CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Mar. 1994, p. 222–226 Vol. 1, No. 2
1071-412X/94/$04.00+0


# Effects of Mild Exercise on Cytokines and Cerebral Blood Flow in Chronic Fatigue Syndrome Patients

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Received 1 November 1993/Returned for modification 30 November 1993/Accepted 13 December 1993

**Chronic fatigue syndrome (CFS) is an idiopathic disorder characterized by fatigue that is markedly exacerbated by physical exertion. In the present study, we tested the hypothesis that mild exercise (walking 1 mph [1 mile = 1.609 km] for 30 min) would provoke serum cytokine and cerebral blood flow abnormalities of potential pathogenic importance in CFS. Interleukin-1β, interleukin-6, and tumor necrosis factor alpha were nondetectable in sera of CFS patients ($n$ = 10) and healthy control subjects ($n$ = 10) pre- and postexercise. At rest, serum transforming growth factor β (TGF-β) levels were elevated in the CFS group compared with the control group (287 ± 18 versus 115 ± 5 pg/ml, respectively; $P < 0.01$). Serum TGF-β and cerebral blood flow abnormalities, detected by single-photon emission-computed tomographic scanning, were accentuated postexercise in the CFS group. Although these findings were not significantly different from those in the control group, the effect of exercise on serum TGF-β and cerebral blood flow appeared magnified in the CFS patients. Results of this study encourage future research on the interaction of physical exertion, serum cytokines, and cerebral blood flow in CFS that will adopt a more rigorous exercise paradigm than the one used in this study.**

Chronic fatigue syndrome (CFS) is a recently defined illness characterized by disabling, unexplained fatigue and a constellation of other symptoms including myalgia, arthralgia, muscle weakness, low-grade fever, painful lymph nodes, headache, sleep difficulties, and a variety of cognitive or psychological complaints (e.g., confusion, concentrating difficulty, memory deficits, depression, or anxiety) (8). A striking feature of the fatigue described by CFS patients is a marked worsening of this symptom following minimal physical exertion. Because of this phenomenon, patients with CFS can walk on average fewer than three blocks (15). The mechanism responsible for this exercise-induced exacerbation of fatigue is unknown.

Although the etiology and pathogenesis of CFS have not been determined, several research groups have hypothesized that cytokines may mediate certain of the symptoms and immunologic disturbances associated with CFS (7, 8, 11, 12, 14). Cytokine abnormalities, however, have been an inconsistent finding, and in some studies, cytokine levels have been completely normal (18). In our laboratory, we found no difference in the serum levels of interleukin-1β (IL-1β), IL-2, IL-4, IL-6, and tumor necrosis factor alpha (TNF-α) of CFS patients and those of healthy control subjects (6). However, serum transforming growth factor β (TGF-β) levels were significantly elevated in the CFS patient group (6). As in the case of many cytokines, a neuropathogenic role has been postulated for the multifunctional cytokine TGF-β (13).

Since previous studies of cytokines in CFS patients have been carried out with resting patients, and physical exertion is known to markedly exacerbate fatigue, the present study was designed to test the hypothesis that mild exercise would provoke serum cytokine abnormalities of potential pathogenic importance in CFS. Aware of the work of other investigators reporting cerebral blood flow abnormalities (i.e., reduced perfusion) in CFS patients compared with healthy control subjects detected through single-photon emission-computed tomographic (SPECT) scanning by using technetium-99m hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) (10), we also investigated whether these abnormalities would be accentuated by mild exercise and their relationship, if any, to serum cytokine levels.

