10^{-4}	13/110 (11.8)
Communication between innate and adaptive immune cells	1.6×10^{-3}	10/90 (11.1)
Biological functions	***P* value**	**Number of differentially expressed molecules**
Inflammatory response	$\leq 5.8 \times 10^{-3}$	132
Infectious disease	$\leq 8.0 \times 10^{-3}$	100
Respiratory disease	$\leq 8.0 \times 10^{-3}$	74
Inflammatory disease	$\leq 7.3 \times 10^{-3}$	193
Immunological disease	$\leq 8.0 \times 10^{-3}$	187
Antigen presentation	$\leq 6.6 \times 10^{-3}$	125
Cell-to-cell signaling and interaction	$\leq 8.0 \times 10^{-3}$	140
Cell-mediated immune response	$\leq 8.0 \times 10^{-3}$	139
Humoral immune response	$\leq 5.4 \times 10^{-3}$	122
Hematological system development and function	$\leq 8.0 \times 10^{-3}$	134
Immune cell trafficking	$\leq 7.4 \times 10^{-3}$	88

Canonical pathways are known pathways for which there is a significant association with the molecules in the data set. Biological functions are composed of the molecules in the data set that are known to be involved in various diseases and functions, and are further divided into statistical significance subcategories

variation. The plot of those components indicated that the primary source of variation among samples correlates with the sample time points, distinguishing the 2 h post-exercise samples from the pre- and immediate post-exercise samples (Fig. 4, PC1, dashed line), while the pre-exercise and immediate post-exercise samples are inseparable along the *x* axis. The distinction of the 2 h post-exercise samples and the lack of separation between the pre- and immediate post-exercise samples are reflective of the number of differentially expressed gene identified by linear modeling between the time points (Fig. 4). The second principal component (PC2), which represents the second greatest source of variation, appears to largely capture differences in expression between participants. This data suggests that the reported change in PBMC population, although significant, is not the largest source of variation rather the change in transcriptional response is directly related to the exercise regime and the time points sampled. This PCA plot presents convincing evidence that the experimental variables (gene response time and participant variation) clustered appropriately and could easily be modeled, indicating good experimental controls with high signal to noise.

Cellular immune response during resistance exercise

Further analysis, comparing the pre- and 2 h post-exercise using ingenuity IPA, consistently identified differential expression of genes related immunological and inflammatory-related responses among the biological processes ($P < 0.01$) (Table 2). Statistically significant changes in gene expression in canonical pathways such as natural killer cell signaling, NF-κB, Cytotoxic T lymphocyte-mediated apoptosis and T cell receptors, suggesting that genes involved in these pathways are critical to mediating the host response to resistance exercise and may be critical targets for future studies (Table 2). As discussed above, this could be partially related to the elevated levels of leukocytes and the decline in lymphocytes but is primarily due to the exercise regime itself.

Whole blood counts showed increases in total leukocyte, lymphocytes, and monocytes immediately after resistive exercise (Fig. 2). Our findings are in agreement with previous studies that have described a mobilization of total leukocytes and their subsets immediately post-exercise, stimulated by an acute bout of resistance exercise (Carlson et al. 2008; Henson et al. 1999; Kraemer et al. 1996;

Simonson and Jackson 2004). The degree of these immune responses was similar to those reported during and after high-intensity prolonged endurance exercise (Nehlsen-Cannarella et al. 1997). Based on the exercise protocol utilized in this study, the 2:2 cadence not only kept all subjects on the same pace, but also may have induced a small degree of muscle damage. The high intensity of the RET bout was evidenced by the approximately sevenfold increase in blood lactate levels immediately following exercise.

Resistance exercise of this nature with pronounced eccentric muscle contraction has been reported to increase inflammation (Simonson and Jackson 2004) in the surrounding tissue, leading to the concomitant inflammatory response as noted by the temporary leukocytosis post-exercise (Malm et al. 2000; Pizza et al. 2001). The recruitment of leukocytes to the peripheral tissues during RET is further supported by the increased expression of tyrosylprotein sulfotransferase 1 (TPST1) by PBMCs (Fig. 3). TSPT1 is necessary for tyrosine O-sulfation, which is integral for protein–protein interactions involving the adherence of leukocytes to P-selectin on activated endothelium (Tu et al. 1999). It has been demonstrated that TSPT1 in immune lineages is essential for recruitment and retention of these cells to peripheral tissue suggesting that in RET leukocyte recruitment is occurring in a similar manner (Westmuckett and Moore 2009).

