Muscle metabolism with blood flow restriction in chronic fatigue syndrome

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PATIENTS DIAGNOSED WITH chronic fatigue syndrome (CFS) report significantly reduced activity levels and increased fatigue without a clear medical explanation (11). A number of studies have examined the question of whether CFS is associated with skeletal muscle dysfunction. These studies have provided mixed results, with some finding CFS patients to have lower maximal oxygen consumption (8), reduced muscle strength (12), and abnormal muscle metabolism (1, 2, 13, 41), whereas other did not (14, 18, 33). Even taking into account differences in methods and in patient populations, it is difficult to interpret the differences between studies.

In two previous studies, our laboratory has found muscle oxidative metabolism and muscle oxygen delivery to be reduced in patients with CFS (23, 24). These differences could be the result of metabolic changes due to deconditioning in the CFS patients (40), who are generally reported to be less active than even inactive control subjects (39). However, because only 24% of CFS patients were determined to be considerably less active than controls (39), other explanations are needed besides deconditioning. It is also possible that the differences in oxygen delivery could be a result of abnormal autonomic nervous system activity. Sympathetic and parasympathetic tone has been reported to be abnormal in CFS patients (7, 10, 30, 43). Previous studies have suggested that abnormal autonomic nervous system function may lead to reduced blood flow to active muscles and result in reduced muscle metabolism and exercise performance (26). In support of this idea, several studies have reported that patients with fibromyalgia (a related syndrome) have abnormal muscle blood flow (20).

The aim of this study was to measure blood flow in patients with CFS and, in particular, to determine whether reduced blood flow in CFS patients could be responsible for reduced oxidative metabolism that was shown in our laboratory’s previous studies (22, 24). To test the hypothesis that blood flow is reduced in CFS, we measured the peak hyperemic flow response after short bouts of intense exercise. To test whether reduced blood flow is responsible for reduced oxidative metabolism in CFS, we partially restricted blood flow during exercise recovery from exercise and measured the degree to which oxidative metabolism was limited. If that hypothesis were true, patients with CFS would have lower peak blood flow values and would show greater reductions in oxidative metabolism for a given level of flow restriction.

METHODS

Subject selection. CFS patients were diagnosed on basis of the case definition of CFS (11). Thus they all reported new onset of fatigue lasting at least 6 mo, not relieved by sleep, and producing a substantial decrease of activity in either social, personal, or occupational spheres. In addition, patients reported problems lasting at least 6 mo in at least four of the following symptoms: lymphadenopathy, headaches, myalgia, arthralgia, unrefreshing sleep, cognitive problems, sore throat, or the report that even minor effort produced an exacerbation of their symptom complex. Common medical causes of fatigue were ruled out by blood tests (34), and no patient had the following psychiatric exclusions: manic-depression, schizophrenia, and drug/alcohol abuse within 2 yr of intake. In addition, we excluded patients who had any psychiatric diagnosis occurring in the 5 yr before onset of CFS; patients with psychiatric illness beginning after their CFS, most often depression and/or anxiety, were excluded. Nineteen CFS patients were tested in this study (age = 39.4 ± 5.4 yr, 12 women, 7 men). Control subjects were healthy and were chosen to be similar in age.

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and to have a sedentary lifestyle by self-report (regular exercise less than once a week for at least 6 mo before testing). Eleven control subjects were tested (age = 37.2 ± 6.9 yr, 5 women and 6 men). The subjects in this study were also used in a companion study (25). This study was approved by the University Committee on Studies Involving Human Beings at the University of Georgia, the New Jersey Medical School, and the University of Pennsylvania. Informed consent was obtained from all subjects prior to testing.

