Immune Cell Inflammatory Cytokine Responses Differ Between Central and Systemic Compartments in Response to Acute Exercise in Mice

Nabeel Pervaiz and Laurie Hoffman-Goetz

School of Public Health and Health Systems, Faculty of Applied
Health Sciences, University of Waterloo, Ontario, Canada

ABSTRACT

Background: *Exhaustive exercise induces apoptosis and oxidative stress in systemic organs and tissues and is associated with increased levels of pro-inflammatory cytokines. The effects of acute exercise on cytokine expression and apoptosis of immune cells in the central nervous system (CNS) have not been well characterized.*
Purpose: *We investigated the effects of a single bout of strenuous exercise on the expression of TNF-α, IL-6, and IL-β, as well as the apoptotic status of cells in the hippocampus of healthy mice. To compare central vs. systemic differences, cytokine expression in the intestinal lymphocytes of a subset of mice were also assessed.*
Methods: *Female C57BL/6 mice were divided into three groups: sedentary controls (NOTREAD) (n = 22), treadmill exercise with immediate sacrifice (TREAD-Imm) (n = 21), or treadmill exercise with sacrifice after 2 hours (TREAD-2h). TNF-α, IL-6, and IL-1β expression in the hippocampus and intestinal lymphocytes were measured by Western blot analysis. Percentages of hippocampal cells undergoing apoptosis (Annexin$^+$) or necrosis (Propidium Iodide$^+$) were determined through flow cytometry. Plasma levels of 8-isoprostane and corticosterone were measured using commercially available EIA kits.*
Results: *Acute treadmill exercise led to significant decreases in TNF-α ($p<0.05$) and increases in IL-6 ($p<0.05$) expression in the hippocampus of healthy mice. No effects of acute exercise on the apoptotic status of hippocampal cells were observed. In intestinal lymphocytes, the exercise bout led to significant increases in TNF-α ($p<0.05$), IL-6 ($p<0.05$), and IL-1β ($p<0.05$). Acute exercise was associated with a significant increase in both plasma 8-isoprostane ($p<0.05$) and corticosterone ($p<0.05$) levels.*
Conclusion: *Acute exercise differentially affects the pattern of pro-inflammatory cytokine expression in the hippocampus compared to intestinal lymphocytes and, further, does not induce apoptosis in hippocampal cells.*

Key words: Acute exercise, hippocampus, intestinal lymphocytes, cytokines, apoptosis, oxidative stress

Correspondence: Dr. Laurie Hoffmann-Goetz, Professor, School of Public Health and
Health Systems, Faculty of Applied Health Sciences, University of Waterloo,
200 University Avenue West, Waterloo, Ontario, Canada N2L 5X4,
Telephone: 1 519 885-1211 ext 33098, E-Mail: lhgoetz@uwaterloo.ca

INTRODUCTION

Exercise can have a positive and a negative impact on the immune system depending on its duration and intensity. Acute exercise is associated with reduced immune function and increased risk of infection (22; 27; 34). Moreover, exhaustive exercise leads to leukocytosis immediately followed by lymphocytopenia (40), which may be due in part to exercise-induced DNA fragmentation and apoptosis (26; 29; 31). Many of these effects are thought to be the result of increased oxidative stress in the affected tissues. Oxidative stress is an imbalance between endogenous antioxidants, such as superoxide dismutase and glutathione peroxidase, and reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radical (48). Acute exercise leads to increases in oxygen consumption, which then results in the formation of ROS and consumption of intracellular antioxidant levels, alterations in mitochondrial membrane potential, and DNA damage leading to apoptosis (29; 42). Essential components of apoptotic cell death are caspase-3 and caspase-7 (2; 15) both of which are significantly elevated after prolonged exhaustive exercise (18; 19; 20). In addition to changes in oxygen metabolism, acute exercise alters the cytokine balance systemically. For example, physical activity at intensities greater than 70% VO_{2max} increases the levels of TNF-α (a cytokine that can activate apoptosis) in plasma (36; 51; 53; 57) and colonic lymphocytes (19; 20). Similar exercise-induced increases were also observed for the cytokines IL-1β and IL-6 (36; 51; 57) suggesting that strenuous exercise (and especially eccentric exercise with muscle damage) results in inflammation.

Few studies, however, have determined if acute exercise affects pro-inflammatory cytokine status in the central nervous system (CNS) and none have examined its effects on apoptosis of immune cells in the brain. Scopel et al. (47) found that in rats two weeks of treadmill exercise at 60% VO_{2max} worsens existing damage to hippocampal mitochondria induced by in vitro oxygen and glucose depletion. In contrast, Ackigoz et al. (1) reported that exhaustive treadmill running (25 m/min, 5° slope) in Wistar rats did not change superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities or thiobarbituric acid reactive susbstance (TBARS) levels in the hippocampus, prefrontal cortex, or striatum; this exercise protocol was therefore not associated with significant lipid peroxidation in these brain compartments. In an older study, Radak et al. (45) reported that exhaustive treadmill exercise at 24 m/min and 15% incline did not alter levels of SOD, catalase, GPx or TBARS in the hippocampus and cerebellum of rats. Somani et al. (49) reported no changes in SOD activity in the cortex, striatum, cerebellum, medulla, and hypothalamus of rats following treadmill exercise at 100% VO_{2max} for 40 min. Together these results imply that the brain is protected from the systemic inflammatory damage related to oxidative stress which occurs with high-intensity aerobic exercise.

However, a recent review by Packer et al. (38) suggests that acute exercise may still pose an inflammatory "threat" to the CNS. Steensberg et al. (52) found that cerebrospinal fluid (CSF) levels of HSP72 (an indicator of oxidative stress) were increased 5-fold in healthy men who underwent 2 hours of cycle ergometry; no changes in CSF IL-6 concentrations were noted and TNF-α levels remained undetectable before and after the exercise bout. In contrast, cerebral IL-6 levels (as

measured by internal jugular venous to arterial differences) were significantly elevated in men who participated in two successive 60 min bouts of cycle ergometry (35). Animal studies (8; 9; 10) also indicate that muscle-damaging downhill treadmill exercise in mice leads to elevations in IL-1β in the cortex and cerebellum through activation of perivascular and meningeal macrophages. Thus, the direction of effects of exhaustive exercise on CNS brain inflammatory cytokine responses is unclear and some of this variation may be due to inter-species differences. This is in contrast to the generally pro-inflammatory and apoptotic responses observed in the peripheral compartments after acute exercise. Moreover, the issue of exercise-induced apoptosis in brain immune cells has gone unexplored.

