Treatment Study of Methylation Cycle Support in Patients with Chronic Fatigue Syndrome and Fibromyalgia

by

Neil Nathan, M.D.
and
Richard A. Van Konynenburg, Ph.D.

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Introduction

At the 2007 conference of the IACFS, one of us (RVK) proposed a hypothesis for the pathogenesis of CFS, called the Glutathione Depletion—Methylation Cycle Block hypothesis [1].

This hypothesis is based on research that had been done by James et al. in autism [2], and a recognition of similarities between the biochemical abnormalities in autism and those in CFS. Deth et al. [3] have published a somewhat similar hypothesis for autism.

Shortly after the conference, with the help of a CFS patient (name withheld to protect privacy), RVK extracted part of the comprehensive treatment program developed by Amy Yasko, Ph.D., N.D., for autism and adult neurological diseases [4], and suggested the use of the resulting seven supplements for treating CFS. Initial experience with this treatment led to a further reduction to five supplements.

After obtaining some positive clinical experience with the five-supplement protocol in his private practice, one of us (NN) proposed this treatment study to evaluate it in a more controlled manner. Funding for lab testing was obtained from a private source.

Objectives

1. To test the Glutathione Depletion—Methylation Cycle Block hypothesis for CFS

2. To assess the potential efficacy of a nonpharmacologic treatment for chronic fatigue syndrome (CFS) that is based on this hypothesis and is designed to support the methylation cycle.

Review of the Glutathione Depletion—Methylation Cycle Block (GD-MCB) Hypothesis for the Pathogenesis of CFS [1]

- An individual inherits a genetic predisposition (polymorphisms in several of certain genes) toward developing CFS. (This genetic factor is more important for the sporadic cases than for the cluster cases of CFS.)

- The person then experiences some combination of a variety of possible stressors (physical, chemical, biological, and/or psychological/emotional) that place demands on glutathione.

- Glutathione levels drop, producing oxidative stress, removing protection from cobalamin (vitamin B12) and allowing toxins to accumulate.

- Toxins react with cobalamin, lowering the rate of formation of methylcobalamin.

- Lack of sufficient methylcobalamin inhibits the activity of methionine synthase, placing a partial block in the methylation and folate cycles.

- Sulfur metabolites drain excessively through the transsulfuration pathway to form cysteine.

- Much of the cysteine is oxidized to cystine because of the state of oxidative stress, and is therefore not available for the synthesis of glutathione. An alternative pathway initiated with catalysis by cystathionine gamma lyase carries the cystine on to form hydrogen sulfide and thiosulfate, and the latter is excreted in the urine.

- An interaction (vicious circle) is established between the partial block in the methylation cycle and glutathione depletion, and the disorder therefore becomes chronic.

- A wide range of symptoms results from these chronic abnormalities in the basic biochemistry of the cells.

- The dysfunction of the detoxication system and the immune system that results from this combination allows toxins and infections to accumulate over time, which increasingly produce effects of their own.

- Treatment should be directed primarily at increasing the activity of methionine synthase. The resulting normalization of the methylation cycle, the folate metabolism and glutathione levels will restore function to the immune system and the detoxication system as well as to a wide range of other parts of the overall biochemistry.

- It can be expected that die-off of pathogens and mobilization of stored toxins will initially produce some exacerbation of symptoms, but improvements will be experienced as the body burdens of toxins and active infections are decreased.

Characteristics of this Study

Type: Open-label clinical study

Setting: A single private practice in Springfield, Missouri

Informed consent: Patients signed forms after explanation of the study and its possible risks.

Duration of treatment: Six months (However, note that after the 6-month study period, individualized treatments were added to the basic protocol for an additional 3 months.

Outcomes after this final period were not analyzed as part of the study, because treatments were not uniform during the final period.)

Outcome measures: Objective testing and self-rating of symptoms (Details are given later in this paper.)

Restrictions on medications and additional supplements: None, except that they and their dosages were not to be changed during the study without the knowledge and agreement of one of us (NN).

Subjects

Number: Thirty patients who presented sequentially to the practice of one of us (NN) were started on the treatment. One dropped out at three months for a reason unrelated to response to the treatment. The remaining 29 patients were treated for the six-month uniform-protocol period of this study. Of these 29 patients, eight did not meet the selection criteria (see below). Therefore, 21 patients were included in the statistical analysis of the results for the six-month period.

Selection criteria: Those selected to be included in the statistical analysis of results were required to satisfy the Fukuda et al. 1994 case definition for CFS [5] and were also required to have experienced post-exertional fatigue and malaise. They were not required to meet the American College of Rheumatology 1990 criteria for fibromyalgia [6], but 18 of the 21 patients who were selected for the statistical analysis also met these criteria.

Sex: All female

Ethnicity: All Caucasian

Ages: 33 to 84 (mean—52) years

Durations of illness: 1 to 20+ years

Additional diagnoses:
Migraine headaches—15 patients
Irritable bowel syndrome—13
Chronic sinus infections—11
Endometriosis—6

Restless leg syndrome—5
Mononucleosis (Epstein—Barr virus)—5
Mold exposure and/or toxicity—5
Multiple chemical sensitivity—4
Lyme disease (previously treated)—2
Interstitial cystitis—1
Mycoplasma infections—1
Chronic vulvitis—0

Histories of previous treatment:
These patients had exhibited partial response to treatment ranging from one to twelve years in duration with a protocol that included evaluation and treatment of adrenal, thyroid and sex hormones; food allergies; intestinal dysbiosis; heavy metal toxicity; infections (EBV, Lyme disease, mycoplasma); mold exposure; magnesium deficiency; and other nutritional imbalances.

Initial General Questionnaire

The patients were given an initial questionnaire that requested the following information:

Starting date of treatment
Name
Age
Date of birth
Sex
    Family history of these conditions (yes or no?), if so, which relatives?
Date of onset of symptoms
Sudden onset (yes or no?)
Cause of onset, if known
Date of diagnosis of chronic fatigue
Date of diagnosis of fibromyalgia
Other diagnoses (listed in previous section above)
Current medications
Current supplements

The initial general questionnaire also included questions to ascertain whether the patients met the Fukuda et al. case definition for CFS [5] and the ACR criteria for fibromyalgia [6], and whether they had experienced post-exertional fatigue and malaise.