## MATERIALS AND METHODS

**Patients and control subjects.** After approval by our institution's Human Subjects Research Committee, 10 CFS patients (eight females and two males; age [mean ± standard deviation], 35.4 ± 9.5 years) were recruited from the Minnesota Regional CFS Research Program at Hennepin County Medical Center (15). Ten age-matched healthy subjects (seven females and three males; age, 34.3 ± 8.3 years [mean ± standard deviation]) were recruited from hospital personnel. CFS was diagnosed after extensive medical, psychometric, and psychiatric evaluations could not establish another explanation for the symptoms and the criteria for a case definition of CFS were satisfied (8, 17). To exclude major depression or other psychiatric disorders as a confounding diagnosis, normal scores were required of all patients and control subjects on the Beck Depression Inventory (1) and the computerized version of the structured psychiatric Diagnostic Interview Schedule (3). At the time of entry into the study, the duration of CFS illness was 44.8 ± 8.8 months (mean ± standard deviation). In all 10 cases, an acute virus-like infection was reported at the onset of illness. None of the patients had received immunosuppressive or psychotropic medications for at least 1 month prior to enrollment, and all patients and control subjects were instructed not to take any anti-inflammatory medications for at least 3 days prior to and during the 1-week study period. To exclude pregnancy, serum β-human chorionic gonadotropin levels were measured and were nondetectable in female CFS patients and control subjects.

**Study design.** Prior to performing the controlled study of the

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hypothesis that mild exercise provokes serum cytokine and cerebral blood flow abnormalities in CFS patients, an open study of four CFS patients was undertaken to ascertain how a mild exercise challenge would be tolerated and whether relevant laboratory abnormalities would be induced by such an exercise paradigm. In this pilot study, patients were asked to walk on a horizontal treadmill moving at 1 mph (1 mile = 1.609 km) and were encouraged to stay on the treadmill for a maximum of 30 min or until exhaustion. Serum cytokine levels and SPECT cerebral perfusion scanning were assessed at rest and after exercise. The patients stayed on the treadmill for a mean of 14.3 min (range, 5 to 29 min); all patients terminated exercise because of a sense of exhaustion. At the end of exercise, TGF-β levels increased at least fourfold in each patient, and abnormalities were detected in the SPECT scans of all four patients. Thus, this mild exercise challenge was considered sufficient to elicit fatigue and abnormalities in the laboratory tests under study in CFS patients.

**Exercise challenge and symptom severity.** On the basis of the results of the pilot study noted above, the 10 CFS patients and 10 healthy control subjects were asked to walk on a horizontal treadmill at a speed of 1 mph for a maximum of 30 min or until exhaustion. All subjects completed a questionnaire at rest (immediately before exercise), immediately after exercise, and 40 min after exercise on which they indicated the severity of each of the five symptoms reported to be most pronounced in CFS (14), namely, fatigue, muscle weakness, muscle pain, joint pain, and headache. Subjects rated the severity of each symptom on a scale from 0 ("not present") to 10 ("couldn't be worse").

**Serum cytokine levels.** Venous blood samples were obtained on the day of exercise challenge at rest (just before exercise), immediately after exercise, and 40 min after exercise. All samples were kept on ice after being drawn and centrifuged within 2 h of collection. Serum was aliquoted and stored frozen (−70°C) prior to being assayed. Cytokines were measured as previously described (6) by using specific enzyme-linked immunosorbent assays (ELISAs; R & D Systems, Inc., Minneapolis, Minn.) to quantify IL-1β, IL-6, and TNF-α. For TGF-β measurement, an IL-4-dependent HT-2 cell proliferation bioassay was used (6). Briefly, murine HT-2 cells were seeded onto 96-well plates at a concentration of $10^4$ cells per well in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 10% fetal calf serum and IL-4 (15 ng/ml; R & D Systems), while heat-inactivated (56°C for 30 min) serum samples were simultaneously incubated in serial dilutions. Serial dilutions of recombinant human TGF-β1 (R & D Systems) were assayed to establish a standard curve. Cells were incubated for 48 h, and 0.25 μCi of [$^3$H]thymidine was added per well during the last 4-h culture period. TGF-β1 (R & D Systems) suppressed HT-2 cell proliferation in a dose-dependent manner, and chicken antibodies specific to TGF-β1 (10 μg/ml) reversed by >85% the TGF-β bioactivity in the serum samples. Assay sensitivities were as follows: IL-1β, 4.5 pg/ml; IL-6, 3.5 pg/ml; TNF-α, 4.8 pg/ml; and TGF-β, 1.0 pg/ml. The inter- and intra-assay variations were less than 5%.