The purpose of this study was to examine the effects of a single bout of acute, strenuous exercise on the expression of classical pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and apoptotic status in the hippocampus of healthy mice. This brain region was chosen because it is involved in cognition, memory, and the stress response (12; 33). In addition, plasma levels of 8-isoprostaglandin $F2_\alpha$ and corticosterone were measured to determine whether the exercise protocol was sufficient to elicit a stress response. A second purpose was to compare these pro-inflammatory cytokine responses centrally vs. peripherally following the acute exercise challenge. We hypothesized that exposure to a strenuous bout of aerobic exercise would lead to increases in the expression of hippocampal TNF-α, IL-1β, and IL-6, coupled with increases in the apoptotic status of hippocampus cells, in a manner similar to intestinal lymphocytes (i.e., a peripheral lymphoid compartment). Through this investigation, the relationship between indicators of exercise-induced oxidative stress in the plasma and apoptosis and central inflammatory processes in hippocampal immune cells was explored.

MATERIALS & METHODS

Animals
Female C57BL/6 mice (n = 63) (Harlan Indianapolis, IN, USA), 4-5 months of age, were housed in individual cages at 21 ± 1 °C, on a 12/12 h reversed light/dark cycle. *Ad libitum* access to a standard rodent diet (Lab Rodent Chow, PMI Feeds, Richmond, IN, USA) and tap water were provided. The experimental procedures adhered to the guidelines established by the Canadian Council on Animal Care and were approved by the University Animal Research Ethics Committee.

Exercise protocol
Mice were matched on weight and randomly assigned to one of three treadmill exercise conditions: (1) treadmill running (90 min, 2° slope) with sacrifice immediately after exercise (TREAD-Imm; n = 21), treadmill running (same duration, speed and grade) with sacrifice 2 h after exercise (TREAD-2h; n = 20), and control animals that were exposed to treadmill noise and vibrations for 90 min, without running, before sacrifice (NOTREAD; n = 22). The running protocol consisted of a 10 min warm-up, 30 min at 22m/min, 30 min at 25 m/min, 30 min at 28 m/min, and a 5 min deceleration to 0 m/min on an Omni-Max metabolic treadmill (Omni Tech Electronics, Columbus, OH, USA). All running took place at the beginning of the

dark cycle (between 7 and 9 am). Mice were motivated to run by gentle prodding using a nylon brush and were fasted overnight prior to the start of exercise.

Plasma collection
Mice were sacrificed by sodium pentobarbital overdose (0.6-0.8 cc per mouse, i.p.). After confirmation of a negative toe pinch response, skin was grasped at the mid-ventral position of the body and an incision was made across the chest to expose the rib cage. This was cut to expose the heart, and blood was collected immediately using a heparinized syringe. Blood was centrifuged at 1500 g for 6 min and plasma was collected and stored at -80 °C until analysis of corticosterone and 8-isoprostaglandin F2$_\alpha$.

Hippocampus removal and single cell suspensions
Excision of mouse hippocampi was performed according to Hassan et al. (16). All brain dissections took place on an ice-mounted stage. Decapitation was completed immediately following sacrifice. A midline incision was made along the skull, granting access to underlying structures, and the brain was excised and washed in cold PBS (0.5% BSA/PBS), transferred to the dissection stage and bisected at the midline. A clean number-1 paintbrush was inserted into the fissure beneath the dorsal cerebral cortex, and the hippocampi from both hemispheres were visualized, isolated, and placed in 1.5 mL RPMI (1640, 2.5% FCS), pressed through a 70 μm cell strainer, and centrifuged at 1500 RPM for 5 min. Cells were resuspended in 5 mL RPMI at room temperature, layered over 5 mL of Lympholyte M (Cedarlane Laboratories, Hornby, ON, Canada), and centrifuged at 1250 g for 20 min. Cells at the interface were recovered, washed, suspended in 300 μL PBS and counted by microscopy. Cell samples were stored at -80 °C until analysis.

Assessments of apoptosis of hippocampal cells
Immediately following the preparation of hippocampal single cell suspensions, 1×10^5 hippocampal cells were incubated for 15 min in the dark with 2.5 μl of Annexin V-FITC (Pharmingen, San Diego, CA, USA), 2.5 μl of Propidium Iodide (PI) (Sigma Chemical, St. Louis, MO, USA), and 100 μl of Annexin binding buffer, in order to obtain percentages of apoptotic and necrotic cells as has been previously described (18).

Protein determination and Western blot analysis of hippocampal cell and intestinal lymphocyte TNF-α, IL-1β, and IL-6
Hippocampal cells were lysed, placed on ice for 45 min, and the lysates centrifuged (10,000 g, 15 min) for protein determination by bicinchoninic acid (BCA) assay. Protein supernatant (40 μg) and molecular weight markers (Full Range Rainbow, Amersham Biosciences, Buckinghamshire, UK) were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE, 12-15%), transferred to a polyvinyldiene fluoride (PVDF) membrane, and stained with Ponceau S to confirm quality of transfer and equal loading. Membranes were then incubated with primary antibody for 1 h (1:200 in 10% FBS-TBST): TNF-α (sc-1350), IL-1β (sc-71435), or IL-6 (sc-1265) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and subsequent incubation with secondary antibody for 1 h: horseradish peroxidase-conjugated anti-goat (TNF-α) or anti-mouse (IL-1β, IL-6)

at 1:2000 in FBS-TBST. Protein quantity was determined using ECL Plus Western blotting detection reagent (Amersham Biosciences, Buckinghamshire, UK) and the ChemiGenius 2 Bio-imaging system (Cambridge, UK). Intestinal lymphocytes were collected as described (20) from a subset of mice (n = 30) for comparison with hippocampal cells and to be used as internal controls to document whether the acute exercise protocol led to previously observed systemic cytokine changes in TNF-α, IL-1β, and IL-6. Western blot analysis of intestinal lymphocytes was performed as described above for hippocampus.