Supplement Protocol

The treatment protocol used in this study was extracted from the full treatment program developed by Yasko for the treatment of autism and adult neurological diseases [4]. It consisted of five supplements:

1. FolaPro [7] (5-methyl tetrahydrofolate): ¼ tablet (200mcg) daily
2. Intrinsi B12/folate [8]: ¼ tablet daily (Combination of [folic acid, 5-methyl tetrahydrofolate, and folinic acid] (200 mcg), cyanocobalamin (125 mcg), calcium (22.5 mg), phosphorus (17.25 mg), and intrinsic factor (5 mg))
3. General Vitamin Neurological Health Formula [9] (a multivitamin, multimineral supplement including antioxidants, trimethylglycine, nucleotides, supplements to support the sulfur metabolism, a high ratio of magnesium to calcium, and no iron or copper): starting with ¼ tablet and increasing the dosage as tolerated, to 2 tablets daily
4. Phosphatidyl Serine Complex [10] (phospholipids and fatty acids): 1 softgel capsule daily
5. Activated B12 Guard [11] (hydroxocobalamin): 1 sublingual lozenge (2,000 micrograms) daily

Objective Testing

1. Methylation pathways panel [12]:
(performed initially and at 3 and 6 months) This panel was the main objective diagnostic used in this study. It evaluates the status of glutathione, the methylation cycle and the folate metabolism, and was used to determine the effects of the treatment on these aspects of the metabolism. This panel includes the following:

Glutathione (plasma)
Oxidized glutathione (plasma)
Adenosine (plasma)
S-adenosylmethionine (RBC)
S-adenosylhomocysteine (RBC)
5-methyl tetrahydrofolate (plasma)
10-formyl tetrahydrofolate (plasma)
5-formyl tetrahydrofolate (plasma)
Tetrahydrofolate (plasma)
Folic acid (plasma)
Folinic acid (whole blood)
Folic acid (RBC)

It is important to note that the sample collection kit for this panel incorporates enzyme inhibitors to prevent the reduced form of glutathione from oxidizing during shipping and storage of samples. This is necessary because glutathione readily oxidizes after removal from the body.

2. Thyroid panel (TSH, total T4, total T3) [13]: (performed initially and at 3 months). Thyroid peroxidase antibody was also measured initially and again at 6 months, if found to be positive. These tests were run to document hypo- or hyperthyroidism and to test for Hashimoto's thyroiditis. Also, it was desired to determine whether the treatment would correct hypothyroidism, as had been reported anecdotally in a small number of cases treated prior to this study.

3. Other Objective Testing: Additional objective testing was performed primarily to further define the characteristics of the tested group in order to facilitate comparison to other patient groups. Another purpose was to assist in choosing individualized treatments for the additional three-month period of treatment beyond the six-month duration of this study. A third purpose was to determine whether the values of the tested parameters would correlate with the responses of the individual patients to the basic protocol, as a means of predicting the applicability of this treatment to other patients. The additional tests are described below, and the results are summarized later in this paper, but some of the analysis and application of these results will be carried out in the future.

a. Characterization of polymorphisms associated with the methylation cycle [14]: (AHCY-01, BHMT-08, CBS C699T, COMT V158M, and MTR A2756G) (one time only).

AHCY-01 is a polymorphism in the gene coding for the enzyme enzyme S-adenosylhomocysteine hydrolase, which catalyzes the removal of adenosine from S-adenosylhomocysteine to form homocysteine, in the methylation cycle.

BHMT-08 is a polymorphism in the gene coding for the enzyme betaine homocysteine methyltransferase, which catalyzes an alternative pathway from homocysteine to methionine in the methylation cycle in the liver and kidneys.

CBS C699T is a polymorphism in the gene coding for the enzyme cystathionine beta synthase, which catalyzes the reaction of homocysteine with serine to form cystathionine, at the junction between the methylation cycle and the transsulfuration pathway.

COMT V158M is a polymorphism in the gene coding for the enzyme catechol-O-methyltransferase, which uses methyl groups from S-adenosylmethionine in the methylation cycle to assist in the metabolism of catecholamines and estrogens.

MTR A2756G is a polymorphism in the gene coding for the enzyme methionine synthase, which catalyzes the reaction of homocysteine and 5-methyltetrahydrofolate to form methionine and tetrahydrofolate, at the junction of the methylation and folate cycles.

These polymorphisms were characterized in response to the work by Yasko [15], who uses polymorphism data to tailor the treatment of individual patients.

c. Human Leukocyte Antigen (HLA) DR DQ typing [16]: (one time only, on 20 of the patients). This panel characterizes inherited polymorphisms in the genes that code for the Class II human leukocyte antigen molecules. These molecules are membrane proteins that are used by cells to display peptide antigens for recognition by helper T cells. This panel was run in response to the work reported by Shoemaker el al. [17] of correlations between particular HLA genotypes and susceptibility to mold illness, Lyme disease and other biotoxin-related disorders that can be associated with CFS.

d. Functional Acuity Contrast Testing (FACT) [18]: (performed initially and at 3 and 6 months). This is a vision test conducted with eye charts that measures the ability to sense contrast over a range of scales in images. This test was run also in response to the work of Shoemaker [16], who finds it to be a sensitive diagnostic for the presence of neurotoxins in the brain, which occurs in biotoxin-related disorders.

e. C4a [19]: (performed at 6 months). C4a is a product of the splitting of the C4 protein as a result of activation of the complement system. The complement system is a collection of circulating and cell membrane proteins that play important roles in host defense against microbes and in antibody-mediated tissue injury. C4a stimulates inflammation. This test was added later in the study in response to the work of Shoemaker et al. [20] and Stricker et al. [21], indicating respectively that its elevation is associated with acute and chronic Lyme disease. Earlier work by Sorenson et al. [22] had also shown elevation of C4a in CFS patients in response to exercise.

f. TGF beta-1 [23]: (performed at 6 months). Transforming growth factor beta-1 is a member of the TGF beta family. These are cytokines produced by activated T cells, mononuclear phagocytes, and other cells, whose principal actions are to inhibit the proliferation and differentiation of T cells, to inhibit the activation of macrophages, and to counteract the effects of proinflammatory cytokines [24]. This test was added later in the study in response to the work of Shoemaker [25]. Earlier measurements of TGF beta or TGF beta-1 in CFS patients have been reported by Chao et al. [26], Peterson et al. [27], MacDonald et al. [28], Bennett et al. [29], Shin et al. [30], Kennedy et al. [31], and Tomoda et al. [32].

Enumeration of Symptoms and Self-Rating of Outcome Measures

The patients were asked to mark their symptoms on a checklist (initially and at 6 months) that included 38 symptoms (see appendix). The patients were also asked to rate five outcome measures initially and at 3 and 6 months on visual analog scales ranging from 1 to 10. These measures consisted of energy, sleep, mental clarity, freedom from pain, and overall feeling of wellbeing. In addition, at 3 and 6 months they were asked to estimate their percentage of improvement.

Conduct of the Clinical Study

The clinical study was conducted by one of us (NN) in his private practice, with the help of administrative and nursing staff.

The study was explained to each of the patients, including the purposes, the protocol, and the possible risks. Each patient signed an informed consent and responded to the initial general questionnaire.

The patients were supplied with the supplements in the protocol at cost.

