A Randomized, Double-blind, Placebo-controlled, Phase III Trial of Coenzyme Q10 in Gulf War Illness

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Background and Rationale

As many as a third of the 700,000 US troops deployed to the Middle East during the 1990-1991 Gulf War are currently ill with a multi-symptom illness termed Gulf War illness (GWI). Investigators have been investigating the cause and potential treatments of the illness or its symptoms for two decades, but to date there have been no successful Phase III trials, and no established treatments beyond palliation of individual symptoms and behavioral aids to coping with chronic illness. There have been significant advances in our understanding of the illness through strong epidemiology, clinical, and basic science studies. However, studies revealed problems with detoxification pathways and clinicians report high levels of drug intolerance in the population, reducing therapeutic options. Gulf War veterans experienced environmental exposures that are known to be oxidative stressors which have contributed to cell injury, resulting in OS-induced mitochondrial dysfunction, findings also supported in pathogenesis studies. This suggested interventions that would support mitochondrial function and prevent or repair active oxidative stress mediators and led to exploratory studies using interventions that target these areas. These early intervention studies included a Phase I/II clinical trial of Coenzyme Q10 (CoQ10) by Dr. Beatrice Golomb and her team at the VA in San Diego, CA. In a double blind, placebo controlled crossover study of CoQ10, 46 veterans with GWI who met both the Kansas and CDC case definition were treated with 100mg or 300mg of a high bioavailability CoQ10 (ubiquinone) product or placebo. They found a significant improvement in men with GWI vs. placebo using their selected primary outcome variable, the general self-rated health (GSRH), in a sex-stratified analysis.

Results revealed that women did not see the same degree of improvement and failed to see significant change, though the sample size was small (7 female veterans). The GSRH showed no significant benefit in the combined-sex sample. The 100 mg product saw a bigger improvement, thought to be secondary to the activating effects of the nighttime dose of the 300mg split dose product when given as a bedtime dose, interfering with quality of sleep. Physical function (summary performance score, SPS) improved on Q100 vs. placebo. A rise in CoQ10 levels approached significance as a predictor of improvement in GSRH and significantly predicted SPS improvement. Among 20 symptoms each present in half or more of the enrolled veterans, direction-of-difference on Q100 vs. placebo was favorable for all except sleep problems; with several symptoms individually significant (Golomb, et al., 2014). Significance for these symptoms, despite the small sample, underscores large effect sizes, and an apparent relation of key outcomes to CoQ10 change increases prospects for causality. The authors concluded that Q100 conferred benefit to physical function and symptoms in veterans with Gulf War illness and that examination in a larger sample is warranted; and that findings from this study could inform the conduct of a larger trial**.** The VA GWI Research Advisory Committee encouraged the research service to consider conducting Phase III studies for any pilot treatment trials that showed initial positive effects on the symptoms of GWI in Gulf War Veterans. Since few treatment studies to date have shown positive results, initiating a Phase III treatment trial of CoQ10 at this time is of the utmost importance to providing some relief to ailing GW Veterans who currently have very few treatment options.

Relevant Literature on CoQ10 Mechanism of action

In an excellent review by Than, et al. (2001), a strong case was made for the use of CoQ10 in cardiovascular disease and illnesses of low cellular energy states. Coenzyme Q10, a lipid-soluble benzoquinone with a 10 isoprenyl unit side chain, is structurally similar to vitamin K (Greenburg et al., 1990). Coenzyme Q is distinguished by the number of isoprenoid subunits in the side-chains. The most common Coenzyme Q in human mitochondria is Q10. Q refers to the quinone head and 10 refers to the number of isoprene repeats in the tail.

The biosynthesis of the compound is complex, with the isoprenyl side chain deriving from mevalonate, the benzoquinone ring structure from tyrosine, and condensation of these structures through polyprenyl transferase

enzyme activity (Olsen, et al., 1983). The primary regulation of CoQ10 biosynthesis is the 3-hydroxy-methylglutaryl coenzyme A (HMG-CoA) reductase reaction, similar to that seen in cholesterol synthesis. Coenzyme Q10 is an essential component in the synthesis of ATP and exhibits both antioxidant and membrane-stabilizing properties. Present in the inner mitochondrial membrane, it serves as an electron transport carrier during the processes of respiration and oxidative phosphorylation, and it has involvement in the manufacture of ATP. Coenzyme Q10 directly regulates NADH and succinate dehydrogenase, enabling reversible reactions between these enzymes in the mitochondrial electron transport chain (Folkers et al, 1993; Greenburg et al, 1990). Coenzyme Q10 also may prevent the depletion of metabolites necessary for the resynthesis of ATP (Greenburg et al., 1990). The compound must be reduced to ubiquinol to wield its antioxidative function, and supplementation may inhibit lipid oxidizability. During supplementation with CoQ10, the level of lipid peroxidation decreased in healthy subjects, yet levels of another antioxidant, vitamin E, remained constant (Weber et al., 1994; Alleva, et al., 1995). Coenzyme Q10 exerts a sparing effect on vitamin E and perhaps more efficiently prevents lipid peroxidation by inhibiting both its initiation and propagation, whereas vitamin E inhibits only propagation (Ermster et al., 1993). The membrane-stabilizing properties of CoQ10 emerge from the reduction of free radicals that may cause damage to structural proteins and lipids found in membranes.