Experimental protocol. Two sets of experiments were performed, one using $^{31}$P-magnetic resonance spectroscopy (MRS) and one using Doppler ultrasound. MRS experiments measured the rate of phosphocreatine (PCr) recovery as an index of the capacity of oxidative metabolism (21, 31, 38). High-intensity exercise (fast repetition, low load) was used to recruit as much of the muscle as possible, and short durations were used to minimize the development of acidosis, which influences PCr levels (23, 38, 42). Seven exercise bouts were performed by one leg. During all exercises, a blood pressure cuff placed proximal to the knee was inflated to 100 mmHg above systolic pressure 10 s before, during, and for ~10 s after exercise. This was done to decrease the likelihood of stored oxygen in the muscle at the start of recovery. Previous studies have reported that metabolic recovery from exercise does not occur when the muscle is ischemic (5, 17, 23). Recovery from the first and last exercise bouts occurred with complete release of cuff pressure. The median five exercise bouts had cuff pressures during recovery that included 50, 60, 70, 80, and 90 mmHg (1 case 40, 50, 60, 70, and 80 mmHg). The same seven exercise bouts were also performed on the same leg and blood flow measured in the femoral artery with Doppler ultrasound. In addition, oxygen saturation and blood volume were measured in the medial gastrocnemius using near-infrared spectroscopy (NIRS). The order of the MRS and Doppler experiments varied, with ~1 h between test sessions. The order of the MRS and Doppler tests did not influence the results. The order of the cuff pressures was usually from 50 to 90, but in some subjects the order was reversed. Again, no order effect was seen in the results.

MRS. Phosphorus metabolites were measured with a home-built spectrometer system in a 78-cm clear bore, 1-T magnet (23, 24). A 6 × 8-cm surface coil tuned to both $^{31}$P and $^1$H frequencies (34.86 and 86.12 MHz, respectively) was placed on the medial gastrocnemius muscle. Phosphorus spectra (3,000-Hz sweep width, 1,024 points) were collected by using pulses to produce maximal signal intensity per pulse. Pulse repetition time was 4 s, and nuclear Overhauser enhancement was used to enhance the $^{31}$P signal. Spectra were Fourier transformed with 5-Hz line broadening and integrated in the frequency domain. Areas of the P, PCr, and β-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 P and PCr.

In this study, PCr values during recovery were fit to a single exponential equation to determine a time constant. Maximal muscle oxidative capacity ($V_{\text{max}}$) was calculated as the inverse of the time constant × resting PCr concentrations. (23, 24).

Doppler ultrasound. Blood flow was measured in the common femoral artery by using quantitative Doppler ultrasound (General Electric Logiq 400CL) (27). A linear-array transducer was used at a frequency of either 6 or 4 MHz. The imaging site was located on the upper third of the thigh and was proximal to the blood pressure cuff and to the exercising muscle. Resting diameter was measured in the longitudinal view during diastole. Pulsed Doppler ultrasound was recorded in the longitudinal view using an insonation angle ±60°. The velocity gate was set to include the entire arterial diameter. Approximately 20 measurements 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 analyzed to determine maximum velocity ($V_{\text{max}}$), minimum velocity, and the time-averaged mean velocity. All velocity calculations were done by General Electric’s advanced vascular program software for the Logiq 400 CL. Waveforms that were not automatically measured by the computer were manually traced 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-averaged mean velocity and the vessel cross-sectional area determined from the diameter. For comparisons between CFS and controls, blood flow was calculated two ways: as the highest flow value (peak blood flow) during each hyperemic condition and for the partial cuff experiments as the integrated flow value over the first 60 s of recovery. Because blood flow was restricted to the exercising muscle, the peak flow response occurred shortly after release of the cuff pressure after exercise had stopped. The half time to recovery was determined as the time where blood flow dropped to one-half the magnitude between maximum flow and resting flow values.

Leg volume. Leg volume measurements were done 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 over the anterior tibialis. Total area of the leg was determined from the circumference measured and fat thickness. On the basis of this information fat volume, lean area volume, and total volume was calculated (28).

NIRS. NIRS measurements used a continuous-light-source, dual-wavelength spectrophotometer (Runman) (22). 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 wavelength light. The difference signal between 760 and 850 nm was used to indicate changes in oxygen saturation. Voltage signals were digitized into a computer with a commercial AD device (Fluke Databucket).

Exercise. Subjects were placed in the supine position with their knees fully extended. The foot was placed in pedal apparatus attached to an air pressure ergometer. Exercise intensity was modulated by changing air pressure in the ergometer. Velcro straps were used to secure the subject to the foot pedal and the platform. Exercise consisted of rapid plantar flexions at a rate of ~2 Hz for 10–16 s. The pressure was chosen to allow the subject to perform the plantar flexions at the desired rate. Exercise bouts were separated by 8–11 min of rest.

