

Chronic fatigue syndrome: new evidence for a central fatigue disorder

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A B S T R A C T

Considerable evidence points towards a prominent role for central nervous system (CNS) mechanisms in the pathogenesis of chronic fatigue syndrome (CFS), a disorder characterized chiefly by persistent, often debilitating, fatigue. We wished to characterize circulating profiles of putative amino acid modulators of CNS 5-hydroxytryptamine (5-HT; serotonergic) and dopaminergic function in CFS patients at rest, as well as during symptom-limited exercise and subsequent recovery. Groups of 12 CFS patients and 11 age- and sex-matched sedentary controls, with similar physical activity histories, underwent ramp-incremental exercise to the limit of tolerance. Plasma amino acid concentrations, oxygen uptake and ratings of perceived exertion were measured at rest, and during exercise and recovery. Peak oxygen uptake was significantly lower in the CFS patients compared with controls. Rating of perceived exertion in the patients was higher at all time points measured, including at rest, relative to controls. Levels of free tryptophan (free Trp), the rate-limiting 5-HT precursor, were significantly higher in CFS patients at exhaustion and during recovery, whereas concentrations of branched-chain amino acids (BCAA) and large neutral amino acids (LNAA) were lower in CFS patients at exhaustion, and for LNAA also during recovery. Consequently, the [free Trp]/[BCAA] and [free Trp]/[LNAA] ratios were significantly higher in CFS patients, except at rest. On the other hand, levels of tyrosine, the rate-limiting dopaminergic precursor, were significantly lower at all time points in the CFS patients. The significant differences observed in a number of key putative CNS 5-HT and dopaminergic modulators, coupled with the exacerbated perception of effort, provide further evidence for a potentially significant role for CNS mechanisms in the pathogenesis of CFS.

INTRODUCTION

Chronic fatigue syndrome (CFS) is a disorder associated with persistent, often debilitating, physical and mental fatigue that can be exacerbated by even modest degrees of physical activity [1,2]. Its prevalence in primary care is now estimated at 5 in 1000 individuals [3]. The aetiology of CFS remains controversial, with considerable debate

as to whether the fatigue is central or peripheral in origin, and resolution of its pathogenesis is compounded by the considerable heterogeneity seen across patients [1].

While early reports indicated abnormalities of muscle metabolism [4] and/or histology [5], inconsistencies in the literature suggest that other mechanisms are also likely to be involved. For example, initial indications of premature blood lactate accumulation during exercise in CFS

Key words: amino acid, chronic fatigue syndrome, exercise, tryptophan, tyrosine.

Abbreviations: BCAA, branched-chain amino acids; CFS, chronic fatigue syndrome; CNS, central nervous system; 5-HT, 5-hydroxytryptamine; LNAA, large neutral amino acids; NEFA, non-esterified ('free') fatty acids; RPE, rating of perceived exertion; $\dot{V}O_2$, oxygen uptake.

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patients [6] were followed by subsequent studies showing considerable variation in the blood lactate response to exercise [7], and the more recent suggestion that lactate metabolism is normal in CFS patients [8]. Furthermore, the characteristic exercise intolerance of patients with CFS has been distinguished from deconditioning [9], which some investigators have argued to be of primary importance in the pathogenesis of CFS (e.g. [10]).

In contrast with the heterogeneity of results from studies on muscle metabolism, an exacerbated perception of effort has been consistently associated with CFS, with patients reporting greater effort perception (compared with healthy controls) during both submaximal and maximal exercise [10,11]. There are several lines of evidence to support the contention that central neural mechanisms of fatigue, including abnormalities of neurotransmission, may be involved in these exacerbated perceptions of tiredness and fatigue. In particular, the 5-hydroxytryptamine (5-HT; serotonergic) system has received considerable attention, through both its association with what Newsholme and colleagues have termed 'central fatigue' [12] and its involvement in the control of hypothalamic functions, abnormalities of which (i.e. sleep, pain, mood and appetite) have been reported in CFS [1]. However, in the absence of direct access to the brain, the associations between 'central fatigue', 5-HT and more recently dopaminergic function in humans [13] remain, at best, tenuous. That is, the majority of studies have relied upon measurement of plasma concentrations of 5-HT precursors and markers in response to targeted drug manipulations (for a review, see [13]).