Corticosterone assessment
Plasma samples were assessed for corticosterone levels using a commercially available enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA). Purification requirements of the samples were determined using the cold spike protocol, and concentration of corticosterone was measured at 412 nm using a PowerWave 340 microplate spectrophotometer (Biotek Instruments, Vermont, USA), according to the manufacturer's protocol. All samples were run in duplicate. The intra-assay % CV was 6.3% and the lower detection limit was 30 pg/ml.

8-isoprostaglandin F2$_\alpha$ assessment
Plasma 8-isoprostaglandin F2$_\alpha$ (8-isoprostane) levels were quantified using a direct EIA kit (Cayman Chemical, Ann Arbor, MI, USA). Samples were hydrolyzed (25 µl 10 N NaOH: 100 µl sample) at 45 °C for 2 h, neutralized with 12 N HCl, centrifuged (5 min, 14,000 g), and supernatant incubated with 8-isoprostane antibody for 24 h at 4 °C. Absorbance was measured at 405 nm at room temperature (PowerWave 340 microplate spectrophotometer, Biotek Instruments, Vermont, USA). All samples were run in duplicate. The intra-assay % CV was 8.6% and the lower limit of detection was 2.7 pg/ml.

Statistical analysis
Cytokine concentrations and measures of apoptosis and necrosis were analyzed using one-way ANOVA with acute treadmill exercise challenge (3 levels: NOTREAD, TREAD-Imm, TREAD-2h) as the independent factor and cytokine protein expression, % Annexin$^+$, and % PI$^+$ as the dependent factors (SPSS Version 18; Chicago, IL, USA). Corticosterone and 8-isoprostane levels were analyzed using one-way ANOVA with acute treadmill exercise challenge (2 levels: NOTREAD, TREAD-Imm) as the independent factor and corticosterone and 8-isoprostane concentrations as the dependent factor (SPSS Version 18; Chicago, IL, USA). Post hoc analysis was determined with Tukey's HSD test and all ANOVAs results were checked for homogeneity of variance with Levene's test). Significant difference from chance alone was accepted if $p < 0.05$; all values are expressed as group means ± 1 SEM for respective units.

RESULTS

Body Mass
At sacrifice, the mean body mass of mice was 26.7 ± 0.8 g (NOTREAD: 27.7 ± 0.7 g; TREAD-Imm: 26.1 ± 0.8 g; TREAD-2h: 26.2 ± 0.8 g) and these groups did not differ (F (2, 62) = 1.309, n.s.).

Apoptosis

No differences were observed with respect to the percentage of Annexin[+] (F (2, 62) = 0.231, n.s.) or PI[+] (F (2, 62) = 0.696, n.s.) hippocampal cells between the NOTREAD (Annexin[+]: 4.0 ± 0.3 %; PI[+]: 3.6 ± 0.3 %), TREAD-Imm (Annexin[+]: 4.2 ± 0.3 %; PI[+]: 4.0 ± 0.3 %), and TREAD-2h (Annexin[+]: 3.9 ± 0.3 %; PI[+]: 3.8 ± 0.3 %) mice.

Hippocampal Cytokines

Figure 1 shows the effects of acute treadmill exercise on the expression of pro-inflammatory cytokines (in Arbitrary Units [AU]) in the hippocampus. There was a significant effect of acute treadmill exercise on TNF-α expression (F (2, 58) = 3.31, p < 0.05) and expression of this cytokine was lower in TREAD-Imm (1.1 ± 0.1 AU) and TREAD-2h (1.1 ± 0.1 AU) compared to NOTREAD (1.4 ± 0.1 AU) mice. Acute exercise significantly affected IL-6 expression in mouse hippocampus (F (2, 58) = 6.23, p < 0.05) with this cytokine being higher in TREAD-2h (1.2 ± 0.06 AU) compared to NOTREAD (0.9 ± 0.06 AU) and TREAD-Imm (1.0 ±
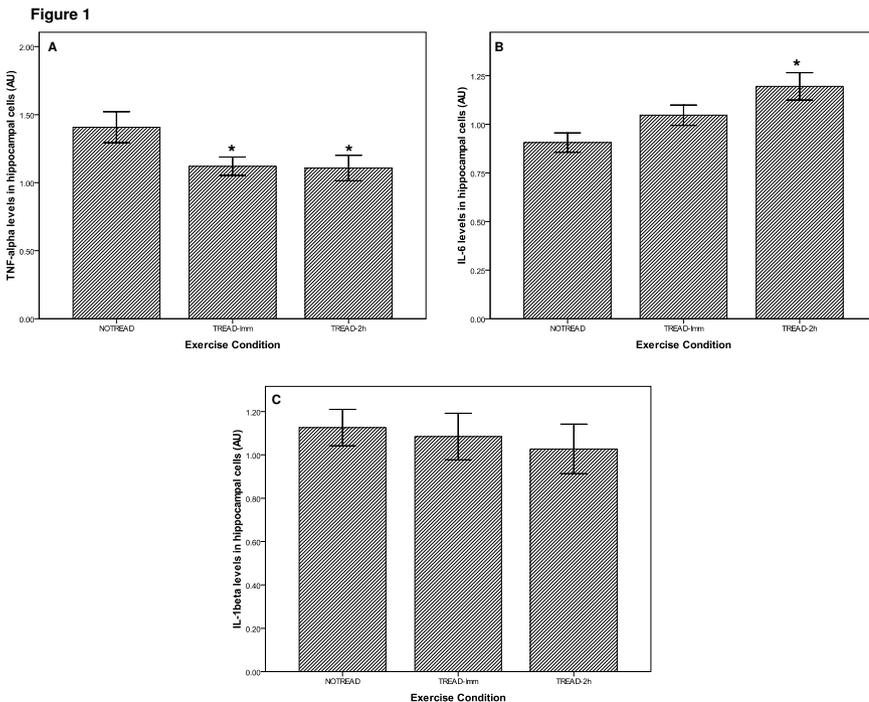
Figure 1

