Psychophysiology, 47 (2010), 615–624. Wiley Periodicals, Inc. Printed in the USA.

DOI: 10.1111/j.1469-8986.2010.00978.x

Severity of symptom flare after moderate exercise is linked to cytokine activity in chronic fatigue syndrome

ANDREA T. WHITE,[a] ALAN R. LIGHT,[b,c] RONALD W. HUGHEN,[b] LUCINDA BATEMAN,[d] THOMAS B. MARTINS,[e,f] HARRY R. HILL,[e,f] AND KATHLEEN C. LIGHT[b]

[a]Department of Exercise and Sport Science, University of Utah, Salt Lake City, Utah, USA
[b]Department of Anesthesiology, University of Utah, Salt Lake City, Utah, USA
[c]Department of Neuroscience, University of Utah, Salt Lake City, Utah, USA
[d]Fatigue Consultation Clinic, Salt Lake City, Utah, USA
[e]Associated Regional & University Pathologists (ARUP), Institute for Clinical and Experimental Pathology, Salt Lake City, Utah, USA
[f]Departments of Pathology, Pediatrics, and Medicine, University of Utah, Salt Lake City, Utah, USA

Abstract

Chronic fatigue syndrome (CFS) patients often report symptom flare (SF) for >24 h after moderate exercise (post-ex). We hypothesized that SF is linked to increases in circulating cytokines and CD40 Ligand (CD40L). In 19 CFS patients and 17 controls, mental and physical fatigue and pain symptom ratings were obtained together with serum for 11 cytokines and CD40L before and at 0.5, 8, 24, and 48 h post-ex. Before exercise, CFS had lower CD40L ($p < .05$) but similar cytokines versus controls. In subgroups based on SF at 48 h, high SF patients ($n = 11$) increased in IL-1β, IL-12, IL-6, IL-8, IL-10, and IL-13 ($p < .05$) 8 h post-ex. Low SF patients ($n = 8$) showed post-ex decreases in IL-10, IL-13, and CD40L, and controls decreased in IL-10, CD40L, and TNFα ($p < .05$). Thus, in CFS, cytokine activity may vary directly with SF, which may explain prior inconsistent findings.

Descriptors: Chronic fatigue syndrome, Cytokines, Exercise, Pain

For over a decade, investigators have examined whether patients with chronic fatigue syndrome (CFS) show altered serum cytokine profiles. Early studies (Gupta, Aggarwal, See, & Starr, 1997; Moss, Mercandetti, & Vojdani, 1999) suggested that CFS patients showed evidence of an enhanced pro-inflammatory state, reflected in higher levels of TNF-α and lower levels of IL-10 together with higher IL-6, which is often interpreted as having mixed pro- and anti-inflammatory effects. Other studies (Borish et al., 1998; Zhang et al., 1999) found elevated levels of both pro-inflammatory cytokines (including TNF-α, IL-2, and IFNγ) and anti-inflammatory cytokines (IL-10) but only in some of the CFS patients, specifically, those with active allergies, psychiatric symptoms, or with Gulf War syndrome. More recent studies have generally not supported the link between CFS and a greater pro-inflammatory cytokine profile. Instead, numerous studies have observed that their CFS samples showed IL-6 and pro-inflammatory cytokines that were lower or not different or even showed higher anti-inflammatory cytokines compared to controls (Amel, Kashipaz, Swinden, Todd, & Powell, 2003; Gaab et al., 2005; Patarca-Montero, Antoni, Fletcher & Klimas, 2001; Skowera et al., 2004; ter Wolbeek et al., 2007; Visser et al., 2001). Prospective studies of several infectious disorders known to lead to CFS in a small subgroup of those who are infected have also yielded mixed observations in regard to whether pro-inflammatory cytokines like IL-1β, TNF-α, IL-2, and IFNγ or IL-6 remain elevated in those patients who do develop CFS in the postinfectious state (Kerr & Tyrrell, 2003; Vollmer-Conna et al., 2007). Nevertheless, antiviral treatments and similar interventions have proven successful in some CFS patients (Kerr, Cunniffe, Kelleher, Bernstein, & Bruce, 2003; Lerner, Beqaj, Deeter, & Fitzgerald, 2007).

Several studies have used exercise as a stimulus when they compared cytokine profiles in CFS patients and healthy individuals. Post-exercise exacerbation of the central symptoms of CFS lasting 24–48 h or longer (including worsening of physical fatigue, problems with memory and concentration, and both muscle and joint pain) is so common that it is part of the Centers for Disease Control (CDC) diagnostic profile for CFS (Fukuda et al., 1994). CFS patients often remark that if they try to follow an exercise regimen, after each bout of exercise there is a significant cost for the next several days in terms of greater symptoms and less ability to function (symptom flare [SF]). Exercise physiologists have documented that several cytokines are increased from 4 to 24 h after sustained or strenuous exercise even in very fit individuals, and these include both pro-inflammatory IL-1β and

The project described was supported by NIH Grant R21 NS057821 from NINDS and NIAMS, with additional support provided by a Catalyst Grant from the University of Utah, by NIH Grant R21 AT002209 from NCAM and by the CFIDS Association.

Address reprint requests to: Kathleen C. Light, Department of Anesthesiology, 3C444 SOM, 30 North 1900 East, Salt Lake City, UT 84132-2304, USA. E-mail: Kathleen.C.Light@hsc.utah.edu

PSYCHOPHYSIOLOGY

anti-inflammatory IL-10, as well as IL-6 and IL-8 (Nielsen & Pedersen, 2007; Patarca-Montero et al., 2001; Peake et al., 2005, 2008; Pedersen & Fischer, 2007). LaManca and colleagues (1999) used a maximal exercise task that did cause a worsening of fatigue and other symptoms in their CFS patients but produced no differences in cytokine responses between CFS and control groups. Although a maximal exercise task may help demonstrate physiological and performance limits in this disorder, this type of challenge is too extreme to generalize to real-life experiences; a mild to moderate exercise task is thus a more appropriate choice to mimic what CFS patients do naturally that can lead to such a SF. Gupta, Aggarwal, and Starr (1999) reported that when CFS patients were tested twice, once when feeling a typical level of fatigue for their condition and once during a natural SF, they showed significantly greater IL-6 levels during the SF. They also attempted to experimentally induce SF through exercise, but only examined cytokine responses immediately after exercise, before the SF was manifested, when CFS patients and controls did not differ. In contrast, Cannon et al. (1999) employed a mild leg exercise task (15 min of stair-step exercise) but examined post-exercise responses for 24 h after exercise, which did induce increases in both IL-6 and IL-1β. They found that although CFS and control groups did not differ in terms of IL-1β, CFS patients did have modestly higher lipopolysccharide-stimulated IL-6 levels if post-exercise levels were combined with pre-exercise levels (Cannon et al., 1999). However, this study was not definitive because their exercise task was so mild or involved such a restricted set of muscles that it failed to induce an increase in fatigue or other symptoms, nor did they examine a full profile of pro-inflammatory and anti-inflammatory cytokines. Because pro-inflammatory and anti-inflammatory cytokines are mutually inhibitory, changes in one type can secondarily lead to changes in the other, indicating the need to study both types (for reviews, see Elenkov & Chrousos, 2002; Elenkov, Wilder, Chrousos, & Vizi, 2000).

