

## Abnormalities in Essential Amino Acids in Patients with Chronic Fatigue Syndrome

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### Abstract

*A laboratory study was performed comparing the patterns of urinary amino acid excretion in patients with chronic fatigue syndrome (CFS) with a control group of subjects without fatigue or allergy. Fifteen out of 21 patients in the CFS Group showed abnormal patterns with both reduced and increased excretion of some acids. In contrast, the control subjects showed only increased excretion of some acids, and a finding present in only four out of 20 subjects.*

**Keywords:** chronic fatigue syndrome, urinary amino acids, abnormality.

### INTRODUCTION

Medical science has been slow to adapt to new developments in biochemistry particularly with regard to nutrition, where the thesis that normal nutrition will always be the consequence of a normal diet has often been repeated, largely without effective challenge. In recent years a codicil has often been added to the above statement, to the effect that a normal diet will result in normal nutrition if the subject is in good health as a result of studies such as the one presented here.

This study does not concern 'normal' subjects, but rather people with chronic fatigue syndrome (CFS). The condition itself is probably not new, but in spite of this has been slow to gain recognition. It is still academically respectable to be uncertain that it exists [1]. Among those who do admit to the existence of the syndrome there are those who would claim that it is purely a psychological entity [2], but an increasing body of evidence exists showing that physical abnormalities can be established in patients at a research level [3]. However, at a routine clinical level, there is not as yet any single laboratory test which provides unequivocal confirmation of a physician's clinical diagnosis. In consideration of this, it is now accepted among researchers that a clinical definition must be drawn and it is the following which have been used to define the group of patients under study.

- (1) A syndrome characterized by fatigue as the principal symptom.
- (2) A syndrome of definite onset that is not life long.
- (3) The fatigue is severe, disabling and affects physical and mental functioning.
- (4) The symptom of fatigue was present for a minimum of 6 months during which time it was present for more than 50% of the time.

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- (5) Other symptoms may be present, particularly myalgia and sleep disturbance.
- (6) Patients with other established medical causes of fatigue are excluded [4].

Amino acid nutrition is certainly one of the areas where the assumption of normality has frequently been made. There are eight amino acids considered biochemically to be essential nutrients which must be provided by the diet and cannot be synthesized metabolically within the body [5] although other amino acids may be conditionally essential in children and in stress situations [6]. Vegetable sources of protein do not individually supply all eight, but a combination of vegetable sources will do so [7]. For those whose diet includes fish and meat a full range of bio-available amino acids is provided by any single source [8]. Therefore, provided that protein nutrition is adequate, any assessment of bodily amino acid status which reveals deficit must result from either a failure to absorb, or increased utilization within the body. In 1988 Buist proposed that the second of these two causes might operate in CFS in relation to the high sulphur amino acids cysteine and methionine which have an important role in detoxifying xenobiotics such as aromatics and organochlorines [9].

As a result of this statement it was decided to use a newly available laboratory test to assess whether amino acid nutrition was abnormal in patients with CFS.

## MATERIALS AND METHODS

### **Selection of Patients**

Subjects for study were drawn from sequential patients referred by their general practitioners to one of the authors (K.K.E.) for management of their chronic fatigue. All were considered to meet the definition quoted in the previous section. Twenty-one patients were tested—10 males and 11 females. The investigation of amino acid status was performed as part of a second tier of laboratory studies on these patients with the exception of the last four in the series (one male, three female) where it was carried out on initial screening. These last four patients were on average UK diets, and were not taking any nutritional supplements. The remainder of the patients had been tested prior to the amino acid assay for B vitamin status (by functional analysis [10]), for common minerals (by sweat analysis [11]) and for gut fermentation by blood alcohol estimation after glucose challenge [12]. Consequent on these tests, all subjects (other than the last four) were taking a daily B vitamin supplement, together with oral mineral supplementation in physiological dosage when appropriate. No supplement contained amino acids. Seven of the males and eight of the females were estimated to have had positive alcohol tests, and therefore at the time of the amino acid testing were on a modified diet, low in refined carbohydrates, yeasts and mould-containing foods, but unrestricted in terms of protein intake [13]. The remainder continued on the diet they had previously been consuming at the time of testing. It was therefore not considered that any of the study group were deficient in amino acid intake.