The assessment of central 5-HT function in patients with CFS has, therefore, been indirect. Typically, this has involved the imposition of neuroendocrine challenge tests and/or measurement of circulating levels of 5-HT modulators, such as the 5-HT precursor tryptophan (Trp) [14]. Based on such approaches, up-regulation of hypothalamic 5-HT receptors in CFS patients at rest has been suggested [15], and increased 5-HT activity has been demonstrated following administration of the selective 5-HT-releasing drug *d*-fenfluramine [16]. As with peripheral indices, however, reports of an association between abnormal 5-HT function and CFS have not been without controversy. For example, Cleare et al. [16] reported enhanced 5-HT-mediated responses to *d*-fenfluramine in patients with CFS (i.e. as reflected in raised levels of cortisol and prolactin), while Bearn et al. [17] found no differences between CFS patients and healthy controls in these indices of 5-HT function. The extent to which such differences are reflective of heterogeneity of patient response is presently unclear.

In order to elucidate further the role of putative central nervous system (CNS) modulators of fatigue in CFS patients, we examined the acute exercise-related responses of those circulating amino acids that have

been demonstrated previously to influence central 5-HT and dopaminergic function, relative to those in matched control subjects with similar physical activity histories.

METHODS

Subjects

A total of 12 patients (ten women and two men) with a diagnosis of CFS [females: age, 39 ± 11 years (mean \pm S.D.); height, 165 ± 8 cm; body mass, 71 ± 11 kg; male 1: age, 45 years; height, 177 cm; mass, 85 kg; male 2: age, 33 years; height, 167 cm; mass, 66 kg] and 11 sedentary controls (nine females: age, 38 ± 12 years; height, 163 ± 8 cm; mass, 62 ± 9 kg; male 1: age, 48 years; height, 168 cm; mass, 76 kg; male 2: age, 33 years; height, 175 cm; mass, 65 kg) provided written informed consent prior to their participation in the study, which was approved by the Glasgow University Ethics Committee. All patients fulfilled the Centers for Disease Control Criteria (CDC) for CFS [2], and were examined to exclude any other medical condition. The control group consisted of healthy individuals, matched to the patients for age, gender, anthropometric characteristics and habitual physical activity status. With regard to the latter, control subjects were selected on the basis that they were office workers who (like the patients) undertook no form of regular recreational physical activity.

Procedures

Following a brief familiarization session with the equipment and procedures, subjects underwent a ramp-incremental exercise test on an electronically braked computer-controlled cycle-ergometer (Excalibur Sport, Lode, The Netherlands) to the limit of tolerance. The incrementation rate for individual subjects varied between 3 and $10 \text{ W} \cdot \text{min}^{-1}$, so as to elicit exhaustion within 10–15 min [18]. Gas exchange variables were determined breath-by-breath, using algorithms developed by Beaver et al. [19]. Respired volumes were measured with a bi-directional turbine transducer (VMM; Alpha Technologies, Laguna Niguel, CA, U.S.A.), calibrated with a 3-litre syringe, using a range of different flow profiles (Hans Rudolph, Kansas City, MO, U.S.A.). Respired gas concentrations were measured every 20 ms by a quadrupole mass spectrometer (QP9000; Morgan Medical, Gillingham, Kent, U.K.), which was calibrated against precision-analysed gas mixtures. Peak oxygen uptake ($\dot{V}_{\text{O}_{2\text{peak}}}$) was calculated as the average value for the final 15 s of the test. Heart rate was derived beat-to-beat from the R-R interval of a 12-lead ECG (Quinton Medical). Rating of perceived exertion (RPE) values were recorded using an electronic visual-analogue scale, where 0 represented 'nothing at all', and 100 represented 'maximum' [20].

