Blood flow and muscle metabolism in chronic fatigue syndrome

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ABSTRACT

The purpose of this study was to determine if chronic fatigue syndrome (CFS) is associated with reduced blood flow and oxidative delivery to skeletal muscle. Patients with CFS according to CDC (Center for Disease Control) criteria (n=19) were compared with normal sedentary subjects (n=11). Muscle blood flow was measured with Doppler ultrasound after cuff ischaemia and exercise. Muscle oxygen delivery was measured as the rate of post-exercise and post-ischaemic oxygen-haem resaturation. Oxygen-haem resaturation was measured in the medial gastrocnemius muscle using continuous wavelength near-IR spectroscopy. Muscle metabolism was measured using 31P magnetic resonance spectroscopy. CFS patients and controls were not different in the peak blood flow after cuff ischaemia, the rate of recovery of phosphocreatine after submaximal exercise, and the rate of recovery of oxygen saturation after cuff ischaemia. In conclusion, CFS patients showed no deficit in blood flow or oxidative metabolism. This suggests that CFS symptoms do not require abnormal peripheral function.

INTRODUCTION

Chronic fatigue syndrome (CFS) is an illness characterized by self-reported fatigue lasting at least 6 months that is medically unexplained [1]. A number of studies have found CFS patients to have reduced muscle strength [2], whereas others do not [3]. Maximal oxygen consumption during treadmill or cycle exercise has also been shown to be decreased in CFS patients in some studies [4], but not others [2,5]. Similarly, abnormal muscle metabolism has been reported to occur in CFS [6–8], whereas some studies have not been able to confirm metabolic abnormalities [3]. In two previous studies [9,10], we found that CFS patients had a moderate reduction in oxidative capacity as measured by the rate of recovery of phosphocreatine (PCr) after submaximal exercise and reduced delivery of oxygen after exercise and cuff ischaemia.

Although the results on strength and metabolism in CFS have been mixed, there have been a number of studies that have shown that CFS is associated with autonomic dysregulation. Both sympathetic and parasympathetic autonomic tone have been reported to be abnormal [11–14]. This autonomic dysregulation could affect blood flow to active muscles [15], and could partially explain the post-exertional fatigue that is a characteristic of the illness.

The aim of this study was to test the hypothesis that patients with CFS would have decreased muscle...
blood flow compared with sedentary controls. To test our hypothesis, we used Doppler ultrasound to measure blood flow in the femoral artery supplying predominately the lower leg. We measured the maximal blood flow response to cuff ischaemia (reactive hyperaemia) and the blood flow response to a short bout of intense exercise (exercise hyperaemia). In addition, we measured muscle metabolism with magnetic resonance spectroscopy (MRS) and oxygen delivery with near-IR spectroscopy (NIRS). We predicted that CFS subjects would show the same decreased muscle oxidative metabolism and oxygen delivery that we had seen in our previous studies.

METHODS

Patient selection

This study was approved by the University Committee on Studies Involving Human Beings at the New Jersey Medical School, the University of Pennsylvania and the University of Georgia. All subjects gave informed consent prior to taking part in the study. Patients were carefully evaluated to fulfil the case definition of CFS [1]; none had evidence of psychiatric disease, as determined by a diagnostic psychiatric interview in the 5 years prior to onset of CFS. Nineteen CFS patients were tested in this study (age, 39.3 ± 5.1 years; 12 females and seven males). Healthy control subjects were chosen to be similar in age and to have a sedentary lifestyle by self-report (regular exercise less than once a week for at least 6 months prior to testing). Eleven sedentary control subjects were tested (age 37.2 ± 6.9 years; five females and six males).

Experimental protocol

Two sets of experiments were performed. Measurements of muscle blood flow and oxygen delivery were made adjacent to the magnet room using Doppler ultrasound and NIRS. The measurements were made at rest and after cuff ischaemia. Measurements of muscle metabolism were made using MRS and short bouts of exercise. Measurements were made on one leg, usually the right leg. All measurements were made on the same day, with a 1 h break between the Doppler and MRS measurements.

Doppler ultrasound

Blood flow was measured in the common femoral artery using quantitative Doppler ultrasound (LogiQ 400CL; GE Medical Systems) [16]. A linear array transducer was used at a frequency of either 4 or 6 MHz. The imaging site was located on the upper third of the thigh and was marked to ensure replication of probe placement. Doppler measurements were made proximal to the cuff to ensure that the vessel placement was maintained throughout the cuff occlusion. Resting diameter was measured in the axial view during diastole. Pulsed Doppler ultrasound was recorded in the longitudinal view using an insonation angle of 60°. The velocity gate was set to include the entire arterial diameter. Measurements (10–20) were made over the course of each trial to capture the peak velocity response as well as the general shape of the blood flow response. Values were averaged over two heartbeats. All data were saved to magnetic optical disks for storage and analysis.