Figure 1

Cytokine (TNF-α, IL-6, IL-1β) expression [AU] in hippocampal cells of mice given a single acute exercise bout and sacrificed immediately (TREAD-Imm) or after 2 hours (TREAD-2h) versus sedentary controls (NOTREAD). *Panel A:* TNF-α expression. *Panel B:* IL-6 expression. *Panel C:* IL-1β expression. Values are means ± one standard error. Significance compared to NOTREAD control indicated by an asterisk (*). See text for details of analysis.
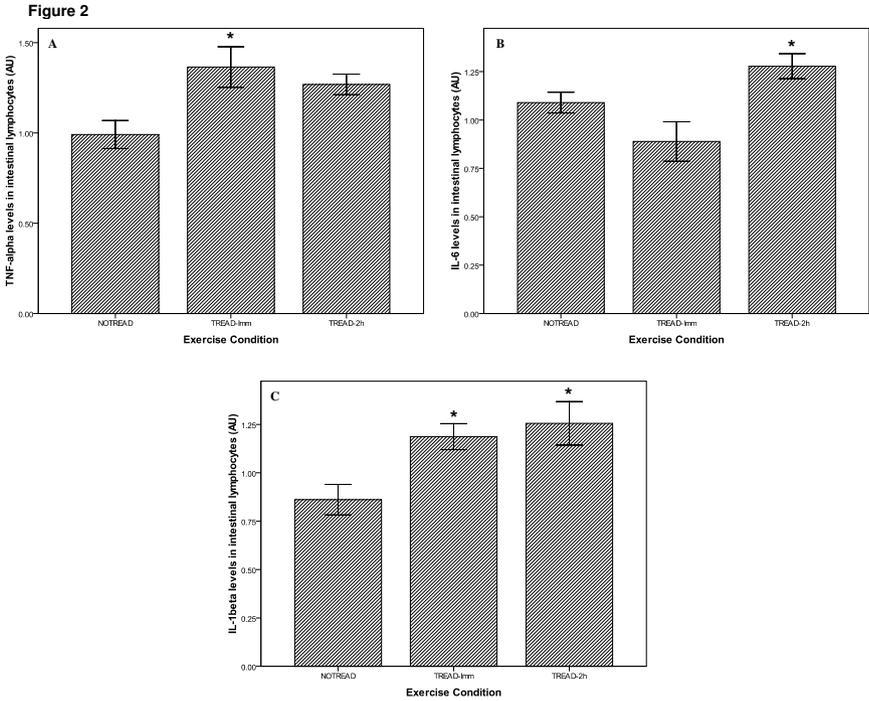
Figure 2

Figure 2
Cytokine (TNF-α, IL-6, IL-1β) expression [AU] in intestinal lymphocytes of mice given a single acute exercise bout and sacrificed immediately (TREAD-Imm) or after 2 hours (TREAD-2h) versus sedentary controls (NOTREAD). *Panel A:* TNF-α expression. *Panel B:* IL-6 expression. *Panel C:* IL-1β expression. Values are means ± one standard error. Significance compared to NOTREAD control indicated by an asterisk (*). See text for details of analysis.

0.06 AU) animals. Expression of IL-1β in mouse hippocampus did not differ as a function of acute treadmill exercise (F (2, 58) = 0.23, n.s.).

Intestinal Lymphocyte Cytokines
Figure 2 shows the effects of acute treadmill exercise on the expression of pro-inflammatory cytokines in intestinal lymphocytes. There was a significant effect of acute treadmill exercise on TNF-α expression (F (2, 28) = 5.21, p < 0.05) due to higher expression in TREAD-Imm (1.4 ± 0.1 AU) compared to the NOTREAD (1.0 ± 0.1 AU) mice. TNF-α expression in intestinal lymphocytes was also elevated in TREAD-2h (1.3 ± 0.1 AU) compared to NOTREAD animals, but this difference only approached significance (p = 0.06). A significant effect of acute exercise on intestinal IL-6 was found (F (2, 28) = 6.60, p < 0.05). A small and non-significant decrease in IL-6 expression occurred in the intestinal lymphocytes from TREAD-Imm (0.9 ± 0.1 AU) mice compared to NOTREAD (1.1 ± 0.1 AU) mice. The TREAD-2h (1.3 ± 0.1 AU) mice, however, had significantly higher expression of IL-6 in intestinal lymphocytes to the NOTREAD animals. Intestinal

lymphocyte IL-1β expression was affected by acute exercise (F (2, 29) = 5.13, p < 0.05) with this cytokine elevated in the TREAD-IMM (1.2 ± 0.1 AU) and TREAD-2h (1.3 ± 0.1 AU) compared to the NOTREAD (0.9 ± 0.1) mice.

Representative immunoblots for TNF-α expression in hippocampal cells and intestinal lymphocytes for NOTREAD, TREAD-Imm, and TREAD-2h mice are shown in **Figure 3.** Note that only the smaller (17 kDa) cleaved form of the cytokine was analyzed and presented in Figure 1 Panel A and Figure 2 Panel A. The larger pro-form (28 kDa) was not analyzed.
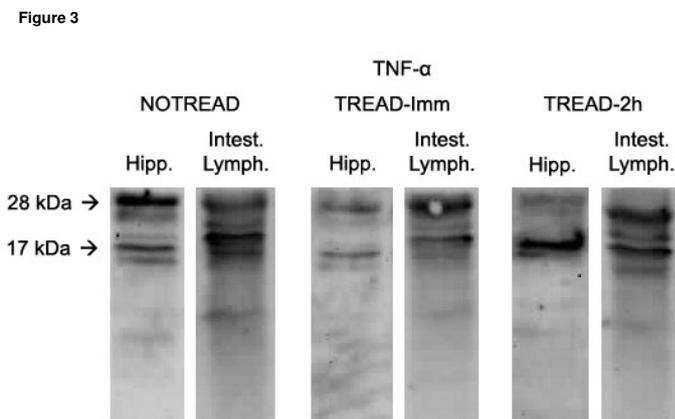
Figure 3

Figure 3
Representative immunoblots for TNF-α in hippocampal cells and intestinal lymphocytes of mice given a single acute exercise bout and sacrificed immediately (TREAD-Imm) or after 2 hours (TREAD-2h) vs. sedentary controls (NOTREAD). Arrows indicate molecular weight (kDa) for the two forms of TNF-α: a larger pro-form at 28 kDa and a smaller cleaved form at ~17 kDa. Only the smaller molecular weight cytokine was analyzed in the experiments.

