Role of dietary modification in alleviating chronic fatigue syndrome symptoms: A systematic review

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Publication Details
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Role of Dietary Modification in Alleviating Chronic Fatigue Syndrome Symptoms: A Systematic Review

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The authors declare they have no conflicts of interest. No funding is declared. KJ carried out analysis and interpretation of data, drafting of the paper, reviewed its content and approved final version of the paper. YP provided advice throughout the research period, provided the structure for the systematic review process and contributed to the editing of the manuscript, reviewing its content and approving the final version of the paper.
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Keywords: chronic fatigue syndrome, diet and supplementation.

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Results: Positive outcomes were highlighted in some included studies for polyphenol intakes in animal studies, D-ribose supplementation in humans and aspects of symptom alleviation for one of three polynutrient supplement studies. Omega three fatty acid blood levels and supplementation with an omega three fatty acid supplement also displayed positive outcomes in relation to chronic fatigue syndrome symptom alleviation.

Conclusions: Limited dietary modifications were found useful in alleviating chronic fatigue syndrome symptoms, with overall evidence narrow and inconsistent across studies.

Implications for Public Health: Due to the individual and community impairment chronic fatigue syndrome imparts on the population, it is vital that awareness and further focused research on this topic is undertaken to clarify and consolidate recommendations and ensure accurate, useful distribution of information at a population level.

Abstract Word Count: 193

Introduction
Chronic fatigue syndrome (CFS) or myalgic encephalomyelitis (ME), recently redefined by the Institute of Medicine as Systemic Exertion Intolerance Disease (SEID)\(^1\), is a chronic multisystemic condition which severely impacts daily life, impairing a persons ability to perform every day tasks due to overwhelming fatigue, in turn affecting quality of life.\(^1\)\(^-\)\(^6\) For the purpose of this review the term CFS will be used. Symptoms characteristic to CFS include immobilising fatigue, sleep disturbance, headaches, difficulty concentrating as well as muscular pain.\(^2\)\(^,\)\(^5\) The aetiology behind CFS is unknown, with new diagnostic criteria\(^1\) and name (SEID) aimed at improving diagnostic time as well as consolidating the focus of new research to address the cause of CFS.\(^1\)\(^,\)\(^3\)\(^,\)\(^4\)\(^,\)\(^6\) Furthermore, recent findings of an objective characteristic chemical signature for CFS are hoping to
also prove useful in eliminating diagnostic uncertainty going forward, highlighting CFS as a pattern of hypometabolic response to environmental stress. CFS community prevalence is estimated to be 0.2% to 0.7% in the United States and United Kingdom, although valid epidemiological data is limited. Not only is the individual and their quality of life impacted but the community itself is as well. The economic impact of CFS within the limited Australian data available is estimated at $13471 annually per case for the year 2000 for individual medical costs and time lost in productivity. The overall annual direct and indirect economic costs being estimated at $525 million. More recent United States data suggests an annual direct and indirect cost estimate of $17-24 billion dollars. Research into effective treatment of CFS symptoms is vital, particularly that addressing treatment using dietary modification rather than pharmacological measures. Previous reviews on CFS have not concentrated solely on the effectiveness of dietary modification, as, to the knowledge of the authors, data specific to this research question is limited. Complementary and alternative medicine therapies were the focus of previously conducted CFS reviews, with some studies briefly discussing dietary modification amongst a vast range of other alternative and pharmacological therapies. This review intends to highlight research in this field with specific focus on dietary modifications alone. By examining the current literature surrounding the topic, this review will identify effective dietary modifications for use. Due to the narrow extent of studies available, literature reviewed in this study is comprised of data on dietary modification by food, supplementation or dietary outcome assessment and its impact on alleviating the symptoms of persons with CFS across a range of study designs. Inclusion across a range of study designs effectively highlights the current state of evidence for this topic. This study aims to review data on dietary modification in food or supplement form or dietary outcome assessment in relation to CFS. The research hypothesis stands on the alternative hypothesis that dietary modifications alone are useful in alleviating the symptoms of CFS.

**Methods:**

The PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis) statement protocol was used to inform the methods used in this systematic review (Supplementary File 1).

**Data Collection**

The scientific databases Scopus, CINAHL Plus, Web of Science and PsychINFO were searched in May 2014 (1994-2014). A date limit was applied due to limited awareness and work surrounding CFS, with the first case definition only developed after 1988. An updated database search was performed for the period of June 2014 to August 2016, limited only by date. The search terms, developed from the initially selected key words, medical subject headings (MeSH) terms and synonyms, were used within Boolean search strategies across the for-mentioned databases. The
search terms included “chronic fatigue syndrome”, “CFS”, “myalgic encephalomyelitis”, “diet” and “supplementation”.

To systematically gather all available evidence, animal as well as human observational and experimental study designs were included if they met inclusion criteria parameters. The broad range of study designs were chosen to be included as the extent of research surrounding this topic is narrow.

**Study Selection**

For inclusion in this review, studies needed to provide data on dietary outcomes assessment or modification in food or supplement form in relation to CFS and its symptoms, or a model of CFS and its accompanying symptoms. For those studies considering supplement use, the Therapeutic Goods Regulations 1990\(^{17}\) was consulted, with a number of designated active ingredients excluded from inclusion in this review. This ensured the review held its focus on dietary modification rather than pharmacological measures. Studies were required to be published studies in the English language, and were excluded if published more than twenty two years ago. Only original studies were included with editorials and letters excluded. Studies were selected ensuring that they met the inclusion criteria, had relevant findings and showed some level of validity through quality assessment methods.\(^{18-20}\)

Relevant studies were selected from the pool of studies returned by the searches performed across each database by initially screening the title and abstract. For preliminary screening, if the title and abstract did not provide sufficient information or the abstract was unavailable the full study was screened. The subsequent full text studies were then reviewed to ensure they met the inclusion criteria, were primary studies and were not reviewing disproved treatment strategies such as homeopathy.\(^{21}\)

**Data Extraction**

Upon gathering data from the included primary studies, a standardised table was developed in order to extract appropriate information (see Supplementary File 2, accessible online). The data extracted from each study pertained to study design, level of evidence\(^{18}\) and use of a control group. It also included the intervention details (range of exposure and length to follow up), study population characteristic (sample size, gender, loss to follow up or non-response), duration, outcome measures, results, confounders, external validity (applicability and generalisability) as well as an overall quality rating\(^{20}\) which was reached through in depth assessment of study validity.

**Quality and Risk of Bias Assessment**
For the included primary studies the level of evidence of each included study was graded according to the National Health and Medical Research Council\textsuperscript{18} A quality rating was assigned according to the Academy of Nutrition and Dietetics American Dietetic Association\textsuperscript{20} quality rating checklist, allowing for an assessment of risk of bias within each included study and fostering systematic and objective quality rating assignment. The checklist addresses relevance, applicability, validity and bias,\textsuperscript{20} These aspects of quality and bias are found within the summary table. Ethics approval of all included studies was also considered.

\textbf{Study Outcomes}

The summary table was used to systematically extract data from each study included in the review in order to make clear comparisons and provide a brief overview of all studies. Results are reported according to prominent data themes which emerged from the review.