A second group was derived by random selection from attenders at the Biolab Medical Unit for a variety of conditions, where allergy and fatigue were not present. Their reasons for attending were for neurological conditions, diabetes mellitus, psychiatric conditions or for a health check. They comprised eight males and 12 females. No attempt was made to match age and sex in the control group. The study group ranged in age from 15 to 62 years (a single subject) with no other subject being over the age of 52. One of the control group was nine-years old and the oldest 52. There were no statistically significant age differences between the two groups.

## Laboratory Study

*Measurement of Urinary Amino Acids.* Amino acid analyses were performed on 24 h urine samples using a fully automated gradient elution HPLC system with fluorimetric detection (Gilson Medical Electronics, Villers-le-Bel, France). The separation was carried out on a C18 microsorb reverse-phase column (Rainin Instrument Co. Inc., Woburn, MA, USA) following pre-column derivatization with the fluorogenic reagent o-phthaldehyde-2-mercaptoethanol (OPA-MCE), as per the method of Turnell & Cooper [14]. Proline and hydroxyproline, which are not detected with this method, were measured using the chloramine-T-oxidation followed by OPA-MCE derivatization technique of Cooper *et al.* [15].

The mean within-run coefficient of variation (CV) was 6%, with a range from 3.2% (Serine) to 12.3% (Ornithine).

Normal values for the test were derived from a group of 38 normal volunteers (20 female, 18 male) who at the time of sampling were healthy, on a normal diet, and receiving no medication or nutritional supplements. Normal values (all in  $\mu\text{mol}$  per 24 h) are as follows: threonine f, 80–450, m, 90–450; valine 10–55; methionine 10–70; leucine 10–100; isoleucine 5–70; phenylalanine 25–145 and tryptophan 30–145.

The values quoted for urinary amino acids are comparable with other laboratory reference ranges. Inter laboratory comparison for amino acid concentrations is also favourable ( $r=0.80$  ornithine to 0.97 phenylalanine [unpublished data]).

## RESULTS

The results for the two study groups are shown in Figs 1 and 2. It will be seen that a modest number of patients in the control group (four out of 20) showed increased excretion of individual amino acids. There were no deficiencies, and the rest were normal. By contrast, the fatigue syndrome patients showed abnormalities in 15 out of 21 patients with both widespread deficiencies as well as raised levels. Methionine, deficient in 11 out of 21 subjects, was the commonest abnormality.

## DISCUSSION

Comparison of the two groups show marked and widespread abnormalities in essential amino acid excretion in the CFS group although this is not a universal finding. This may

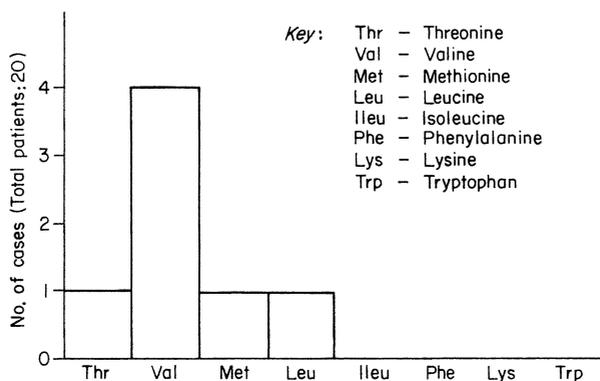


FIG. 1. Abnormalities in essential amino acid excretion in control group.