The present study used a moderate exercise task to examine worsening of the central symptoms of fatigue and pain in CFS patients for 48 h after the exercise task while also monitoring pre- and post-exercise cytokine responses for this full period. Our protocol improves upon earlier research in the following ways: (a) We attempted to control for variables that might have caused SF at baseline by asking both CFS and control subjects to refrain from all exercise for 48 h prior to the study and by not testing patients who recently were diagnosed or needed additional medical intervention. (b) We employed a moderate whole-body exercise task (working both arms and legs) for 25 min that was mild enough that all CFS patients were able to complete it successfully but did induce a flare of fatigue and pain symptoms that remained above pre-exercise levels for 48 h post-exercise in the majority of patients. (c) Physical fatigue, mental fatigue, and overall pain symptoms were obtained concurrently with blood samples for cytokines at five times: pre-exercise and at 0.5, 8, 24, and 48 h post-exercise. (d) Whereas prior studies in CFS typically assessed only one or two cytokines, our cytokine profile included six pro-inflammatory cytokines/ligands, four anti-inflammatory/regulatory cytokines, and two exercise-responsive cytokines that have mixed effects. We included one new pro-inflammatory marker, CD40 ligand (CD40L), that has not been previously examined in this population. Genetic alterations that reduce levels of CD40L have been shown to enhance vulnerability to opportunistic infections (Danielian, Oleastro, Rivas, Cantisano, & Zelazko, 2007), a pattern typical of a subgroup of CFS patients.

Methods

Participants

Nineteen CFS patients and 17 healthy controls aged 19–65 years participated in this study. Following the recommendation of Booth, Gordon, Carlson, and Hamilton (2000), we did not restrict our control sample to a completely sedentary population, who may have underlying health abnormalities; therefore, the control group included 9 who regularly exercised for 20 min or more 1–3 times/week and 8 who were sedentary, thus representing the range in fitness typical of the healthy adult population. The CFS sample included 8 who also reported exercising every week (if symptoms permitted) and 11 who did not. All patients met the CDC 1994 diagnostic criteria for CFS (Fukuda et al., 1994) and had been evaluated previously by a practitioner who excluded all other causes for their severe persistent or relapsing fatigue and related symptoms before assigning a diagnosis of CFS. Subjects taking corticosteroids, sympathetic agonists, or prescription analgesics known to affect sympathetic nervous system (SNS), hypothalamic-pituitary axis (HPA), or cytokine activity were excluded, as were subjects with uncontrolled cardiovascular or pulmonary disease. Any pain medications were stopped for 48 h before and during testing. Due to the 4- to 6-week gradual tapering required, use of antidepressants was not an exclusion criterion, nor were these drugs stopped in patients or controls. At the time of testing, 33% of controls and 42% of CFS patients were taking antidepressants as prescribed by their own physicians for treatment of depression. In addition, all patients underwent screening for fibromyalgia (FMS) using the American College of Rheumatology (ACR) criteria, including presence of widespread pain symptoms for at least 6 months and pain reported at 11 or more of 18 sites during Tender Point examination. Fourteen of the CFS patients also met ACR diagnostic criteria for FMS. Subject characteristics are presented in Table 1.

Results are presented for all CFS patients versus controls as well as for patients subgrouped into those showing high versus low SF. Those CFS patients demonstrating increases in physical fatigue ratings at or above the group median were classified as high SF whereas those patients below the median were classified as low SF. Figure 1 illustrates the results of this subgrouping and also shows that, relative to the low SF patients as well as to controls, the high SF patients had greater increases in mental fatigue and pain ratings as well as physical fatigue 8–48 h post-exercise, which supports this definition of SF.

Protocol Overview

All participants refrained from formal exercise other than the required exercise test for a period of 4 days beginning 48 h preceding the exercise test until after the final 48-h post-exercise blood sample had been drawn. Upon arrival at the laboratory, subjects provided written informed consent for our protocol that was approved by the University of Utah Institutional Review Board. Venous blood samples were obtained at the end of a 15-min seated baseline condition and at 0.5, 8, 24, and 48 h post-exercise. To minimize degradation of samples, they were centrifuged immediately and serum was flash frozen within 18 min and then kept at -80°C until assay. Leukocytes were also obtained from most of these same subjects (and from some additional subjects) which were used to determine gene expression of ion channel and adrenergic receptors and a small subset of immune markers; these gene expression results are reported in a separate paper (Light, White, Hughen, & Light, 2009). To assess severity

Table 1. *Subject Characteristics for Controls, All Patients, and Patient Subgroups*

	Controls ($n = 17$; 11 F)	All patients ($n = 19$; 15 F)	CFS high SF ($n = 11$; 8 F)	CFS low SF ($n = 8$; 7 F)
Age (years)	33.6 ± 2.7	40.1 ± 2.8	42.9 ± 3.5*	38.2 ± 2.8
BMI ($kg \cdot m^2$)	22.9 ± 0.65	26.9 ± 1.36*	28.4 ± 1.77*	25.0 ± 2.07
Resting SBP (mm Hg)	123 ± 3.8	122 ± 3.5	130 ± 4.1	112 ± 3.9*†
Resting DBP (mm Hg)	77 ± 2.1	80 ± 2.4	85 ± 2.7	72 ± 2.8†
Exercise HR (% PMHR)	71.4 ± 0.76	69.9 ± 1.13	71.6 ± 1.40	67.6 ± 1.71
Exercise WR ($kcal \cdot kg^{-1} \cdot min^{-1}$)	6.9 ± 0.33	4.2 ± 0.35*	4.4 ± 0.49*	4.0 ± 0.49*
Exercise RPE (0–10)	2.8 ± 0.15	4.6 ± 0.35*	4.1 ± 0.36*	5.2 ± 0.63*
Exercise SBP (mm Hg)	146 ± 5.8	147 ± 6.1	157 ± 10.2	136 ± 5.8
Exercise DBP (mm Hg)	86 ± 1.7	89 ± 2.4	92 ± 3.8	85 ± 3.0

Values are means ± *SE*. CFS high SF: CFS patients with physical fatigue scores at or above patient median at 48 h post-exercise (high symptom flare); CFS low SF: CFS patients with physical fatigue scores below the median at 48 h post-exercise (low symptom flare); BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; %PMHR: percent of age-predicted maximal heart rate; WR: work rate; RPE: rating of perceived exertion.
*Significant difference from controls, $p < .05$.
†Significant difference between CFS high SF and CFS low SF, $p < .05$.

of preexisting and exercise-related fatigue and myalgia symptoms, at each of these blood sampling times, the subject also provided numerical ratings of mental fatigue, physical fatigue, and overall body pain using a 0–100 scale where 100 was defined as the greatest level of fatigue or pain the subject could ever imagine experiencing. Immediately following the baseline blood draw, participants began the exercise session, as described below.

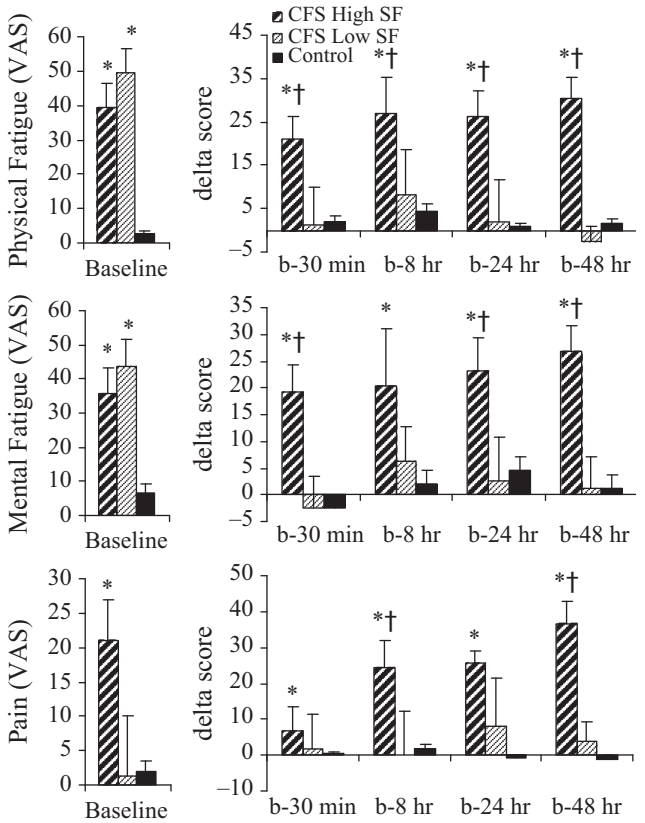

Figure 1. Scores for physical fatigue, mental fatigue, and pain by group. Left panels are baseline scores and right panels represent changes from baseline to 30 min and 8, 24, and 48 h post-exercise. *Significant difference from controls, $p < .001$. †Significant difference from low SF, $p < .01$.