Before the exercise test, a 21 G cannula was introduced into a superficial vein on the dorsal surface of the subject's pre-heated hand [21]; the cannula was kept patent by a slow ($\sim 0.5 \text{ ml} \cdot \text{min}^{-1}$) infusion of isotonic saline. Arterialized-venous blood samples were subsequently drawn at rest, during unloaded exercise, at exhaustion and after 6 min of recovery, and analysed for plasma concentrations of amino acids by HPLC, using fluorescence detection and pre-column derivatization with *o*-phthalaldehyde (Hypersil amino acid method; ThermoHypersil-Keystone, Runcorn, U.K.). Free Trp was separated from protein-bound Trp by filtering plasma through 10 000 NMWL ('nominal molecular weight limit') cellulose filters (Ultrafree-MC filters; Millipore Corp.) during centrifugation at 5000 *g* for 60 min at 4 °C. Concentrations of blood glucose and lactate [22] and of non-esterified ('free') fatty acids (NEFA) (colorimetric method; Boehringer Mannheim Biochemica, London, U.K.) were determined by standard enzymic methods, using an automated spectrophotometer (ABX Diagnostics).

Statistical analyses

Data are expressed as mean \pm S.D. or median (range) as appropriate, following a test for the normality of distribution. Statistical analysis of the data was carried out using independent Student's *t* tests and Mann-Whitney tests to assess differences for the measured variables between the two groups for each time point, as appropriate. Correlation analysis was carried out using Pearson's (product moment; *r*) correlation coefficient. Statistical significance was declared at $P \leq 0.05$.

RESULTS

$\dot{V}_{O_{2\text{peak}}}$ in the CFS group ($21.2 \pm 6.3 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) was significantly lower than in the control group ($28.3 \pm 6.4 \text{ ml} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). Peak heart rate was lower in the CFS group than in the control group, although this difference was not statistically significant (CFS, $160 \pm 29 \text{ beats} \cdot \text{min}^{-1}$; control, $176 \pm 13 \text{ beats} \cdot \text{min}^{-1}$; $P = 0.12$); this corresponded to 88 % of the maximal age-predicted value for the CFS patients, compared with 97 % for the controls. The peak respiratory exchange ratio was significantly lower in the patients (0.98 ± 0.06) than in the control group (1.15 ± 0.40). RPE was significantly higher in the CFS group at all time points, compared with controls: rest: CFS, 14 (2–63) [median (range)]; control, 2 (0–6); unloaded exercise: CFS, 24 (2–99), control, 2 (0–8); at exhaustion (near-maximal values): CFS, 98 (91–100), control, 90 (51–100).

Plasma levels of free Trp, the rate-limiting 5-HT precursor, were elevated in the CFS group throughout the protocol; these increases were highly significant both

Table 1 Plasma concentrations of amino acid 5-HT and dopaminergic precursors in the CFS and control groups

Values are means \pm S.D.; * indicates a significant difference between the CFS and control groups ($P < 0.05$).

Amino acid	Concentration ($\mu\text{mol} \cdot \text{l}^{-1}$)			
	Rest	Unloaded exercise	Exhaustion	Recovery
Total Trp				
CFS	41.5 ± 6.6	38.3 ± 6.5	38.8 ± 5.6	37.9 ± 7.6
Control	42.2 ± 4.3	41.3 ± 5.2	41.2 ± 4.8	37.4 ± 5.1
Free Trp				
CFS	4.2 ± 1.4	4.7 ± 1.1	5.1 ± 1.4	5.7 ± 1.2
Control	3.9 ± 0.9	3.8 ± 1.1	$3.8 \pm 0.8^*$	$4.6 \pm 1.0^*$
BCAA				
CFS	351.9 ± 74.2	338.9 ± 71.6	348.7 ± 50.7	343.5 ± 73.5
Control	391.9 ± 68.6	391.6 ± 78.9	$394.4 \pm 51.1^*$	397.8 ± 74.7
LNAA				
CFS	531.6 ± 97.5	506.8 ± 95.1	526.5 ± 61.3	516.9 ± 95.8
Control	587.4 ± 87.9	584.4 ± 103.7	$596.1 \pm 66.4^*$	$602.8 \pm 100.5^*$
Tyrosine				
CFS	49.4 ± 14.3	45.8 ± 12.9	49.5 ± 11.0	47.1 ± 11.0
Control	$62.8 \pm 9.1^*$	$61.9 \pm 10.0^*$	$66.1 \pm 8.8^*$	$65.0 \pm 13.1^*$