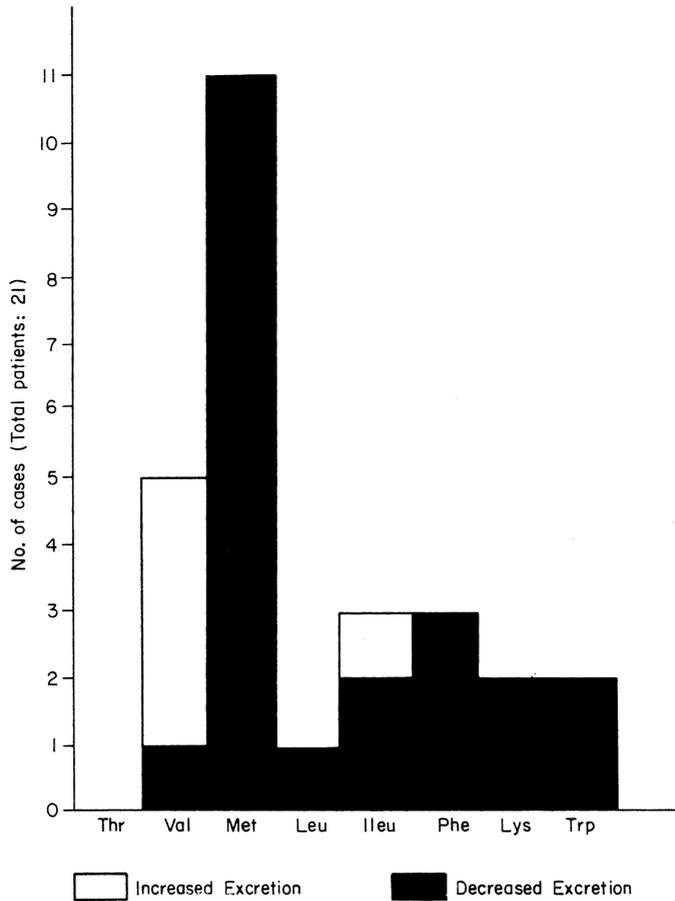


FIG. 2. Abnormalities in essential amino acid excretion in chronic fatigue syndrome group. Key is as in Fig. 1.

reflect the imprecise nature of the diagnosis. Abnormalities may have been present at some other stage in the disease, but not at the time of study.

Traditionally it is assumed that deficiencies of essential nutrients are not to be expected in patients living in an adequately fed environment in the Western world. However, modern nutritional research is increasingly challenging this assumption, and now many studies can be quoted indicating that deficiencies of trace minerals [16], essential fatty acids [17] and vitamins [18] do occur in subjects ostensibly consuming a nutritionally adequate and balanced diet. However, in the majority of cases it cannot yet be stated as to whether these constitute a failure of absorption, increased metabolic demand or increased excretion. In view of the fact that we have demonstrated deficiencies in amino acid status in CFS, deficiencies of other nutrients are also likely and this is an appropriate field for further study.

Having found such abnormalities it is necessary to consider possible causes. This study did not seek to establish a cause, but solely to determine whether a problem was present. Many cases of CFS are post-viral, and enteroviruses have been incriminated [19]. Such viruses will cause an element of gut damage, and it may well be that implicit in this damage will be diminution of normal small bowel absorptive capacity. This could be investigated

by further biochemical tests but so far this work has not been done, and such a conclusion is therefore at present speculative. Could the specific nature of the findings yield an alternative explanation in relation to the roles of individual amino acids, and their possible utilization in gut mucosa? Low status has been found in this present study for all essential amino acids except threonine, although most are only low in single cases, methionine clearly stands out as being deficient in half the patients, with phenylalanine being next frequent, but only in three out of 21. In terms of raised levels only valine stands out, as being raised in four subjects: however, it can also be deficient. A possible explanation may be advanced for the low status of methionine, which is concerned in detoxifying xenobiotics such as aromatics and organochlorines [9]. It is also involved in anti-oxidant processes in free radical scavenging. Methionine (Met) is an essential sulphur-containing amino acid in human metabolism where it performs three major functions.

As S-adenosyl-L-methionine (the first step of Met metabolism) it acts as a methyl ( $-\text{CH}_3$ ) group donor for a wide variety of transmethylation reactions in the body, particularly in the brain [20]. It can also act as a donor of sulphhydryl groups ( $-\text{SH}$ ), helping in the detoxification of xenobiotics etc. within the liver. Finally, it is the essential precursor of other sulphur-containing amino acids, namely cysteine and taurine. It is also a precursor for the synthesis of the tripeptide glutathione (L-glutamyl-L-cysteinyl-glycine). This function is very important as these molecules play an important part in the antioxidant capacity of the body [21].

Methionine has been shown to reduce circulating histamine levels by increasing the rate of its breakdown. This feature has been exploited in the treatment of allergy and also in the treatment of depressive illness [22].

In the context of CFS, it is conceivable that a significant fall in Met concentrations may be, in part at least, responsible for the depressive and allergic aspects of the illness. In addition, patients suffering with CFS do appear to have a reduced antioxidant capacity as measured by such indices as erythrocyte glutathione peroxidase (GSHPx), a selenium-dependent antioxidant enzyme (Hunnisett, unpublished observations). Such a situation would be exacerbated by a fall in the concentration of the precursors of antioxidant molecules. Clearly there is an indication for supplementation of Met in this situation.

Vitamin B6 deficiency may increase valine excretion, while zinc deficiency may cause low absorption of branch chain amino acids with consequent reduced output. However, this does not explain fully the scattered pattern of low levels of other amino acids, nor the fact that valine and isoleucine could be either depleted or raised.