Cortisterone and 8-isoprostaglandin F2$_\alpha$ (8-isoprostane)

Figure 4 shows the plasma corticosterone (Panel A) and 8-isoprostane (Panel B) responses to acute treadmill exercise. The treadmill running led to a significant increase in plasma corticosterone concentration (F (1, 39) = 60.25, p < 0.05) in TREAD-Imm (99.8 ± 5.7 ng/ml) compared to NOTREAD (37.5 ± 5.7 ng/ml) mice. Acute treadmill exercise also was associated with a significant increase in plasma 8-isoprostane levels (F (1, 32) = 7.53, p < 0.05) in TREAD-Imm (97.3 ± 6.2 pg/ml) compared to NOTREAD (73.5 ± 6.0 pg/ml) mice. Plasma corticosterone and 8-isoprostane levels were not assessed in the TREAD-2h group as previous studies (17; 20) have shown that differences in these measures are no longer significant after a 2 hour rest period.
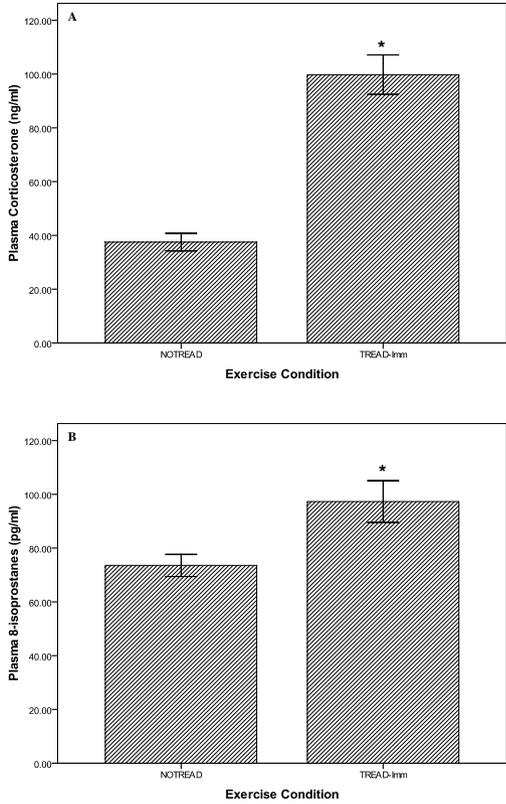
Figure 4

Figure 4
Plasma corticosterone and 8-isoprostane concentrations in mice given a single acute exercise bout and sacrificed immediately (TREAD-Imm) or after 2 hours (TREAD-2h) versus sedentary controls (NOTREAD). *Panel A:* Plasma corticosterone concentrations [ng/ml]. *Panel B:* Plasma 8-isoprostane concentrations [pg/ml]. Values are means ± one standard error. Significance compared to NOTREAD control indicated by an asterisk (*). See text for details of analysis.

DISCUSSION

We determined the effects of a single bout of strenuous aerobic exercise on cellular apoptosis and necrosis, and on the expression of pro-inflammatory cytokines in the hippocampi of healthy mice. A secondary objective was to evaluate intestinal lymphocyte pro-inflammatory cytokine expression in a subset of the experimental groups, along with physiological markers of stress, to determine that the exercise protocol was sufficient to elicit inflammatory cytokine changes as we previously reported (19; 20).

Although several studies have investigated the phenomenon of acute exercise-induced apoptosis in systemic lymphoid compartments, no previous studies, to our knowledge, have explored such effects in the healthy brain. Our novel results suggest that the hippocampus may be protected against the loss of cells incurred as a result of intense physical activity. This perspective is suggested by related findings in the literature. For example, Kim et al. (23) investigated the hippocampi obtained from rats undergoing 10 days of treadmill exercise following induced traumatic brain injury (TBI). TBI was found to impair short-term memory, increase DNA fragmentation, elevate caspase-3 and Bax expression, and decrease Bcl-2 protein expression in the hippocampus. However, in the exercised animals, there was less memory impairment, DNA fragmentation, and caspase-3 and Bax expression, indicating that physical activity reduces the apoptosis associated with central trauma. Although this study examined repeated bouts of treadmill running over a 10 day period, each "session" of forced exercise did not exacerbate traumatic damage. Um et al. (54) found that long-term treadmill running inhibits the apoptotic cascade in the brain by reducing cytochrome c, caspase-9, and caspase-3 protein levels, while inducing the expression of superoxide dismutase-1, catalase, and Bcl-2 to combat the effects of oxidative stress. Radak et al. (46) examined the effects of a prolonged acute exercise bout on markers of oxidative damage in the hippocampus of rats following a period of stress; immobilization increased lipid peroxidation, carbonylated protein concentration, DNA damage, and reduced glutamine synthetase activity. A single bout of acute exercise was able to restore levels to those observed in control animals. We suggest that intense aerobic exercise (at least at the intensity and duration used in this study) may not be sufficient to generate apoptotic conditions in the brain, and instead initiates the generation of conditions that may even be anti-apoptotic. Nevertheless, the literature in this area is limited (38), and caution must be used when comparing these studies: some utilized training (54), others repeated acute exercise (23), and still others a single-bout of acute exercise (46).

In contrast to our initial hypothesis, high-intensity exercise decreases the expression of TNF-α and increases the expression of IL-6 in the hippocampus. IL-6 has both pro- and anti- inflammatory functions, depending on the surrounding cytokine milieu, and elevated plasma IL-6 inhibits circulating levels of TNF-α both directly and through up-regulation of the soluble TNF receptor and IL-1ra (39; 41; 50). Thus, the higher IL-6 and lower TNF-α expression observed in our study may be a "mechanism" not only where acute exercise leads to decreased immunity to infection, but also preserves cognitive capacity, immediately after the physical stressor. TNF-α has a largely anti-pathogenic activity and is found throughout many areas of the central nervous system. It is responsible for MHC I and II expression in the glia and is highly involved in nitric oxide production in the CNS as a means of eliminating infectious agents. In contrast, IL-6 is primarily localized within the hippocampus and prefrontal cortex, promotes neuron survival factor, protects against excitotoxic brain damage, and has stress modulating effects on cognition (56). Acute exercise-induced increases in central IL-6 expression may also be responsible for greater cognitive task performance during, and immediately after, exercise bouts of varying intensity (5; 24).