Other important activities may include stabilization of calcium-dependent slow channels, inhibition of intracellular phospholipases, and alteration of prostaglandin metabolism (Greenberg et al. 1990). The positive inotropic effect of CoQ10 is reported to be similar to the effects of digoxin (Murray et al., 1996). Coenzyme Q10 was also recognized to have an effect on gene expression that might account for its effects on overall tissue metabolism and inflammation. (Groneberg et al., 2005; Schmeizer et al., 2008). This is relevant, considering the observation of a reduced cardiac output in GWI subjects a finding more severe in those with comorbid PTSD (Peckerman et al., 2003).

Pharmacokinetics of CoQ10

A pharmacokinetic study showed oral administration of CoQ10 30 mg in healthy subjects can significantly increase the mean peak blood level to about 1 µg/ml within an average of 6 hours, with a second peak occurring again at 24 hours after dosing (Lucker, 1984). These findings corroborate those of studies in the existing pharmacokinetic literature that examined higher dose (Tomono, et al., 1986; Yuzuhiro, et al., 1983). The increase observed in plasma CoQ10 levels 24 hours after dosing may suggest enterohepatic recycling (Tomono et al., 1986). Maximum concentrations achieved from the administration of 30-mg doses either as a meal or capsule in healthy subjects did not differ significantly (Lucker, 1984). Administration of 100 mg 3 times/day achieved a mean steady-state level that was estimated at 5.4 µg/mL, which may be up to 7 times higher than endogenous levels of CoQ10 (Murray,1996). The plasma half-life is approximately 34 hours and is indicative of the low clearance rate from plasma and would support once a day dosing strategies (Overvad, 1999; Greenberg, 1990).

On deposition in the liver, exogenous CoQ10 is packaged into low-density lipoprotein and very low-density lipoprotein fractions of cholesterol and highly concentrates in tissues of the heart, liver, and kidneys (Weis, 1994). The slow absorption of CoQ10 from the gastrointestinal tract can be attributed to the coenzyme's high molecular weight and low water solubility. Coenzyme Q10 is a lipophilic molecule and therefore can display variability in absorption depending on the formulation. In a trial comparing different formulations that included combinations of polysorbate, lecithin, or soybean oil, results indicated highest bioavailability with the soybean oil-only formulation (Weis, 1994). Preparations of CoQ10 that contain lipid vehicles, such as vegetable oil and vitamin E, increase solubility and yield more efficient absorption rates than that of the purified coenzyme alone (Chopra, 1995). The extent of hepatic metabolism is unknown, and the excretion of CoQ10 is mainly through the biliary tract, with over 60% of an oral dose recovered unchanged in the feces (Greenberg, 1994).

The available information would argue for an oil based CoQ10 supplement, in either the reduced form (ubiquinol) or precursor (ubiquinone). Because Dr. Golomb's study of CoQ10 in higher doses (300 mg bid) saw a loss of efficacy with bid dosing, thought to be a result of increased evening energy and reduced sleep (personal communication), and because they saw the best responses in those with the biggest change from baseline CoQ10 plasma levels, we would propose a daily morning dosing strategy with the more highly bioavailable formulation, ubiquinol, in a dose substantial enough to have proven health impact in other conditions of low cellular energy and chronic oxidative stress.

The role of CoQ10 in chronic illnesses and symptom management: The role of CoQ10 can be rationalized in numerous conditions based on its involvement in the mitochondrial electron transport chain leading to synthesis of adenosine 5´-triphosphate (ATP) and on its possession of antioxidant and membrane-stabilizing properties The health benefits of CoQ10 have been recognized in the medical literature to ameliorate symptoms experienced by Gulf War veterans with GWI such as headaches, muscle pain, or weakness and cognitive symptoms. CoQ10 has shown benefits for medical conditions such as congestive heart failure, essential hypertension, stable angina, doxorubicin cardiotoxicity, ventricular arrhythmias, cardiomyopathy, breast cancer, acquired immunodeficiency syndrome, diabetes, muscular dystrophy, and periodontal disease and as prophylaxis for heart surgery, with differing degrees of efficacy (Jellin et al., 1993). Furthermore, the existence of deficient endogenous levels of CoQ10 is associated with a variety of these disorders and is particularly well documented in heart failure. A deficiency of CoQ10 appears to be directly related to severity of symptoms and disease (Mortenson, 1993). Either an increased requirement or a decreased production of CoQ10 with advancing age contributes to a reduction of the endogenous level of this pro-vitamin. Low endogenous levels of CoQ10 can also be the result of inadequate nutritional intake, a genetic defect in biosynthesis, or a depletion of vitamins, trace elements, or other precursors necessary for CoQ10 biosynthesis (Murray, 1996; Greenberg, 1990). The CoQ10 levels in GWI have not been reported except in the Golomb study, which showed highly variable baseline levels. While an analysis was not provided on response as it related to baseline levels, degree of change from baseline seemed predictive of best responders (personal communication).