The patients were given the following instructions with respect to the supplement protocol:

- The first two supplement tablets are difficult to break into quarters. We recommend that you obtain (from any pharmacy) a good-quality pill splitter to assist with this process. They can, alternatively, be crushed into powders, then separated on a flat surface, and the powders can be mixed together. They can be taken orally with water, with or without food.
- Occasionally these can make patients sleepy, so some take them at bedtime. They can be taken any time of day, with or without food.
- GO SLOWLY. Occasionally, as the methylation cycle blockages are released, toxins are released and processed by the body, and this can lead to an exacerbation of symptoms. IF THIS HAPPENS, try smaller doses, every other day. SLOWLY work up to the full dosages. If you have questions, please call our office to discuss them.

The objective testing as described above was performed on the patients initially and after 3 and 6 months of treatment. After 3 and 6 months of treatment, they also responded to follow-up questionnaires that included general questions about response to treatment, a symptom checklist and self-rated visual analog scales for the outcome measures described earlier.

As noted earlier, one patient dropped out of the study at 3 months for a reason not related to response to the treatment. The remaining 29 patients completed 6 months of treatment.

Results

Various patients reported some early exacerbation of symptoms, which in most cases was followed by a greater improvement in symptoms. Three of the patients found it necessary to decrease their dosage frequency to every second or third day for several days, until they could tolerate the full daily dosage schedule.

Sixteen of 30 patients (53%) reported an initial worsening of symptoms, beginning in most of these cases within 3 or 4 days, but in some cases beginning at up to 2 weeks. Most of the symptoms were mild, and none of the patients discontinued usage of the supplements during the first 3 months. The most common side effects were gastrointestinal (pain, cramps, constipation, or diarrhea), reported by 6 out of 30 patients or 20%; increase in pain, reported by 4 out of 30 or 13%; and increase in fatigue, reported by 3 out of 30 or 10%. Other symptoms, reported by one patient each, were a decrease in appetite, poor sleep, weak legs, flu-like symptoms, and an increase in anxiety and depression.

For those who experienced improvement, the time to self-reported improvement on the protocol was an average of 5.6 weeks, with a range from immediate improvement (which was rare) to as long as 8 weeks before improvement was experienced.

Results of the methylation pathways panel are shown in Table 1.
The numbers shown are mean values for 21 patients, with the standard deviations in parentheses (except that the values for one patient were omitted from the red blood cell folate
Table 1. Methylation Pathways Panel Results

Metabolite	Reference value	Time on treatment (months)		
		0	3	6
Glutathione (GSH) (plasma) (nmol/mL)	4.65 (0.42)	3.31* (0.49)	3.97** (0.57)	4.34** (0.67)
Glutathione (oxidized) (GSSG) (plasma) (nmol/mL)	0.33 (0.09)	0.48* (0.15)	0.50 (0.14)	0.53 (0.15)
Adenosine (plasma) (nmol/L)	191 (11.5)	178 (80)	202 (76)	221# (64)
S-adenosylmethionine (SAM) (RBC) (mcmol/dL)	238.5 (8.8)	214* (20)	227**** (16)	234** (14)
S-adenosylhomocysteine (SAH) (RBC) (mcmol/dL)	43.5 (2.8)	45.8 (13.2)	48.4 (11.0)	51.7 (10.9)
5-Methyl-tetrahydrofolate (plasma) (nmol/L)	8.4 to 72.6	14.2 (9.6)	17.2 (10.2)	20.9*** (11.4)
10-Formyl-tetrahydrofolate (plasma) (nmol/L)	4.8 (1.7)	1.1* (0.5)	1.5**** (0.5)	1.9*** (0.9)
5-Formyl-tetrahydrofolate	6.4	1.3*	1.5	2.0

(plasma) (nmol/L)	(2.6)	(1.4)	(1.2)	(1.8)
Tetrahydrofolate (plasma) (nmol/L)	3.7 (1.6)	2.2* (1.8)	2.4 (1.9)	3.2**** (2.0)
Folic acid (plasma) (nmol/L)	16.8 (3.9)	19.9 (13.0)	24.0 (12.2)	27.7**** (10.7)
Folinic acid (whole blood) (nmol/L)	22.2 (6.6)	9.1* (3.2)	10.4 (3.1)	11.5**** (3.3)
Folic acid (RBC) (nmol/L)	950 (275)	427 (109)	457 (121)	476 (132)
GSH / GSSG	14.1	7.48 (2.47)	8.54 (2.51)	8.75 (2.91)
SAM / SAH	5.48	5.01 (1.35)	4.93 (1.10)	4.69 (0.86)

Numbers shown in parentheses are standard deviations.
* $p < 0.0005$ with respect to the reference value.
The following symbols indicate the p values of measured parameters with respect to their values at time 0: ** $p < 0.0005$; *** $p < 0.005$; **** $p < 0.02$;
# $p < 0.04$.

distributions, because of a clearly spurious value). The laboratory reference values shown in this table were determined by Vitamin Diagnostics, Inc. They are each based on measurements in at least 120 volunteer male and female medical students between the ages of 20 and 40, nonsmoking and with no known chronic diseases [33]. They are shown as mean values with standard deviations, except that the lab reference range is given for 5-methyl-tetrahydrofolate. This folate vitamer's reference range is quite broad, presumably because of variations between individuals in the prominence of the so-called folate trap phenomenon, in which lowered activity of methionine synthase causes 5-methyl-tetrahydrofolate to leak out of cells into the blood plasma.
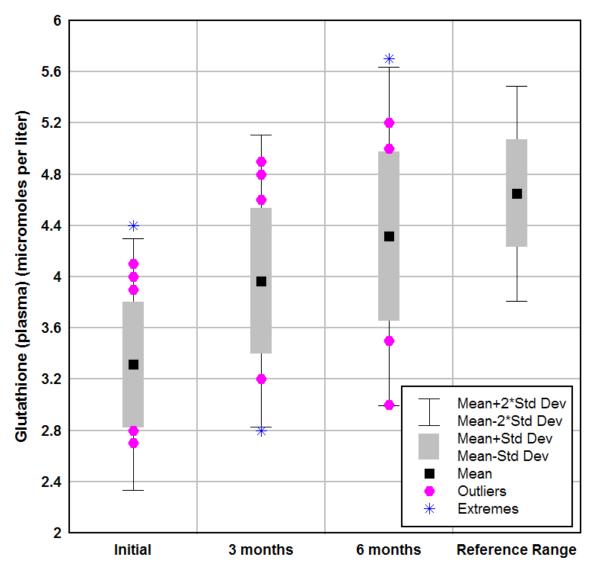
The reference values for the ratios GSH/GSSG and SAM/SAH were calculated from the mean laboratory reference values of the parameters, but standard deviations for these ratios were not known. The p-values were determined using the one-tailed Student's t distribution. Because the distributions of values for 5-methyl-tetrahydrofolate and tetrahydrofolate were highly skewed, these values were transformed to their reciprocals before the statistical analysis was done on them, in order to obtain distributions more nearly normal. Note that folinic acid and 5-formyl-

tetrahydrofolate are the same chemical species, measured both in plasma and in whole blood in this panel.