Exercise Protocol

A combined arm–leg cycle ergometer (Schwinn Airdyne) was used for the 25-min exercise test. In the first 5 min of exercise, subjects were asked to increase pedaling rate until 70% age-predicted maximal heart rate was achieved. Thereafter, work rate was adjusted in order to maintain this target heart rate throughout the protocol. Rating of perceived exertion (RPE) was obtained on a scale of 1 to 10 (Borg, 1982) every 5 min, and blood pressure was measured at baseline, every 10 min during exercise, and upon completion of the exercise.

Cytokine Assays

A multiplexed fluorescent microsphere immunoassay developed at the ARUP Institute for Clinical and Experimental Research (Salt Lake City, UT) was used to simultaneously assess the concentration of 12 cytokines/ligands, including 6 pro-inflammatory (IL-1β, IL-2, IL-12, TNFα, soluble CD40L, and IFNγ), 3 anti-inflammatory/regulatory (IL-4, IL-10, IL-13), and 2 categorized as exercise responsive with mixed pro- and anti-inflammatory effects (IL-6 and IL-8), all from only 75 μl of serum (Martins, Pasi, Litwin, & Hill, 2004). The Luminex Multi-Analyte Profiling system (Luminex 100, Luminex Corp, Austin, TX) is a flow cytometry based instrument that allows multiple analytes to be assayed simultaneously in a single sample (Fulton, McDade, Smith, Kienker, & Kettman, 1997).

Complete Blood Cell Counts (CBC)

Complete blood cell counts with differential subtyping were obtained by standard laboratory assays from separate blood samples collected in EDTA tubes. Measures obtained included total red blood cells, hematocrit and hemoglobin, platelets, and total white blood cells plus subtypes including monocytes, eosinophils, lymphocytes, and granulocytes.

Statistical Analysis

Raw cytokine data were nonnormally distributed, and, in some instances, values were less than 1.0; therefore, a constant was added to the actual value at each time point for each cytokine to ensure that all values exceeded the minimum of 1.0 required for transformation, and then all cytokine data were log transformed to yield distributions that could be appropriately analyzed with parametric statistics. Baseline differences between controls and patients and patient subgroups were determined with one-way analysis of variance (ANOVA; SPSS v. 16). When group effects

were significant, post hoc Games–Howell analyses were performed to identify which groups differed.

Two (CFS patients vs. controls) × 4 (baseline to 0.5, 8, 24, and 48 h post-exercise) repeated measures ANOVAs were used to analyze changes in cell counts and cytokines. In the second set of analyses, high and low SF patients were compared to each other and to CONTROLS using 3 (group) × 4 (time) repeated measures ANOVAs. For effects involving the time factor, the conservative Huynh Feldt correction factor was chosen to correct for sphericity issues; this yields different fractional degrees of freedom depending on the data array involved. Simple contrasts comparing each post-exercise time point to baseline were built into the model to determine which time points were significantly different from baseline, and Games–Howell post hoc analyses were used to determine group differences. Relationships between baseline and post-exercise cytokines versus pain and fatigue symptoms were examined using Pearson correlations. Significance level was set at .05. Data are presented as means and standard errors.

Results

Compliance and Exercise Performance

All subjects, including the most deconditioned patients, completed the 25-min exercise protocol, which was individually adjusted to attain the same relative exercise intensity of 70% of age-predicted maximal heart rate (PMHR). Although controls and CFS patients exercised at the same relative intensity (see Table 1), absolute work rate for all patients and both patient subgroups was significantly lower, $F(2,35) = 15.79$, $p < .001$, and their RPE was significantly higher compared to controls, $F(2,35) = 13.28$, $p < .001$. There were no overall group differences for age or exercise blood pressure, but the subgroup analysis revealed that the low SF group had significantly lower resting blood pressure than controls ($p < .05$). All patients and specifically the high SF subgroup had higher body mass index (BMI) than controls, $F(2,35) = 4.62$, $p < .05$.

Baseline Blood Cell Counts and Cytokines

At baseline, the high SF patients showed significantly higher total white cells than controls, suggestive of preexisting immune activation, whereas the low SF patients had normal white cells (see Table 2). Subtypes that were elevated in the high SF patients included eosinophils, granulocytes, and basophils whereas monocytes and lymphocytes were not reliably increased. In the low SF but not the high SF patients, baseline hemoglobin levels were significantly lower than controls, whereas platelet counts did not differ by group.

For baseline cytokines, group differences were seen for only one pro-inflammatory marker, CD40L, which was lower in all patients than in controls, $F(2,35) = 3.66$, $p < .05$, an effect principally due to the high SF subgroup, which had the lowest CD40L (see Table 3).

Effect of Moderate Exercise on Perceptions of Pain and Fatigue

At baseline, both the high SF and the low SF patients with CFS demonstrated significantly higher scores for mental fatigue and physical fatigue, and the high SF subgroup also showed higher overall body pain compared to controls, $F(2,35) > 26.24$, $p < .001$ (see Figure 1). After exercise, only the high SF patients showed significant worsening of their fatigue and pain symptoms, differing significantly from controls at all post-exercise times for all three symptoms, $F(2,35) > 7.04$, $p < .003$. Compared to the low SF patients, high SF patients also showed greater increases in physical fatigue at all post-exercise times, greater increases in mental fatigue at 30 min and 24 and 48 h, and greater increases in pain at 8 and 48 h ($p < .01$). In the high SF patients, peak fatigue and pain ratings occurred at 48 h after exercise.

Effect of Moderate Exercise on Blood Cell Counts

Post-exercise changes in blood cell counts did not differ between high and low SF patients, although the high SF subgroup maintained their higher white cell counts relative to controls. Thus, analyses involving post-exercise changes compared controls versus all CFS patients combined. For white cell counts, a significant time effect was observed, in which patients and controls demonstrated increases at 8 h post-exercise, $F(3.4,92.7) = 16.38$, $p < .001$ (see Figure 2), marking this point as the likeliest time for enhanced immune activation. A significant Group × Time interaction and time effect were observed for post-exercise changes in red cell counts, $F(4,108) = 3.14$, $p < .02$ and $F(4,108) = 4.55$, $p < .002$, respectively, showing a reduction for controls and patients at 8 h post-exercise, with controls returning to baseline by 48 h post-exercise whereas patients' red cell levels remained low during the same time period (Figure 2). Because females typically have lower red cell counts, we repeated these analyses with sex as a covariate and found the results were not changed. The 8-h post-exercise changes in white and red cells may have been due to diurnal variations, as they match the profile reported by Pocock, Ashby, Shaper, Walker, and Broughton (1989).