The consistent elevation in leukocytes is in contrast to the decline in lymphocytes post RET (Fig. 2). This decline in lymphocyte population has been previously shown after either prolonged endurance or exhaustive resistance exercises (Nehlsen-Cannarella et al. 1997; Carlson et al. 2008; Nieman et al. 1995b). The decline in lymphocyte population is also consistent with the measured changes in gene transcription that identified a dramatic decrease in both CD160 and XCL1 and an increase in orosomucoid 1 (ORM1). CD160 is a cell membrane receptor, also referred to as BY55 (Nikolova et al. 2002). CD160 is a 27 kDa glycoprotein whose expression is linked to peripheral blood natural killer cells, as well as CD8+ T lymphocytes. Much like the other genes implicated in our study, CD160 is linked to immunity, which can be seen through its expression, and the resulting decrease in expression may be correlated to the displayed decrease in circulating lymphocytes 2 h post-exercise. According to Nikolova et al. (2002), CD160 is expressed on intestinal intraepithelial T lymphocytes, circulating natural killer cells ($CD56^{DIM+}$ and $CD16^{+}$), and small amount of other circulating lymphocytes such as $CD8^{bright+}$, responsible for mediating cytotoxic activity. Likewise, XCL1 is chemokine secreted from CD8+ cells and its decline may be directly correlated to the decrease in lymphocyte populations. Interestingly, XCL1 transcript remained unchanged in response to aerobic exercise (1 h recovery time point) despite similar increase in lymphocyte populations in response to both RET and aerobic exercise (Connolly et al. 2004).

The displayed decrease in CD160 and in the increase of ORM1 shed light on the regulatory mechanisms of lymphocyte populations in the blood following exercise. A more extensive examination of the time course of PBMCs transcriptional response to RET would be useful in order to determine the relationship between these two genes and whether they are influenced by high lactate or other regulatory events.

Differential expression of genes related to cellular signaling

Signaling cascades related to inflammatory disease, immunological disease and cell-to-cell signaling showed the greatest changes as shown by number of genes differentially expressed and the most significant changes from pre-exercise to 2 h post-exercise (Table 1; Fig. 3). It is important to note that that pathways most affected by RET encompass many cellular and whole body systems. These changes involve genes specific to immune response (ORM1, XCL1, CD160), cell communication (DUSP2, DAAM2, BMX, TSPT1), development (HOPX), and matrix remodeling (MMP9) under both stress (ARG1) and disease conditions. This global transcriptional changes identified in PBMCs suggests that these variations in expression greatly impact systemic physiological changes in response to exercise rather than just localized changes in the sample population. Others have noted changes in these cellular pathways; however, it is important to note that there are distinct differences in the expression profiles as a result of the different exercise regimes. Differential expression of transcripts compared pre-, post- and 2 h post-exercise are summarized in Fig. 3.

Transcripts of interest showing differential expression at the 2 h post-exercise recovery time point were matrix metalloproteinase 9 (MMP9), orosomucoid (ORM1), dishevelled-associated activator of morphogenesis (DAAM) and arginase 1 (ARG1), showing significant upregulation, while CD160 was significantly down-regulated. These transcripts show consistent changes across the data set suggesting that the transcriptional response is immediate in response to exercise and persists 2 h post-exercise. This static change is in contrast to the dynamic transcriptional changes of dual specificity phosphatase 2 (DUSP2) that has been previously reported (Connolly et al. 2004) (Fig. 3). DUSP2 has many roles in stress and inflammatory related pathways and the early induction of the transcript may function in chronic inflammation. Changes in expression of CD160 are discussed above and most likely reflect the changes in cell population where as alterations in ORM1,

MMP9 and ARG1 may be related to dynamic changes influencing peripheral tissues during RET.

ORM1 or α 1-acid glycoprotein (αAGP) was also significantly increased in response to RET. ORM1 is a plasma protein secreted under stressful conditions, injury, infection, inflammation, and has been implicated is suggested to have roles in immunomodulatory, barrier and carrier functions (Lecchi et al. 2009). ORM1 has been shown to inhibit mitogen-induced proliferation of lymphocytes and this may be partially responsible for the reduction in lymphocytes observed in this study. The resulting decrease in lymphocytes could be a direct result of an increase in circulating ORM1 from the PBMCs and continued studies would be of interest to demonstrate that ORM1 is or is not responsible for halting proliferation of resident lymphocyte populations in the case of RET. Interestingly, other studies in both aerobic exercise and tumor progression experiments demonstrated that an increase of lactate in the blood stream may cause a regression or inhibition of TCR, lymphocytes, and associated pathways (Mihm and Droge 1985; Fischer et al. 2007). However, this has not yet been show in RET, but may not be surprising as the levels of lactate in this study were significantly elevated (Fig. 2).