at exhaustion and during recovery, with a non-significant tendency during unloaded exercise ($P < 0.1$). However, the total concentration of Trp was not significantly different between the groups at any of the time points (Table 1). Concentrations of branched-chain amino acids (BCAA) and large neutral amino acids (LNAA), both of which compete with free Trp for entry into the brain, were lower in the CFS patients throughout the protocol, with highly significant differences at exhaustion, and for LNAA also during recovery (Table 1). Consequently, the concentration ratios of the central 5-HT modulators [free Trp]/[BCAA] and [free Trp]/[LNAA] were higher in the CFS group, significantly so at all time points except at rest (Figure 1). On the other hand, significantly lower levels of tyrosine, the rate-limiting dopaminergic precursor, were found in the CFS patients at all time points (Table 1). As a result, the [free Trp]/[tyrosine] ratio was significantly higher in the CFS group at all time points (Figure 2).

There were no differences between the CFS and control groups in concentrations of blood glucose, plasma NEFA or blood lactate (with the exception of a significantly lower recovery value in the CFS group) (Table 2). Significant correlations were found between [free Trp] and [NEFA] for all time points collectively, for both the CFS group ($r = 0.44$, $n = 12$, $P = 0.003$) and the control group ($r = 0.47$, $n = 11$, $P = 0.002$).

Significant positive correlations were found between $\dot{V}_{O_{2\text{peak}}}$ and the putative central 5-HT modulators, i.e. [free Trp] ($r = 0.73$, $n = 12$, $P = 0.011$), [free Trp]/[BCAA]

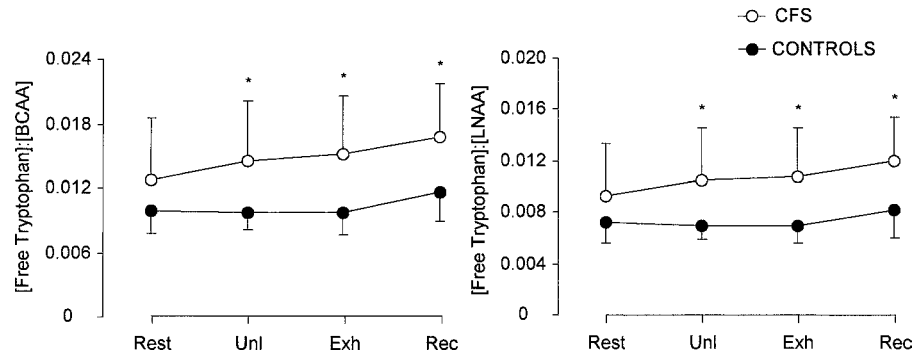


Figure 1 [Free Trp]/[BCAA] and [free Trp]/[LNAA] ratios in the CFS (○) and control (●) groups

Ratios are given at rest, during unloaded exercise (Unl), at exhaustion (Exh) and during recovery (Rec). Values are means \pm S.D.; * indicates a significant difference between groups.

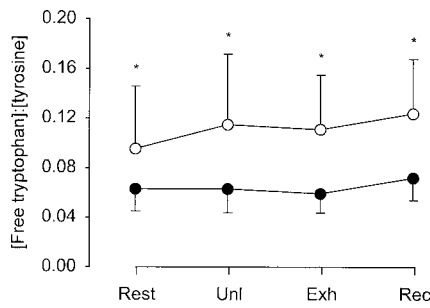


Figure 2 [Free Trp]/[tyrosine] ratios in the CFS (○) and control (●) groups

Ratios are given at rest, during unloaded exercise (Unl), at exhaustion (Exh) and during recovery (Rec). Values are means \pm S.D.; * indicates a significant difference between groups.

Table 2 Blood lactate, glucose and NEFA concentrations in the CFS and control groups

Values are means \pm S.D. or median (range); * indicates a significant difference between the CFS and control groups ($P < 0.05$).

