Cytokine responses to acute and chronic exercise in multiple sclerosis

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Castellano V, Patel DI, White LJ. Cytokine responses to acute and chronic exercise in multiple sclerosis. J Appl Physiol 104: 1697–1702, 2008.—Regular exercise reduces functional loss associated with multiple sclerosis (MS). However, the impact of exercise on inflammatory mediators associated with disease activity remains relatively unexplored. The purpose of this study was to determine whether ambulatory MS subjects would respond similarly to aerobic cycle training compared with matched controls on circulating immune variables, interleukin (IL)-6, tumor necrosis factor (TNF)-α and interferon (IFN)-γ. Eleven MS and 11 non-MS control subjects (8 women and 3 men in both groups) matched in age, height, body mass, body fat, and peak O2 uptake completed the study. Subjects completed 30 min of cycle ergometry at 60% of peak O2 uptake, 3 day/wk for 8 wk. Plasma cytokine concentrations were determined before and after exercise at weeks 0, 4, and 8. MS and control subjects showed a similar cytokine response to exercise. IL-6 at rest tended to decrease (P = 0.08) with training in both groups. Resting plasma TNF-α tended to be higher in MS controls compared with controls throughout the study (P = 0.08). MS subjects showed elevated resting TNF-α in MS at the end of the 8-wk program (P = 0.04), whereas resting TNF-α remained unchanged in controls (P > 0.05). Resting plasma IFN-γ at rest was elevated in MS subjects (P = 0.008) and unchanged in controls at the end of the intervention (P > 0.05). The response of plasma IL-6, TNF-α, and IFN-γ after a single bout of exercise was similar between MS and control subjects (P > 0.05). Additional research to understand the impact of exercise on immune variables in MS is warranted.

cytokines; exercise; interferon-gamma; interleukin-6; tumor necrosis factor-alpha

MULTIPLE SCLEROSIS (MS) is an autoimmune degenerative disease of the central nervous system characterized by different patterns of inflammation, demyelination, and axonal loss. Cytokines play an important role in the pathogenesis of MS and are a major target for treatment interventions. Interleukin (IL)-6, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ have a prominent role in the process of demyelination and axonal damage experienced by persons with MS (8). Moreover, exercise has been shown to modulate immunological responses through cytokine production in short bouts of exercise in healthy populations (22, 31, 32), but the influence of exercise on cytokine concentration in people with MS remains mostly unexplored. Given the health benefits associated with regular physical activity, further study to understand the impact of exercise on immune variables in MS is warranted.

It has been proposed that short-term release of cytokines during acute exercise may contribute to the maintenance of an immune homeostatic environment (23). In addition, many acute-phase proteins released in response to cytokine fluctuations are protease inhibitors or free-radical scavengers that diminish tissue damage by activated neutrophils (23). Therefore, a single bout of exercise is a mild physical stressor that exerts an array of effects on immune parameters (36, 38).

Heesen and colleagues (17) found that sedentary MS subjects showed a blunted cytokine response (IFN-γ, TNF-α, IL-10) to a single bout of exercise (30 min of cycling at 60% of their maximal O2 uptake) compared with aerobically trained MS subjects. In the trained state, MS subjects had a similar cytokine response to a single bout of exercise when compared with healthy controls, suggesting that training status may have immunomodulatory outcomes (17). However, resting circulating concentration of cytokines were not different between trained and untrained MS subjects (17). Previously, our group found that 8 wk of progressive resistance training was associated with decreased resting plasma concentration of IL-4, IL-10, C-reactive protein (CRP), and IFN-γ and with a tendency for TNF-α to decrease while IL-2 and IL-6 remained unchanged (41). These data suggest that strength training may also be immunomodulatory. Schultz and colleagues (36) found no changes in resting plasma IL-6 and IL-6ra following 8 wk of aerobic exercise. These data suggest that exercise stress and training status may be immunomodulatory, but these findings need to be corroborated. To fully appreciate the influence of exercise on immune status in individuals with MS, additional research is needed. Therefore, the purpose of this study was to determine whether MS subjects would respond similarly or differently to both acute and chronic exercise stress with respect to the cytokines IFN-γ, TNF-α, and IL-6 compared with controls. Based on previous findings, we hypothesized that aerobic exercise training would be associated with reduced resting pro-inflammatory cytokines in both MS and controls and that fitness status would influence the cytokine response to an acute exercise stress.