As can be seen from the table, the initial mean values of glutathione (GSH), oxidized glutathione (GSSG), S-adenosylmethionine (SAM), and four of the folate vitamers were significantly different from their laboratory reference values. In particular, GSH was significantly depleted, as was SAM.
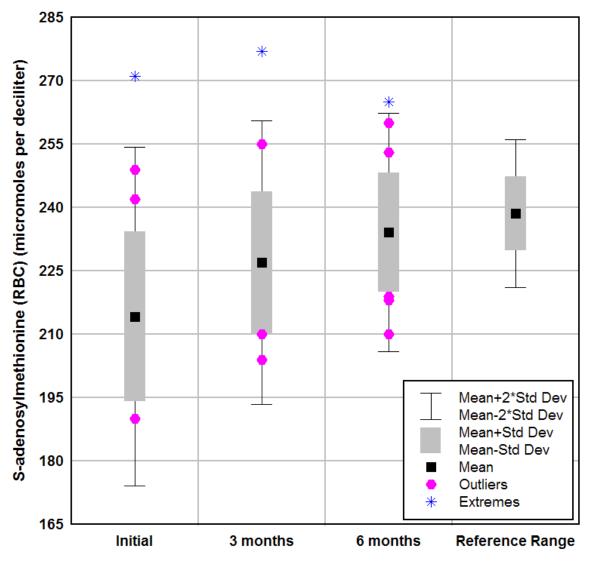
It can also be seen that significant increases in the levels of GSH, SAM and 10-formyl-tetrahydrofolate were observed after 3 months of treatment. After 6 months of treatment, significant increases were observed in GSH, adenosine, SAM, 5-methyl-tetrahydrofolate, 10-formyl-tetrahydrofolate, tetrahydrofolate, plasma folic acid and red blood cell folinic acid. Adenosine and plasma folic acid started below, and rose to values above, their laboratory reference values. The ratios GSH/GSSG and SAM/SAH were initially below their laboratory reference values. Although the former rose and the latter decreased during the treatment, these changes were not statistically significant.

Glutathione is plotted in Figure 1, S-adenosylmethionine in Figure 2, and tetrahydrofolate in Figure 3.

Time on treatment

Figure 1. Plasma reduced glutathione in 21 CFS patients
initially and after 3 and 6 months of treatment,
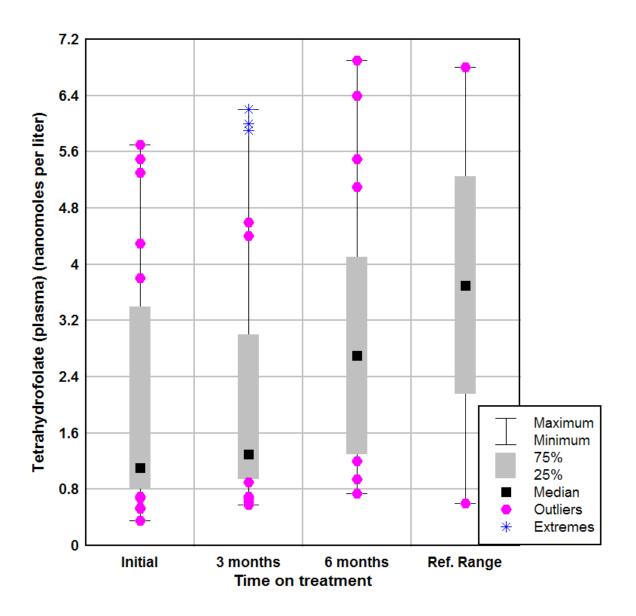compared to the laboratory reference range

Figure 2. Plasma S-adenosylmethionine in 21 CFS patients
initially and after 3 and 6 months of treatment,
compared to the laboratory reference range

Tetrahydrofolate is plotted because of its role as the hub of the folate metabolism. The coenzyme forms of folate are derived from it. Its level can therefore be used as a gauge of the degree of normalcy of the folate metabolism. Because its distribution is highly skewed, as noted earlier, the median values rather than the mean values are shown. After 6 months of treatment, though there was a large spread in the values, the mean value of tetrahydrofolate had risen significantly.

Figure 3. Plasma tetrahydrofolate in 21 CFS patients
initially and after 3 and 6 months of treatment,
compared to the laboratory reference range

As these figures show, glutathione, S-adenosylmethionine, and tetrahydrofolate all monotonically approached their laboratory reference values during the 6 months of treatment, but it appears that this period of treatment was not long enough for their mean values to reach the mean laboratory reference values. (As mentioned earlier, in fact treatment was continued for an additional 3 months, but since individualized treatments were added during this period, the results at 9 months were not included in the statistical analysis. It is interesting to note, however, that after 9 months of treatment the mean glutathione and SAM levels had reached and exceeded their laboratory reference values.)

With regard to the thyroid testing, 10 of the 21 patients were on thyroid hormone supplementation at the beginning of the study, and remained on it during the study. The initial total T3 levels were found to converge toward the mean reference value at 3 months, as seen in a shift in the mean and a decrease in the standard deviation. The initial and final mean and standard deviation values were 156.9 (48.9) and 136.1 (29.9) ng/dL, compared to the reference mean and standard deviation of 120.5 (30.2) ng/dL. This shift barely missed achieving the $p<0.05$ criterion of significance, but in 19 out of the 21 patients, the shift in total T3 was in the direction toward the reference mean. Two out of the 21 patients had elevated thyroid peroxidase antibodies initially. In one of them the level decreased significantly after 6 months of treatment, while the level in the other remained nearly the same.

The allele frequencies of the polymorphisms associated with the methylation cycle in the group of 21 patients were as follows: AHCY-01: 17%; BHMT-08: 48%; CBS C699T: 38%; COMT V158M: 60%; and MTR A2756G: 19%. None of the patients was homozygous for AHCY-01 or MTR A2756G. There were both homozygous and heterozygous cases for the other three polymorphisms.