\textbf{Results}

\textbf{Study Retrieval}

Across the identified databases 435 potential primary research papers were identified (see Supplementary File 3 for expanded search details, viewable online). Of these, 22 primary research papers were included in the systematic review.

\textbf{Animal studies and polyphenols}

Three animal studies were included examining the effect of differing polyphenols and their impact on induced CFS symptoms in mice or rats.\textsuperscript{22-24} These polyphenols included astragalus membranaceus flavonoids (AMF), epigallocatechin gallate (EGCG) and naringin.

The effect of AMF oral administration in rats highlighted statistically significant increases in endurance through forced swimming compared to rats not administered with AMF (p<0.05) in the chronically fatigued groups.\textsuperscript{22} Spleen cell proliferation was statistically significantly increased with AMF supplementation (p<0.05) as well as abnormal cytokine production levels being regulated (p<0.05) in chronically fatigued groups.\textsuperscript{22}

Daily EGCG oral administration highlighted a significant increase in endurance capacity during forced swimming and decreased post swim fatigue levels compared to the chronic fatigue rat group at 28 days (p= <0.05). Greater endurance and decreased post fatigue levels were displayed with the administration of 100 mg/kg compared to 50 mg/kg (p<0.05).\textsuperscript{23} Use of EGCG prevented reductions in body weight and restored spleen and thymus induced hypotrophy compared to the chronic fatigue rat group (p<0.05), with significantly greater prevention of body weight reduction and spleen hypertrophy restoration using 100mg/kg dose verse 50mg/kg dose (p<0.05).\textsuperscript{23} In an elevated plus maze learning task administration of EGCG decreased initial transfer latency compared to the
chronic fatigued group (p= <0.05). Use of an oral dose of EGCG also showed favourable statistically significant changes in measures of oxidative and nitrosative stress and pro-inflammatory cytokine levels, factors which may contribute to fatigue development. For the polyphenol naringin, differing oral doses were administered to mice treated with lipopolysaccharide (LPS) or brucella abortus (BA) antigen which induced immunological fatigue. Naringin use showed a statistically significant and dose-dependent decrease in the duration of immobility in immunologically induced fatigued mice (p= <0.05) and a significant increase in mean tail withdrawal latency compared to BA or LPS injected mice (p= <0.05). A statistically significant dose dependant increase in reduced glutathione levels was also seen through naringin administration compared to BA or LPS injected mice (p= <0.05). Again, as seen with use of the other polyphenols, statistically significant favourable changes in measures of oxidative and nitrosative stress and a pro-inflammatory cytokine were highlighted.

**Dietary interventions and CFS symptom alleviation**

Only one eligible study assessed a dietary intervention with reference to CFS symptoms alleviation. The experiment applied a twenty-four week intervention of a low sugar low yeast diet (LSLY), with intention to treat analysis showing no statistically significant difference for the intervention and guideline based healthy eating control between the primary outcome measures. Compliance was poor, compliant analysis was performed only on baseline characteristics comparing those who completed their role in the experiment and those that did not.

**Dietary intake and poor vitamin or mineral status and CFS**

Three studies examined dietary intake and associated correlations to CFS. Differing dietary intake patterns were highlighted through a descriptive observational study design, with findings of dairy and grain avoidance most prominent in the CFS cohort evaluated. These dietary restrictions in many cases were reported to be undertaken due to provision of non-evidence based information and diagnostic testing from alternative therapy practitioners. Very little correlation of abnormal biomarkers with inadequate nutritional intake in CFS patients was displayed in another descriptive observational study. The findings continued in a consistent manner highlighting through an analytical observational study 95% had poor fibre intake and 70% had unhealthy fat, fruit, and vegetable intake measured through defined cut off limits from questionnaire assessment tools. Yet, lifestyle factors including overweight and unhealthy dietary intake were not significantly associated with fatigue severity or functional impairment. One study considered the vitamin status and direct association of CFS symptoms alleviation. The analytical observational study examined mean serum 25 OH vitamin D levels. Levels were highlighted to be moderately to severely suboptimal in CFS participants at 60.1% for those not
taking supplements. This finding was statistically significantly lower over winter/spring (p = 0.04) and summer/autumn (p = 0.003) compared to the general population control study arm, yet no observed relationship for alleviation of symptoms of CFS was seen.

Supplements and CFS symptom alleviation

Three experimental studies examined use of single nutrient supplements. Following on from the aforementioned serum 25 OH vitamin D analysis, an experimental trial investigated the use of an oral vitamin D3 supplement. The measured outcomes from this trial pertinent to the review highlighted no statistically significant improvement for both fatigue or for the selected markers of inflammatory and oxidative stress.

Secondly, probiotic supplementation was examined finding no significant change. Six of fifteen participants in this study did report subjective improvement after the trial and statistically significant improvement was seen for the neurocognitive function measures (p = 0.04), yet no significant changes in aerobic intestinal microflora were found.

Another study observed supplementation using D-ribose. Significant improvement for energy levels between pre and post enrolment in the compliant analysis was seen (p<0.0001, CI; 1.1-2.2) and 65.7% of participants indicated improvement in regard to their overall subjective feelings. Regarding the experimental studies examining polynutrient supplements, one vitamin and mineral supplement study highlighted statistically significant improvements for before and after measurements of superoxide dismutase (SOD) antioxidant enzyme activity (p ≤ 0.005). Aspects of fatigue measures showed statistically significant improvement in fatigue itself (p = 0.0009), sleep disorder (p = 0.008), autonomic nervous system symptoms (p = 0.018), headaches (p = 0.0001), and subjective feelings surrounding infection (p = 0.0002). Some aspects of quality of life measures were also statistically significant including total physical function (p = 0.053), physical role (p = 0.031), pain (p = 0.01), mental vitality (p = 0.029) and mental health function scores (p = 0.014).

Yet, in another study trialing a vitamin and mineral supplement no statistically significant relationship was seen. Furthermore, another study also experimentally addressed polynutrient supplementation seeing no statistically significant results with fatigue, disability and symptoms assessed through the primary measured outcomes.

Lastly, practices of mixed self-applied supplementation were observed in an analytical observational study. Minimal changes in fatigue were shown over the follow up period in response to a range of different self-applied therapies, including nutritional therapies. A small number of the total cohort found a nutritional therapy useful, with ginseng found to be most helpful (56%) within users. Measured outcomes compared to effect of different nutritional therapies revealing inconsistent results. Magnesium (no specified dose) at six months was statistically significant with improvement in fatigue (p = 0.002), yet at two years showed no significant
Calcium (no specified dose) was introduced by participants at six months to two years and showed statistically significant improvement in fatigue (p= 0.01), yet was based on five participants.  

**Fatty acids and CFS symptom alleviation**

Three eligible studies regarding fatty acids were considered in relation to CFS symptoms. In an analytical observational study a statistically significant reduction was shown in the CFS cohort for the omega three (n-3) to omega six (n-6) polyunsaturated fatty acid (PUFA) ratio (p= 0.0003) and eicosapentaenoic acid (EPA) to arachidonic acid (AA) ratio (p= 0.02). A statistically significant positive relationship was also observed between n-3 to n-6 PUFA ratio and serum zinc (p= 0.009), an indicator of the inflammatory response. A statistically significant relationship between lowered n-3 PUFA and the expression of CD69 on activated T cells (p= <0.05) was seen. There was also a statistically significant positive relationship between the severity of CFS symptoms and certain n-6,PUFA, n-9 PUFA, oleic and palmitic acid (p= <0.05) and a statistically significant negative relationship between n-3 to n-6 ratio and severity of CFS symptoms (p= 0.027).