Table 2. *Baseline Cell Counts for Controls, All Patients, and Patient Subgroups*

	Controls ($n = 17$; 11 F)	All Patients ($n = 15$; 12 F)	CFS High SF ($n = 7$; 5 F)	CFS Low SF ($n = 8$; 7 F)
White cells ($K \cdot \mu l^{-1}$)	5.99 ± 0.35	6.89 ± 0.48	8.08 ± 0.59*	6.59 ± 0.46
Platelets ($K \cdot \mu l^{-1}$)	271 ± 13	283 ± 17	293 ± 24	277 ± 24
Monocytes ($K \cdot \mu l^{-1}$)	0.31 ± 0.02	0.37 ± 0.03	0.41 ± 0.07	0.35 ± 0.04
Eosinophils ($K \cdot \mu l^{-1}$)	0.16 ± 0.04	0.27 ± 0.04	0.36 ± 0.08*	0.21 ± 0.04
Lymphocytes ($K \cdot \mu l^{-1}$)	1.88 ± 0.12	2.24 ± 0.18	2.37 ± 0.33	2.00 ± 0.19
Granulocytes ($K \cdot \mu l^{-1}$)	3.45 ± 0.25	4.27 ± 0.30*	4.92 ± 0.51*	3.84 ± 0.32
Basophils ($K \cdot \mu l^{-1}$)	0.04 ± 0.01	0.06 ± 0.01	0.10 ± 0.00*§	0.03 ± 0.02
Red cells ($M \cdot \mu l^{-1}$)	4.72 ± 0.12	4.66 ± 0.11	4.78 ± 0.19	4.58 ± 0.12
Hemoglobin ($g \cdot dl^{-1}$)	14.8 ± 0.31	14.1 ± 0.32	14.3 ± 0.59	13.9 ± 0.35*

Values are means ± *SE*. CFS high SF: CFS patients with physical fatigue scores at or above patient median at 48 h post-exercise (high symptom flare); CFS low SF: CFS patients with physical fatigue scores below the median at 48 h post-exercise (low symptom flare).
*Significant difference from controls, $p < .05$.
§Significant difference from CFS low SF, $p < .05$.

Table 3. *Baseline Cytokines for Controls, All Patients, and Patient Subgroups*

	Controls ($n = 17$; 11 F)	All Patients ($n = 19$; 15 F)	CFS High SF ($n = 11$; 8 F)	CFS Low SF ($n = 8$; 7 F)
Pro-inflammatory				
IL-1β	2.04 ± 0.02	2.02 ± 0.01	2.00 ± 0.01	2.04 ± 0.02
IL-2	1.89 ± 0.02	1.88 ± 0.01	1.87 ± 0.01	1.88 ± 0.01
IL-12	1.50 ± 0.02	1.48 ± 0.01	1.46 ± 0.01	1.49 ± 0.02
IFNγ	1.05 ± 0.02	1.02 ± 0.01	1.01 ± 0.01	1.02 ± 0.01
CD40L	2.29 ± 0.05	1.94 ± 0.12*	1.89 ± 0.16*	2.02 ± 0.17
TNFα	1.68 ± 0.02	1.64 ± 0.01	1.63 ± 0.01	1.66 ± 0.01
Anti-inflammatory				
IL-4	0.61 ± 0.02	0.61 ± 0.01	0.59 ± 0.01[§]	0.64 ± 0.02
IL-10	1.36 ± 0.05	1.18 ± 0.11	1.03 ± 0.15*	1.35 ± 0.16
IL-13	2.60 ± 0.02	2.63 ± 0.03	2.59 ± 0.02	2.67 ± 0.05
Mixed response				
IL-6	1.34 ± 0.02	1.34 ± 0.01	1.33 ± 0.01	1.36 ± 0.03
IL-8	1.03 ± 0.02	1.04 ± 0.03	1.00 ± 0.01	1.05 ± 0.06

Values are log transformed means ± *SE*. Raw *SE*s of 1.0 or less (where log = 0) were rounded up to raw value of 1.025 where log = 0.01.
*Significant difference from controls, $p < .05$.
[§]Significant difference from CFS low SF, $p < .05$.

Effect of Moderate Exercise on Post-Exercise Changes in Cytokines

A significant main effect of time was seen for post-exercise changes from baseline in IL-10, $F(2,35) = 2.87$, $p < .05$, which decreased from baseline in controls at 0.5, 8, and 24 h ($p < .05$; see Figure 3). For low SF patients, IL-10 levels were similar to the controls, whereas high SF patients showed a trend ($p < .07$) to have lower overall IL-10 levels than controls.

As indicated by the increase in white cells at 8 h post-exercise, the largest increases in cytokines were observed at this time point (see Figure 4). A significant main effect of time was obtained for IL-8, $F(2.4,79.7) = 11.34$, $p < .01$, revealing a significant increase at 8 h in both CFS subgroups ($p < .01$) and a nonsignificant increase in controls. High SF patients also showed significant increases at 8 h in five other cytokines: IL-6, IL-1β, IL-12, IL-10, and IL-13, $F(2,35) > 3.57$, $p < .05$. In contrast, low SF patients showed decreases in IL-10 at 8 h post-exercise as well as consistent overall decreases in IL-13 and CD40L, $F(2,35) > 3.32$, $p < .05$, from 0.5 through 48 h (see Figure 5). Controls showed gradually decreasing levels of both CD40L and TNFα after exercise, becoming significantly lower than baseline by 24 h and showing no recovery even at 48 h. In sum, low SF patients and controls showed a pattern of post-exercise decreases in both pro- and anti-inflammatory cytokines (with the exception of increases in IL-8), whereas the high SF patients showed a pattern of increases in both cytokine types at 8 h and no decreases at any time.

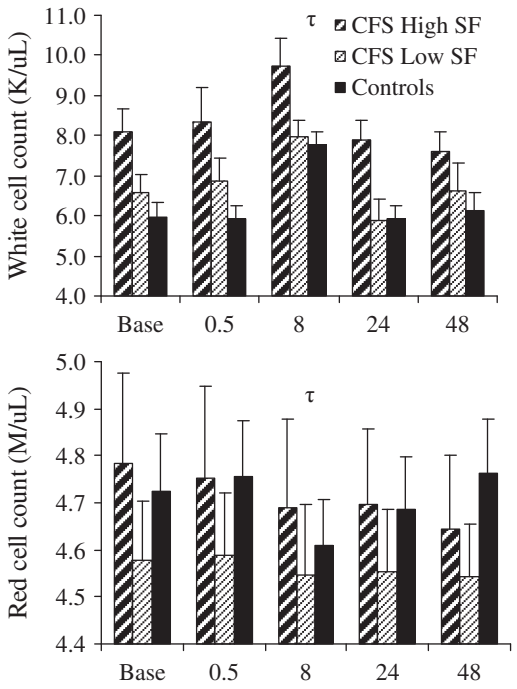

Figure 2. White cell counts (top panel) and red cell counts (bottom panel) for CFS patients and controls at baseline and at 0.5, 8, 24, and 48 h following exercise. [τ]Time point significantly different from baseline across groups, $p < .05$.

Linear Relationships between Patient Cytokine Responses and Symptoms of Fatigue and Pain

At baseline, the cytokine that had the strongest association with higher baseline fatigue in CFS patients was higher IL-6 ($r = +.43$ and $+.57$ with physical fatigue and mental fatigue ratings, $p < .05$). Higher baseline IL-6 was also associated with higher reported levels of physical fatigue at 48 h ($r = +.48$, $p < .05$). Predictive relationships between changes in cytokines/ligands at 8 h post-exercise (when greatest cytokine changes usually occurred) and SF severity at 48 h were also examined in all CFS patients using linear regression. Greater increases in IL-6 and in CD40L predicted greater increases in physical fatigue, accounting for 43% and 31% of the variance in fatigue increases, respectively ($p < .01$; see Figure 6), and increases in IL-6 also accounted for 29% of the variance in increased pain ($p < .01$, not depicted). However, although the relationship with CD40L was robust, the relationships between IL-6 and both physical fatigue and pain increases were strongly influenced by the patient showing the largest IL-6 increase. When reexamined after removal of that individual, these relationships were no longer significant ($r = +.36$, $R^2 = 13\%$). Still, when considered together with the associations between higher baseline IL-6 and greater fatigue, these observations encourage continued research attention to IL-6 as well as CD40L in relation to SF.