Doppler waveforms were analysed to determine maximum (\(V_{\text{systolic}}\)) and minimum (\(V_{\text{diastolic}}\)) velocity, and the time-average mean velocity (blood velocity). All velocity calculations were performed by GE Medical Systems’ advanced vascular program software for the LogiQ 400 CL. Waveforms that were not automatically measured by the computer were manually traced on the GE Medical Systems software to determine velocities and flows. B mode images were marked and measured to determine the diameter throughout the test.

Blood flow values were calculated as the product of the time-average mean velocity, and the vessel cross-sectional area was determined from the diameter. The peak blood flow response was the highest blood flow value for each ischaemic test. The highest flow value for each person was used as their peak flow value for comparisons between CFS patients and controls. This was usually the value from 10-min cuff duration experiment, but was sometimes the value from the 4-min experiment. The half time to recovery was determined as the time at which blood flow had dropped to one-half the magnitude between maximum flow and resting flow values.

Leg volume

Leg volume measurements were performed by measurements of fat thickness by Doppler ultrasound and by circumference measurements of the lower leg. Doppler images of the thickness between skin to muscle fascia were attained every 3 cm over the medial gastrocnemius and anterior tibialis to determine the amount of subcutaneous fat. Total area of the leg was determined from the circumference measurement and fat thickness. Based on this information, fat volume, non-fat volume and total volume were calculated.

NIRS

The NIRS measurements used a continuous light source, dual-wavelength spectrophotometer (Runman™; NIM, Inc.). The separation distance between the light sources and detectors was 3 cm. Light photons migrate through the tissue and are collected by the detectors with optical filters set at 760 and 850 nm. Oxygen-haem groups have a greater absorbance at 850 nm compared with 760 nm, with deoxy-haem groups absorbing more at 760 nm than at 850 nm. The difference signal between 760 and 850 nm was used to indicate changes in oxygen saturation. Voltage signals were digitized into a computer by a commercial AD device (National Instruments A/D board). Values were averaged over two heartbeats. All data were saved to magnetic optical disks for storage and analysis.

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for the difference signal are converted into a relative scale of 0–100%. Oxygen delivery capacity was measured as the half-time to recovery after either exercise or cuff ischaemia [17].

**MRS**

Phosphorus metabolites were measured with a 78 cm clear bore, 2.1 Tesla magnet with a home-built spectrometer system [9,10]. The lateral gastrocnemius muscle was examined using a 6 cm × 8 cm surface coil tuned to both 31P and 1H frequencies (34.86 and 86.12 MHz respectively). Phosphorus spectra (3000 Hz sweep width, 1024 points) were collected using pulses to produce maximal signal intensity/pulse. Resting measurements were made from the sum of 10 pulses collected with a 20-s repetition time. Exercise and recovery values were obtained from single pulses collected with 4-s repetition respectively. Nuclear Overhauser enhancement was used to enhance the 31P signal during exercise and recovery. Spectra were Fourier transformed with 5 Hz line broadening and integrated in the frequency domain. Areas of the Pi, Pcr, and 3-ATP peaks were computer-integrated and corrections made for differences in saturation and nuclear Overhauser enhancement between the peaks. Muscle pH was calculated from the frequency difference between P1 and Pcr. Pcr values during recovery were fitted to a single exponential equation to determine a time constant (PcrTc). ADP levels were calculated using the creatine kinase equilibrium equation:

\[ [\text{ADP}] = ([\text{creatinine}][\text{ATP}] / ([\text{Pcr}] [\text{H}^+] )) \times K_{eq} \]

assuming the equilibrium constant of the reaction \( (K_{eq}) = 1.66 \times 109 \text{ kg/mol} \), \( [\text{ATP}] = 9.2 \text{ mM} \) and total creatine = 42.5 mM [18]. Maximal muscle oxidative capacity (Vmax) was calculated as the inverse of the time constant × resting Pcr concentrations [9,10].