An essential fatty acid (EFA) supplement rich in EPA was individually examined in a case study. Depression scores dropped from 27 at baseline to 3 at 16 weeks and the lateral ventricular volume of the brain had decreased at 16 weeks for the CFS participant. Yet, another experimental trial of an EFA supplement verse a placebo supplement of sunflower oil showed no statistically significant difference in the RBC membrane fatty acid profile and symptoms scores between the intervention and control group.

**Tryptophan and glutamine amino acids and CFS**

The amino acid tryptophan (Trp) and its potential role in CFS symptoms expression was examined in two studies. Within one, the selected CFS cohort displayed a significantly higher mean free (CAA) ratio was significantly higher (43% p= 0.0000) in the CFS cohort and the serum total Trp to CAA ratio was only significantly higher when co-varied with age and gender (p= 0.0562). Non-esterified fatty acid (NEFA) levels were significantly higher in the CFS cohort (p= 0.0192), as was serum glucose (p= 0.0058) and a slightly higher albumin concentration was also seen in the CFS cohort (p= 0.0016). Total CAA concentration though was not significantly different between the healthy control cohort and CFS cohort. Within CFS participants if suggested serotonin parameters are applied there was a significant division in this cohort between those with high or normal serotonin status shown in free Trp (p= 0.0002) and total Trp to CAA ratio (p= 0.0195) and free Trp to CAA ratio (p= 0.0000).
Within the second study, the amino acid blood sample findings were related to the research question. These findings highlighted baseline free Trp was significantly lower in the CFS cohort (p= 0.033) with no difference seen in neutral amino acid (NAA) concentration between the two groups. Although lowered, there was no significant difference between groups for serum free Trp to NAA ratio and there was no difference for total Trp between the groups.

For the amino acid glutamine, the one experimental study revealed baseline measurements of participants with CFS had significantly lower mean plasma (p= <0.001) and muscle (p= 0.027) glutamine concentrations. Post trial outcomes for those with CFS in the intervention group receiving a glutamine supplement highlighted there was a significant increase in plasma and muscle glutamine concentration (p= <0.05). Those with CFS in the placebo group saw no significant increase in plasma or muscle glutamine concentrations (p= >0.05). Although supplementation increased stores of glutamine for CFS participants, those with CFS in the intervention group showed no statistically significant change in their five category questionnaire scores assessing symptoms, whereas CFS participants in the placebo group showed a significant decrease in the two categories of emotionality (p= 0.02) and sleep problems (p= 0.02). Grouping CFS participants to show clinical improvement or no clinical improvement displayed both the intervention and control groups in each category.

The CFS participants in the group which showed no clinical improvement did display significant increases in mean plasma glutamine concentration (p= 0.013) post trial as well as increases in their T-cell counts (p= 0.010), T-helper cells (p= 0.002) and a decrease in natural killer cell counts (p= 0.019) and activated T-cells (p= 0.015). These outcomes oppose the reported baseline measures in the initial assessment, yet were not seen in the group which displayed clinical improvement.

**Phosphate diabetes and consideration of differential diagnosis to CFS**

Mean serum phosphate concentration was outlined to be significantly lower in the CFS cohort (p= 0.01) but still within reference range in the single included analytical observational study. Fourteen percent of participants in the CFS cohort fulfilled diagnostic criteria for phosphate diabetes (PD), yet did not show a significant difference in symptoms when compared to those with CFS without a PD diagnosis. No statistically significant difference was seen between the intervention and control for mean overall values such as phosphate clearance, mean renal threshold phosphate concentration (TmPO4/GFR) and mean phosphate tubular re-absorption (PTR) rate values.