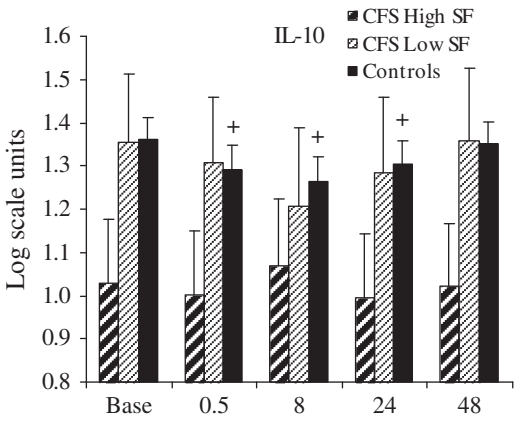

Figure 3. IL-10 levels at baseline and at 0.5, 8, 24, and 48 h post-exercise by group. $^{+}$Within group difference from baseline, $p < .05$.

Discussion

The present investigation was designed to assess whether patients with CFS or subgroups of these patients demonstrate altered patterns of pro- and anti-inflammatory cytokines before and for up to 48 h after a moderate whole-body exercise task and whether these patterns may relate to severity of postexertional fatigue and pain symptoms. When the CFS group as a whole was compared to healthy controls, the only immune marker that differed at baseline was CD40L, which was lower in CFS patients than controls. This difference was maintained following exercise. Part of the pro-inflammatory TNF family, CD40L may be produced primarily from platelets (which did not differ between groups), it is considered a marker of platelet activation, and high levels have been linked to cardiovascular disorders (Henn, Steinbach, Buchner, Presek, & Kroczek, 2001). Genetic X-chromosome-linked mutations associated with absent CD40L or milder mutations causing moderate CD40L deficiency have been associated with enhanced vulnerability to opportunistic infections (Danielian et al., 2007; Ferrari et al., 2001). It is possible that decreased CD40L in some CFS patients may have a genetic basis and contribute to pathogenesis.

Exercise-Induced Changes in Cytokines

Consistent with prior studies in athletes and other healthy adults (Nielsen & Pederson, 2007; Pedersen & Fischer, 2007), sustained arm and leg exercise for 25 min led to increases in circulating levels of the more exercise-responsive cytokines, but only at one particular time point. This is in contrast to Cannon and colleagues (1997, 1999), who found no changes in plasma IL-6 or IL-1β levels in CFS patients at 6 and 24 h after a different moderate exercise task that only involved leg activity. However, these investigators did note a significant increase in LPS-induced IL-6 levels at 6 h post-exercise. In the present study all groups showed post-exercise increases in IL-8 at 8 h post-exercise, the same time point when significant increases in total white cells were seen. In contrast to other reports of anti-inflammatory IL-10 increases only after strenuous or prolonged exercise (Peake et al., 2005, 2008), our shorter and more moderate exercise task led to significant decreases in IL-10 levels at 0.5, 8, and 24 h but recovery to pre-exercise levels by 48 h in controls and low SF patients. It deserves mention that, although actual work rate was not correlated with post-exercise cytokine changes, perceived exertion was correlated with the peak increase in IL-8 at 8 h ($r = +.33$, $p < .05$). Perceived exertion reflects the levels of psychological as well as physical stress and was higher in all of the CFS patients compared to controls.

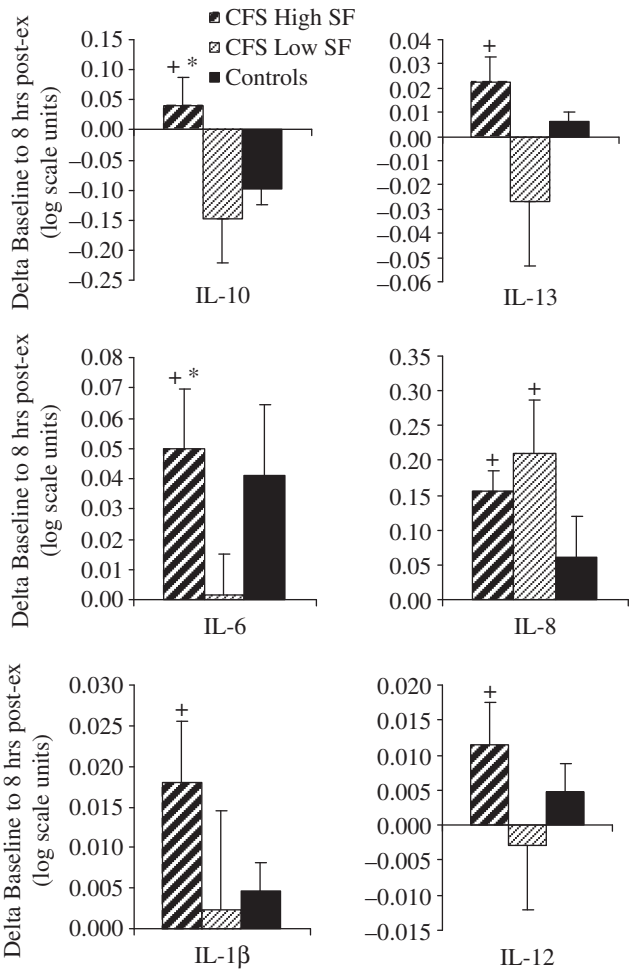

Figure 4. Changes from baseline to 8 h post-exercise by group for IL-10 and IL-13 (top panels), IL-6 and IL-8 (middle panels), and IL-1β and IL-12 (bottom panels). $^{+}$Within group difference from baseline, $p < .05$. *Significant difference from low SF group, $p < .05$.

In controls, the earliest post-exercise cytokine changes were decreases in IL-10 and total anti-inflammatory cytokines beginning at 30 min, followed by increases in IL-8 at 8 h, and finally by decreases in CD40L and TNF-α at 24 h that did not recover by 48 h. Such long-lasting decreases in pro-inflammatory cytokines/ligands may be one reason why even moderate exercise has beneficial effects in healthy adults.

Cytokines and Postexertional Symptom Flare in CFS Patients

The exercise task selected for this study was deliberately chosen to be moderate in order to observe different post-exercise symptoms in CFS patients and controls. It should be noted that the use of age-predicted maximal heart rate as a basis for determining the same relative exercise intensity for all subjects has potential for error. However, the observed consistency of heart rates during the 25-min exercise sessions suggests that all subjects obtained a submaximal steady state. Sargent, Scroop, Nemeth, Burnet, and Buckley (2002) determined that CFS patients had normal maximal oxygen uptake and lactate metabolism compared to activity-matched controls. Further, they showed that actual maximal