Migraines are a common feature of GWI, and there is some evidence that migraines may be a mitochondrial disorder (Markley, 2012). Supplementation of CoQ10 appears to give relief from migraines, as suggested by a study where patients who experience migraines took 150 mg CoQ10 daily for three months and demonstrated a 50-percent reduction in number of days with migraine headache. (Rozen et al., 2002). Coenzyme Q10 was superior to placebo for attack-frequency, headache-days and days with nausea in another randomized controlled trial (Sandor et al., 2005). A study of patients with cardiovascular disease highlighted the anti-inflammatory and antioxidant properties of CoQ10. In a randomized, placebo-controlled trial of coronary artery disease patients, study results revealed that following use of CoQ10, antioxidant enzymes activities were significantly higher, and markers of inflammation were significantly lower (Lee et al., 2013).

In another study, patients with fibromyalgia participated in a Phase II randomized, double-blind, placebo-controlled trial where CoQ10 supplementation induced a recovery of inflammation, antioxidant enzymes, mitochondrial biogenesis and AMPK (5' adenosine monophosphate-activated protein kinase) gene expression levels. The study participants demonstrated a reduction in pain, tender points, fatigue, and morning tiredness. The investigators concluded that CoQ10 may have a potential therapeutic effect in fibromyalgia and may indicate new potential molecular targets for the therapy of this disease (Cordero et al., 2013). Further support of CoQ10 supplementation to increase antioxidant enzyme activity, reduce oxidative stress, and improve symptoms of fatigue and depression was demonstrated in a study of patients diagnosed with multiple sclerosis (Sanoobar et al., 2013).

The use of CoQ10 on the treatment of neurodegenerative diseases such as Parkinson's and Huntington's that focus on symptoms reveal disappointing results in recent studies (Lin and Wang,2014). Clinical trials of CoQ10 for Huntington's disease and ALS patients did not show positive results (Kaufman et al., 2009; Mestre et al., 2009) A large multicenter study of escalating high dose up to 2400 mg CoQ10 for patients with Parkinson's disease saw no difference in their progression rates symptom or scores, although the treatment was well tolerated without significant side effects. (Parkinson's Study Group QE3 Investigators, 2014). In terms of the effects of CoQ10 on cognitive performance, several recent animal studies indicate benefits that encourage further research on human subjects. The beneficial effects of CoQ10 were demonstrated in a recent study of rats against organophosphate-induced cognitive impairments and hippocampal neuronal degeneration (Binukumar et al., 2012), in a GWI animal model. In another study of mice to evaluate the effect of CoQ10 on the role of mitochondrial functions in cognitive impairment, the study results indicate that CoQ10 improves cognitive decline in post-menopausal state by modulating mitochondrial functions and oxidative stress (Sandhir et al., 2014). Improvements in cognitive performance were indicated in a mouse model of Alzheimer's disease that showed that CoQ10 also reduces oxidative stress and amyloid pathology (Dumont et al., 2011). Poorer cognition was correlated with lower levels of CoQ10 in the parietal cortex in a study of aged dogs (Martin et al., 2011).

Recognizing the antioxidant properties of CoQ10 and its role in proper mitochondrial function, these studies support further research of CoQ10's role in cognitive health.

Safety and Tolerability and Dosage

Excellent safety and tolerability is a common feature of CoQ10 clinical trials results across multiple disease states, formulations, and dose ranges from 50 to 3600 mg daily. The most common adverse effects are gastrointestinal in nature and can include nausea, epigastric pain, diarrhea, heartburn, and appetite suppression. However, the prevalence of these adverse effects was less than 1% in reported studies (Jelin, 1999). Gastrointestinal effects of CoQ10 may be lessened with a dosage reduction or may subside with continued therapy. Asymptomatic elevations in serum lactate dehydrogenase and hepatic enzymes were observed and may occur with oral dosages of CoQ10 in excess of 300 mg/day; however, cases of serious hepatotoxicity have not been reported (Greenberg, 1990; Micromedix, 2014). In congestive heart failure studies, clinical relapse was noted on withdrawal of CoQ10, whereas reinstatement of therapy resulted in improvement (Mortenson, 1990; Mortenson, 1985). We selected a formulation that has been used in prior clinical trials in other disease states and have published safety data, bioavailability data as well as efficacy data (Chandran et al., 2012; Sanmukhani et al., 2014; Kizhakkedath et al., 2013; Kern, 2011). Based on the literature review of treatment responses in other illnesses and Dr. Golomb's experience we will use ubiquinol 200 mg (the equivalent of ubiquinone 600mg) as a morning dose, to avoid evening side effects of sleeplessness and to optimize its bioavailability, after a 2 month loading dose of 400 mg to restore any deficiency states.