Although it appears from the above that we cannot yet give a full explanation for the findings of this survey, in view of the fact that patients with CFS are ill, and suffering at the present time, we should also consider treatment. Anecdotally, many patients report help from 'cocktail' amino acid preparations put together by commercial organizations supplying nutritional supplements. From our study it would appear that such a policy may be therapeutically justified, provided that the preparations are safe. Tryptophan preparations have been implicated in eosinophilia myalgia syndrome, with eosinophil counts in excess of  $2000 \text{ mm}^{-3}$ , myalgia, arthralgia, fever, oedema and rash, and in August 1990 they were withdrawn from sale in the UK both from nutritional supplements and licensed pharmaceutical preparations [23]. It has never been contended that the essential amino acid itself is toxic at physiological levels, as this must be by definition impossible, and the toxicity must therefore result from a contaminant [24]. At present, therefore, it would seem that if supplements are a justified treatment for established deficiency there are no products available for treatment of tryptophan deficiency which would be acceptable to the Medicines Inspectorate. This situation requires urgent consideration.

There may however be an alternative treatment if the problem results from gut damage. If this is the case, since the dietary intake is likely to be adequate, a better therapeutic result

might be achieved by steps which help to heal the damaged organ, and the role of cytoprotective agents, such as tri-potassium dicitrato bismuthate, needs to be explored.

A literature search has been made of all world references, in any language, to deficiencies of essential amino acids in subjects with a normal nutritional intake [25] and references are remarkably scant. A single report in 1989 exists to a related enzyme deficiency (methionine synthase) [26] and tryptophan deficiency stupor is the subject of a single report from Sweden as a new psychiatric syndrome [27] in 1982. In this instance, diet may have been inadequate as the patients suffered either from Carcinoid or Parkinsonism of such severity that one subject could not feed herself. A total of four cases are reported. In addition, Truss [28] reported abnormalities in 24 patients with "symptoms typical of mould sensitivity and yeast susceptibility". Urinary amino acid excretion over 24 h was measured using a Beckman Amino Acid Analyzer. Normal values for the estimations were taken from figures obtained by Bio Science Laboratories, Los Angeles, CA. A total of 41 amino acids were studied, from which we have abstracted seven out of the eight essential amino acids. Tryptophan was not estimated. The results show (for essential amino acids) deficiencies only and are presented in Fig. 3. Truss did not find the raised levels seen in some of our patients. Both the laboratory normals and laboratory methodology were different, and the patients were not an identical group, although many of our patients may well have had mould and yeast problems. The feature to which we would call attention is that both his patients and our CFS patients are more nearly alike to each other than to the control group or to normal subjects. Amino acid levels require more study, both in CFS and established allergic groups as this may illuminate the nature of the disease process.

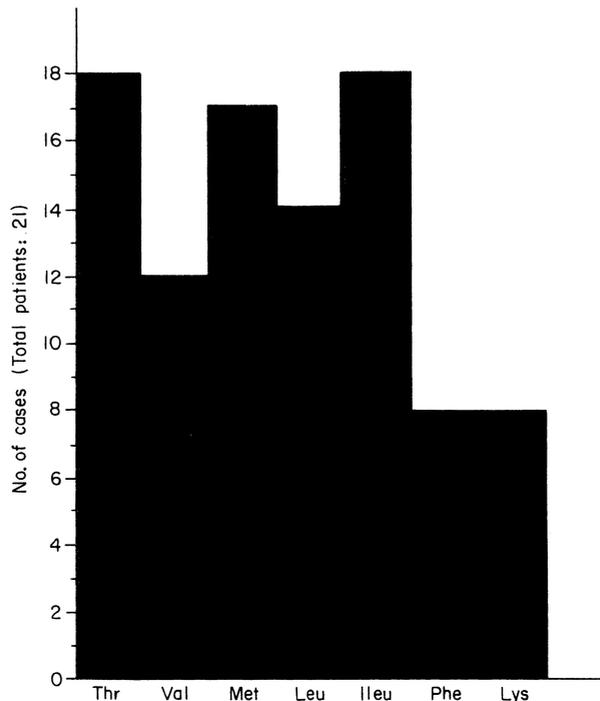


FIG. 3. Abnormalities in essential amino acid excretion in a group of mould and yeast sensitive patients. After Truss [28]. NB: Tryptophan not measured. Key is as in Fig. 1.

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