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Plasma and urinary carnitine and acylcarnitines in chronic fatigue syndrome

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Abstract

Contradictory reports have suggested that serum free carnitine and acylcarnitine concentrations are decreased in patients with chronic fatigue syndrome (CFS) and that this is a cause of the muscle fatigue observed in these patients. Others have shown normal serum free carnitine and acylcarnitines in similar patients. We report here studies on free, total and esterified (acyl) carnitines in urine and blood plasma from UK patients with CFS and three control groups. Plasma and timed urine samples were obtained from 31 patients with CFS, 31 healthy controls, 15 patients with depression and 22 patients with rheumatoid arthritis. Samples were analysed using an established radioenzymatic procedure for total, free and esterified (acyl) carnitine. There were no significant differences in plasma or urinary total, free or esterified (acyl) carnitine between UK patients with CFS and the control groups or in renal excretion rates of these compounds. The data presented here show that, in the CFS patients studied, there are no significant abnormalities of free or esterified (acyl) carnitine. It is thus unlikely that abnormalities in carnitine homeostasis have any significant role in the aetiology of their chronic fatigue.

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1. Introduction

Chronic fatigue syndrome (CFS) [often linked with myalgic encephalomyelitis (ME)] is character-

ised by severely disabling fatigue, both mental and physical, combined with a variety of other symptoms [1–4] and with a relatively high prevalence in primary care patients [3]. ME/CFS patients describe a variety of infectious and non-infectious antecedents often including a stressful situation and with primary or secondary psychological problems which may interact in a common pathway [5]. This pathophysiology involves elements of neuroendocrine, neurotransmitter and immune function, all of which individually may be sub-clinical and thus difficult

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to precisely identify or establish. The high prevalence and debilitating nature of the disorder have provoked extensive scientific and clinical studies in the search for a unifying physiological or metabolic cause. Despite this, the aetiology of the disease remains obscure.

Patients with CFS show reduced exercise tolerance and post-exercise fatigue induced by minimal physical activity, suggesting abnormal muscle function as one cause of the syndrome. Abnormal mitochondria have been observed in muscle of some patients with CFS [6], suggesting underlying abnormalities in muscle mitochondrial energy production. L-Carnitine has major importance in mitochondrial energy metabolism, being essential for the transport of long chain fatty acids across the inner mitochondrial membrane, in removal of accumulating acyl moieties from the mitochondria and in maintenance of mitochondrial homeostasis by regulation of the inner mitochondrial acyl CoA/free CoASH ratio [7,8]. Thus, it has been suggested that L-carnitine may have a role in the aetiology of CFS. Some studies have shown decreased serum free carnitine [9] and decreased serum acylcarnitine [10,11] in patients with CFS while others have shown normal levels of serum free carnitine and acylcarnitines [12]. These reports are contradictory and no conclusions can be made regarding the carnitine status of patients with CFS. None of these groups studied carnitine or acylcarnitines in urine from their patients or control subjects although urinary levels may provide a better reflection of apparent carnitine deficiency or insufficiency [8]. We report here studies on free, total and esterified (acyl) carnitines in urine and blood plasma from UK patients with CFS and three control groups.

2. Patients and materials

These studies were approved by the Wandsworth Local Research Ethics Committee and informed consent was obtained from all participants before involvement in the studies.

Patients with ME/CFS were defined according to the International Oxford and CDC criteria. The CDC criteria of chronic fatigue syndrome encompass the symptoms of impaired memory and concentration, sore throat, tender cervical or axillary lymph nodes,

muscle pain, headaches of a new type or severity, unrefreshing sleep, post-exertional malaise for >24 h and multi-joint pain. Patients with four or more of these symptoms were judged as meeting the criteria for CFS. None of the patients showed symptoms of depression. A total of 31 patients were recruited, 12 male and 19 female with an overall age range of 21–84 years (Table 1).

Fifteen patients with depression but without CFS and 22 patients with rheumatoid arthritis as a representative inflammatory disease were recruited as control patients (Table 1). All were free from concomitant diseases of liver, kidney and heart and were not receiving medications that would interfere with the analyses being undertaken.

31 healthy subjects were also recruited as a further control group, matched as closely as possible to age and sex distribution of the patients with CFS (Table 1) and also with their general lifestyle. Subjects on medications and/or who were smokers were excluded.