FDA Approvals

We requested a pre-IND meeting with the FDA. As we anticipated the FDA waived the need for a full IND review. The nutraceutical CoQ10 is widely used, and the target of treatment focuses on a reduction in symptom severity. CoQ10 is a nutraceutical that has been evaluated by the United States Food and Drug Administration and approved as being classified as "GRAS" (generally recognized as safe), available in this country without prescription at the doses being studied.

Study Objectives and Specific Aims

In this study, we will assess the clinical efficacy and safety of CoQ10 (ubiquinol) in 200 veterans with GWI, in a classic double-blind, randomized, placebo-controlled trial, with 100 participants per treatment arm of the study. Subjects will be recruited at four geographically different sites.

Objectives:

1. To determine the efficacy of CoQ10 in GWI related symptom control;
2. To evaluate putative biomarkers for their ability to predict severity and response to therapy;
3. To test the utility of measures of illness severity and function as outcome variables in GWI.

Specific Aims:

1. Perform a randomized, double blind, placebo control, Phase III study comparing CoQ10 (ubiquinol) (200 mg q am) to placebo, with a 6 month intervention and assessment of safety, efficacy and biomarker response to therapy;
2. Perform biomarker studies before and after 2, 4, and 6 months of therapy, with blood and saliva collections and laboratory assessments of oxidative stress and mitochondrial function, CoQ10 levels, cytokine, neuropeptide, hormone and cell population studies;
3. Assess the domains of illness and illness severity and evaluate the utility of the selected instruments for future clinical trials use in GWI.

The **primary objective** is to assess efficacy. The **primary endpoint** will be the SF36 physical subscore, selected for its sensitivity to the impact of this illness. We will assess a number of secondary outcome variables, as there are few large longitudinal studies in GWI and this study can provide data on the best instruments to utilize in the clinical trials setting, covering the key domains of the illness. Therefore, the **secondary endpoint instruments** will include objective measures of activity and cognitive function, the Conners CPT-3, Brief Visual Memory Test, and the California Verbal Learning Test-I, as well as self-report instruments to include the SF36 mental and total scores, the Health Symptom Checklist (HSC) adapted for severity and frequency scoring, the Multidimensional Fatigue

Inventory, Checklist Individual Strength (CIS), Cognitive Difficulties Scale, Pittsburgh Sleep Inventory, and Brief Pain Inventory. Each of these instruments are well established assessment tools that are validated in complex medical illnesses. The HSC is unique to GWI but is quite similar to the instrument validated in ME/CFS, the DePaul Symptom Survey (Jason et al 2010). The HSC is a list of 34 frequently reported health and mental health symptoms rated as how often in the past 30 days the symptoms were experienced. Symptoms from 9 body systems are assessed (cardiac, pulmonary, dermatological, gastrointestinal, genitourinary, musculoskeletal, neurological, and psychological). The HSC classification method was developed by VA Boston investigators and has been used in the longitudinally following 2,949 member Ft. Devens, MA Gulf War veteran cohort to assess self-reported health symptoms incrementally over the past 24 years (Proctor et al., 1998; Wolfe et al., 1998). It was originally adapted from the 20-item health symptom checklist of Bartone et al., (1989). **Biomarkers**, identified in our earlier GWI studies, will also serve as **secondary outcome variables** to better design future clinical trials with surrogate markers (Whistler et al., 2009; Broderick et al.; 2011; Broderick, et al., 2012; Broderick, et al., 2013). We have reported that SF36 measures for physical function, physical limit, pain, and vitality cluster together and were linked to IL-1α, IL-2 and IL-5 levels. MFI indicators were more diffuse in their association with reduced motivation, for example, standing apart and correlating positively with changes in IL-4, 12 and IL-10. SF36 general score also stood apart and correlated negatively with levels of IL-10. Of the 6 cytokines that remained in the sub-network, IL-10 had the broadest effects on severity with direct connections to 6 symptom constructs including SF36 social function which itself supported a cluster of 10 symptoms. We propose to include a cytokine multiplex assay to assess pro-inflammatory, TH1, Th2, Th17 and anti-inflammatory cytokines as putative biomarkers in GWI. We will also assay CoQ10 levels, oxidative stress and other mitochondrial measures. Two other objective domains of illness will be assessed. Activity measurement using the **FitBit** will be utilized as an objective marker of activity and an indirect measure of sleep efficiency. This measurement can also be used to assess relapse after activity rates by looking for rest pattern responses after bursts of sustained activity. Secondary outcomes will include measures from a previously validated assessment of cognitive function in GW veterans (Sullivan et al., 2003).