**In-magnet exercise**

Pcr recovery values were measured after short duration exercise. Subjects were placed in the prone position with their knees fully extended using a foot pedal attached to an air pressure ergometer. Buckled straps were used to secure the subject to the platform and Velcro straps were used to secure the leg and foot. Exercise consisted of rapid plantar flexions at a rate of approx. 2 Hz for 10–16 s. Exercise intensity was modulated by changing air pressure in the ergometer. The pressure was chosen to allow the subject to perform the plantar flexions at the desired rate. Occasionally the chosen pressure for the first test was inappropriate and the test was repeated with a different pressure level. Recovery for 7–9 min was allowed between each trial.

**Recovery from cuff ischaemia**

The peak blood flow and the rate of recovery of oxygen saturation were measured after 2, 4 and 10 min of cuff ischaemia. These measurements were made on the opposite leg from the exercise measurements. A standard blood pressure cuff was placed just above the knee on the leg being tested. Ischaemia was produced by rapidly inflating the cuff (1–2 s) with a rapid cuff inflator (DE Hokkans Inc.) to suprasystolic pressures (> 100 mmHg above systolic pressure). A rest of 5 min was allowed between each trial.

**Statistical analysis**

Data are presented as means ± S.D. Comparison of baseline data between CFS patients and controls used two-tailed unpaired Student’s t tests for values that were not different between males and females. For values that were different between males and females, ANOVA with group (CFS and controls) and sex was used. Significant differences were assumed with \( P < 0.05 \). Two potential CFS patients were tested, but were later excluded from the analysis as having either idopathic fatigue or medically explained fatigue. Including or removing these subjects from the analysis did not alter any of the statistical conclusions of the study.

**RESULTS**

Data were obtained from 19 CFS patients and 11 control subjects. An additional CFS patient was not tested, due to previous history of venous problems in the lower leg. All of the remaining subjects performed the tests without incident. No differences were found between CFS patients and control subjects in age, height, weight, resting blood pressures and resting heart rates (Table 1). Lean leg volume was also not different between CFS patients and control subjects, although there was a tendency for CFS patients to have slightly smaller legs (Table 2).

<table>
<thead>
<tr>
<th>Table 1 Subject demographics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are means ± S.D. BMI, body mass index; f/m, female/male.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (years)</th>
<th>Gender (f/m)</th>
<th>Height (m)</th>
<th>Weight (kg)</th>
<th>BMI (kg/m²)</th>
<th>Systolic pressure (mmHg)</th>
<th>Diastolic pressure (mmHg)</th>
<th>Mean pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>39.3 ± 5.1</td>
<td>12/7</td>
<td>1.70 ± 0.11</td>
<td>73.1 ± 11.4</td>
<td>25.3 ± 4.2</td>
<td>120 ± 10.9</td>
<td>71.6 ± 9.2</td>
<td>88.4 ± 8.9</td>
</tr>
<tr>
<td>Control</td>
<td>37.2 ± 6.9</td>
<td>5/6</td>
<td>1.72 ± 0.10</td>
<td>76.2 ± 16.9</td>
<td>25.4 ± 3.4</td>
<td>118.5 ± 11.7</td>
<td>69.0 ± 9.4</td>
<td>87.0 ± 10.9</td>
</tr>
</tbody>
</table>

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Table 2  Leg volume and femoral artery diameter
Values are means ± S.D.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Lean (cm$^3$)</th>
<th>Fat (cm$^3$)</th>
<th>Total (cm$^3$)</th>
<th>Femoral artery diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Men (cm)</td>
</tr>
<tr>
<td>CFS</td>
<td>1810 ± 395</td>
<td>890 ± 359</td>
<td>2700 ± 593</td>
<td>0.559 ± 0.054</td>
</tr>
<tr>
<td>Control</td>
<td>2003 ± 511</td>
<td>777 ± 349</td>
<td>2781 ± 714</td>
<td>0.587 ± 0.037</td>
</tr>
</tbody>
</table>

Blood flow
No difference in resting blood velocities (Figure 1) or resting femoral artery diameters (Table 2) were observed between CFS patients and control subjects. As lean leg volume and resting blood pressure were not different between the two groups, we also found no differences in resting blood flow normalized per lean tissue mass or in resting conductance.

Peak blood flow responses usually occurred with 10 min of cuff ischaemia (Figure 2). Peak blood velocity after cuff release was similar between CFS patients and control subjects (Figure 2, lower panel). The peak blood flow response was 7- to 8-fold higher than resting flow, and was not different between CFS patients and control subjects at all cuff durations (Figure 3). This was also true when peak blood flow was normalized to lean tissue and used to calculate conductance.