We also found that the pattern of cytokine expression in the CNS differs from that of intestinal (systemic) tissues after an acute exercise challenge. In the hippocampus there were decreases in TNF-α, increases in IL-6, and no change in IL-1β expression after an acute exercise challenge in mice. In the intestine, however, all of the pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) showed increased expression. The intestinal lymphocyte cytokine pattern confirms earlier research that acute exercise leads to increases in TNF-α, IL-6, and IL-1β expression in intestinal lymphocytes, blood, and muscle (20; 25; 32). The acute exercise-induced increase in intestinal TNF-α expression is accompanied by elevations in pro-apoptotic proteins and lymphocytosis, which are thought to be a result of the oxidant stress generated by the stressor (20). Administration of an anti-oxidant prevented exercise-induced lymphocyte apoptosis (44), establishing the fact that acute exercise can have damaging effects in the intestine (and likely other systemic immune compartments) as a result of oxidative stress. The mechanism of oxidative stress in the periphery is also indicated by the lack of apoptotic responses in intestinal lymphocytes following injection of corticosterone at concentrations observed with intense treadmill exercise (43).

Acute high-intensity treadmill exercise leads to systemic elevations in markers of oxidative stress, including plasma 8-isoprostanes and corticosterone concentrations (20; 21; 52). Glucocorticoids, in particular, are modulators of cytokine activity (37) and cortisol secretion is correlated with IL-6 release and TNF-α suppression in blood obtained from major depression patients after an endotoxin challenge (55). Audet et al. (3) examined these phenomena in male mice, utilizing an acute psychosocial stressor (pairing of submissive and dominant mice) to evaluate corticosterone and cytokine responses. Mice with high plasma corticosterone responses to stress also had elevated expression of IL-6 (but not IL-1β) and of mRNA for IL-6 and IL-1β in the hippocampus. The authors suggested that IL-1β protein expression increases at a later time-point not considered in their study. In addition, hippocampal TNF-α mRNA in response to stress was unchanged, but pre-frontal cortex TNF-α mRNA production was lower in corticosterone high responders. Circulating levels of corticosterone are thus increased under immunological or psychological stress (14) and in response to intense exercise (7); this is coupled with increases in brain IL-6 in rats in response to chronic mild stress (30).

In addition, glucocorticoid administration inhibits plasma TNF-α increases that occur following an endotoxin challenge in healthy humans (4; 6). LPS-induced serum corticosterone levels are positively correlated with brain IL-6 and IL-1β concentrations 2 hours after endotoxin challenge (11). Central TNF-α levels were found to be elevated 16 hours after LPS stimulation whereas plasma (peripheral) corticosterone, IL-6, and IL-1β levels had already returned to baseline concentrations. Moreover, corticosterone administered intraperitoneally to adrenalectomized rats crosses the blood-brain-barrier with uptake and retention of the hormone in the hippocampus (28). These studies suggest that systemic glucocorticoids (whether from endogenous or exogenous sources), affect central and peripheral cytokine expression. Thus, it may be the case that exercise-induced changes in central pro-inflammatory cytokine synthesis or balance are influenced by corticosterone rather than oxidative responses to the exercise.

This study is not without limitations. Firstly, we did not separate cell subsets in the hippocampus in order to determine whether or not there were differential effects of acute exercise depending on cell type. Instead, our hippocampal single cell suspensions consisted of a mixture of microglia and other non-immune cells, making it difficult to interpret the source of cytokines observed, as well as impact of apoptotic changes in specific cell populations. Another limitation is that only a single bout of exercise was given prior to sacrifice. Human studies have shown that successive exercise bouts can lead to major increases in central IL-6 levels (35) and this must also be addressed in future animal investigations. It is unclear whether repeated exercise will lead to similar cytokine changes in the mouse hippocampus. Furthermore, this study was cross sectional, as we only measured changes immediately and two hours after the exercise bout. It cannot be determined from our results whether the differences in cytokine concentrations observed in the hippocampus and intestine were transient or more long-lasting. Studies will be needed with additional post-exercise time points to provide this clarification and to assess the kinetics of central vs. systemic cytokine expression in immune cells. As such, future experiments should include timed resting controls to address potential temporal effects. In addition, we report on levels of corticosterone in the plasma, but did not measure brain glucocorticoid expression. If corticosterone is affecting the concentration of central pro-inflammatory cytokines, it is essential to determine if this is a result of systemic or central sources of this stress hormone. Furthermore, it needs to be clarified whether corticosterone is actually responsible for the observed cytokine changes. Future investigations may involve repeating the exercise protocol with adrenalectomized mice, or with administration of cortisol receptor antagonists, to test this hypothesis. Studies must also include testing of other stress hormones, such as catecholamines, and additional markers of oxidative stress, including dichlorofluorescein diacetate. We did not measure apoptotic status (i.e., Annexin V positive) in intestinal lymphocytes because of limited tissue availability. However, we have shown elsewhere that aerobic exercise leads to a loss of intestinal lymphocytes and accompanying increases in apoptotic cells (17). Our experiments were conducted only with female C57BL/6 mice, which did not allow for the determination of any gender-specific effects in the hippocampus or intestine due to acute exercise. Females were chosen in order to 1) allow comparison with earlier studies from our lab (17; 19; 20), and 2) because they are better runners and show less bout-length attrition than males of this strain (13).

In conclusion, a single bout of intense aerobic treadmill running in healthy female C57BL/6 mice does not affect the percentage of apoptotic hippocampal cells, but alters the expression of pro-inflammatory cytokines by decreasing TNF-α and increasing IL-6 in the hippocampus immediately and 2 hours after cessation of exercise. These changes in the brain do not mirror the cytokine changes observed in intestinal lymphocytes. In the intestine, the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) is increased after intense exercise. We suggest that the oxidative stress that accompanies acute exercise is not sufficient to generate damaging (apoptotic) effects in the central nervous system, as this compartment may be protected to preserve cognitive function during physical activity. We also tentatively propose that exercise-induced elevations in circulating corti-

costerone levels may be one mechanism to explain the pattern of hippocampal cytokine expression. Glucocorticoids are known to increase and reduce central levels of IL-6 and TNF-α, respectively. Future studies on physical activity and the central expression of specific pro- (e.g., caspases, Bax) and anti- (e.g., Bcl-2) apoptotic proteins and other pro-inflammatory cytokines will be necessary. Whether reducing or blocking glucocorticoid release (e.g., adrenalectomy) affects brain pro-inflammatory cytokine response to acute exercise stress remains to be determined.