**Discussion**

Within this review no definitive dietary modification was found for alleviation of the symptoms for individuals with CFS. Some promising research though has been examined. The alternative
hypothesis that dietary modifications are useful in alleviating the symptoms of CFS has not been substantiated within this review. The included animal studies set a good platform for further human studies to be conducted in these areas of interest, with some encouraging significant results highlighted through polyphenol use in mice or rats displaying induced fatigue.\textsuperscript{22-24} Further studies in humans should consider supplementation of these isolated polyphenol compounds, as the results highlighted are unlikely to be obtained through normal dietary intake, for example, to obtain the effects seen from naringin around 50-200 mg/kg was administered.\textsuperscript{24} Mean values of several fruit varieties high in naringin, including red grapefruit, blond grapefruit and sweetie fruit, showed around 50 mg/100 g of fresh weight from fruit identified, further impeded by it’s bioavailability in the food matrix.\textsuperscript{43} Experimental LSLY dietary intervention was inconclusive. Although, bias from poor compliance must be considered when interpreting these findings, with compliance not analysed against the measured outcome scores. Other reasons for such a finding may be potentially due to lack of power masking a result.\textsuperscript{4} The results may have been clouded from the beginning as the studies hypothesis relied on dietary treatment of a suggested cause of CFS. Both the suggested cause and use of dietary modification for this suggested cause (LSLY diet) lacked any supporting scientific data, are controversial and to date have not been linked directly to CFS.\textsuperscript{4} Dietary intake patterns and poor vitamin or mineral status were not found to be associated with symptoms alleviation in the observational studies reviewed.\textsuperscript{25-28} Some important trends were found within these studies. One study notably observed in their selected CFS cohort almost half of the dietary exclusions were made based on information from practitioners of alternative therapy or experience.\textsuperscript{25} Those following this advice tended to have higher vitamin supplement intake which may be detrimental as discussed by Mursu et al.\textsuperscript{44}, who noted that vitamin and mineral supplements increased total mortality risk in a cohort of generally healthy women with a mean age of 60 years. Although this study displays a low level of evidence and had many limitations\textsuperscript{25}, these trends regarding food avoidance and its influence on the diets of those with CFS are important to note for research and clinical practice in order to ensure the information being distributed and followed within CFS populations is correct and has a sound evidence base. Some supplementation recommendations were highlighted in light of other findings. Due to the nature of CFS with increased in-door dwelling, potential increased osteoporosis risk and significantly lower mean serum 25-OH vitamin D, vitamin D intake through the diet and supplements should be considered for general health, regardless of the study findings\textsuperscript{26}. The risk of chronic vitamin D deficiency mimicking CFS symptoms is also noted in two studies.\textsuperscript{26,28} One study continues, suggesting that an underlying pathological process may be responsible for abnormal biomarkers which showed very little correlation with inadequate nutritional intake\textsuperscript{28}, an explanation that may also explain lifestyle factors of weight and dietary intake not significantly correlating with CFS symptoms.\textsuperscript{27}
For the three experimental studies of single nutrient supplements varied results were seen.\textsuperscript{33-35} For the study considering probiotic supplementation it was not clear whether participant compliance was measured and this may have influenced the inconsistent results.\textsuperscript{33} In part, this result may have been seen as this trial might not have used a high enough dose or may have focused on too many different strains of bacteria at once, with Rao et al.\textsuperscript{45} supporting this hypothesis, highlighting positive outcomes for anxiety and intestinal microflora using a higher single strain probiotic dose. With this understanding of the included probiotic experiment, the potential relationship between the gut-brain axis and probiotic use and the gut microbiota for CFS is further reinforced and needs continued investigation, D-ribose was highlighted to have positive preliminary results although further investigation should be made as the quality rating identified a potential conflict of interest.\textsuperscript{34} Polynutrient vitamin and mineral supplements again displayed inconsistent results across the studies included.\textsuperscript{30-32} Data from Brouwers et al.\textsuperscript{30} and Martin et al.\textsuperscript{32} showed no statistically significant relationship between CFS symptoms and polynutrient supplementation, respectively.\textsuperscript{30, 32} One of these experimental studies\textsuperscript{32} did show better non significant scores than those not using the vitamin and mineral supplement. Regardless, these studies do not support general prescription of polynutrient supplements to individuals with CFS. Marie et al.\textsuperscript{31} highlighted a number of statistically significant results, yet the overall inconsistent results were displayed across the measured outcomes. It was suggested that potentially the vitamin and mineral supplement was working on an inflammatory level, with symptoms related to inflammation significantly improving alongside superoxide dismutase (SOD) activity.\textsuperscript{31} The change in SOD activity could possibly imply an effect of lowering inflammatory cytokine concentration, overall suggesting decreased levels of oxidative stress could perhaps influence CFS symptoms.\textsuperscript{31} This suggestion is complemented by Morris et al.\textsuperscript{46} in sighting that low grade inflammation from heightened pro-inflammatory cytokines are characteristic in those with CFS and that antioxidant use may be helpful. These antioxidant/cytokine hypotheses align with the results seen in the animal models of polyphenols and their positive changes to induced fatigue and corresponding cytokine measured outcomes.\textsuperscript{22-24} Mixed self-applied supplementation gave inconsistent results. Within the included analytical observational study a list of different therapies used over the study duration was cited by noting the most commonly used therapies amongst participants.\textsuperscript{29} This allows for many other potential treatments to also have been in use concurrently to the listed therapies and dramatically decreases the accuracy of the results.\textsuperscript{29} Studies examining the role of fatty acids and CFS highlighted some positive outcomes, yet overall inconsistent results were seen.\textsuperscript{36-38} One experimental study returned no significant results, although, the supplements used may have acted as a confounder to these results.\textsuperscript{38} An EFA supplement containing a range of fatty acids, both n-3 and n-6 PUFA was used although the control supplement did not contain EPA and DHA. Mixing n-3 and n-6 PUFA could have negatively contaminated the
results as highlighted by Maes et al. who made observations regarding plasma free fatty acids. Although, Puri et al. did see significant results within their case study from a mixed EFA supplement (predominately n-3 PUFA), the previous hypothesis that n-6 PUFA may negatively impact results should still be taken into consideration for further research work. The positive outcomes seen from supplementation with predominately omega three fatty acids and observations in plasma free fatty acids could indicate the anti-inflammatory role of omega three fatty acids imparting positive effects for inflammatory response in CFS as well as some symptoms reduction. Findings again were inconsistent for those studies examining the role of tryptophan and glutamine amino acids and CFS. CFS participants displayed a significantly higher serum free Trp to CAA ratio with increased Trp available to the brain, highlighting an excess of central serotonin by one analytic observational study. Although, this was only due to higher serum free Trp as CAA concentration was not different between the two groups. Unfortunately some of the CFS cohort had not fasted and this confounder significantly increased the concentration level of serum free Trp, hence affecting the validity of the serum free Trp to CAA ratio. In light of this result, conflicting findings are understood from another included analytical observational study. This data highlights potential implications for the management of CFS, proposing that CFS patients may be divided into sub-groups of those with normal or those with high serotonin status. It must be noted that within Vassallo et al. observational study, the amino acid level investigation and results observed were reviewed, yet the other sub sectors of this study were not relevant to the research question having largely a neuroendocrine focus. Glutamine amino acids findings showed experimentally that a CFS cohort at baseline displayed significantly lower mean plasma and muscle glutamine concentration, yet it is stated this is likely because of four participants low values. Post trial outcomes show those with CFS in the intervention group saw a significant increase in plasma and muscle glutamine concentration as compared to CFS participants in the placebo group who saw no significant increases. This indicates the effectiveness of glutamine supplementation in increasing the bodies supply of the amino acid. Inconsistent results though were highlighted with use of categorisation of CFS participants into clinical improvement or no clinical improvement groupings, with a statistically significant increase for those who did not show clinical improvement post trial in mean plasma glutamine concentration. It is proposed that a rise in only plasma glutamine for this group indicates an issue of glutamine regulation rather than decreased availability of glutamine in those with CFS. Overall it can be stated glutamine supplementation increased body supplies of glutamine but demonstrated inconsistent effects on reported symptoms or clinical improvement. Phosphate diabetes (PD) CFS cohort findings were contradictory and although still within reference range, the CFS group did display a significantly lower mean serum phosphate concentration in the included analytical observational study. Fourteen percent of the CFS cohort did meet the PD
diagnostic criteria, whereas no one in the healthy control group met the diagnostic criteria. Further studies therefore should rule out the need for consideration of PD upon clinical presentation of CFS symptoms to ensure the diagnosis and management are correct.

Restricted primary research regarding this topic is a limitation of this review, not only is the data of lower quality and evidence level, the extent of research currently is narrow. Weaknesses exist within the body of evidence and add to the complexity of this topic. Weaknesses include a variety of differing case definitions and diagnostic criteria for CFS, with the Institute of Medicine redefining CFS highlighting this weakness and aiming to address it. Furthermore, a vast number of assessment tools and measures, displaying varying levels of validity are in use for CFS.

Relevant insights of study limitations are provided in this review, yet the aforementioned weaknesses meant that assessment of bias had to be carefully considered. Rather than a uniform assessment of bias be made across the included studies, an overview of individual assessments of limitations, validity and bias was made, provided in Supplementary File 2 available online.

Limitations of relatively small sample sizes across much of the included primary research existed, with none of the included studies using an Australian cohort. Further research may benefit from including larger numbers and potentially multi country study designs to ensure greater generalisability to the CFS population. As commonly evident in systematic reviews some level of publication bias may be present as only published primary research met the strict inclusion criteria. Not only is there a need for stronger study design and larger sample size use, but also a need for research to assess varying whole diet interventions being applied, as currently from included data in this review only one primary research paper included a specific dietary intervention trial with more emphasis in the current body of evidence on supplementation or single nutritive factors.

**Conclusion**

Overall the evidence displayed inconsistent results across data themes which emerged, with only limited dietary modifications shown to be useful in alleviating symptoms of CFS. The evidence-base included many studies of lower levels of evidence according to the National Health and Medical Research Council. At present, effective dietary changes have not yet been substantiated within this review, including effective communication and implementation of dietary interventions tailored to meet the needs of the CFS population. Individualized clinical recommendations still need to focus on evidence-based advice and dietary counselling, alongside general promotion of healthy eating habits across all medically tolerable food groups in order to also reduce other chronic disease risks and avoid development of deficiencies.