Figure 4 shows the distributions of the values of the sum of S-adenosylmethionine and S-adenosylhomocysteine (the two methylation cycle metabolites measured in this study) as a function of time on treatment for patients who do not have the CBS C699T polymorphism as well as those who are heterozygous and homozygous for it. As can be seen, those having either one or two copies of this polymorphism have lower values of SAM + SAH than those who do not have it. The differences are statistically significant ($p<0.04$) between the (-/-) and the (+/-) groups for all three times.
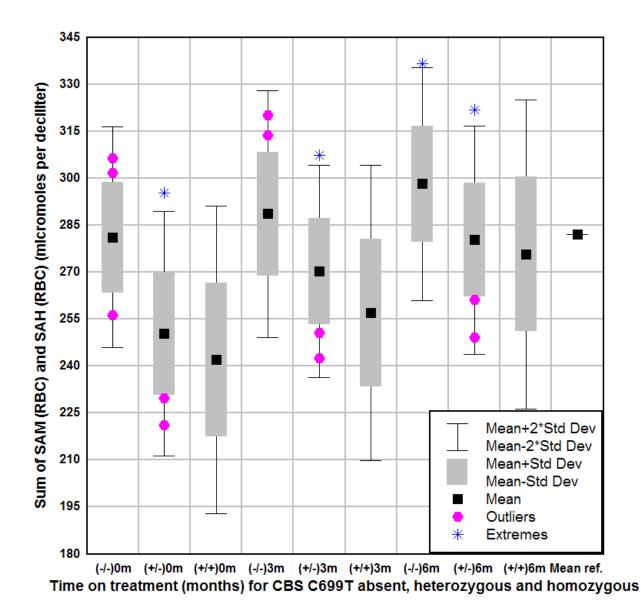
Figure 4. Sum of SAM (RBC) and SAH (RBC) for patients who do not have the CBS C699T polymorphism (-/-), those who are heterozygous (+/-), and those who are homozygous (+/+), at 0, 3, and 6 months of treatment.

The difference between the (-/-) and the (+/+) groups is statistically significant at 0 months (p<0.04), but not at 3 and 6 months. (Even though the sums of SAM and SAH were lower at all three times for the (+/+) group, there were only two patients in this group, so the statistical power was low in comparing it to the (-/-) group.)

The HLA allele frequencies for the association categories identified by Shoemaker et al. [17] in 20 of the 21 patients for which these alleles were characterized were as follows:

"Multisusceptible"-- 30%; "Mold"-- 28%; "Low MSH"-- 15%; and "Borrelia, Post Lyme Syndrome"-- 8%.

The results of the Functional Acuity Contrast Testing (FACT) of 21 patients were as follows: clearly negative on all three tests (performed at 0, 3 and 6 months)—3; clearly positive on all three tests—8; positive or borderline on one or more of the three tests—10.

The results of the C4a measurements were that 15 of 21 patients had values in excess of the laboratory reference range. The mean and standard deviation of the laboratory reference values were 1120 (381). The mean and standard deviation of the distribution of C4a values of the 21 patients were 6086 (7767). The patient values were significantly higher than the reference values, with $p < 0.005$.

The results of the TGF beta-1 measurements were that 20 of 21 patients had values above the laboratory reference range. The mean and standard deviation of the laboratory reference range values were 1,363 (510). The mean and standard deviation of the distribution of TGF beta-1 values of the 21 patients was 6,935 (4,274). The patient values were significantly higher than the reference values, with $p < 0.0005$.

In response to the question asking for a subjective estimate of percentage of improvement after 6 months of treatment, 15 out of 21 (71%) reported improvement, 5 reported no improvement (24%), and one did not respond. Of those who reported a percentage of improvement, the percentages ranged from 5 to 98%, with a mean value of 47.5% and a standard deviation of 25%.

In response to the symptoms checklist, twenty out of 21 patients (95%) reported a decrease in their number of symptoms at 6 months, compared to their number at the start of treatment, and one reported an increase from 19 to 20 symptoms.

In the self-rating of outcome measures at 6 months, 16 of 21 (76%) reported improvement in energy, 16 of 21 (76%) reported improvement in sleep, 15 of 21 (71%) reported improvement in mental clarity, 15 of 21 (71%) reported greater freedom from pain, and 14 of 21 (67%) reported improvement in their overall feeling of wellbeing.

Table 2. Enumeration of Symptoms
and Self-Rating of Outcome Measures

	Time on treatment (months)		
	0	3	6
Number of symptoms	22.1 (6.2)	--	11.8** (7.6)

Outcome measures (rated from 1 to 10)			
Energy	4.0 (1.7)	5.8*** (2.0)	6.0*** (2.1)
Sleep	4.6 (1.6)	5.7## (2.2)	6.4*** (2.0)
Mental clarity	5.0 (2.0)	6.3# (2.0)	6.7*** (1.7)
Freedom from pain	4.3 (1.8)	5.6**** (1.9)	5.8**** (2.0)
Overall feeling of wellbeing	4.5 (1.3)	6.7** (1.9)	6.3*** (2.1)

The following symbols indicate the p values of measured parameters with respect to their values at time 0: ** p<0.0005; *** p<0.005; **** p<0.02;
# p<0.025; ## p<0.04

Results of statistical analysis of the enumeration of symptoms and the self-rating of outcome measures are shown in Table 2 as mean values and standard deviations, and are plotted in Figures 5 through 10.
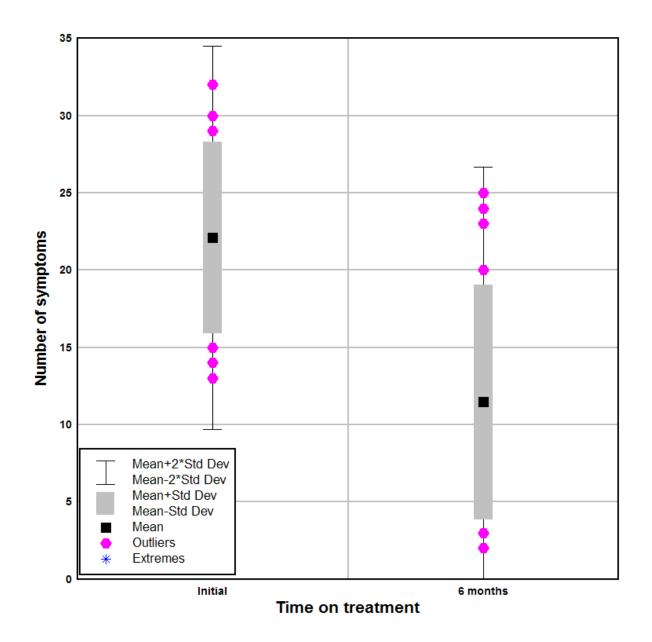
Figure 5. Number of symptoms reported by 21 patients before and after 6 months of treatment
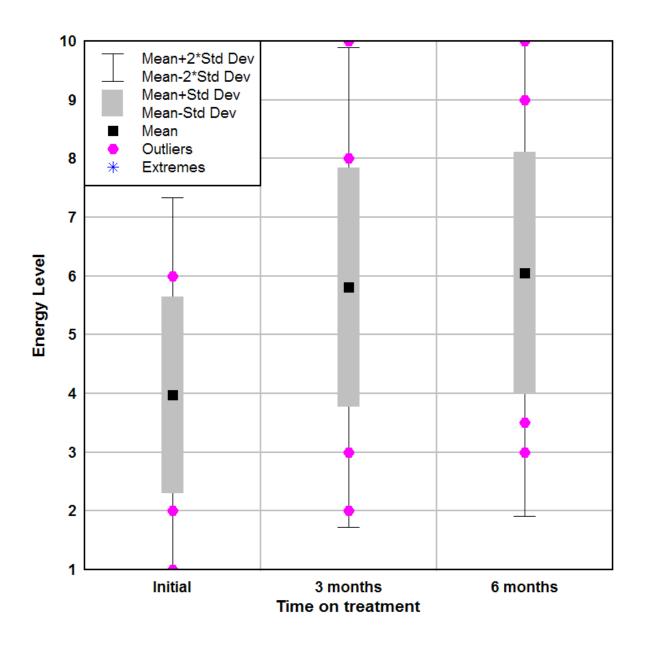
Figure 6. Self-rated energy level for 21 patients, initially and after 3 and 6 months of treatment