ACKNOWLEDGEMENTS

REFERENCES

1. Ackigoz O, Aksu I, Topcu A, and Kayatekin BM. Acute exhaustive exercise does not alter lipid peroxidation levels and antioxidant enzyme activities in rat hippocampus, prefrontal cortex and striatum. Neurosci Lett 406: 148-151, 2006.
2. Adrain C, Murphy BM, and Martin SJ. Molecular ordering of the caspase activation cascade initiated by the cytotoxic T lymphocyte/natural killer (CTL/NK) protease Granzyme B. J Biol Chem 280: 4663-4673, 2005.
3. Audet M, Mangano EN, and Anisman H. Behavior and pro-inflammatory cytokine variations among submissive and dominant mice engaged in aggressive encounters: moderation by corticosterone activity. Front Behav Neurosci 4: 1-12, 2010.
4. Barber AE, Coyle SM, Marano MA, Fischer E, Calvano SE, Fong Y, Moldawer LL, and Lowry SF. Glucocorticoid therapy alters hormonal and cytokine response to endotoxin in man. J Immunol 150: 1999-2006, 1993.
5. Barella LA, Etnier JL, and Chang YK. The immediate and delayed effects of an acute bout of exercise on cognitive performance of healthy older adults. J Aging Phys Act 18: 87-98, 2010.
6. Beishuizen A and Thijs LG. Endotoxin and the hypothalamo-pituitary-adrenal (HPA) axis. J Endotoxin Res 9: 3-24, 2003.
7. Brown DA, Johnson MS, Armstrong CJ, Lynch JM, Caruso NM, Ehlers LB, Fleshner M, Spencer RL, and Moore RL. Short-term treadmill running in the rat: what kind of stressor is it? J Appl Physiol 103: 1979-1985, 2007.
8. Carmichael MD, Davis JM, Murphy EA, Brown AS, Carson JA, Mayer EP, and Ghaffar A. Recovery of running performance following muscle-damaging exercise: relationship to brain IL-1 beta. Brain Behav Immun 19: 445-452, 2005.
9. Carmichael MD, Davis JM, Murphy EA, Brown AS, Carson JA, Mayer EP, and Ghaffar A. Role of brain IL-1 beta on fatigue after exercise-induced muscle damage. Am J Physiol Regul Integr Comp Physiol 291: R1344-R1348, 2006.
10. Carmichael MD, Davis, JM, Murphy EA, Carson J, Van Rooijen N, Mayer E, and Ghaffar A. Role of brain macrophages on IL-1 beta and fatigue following eccentric exercise-induced muscle damage. Brain Behav Immun 24: 564-568, 2010.

11. Chen R, Zhou H, Beltran J, Malellari L, and Chang SL. Differential expression of cytokines in the brain and serum during endotoxin tolerance. J Neuroimmunol 163: 53-72, 2005.

12. Cotman CW, Berchtold NC, and Christie LA. Exercise builds brain health: key roles of growth factor cascades and inflammation. Trends Neurosci 30: 464-472, 2007.

13. De Bono JP, Adlam D, Paterson DJ, and Channon KM. Novel quantitative pheno-types of exercise training in mouse models. Am J Physiol Reg I 290: R296-R934, 2006.

14. De Kloet ER. Hormones and the stressed brain. Ann NY Acad Sci 1018: 1-15, 2004.

15. Degterev A, Boyce M, and Yuan J. A decade of caspases. Oncogene 22: 8543-8567, 2003.

16. Hassan NF, Rifat S, Campbell DE, McCawley LJ, and Douglas SD. Isolation and flow cytometric characterization of newborn mouse brain-derived microglia main-tained in vitro. J Leukocyte Biol 50: 86-92, 1991.

17. Hoffman-Goetz L and Quadrilatero J. Treadmill exercise in mice increases intestinal lymphocyte loss via apoptosis. Acta Physiol Scand 179: 289-297, 2003.

18. Hoffman-Goetz L and Spagnuolo PA. Effects of repeated exercise stress on caspase 3, Bcl-2, HSP 70 and CuZn-SOD protein expression in mouse intestinal lympho-cytes. J Neuroimmunol 187: 94-101, 2007.

19. Hoffman-Goetz L, Pervaiz N, and Guan J. Voluntary exercise training in mice increases the expression of antioxidant enzymes and decreases the expression of TNF-α in intestinal lymphocytes. Brain Behav Immun 23: 498-506, 2009.

20. Hoffman-Goetz L, Pervaiz N, Packer N, and Guan J. Freewheel training decreases pro- and increases anti-inflammatory cytokine expression in mouse intestinal lym-phocytes. Brain Behav Immun 24: 1105-1115, 2010.

21. Huang CJ, Webb HE, Evans RK, McCleod KA, Tangsilsat SE, Kamimori GH, and Acevedo EO. Psychological stress during exercise: immunoendocrine and oxidative responses. Exp Biol Med 235: 1498-1504, 2010.

22. Kakanis MW, Peake J, Brenu EW, Simmonds M, Gray B, Hooper SL, and Marshall-Gradisnik SM. The open window of susceptibility to infection after acute exercise in healthy young male elite athletes. Exerc Immunol Rev 119: 119-137, 2010.

23. Kim D, Ko I, Kim B, Kim T, Kim S, Shin M, Kim C, Kim H, Kim K, and Baek S. Treadmill exercise inhibits traumatic brain injury-induced hippocampal apoptosis. Physiol Behav 101: 660-665, 2010.

24. Lambourne K and Tomporowski P. The effect of exercise-induced arousal on cogni-tive task performance: a meta-regression analysis. Brain Res 1341: 12-24, 2010.

25. Liburt NR, Adams AA, Betancourt A, Horohov DW, and McKeever KH. Exercise-induced increases in inflammatory cytokines in muscle and blood of horses. Equine Vet J 42: 280-288, 2010.