Overall further research surrounding the research question and data themes presented in this review is needed. It is hoped that further research will focus on strengthening the level of evidence.
contributing to future research in this area in order to clarify and consolidate recommendations as well as ensure the distribution of accurate and useful information at a population level.

Conflict of interests, source of funding and authorship
See Title Page.

Word Count (including title, abstract and reference list): 6525

Reference List


**Figure 1** Flow diagram of database searches

**Search One:** Scopus  
Total Records Retrieved: 142  
Example Search Strategy: “chronic fatigue syndrome” OR “CFS” AND “diet*” AND “supplementation*”

**Search Two:** Cinahl Plus with Full Text  
Total Records Retrieved: 243

**Search Three:** Web of Science  
Total Records Retrieved: 31

**Search Four:** PsycINFO  
Total Records Retrieved: 19

Records screened: 435  
Records excluded: 387

Full-text articles assessed for eligibility: 48

Full-text articles excluded:  
21 articles did not meet inclusion criteria components, were not a primary study or were reviewing disproved treatment strategies i.e. homeopathy (21)

Total number of studies included: 27  
Duplicates removed: 5

Total number of studies included: 22
## Supporting Information: Summary Table of Included Primary Research Papers for:
”Role of Dietary Modification in Alleviating Chronic Fatigue Syndrome Symptoms: A Systematic Review”

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design (Level of Evidence)</th>
<th>Control</th>
<th>Intervention: exposure and duration</th>
<th>Duration</th>
<th>Outcome measures</th>
<th>Results: effect size and statistical significance/risk reduction</th>
<th>Confounders</th>
<th>External validity: applicability and generalisability</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal studies and polyphenols</strong></td>
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<tr>
<td>Kuo et al.</td>
<td>Animal study (N/A)</td>
<td>Not manipulated (n=8, rats)</td>
<td>Four groups (n=8) chronically fatigued rats through food intake restrictions and forced swimming. Some mice received treatment with astragalus membranaceus flavonoids (AMF)</td>
<td>6 weeks</td>
<td>- Endurance by forced swimming - Atrophied spleen as measured through decrease in spleen/body weight ratio - Spleen cell proliferation - Cytokine production</td>
<td>- AMF use (100mg/kg body weight) significantly increased endurance (p&lt;0.05) compared to rats without AMF - Spleen/ body weight ratio not significantly increased with supplementation - Spleen cell proliferation significantly increased with supplementation (p&lt;0.05) - Abnormal cytokine production levels regulated with supplementation (p&lt;0.05)</td>
<td>Not comparable to human evidence, yet provides a platform for further studies.</td>
<td>Not applicable or generalisable to the CFS population.</td>
</tr>
<tr>
<td>Sachdeva et al.</td>
<td>Animal study (N/A)</td>
<td>Not manipulated (5-6 rats)</td>
<td>Three intervention groups (n=5-6) One group subject to weight loaded forced swimming. Two other groups also received epigallocatechin gallate (EGCG) polyphenol.</td>
<td>28 days</td>
<td>- Endurance by forced swimming - Cognitive behaviour using elevated plus maze learning task - Grip strength using rota rod test - Locomotor activity using atophotometer and hyperalgesia - Brain homogenate oxidative stress, nitrosative stress, blood tumour necrosis factor-a (TNF-a) glutathione, superoxide dismutase,</td>
<td>- EGCG administration significantly increased endurance capacity and decreased fatigue levels (p&lt;0.05) - Greater endurance and decreased post fatigue levels displayed with the administration of 100mg/kg (p&lt;0.05) - Prevented reductions in body weight and restored spleen and thymus induced hypotrophy (p&lt;0.05), - Significantly greater prevention of body weight reduction and spleen hypertrophy restoration using100mg/kg (p&lt;0.05). - Decreased locomotive scores and increased time for the rota rod and hyperalgesia tests (p&lt;0.05) - Elevated plus maze learning task</td>
<td>Not comparable to human evidence, yet provides a platform for further studies.</td>
<td>Not applicable or generalisable to the CFS population.</td>
</tr>
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<tr>
<td>Vij et al.</td>
<td>Animal study</td>
<td>Injected with carboxymethyl cellulose (drug transport substance) and lipopolysaccharide (LPS) or brucella abortus (BA) antigen to induce immunological fatigue</td>
<td>Naringin (natural polyphenol) administered orally at differing doses</td>
<td>19 days</td>
<td>- Im mobility time</td>
<td>- Significant and dose-dependent decrease in the duration of immobility (p&lt;0.05)</td>
<td>Not comparable to human evidence, yet does provide a platform for further studies - To obtain these effects around 50-200mg/kg naringin was administered. Mean values of several fruit varieties high in naringin showed around 50mg/100g of fresh weight. The levels tested may not be obtainable through diet alone.</td>
<td></td>
</tr>
</tbody>
</table>

**Dietary interventions and CFS symptom alleviation**

| Hobday et. | Randomised    | Healthy eating | Low sugar, low | 24 weeks | Chalder Fatigue Scale | Intention to treat analysis showed no  | - The suggested cause | - Applicable to |

**Quality rating**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Level of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vij et al.</td>
<td>(N/A)</td>
</tr>
</tbody>
</table>

**Control**

- N/A

**Intervention: exposure and duration**

- N/A

**Population:** sample size.

- N/A

**Outcome measures**

- N/A

**Results:**

- N/A

**Confounders:**

- N/A

**External validity:** applicability and generalisability

- N/A

**Not applicable or generalisable to the CFS population.**

**Statistically significant changes in oxidative and nitrosative measures such as decreases in MDA, nitrite, and serum TNF-a levels and increased reduced glutathione, superoxide dismutase and catalase activity (p<0.05)**

**Further decreases for MDA, nitrite, and serum TNF-a levels and increases for reduced glutathione, superoxide dismutase and catalase activity for the 100mg/kg dose (p<0.05)**

**Significant and dose-dependent decrease in the duration of immobility (p<0.05)**

**Significant increase in mean tail withdrawal latency (p<0.05)**

**A significant dose dependant increase in reduced glutathione levels (p<0.05)**

**Oxidative and nitrosative stress measures such as MDA measures, brain nitrite levels and serum TNF-a were statistically significantly reduced (p<0.05)**

**Not comparable to human evidence, yet does provide a platform for further studies**

**To obtain these effects around 50-200mg/kg naringin was administered. Mean values of several fruit varieties high in naringin showed around 50mg/100g of fresh weight. The levels tested may not be obtainable through diet alone.**
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design (Level of Evidence)</th>
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<th>Results: effect size and statistical significance/risk reduction</th>
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</tr>
</thead>
<tbody>
<tr>
<td>al. (2008)*</td>
<td>controlled trial (level II)</td>
<td>- n=52 participants diagnosed with CFS (9 males and 43 females).</td>
<td>self report questionnaire for fatigue, quality of life (QoL) using medical outcomes survey short form-36 (MOS SF-36). hospital anxiety and depression score (HADS) to assess mood.</td>
<td>statistically significant difference between the primary outcome measures.</td>
<td>and use of dietary modification for this study lack scientific evidence and are controversial. - Underpowered - Poor compliance, compliant analysis on baseline characteristics.</td>
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<tr>
<td>Dietar intake and poor vitamin or mineral status and CFS</td>
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<tr>
<td>Trabai et al. (2012)**</td>
<td>Cross-sectional pilot study (level IV)</td>
<td>- Study duration N/A</td>
<td>Food frequency questionnaires (FFQ) and three day food records were collected and analysed for dietary habits and food restrictions</td>
<td>Patterns of dairy and grain avoidance were described as most prominent in the study cohort</td>
<td></td>
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<tr>
<td>Jenkins and Rayman (2005)**</td>
<td>Case series (level IV)</td>
<td>Participants with CFS displaying low values for minerals and B vitamins.</td>
<td>Low intakes of vitamin D and E, calcium selenium, iodine when compared to UK reference nutrient intakes.</td>
<td>Time lapse between initial blood work and undertaking of the dietary assessment - The use of a food diary &lt;7 days compromised some accuracy and imposed</td>
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</tbody>
</table>