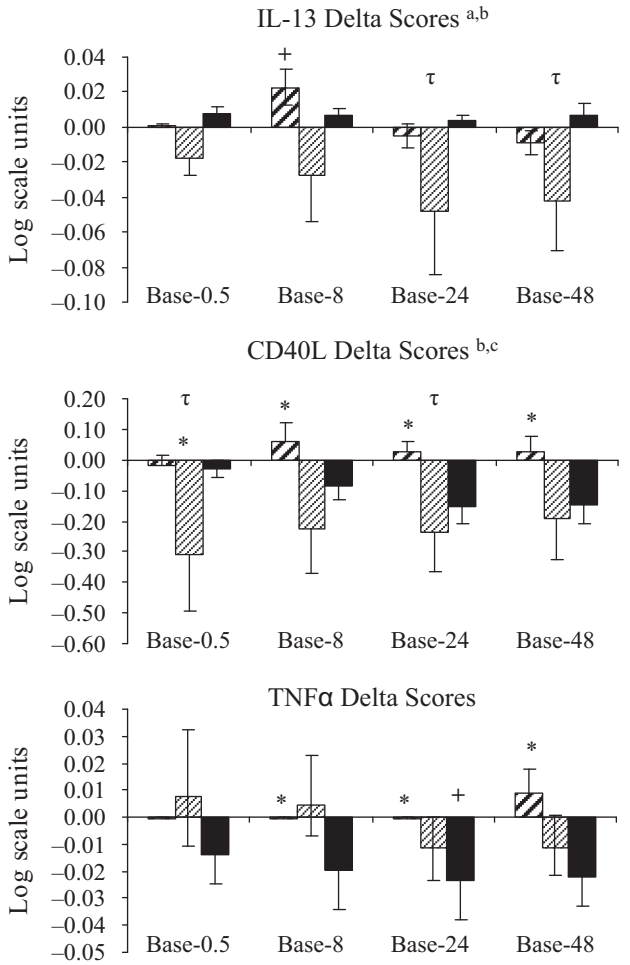

Figure 5. Group changes following exercise for IL-13 (top panel), CD40L (middle panel), and TNFα (bottom panel). Contrasts indicated significant across-group decreases in IL-13 at 24 and 48 h post-exercise. For the high SF group, a within-group increase in IL-13 was noted at 8 h post-exercise. Across group decreases in CD40L were observed at 0.5 and 24 h post-exercise. [a]Significant Group × Time interaction, $p < .05$. [b]Significant overall time effect, $p < .05$. [c]Significant overall group effect, $p < .05$. [τ]Time point significantly different from baseline across groups, $p < .05$. [+]Significant within-group change from baseline, $p < .05$. *Significant difference from controls at that time point, $p < .05$.

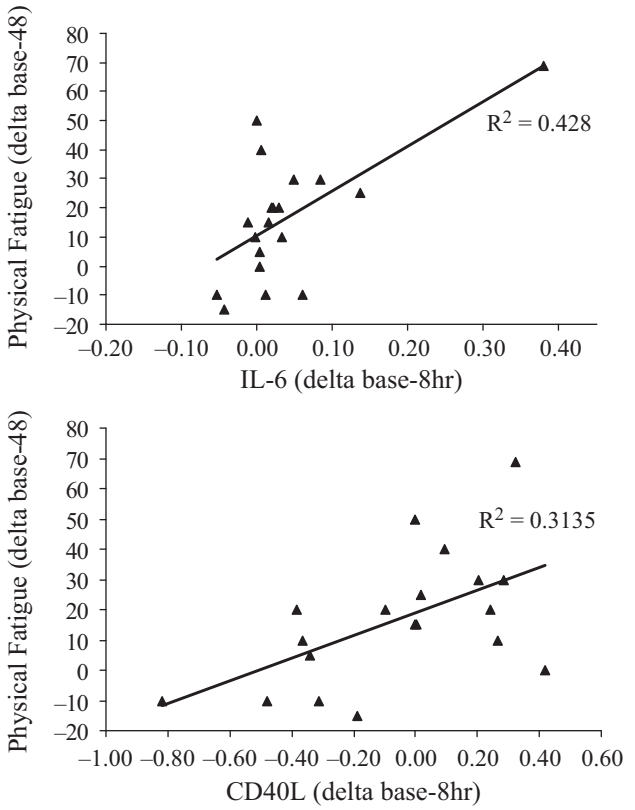

Figure 6. Relationship between increases in IL-6 from baseline to 8 h post-exercise (top panel) and increases from baseline to 8 h post-exercise in CD40L (bottom panel) as they relate to increases in physical fatigue from baseline to 48 h post-exercise in CFS patients.

heart rate obtained for CFS patients during graded exercise testing was 99% of age-predicted maximal heart rate, supporting the latter measure as a valid index in these patients. Thus, we are confident that the CFS and control groups exercised at the same relative intensity. As we expected, the exercise task elicited only mild and transitory increases in fatigue in the controls. Although both groups reflected a wide range of physical activity levels, the fact that controls attained 70% of their age-predicted maximal heart rate at a higher mean absolute work rate suggests that they had a higher exercise capacity than the patients. This was likely due to differences in exercise routines (intensity, frequency, or duration), but could also reflect intrinsic differences between controls and patients in the ability to tolerate exercise due to disease-related alterations in ion channel receptor sensitivity (Light, White, et al., 2009). Even though the CFS patients exercised at lower absolute work rates than controls, they suffered much greater symptomatic consequences. Their mental fatigue, physical fatigue, and pain symptoms all became worse during the 48 h after exercise. The 11 patients identified as having high SF were the only group to show significant increases in multiple pro- and anti-inflammatory cytokines after exercise, even though their exercise work rates were identical to the low SF patients. At 8 h post-exercise, the high SF patients showed increases in pro-inflammatory IL-1β and IL-12 as well as in anti-inflammatory IL-10 and IL-13 and in IL-6, which may have mixed effects. The high SF group also failed to show post-exercise decreases in CD40L, TNFα, or IL-13, as shown by controls or by the other CFS patients who experienced less SF. This is similar (but reveals more cytokine differences) to prior reports of cytokines during naturally occurring SF in patients with FMS (Bazzichi et al., 2007) or with CFS (Gupta et al., 1999).

At baseline, higher IL-6 was linked to greater mental and physical fatigue measures, and high SF patients had greater baseline white cell counts, suggesting that even before our task, the more symptomatic CFS patients had slightly greater immune activity. Likewise, greater increases in CD40L and IL-6 at 8 h post-exercise were predictors of increases in physical fatigue and pain at 48 h, although patients with high SF at 48 h also had increases in five other cytokines, indicating generalized immune activation. Gaab et al. (2005) previously noted significant correlations between baseline LPS-induced IL-6 and TNF-α levels and both mental and physical fatigue scores in CFS patients following a psychosocial stress test. IL-6 has also been associated

with the fatigue of overtraining and impaired performance in athletes (Robson, 2003; Robson-Ansley, Blannin, & Gleeson, 2007), and with the fatigue from chronic insomnia (Vgontzas et al., 2002).

It is notable that not just fatigue but also pain ratings were much higher in our high SF patients. Although our definition of severity of SF was based only on physical fatigue changes at 48 h post-exercise, 10 of the 11 CFS patients in the high SF group had comorbid FMS, and this group showed greater increases in both fatigue and pain symptoms beginning at 30 min after the exercise test right through 48 h. In contrast, 4 of 8 CFS patients in the low SF group had comorbid FMS. These results suggest that the same type of post-exertional SF including enhanced pain may be linked to increased cytokines in patients with FMS as well. Future studies of patients with FMS but not CFS will be necessary to confirm this.

As summarized earlier, previous studies on cytokine responses in CFS patients have yielded contradictory results, with some showing higher pro-inflammatory cytokine levels and other showing no differences or higher anti-inflammatory cytokines (Amel et al., 2003; Borish et al., 1998; Gaab et al., 2005; ter Wolbeek et al., 2007; Zhang et al., 1999). Consistent with Gupta and colleagues (1999), we suggest that these differences may be due in large part to whether or not most of the CFS patients who were tested in each prior study were experiencing SF. In several of the earlier reports where retrospective data were used, it is likely that blood samples came from CFS patients who were in clinic because of worsening symptoms. This may also be the case for Gulf War syndrome patients. In some of the more recent studies, where participants were recruited by advertisement or from medical records of previously treated patients and where patients may have been more likely to participate when they were feeling less symptomatic, the CFS patient samples may have included a lower percentage with a severe SF. Another explanation for the inconsistencies is that CFS is a heterogeneous, multiply determined disorder and that specific patterns of immune responses may occur (with or without SF) only in subgroups of these patients (Fostel, Boneve, & Lloyd, 2006; Siegel et al., 2006). Gene expression studies have recently identified 88 genes, including some related to cytokines TNFα, IL-10, and IL-6, which are either over- or underexpressed in CFS, but only in gene cluster-defined subgroups of patients, not in the CFS sample as a whole (Kerr et al., 2008).