Metabolite	Concentration ($\text{mmol} \cdot \text{l}^{-1}$)			
	Rest	Unloaded exercise	Exhaustion	Recovery
Lactate				
CFS	0.6 ± 0.3	0.8 ± 0.2	5.6 ± 3.5	4.8 ± 3.3
Control	0.6 ± 0.1	0.9 ± 0.2	7.3 ± 1.3	$8.7 \pm 2.2^*$
Glucose				
CFS	4.7 (4.1–6.4)	4.8 (4.0–6.2)	4.7 (4.0–6.1)	4.6 (4.1–6.7)
Control	4.5 (4.1–5.6)	4.5 (4.1–5.4)	4.7 (4.4–5.3)	5.1 (4.3–6.3)
NEFA				
CFS	0.7 ± 0.4	0.7 ± 0.3	0.7 ± 0.4	0.7 ± 0.4
Control	0.5 ± 0.2	0.6 ± 0.3	0.4 ± 0.2	0.6 ± 0.3

($r = 0.80$, $n = 12$, $P = 0.003$) and [free Trp]/[LNAA] ($r = 0.78$, $n = 12$, $P = 0.005$), at exhaustion in the CFS group, but not in the control group. On the other hand, a

significant negative correlation was found between RPE and [tyrosine] at rest ($r = -0.60$, $n = 12$, $P = 0.05$) and during unloaded exercise ($r = -0.65$, $n = 12$, $P = 0.029$) in the CFS group only. $\dot{V}_{O_{2\text{peak}}}$ and [free Trp]/[tyrosine] were also significantly correlated at exhaustion in the CFS group ($r = 0.69$, $n = 12$, $P = 0.018$), but not in the control group.

DISCUSSION

As in previous studies (e.g. [10]), our patients with CFS showed marked exercise intolerance, associated with an exacerbated perception of effort. The demonstration of lower values for heart rate and respiratory exchange ratio at peak exercise in the CFS patients, compared with the controls, in conjunction with an almost maximal value for RPE, is consistent with the involvement of a centrally mediated increase in effort perception (rather than O_2 transport) being the limitation to exercise performance (e.g. [23]). That is, it appears likely that the abnormally high perception of effort may translate into an inability or unwillingness to effect the power outputs necessary to achieve maximal performance [24], thus precluding the 'classic' criteria for attainment of maximal effort from being fulfilled. Thus the limit of tolerance provides a symptom-limited peak \dot{V}_{O_2} value, rather than a true maximum.

Fundamental to the interpretation of the present study is the extent to which the CFS patients and the control subjects were matched for habitual physical activity status. The provision of appropriate controls for patients with CFS can be problematic when the patient group manifests a high degree of exercise intolerance (as was the case in our present study). That is, the available pool of healthy subjects whose lifestyle is characterized by a corresponding degree of sedentarity is small in many communities, owing to the growing popularity

of recreational fitness activities; this was compounded by the requirement for serial blood sampling. To match the control subjects with the patient group for physical activity history, we therefore recruited office-based workers who undertook no regular physical activity in their free time, apart from leisurely and modest walking. The control subjects' marked sedentarity was affirmed by a $\dot{V}O_{2\text{peak}}$ that was 92 % of predicted [25].

The demonstration of significant differences between our CFS patients and the healthy sedentary controls for a number of key putative CNS 5-HT and dopaminergic modulators, both at rest and during exercise, points towards a potentially important role for central neural mechanisms of fatigue in the pathogenesis of CFS. This argument depends on the assumption that the peripheral precursors that we measured in the present study do indeed initiate an increase in brain 5-HT and dopaminergic activity. Although still considered by some as controversial, the raised concentration of plasma free Trp in our CFS patients during exercise and subsequent recovery, in combination with the high baseline levels of free Trp reported by Castell et al. [14], imply increased concentrations of Trp in the brain [26] and, potentially, an increase in central 5-HT turnover. The association between increased 5-HT turnover and the subjective feeling of fatigue is one that is widely documented. Increases in brain 5-HT concentration are associated with lethargy, sleepiness and mood changes, all of which have also been linked to heightened effort perception (e.g. [13]). It is also well established that elevated levels of plasma free Trp and, consequently, of brain 5-HT are associated with decrements in physical performance [27], albeit only in animal models. Free Trp, however, shares the same mechanism for transport into the brain as other LNAAs, most notably the BCAAs (i.e. valine, leucine and isoleucine), with consequent competition for entry into the brain. In our CFS patients, we found decreased plasma LNAAs and BCAA levels at exhaustion, and also during recovery for LNAAs. The resulting increases we observed in plasma [free Trp]/[BCAA] and [free Trp]/[LNAAs] ratios in the CFS group strengthen the proposed hypothesis linking fatigue in CFS with abnormally high brain 5-HT activity. On the other hand, however, the unexpected positive correlation between $\dot{V}O_{2\text{peak}}$, a measure of exercise tolerance, and the putative central 5-HT modulators at exhaustion in the CFS group, but not in the control group, would appear to contradict any involvement of the 5-HT system in the pathogenesis of CFS.