**Dietary intake and poor vitamin or mineral status and CFS**

- Patients with CFS displaying low values for minerals and B vitamins.
- Participants with CFS (9 males, 42 females).
- 4 day diet diary
- Routine blood sample
- Participants clinical evaluation using Chalder Fatigue Scale, the General Health Questionnaire (GHQ-12), HADS, the Work...
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design (Level of Evidence*)</th>
<th>Control</th>
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<tbody>
<tr>
<td>Goedendorp et al. (2009)**</td>
<td>Case-control study (level III-3)</td>
<td>Dutch population</td>
<td>Questionnaire data from 247 CFS participants</td>
<td>- No duration is given for the collection of data</td>
<td>- A lifestyle questionnaire to measure smoking and alcohol use</td>
<td>- Significantly less participants in the CFS cohort were overweight (18-45 years) (p&lt;0.01), almost one-third CFS cohort had an unhealthy BMI</td>
<td>bias to the participant,</td>
<td>- As the comparisons were made to the UK reference nutrient intakes and set in the setting must be considered</td>
</tr>
<tr>
<td>Berkovitz et al. (2009)**</td>
<td>Retrospective survey (level III-3)</td>
<td>- A single arm study of patients with a variety of chronic diseases used as control for serum 25OH vitamin D</td>
<td>CFS comparison study arm 2 years of laboratory data on serum 25OH vitamin D and case notes, n=324 participants. CFS comparison study arm n=16 males and n=84</td>
<td>- Serum 25 OH vitamin D levels</td>
<td>- Severity of fatigue assessed from case notes using the Myalgic Encephalomyelitis Encephalomyelitis Disability Score (MEDS) and the Chalder Fatigue Scale</td>
<td>Mean serum 25 OH vitamin D levels were moderately to severely suboptimal in CFS participant at 60.1% for those not taking supplements, statistically significantly lower over winter/spring (p&lt;0.04) and summer/autumn (p&lt;0.003)</td>
<td>Case notes were used to assess fatigue and may not have been concurrent to when the serum 25 OH vitamin D sample was taken</td>
<td>- The sample was 95% Caucasian, making it less applicability to the CFS population as a whole. - Weighted</td>
</tr>
<tr>
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<tr>
<td>Wilham et al. (2015)</td>
<td>- Randomised controlled trial (level II)</td>
<td>Half of the study population acted as a control receiving a parallel placebo</td>
<td>100,000 units of oral vitamin D3 or matching myglol oil placebo</td>
<td>- 6 months</td>
<td>- Arterial stiffness</td>
<td>Results pertinent to this review highlighted no statistically significant effects in any aspects of the Piper Fatigue Scale. No statistically significant changes for all metabolic, inflammatory and oxidative stress outcome measures.</td>
<td>- Funding from ME research UK though the funder had no role in the undertaking of the trial</td>
<td>- Not fully applicable to the CFS population, weighted toward the predominately Caucasian, female adult CFS population</td>
</tr>
<tr>
<td>Sullivan et al. (2009)</td>
<td>- Case series study with pre and post test outcomes (level IV)</td>
<td>No control group</td>
<td>Probiotic supplement (Cultura Dofilus Natural Yoghurt)</td>
<td>- 10 weeks (4 weeks of intervention)</td>
<td>- Symptom intensity including neurocognitive symptoms using the Visual Analogue Scales (VAS)</td>
<td>A statistically significant improvement for VAS neurocognitive symptoms (p=0.04)</td>
<td>- Studyparticipants acted as their own control, though they did not undertake a placebo treatment with wash out period</td>
<td>- The study was applicable to the general CFS population</td>
</tr>
</tbody>
</table>

**Single nutrient supplements**

- Wilham et al. (2015)
  - Study design: Randomised controlled trial
  - Duration: 6 months
  - Intervention: 100,000 units of oral vitamin D3 or matching myglol oil placebo
  - Population: n=50 participants diagnosed with CFS; (12 males and 38 females).
  - Results: No statistically significant changes for all metabolic, inflammatory and oxidative stress outcome measures.

- Sullivan et al. (2009)
  - Study design: Case series study with pre and post test outcomes
  - Duration: 10 weeks (4 weeks of intervention)
  - Intervention: Probiotic supplement (Cultura Dofilus Natural Yoghurt)
  - Population: 15 participants with CFS; (5 male, 10 females).
  - Results: A statistically significant improvement for VAS neurocognitive symptoms (p=0.04).

**Notes:**
- *Experimental study*
- CFS population is in Britain where factors such as sun exposure are widely different.
- The setting is weighted toward females.
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Teitelbaum et al. (2006)</td>
<td>- Case series pilot study with pre and post test outcomes (level IV)</td>
<td>No control</td>
<td>Supplement of 5g D-ribose 3x/day</td>
<td>17 to 35 days</td>
<td>- Pre and post enrolment discrete visual analogue scale (DVAS) and/or CFS questionnaire describing energy levels, sleep patterns, mental clarity, pain threshold and state of well-being</td>
<td>- Statistically significant improvement for energy levels ($p&lt;0.0001$, CI; 1.1-2.2, all other areas spanning 1 for the 95% confidence intervals [CI])</td>
<td>- Gender was associated in some areas of the DVAS</td>
<td>a cohort from Sweden</td>
</tr>
<tr>
<td></td>
<td>- Negative</td>
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<td>- Post enrolment subjective feelings</td>
<td>- 65.7% experienced some level of improvement in regard to their overall subjective feelings.</td>
<td>- Study sample mainlyfemales</td>
<td>- Proper analysis was not given to CFS patients alone to display a significant result.</td>
</tr>
<tr>
<td></td>
<td>* 'experimental study'</td>
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<td></td>
<td>- Post enrolment discrete visual analogue scale (DVAS) and/or CFS questionnaire describing energy levels, sleep patterns, mental clarity, pain threshold and state of well-being</td>
<td>- Intention to treat analysis was not performed</td>
<td>- Two of the three researchers worked for the company producing the D-ribose- may have affected the interpretation of the results</td>
<td>- Participants were from the Annapolis region, USA where access to health care may vary from other areas</td>
</tr>
<tr>
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<td></td>
<td>- Pre and post enrolment discrete visual analogue scale (DVAS) and/or CFS questionnaire describing energy levels, sleep patterns, mental clarity, pain threshold and state of well-being</td>
<td>- CFS participants saw improvement in pre and post DVAS scores for all areas, with no reported p value or 95% CI</td>
<td>- Relatively small study sample size</td>
<td></td>
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<tr>
<td>Polynutrient vitamin and mineral supplements</td>
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<tr>
<td>Maric et al. (2014)</td>
<td>- Case series study with pre and post test outcomes (level IV)</td>
<td>No control</td>
<td>Vitamin and mineral supplement</td>
<td>2 months</td>
<td>- Before and after superoxide dismutase (SOD), Fibro Fatigue Scale (FFS) for symptom severity and the Quality of Life Short Form-36 (QOL SF-36) for physical, psychological and social functioning were completed</td>
<td>- Statistically significant improvement in SOD antioxidant enzyme activity ($p&lt;0.005$) (decreased oxidative stress showing decreased SOD)</td>
<td>- The small sample size may have impacted the ability to find further significant results</td>
<td>Applicable only to the female CFS population</td>
</tr>
<tr>
<td></td>
<td>- Neutral</td>
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<td></td>
<td>- FFS scores showed improvement in fatigue ($p=0.0009$), sleep disorder ($p=0.008$), autonomic nervous system symptoms ($p=0.018$), headaches ($p=0.0001$), and subjective feelings surrounding infection ($p=0.0002$).</td>
<td>- QOL SF-36 showed statistically significant total physical function ($p=0.053$), physical role ($p=0.031$), pain ($p=0.01$) and mental vitality ($p=0.029$)</td>
<td>- Conducted within a cohort from Sweden</td>
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<tr>
<td></td>
<td>* 'experimental study'</td>
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<td>- Physical fatigue ($p=0.01$), and subjective feelings surrounding infection ($p=0.002$).</td>
<td>- QOL SF-36 showed statistically significant total physical function ($p=0.053$), physical role ($p=0.031$), pain ($p=0.01$) and mental vitality ($p=0.029$)</td>
<td>- Conducted within a cohort from Sweden</td>
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</table>