**Scintigraphy.** At rest and after exercise, each subject had SPECT cerebral perfusion scanning using 30 to 35 mCi (1,110 to 1,295 MBq) of $^{99m}$Tc-HMPAO. $^{99m}$Tc-HMPAO was prepared from a commercially available kit (Ceretec; kindly provided by Amersham, Arlington Heights, Ill.). Scanning began 15 min after intravenous injection. Images were acquired with a rotating gamma camera (Siemens Orbiter 7500) equipped with a low-energy, all-purpose collimator with a 20% window centered at 140 keV. Data acquisition lasted about 20 min and required 64 projections with a 360° rotation of the gamma camera. The projections were prefiltered with a Butterworth filter (cutoff frequency, 0.5). Images were reconstructed in the transaxial, sagittal, and coronal planes.

The resting scans were performed with the subject lying supine in a darkened, quiet setting. After 30 min in this environment, $^{99m}$Tc-HMPAO was administered, and scanning began about 15 min later. To allow for elimination of the radioisotope after the resting scan, the exercise scan was performed 1 week later. On this day, symptom severity and serum cytokine levels were also simultaneously assessed, as described above. After the first 1 min of treadmill exercise, $^{99m}$Tc-HMPAO was administered through a heparin lock. Scanning began within 15 min after the exercise challenge was completed.

All 40 scans were randomly sorted and then interpreted by a board-certified radiologist with special competence in nuclear radiology (S. A. Sirr), who was blind to patient versus healthy subject identity and to the scan sequence (rest or postexercise). The scans were scored semiquantitatively: perfusion abnormalities were graded by size (1, small; 2, medium; or 3, large), side (right or left), and location (frontal, parietal, temporal, occipital, or cerebellar) of each defect. Scoring criteria were established by consensus between two radiologists (S. A. Sirr and F. C. Grammith).

**Statistical analyses.** For the analysis of symptoms in the patient and control groups, differences between means at each postexercise time point were compared with preexercise values by using the Wilcoxon sign rank test. For analysis of TGF-β values, a repeated-measure analysis of variance was used to examine differences between the two groups across observation periods. Logistic regression was used to model the relationship between TGF-β levels and SPECT findings, after ensuring that the TGF-β distribution was normal. Where appropriate, data are presented as means ± standard errors of the means.

## RESULTS

**Symptom severity.** Eight of the 10 CFS patients were able to complete 30 min of treadmill exercise (1 mph); the other 2 patients stopped after 16 and 19 min because of a sense of exhaustion. All 10 of the healthy subjects met this mild exercise challenge.

Symptom severity results for both the patients and the healthy subjects are shown in Table 1. Compared with the healthy subjects, the patients with CFS reported a higher degree of fatigue at rest, immediately after exercise, and 40 min after exercise. The level of fatigue was significantly increased ($P < 0.01$) after exercise in the CFS patients but not in the controls. All 10 of the CFS patients complained of increased fatigue immediately and 40 min after exercise, whereas none of the control subjects complained of fatigue after exercise. Although CFS patients also complained of more severe muscle pains, joint pains, and headaches than the healthy subjects, their symptoms, with the exception of the feeling of muscle weakness, did not significantly increase after exercise.

**Serum cytokine levels.** IL-1β, IL-6, and TNF-α were not detectable in the serum of any of the CFS patients or the healthy control subjects at rest, immediately after exercise, or 40 min after exercise. TGF-β was measured at each sampling time in the sera of all of the patients and control subjects, and levels of this cytokine were higher in the CFS patient group than the healthy subject group (Fig. 1A). Prior to exercise, serum TGF-β levels were elevated ($P < 0.01$) in the CFS group (287 ± 18 pg/ml) compared with those in the control group