Statistical analysis. Data are presented as means ± SD. Comparison of baseline data between CFS and normal subjects used two-tailed unpaired t-tests for values that were not different between men and women. For comparisons between CFS and control subjects during the partial cuff experiments, ANOVA with group and condition was used. For values that were different between men and women, ANOVA with group (CFS and control subjects) and sex was used. Significant differences were assumed with $P < 0.05$.

RESULTS

All of the subjects performed the tests without incident, and we obtained complete data on all 30 subjects. Two potential CFS subjects 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. Some but not all of the CFS patients reported the testing protocol as tiring. No differences were found between CFS and control subjects in age, height, weight, resting blood pressures, resting heart rates, and lean leg volume (Table 1).

Blood flow. Resting blood velocities or resting femoral artery diameters were not different between CFS and control subjects (Table 2). Because lean leg volume and resting blood pressure were not different between CFS and control subjects,
we also found no differences in resting blood flow normalized per lean tissue mass or in resting conductance.

Peak flow responses to exercise usually occurred \( \sim 10 \) s after release of the cuff. Representative examples of femoral artery blood velocities are shown in Fig. 1A. There were no differences in peak blood flow between CFS and control subjects \( (P = 0.095; \text{Fig. 2, top}) \). However, CFS patients did have significantly longer half times of recovery of oxygen saturation compared with control subjects \( (P = 0.024; \text{Fig. 2, bottom}) \).

Partial cuff inflation resulted in a reduction in peak blood flow with unusual flow patterns after the first 40–60 s (Fig. 1B). Figure 3 shows an example of a change in blood flow after exercise with partial cuff inflation. This integrated blood flow over the first 60 s of recovery showed a progressive decline with increasing cuff pressure (Fig. 4A). As demonstrated in Fig. 3, after final release of the cuff, there was an additional bolus of blood flow that was proportional to cuff occlusion pressure (Fig. 4B). CFS patients had significantly lower integrated blood flows across all the partial cuff pressures compared with control subjects \( (P < 0.001) \). The peak flow responses after partial cuff pressures were not different between groups \( (P = 0.26) \). There were no differences in the in the bolus increase in flow after release of occlusion pressure.

**Oxidative metabolism.** In response to the short exercise bouts, PCr levels were, on average, 54% of resting for CFS patients and 50% of resting for controls. Representative examples of changes in PCr during the exercise bouts are shown in Fig. 5. End-exercise pH was 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 (Fig. 6). The time constant of PCr recovery after exercise was not different between CFS and control subjects (40.6 ± 12.6 s for CFS and 40.1 ± 14.0 s for control subjects). Calculated \( V_{\text{max}} \) values were not different between CFS and control subjects \( (P = 0.20; \text{Fig. 6}) \).

Partial cuff ischemia resulted in a progressive impairment of PCr recovery. This is demonstrated in Fig. 5C. Interestingly, in most subjects partial cuff inflation resulted in not only a slower PCr recovery but also, as shown in Fig. 5C, a decrease in PCr levels that persisted until the final cuff release. This was interpreted as “dilution” of the PCr signal due to the accumulation of blood in the limb (from total occlusion of major veins with continued inflow of blood). This was corrected for by assuming a linear accumulation of blood in the sample area over the time period of partial cuff occlusion. PCr curve fitting was performed on these corrected values. By using the corrected values, calculated \( V_{\text{max}} \) values progressively declined

### Table 1. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>Age, yr</th>
<th>Height, m</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Gender, F/M</th>
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</thead>
<tbody>
<tr>
<td>CFS</td>
<td>38.9±5.4</td>
<td>1.70±0.10</td>
<td>73.4±10.9</td>
<td>25.4±4.0</td>
<td>14/7</td>
</tr>
<tr>
<td>Control</td>
<td>37.2±6.9</td>
<td>1.72±0.10</td>
<td>76.2±16.9</td>
<td>25.4±3.4</td>
<td>5/6</td>
</tr>
</tbody>
</table>

Values are means ± SD. CFS, chronic fatigue syndrome; BMI, body mass index; F/M, female/male.