All patients and control subjects were provided with a questionnaire, a shortened form of that of Ray et al. [13], to assess (A) their somatic symptoms and cognitive difficulties and (B) disability and recent course of illness. The scores for each section were combined to provide a Sickness Impact Profile Score (SIPS). The average SIPS for patients with ME/CFS was 65, compared to 12 for the age and sex-matched healthy controls (Table 1).

Table 1
Patient and control groups

Patient group	Number (M=male; F=female)	Age range (median)	Sickness impact profile score ^a (Median and range)
ME/CFS	12 M 19 F	26–63 (42) 21–84 (42)	65 (35–98)
Healthy	12 M 19 F	20–66 (46) 26–79 (42)	12 (0–34)
Depression	6 M 9 F	34–71 (43) 24–59 (47)	**
Rheumatoid arthritis	5 M 17 F	43–67 (59) 36–65 (55)	**

**No scores are given for the latter two groups of control patients because of small numbers and returns of incomplete questionnaires from several patients.

^a Based upon a questionnaire (Ray et al. [12]) to assess (A) their somatic symptoms and cognitive difficulties and (B) disability and recent course of illness. The scores for each section were combined to provide the Sickness Impact Profile Score (SIPS).

Table 2
Carnitine concentrations in plasma from patients with CFS and control groups

	CFS patients	Healthy control subjects	Patients with depression	Patients with rheumatoid arthritis
Number in group	31	31	15	22
Total carnitine	40.5 ± 8.7 (37.5–43.6)	43.1 ± 9.8 (39.6–46.5)	43.1 ± 11.7 (37.2–49.0)	48.6 ± 11.5 (43.8–53.4)
Free carnitine	33.2 ± 7.9 (30.4–36.0)	36.6 ± 9.5 (33.2–39.9)	37.5 ± 9.3 (32.8–42.2)	41.2 ± 10.2 (36.9–45.4)
Acylcarnitines	7.4 ± 3.0 (6.3–8.4)	6.5 ± 3.7 (5.2–7.8)	5.6 ± 3.4 (3.9–7.3)	7.4 ± 4.3 (5.6–9.2)

Expressed in $\mu\text{mol/L}$; mean \pm standard deviation (95% confidence limits).

Patients were asked to provide two samples of urine, the first sample passed in the morning for qualitative metabolite analysis (Chalmers et al., in press) and a timed 6-h collection while continuing the overnight fast, for quantitative metabolite analysis. On presentation to the clinic with the latter urine collection, and while the patients or subjects were still fasting, a sample of blood was collected into heparinised tubes kept on ice. The blood plasma and cells were separated as soon as possible after collection by centrifugation in a refrigerated centrifuge at 4 °C. Samples of urine and blood plasma were stored deep frozen at –20 °C until analysed.

3. Methods

Plasma and urine total and free carnitine were measured using an established radio-enzymatic assay, the free directly and the total following alkaline hydrolysis. Esterified (acyl) carnitine was calculated as the difference between total and free carnitine concentrations [14].

Urinary creatinine was assayed using a modified Jaffé method.

Data were analysed using SPSS for MS Windows software (SPSS UK Ltd., Woking, UK); differences between groups were tested using unpaired independent *t*-tests and Mann–Whitney *U* tests; $p < 0.05$ was taken as indicating significance between means.

4. Results and discussion

Table 2 shows the concentrations of total, free and esterified (acyl) carnitine in plasma from patients with CFS and the control groups (healthy subjects, patients with depression and patients with rheumatoid arthritis), expressed in $\mu\text{mol/L}$ (mean \pm standard deviation and 95% confidence limits). Comparison of CFS patients with healthy controls and patients with depression showed no significant differences between the groups. Comparison with patients with rheumatoid arthritis showed significant differences for total carnitine ($p < 0.01$) and for free carnitine ($p < 0.005$). However, comparison between healthy control subjects and patients with rheumatoid arthritis showed no significant differences. Patients with CFS showed lower levels of plasma free carnitine whereas those with rheumatoid arthritis showed higher levels; thus, the significant differences between these groups reflect the two extremes of the data rather than differences of clinical significance. Patients with rheumatoid arthritis, a chronic inflammatory disease, may have some degree of tissue damage that will lead to release of free carnitine from the tissues, resulting in comparatively higher levels of plasma free (and total) carnitine. Thus, the differences observed reflect clinical differences in the rheumatoid patient group rather than in those with CFS.