Oxidative metabolism
Resting metabolite levels were not different between CFS patients and control subjects (Table 3). In response to the short exercise bout, PCr levels decreased to 54 % of resting for CFS patients and 50 % of resting for controls. End-exercise pH was also not different between groups (6.97 ± 0.08 for CFS and 6.97 ± 0.09 for controls). This resulted in end-exercise ADP levels that were not significantly different between groups (57.4 ± 18.5 for CFS patients and 65.8 ± 11.7 µM for controls). PCr recovery after exercise was not different between CFS patients and control subjects (Figure 4). This was based on a lack of difference between PCrTc values and in calculated V$\text{max}$ values between CFS patients and control subjects.

Oxygen delivery
The rate of recovery of oxygen saturation after cuff ischaemia was not significantly different between CFS
patients and control subjects. CFS patients had a trend towards recovery rates that were 15% slower across the three different cuff durations (Figure 5); however, this was not significant based on a repeated measures ANOVA ($P = 0.128$).

**DISCUSSION**

The present study found no deficits in muscle blood flow in patients with CFS compared with sedentary controls.

CFS patients may have decreased muscle blood flow either at the onset of exercise or during exercise, which could contribute to their muscle fatigue. The present study measured the magnitude of reactive hyperaemia to three different durations of cuff ischaemia. CFS patients were not different in the maximal hyperaemic blood flow or in the ratio of blood flow at the different cuff durations. This was true when the blood flow was corrected for differences in lean leg volume and blood pressure. Impairment in the onset of blood flow might show up as decreased blood flow at shorter cuff durations relative to longer cuff durations.

The lack of any differences in blood flow between the CFS patients and control groups was not expected. A number of previous studies have shown that CFS patients have a problem in cardiovascular control manifested in some by delayed orthostatic intolerance [14] and subtle autonomic abnormalities [11,12]. Patients with complaints of syncope and abnormal responses to orthostatic challenge (not necessarily CFS patients) have been found to have increased forearm vascular resistance [19], an indicator of abnormal muscle blood flow. However, we found that resting blood flow, peak blood flow in response to cuff ischaemia and post-exercise blood flow were not reduced in CFS patients. And, although reduced blood flow at the start of exercise has been hypothesized in CFS patients, we did not see any evidence in the

**Table 3 Resting muscle metabolites**

Measurements of phosphomonoesters (PME), phosphodiesteres (PDE), total phosphate (TP) and ADP are based on ATP levels of 9.2 mM and total creatine levels of 42.5 mM. There were no significant differences between CFS and control subjects. Values are means ± S.D.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>$P_i$/PCr</th>
<th>PCr/ATP</th>
<th>PME/TP</th>
<th>PDE/TP</th>
<th>$pH$</th>
<th>PCr (mM)</th>
<th>ADP (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFS</td>
<td>0.087 ± 0.015</td>
<td>4.25 ± 0.40</td>
<td>0.018 ± 0.014</td>
<td>0.044 ± 0.014</td>
<td>7.05 ± 0.02</td>
<td>34.8 ± 3.2</td>
<td>13.0 ± 6.6</td>
</tr>
<tr>
<td>Control</td>
<td>0.089 ± 0.017</td>
<td>4.25 ± 0.39</td>
<td>0.012 ± 0.005</td>
<td>0.041 ± 0.019</td>
<td>7.06 ± 0.02</td>
<td>34.9 ± 3.2</td>
<td>13.0 ± 7.0</td>
</tr>
</tbody>
</table>

**Figure 4** $P_{i}$/PCr and calculated $V_{max}$ values for CFS and control subjects

Values are means ± S.D.

**Figure 5** Half-time ($T_{1/2}$) of recovery of oxygen saturation measured with NIRS after three different durations of cuff ischaemia

Values are means ± S.D. Hatched bars, CFS subjects; and black bars, controls.
present study to support this. The blood flow response to short cuff durations was not proportionally lower in CFS patients.

A number of studies have examined blood flow in patients with fibromyalgia, which may overlap with CFS. Bennett et al. [20] measured muscle blood flow using a Xenon133 washout method in 16 fibromyalgia patients and 16 sedentary controls and found muscle blood flow in the fibromyalgia group to be 76% of that in the sedentary controls. Interestingly, there was no difference in maximal oxygen consumption between these groups, suggesting that the groups were adequately matched for fitness level. Lindh et al. [21] found fibromyalgia patients to have reduced capillary densities in the vastus lateralis muscles compared with control subjects (271 versus 309 capillaries/mm respectively). It is thought that at least some fibromyalgia patients have abnormal up-regulation of α2-adrenergic receptors, which could reduce blood flow [20].