**Quality rating**

† Level of Evidence

* 'experimental study'
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</thead>
<tbody>
<tr>
<td>Martin et al. (1994)</td>
<td>Placebo controlled, double blind, cross over study (level II)</td>
<td>Placebo supplement</td>
<td>Vitamin and mineral supplement</td>
<td>6 months</td>
<td>n=42 participants with CFS</td>
<td>General Health Questionnaire (GHQ) for physical symptoms, Physical Questionnaire (PQ) scores</td>
<td>The data analysis showed no statistically significant relationship between CFS measured outcomes and the vitamin and mineral supplement</td>
<td>Nutritional status was not assessed prior to the study commencement to establish possible nutrient deficiency</td>
<td>- Applicability to the CFS population is likely to be high including participants with a sum score of measuring outcomes of 12 or over. - Conducted within a cohort from Scotland where factors such as sun exposure are widely different to other contexts</td>
</tr>
<tr>
<td>Brouwers et al. (2002)</td>
<td>Randomised control trial (level II)</td>
<td>Placebo supplement</td>
<td>Polynutrient antioxidant supplement of a range of macro and micro nutrients</td>
<td>10 weeks</td>
<td>n=53 participants with CFS (16 males, 37 females).</td>
<td>Fatigue severity using the Checklist Individual Strength (CIS-fatigue) score - Disability through Sickness Impact Profile (SIP-8) score - CDC checklist to assess CFS symptoms.</td>
<td>No statistically significant result was displayed between the control or intervention group in any of the outcomes measured</td>
<td>Effect on CFS symptoms, nutritional status was not assessed prior to the study commencement to establish if possible nutrient deficiency - The study doesn’t mention whether supplement containers</td>
<td>- The applicability of the cohort to the CFS population was good yet weighted toward the female CFS population - Conducted within a cohort</td>
</tr>
</tbody>
</table>
### Mixed self applied supplementation

**Bentler et al. (2005)**
- Case series study (level IV)
- Negative
- ‘analytical observational study’
- No control group
- No intervention, group
- 2 years
- n=159 participants with CFS (20 male, 135 female)
- Physical activity was measured through actometer
- Daily fatigue through Daily Observed Fatigue (DOF) rating
- Individual self improvement rating
- Fatigue intensity using a mix of the Rand Vitality Index and the Fatigue Severity Scale.
- Somatic symptoms through non-validated questions
- Depression was assessed using the Zung Self-Rating Depression Scale and the MOS SF-36
- Minimal change in fatigue score - A small number of the total cohort found a nutritional therapy useful, of that proportion ginseng was found to be most helpful within users (out of 18 subjects, 56% found this helpful) of the nutritional supplements considered appropriate for analysis within this review.
- Inconsistent results where information was provided
- Magnesium (no specified dose) at 6 months was statistically significant with improvement in fatigue (p=0.002)
- Calcium (no specified dose) at 6 months to 2 years showed statistically significant improvement in fatigue (p=0.01), yet was based on 5 participants
- Different therapy used over the study duration
- Treatments may have been in use concurrently to the listed therapies
- The study sample was heavily weighted toward the female.
- Caucasian CFS therefore not highly applicable to the general CFS population.
- The study was performed within an American cohort

### Fatty acids and CFS symptom alleviation

**Maes et al. (2005)**
- Case-control study (level III-2)
- Neutral
- N=12 normal control participants
- N=22 CFS participants
- No exact duration mentioned; -
- n=34 participants
- Plasma free fatty acids, serum zinc, serum alpha-2 protein fraction.
- CFS cohort showed a statistically significant reduction in the omega three (n-3) to omega six (n-6) polyunsaturated fats (despite health status was not described)
- Applicable to the general CFS population,
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Puri et al. (2004)</td>
<td>Single case study (level IV)</td>
<td>No control group</td>
<td>Essential fatty acid supplement rich in eicosapentaenoic acid (EPA)</td>
<td>- 16 weeks</td>
<td>CD96 expression on T lymphocytes and T helper and suppressor cells - In the CFS cohort FMS and CFS Rating Scale to measure severity of symptoms</td>
<td>fatty acid (PUFA) ratio (p=0.0003) and eicosapentaenoic acid (EPA) to arachidonic acid (AA) ratio (p=0.02) - Statistically significant positive relationship between n-3:n-6 PUFA ratio and serum zinc, an indicator of the inflammatory response (p=0.009) - Statistically significant relationship between lowered n-3 PUFA and the expression of CD69 on activated T cells (for CD3+ p=0.005, CD3+CD4+ p=0.04, CD3+CD8+ p=0.01) - Statistically significant positive relationship between the severity of CFS symptoms and certain n-6 PUFA (LA p=0.005, AA p=0.038) and n-9 PUFA (p=0.02), oleic (p=0.03) and palmitic acid (p=0.02) - Statistically significant negative relationship between n-3:n-6 ratio and severity of CFS symptoms was seen (p=0.027)</td>
<td>- No analysis of dietary intake was recorded although gender was not quantified - This study was conducted within a cohort from Belgium</td>
<td></td>
</tr>
</tbody>
</table>

- *‘analytical observational study’

- Provided blood samples

- Depression outcome using the Montgomery and Asberg Depression Rating Scale.

- Lateral ventricular brain size was assessed through MRI scan

- Depression scores dropped from 27 at baseline to 3 at 16 weeks.