Table 3 shows the concentrations of total, free and esterified (acyl) carnitine in urine from patients with

Table 3
Carnitine concentrations in urine from patients with CFS and control groups

	CFS patients	Healthy control subjects	Patients with depression	Patients with rheumatoid arthritis
Number in group	31	31	15	21
Total carnitine	16.8 ± 7.9 (14.0–19.5)	18.8 ± 9.9 (15.3–22.3)	18.6 ± 15.5 (10.8–26.5)	15.1 ± 8.1 (11.7–18.5)
Free carnitine	6.8 ± 5.7 (4.7–8.8)	7.8 ± 6.6 (5.4–10.1)	9.2 ± 11.5 (3.4–15.0)	6.2 ± 5.6 (3.9–8.6)
Acylcarnitines	10.0 ± 2.7 (9.1–11.0)	11.0 ± 4.5 (9.4–12.6)	9.4 ± 4.3 (7.2–11.6)	8.9 ± 3.6 (7.4–10.4)

Expressed in mmol/mol creatinine; mean \pm standard deviation (95% confidence limits).

Table 4
Carnitine excretion rates from patients with CFS and control groups

	CFS patients	Healthy control subjects	Patients with depression	Patients with rheumatoid arthritis
Number in group	31	31	15	21
Total carnitine	7.09 ± 3.68 (5.79–8.39)	8.87 ± 5.85 (6.81–10.93)	7.81 ± 5.57 (4.99–10.62)	5.52 ± 3.18 (4.19–6.85)
Free carnitine	2.81 ± 2.42 (1.95–3.66)	3.83 ± 3.70 (2.52–5.13)	3.77 ± 4.06 (1.71–5.82)	2.32 ± 2.14 (1.42–3.21)
Acylcarnitines	4.28 ± 1.75 (3.67–4.90)	5.04 ± 2.51 (4.16–5.93)	4.04 ± 1.83 (3.11–4.97)	3.20 ± 1.44 (2.60–3.81)

Expressed in as $\mu\text{mol/h}$; mean \pm standard deviation (95% confidence limits).

CFS and the control groups (healthy subjects, patients with depression and patients with rheumatoid arthritis), expressed in mmol/mol creatinine (mean \pm standard deviation and 95% confidence limits). Comparison of CFS patients with all three control groups (healthy subjects, patients with depression and patients with rheumatoid arthritis) showed no significant differences between the groups. Similarly, comparison of the healthy control group with patients with depression or with rheumatoid arthritis also showed no significant differences.

The urinary carnitine data in Table 3 are based upon the urinary creatinine output of the subjects studied. Urinary creatinine reflects the mean muscle mass of the subjects and because the latter might be decreased in patients with CFS and others because of reduced physical activity, data were also calculated in terms of urinary excretion rates, expressed as $\mu\text{mol/h}$. These data are shown in Table 4. Comparison of data for CFS patients with all three control groups showed no significant differences for total carnitine or for free carnitine excretion rates. Comparison of healthy subjects with patients with rheumatoid arthritis showed a significant difference for total carnitine ($p < 0.05$) but no significant difference for free carnitine. Significant differences were observed in excretion rates of acylcarnitines when patients with rheumatoid arthritis were compared with healthy controls ($p < 0.001$) and patients with CFS ($p < 0.05$), but not with patients with depression. Thus, patients with rheumatoid arthritis show lower rates of excretion of acylcarnitines although urinary carnitine concentrations were not significantly different and this suggests possibly slightly reduced or altered renal function in patients with rheumatoid arthritis. Finally, comparison of urinary creatinine concentrations (expressed as mmol/L ; data not shown) showed no significant differences between any of the groups studied suggesting any renal impairment in the rheumatoid group was minimal.

5. Discussion and conclusions

Kuratsune et al. [10] showed, using an enzymatic cycling method utilising NADH-dependent carnitine dehydrogenase, that free and total carnitine levels in serum of Japanese patients with chronic fatigue syndrome were similar to those in healthy control subjects but those of acylcarnitines (esterified carnitines) were decreased. They showed a correlation with ‘performance levels’ (a measure of their patients’ daily activity) in their patients and suggested this reflected abnormality in mitochondrial homeostasis as a basis for the multiple symptoms observed in CFS. Plioplys and Plioplys [9], using a radioenzymatic procedure, found that levels of total, free and acyl carnitine in serum from patients with chronic fatigue syndrome were decreased and found significant correlations between levels of total and free carnitine and symptomatology. They also suggested that this reflected mitochondrial dysfunction, although their results were in contrast to those of Kuratsune et al. [10]. The latter workers confirmed their findings in a group of Swedish patients with CFS although they also observed different levels of free carnitine and of acylcarnitines in serum between Japanese and Swedish patients and control subjects [11]. However, Soetekouw et al. [12], using a radioenzymatic procedure, found no differences between Dutch patients with CFS and age, sex and life-style matched healthy subjects for total, free or acylcarnitines in serum. They also showed, using tandem mass spectrometry, that the concentrations of individual acylcarnitine species did not differ between the two groups.