The dopaminergic system has also been implicated in central fatigue during exercise [28]. Increased brain dopamine levels have been linked with increased arousal, motivation, muscular co-ordination and physical performance [13]. It is of interest, therefore, that we found significantly lower levels of tyrosine, the rate-limiting dopaminergic precursor, in the CFS group at all

time points, including at rest. To our knowledge, this is the first study to demonstrate low plasma dopaminergic precursor levels in patients with CFS. The significant negative correlation found between RPE and plasma tyrosine levels at rest and during unloaded exercise in the CFS group provides additional support for involvement of the dopaminergic system in CFS. Since fatigue during prolonged physical activity has also been shown to be associated with increased brain 5-HT and reduced brain dopamine levels [29], a high [5-HT]/[dopamine] ratio should favour reduced exercise performance. Indeed, the ratio of the precursors of 5-HT and dopamine, i.e. plasma [free Trp]/[tyrosine], was consistently higher in our CFS patients at all time points when compared with the control group. The increased baseline [free Trp]/[tyrosine] ratio may also explain the evident fatigue and associated high effort perception, even prior to physical exertion, in this patient population.

Interestingly, the CFS patients exhibited a higher variance in the measured plasma 5-HT and dopamine precursor concentrations than did the control subjects (Figures 1 and 2). This may well reflect the heterogeneity that is frequently associated with CFS, as variation has been reported previously in cardiovascular, histological and metabolic attributes. The heterogeneous nature of CFS is likely to be important in understanding its pathogenesis; for example, it is possible that CFS is actually a multiple-stage disorder and/or one that consists of multiple pathological conditions.

At present, the physiological mechanisms underlying the apparent 5-HT overactivity and the concurrent depression of the dopaminergic system in patients with CFS (the latter possibly being a direct result of the inhibitory effect of increased 5-HT activity [29]) cannot be readily explained. The altered levels of circulating amino acids demonstrated in the present study may indicate a dysfunction in amino acid metabolism, either within the CNS or peripherally (e.g. uptake by liver or skeletal muscle). One cannot, therefore, entirely exclude the involvement in CFS pathogenesis of peripheral mechanisms, which could ultimately affect levels of those amino acids measured in the present study. Although plasma concentrations of 5-HT and dopaminergic modulators have been shown to be closely associated with brain 5-HT and dopamine levels respectively [13], we were unable to make any measurements within the CNS and, thus, can only speculate on a 'true' central component in the disorder. Plasma levels of free Trp are highly dependent on plasma concentrations of NEFA, which have been shown to displace Trp from binding to albumin [30]. However, we could discern no difference in the correlations of concentrations of plasma free Trp with NEFA between the CFS and control groups. The close association between free Trp and NEFA concentrations in both groups would imply normal Trp-albumin binding properties.

In conclusion, the significant differences between patients with CFS and healthy controls that we observed in several key CNS 5-HT and dopaminergic modulators, assuming that they are indeed reflective of brain 5-HT and dopamine levels, suggest that central neural mechanisms may contribute to the increased perception of effort and impaired exercise tolerance in CFS. However, the significance of the observed associations between 5-HT and dopamine modulators and exercise intolerance/effort perception, found only in the CFS patients, is not presently understood. The precise role, therefore, of the 5-HT and dopaminergic systems in the pathogenesis of CFS remains to be elucidated, and future studies using larger populations of patients with CFS, and more direct measurements, are required.

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