Research Design and Methods

Study Design and Population. The proposed project utilizes a multi-site, Phase III double blind, placebo controlled design of CoQ10 in a total of 200 1991 Gulf War veterans at four VA clinical study sites: Miami (n=50 veterans), Boston (n=50 veterans), Bronx (n=50 veterans), and Minneapolis (n=50 veterans) recruited from among veterans attending VA GW clinics The study includes a large number of GWI cases to enhance the study's power to evaluate GWI subgroups of potential importance (e.g. subgroups associated with illness severity, exposure history, and gender). The Phase III placebo control clinical trial will be designed as a 6-month intervention in a group A to group up B comparison. With this study design we will compare groups A and B, in addition response rate across 3 assessment times and/or recovery from any adverse events will be studied. Dosing will reflect the standard dosing used in illnesses that presume mitochondrial dysfunction or oxidative stress mechanisms for relevant and associated conditions we are using a nutraceuticals (ubiquinol) with established safety profiles at doses that exceed the proposed treatment range, and efficacy profiles established in other diseases.

Study Surveys and Questionnaires

The **Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID-I**) (First, et al., 1996): Computer driven interview format assessment to diagnose current and lifetime DSM-IV Axis 1 psychiatric disorders. These include mood, anxiety, psychotic, substance abuse, eating and somatoform disorders. (Staff administered, staff trained by Dr. Krengel and Sullivan's group, Boston VAMC/BU) In this Phase III study, there will be a broad panel of variables measured. These will include the GWI **Health Symptom Checklist (HSC),** a list of the domains noted in the GWI case definitions (previously determined by factor analysis), with a visual analog severity scale, including current, worst day and best day scales which we have further adapted to include frequency of symptom. Further emphasis on the domains of GWI will include measures of biomarker studies. **Multidimensional Fatigue Inventory (MFI)** (Smets, et al, 1995). The **Pittsburgh sleep inventory** (short form) measuring quality of sleep, patient assessment of **Pain** (by a 10-cm visual analog scale, in which 0 = no pain and 10 = the worst pain possible), (Note that the PE also includes a quantitative tender point evaluation), **Medical Outcomes Study 36-item short-form health survey (SF-36)** (Ware and Sherborne, 1992): This instrument is a general indicator of

health status (function and well-being) with physical and emotional subscales. The SF-36 assesses health-related quality of life in 8 areas: 1.) Limitations in physical activities because of health problems; 2.) Limitations in social activities because of physical problems; 3.) Limitations in usual role activities because of physical health problems; 4.) Bodily pain; 5.) General mental health; 6.) Limitations in usual role activities because of emotional problems; 7.) Vitality (energy and fatigue); and 8.) General health perceptions. For the **Gynecologic Questionnaire**, female subjects are asked to complete this questionnaire to assess routine gynecologic parameters and time of assessment as it relates to menstrual cycle. In our operationalization, we sync the first on site (T0) assessment with estrous phase in menstruating subjects. **Ham-D, Ham-A (**Hamilton, 1959) measuring depression and anxiety; **Davidson Trauma Scale** (Davidson et al, 1997) is designed to assess symptoms of PTSD in three clusters: intrusion, avoidance, and hyperarousal. While not exclusion criteria unless hospitalized for PTSD in the prior 3 years, this information is collected to establish subpopulations and inform the understanding of results. **Gulf War Exposures Questionnaire** includes questions regarding military service and career and experiences in the Persian Gulf region. Cognitive function will be assessed, as described in Table 2. Another objective measure, the **activity monitor, Fitbit** counts steps, stairs, heart rate, periods of inactivity, that can tabulate this indirect measure of hours of sleep, restless periods of sleep, awake periods during sleep, and naps. For the purpose of this study, it will be worn on the wrist continuously for 28 weeks. It does not need to be removed during water exposure (swimming, showering, etc.), and will be given to the participant as a gift for participating in the study. In earlier studies using actigraph, non-adherence due to forgetfulness after bathing compromisted the results. The Fitbit data has near 100% compliance in current studies (Klimas, personal communication) and has the advantage of continuously updating the data in Wi-Fi environments, reducing the risk of lost data due to lost devices. Participants will also keep a sleep and activity diary as a validation step. **Autonomic nervous system measures** include measurement of blood pressure and heart rate in laying and standing positions. Subjects are recumbent for 30 minutes and stand for five minutes and ten minutes. Blood pressure and heart rate are measured at lying and after five and ten minutes of standing. For the virtual platform, autonomic measures, pressure point, lymph node and physical exam will not be performed and laboratory tests will be combined for the screening and baseline visits. During the screening visit, (after signed consent is received), the tests will include all baseline tests and the exclusion tests. The Coordinator will instruct the virtual participant on use of a personal blood pressure monitor.