As can be seen, the mean number of symptoms reported by the patients dropped by nearly half after 6 months of treatment, and the mean ratings of all five of the symptomatic outcome measures were significantly improved at both 3 months and 6 months of treatment. The rating of overall feeling of wellbeing decreased between 3 and 6 months, but this decrease was not statistically significant.
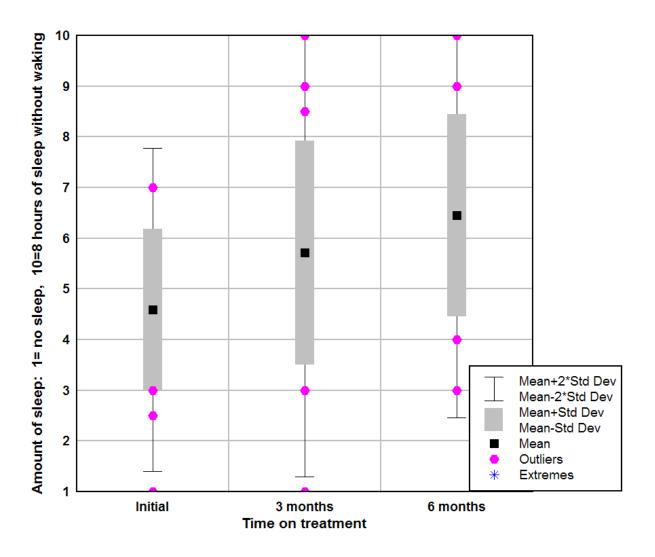
Figure 7. Self-rated amount of sleep for 21 patients, initially and after 3 and 6 months of treatment
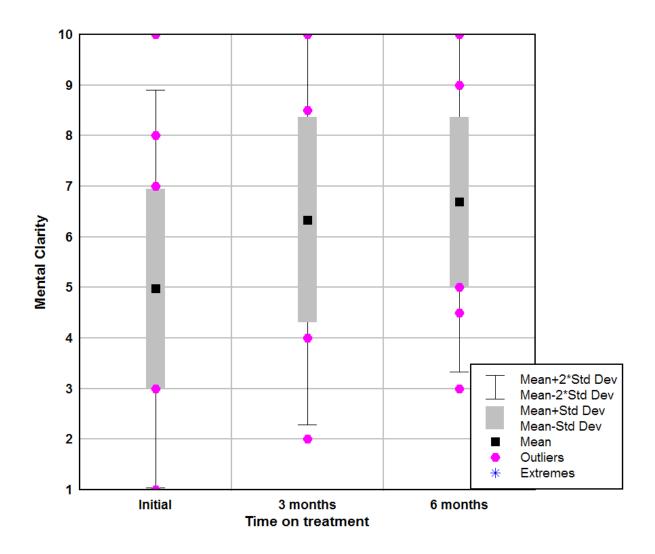
Figure 8. Self-rated mental clarity for 21 patients, initially and after 3 and 6 months of treatment
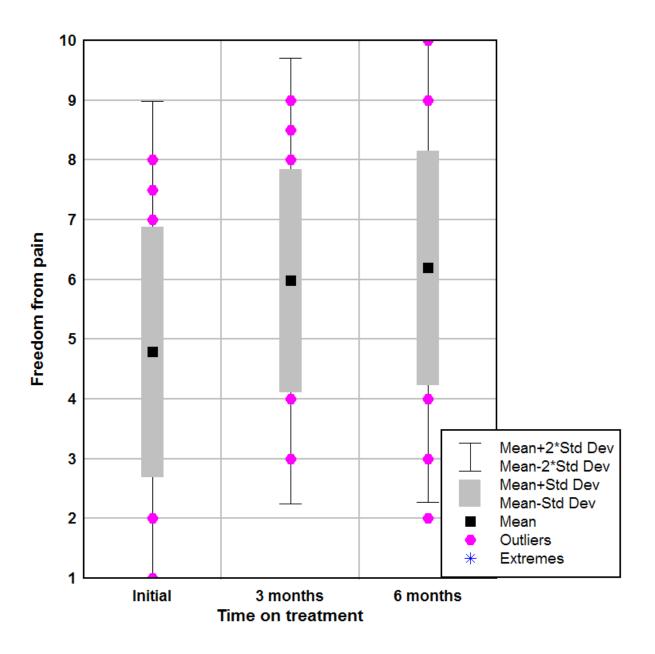
Figure 9. Self-rated freedom from pain for 21 patients, initially and after 3 and 6 months of treatment
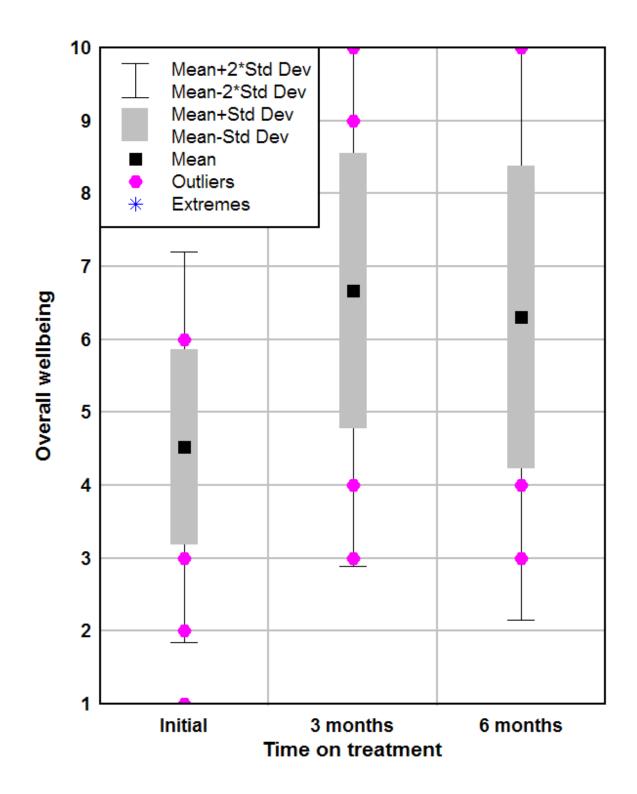
Figure 10. Self-rated overall feeling of wellbeing for 21 patients, initially and after 3 and 6 months of treatment

Discussion

The objectives of this study were to test the Glutathione Depletion—Methylation Cycle Block hypothesis and to assess the potential efficacy of a treatment based upon it.