### Table 2. Blood pressure and leg size

<table>
<thead>
<tr>
<th>Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Leg Volume, cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>Diastolic</td>
<td>Mean</td>
</tr>
<tr>
<td>CFS</td>
<td>119.1±10.5</td>
<td>71.7±9.1</td>
</tr>
<tr>
<td>Control</td>
<td>118.5±11.7</td>
<td>69.0±9.4</td>
</tr>
</tbody>
</table>

Values are means ± SD.
with increasing cuff occlusion pressure, with 90 mmHg pressures reducing $V_{\text{max}}$ to 30.7 and 35.3% of nonoccluded $V_{\text{max}}$ for CFS and controls, respectively (Fig. 5). Again, there was no difference in calculated $V_{\text{max}}$ values between CFS and control subjects.

The main hypothesis of the study was that partial occlusion of blood flow would reduce $V_{\text{max}}$ to a greater degree in CFS compared with control subjects. This was not seen, because ANOVA of $V_{\text{max}}$ measurements across the different cuff pressures resulted in a $P$ value of 0.20. However, the integrated blood flow during the first 60 s after exercise was significantly lower in the CFS group compared with controls ($P < 0.001$). The relationship between integrated blood flow and $V_{\text{max}}$ is shown in Fig. 7.

DISCUSSION

This study found that hyperemic blood flow after exercise during partial cuff restriction was reduced in CFS patients compared with controls. Blood flow was measured as the integrated flow over the first 60 s of recovery, and the measurement took into account lean leg mass with no group differences in blood pressure. We also found that recovery of
Oxygen saturation was reduced in CFS patients after exercise, consistent with our previous studies (23). CFS patients have been reported to have altered sympathetic activity and impaired autonomic tone (7, 10, 30, 43). The measurements of integrated flow and oxygen delivery might reflect autonomic impairment. Although there are no available studies on CFS to compare our blood flow measurements, previous studies have suggested that CFS patients have cholinergic abnormalities in peripheral microcirculation (19, 35). Patients with fibromyalgia, a related syndrome, have reported reduced blood flow (3) and reduced capillary densities (20) relative to control subjects.

The peak blood flow response for the no cuff exercise conditions was not reduced in the CFS patients compared with controls, in contrast to the integrated flow responses. The lack of a difference in peak blood flow responses after exercise was similar to our most recent study, which found no differences in peak blood flow after cuff ischemia (25). It is not clear why we would obtain different conclusions for peak and integrated blood flow. One possibility is that integrated flow values might be more sensitive markers of flow abnormalities in CFS patients than peak flow. Our laboratory has found altered rates of recovery of blood flow after ischemia in both older and spinal cord-injured subjects (27, 29), consistent with altered autonomic tone in these groups (9, 36). These results suggest that the capacity for peak blood flow was not reduced in CFS patients compared with controls but that the actual delivery of oxygen might be reduced because of alterations in the integrated flow response.

The question that comes from our results is what is the importance of the reduced integrated flow and oxygen delivery responses? Our primary hypothesis was that restricting blood flow during recovery would impair oxidative metabolism in CFS patients to a greater extent than in control subjects. We found no evidence that this was true. Oxidative capacity measured with MRS during recovery from exercise with partial.
cuff occlusion was not impaired in CFS subjects. We did see a graded decrease in blood flow and oxidative metabolism with increasing cuff pressure, suggesting that partial cuff inflation did restrict blood flow and did reduce oxidative metabolism. As shown in Fig. 1, we took care to design the experiment to minimize residual levels of muscle oxygen before the partial cuff occlusion. This included applying the cuff before exercise to assist in reducing stored oxygen levels in the muscles and keeping the cuff on the muscle for ~10 s after cessation of exercise to ensure that PCr recovery was stopped. So, when flow restriction did reduce oxidative metabolism, there was no evidence that CFS patients were more sensitive to flow restriction than control subjects.

A number of previous studies have examined the effects of flow restriction on muscle metabolism and function. Conrad and Green (6) found that cuff pressures of 50–60 mmHg reduced direct measures of resting arterial flow by 5–9% in a cat hindlimb preparation. In a study on humans, Hiatt et al. (15) found much larger changes with cuff pressures of 50 mmHg, including 44% decreases in femoral artery diameter directly under the cuff and a 38% decrease in blood velocity downstream of the cuff. These measurements were made under stable conditions, and it is not clear how they compare with our measurements, because we were primarily concerned with changes in blood flow during the first minute after partial cuff occlusion. Sundberg and Kaijser (37) found that lower body negative pressures of 50 mmHg reduced arterial blood flow by 16% during exercise and also resulted in lower venous oxygen saturation and higher venous lactate levels during exercise. Cole and Brown (4) used cuff occlusion during electrical stimulation, and found that cuff pressures of 50 and 80 mmHg resulted in force reductions of 15–22%. Iwanaga et al. (17) found cuff occlusion at systolic and diastolic blood pressures to have pressure-dependent effects on work rate, PCr levels, and intracellular pH during exercise. Taken together, these studies support our findings that even relatively low occlusion pressures can restrict blood flow and impair muscle metabolism.