Table 1. Assessment Platform and Timeline

Laboratory Variables	**Screening on-site**	**T0 on-site**	**4 wk on-line**	**8 wk on-site**	**12 wk on-line**	**16 wk on-site**	**20 wk on-line**	**24 wk on-site**	**28 wk on-line**
CBC with diff		✓		✓		✓		✓	
CMP	✓	✓				✓		✓	
U/A	✓	✓				✓		✓	
Cortisol series		✓		✓		✓		✓	
Autonomic measures		✓				✓		✓	
HPG axis		✓				✓		✓	
HPT axis		✓				✓		✓	
CoQ10		✓		✓		✓		✓	
Exclusion HIV, Hep B, C, RA, CBC, RF, LFTS, CRP, HbA1c	✓								
Stored sample for biomarker studies	✓	✓		✓		✓		✓	

Self-Report Variables	on-site	on-site	on-line	on-site	on-line	on-site	on-line	on-site	on-line
MFI	✓	✓	✓	✓	✓	✓	✓	✓	✓
Sx checklist	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pain	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pittsburgh Sleep	✓	✓	✓	✓	✓	✓	✓	✓	✓
SF 36	✓	✓	✓	✓	✓	✓	✓	✓	✓
HAM A		✓	✓	✓	✓	✓	✓	✓	✓
HAM D		✓	✓	✓	✓	✓	✓	✓	✓
Davidson Trauma		✓		✓		✓		✓	
SCID	✓								
Gyn Questionnaire		✓		✓		✓		✓	

Objective Clinical Measures	on-site	on-site	on -	on-site	on-lin	on-site	on-line	on -	on-line
Cognitive Testing		✓		✓		✓		✓	
Fitbit Activity measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓

The cognitive test battery to be used assesses the functional domains of sustained attention, psychomotor function, visual memory and verbal memory. The battery includes tests shown to have high specificity and sensitivity for detecting changes in cognitive function between veterans with and without Gulf War Illness. Sustained attention is measured by the number of errors on a test of continuous performance (CPT), a computer-assisted test from Conners et al., 2013. This instrument is widely used in the field of occupational health, and represents adaptations of traditional cognitive instruments for computerized stimulus presentation and recording of responses. The Conners CPT-3 has reliable psychometric properties and has demonstrated validity in epidemiological and laboratory studies. Psychomotor functioning is assessed by reaction time on the CPT test. Previous studies of Gulf War veterans have documented changes in perceptual motor functions and motor speed (Proctor et al., 2006; Toomey et al., 2009; Chao et al., 2010).

Treatment-seeking GWVs have had difficulty in the areas of acquisition and retrieval (Sullivan et al., 2003). Therefore, we will examine verbal and nonverbal memory with the use of the Brief Visual Memory Test (BVMT; Benedict, 1996) and the California Verbal Learning Test-II (CVLT-II) and an alternate version (Delis et al., 2000) that measures total recall trials 1 to 5 (raw score) and long-delay free recall (raw score). For the virtual platform, the BVMT will not be performed, as it must be administered in person.

The advantage of these memory tests is that they have multiple alternate but psychometrically comparable versions that can be used during the different follow-up sessions, thus reducing practice effects. Differences in cognitive measures will be considered secondary outcome measures in this CoQ10 treatment trial. A description of the cognitive domains, the complete cognitive test battery, and the study instruments and procedures is presented in **Table 2.**

Table 2. Cognitive Test Battery

Test Name	Description	Outcome
I. Tests of Attention, Vigilance and Tracking		
Conners Continuous Performance Test (CPT-3; Conners et al., 2013)	Target letter embedded in series of distractors; to assess sustained attention and reaction time.	Reaction Time Total Omission
II. Tests of Verbal and Visual Memory		
California Verbal Learning Test (CVLT-II; Delis et al., 2000)	List of 16 nouns from 4 categories presented over multiple learning trials with recall after interference; assesses memory and learning strategies.	Total Trials 1-5 Long Delay
Brief Visual Memory Test (Benedict, 1996)	Drawing test of 6 geometric figures; measures visual memory recall.	Total recall and placement score.