The hypothesis predicts that CFS patients are depleted in glutathione and have a partial block in their linked methylation cycle and folate metabolism. It furthermore predicts that the abnormalities in glutathione, methylation, and the folate metabolism are linked together, and that the key to correcting these abnormalities is to stimulate the activity of the enzyme methionine synthase.

The reduced glutathione level in the blood plasma was found to be significantly depleted in the patients before the treatment was begun, which is consistent with the hypothesis. It is important to consider how this result compares to past reports of measurements of glutathione in CFS patients. Some background information may be helpful in making this comparison:

Glutathione is present in all cells of the body, and in the blood plasma, the bile and the epithelial lining fluid of the lungs, but is compartmentalized, having different concentrations in different locations in the body. Of most interest in testing the GD-MCB hypothesis would be the levels of reduced glutathione inside the cells that are most closely associated with the symptoms of CFS, particularly those of the skeletal muscles, the heart muscle, the brain and nervous system, the immune system, and glands that secrete hormones found to be deficient in CFS.

However, though muscle biopsies can be performed, the most practical medium for clinical testing is blood. Within blood, the concentration of glutathione is about three orders of magnitude higher in the red blood cells than in the blood plasma, and it is also much easier to evaluate the glutathione level in whole blood or in red blood cells than in plasma. As a result, this is what has most commonly been done. Because of the great abundance of red blood cells in whole blood and the much higher concentration of glutathione in red blood cells than in plasma, the whole blood glutathione level is dominated by the red blood cell glutathione, so that a whole blood measurement of glutathione is essentially equivalent to a measurement of red blood cell glutathione.

In evaluating the relevance of measurements of glutathione in red blood cells or whole blood to CFS, it is necessary to consider to what degree these measurements reflect the levels of glutathione inside the cells that are actually of interest. Red blood cells normally have excess capacity for producing glutathione, and they are net exporters of it, as are the cells of the liver [34]. Red blood cells have systemic roles to play in controlling oxidative stress and in conjugating toxins in addition to controlling their own redox status, and this probably accounts for their excess capacity.

This is in contrast to the situation of the cells of interest, which are net users of glutathione. These cells utilize reduced glutathione from the blood plasma, or cysteine or cystine that originated in glutathione, and they export oxidized glutathione when the amounts are in excess of

their abilities to recycle it [35]. As a result, the levels of reduced and oxidized glutathione in the blood plasma can be expected to more closely reflect the glutathione status in the cells of interest than will the levels in the red blood cells. The overall system of producing and distributing glutathione in the body should be kept in mind when comparing the present glutathione measurements in blood plasma to literature reports of past glutathione measurements in red blood cells or whole blood in CFS patients.

With this background, here is a brief review of the relevant literature:

In 1999, Droge and Holm [36] suggested that CFS is among several "low cystine and low glutamine syndromes" in which glutathione is depleted.

Also in 1999, Cheney reported in public talks that glutathione depletion was "almost universal" in his CFS patients [37,38]. Enlander [39] and Salvato [40] had also been treating CFS patients with glutathione for some years at that time.

In 2000, four papers were published reporting measurements of glutathione in CFS patients. Richards et al. [41,42] found that the patients they studied could be divided into two distinct groups, one having significantly elevated erythrocyte (red blood cell) reduced glutathione relative to a healthy control group, and the other having significantly lower values than the control group. Manuel y Keenoy et al. [43] found that a subgroup of fatigued patients having low magnesium, which did not improve with supplementation, had significantly low erythrocyte glutathione levels. Fulle et al. [44] found elevated total (reduced plus oxidized) glutathione in skeletal muscle biopsy specimens.

In 2001, Manuel y Keenoy at al. [45] reported that they did not find a significant difference between CFS patients and non-CFS fatigued controls in terms of whole blood glutathione.

In 2003, Kurup and Kurup [46] reported finding significantly lower erythrocyte reduced glutathione in myalgic encephalomyelitis patients compared to healthy controls.

In 2005, Kennedy et al. [47] reported finding low red blood cell glutathione in a subset of CFS patients who had cardiovascular risk factors of obesity and hypertension, but not in a subset who did not have these risk factors. Also in 2005, Jammes et al. [48] did not find a significant difference between the resting erythrocyte reduced glutathione levels in CFS patients and controls.

In 2007, Richards et al. [49] also reported finding no significant difference in erythrocyte reduced glutathione levels between CFS patients and controls.

As can be seen, all but one of these reported measurements utilized red blood cells or whole blood, and they obtained a variety of results. The measurements using skeletal muscle biopsies measured total glutathione.

It is our view that the present measurements of reduced glutathione in the blood plasma are more indicative of the reduced glutathione levels in the cells of interest in CFS than are the measurements reported in the literature, for the reasons discussed earlier.

The observation that the level of reduced glutathione had risen by over 30% after six months of treatment, while the level of oxidized glutathione had risen by only 10% (the latter not statistically significant) suggests that the initial glutathione depletion was principally due to a deficit in its synthesis, rather than a deficit in its recycling via the glutathione reductase reaction. In other results in the present study, S-adenosylmethionine was found to be significantly low initially, suggesting dysfunction in the methylation cycle, and there were also significant initial abnormalities in several of the folate vitamers, indicating problems in the folate metabolism as well. These results are also consistent with the GD-MCB hypothesis.

It can be seen from the results of the present study that treatment directed specifically at increasing the activity of methionine synthase brought significant improvement in S-adenosylmethionine and several folate vitamers. This is evidence that a partial block of this enzyme was responsible for the observed methylation cycle and folate cycle dysfunctions in CFS, as proposed in the GD-MCB hypothesis.

It is particularly noteworthy that treatment directed at assisting the methylation cycle produced a significant increase in the level of glutathione, even though glutathione was not supplemented directly. This is evidence for a vicious circle type of interaction between these two phenomena, as predicted by the GD-MCB hypothesis, and as consistent with what was found earlier by James et al. in autism [2].

As noted in the results above, the ratios GSH/GSSG and SAM/SAH were below normal initially and did not change significantly during the treatment period. A low value of GSH/GSSG indicates a state of oxidative stress, and a low value of SAM/SAH indicates lower than normal capacity for performing methylation reactions. The observation that these ratios remained low at the end of the 6-month period is evidence that the recovery is not yet complete at this time.

The treatment was not found to completely correct hypothyroidism in this group of patients, though it had been reported to do so in a small number of patients prior to this study. However, the improvement in the values of total T3, the most active effector hormone in the hypothalamus-pituitary-thyroid axis, suggests that the treatment did act in the direction of normalizing the operation of this axis.