METHODS

Subjects

Twenty-seven subjects volunteered for study participation. Eleven individuals with MS and 11 healthy controls (8 women and 3 men in each group) completed the study. Each subject had physician clearance and signed a consent form approved by the University Institutional Review Board. The experimental protocol was submitted to and approved by the University of Florida Institutional Review Board.

Subject Inclusion/Exclusion Criteria

MS subjects with clinically diagnosed relapsing remitting disease (34) and who were clinically stable and had minimal to moderate disability [expanded disability status scale (EDSS) score of 0–5.5]...
were included in the study. Subjects with known cardiovascular disease, diabetes, thyroid disorders, gout, and orthopedic limitations were excluded from the study (1). Additionally, individuals using prednisone or antispasmodics drugs reported experiencing a relapse or unable to cycle at the time/target intensity were excluded.

Experimental Design

The study consisted of an 8-wk aerobic exercise training program wherein subjects exercised on a cycle ergometer three times per week for 30 min at 60% peak O2 uptake (V\textsubscript{O2peak}).

Resting and postexercise plasma samples were acquired before and following exercise at the beginning (baseline), midpoint (week 4), and end (week 8) of the study and analyzed for IL-6, TNF-\textgreek{a}, and IFN-\textgreek{g} concentration. Assessments of aerobic fitness and body composition were acquired at baseline and following the intervention. Figure 1 displays the experimental design. An 8-wk training program was selected because it has been previously found to provide a sufficient stimulus to alter cardiovascular fitness (33), muscular endurance (25), and immune function (17) in MS subjects, whereas cycle ergometry was selected to accommodate for varying levels of physical impairment and balance.

Exercise Training Protocol

Each exercise session consisted of a 3-min warm-up at a self-assessed comfortable submaximal intensity followed by 30 min of cycle ergometry at 60% of V\textsubscript{O2peak} (3 times/wk). Each training session was supervised. In some instances, cycling intensity was temporarily reduced (30–90 s) to accommodate for leg fatigue.

Graded Exercise Testing

The subjects were asked to visit the laboratory for testing in the morning (8–11 AM) after abstaining from physical activity for 24 h and abstaining from alcohol, caffeine, or food for the previous 12 h before the exercise program started, at week 4, and at week 8. The exercise test was initiated with a resistance of 25 W, and resistance was increased 10–25 W every 2 min until the subject reached 85% of estimated maximum heart rate. Expired gas concentrations were recorded continuously using a metabolic cart (Parvomedics, Salt Lake City, UT) and maximum O2 consumption was predicted (1).

Single Bout of Aerobic Exercise

A standardized bout of aerobic exercise was performed 72 h after the completion of the graded exercise test to assess acute cytokine response and plasma volume changes. For this test, subjects cycled at 60% of their measured V\textsubscript{O2peak} for 30 min as previously reported (17, 36).

Blood Collection and Processing

Blood samples (20 ml) were obtained by venipuncture before the single bout of exercise and at 30 min, 2 h, and 3 h postexercise. This blood collection protocol was selected because of the variation in cytokine dynamics (31, 32). All blood samples were acquired from the antecubital vein in a seated position. The subjects were asked to visit the laboratory at the same time of day (8–11 AM) after abstaining from physical activity, alcohol, caffeine, or food for 12 h. Blood samples were collected 48 h after any MS-related interferon drug administration to control for the possible impact of the drug on cytokine regulation (11). Plasma samples were immediately centrifuged at 3,000 g for 15 min at 4°C and then stored at −80°C for subsequent analyses.