Selection of CoQ10 product study medication and composition of placebo
The decision to use the reduced form of CoQ10 (ubiquinol) over the oxidized form (ubiquinone) was based on the following factors: (1) Ubiquinol accounts for approximately 95% of the total CoQ10 plasma concentration; (2) Ubiquinol is responsible for the antioxidant activity in the mitochondria and lipid membranes; (3) Ubiquinol has a higher oral bioavailability than ubiquinone resulting in higher plasma concentrations. The active formulation will be soft gelatin capsules containing 200 mg of CoQ10 (ubiquinol) emulsified with diglycerol monooleate, rapeseed oil, soy lecithin, and beeswax and the matching placebo formulation will be soft gelatin capsules containing all the above ingredients excluding CoQ10. We will be administering one 400 mg ubiquinol capsules for 2 months followed by 200 mg per day for the remaining 4 months of the clinical trial. The active and placebo soft gelatin capsules will be provided by Kaneka Nutrients, an FDA registered, NSF International and Natural Products Association (NPA) Good Manufacturing Practices (GMP) certified facility that manufactures United States Pharmacopeia (USP) compliant supplements. The purity and stability of the CoQ10 soft gelatin capsules will be monitored by the VA CSP CRPCC Laboratory following USP standards.

Pre-Enrollment Screening: Case definition: In our prior work and in Beatrice Golomb' s work using CoQ10 in GWI, we have used concurrent CDC and Kansas case definitions (Steele, 2000; Fukuda et al., 1998). The more rigorous Kansas case definition captures at least a third of the deployed veterans from the first Gulf War (Research Advisory Committee Report, 2014, page 18), and the CDC captures about 10% more. The key difference between the two is the factor analysis based Kansas definition weighted severity and frequency of symptoms. The Kansas definition requires deployment to the theater of operations between August 8, 1990 and July 31, 1991 with multiple and moderately severe symptoms (> 6 months) in at least 3 of 6 symptom domains; excluding serious medical/psychiatric diagnoses that account for symptoms or preclude accurate symptom reporting. The 6 domains are fatigue/sleep problems; somatic pain symptoms; neuro/cognitive/mood symptoms; gastrointestinal symptoms; respiratory symptoms, and skin symptoms. The exclusion criteria as modified by Steele in 2014 allow for common comorbid conditions.

Due to the aging of the Gulf War I cohort, we will adjust the restrictions of the exclusion criteria imposed by the Kansas case definition to allow for recruitment of veterans who are diagnosed with specific well-controlled, chronic conditions that may be typical of an aging population. We will defer to the clinician at each site to decide whether the conditions meet the relaxed Kansas exclusions,

according to specific guidelines. This change follows the principles recommended by experts and funded Investigators in Gulf War Illness research.

The relaxed Kansas exclusions include the following:

1) Allowance for normal illnesses of aging, if the conditions are treated and are in demonstrable stable and normal ranges at the time of screening and assessment.
2) Allowance of stable comorbid conditions include PTSD, MDD, and mild TBI that have not required hospitalization in the 2 years prior to recruitment. Severe TBI is excluded.

The changes to the entry criteria include:1) Diabetes—include if blood sugar controlled over the past 2 years; 2) Heart disease (stable hypertension is acceptable)—if not had condition in the past 2 years; 3) Arthritis—depending on type: include for osteoarthritis and exclude for rheumatoid arthritis;4)Seizure disorder—include if not treated or not had any seizures in the past 2 years; 5) Cancer—include if not treated in the past 3 years; 6) Skin cancer—include if Stage 1 or treated by excision only; 7) Liver disease—include if not had condition in the past 2 years; 8) Kidney disease—include if not had this condition in the past 2 years.

Entry Criteria

We will study 200 veterans with GWI, 100 in each group (intervention or placebo). The inclusion criteria will follow the case definition by Steele (Kansas Case Definition described) but will be expanded to accept veterans as eligible participants who possess specific, well-controlled chronic conditions. All subjects will be between 35 and 70 years old, in good health by medical history prior to 1990, and currently have no exclusionary diagnoses that could reasonably explain the symptoms of their fatiguing illness and their severity, using the exclusion criteria best described in the paper by Reeves et al. 2003, which clarifies exclusionary conditions for complex multisymptom illness and agrees with Steele's recent unpublished modification. This includes exclusion of major depression with psychotic or melancholic features, schizophrenia, bipolar disorder, delusional disorders, dementias of any type and no hospitalization in the past 2 years for alcoholism or drug abuse. Medical conditions excluded include organ failure, defined rheumatologic inflammatory disorders, chronic active infections such as HIV, hepatitis B and C, transplant, and primary sleep disorders. Medications that could potentially impact immune function will be excluded (e.g., steroids, immunosuppressives; as well as nutraceuticals that are formulated to impact mitochondrial function or oxidative stress). Morphine derivatives that are used daily will be disqualifiers in in this study. PRN use of less strong pain medications is one of the variables that can be studied, thus medications such as tramadol or codeine can be monitored for frequency of use as an indication of CoQ10's impact on chronic pain. Common multivitamin preparations will be allowed if taken without change throughout the protocol. Known allergy to CoQ10 and/or inactive ingredients of active and placebo soft gelatin capsules or current use of Coumadin (given the vitamin K structural similarity of CoQ10) will also be excluded. In the event a subject tests positive for an infectious disease or medical condition at the screening visit, he/she will be counseled and referred to the appropriate service within the Veterans Administration Medical Center (VAMC) or to their primary health care provider. All reportable diseases will be reported as required by hospital and state guidelines. If our psychological exams indicate that the participant is at risk from a mental health perspective, the individual will be referred for further care. Subjects need to not take the supplement CoQ10 (ubiquinone or ubiquinol) for at least 3 months before study entry; as well as other nutraceuticals formulated to impact mitochondrial function. They agree to leave any other supplements unchanged in their regimen throughout the course of the 6 month intervention.