TABLE 1. Effect of exercise on symptom severity

| Group and symptom | Severity[a] | | |
|---|---|---|---|
| | Preexercise | Postexercise | |
| | | Immediate | 40 min |
| CFS patients ($n$ = 10) | | | |
| Fatigue | 5.8 ± 0.8 | 7.4 ± 0.7[b] | 7.6 ± 0.7[b] |
| Muscle pain | 4.5 ± 1.3 | 5.0 ± 1.3 | 5.3 ± 1.4 |
| Muscle weakness | 4.9 ± 1.0 | 6.3 ± 1.1[b] | 6.5 ± 1.0[b] |
| Joint pain | 4.1 ± 1.3 | 5.2 ± 1.3 | 4.5 ± 1.4 |
| Headache | 2.1 ± 0.9 | 1.9 ± 0.9 | 2.5 ± 1.3 |
| Control subjects ($n$ = 10) | | | |
| Fatigue | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Muscle pain | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| Muscle weakness | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Joint pain | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| Headache | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.2 ± 0.1 |

[a] Subjects rated the severity of each symptom on a scale of 0 (not present) to 10 (couldn't be worse) and were instructed to walk on a treadmill at a speed of 1 mph for a maximum of 30 min or to the time of exhaustion. Values are means ± standard errors of the means.
[b] $P < 0.01$ versus corresponding preexercise values.

(115 ± 5 pg/ml). Although serum TGF-β levels in the CFS group were higher than the corresponding control values at each of the three sampling times, the mean TGF-β levels in the CFS group did not change significantly after exercise. However, large individual variations in response to the exercise challenge were observed in the CFS group (Fig. 1B). In nine of the CFS patients, the postexercise levels were elevated above the preexercise values either immediately postexercise (six patients) or 40 min after exercise (three patients). In three CFS patients, serum TGF-β levels dropped immediately after exercise, and a rebound followed 40 min after exercise. In the six CFS patients with increased serum TGF-β levels immediately after exercise, there was a fall toward resting values 40 min after exercise. In the control subjects, on the other hand, there was a more uniform increase in serum TGF-β levels immediately after exercise (164 ± 8 versus 115 ± 5 pg/ml preexercise; $P < 0.05$), with a reduction toward baseline 40 min after exercise (Fig. 1C).

**Scintigraphy.** The results of the assessments of the effects of exercise on cerebral blood flow perfusion scans are given in Table 2. In the CFS group, 30% (3 of 10) of the patients had abnormal scans at rest, and 60% (6 of 10) had abnormal scans after exercise. All three CFS patients with abnormal scans at rest also had abnormal scans after exercise. Three patients with normal scans at rest had abnormal scans after exercise; however, no patient had an abnormal scan at rest and then had a normal scan after exercise. In the healthy control subjects, 20% (2 of 10) had abnormal scans at rest, and 20% (2 of 10) had abnormal scans after exercise. In one subject, a normal scan at rest became abnormal after exercise, whereas in another subject, an abnormal scan at rest became normal after exercise. One healthy subject had abnormal scans both at rest and after exercise. When comparing the postexercise scans only, 60% of the CFS patients versus 20% of the healthy subjects had abnormal scans ($P = 0.17$; Fisher's exact test, two-tailed). All abnormal scans in both groups involved reduced cerebral perfusion and small to medium-size defects preferentially affecting the left temporal-parietal regions. By using logistic regression, TGF-β values did not significantly predict SPECT outcome independently or after controlling for CFS condition.