One of the complications of our approach is that partial arterial occlusion is also associated with venous occlusion. This dramatically altered the arterial velocity waveform (Fig. 1), most likely due to back pressure associated with the accumulation of blood in the venous system. The accumulation of blood also influenced the PCr measurements because we could detect changes in PCr concentration in association with the change in cuff pressure (Fig. 5). Partial cuff ischemia reduced PCr values and altered the shape of the PCr recovery curves. We chose to correct our PCr values by using a linear increase in blood volume during the time period that the partial cuff pressure was applied. We based this in part on the NIRS results, which showed that partial cuff ischemia increased blood volume. Interestingly, the increase in blood volume appeared to be primarily deoxygenated blood because the level of oxygen saturation was progressively reduced during partial cuff ischemia. These results suggest that alterations in blood volume need to be taken into account when evaluating the vascular response to partial cuff ischemia.

Another limitation of our study was the measurement of peak blood flow with the use of two cardiac cycles. Although the short measurement time interval allowed us to track the rapidly changing blood flows needed to measure a peak flow value, it does introduce some added variability. For example, we did not control for the effects of respiration on time-averaged blood flow. We did use integrated flow over 60 s when comparing blood flow during partial cuff ischemia with PCr recovery rates. We believed that blood flow over 60 s would more accurately reflect oxygen delivery while PCr was being resynthesized. This would also reduce variability of the measurements due to respiration and other transient effects.

Other approaches have been used to evaluate muscle metabolism in response to altered oxygen delivery. Studies by Hogan et al. (16) showed that PCr levels during exercise were sensitive to the concentration of oxygen during exercise. The rate of PCr resynthesis was also altered by altering oxygen concentrations in inspired air, but this result was found only in well-trained subjects (32). It is hard to compare the magnitude of reduction in oxygen delivery in the studies that altered the concentrations of inspired oxygen with our study where we reduced blood flow. However, their results are consistent with ours and support the idea that oxidative metabolism is linked to oxygen delivery during exercise.

Oxidative metabolism, as measured by the rate of PCr recovery after exercise, was not different between the CFS patients and our controls in this study. This was reported in an earlier manuscript (25). In addition, CFS patients showed the same degree of reduction of oxidative metabolism as control subjects with partial cuff occlusion to reduced blood flow. The lack of impairment of oxidative metabolism in CFS patients was consistent with some previous studies (18) but not with others (23, 24). It is not clear why the different studies have different conclusions, especially our present study and our laboratory’s previous study, which evaluated CFS and controls subjects from the same source and used basically the same equipment and protocols (22, 24). Most of the subjects in our study reported “severe” CFS symptoms, so we believe that altered muscle metabolism is not a requirement for CFS symptoms to be present. It is possible that our lack of change in blood flow responses was due to the lack of metabolic differ-
ences and that CFS patients with clearly abnormal oxidative metabolism would have abnormal blood flow.

In summary, CFS patients had rates of oxidative metabolism that were not different from those of control subjects, even with partial restriction of blood flow. This suggests that the CFS symptoms that these patients reported were not caused by peripheral abnormalities in oxidative metabolism. However, we did find evidence that CFS patients had reduced oxygen delivery, on the basis of both on NIRS measurements of the rate of recovery after exercise, and reductions in the integrated flow response to partial arterial occlusion. These measurements do support the hypothesis that CFS patients have abnormal control of circulation, perhaps due to altered sympathetic and/or parasympathetic tone. However, it is not clear how significant the changes in control of circulation are as they were not associated with changes in muscle oxidative metabolism, which is normally highly sensitive to oxygen delivery. Partial cuff occlusion resulted in graded reductions in oxidative metabolism, even with occlusion pressure below diastolic pressure. This suggests that partial occlusion of blood flow could be a useful method of evaluating oxygen delivery and muscle metabolism as long as the effects of venous filling are taken into account.

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