As indicated, both MMP9 and ARG1 were significantly increased in response to RET suggesting that there is significant muscle damage and associated cell recruitment and repair occurring. Neither of these transcripts were shown to be upregulated in response to 30 min of nonimpact exercise (Connolly et al. 2004); this suggests that the exercise regime implement here has different ramification for muscle remodeling.

Functionally, MMPs are enzymes responsible for the destruction of extracellular matrix, and are linked to inflammation and tissue remodeling (Kanbe et al. 1999). MMP9, also referred to as gelatinase B, has been classified by its protein and domain structure as the largest and most complex of the MMP family (Dubois et al. 1999). MMP9 is quite complex and elicits a widespread effect, including activation of other MMPs, regulating gene transcription and protein secretion by both cytokines and chemokines, proenzyme activation by components of the plasminogen activation system and action of tissue inhibitors of matrix metalloproteinases. Interplay between MMP9 and transforming growth factor beta (TGF-β) signaling is integral to macrophage recruitment to the vasculature allowing for matrix remodeling and promoting of angiogenesis (Stamenkovic 2000). This signaling cascade has been well characterized in tumorogenic tissues and a similar mechanism may be occurring to enhance perfusion of the skeletal muscle during RET. Additionally, high levels of circulating MMP9 have been correlated with an increase in left ventricular remodeling (Sundstrom et al. 2004). This suggests that the RET training used may be significant enough to induce cardiac stress translating into cardiac matrix remodeling. Levels of circulating MMP9 may be a key indicator of stress induced as a result of RET and could assist in the development of optimal exercise regimes allowing for cardiac perfusion rather than damage.

The increase in protein associated with cellular remodeling is consistent with an increase in DAAM, a regulatory protein in Wnt signaling family, playing a role in directing signals necessary for directing both endothelial cell proliferation and migration during this process (Cirone et al. 2008). Muscle biopsies following RET demonstrated a change in transcription calpain-1 and myostatin (Louis et al. 2007), both of which have been implicated in Wnt signaling and could be downstream targets of DAAM within the peripheral tissues. Although correlative, the data from this and previous work suggests that the transcriptional changes in the PBMCs will direct proteome changes in the active tissues.

Likewise, the increase in ARG1 expression in response to RET suggests and influx of intermediates to the urea cycle in response to amino acid breakdown from skeletal muscle. Given the engagement of large muscle groups from RET this is expected and consistent with the increase in lactic acid (Fig. 2). ARG1 has also been shown to reduce activity of MMP9 by reduction in reactive oxygen species suggesting that in this study the upregulation of both ARG1 and MMP9 activity may facilitate the increase in muscle remodeling as a result of RET (Xu et al. 2003). Interestingly, ARG1 is also able to elicit its function on the body through regulation of nitric oxide because it competes with nitric oxide synthase (NOS) for arganine (Xu et al. 2003). According, to Bansal and Ochoa (2003) the competition for arginine between ARG1 and NOS may lead to macrophage and T-lymphocyte activation (Bansal and Ochoa 2003). This activation of T-lymphocytes also correlates well with the decrease in circulating lymphocytes (Fig. 2) and it is possible that the increase in ARG1 expression facilitates the movement of this cell population to the peripheral tissue to aide in muscle remodeling. It is for this reason that arginase expression is implicated in both the immune response and inflammation associated with disease process (Xu et al. 2003).

Conclusions

In summary, an acute bout of resistance exercise disrupts cellular homeostasis, and induced a transient redistribution of certain immune cell populations. The exercise bout also altered the gene expression in the peripheral blood which in turn appeared to modulate both PBMC population and systemic processes occurring in the peripheral tissues in response to RET. Future studies should validate the

differentially expressed genes with RT-qPCR and determine whether this transcriptional increase translates to changes in protein expression in both PBMCs and sites of tissue remodeling. Detailed understanding of the effects of RET on PBMCs and how that translates into skeletal or cardiac adaptation, vascularization and optimal immune response may help optimize exercise regimes and impact how exercise is used for prescribed health.

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Conflict of interest None.

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