26. Lin Y, Kuo H, Kuo C, Wang S, Yang B, and Chen H. Antioxidant administration inhibits exercise-induced thymocyte apoptosis in rats. Med Sci Sports Exer 31: 1598-1598, 1999.

27. Mars M, Govender S, Weston A, Naicker V, and Chuturgoon A. High intensity exer-cise: a cause of lymphocyte apoptosis? Biochem Bioph Res Co 249: 366-370, 1998.

28. McEwen BS, Weiss JM, and Schwarz LS. Uptake of corticosterone by rat brain and its concentration by certain limbic structures. Brain Res 16: 227-241, 1969.

29. Mooren FC, Blöming D, Lechtermann A, Lerch MM, and Völker K. Lymphocyte apoptosis after exhaustive and moderate exercise. J Appl Physiol 93: 147-153, 2002.

30. Mormède C, Castanon N, Médina C, Moze E, Lestage J, Neveu PJ, and Dantzer R. Chronic mild stress in mice decreases peripheral cytokine and increases central cytokine expression independently of IL-10 regulation of the cytokine network. Ann NY Acad Sci 10: 359-366, 2002.

31. Navalta JW, McFarlin, BK, and Lyons TS. Does exercise really induce lymphocyte apoptosis? Front Biosci 2: 478-488, 2010.

32. Nemet D, Oh Y, Kim H, Hill MA, and Cooper DM. Effect of intense exercise on inflammatory cytokines and growth mediators in adolescent boys. Pediatrics 110: 681-689, 2002.

33. Neves G, Cooke SF, and Bliss TVP. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat Rev Neurosci 9: 65-75, 2008.

34. Nieman D. Current perspective on exercise immunology. Cur Sports Med Rep 2: 239-242, 2003

35. Nybo L, Nielsen B, Pedersen BK, Møller K, and Secher NH. Interleukin-6 release from the human brain during prolonged exercise. J Physiol 542: 991-995, 2002.

36. Ostrowski K, Rohde T, Asp S, Schjerling P, and Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515.1: 287-291, 1999.

37. Pace TWW and Miller AH. Cytokines and glucocorticoid receptor signalling – relevance to major depression. Ann NY Acad Sci 1179: 86-105, 2009.

38. Packer N, Pervaiz N, and Hoffman-Goetz L. Does exercise protect from cognitive decline by altering brain cytokine and apoptotic protein levels? A systematic review of the literature. Exerc Immunol Rev 16: 138-162, 2010.

39. Pedersen BK and Febbraio M. Muscle-derived interleukin-6 – a possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun 19: 371-376, 2005.

40. Pedersen BK and Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80: 1055-1081, 2000.

41. Pedersen BK. Edward F. Adolph distinguished lecture: Muscle as an endocrine organ: IL-6 and other myokines. J Appl Physiol 107: 1006-1014, 2009.

42. Phaneuf S and Leeuwenburgh C. Apoptosis and exercise. Med Sci Sport Exer 33: 393-396, 2001.

43. Quadrilatero J and Hoffman-Goetz L. In vivo corticosterone administration at levels occurring with intense exercise does not reduce intestinal lymphocyte apoptosis in mice. J Neuroimmunol 162: 137-148, 2005.

44. Quadrilatero J and Hoffman-Goetz L. N-acetyl-L-cysteine prevents exercise-induced intestinal lymphocyte apoptosis by maintaining intracellular glutathione levels and reducing mitochondrial membrane depolarization. Biochem Bioph Res Co 319: 894-901, 2004.

45. Radák Z, Katsumi, A, Kizaki T, Shuji O, Masaya I, and Hideki O. Acute bout of exercise does not alter the antioxidant enzyme status and lipid peroxidation of rat hippocampus and cerebellum. Pathophysiol 2: 243-245, 1995.

46. Radák Z, Sasvari M, Nyakas C, Kaneko T, Tahara S, Ohno H, and Goto S. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. Neurochem Int 39: 33-38, 2001.

47. Scopel D, Fochessato C, Cimarosti H, Rabbo M, Belló-Klein A, Salbego C, Netto CA, and Siqueira IR. Exercise intensity influences cell injury in rat hippocampal slices exposed to oxygen and glucose deprivation. Brain Res Bull 71: 155-159, 2006.

48. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 82: 291-295, 1997.

49. Somani SM, Husain K, Diaz-Phillips L, Lanzotti, DK, Kareti KR, and Trammell GL. Interaction of exercise and ethanol on antioxidant enzymes in brain regions of the rat. Alcohol 13: 603-610, 1996.

50. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, and Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. FASEB J 17: 884-886, 2003.

51. Starkie RL, Angus DJ, Rolland J, Hargreaves M, and Febbraio MA. Effect of prolonged submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. J Physiol 528.3: 647-655, 2000.

52. Steensberg A, Dalsgaard MK, Secher NH, and Pedersen BK. Cerebrospinal fluid IL-6, HSP72, and TNF-alpha in exercising humans. Brain Behav Immun 20: 585-589, 2006.

53. Steinacker JM, Lormes W, Reissnecker S, and Liu Y. New aspects of the hormone cytokine response to training. Eur J Appl Physiol 87: 38-42, 2004.

54. Um HS, Kang EB, Leem YH, Cho IH, Yang CH, Chae KR, Hwang DY, and Cho JY. Exercise training acts as therapeutic strategy for reduction of the pathogenic phenotypes for Alzheimer's disease in an NSE/APPsw-transgenic model. Int J Mol Med 22: 529-539, 2008.

55. Vedder H, Schreiber W, Schuld A, Kainz M, Lauer CJ, Krieg J, Holsboer F, and Pollmächer T. Immune-endocrine host response to endotoxin in major depression. J Psychiat Res 41: 280-289, 2007.

56. Wilson CJ, Finch CE, and Cohen HJ. Cytokines and cognition – the case for a head-to-tose inflammatory paradigm. J Am Geriatr Soc 50: 2041-2056, 2002.

57. Zaldivar F, Wang-Rodriguez J, Nemet D, Schwindt C, Galassetti P, Mills PJ, Wilson, LD, and Cooper DM. Constitutive pro- and anti-inflammatory cytokine and growth factor response to exercise in leukocytes. J Appl Physiol 100: 1123-1133, 2006.