We must acknowledge that, in general, the magnitude of the increases in cytokines shown by the CFS patients with high SF were small, not even approaching the high levels that occur during an active infection. Nevertheless, because so many different cytokines were increased along with significant increases in total white cells at 8 h post-exercise, it is reasonable to assume that a sufficient activation of the immune system was evoked in these patients by this mild but sustained exertion. This immune activation combined with other factors (including enhanced sensitivity of ion channel and adrenergic receptors that we have also observed in these CFS patients) may lead to worsening of muscle fatigue and pain (Light, White, et al., 2009).

Possible Involvement of Dysregulated SNS-Immune Interactions

Increases in circulating levels of IL-6 and IL-8 after exercise may be influenced by production of these cytokines by muscle, but that is unlikely to explain our findings at 8 h post-exercise. Muscle activity (as assessed by actual work performed) was greater in controls, yet the patients showed equal or greater increases in IL-8 and IL-6. Also, it might be expected that increases in these cytokines would be observed very soon after exercise if the muscle activity itself was the central stimulus; however, no increase in these cytokines was seen in the first post-exercise sample in any group. If not directly produced by muscles, what is the likeliest explanation for the increase in circulating levels of these cytokines at 8 h? Elenkov et al. (2000) have documented that activation of beta-2 adrenoceptors on leukocytes through generalized increases in SNS activity (such as occurs during aerobic exercise or psychological stress) enhances IL-6 and IL-8 while it reduces pro-inflammatory cytokines. This would explain the increases in IL-6 and IL-8 seen in all groups as well as the significant decreases in CD40L and other cytokines evident in healthy participants and the low SF patients during the post-exercise period.

Greater overall sympathetic activation of beta-2 adrenoceptors may also explain part of the low baseline CD40L and overall pro-inflammatory cytokine profiles shown by the CFS patients, especially those who also have FMS. Our results showed substantially higher baseline and post-exercise increases in pain in the CFS subgroup with high SF, and they also had higher pre-exercise blood pressure levels. Cardiovascular signs of sympathetic dysregulation, including decreased heart rate variability, abnormal vasomotor responses, and altered catecholamine levels before and during stressors, have previously been reported in CFS and FMS patients (see Light & Vierck, 2009). Chronic stress may enhance pain, and chronic pain and stress may enhance SNS activity. Pain in FMS has been shown to worsen after administration of sympathetic agonists (Martinez-Lavin et al., 2002), and we have recently shown that pain in FMS decreases after acute administration of low doses of a beta-receptor antagonist (Light, Bragdon, et al., 2009). Finally, our research group has obtained preliminary evidence that CFS patients with and without FMS show greatly exaggerated increases in alpha-2A, beta-1, and beta-2 adrenergic receptor mRNA expression after exercise (Light, White, et al., 2009).

Conclusion

In summary, CFS patients as a total group demonstrated only one immune difference from healthy controls, lower levels of pro-inflammatory CD40 ligand, although CFS patients with higher baseline levels of physical and mental fatigue did have higher pre-exercise IL-6 levels. After 25 min of moderate arm and leg exercise, healthy controls and the CFS patients who experienced milder SF showed increases in only one cytokine, IL-8 at 8 h post-exercise, while showing decreases in several pro- and anti-inflammatory cytokines (CD40L, TNFα, IL-10, and IL-13). In contrast, those CFS patients experiencing a greater SF lasting through 48 h post-exercise showed increases in six cytokines at 8 h post-exercise (pro-inflammatory IL-1β, IL-8, and IL-12, anti-inflammatory IL-10 and IL-13, and in IL-6). The high SF group also failed to show post-exercise decreases in CD40L, TNFα, or IL-13 as shown by controls or by the less symptomatic CFS patients. Higher baseline IL-6 was associated with higher baseline fatigue, and greater IL-6 and CD40L increases at 8 h post-exercise (when white cell counts increased significantly) were related to greater fatigue and pain increases at 48 h, although the IL-6 effect was strongly influenced by one patient. Whether these alterations in cytokines are causally related to symptoms or represent parallel alterations in immune function and behavioral symptoms caused by a common upstream factor will require further investigation.

REFERENCES

Amel Kashipaz, M. R., Swinden, D., Todd, I., & Powell, R. J. (2003). Normal production of inflammatory cytokines in chronic fatigue and fibromyalgia syndromes determined by intracellular cytokine staining in short-term cultured blood mononuclear cells. *Clinical and Experimental Immunology*, *132*, 360–365.

Bazzichi, L., Rossi, A., Massimetti, G., Giannaccini, G., Giuliano, T., De Feo, F., et al. (2007). Cytokine patterns in fibromyalgia and their correlation with clinical manifestations. *Clinical & Experimental Rheumatology*, *25*, 225–230.

Booth, F. W., Gordon, S. E., Carlson, C. J., & Hamilton, M. T. (2000). Waging war on modern chronic diseases: Primary prevention through exercise biology. *Journal of Applied Physiology*, *88*, 74–87.

Borg, G. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sport and Exercise*, *14*, 377–381.

Borish, L., Schmaling, K., DiClementi, J. D., Streib, J., Negri, J., & Jones, J. F. (1998). Chronic fatigue syndrome: Identification of distinct subgroups on the basis of allergy and psychologic variables. *Journal of Allergy and Clinical Immunology*, *102*, 222–230.

Cannon, J. G., Angel, J. B., Abad, L. W., Vannier, E., Mileno, M. D., Fagioli, L., et al. (1997). Interleukin-1β, interleukin-1 receptor antagonist and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. *Journal of Clinical Immunology*, *17*, 253–261.

Cannon, J. G., Angel, J. B., Ball, R. W., Abad, L. W., Fagioli, L., & Komaroff, A. L. (1999). Acute phase responses and cytokine secretion in chronic fatigue syndrome. *Journal of Clinical Immunology*, *19*, 414–421.

Danielian, S., Oleastro, M., Rivas, M. E., Cantisano, C., & Zelazko, M. (2007). Clinical follow-up of 11 Argentinian CD40L-deficient patients with 7 unique mutations including the so-called "milder" mutants. *Journal of Clinical Immunology*, *27*, 455–459.

Elenkov, I. J., & Chrousos, G. P. (2002). Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Annals of the New York Academy of Science*, *966*, 290–303.

Elenkov, I. J., Wilder, R. L., Chrousos, G. P., & Vizi, E. S. (2000). The sympathetic nerve—An integrative interface between two supersystems: The brain and the immune system. *Pharmacological Reviews*, *52*, 595–638.

Ferrari, S., Gilliani, S., Insalaco, A., Al-Ghonaium, A., Soresina, A. R., Loubser, M., et al. (2001). Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM. *Proceedings of the National Academy of Sciences, USA*, *98*, 12614–12619.

Fostel, J., Boneva, R., & Lloyd, A. (2006). Exploration of the gene expression correlates of chronic unexplained fatigue using factor analysis. *Pharmacogenomics*, *7*, 441–454.

Fukuda, K., Straus, S. E., Hickie, I., Sharp, M. C., Dobbins, J. G., & Komaroff, A. (1994). The chronic fatigue syndrome: A comprehensive approach to its definition and study. *Annals of Internal Medicine*, *121*, 953–959.

Fulton, R. J., McDade, R. L., Smith, P. L., Kienker, L. J., & Kettman, J. R. Jr. (1997). Advanced multiplexed analysis with the FlowMetrix system. *Clinical Chemistry*, *43*, 1749–1756.

Gaab, J., Rohleder, N., Heitz, V., Engert, V., Schad, T., Schürmeyer, T. H., et al. (2005). Stress-induced changes in LPS-induced pro-inflammatory cytokine production in chronic fatigue syndrome. *Psychoneuroendocrinology*, *30*, 188–198.

Gupta, S., Aggarwal, S., See, D., & Starr, A. (1997). Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. *Journal of Psychiatric Research*, *31*, 149–156.