We have studied here carnitine levels in plasma and urine of UK patients with CFS and compared these to levels in healthy control subjects, patients with depression and patients with rheumatoid arthritis. The results show that there are no significant differences in plasma or urinary total, free or esterified (acyl) carnitine be-

tween patients with CFS and the control groups, or in renal excretion rates of these compounds, other than those that may be ascribed to minor abnormalities in patients with rheumatoid arthritis. The latter may be ascribed to slight tissue damage and to minor impairment of renal function and are in accord with other reports on carnitine homeostasis in patients with rheumatoid arthritis [15]. The data presented here show that, in the CFS patients studied, there are no significant abnormalities of free or esterified (acyl) carnitine. It is thus unlikely that abnormalities in carnitine homeostasis have any significant role in the aetiology of their chronic fatigue.

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References

- [1] Fukuda K, Straus SE, Hickie I, Sharpe MC, Dobbins JG, Komaroff A. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994; 121:953–9.
- [2] Bearn J, Wessely S. Neurobiological aspects of the chronic fatigue syndrome. *Eur J Clin Invest* 1994;24:79–90.
- [3] Sharpe M, Wilks D. ABC of psychological medicine. *Br Med J* 2002;325:480–3.
- [4] Chaudhuri A, Behan PO. Fatigue in neurological disorders. *Lancet* 2004;363:978–88.
- [5] Demitrack MA. Chronic fatigue syndrome: a disease of the hypothalamic–pituitary–adrenal axis? *Ann Med* 1994;26: 1–5.
- [6] Behan WMH, More IAR, Behan PO. Mitochondrial abnormalities in the post-viral fatigue syndrome. *Acta Neuropathol* 1991;83:61–5.
- [7] Chalmers RA, Roe CR, Tracey BM, Stacey TE, Hoppel CL, Millington DS. Secondary carnitine insufficiency in disorders of organic acid metabolism: modulation of acyl-CoA/CoA ratios by L-carnitine in vivo. *Biochem Soc Trans* 1983;11: 724–5.
- [8] Chalmers RA, Roe CR, Stacey TE, Hoppel CL. Urinary excretion of L-carnitine and acylcarnitines by patients with disorders of organic acid metabolism: evidence for secondary insufficiency of L-carnitine. *Pediatr Res* 1984;18: 1325–8.
- [9] Plioplys AV, Plioplys S. Serum levels of carnitine in chronic fatigue syndrome: clinical correlates. *Neuropsychobiology* 1995;32:132–8.
- [10] Kuratsune H, Yamaguti K, Takahashi M, Misaki H, Tagawa S, Kitani T. Acylcarnitine deficiency in chronic fatigue syndrome. *Clin Infect Dis* 1994;18(suppl. 1):S62–7.
- [11] Kuratsune H, Yamaguti K, Lindh G, Evengard B, Takahashi M, Machii T, et al. Low levels of serum acylcarnitine in chronic fatigue syndrome and chronic hepatitis type C, but not seen in other diseases. *Int J Mol Med* 1998;2:51–6.
- [12] Soetekouw PMMB, Wevers RA, Vreken P, Elving LD, Janssen AJM, van der Veen Y, et al. Normal carnitine levels in patients with chronic fatigue syndrome. *Neth J Med* 2000;57:20–4.
- [13] Ray C, Weir WR, Cullen S, Phillips S. Illness perception and symptom components in chronic fatigue syndrome. *J Psychosom Res* 1992;36:243–56.
- [14] de Sousa C, English NR, Stacey TE, Chalmers RA. Measurement of L-carnitine and acylcarnitines in body fluids and tissues in children and in adults. *Clin Chim Acta* 1990; 187:317–28.
- [15] Krahenbuhl S, Willer B, Bruhlmann P, Hoppeler H, Stucki G. Carnitine homeostasis in patients with rheumatoid arthritis. *Clin Chim Acta* 1999;279:35–45.