- The lateral ventricular volume of the brain had decreased at 16 weeks

- Only one participant was included

- Not applicable to the CFS population as only one participant was included

- This study was conducted within a UK participant
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Warren et al. (1999)</td>
<td>Randomized control trial (level II)</td>
<td>Placebo supplement of sunflower oil</td>
<td>Essential fatty acid (EFA) supplementation</td>
<td>3 months</td>
<td>Physical Symptom (PS) Checklist adapted from another study, The Beck Depression Inventory (BDI) for assessment of depression</td>
<td>No statistically significant differences in the RBC membrane fatty acid profile and symptoms scores from PS and BDI between the intervention and control group were displayed</td>
<td>- The EFA supplement contained a range of fatty acids, both n-3 and n-6 polyunsaturated fatty acids (PUFA), although the control supplement did not contain EPA and DHA.</td>
<td>- The study was applicable to the general CFS population.</td>
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<td>- The study was conducted within a cohort from England where factors such as sun exposure are widely different to other contexts</td>
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<td>- Compliance of participants in the intervention group is not mentioned</td>
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<tr>
<td>Tryptophan and glutamine amino acids and CFS</td>
<td>Case-control study (level III-2)</td>
<td>42 healthy participants (26 males, 16 females)</td>
<td>N=23 participants with CFS</td>
<td>2 year, 7 month period</td>
<td>Serum free and total tryptophan (Trp), cortisol, and amino acids, albumin, non-esterified fatty acids (NEFA), glucose and kynurenine, all exhibiting some role in Trp metabolism were measured</td>
<td>The CFS cohort had significantly higher mean free (48%, p=0.0000) and total (19%, p=0.0016) Trp</td>
<td>Some of the CFS cohort were not fasted outcomes indicated significantly higher serum free Trp to CAA ratio only due to higher serum free Trp</td>
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<tr>
<td>Badawy et al. (2005)</td>
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<td>- This study is applicable to the CFS population.</td>
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<td>- This study was conducted within a cohort from the United Kingdom where factors such as sun exposure are widely different to other contexts</td>
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<tr>
<td>Reference</td>
<td>Study design</td>
<td>Control</td>
<td>Intervention:</td>
<td>Duration</td>
<td>Outcome measures</td>
<td>Results:</td>
<td>Confounders</td>
<td>External validity:</td>
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<td>Vassallo et al. (2001) ⁴⁰</td>
<td>Case-control study (level III-2)</td>
<td>N=21 healthy participants (5 males, 16 female)</td>
<td>N=20 participants with CFS (4 males, 16 female)</td>
<td>A duration not mentioned</td>
<td>Relevant to the research question - plasma neutral amino acid concentration (NAAs) - plasma total and free Trp was measured - from these measures the ratio of free Trp to NAA was given</td>
<td>Relevant to the research question were the amino acid findings: - baseline free Trp was significantly lower in the CFS cohort (p=0.033) - no difference was seen in NAA concentration between the two groups - although lowered there was no significant difference between groups for serum free Trp to NAA ratio</td>
<td>One participant who did not meet the criteria for CFS</td>
<td>different to other contexts</td>
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<tr>
<td>Rowbottom et al. (1998) ⁴¹</td>
<td>Randomised double blind case-controlled trial (level II)</td>
<td>Placebo supplement</td>
<td>L-glutamine supplement (2000mg/day for trial duration)</td>
<td>26 weeks</td>
<td>- 26 weeks</td>
<td>- CFS group reported more frequently the occurrence of symptoms for all five categories of the questionnaire (p&lt;0.001)</td>
<td>Relatively small study sample size</td>
<td>The study population in this trial is applicable to the CFS</td>
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</table>

Higher albumin concentration was also seen in the CFS cohort (p=0.0016). - Total CAA concentration though was not significantly different between the control and CFS groups - This study was conducted within a cohort from the United Kingdom where factors such as sun exposure are widely different to other contexts.
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design (Level of Evidence)</th>
<th>Control</th>
<th>Intervention: exposure and duration</th>
<th>Duration</th>
<th>Outcome measures</th>
<th>Results: effect size and statistical significance/ risk reduction</th>
<th>Confounders</th>
<th>External validity: applicability and generalisability</th>
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</thead>
<tbody>
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<td></td>
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<td>16 participants with CFS (6 males, 10 females)</td>
<td>sample for plasma glutamine levels, complete haematology, five cell differential leucocyte counts and cortisol and testosterone analysis.</td>
<td>sample size.</td>
<td>- The CFS group showed significantly lower mean T-cell counts (p=0.002), T-helper cell counts (p=0.018) and higher activated T-cells (p=0.027)</td>
<td>- The CFS group showed significantly lower mean plasma (p=&lt;0.001) and muscle (p=0.027) glutamine concentrations pre trial</td>
<td>- CFS participants in the intervention group showed no statistically significant change in their five category questionnaire scores, whereas CFS participants in the placebo group showed a significant decrease in the two categories of emotionality (p=0.02) and sleep problems (p=0.02). - For those in the intervention group with CFS a significant increase in plasma and muscle glutamine concentration was seen (p=&lt;0.05). - CFS participants who did not show clinical improvement showed significant increases in mean plasma glutamine concentration (p=0.013) post trial and significant increases in T-cell counts (p=0.010), T-helper cells (p=0.002) and a decrease in natural killer cell counts (p=0.019) and activated T-cells (p=0.015).</td>
<td>population and generalisability is dependant on the context</td>
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<td>Reference</td>
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<td>De Lorenzo et al. (1998)</td>
<td>Case-control study (level III-2)</td>
<td>N=37 healthy control participants</td>
<td>N=87 participants with CFS</td>
<td>No duration mentioned; - Participants provided blood and urine samples - n=124 participants (51 males, 46 females)</td>
<td>Urinary sodium, potassium, calcium and phosphate, b serum calcium and phosphate, parathyroid hormone and vitamin D were measured - Phosphate clearance, phosphate tubular re-absorption (PTR) and renal threshold phosphate concentration (TmPO4/GFR) were all measured</td>
<td>Mean serum phosphate concentration was significantly lower in the CFS cohort (p=0.01) but within reference range - 14% of participants in the CFS cohort fulfilled diagnostic criteria for phosphate diabetes (PD), yet no significant difference in symptoms - no statistically significant difference for overall mean values, such as phosphate clearance, mean TmPO4/GFR and mean PTR rate values</td>
<td>Analysis of PD was performed among the cohort – no analysis of dietary intake to reduce the impact that poor dietary intake</td>
<td>- This study is applicable to the CFS population - Considered as this study was conducted within a cohort from London where factors such as sun exposure are widely different to other contexts</td>
</tr>
</tbody>
</table>

* 'analytical observational study' | Neutral | Control participants | Case-control study | N=37 healthy control participants | N=87 participants with CFS | | | |

† The evidence level of each included study was graded according to the National Health and Medical Research Council.¹⁹
# A quality rating was assigned according to the Academy of Nutrition and Dietetics American Dietetic Association²⁰ quality rating checklist allowing assessment of risk of bias within each included study and fostering systematic and objective quality ratings assignment. The checklist addresses relevance, applicability, validity and bias.
* Study title descriptor given to the references when described within the systematic literature review itself to allow the reader quick reference and comparison of study designs
FMS: fibromyalgia, CFS: chronic fatigue syndrome