Gupta, S., Aggarwal, S., & Starr, A. (1999). Increased production of interleukin-6 by adherent and non-adherent mononuclear cells during 'natural fatigue' but not following 'experimental fatigue' in patients with chronic fatigue syndrome. *International Journal of Molecular Medicine*, *3*, 209–213.

Henn, V., Steinbach, S., Buchner, K., Presek, P., & Kroczek, R. A. (2001). The inflammatory action of CD40 ligand (CD154) expressed on activated human platelets is temporarily limited by coexpressed CD40. *Blood*, *98*, 1047–1054.

Kerr, J. R., Cunniffe, V. S., Kelleher, P., Bernstein, R. M., & Bruce, I. N. (2003). Successful intravenous immunoglobulin therapy in 3 cases of parvovirus B19-associated chronic fatigue syndrome. *Clinical Infectious Diseases*, *36*, 100–106.

Kerr, J. R., Petty, R., Burke, B., Gough, J., Fear, D., Sinclair, L. I., et al. (2008). Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. *Journal of Infectious Disease*, *197*, 1171–1184.

Kerr, J. R., & Tyrrell, D. A. (2003). Cytokines in parvovirus B19 infection as an aid to understanding chronic fatigue syndrome. *Current Pain and Headache Reports*, *7*, 333–341.

LaManca, J. J., Sisto, S. A., Zhou, X. D., Ottenweller, J. E., Cook, S., Peckerman, A., et al. (1999). Immunological response in chronic fatigue syndrome following a graded exercise test to exhaustion. *Journal of Clinical Immunology*, *19*, 135–142.

Lerner, A. M., Beqaj, S. H., Deeter, R. G., & Fitzgerald, J. T. (2007). Valacyclovir treatment in Epstein-Barr virus subset chronic fatigue syndrome: Thirty-six months follow-up. *In Vivo*, *21*, 707–713.

Light, A. R., White, A. T., Hughen, R. W., & Light, K. C. (2009). Moderate exercise increases expression for sensory, adrenergic and immune genes in chronic fatigue patients, but not in normal subjects. *Journal of Pain*, *10*, 1099–112.

Light, K. C., Bragdon, E. E., Grewen, K. M., Brownley, K. A., Girdler, S. S., & Maixner, W. (2009). Adrenergic dysregulation and pain with and without acute beta-blockade in women with fibromyalgia and temporomandibular disorder. *Journal of Pain*, *10*, 542–552.

Light, K. C., & Vierck, C. J. (2009). HPA and sympathetic influences on pain and fatigue in fibromyalgia, chronic fatigue and overlapping functional pain syndromes. In E. A. Mayer & M. C. Bushnell (Eds.), *Functional pain syndromes: Presentation and Pathophysiology* (pp. 301–317). Seattle: IASP Press.

Martinez-Lavin, M., Vidal, M., Barbosa, R. E., Pineda, C., Casanova, J. M., & Nava, A. (2002). Norepinephrine-evoked pain in fibromyalgia. A randomized pilot study. *BMC Musculoskeletal Disorders*, *3*, 2.

Martins, T. B., Pasi, B. M., Litwin, C. M., & Hill, H. R. (2004). Heterophile antibody interference in a multiplexed fluorescent microsphere immunoassay for quantitation of cytokines in human serum. *Clinical and Diagnostic Laboratory Immunology*, *11*, 325–329.

Moss, R. B., Mercandetti, A., & Vojdani, A. (1999). TNF-alpha and chronic fatigue syndrome. *Journal of Clinical Immunology*, *19*, 314–316.

Nielsen, A. R., & Pedersen, B. K. (2007). The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Applied Physiology Nutrition and Metabolism*, *32*, 833–839.

Patarca-Montero, R., Antoni, M., Fletcher, M. A., & Klimas, N. G. (2001). Cytokine and other immunologic markers in chronic fatigue syndrome and their relation to neuropsychological factors. *Applied Neuropsychology*, *8*, 51–64.

Peake, J., Peiffer, J. J., Abbiss, C. R., Nosaka, K., Okutsu, M., Laursen, P. B., et al. (2008). Body temperature and its effect on leukocyte mobilization, cytokines and markers of neutrophil activation during and after exercise. *European Journal of Applied Physiology*, *102*, 391–401.

Peake, J. M., Suzuki, K., Hordern, M., Wilson, G., Nosaka, K., & Coombes, J. S. (2005). Plasma cytokine changes in relation to exercise intensity and muscle damage. *European Journal of Applied Physiology*, *95*, 514–521.

Pedersen, B. K., & Fischer, C. P. (2007). Beneficial health effects of exercise—The role of IL-6 as a myokine. *Trends in Pharmacological Science*, *28*, 152–156.

Pocock, S. J., Ashby, D., Shaper, A. G., Walker, M., & Broughton, P. M. (1989). Diurnal variations in serum biochemical and haematological measurements. *Journal of Clinical Pathology*, *42*, 172–179.

Robson, P. (2003). Elucidating the unexplained underperformance syndrome in endurance athletes: The interleukin-6 hypothesis. *Sports Medicine*, *33*, 771–781.

Robson-Ansley, P. J., Blannin, A., & Gleeson, M. (2007). Elevated plasma interleukin-6 levels in trained male triathletes following an acute period of intense interval training. *European Journal of Applied Physiology*, *99*, 353–360.

Sargent, C., Scroop, G. C., Nemeth, P. M., Burnet, R. B., & Buckley, J. D. (2002). Maximal oxygen uptake and lactate metabolism are normal in chronic fatigue syndrome. *Medicine & Science in Sports & Exercise*, *34*, 51–56.

Siegel, S. D., Antoni, M. H., Fletcher, M. A., Maher, K., Segota, M. C., & Klimas, N. (2006). Impaired natural immunity, cognitive dysfunction, and physical symptoms in patients with chronic fatigue syn-

drome: Preliminary evidence for a subgroup? *Journal of Psychosomatic Research*, *60*, 559–566.
Skowera, A., Cleare, A., Blair, D., Bevis, L., Wessely, S. C., & Peakman, M. (2004). High levels of type 2 cytokine-producing cells in chronic fatigue syndrome. *Clinical & Experimental Immunology*, *135*, 294–302.
ter Wolbeek, M., van Doornen, L. J., Kavelaars, A., van de Putte, E. M., Schedlowski, M., & Heijnen, C. J. (2007). Longitudinal analysis of pro- and anti-inflammatory cytokine production in severely fatigued adolescents. *Brain Behavior and Immunity*, *21*, 1063–1074.
Vgontzas, A. N., Zoumakis, M., Papanicolaou, D. A., Bixler, E. O., Prolo, P., Lin, H. M., et al. (2002). Chronic insomnia is associated with a shift of interleukin-6 and tumor necrosis factor secretion from nighttime to daytime. *Metabolism*, *51*, 887–892.
Visser, J., Graffelman, W., Blauw, B., Haspels, I., Lentjes, E., de Kloet, E. R., et al. (2001). LPS-induced IL-10 production in whole blood cultures from chronic fatigue syndrome patients is increased but supersensitive to inhibition by dexamethasone. *Journal of Neuroimmunology*, *119*, 343–349.
Vollmer-Conna, U., Cameron, B., Hadzi-Pavlovic, D., Singletary, K., Davenport, T., Vernon, S., et al. (2007). Postinfective fatigue syndrome is not associated with altered cytokine production. *Clinical Infectious Diseases*, *45*, 732–735.
Zhang, Q., Zhou, X. D., Denny, T., Ottenweller, J. E., Lange, G., LaManca, J. J., et al. (1999). Changes in immune parameters seen in Gulf War veterans but not in civilians with chronic fatigue syndrome. *Clinical and Diagnostic Laboratory Immunology*, *6*, 6–13.

(Received April 13, 2009; Accepted August 17, 2009)

[Correction added after online publication March 4, 2010: in Figure 1 and Table 2, several asterisks were missing.]