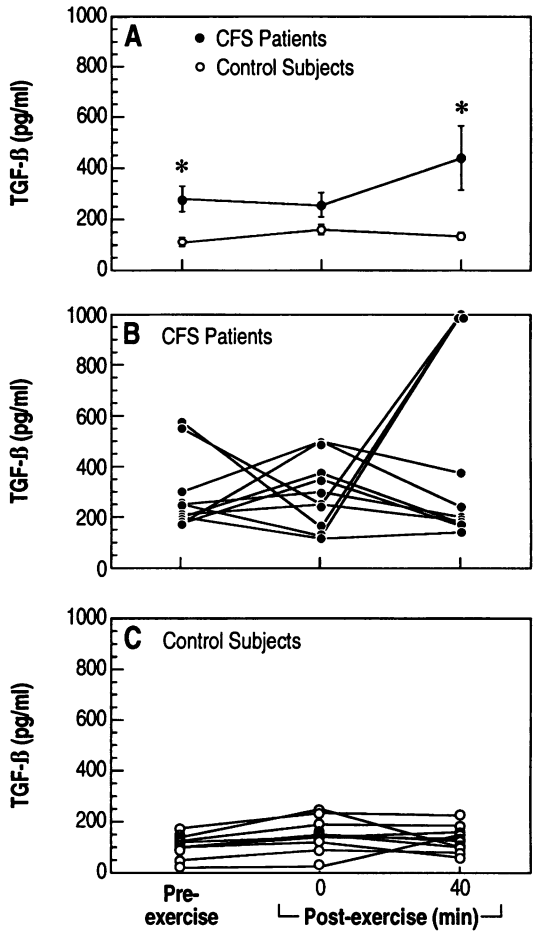


FIG. 1. Effects of exercise on serum TGF-β levels. Serum samples were obtained from 10 CFS patients and 10 healthy control subjects preexercise, immediately after exercise, and 40 min after exercise. (A) Data expressed as means ± standard errors of the means; $P < 0.01$ versus corresponding control values; (B) individual values of CFS patients; (C) individual values of control subjects.

## DISCUSSION

Cytokines are considered to be the principal immune system mediators of many of the central nervous system manifestations of infectious and autoimmune diseases (e.g., fever, anorexia, and sleep disorders). Fatigue is also a common accompaniment of these diseases; however, the pathogenesis of this symptom is unknown. After finding that TGF-β was elevated in the sera of patients with CFS (6), we subsequently determined that serum levels of this cytokine were also elevated in a murine model of immunologically mediated fatigue (4). Moreover, in murine astrocyte cultures, TGF-β suppressed the activity of glutamine synthetase (5), a critical enzyme in neurotransmitter metabolism. On the basis of these observations, we hypothesized in the present study that a mild exercise challenge of patients with CFS, which as expected provoked a significant increase in their sense of fatigue, would be associated with an exaggerated increase in serum TGF-β levels.

Serum TGF-β levels were found to be higher in the CFS patient group than in the healthy control group, and an increase in the TGF-β level was observed within 40 min after exercise challenge in 9 of 10 patients. However, an increased serum TGF-β level was observed immediately after exercise in

TABLE 2. Effect of exercise on $^{99m}$Tc-HMPAO SPECT cerebral blood flow perfusion scans

| Case no. | Sex | Age (yr) | Scan results[a] | |
|---|---|---|---|---|
| | | | At rest | After exercise |
| CFS patients | | | | |
| 1 | F | 43 | N | 1, R, T |
| 2 | F | 32 | N | 2, L, F |
| 3 | F | 39 | N | N |
| 4 | M | 44 | 1, L, T | 1, L, T; 1, R, T |
| 5 | M | 53 | 2, R, P | 1, R, P; 1, L, P |
| 6 | F | 25 | N | N |
| 7 | F | 33 | N | 2, L, T |
| 8 | F | 35 | N | N |
| 9 | F | 22 | N | N |
| 10 | F | 28 | 1, L, F | 1, L, P |
| Controls | | | | |
| 11 | F | 34 | N | N |
| 12 | M | 38 | N | 1, R, P; 1, L, P |
| 13 | F | 30 | 1, L, P | N |
| 14 | F | 22 | N | N |
| 15 | F | 40 | N | N |
| 16 | M | 47 | N | N |
| 17 | F | 24 | 1, L, P; 2, R, O | 1, L, P |
| 18 | F | 42 | N | N |
| 19 | M | 41 | N | N |
| 20 | F | 32 | N | N |

[a] SPECT scans were performed a week apart in subjects at rest and immediately after exercise (walking on a treadmill at a speed of 1 mph). N, normal. Abnormalities were graded by size (1, small; 2, medium; 3, large), side (R, right; L, left), and location (F, frontal; P, parietal; T, temporal; O, occipital; C, cerebellar) of the defects.