Cytokine Assessment

Cytokines IL-6, TNF-\textgreek{a}, and IFN-\textgreek{g} were analyzed using a multiplex immunoassay utilizing fluoroscendently labeled microsphere beads and laser-based fluorescent detection (Lincor Research, St. Charles, MO) for each acquired blood sample. Plasma samples were analyzed in duplicate. The intra-assay coefficient of variability for IL-6, TNF-\textgreek{a}, and IFN-\textgreek{g} were 4.9, 6.1, and 6.0%, respectively, as provided by the manufacturer. The individual sensitivities (pg/ml) of IL-6, TNF-\textgreek{a}, and IFN-\textgreek{g} were 1.7, 0.7, and 1.7, respectively, as provided by the manufacturer. There was no significant cross reactivity between other cytokine antibodies in this panel.

Plasma Volume Assessment

Plasma volume was assessed before and following the standardized bout of exercise performed at weeks 0, 4, and 8 using hemoglobin and hematocrit concentration determined from whole blood samples (9).

Disability Status

Subjects completed a MS self-assessed standardized EDSS (21) before and after the completion of the study.

Statistical Analysis

All analyses were performed using SPSS 12.0. A multivariate ANOVA with time (pre, mid, and post) as the within factor and group (MS vs. control) entered as the between-subjects factor was used to assess the effect of the exercise training program on IL-6, TNF-\textgreek{a}, and IFN-\textgreek{g}. An ANOVA with repeated measures for each blood collection point was used to assess changes in cytokine dynamics after the standardized bout of exercise. When necessary, Tukey’s post hoc analysis was implemented. Correlations were analyzed using a Pearson’s correlation analysis. A value of P < 0.05 was considered significant. All values are expressed as means ± SD.

RESULTS

Subjects

There were no significant differences between groups in age, height, body mass, percent fat, or V\textsubscript{O2peak} (P > 0.05) (Table 1). There was a significant increase in absolute V\textsubscript{O2peak} (l/min) after 8 wk of aerobic exercise training in both groups (P < 0.05). There were no significant changes in plasma volume, body mass, body mass index, waist-to-hip ratio, relative V\textsubscript{O2peak} (ml·kg\textsuperscript{-1}·min\textsuperscript{-1}), or percent body fat after the training program in either group (P > 0.05). The MS subjects decreased their perceived disability by 24% (P = 0.04) after 8 wk of aerobic exercise training (3.4 vs. 2.6 EDSS scores). Control subjects had a score of 0 at the beginning of the study, and it remained unchanged after 8 wk of aerobic exercise training.
(P > 0.05). Table 1 describes the characteristics of the subjects before and after the training intervention.

**Resting Plasma Cytokine Concentration**

**IL-6.** Plasma IL-6 at rest was similar between groups at weeks 0, 4, and 8 (P > 0.05) (Fig. 2). Following the 8-wk cycling intervention, plasma IL-6 at rest tended to decrease compared with week 0 in both groups (P = 0.075).

**TNF-α.** Plasma TNF-α concentration at rest tended to be higher in MS compared with controls throughout the study (P = 0.08) (Fig. 3). MS subjects showed increased TNF-α levels at rest from week 0 to week 8 and from week 4 to week 8 (P = 0.04), whereas TNF-α plasma concentration in control subjects remained unchanged at rest (P > 0.05).

**IFN-γ.** There was an interaction between plasma IFN-γ concentration at rest between groups and week of training (weeks 0, 4, and 8) (P = 0.03) (Fig. 4). IFN-γ concentration at rest increased significantly in MS subjects from week 0 to week 8 (P = 0.008) and from week 4 to week 8 (P = 0.01). IFN-γ at rest was increased in control subjects from week 0 to week 4 (P = 0.02) followed by a tendency for IFN-γ at rest to decrease from week 4 to week 8 (P = 0.07). In addition, control subjects had similar IFN-γ concentration at rest for week 0 and week 8 (P = 0.3). IFN-γ was significantly correlated with TNF-α (r = 0.932; P = 0.001).