The lack of a deficit in blood flow in CFS patients found in this study was consistent with the finding of a lack of deficit in muscle oxidative metabolism and muscle oxygen delivery measured with MRS and NIRS respectively. The lack of metabolic abnormalities in CFS subjects is consistent with studies by other investigators, which have reported no differences in oxidative muscle metabolism [3]. However, the present study is in contrast with our two previous studies [9,10]. Our previous studies found significant reductions of 25% [9] and 20% [10] in Vmax, whereas the present study found a non-significant 6% decrease. The measurements were made in a similar fashion and we found similar overall Vmax values. The signal-to-noise values and co-efficients of variation in our measurements (approx. 11–13%) were similar between studies. Recovery rates of oxygen saturation were also not slower in CFS patients compared with controls. This is consistent with the lack of difference in muscle Vmax, although again different from our previous study [10]. As oxygen delivery and muscle oxidative metabolism are linked [23–25], our findings in the present study are internally consistent.

We do not have a clear explanation for why our present study produced different results from our previous ones. One possibility is that the CFS subjects in our present study were different from those in our previous studies. CFS has the potential to be a heterogeneous disorder and previous studies, including ours [9,10,22], have reported increased variance in the measurements on CFS patients. The current study did not find a wider range of values in our CFS subjects, and it is possible that the current group of CFS patients did not include a potential subpopulation of CFS patients with abnormal muscle metabolism. The solution to this question is to perform larger studies, which can be impractical with complicated methodologies on a rigidly defined group of subjects. Another explanation is that the current group of CFS patients were, as a whole, less affected than the CFS patients in our previous studies. However, CFS severity scores (results not shown) were not different between the CFS subjects in the present study and those from previous studies. As a whole, our results are consistent with the inconsistent findings of peripheral deficits in patients with CFS from other studies. Previous studies have varied from those that have found profound deficits in function [4] to those, similar to ours, that have found no deficit [3]. Indeed, evidence reported by Byrne and co-workers [26] initially concluded that CFS patients had abnormal mitochondria using electron microscopy, but subsequently reported no group abnormalities in CFS patients [27].

The lack of peripheral metabolic differences seen in the present study raises several very interesting points. One possibility is that CFS symptoms do not result from peripheral deficits. Abnormalities in brain hormonal regulation [28] and/or cytokine levels [29] could result in fatigue symptoms despite normal peripheral function. Hypotheses concerning CFS that involve abnormal vascular responses, as mentioned earlier, should reduce blood flow, oxygen delivery and, potentially, muscle metabolism. We found no evidence of this in our present study. Thus our results might suggest that, although peripheral dysfunction might occur in some CFS patients, it does not have to occur in order to have CFS symptoms.

One of the key issues in the study of peripheral deficits in CFS is the role of physical activity levels. By definition, CFS patients have activity levels less than 50% of what they were prior to their illness, and it can be expected that their activity levels would be less than sedentary control subjects. Several studies have shown that CFS patients have activity levels that are 60–85% of normal sedentary levels [30,31]. Inactivity has been shown to have negative effects on muscle metabolism and muscle blood flow [32]. However, it is not clear what the importance of activity level is in the range between CFS and sedentary controls. As much as 30–50% of oxidative metabolism maybe genetically determined [33], and differences in genetic aptitude may dominate inter-subject differences in subjects that have small differences in activity levels, such as the subjects in the present study. Although we did not measure activity levels in our present study, the experimental burden of accurately measuring activity levels results in most studies of CFS patients not including this measurement. Clearly, further studies measuring activity levels in CFS and studying the influence of altering activity levels in CFS patients are needed to address this issue.

In summary, this study demonstrates a lack of impairment in blood flow, muscle metabolism and oxygen delivery in CFS patients compared with normal subjects. Although differences in activity level or, in the present study, a lack of difference in activity level might help to explain the results, this is most unlikely
to be the explanation. The specific population of CFS patients might also be important. However, the clear implication of the present study is that, although muscle abnormalities may be associated with CFS, they are not an essential part of the syndrome. This suggests that further studies on CFS patients should focus on issues directly related to the primary symptoms, which is the reduced ability to sustain normal activity levels.

ACKNOWLEDGMENTS

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