only 6 patients, despite a sense of increased fatigue in all 10 patients. On the other hand, a modest but consistent increase in serum TGF-β levels was observed immediately after exercise in the healthy control subjects, none of whom complained of fatigue. The source of the serum TGF-β that was measured in the present study is unknown. This cytokine is produced by many cell types and is secreted in a latent form which must be activated by proteolysis (16). Since a biological assay was used to measure TGF-β in this study, it is possible that CFS patients have increased TGF-β activation rather than increased TGF-β secretion relative to normal individuals. Although little is known about the process of TGF-β activation, future studies could address this possibility.

Since TGF-β is known to affect the function of many cell types (16), we considered the possibility in the present study that the cerebral blood flow abnormalities which had been reported in CFS patients (9) might be governed by endovascular effects of TGF-β. After a mild exercise challenge, the CFS patients had a higher incidence of cerebral perfusion defects than the healthy control subjects (60 versus 20%, respectively). Although this difference approached statistical significance, we were unable to find an association between serum TGF-β levels and abnormalities of cerebral perfusion as detected by SPECT scanning.

Thus, the results of this study did not support our primary hypothesis that mild exercise would reveal serum cytokine abnormalities of potential pathogenic importance in the fatigue associated with CFS. Before discounting the role of cytokines such as TGF-β in the pathogenesis of fatigue in CFS, however, several caveats should be considered. First, the small number of subjects evaluated in the present study may have given a sample size that lacked sufficient power to identify biologically significant differences between the CFS patients and healthy controls. Second, in this controlled study, we may have unwittingly selected for a group of patients with relatively mild illness, 80% of whom could complete the 30-min exercise challenge. In the open pilot study of four CFS patients which preceded the controlled study, none could walk for 30 min, and in all four cases, serum TGF-β levels increased by at least fourfold immediately after exercise. It is possible that a more rigorous exercise challenge in our controlled study would have uncovered more consistent increases of serum TGF-β or of the other cytokines tested in this study (i.e., IL-1β, IL-6, and TNF-α). Third, marked discrepancies recently have been found in measurements of IL-6 and TNF-α with different commercially available ELISA kits (2). It is possible that we would have detected an increase in one of these cytokines in the sera of the CFS patients had we used another ELISA kit or a biological assay for IL-6, TNF-α, or IL-1β. Fourth, we did not study serum levels of other cytokines that have been implicated in CFS, e.g., alpha interferon (9, 12), IL-1α (11), or IL-2 (7). Finally, the finding of normal cytokine levels within the systemic circulation does not exclude a neuropathogenic role for local production of these mediators by glial cells within the central nervous system itself.

Presently, there are no laboratory tests that have proved to be useful in confirming a diagnosis of CFS. The finding of elevated serum TGF-β in the CFS patient group versus the healthy control group in the present investigation is consistent with results of an earlier study (6). Additional studies are needed to ascertain whether CFS patients with elevated serum TGF-β levels have any distinguishing clinical features and to determine whether serum TGF-β levels are increased in other patient groups (e.g., patients with autoimmune diseases or major depression). Also, studies of the relationship between physical conditioning and serum TGF-β levels in healthy subjects should be carried out to determine whether the association between elevated serum TGF-β levels and CFS might be explained by deconditioning in these patients. If elevated serum TGF-β levels prove to be unique to CFS and a more rigorous exercise challenge than the one used in the present study yields a clearer separation between CFS patients and controls, then measurement of this cytokine could be a useful diagnostic marker.

## ACKNOWLEDGMENTS

This work was supported in part by the National Institute of Allergy and Infectious Diseases and by a grant from Hennepin Faculty Associates.

We thank Julie Peterson and Sharron Coulter for their technical assistance, Suk Won Kim for providing the computerized Diagnostic Interview Schedule, and Stacey Ulen for help in the preparation of the manuscript.

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