**Table 1. Subject characteristics pre and post 8 wk of aerobic exercise**

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Age, yr</td>
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<td>40±10</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.68±0.1</td>
<td>1.68±0.1</td>
</tr>
<tr>
<td>Mass, kg</td>
<td>72±14</td>
<td>73±15</td>
</tr>
<tr>
<td>% Body fat</td>
<td>35.6±8</td>
<td>34.6±8</td>
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<tr>
<td>VO_{2peak}, l/min</td>
<td>2.2±0.4</td>
<td>2.5±0.4</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24±4</td>
<td>26±5</td>
</tr>
</tbody>
</table>

Values are means ± SD. MS, multiple sclerosis; %Δ, percent change; VO_{2peak}, peak O₂ uptake; BMI, body mass index. *Significant difference after 8 wk of aerobic exercise (P < 0.05). **Significant difference after 8 wk of aerobic exercise (P < 0.001).

**Standardized Exercise Bout**

**IL-6.** Plasma IL-6 concentration following the standardized bout of exercise at the beginning, midpoint, and end of the study was similar between groups (P > 0.05). Specifically, both groups displayed similar significant increases in plasma IL-6 concentration following the 30 bouts of cycle ergometry at 60% of VO_{2peak} (Fig. 5). Although it was not statistically different due to the large standard deviations in both groups, the magnitude of increase of IL-6 at 30 min into recovery in MS was 7%, whereas controls increased IL-6 by 20%. Since the results were not significantly different at any time point between groups, the following results are averages from both groups at each time point. Plasma IL-6 concentration at baseline increased significantly 30 min postexercise (13%; P = 0.021), tended to stay elevated 2 h postexercise (9%; P = 0.09), and returned to baseline 3 h postexercise in both groups.

**TNF-α.** Plasma TNF-α response to the standardized bouts of exercise were similar between MS and control subjects (P > 0.05) and did not significantly change throughout the training program (P > 0.05) (Fig. 6). Both groups experienced signif-
significant decreases in TNF-α plasma concentration following 30 min of aerobic exercise at 60% of $V_O^{\text{peak}}$. However, TNF-α decreases were not significantly different between groups. Specifically, TNF-α plasma concentration at baseline (7.6 pg/ml) decreased significantly 2 h (6.4 pg/ml; −16%) and 3 h (4.6 pg/ml; −39.5%) following exercise in the MS group. In the control group, TNF-α plasma concentration at baseline (5.3 pg/ml) also decreased significantly 2 h (3.9 pg/ml; −26%) and 3 h (3.0 pg/ml; −43%) following exercise.

**IFN-γ.** Plasma IFN-γ response to the standardized exercise bouts were also similar between MS and control subjects ($P > 0.05$) and did not significantly change throughout the training program ($P > 0.05$). Both groups displayed similar significant decreases in IFN-γ plasma concentration following 30 min of aerobic exercise at 60% of $V_O^{\text{peak}}$ (Fig. 7). However, IFN-γ decreases were not significantly different between groups. IFN-γ plasma concentration at baseline (31 pg/ml) decreased significantly 2 h (21.3 pg/ml; −31%) and 3 h (23 pg/ml; −26%) following exercise in the MS group. In the control group, IFN-γ plasma concentration at baseline (19 pg/ml) also decreased significantly 2 h (12 pg/ml; −36%) and 3 h (13 pg/ml; −31%) following exercise.

DISCUSSION

Clinical studies investigating the impact of exercise training on cytokine levels in individuals with MS are limited. Our data suggest that exercise training may influence resting cytokine concentrations. In addition, our study also provides preliminary evidence that a single bout of exercise appears to influence plasma cytokine levels, but the response is similar between MS and matched controls, independent of training status.