The data concerning the polymorphisms associated with the methylation cycle as well as the HLA, FACT, C4a and TGF beta-1 data were used to help guide the choice of individualized treatment for the additional 3-month treatment period that was not part of the present analysis. They will also be used in the future to relate the patients who participated in this study to patient groups in other studies as well as to suggest how patient characteristics correlate with their response to this treatment.

One interesting feature of the polymorphism data associated with the methylation cycle is the effect of the presence of the CBS C699T polymorphism on the levels of the metabolites in this cycle. Yasko [4] has claimed that this polymorphism increases the activity of cystathionine beta synthase, and thus causes the metabolites in the methylation cycle to drain more rapidly than normal into the transsulfuration pathway.

According to Yasko, this depletes the methylation cycle when there is a partial block of methionine synthase, and it promotes excessive synthesis of toxic sulfur-containing species (such as sulfite) and ammonia if efforts are not made to compensate for it before support is given to the methylation cycle.

The data in Figure 4 support Yasko's claim that the CBS C699T polymorphism significantly drains metabolites from the methylation cycle when there is a partial block of methionine synthase. However, during the treatment, though there continued to be differences in the values of SAM +SAH between those who had the polymorphism and those who did not, even those who were homozygous for this polymorphism attained levels of SAM + SAH averaging near the reference value after 6 months of treatment, without compensatory treatment for the presence of this polymorphism. We did not measure sulfite or ammonia in this study, and we have not compared the symptomatic responses to treatment of those with the polymorphism to those without.

As noted in the results above, the ratios GSH/GSSG and SAM/SAH were below normal initially and did not change significantly during the treatment period. A low value of GSH/GSSG indicates a state of oxidative stress, and a low value of SAM/SAH indicates lower than normal capacity for performing methylation reactions.

The fact that improvements in several of the measured parameters were still occurring after 6 months of treatment and that the GSH/GSSG and SAM/SAH ratios continued to be low at that time suggests that a longer duration of treatment would produce additional benefit (and in fact, this was observed in the subsequent 3-month treatment period that included additional individualized treatments beyond the protocol used in this study).

The initial exacerbation of symptoms that was experienced by over half of the patients may have resulted from mobilization of stored toxins, and well as toxins produced in the die-off of pathogens. Since the methylation cycle operation, the folate cycle operation, and the glutathione status were all improved, it can be expected that both the detoxication system and the immune system moved toward more normal operation, because their operation depends to a large degree on these parts of the overall biochemistry. During their extended Illnesses, it can be expected that toxins and pathogens had accumulated in the bodies of the patients, and that there would therefore have been considerable mobilization of toxins into the blood stream when these systems began functioning more normally. Because the rates of excretion into the stools, urine and perspiration are limited, the resulting elevation in toxin levels in the blood can be expected to impact tissue cells, leading to a variety of detoxication-related symptoms.

Though there was (mostly mild) initial exacerbation of symptoms in over half the patients, the symptomatic improvement in at least two thirds of the patients was the dominant effect of the treatment. There was a significant decrease (by nearly half) in the average number of symptoms after 6 months of treatment, and significant improvements in all five of the self-rated symptomatic outcome measures. Four of the self-rated measures showed monotonic improvement with treatment time. The rating of overall feeling of wellbeing at 6 months was lower than the value to which it had risen at 3 months, but this decrease was not statistically significant.

The fact that treatment of this type produced improvement in the whole range of symptoms experienced in CFS is evidence that the partial block at methionine synthase is fundamental to the pathophysiology of CFS, and this is consistent with the central feature of the GD-MCB hypothesis.

Limitations of this Study

The principal limitations of this study, being a preliminary, open label study, were that it was not blinded, randomized or placebo-controlled, it was limited in size to 21 patients (who met the strict selection criteria) in a single practice, and the duration of uniform treatment was limited to 6 months.

Because they funded their own office visits and supplements, the patients may have been biased toward achieving a positive outcome.

However, objective testing was used in addition to self-evaluation of symptoms, and the magnitudes of the improvements observed were sufficient to produce statistically significant results even in a study with relatively modest statistical power.

Additional, more controlled study will be necessary to determine whether these improvements will continue to stand when possible sources of bias that are inherent in a preliminary study of this type are subject to control, and also to determine to what degree the results can be generalized to the CFS population as a whole.

It is not possible to determine from this study what the ultimate degree of improvement might be from this treatment, or how long a duration of treatment would be required before either no further improvement (or, more optimistically, even complete recovery) would be observed. As noted above, it appears that longer duration of treatment would be desirable in future studies in order to realize the full potential of this treatment.

It should also be pointed out that this treatment has not been optimized for CFS, and it is therefore likely that it could be improved by controlled studies. Nevertheless, the results of this study suggest that the treatment that was used does contain the essence of what is needed to correct what appears to be a fundamental aspect of the biochemical abnormalities of CFS.

Conclusions

The results of this study are consistent with the predictions of the Glutathione Depletion—Methylation Cycle Block hypothesis for the pathogenesis of chronic fatigue syndrome. This hypothesis appears to be a good candidate for more detailed testing.

A treatment based on this hypothesis and directed at supporting the methylation cycle was found to produce significant improvements in the levels of glutathione, metabolites in the methylation cycle and folate vitamers in this group of CFS patients. There was also a significant decrease in the number of symptoms and significant self-rated improvements in energy, sleep, mental clarity, freedom from pain, and overall feeling of wellbeing. Treatment to support the methylation cycle in CFS is promising, and should receive more controlled study.

Acknowledgments

Disclaimers: The authors have no financial interest in the tests or supplements discussed in this paper. Although the treatment described in this paper consists only of food supplements, it has not yet been widely tested in chronic fatigue syndrome, and it must be entered upon only under the supervision of a licensed physician, because adverse effects are possible in patients whose general health has become quite debilitated, or those who have certain respiratory, cardiac, endocrine or autoimmune conditions.

Appendix—Symptom Checklist

Confusion, disorientation
Difficulty in word finding
Impairment of concentration, difficulty assimilating new
information
Reduced task completion
Hypersensitivity to bright light
Night blindness
Tearing, redness of eyes
Blurred vision
Chronic aching muscles
Joint pain, morning joint stiffness

Pain in weight bearing joints
Nausea
Loss of appetite
Weight gain (How much, and over what period of time?)
Abdominal pain
Chronic sinus congestion
Chronic cough that mimics asthma
Shortness of breath
Ice-pick like pain, or electrical pain that shoots into a muscle
Nosebleeds
Metallic taste or other unusual taste
Vertigo, dizziness
Ringing in the ears (tinnitus)
Rage or inappropriate anger
Panic attacks or anxiety
Depression
Tingling, "needles and pins" sensation
Increased sensitivity to touch
Difficulty with sleep
Difficulty with getting to sleep
Difficulty with staying asleep
Mood swings
Excessive thirst or frequent urination
Impotence
Irregular vaginal bleeding
Low body temperature
Chronic yeast infections
Onset of menopause (if appropriate)

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