We observed a tendency for resting IL-6 to decrease across weeks of training in both MS and control subjects. Schulz and colleagues (36) investigated the impact of chronic aerobic exercise on IL-6 and found that, with a similar training regimen, no changes in resting concentration of IL-6 were observed. Perhaps larger sample sizes would yield more conclusive results since our results vary slightly from those of Schulz et al. (36). Another explanation may be that IL-6 levels need longer training programs to vary or that, in the MS population, IL-6 is maintained at higher levels to counteract other inflammatory cytokine activity. Considering that regulatory changes of systemic IL-6 may be important in lesion formation in the central nervous system (37), decreases in this cytokine may have important clinical outcomes in individuals with MS. Previous observations suggest that abnormally high IL-6 concentrations in the periphery may result in excess inflammation that may exacerbate disease activity in MS (27). Also, elevated IL-6 may disrupt the clearance of microbial pathogens (27) and participate in T-cell activation, potentially contributing to MS disease processes (26, 27).

Additionally, plasma IL-6 levels may also be an indicator of skeletal muscle controlled metabolic regulation. IL-6 can act in both a paracrine and endocrine manner. IL-6 may impact the release of additional IL-6 from the local skeletal muscle (30) or travel in circulation and impact hepatic glucose release (13). Moreover, resting basal IL-6 concentrations have been shown to decrease with training as glucose dependence decreases (15). Therefore, decreases in IL-6 may be a training response and could possibly reflect metabolic changes of our subjects. Further research that clearly identifies the significance of changes in IL-6 in people with MS would be valuable.
Resting concentrations of TNF-α and IFN-γ increased in our MS subjects following 8 wk of training. Previous work from our laboratory, utilizing 8 wk of progressive resistance training, showed a tendency for resting TNF-α to decrease in MS subjects (41). Given the complex roles of both cytokines, our findings are difficult to fully interpret. Previous research suggests that elevated TNF-α concentration in blood may have beneficial (19) or detrimental effects (37) in people with MS. For example, although increased TNF-α concentrations in blood and cerebrospinal fluid may correlate with the degree of blood brain barrier dysfunction (37), it may also be associated with favorable decreases in disease relapses while on interferon-β treatment (19). In our study, perceived disability decreased and physical fitness improved with exercise training, suggesting that changes in resting circulating pro-inflammatory cytokines may not be linked to negative short-term disease outcomes and that quality of life improved regardless of major changes in cytokine regulation. These quality of life changes may be of great importance and should be studied further. Moreover, we cannot rule out the possibility that elevated TNF-α could have deleterious consequences.

The role of TNF-α in MS is complicated by the observation that TNF-α has dual roles (2, 3, 35, 37, 39) that may be unique to autoimmune diseases such as MS. Although TNF-α has been linked to inflammatory demyelination in MS (5, 12, 18), recent reports show strong evidence that TNF-α may also be neuroprotective through enhancement of oligodendrocyte proliferation and stimulation of remyelination (2, 3, 37). In fact, intravenous anti-TNF-α therapy was ineffective in MS patients and may actually worsen MS symptoms (2, 40). It is therefore difficult to resolve the contradictory roles of TNF-α on disease activity. One possible explanation may be the existence of two different signaling pathways mediated by two different TNF-α receptors (p55 and p75) (2, 37). It is possible that exercise can induce activation of the “good” inflammatory TNF-α p75 receptor pathway that promotes cell growth and proliferation (2). Possible mechanisms of action include neuroprotection of the TNF-α p75 receptor through the induction of superoxide dismutase (4, 42), protecting neurons from reactive oxygen species, and calbindin stabilization of calcium homeostasis in the central nervous system (6). Our study provides preliminary data suggesting that exercise may modulate cytokines associated with MS disease activity.

Similar to TNF-α, plasma concentration of IFN-γ also increased following 8 wk of aerobic exercise in MS subjects. To date, IFN-γ is thought to be present during relapses, and it is considered detrimental to the central nervous system of individuals with MS (10, 20, 24). However, the role of IFN-γ in the periphery remains unknown. In our study, IFN-γ and TNF-α were highly correlated and seem to follow similar dynamics throughout the study (r = 0.932, P = 0.001). Work by Moldovan et al. (24) showed that T-cell secreting IFN-γ ex vivo correlated with functional impairments in MS patients. In contrast, Kraus et al. (20) found that circulating pro-inflammatory cytokines did not correlate with disease activity and severity as assessed by lesion load in the brain. As mentioned earlier, it remains to be elucidated whether exercise plays a positive or negative role in the pathophysiology of the disease. Clearly, further investigations are needed to clarify the roles of exercise-mediated changes in pro-inflammatory cytokines in individuals with MS.

In addition to measuring resting cytokine concentration in response to aerobic exercise training, we also investigated the response of immune factors following a single bout of aerobic exercise before and after the intervention. It has been proposed that the short-term release of cytokines during acute exercise may contribute to the maintenance of an immune homeostatic environment. It is well known that skeletal muscle contractions stimulate IL-6 production and may increase circulating IL-6 concentration via complex signaling cascades initiated both by Ca2+-dependent and -independent stimuli (28). Plasma IL-6 increases in exponential fashion with exercise and is intensity and duration dependent (14, 31, 32).

In our study, MS and control subjects experienced similar significant increases in plasma IL-6 concentration following 30 min of aerobic exercise at 60% of Vo2peak as reported in the literature by others [see review by Pedersen and Febbraio (29)]. Specifically, IL-6 increased significantly 30 min postexercise and tended to stay elevated for 2 h while returning to baseline 3 h postexercise in both groups. Although not significantly different, the IL-6 response to exercise seemed blunted in the MS group (7% increase) compared with the control group (20% increase). Interestingly, Schulz et al. (36) found that IL-6 remained unchanged immediately after a single bout exercise, but no additional postexercise measures were reported. Considering the dynamics of each cytokine can vary considerably in response to exercise (22), our findings provide some additional information on the postexercise cytokine dynamics in MS and controls. Our results provide preliminary data suggesting that 2–3 h of postexercise data are needed to capture the exercise-mediated IL-6 response to a single bout of moderate-intensity exercise.

In addition to IL-6, we assessed the TNF-α and IFN-γ response following a standardized bout of exercise in both groups. TNF-α and IFN-γ plasma concentrations decreased in similar fashion in MS and control subjects during the recovery period after a single bout of exercise. The response after exercise of both cytokines did not change after training in either group. Our results are in contrast to Heesen et al. (17), who reported increased TNF-α and IFN-γ concentrations 30 min postexercise (30 min of cycle ergometry at 60% Vo2peak) with no additional postexercise assessment. In our study, TNF-α and IFN-γ concentrations 30 min postexercise were similar to baseline values, with a marked significant decrease 2 and 3 h postexercise. The kinetic profile of TNF-α and IFN-γ follows the opposite dynamics to IL-6 following a single bout of exercise and provides further information on the impact of exercise on immune markers.

In our study, MS subjects had a similar cytokine response (IL-6, TNF-α, and IFN-γ) compared with control subjects before the initiation of the exercise training program, which remained statistically unchanged at our three measurement time points. Limited information is available on the influence of exercise on immune variables that are known to impact disease activity in MS. These findings suggest that individuals with MS may respond to physical stress similarly to matched healthy controls. In fact, stabilized levels of interacting Th1/Th2 cytokines are maintained in the benign course of MS, and it is hypothesized that benign MS (EDSS < 2) is characterized by a fairly balanced cytokine and neuroendocrine network (16). Perhaps MS subjects with higher disability (EDSS > 5) may exhibit a different response due to a stronger immune dysregulation. Our subjects reported slightly higher EDSS.
score than benign MS (EDSS = 3.4) but may still maintain a balanced cytokine network when reacting to a physical stress.

In conclusion, additional studies are needed to provide a more complete and comprehensive understanding of the dynamic cytokine response to physical stress in MS and its implications on disease activity. Future research focused on the dynamic cytokine response to physical stress in MS and its implications on disease activity. Future research focused on

### References


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