The clinical and laboratory testing is performed without knowledge of the subjects' group membership, the group A vs B true condition will remain blinded throughout the study until its conclusion. There are two treatment conditions: CoQ10 (ubiquinol) 400mg for 2 months followed

by 200mg maintenance for 4 months and placebo taken once a day in the morning with food. The subject schedule includes 5 on-site assessments. On-site assessments include the screening visit with informed consent, the drug initiation visit, an 8 week visit and the 16 and 24-week visits. In addition, there is a web based self-report assessment given every 4 weeks throughout the treatment period and at 4 weeks after completion of the study drug. A member of the research team may contact the participant by phone to remind him or her to complete the surveys on the computer. Subjects will also utilize an activity monitor (FitBit) device to assess activity and sleep throughout the study period through 7 months. **(Assessments, see Table 1.)**

The CSR&D Data Monitoring Committee (DMC) and the local VA pharmacist will have the authority to break the blind and recommend stopping the study should one group have evidence of adverse effects or significant efficacy in group A or B that would ethically require the blind to be broken. Further details regarding the interim efficacy analysis and formal efficacy stopping rules are provided in the statistical section below. There will be no formal stopping rules for safety; the DMC may recommend stopping the study for any concerns they feel jeopardize the safety of patients in the trial regardless of resulting p-values. The research pharmacist will also have access to an electronic emergency unblinding system developed by the CSP Pharmacy Center should an individual subject event require the blind be broken to access appropriate care (e.g., a toxic reaction to a product requiring treatment). This decision would follow institutional guidelines, following a discussion with the treating clinician. All subjects will have a physical examination, medical history, and will be given a battery of psychometric questionnaires.

Recruitment Process and Informed Consent

Gulf War veterans will be recruited at all four VA clinical sites: Miami, Boston, Bronx, and Minneapolis. Miami VA study staff also will recruit participants using a virtual platform. Subject recruitment will be the responsibility of the clinical research staff and each VA site investigator. Subjects are recruited by several methods: discussions with their physician in the GWI clinic,; in response to brochures that are handed out by study staff at veteran events in the community; directed to our research personnel by their primary care providers who are made aware of our work in CME lectures or seminars; through the use of social media as described by Dr. Golomb' s group in a recent paper (Erickson 2013); and through direct contact with veterans who have been assessed through the VA registry, using a phone screen (scripted) contact. Prior to enrollment in the study, all subjects will be fully informed about the nature of the study and sign an informed consent document. The initial contact will include a description of the study protocol including surveys, cognitive assessments, blood draws (approximately ½ cup), time required, and reimbursement for their time and effort.

Informed consent will be obtained by the study investigator or by an IRB-approved delegate after a screening interview, but prior to the initiation of any study procedures. Subjects will be given a local telephone number and will have an opportunity to ask questions about the procedures. They will be informed that their decision to or not to participate will have no bearing on their medical care and that, if they choose to participate, they may withdraw at any time without loss of VA benefits to which they are entitled. There is no coercion; potential subjects are given the opportunity to ask questions, take home materials that accurately reflect the study protocol, and volunteer or not without impacting their relationship with providers. For the virtual platform, Miami VA research staff will mail 2 copies of the informed consent to the prospective participant. Once the signed copy is received, the Miami VA research staff will contact the participant to initiate study procedures. A consent form will be mailed to a potential participant only if he/she is interested in the study. If the individual does not return a signed consent form to the Coordinator, the individual will demonstrate that he/she has opted to not participate in the study. Subjects are compensated for their travel and effort in small part.

After a screening visit, they are compensated $125 for assessment at Baseline and 3 follow-up visits that involve relatively lengthy self-report instruments, a physical exam, a cognitive assessment fitting, given an activity meter with instructions and a blood draw. This initial visit requires 3-4 hours to complete. They return for additional visits that focus on cognitive testing and a brief physical exam at month 2, 4, and 6. For the virtual platform, Miami VA research staff will ask the participant to complete the cognitive testing online at baseline, 2, 4, and 6. At these visits, the Miami VA Nurse Coordinator will ask the same health status and medication questions via telephone, in place of the physical exam procedures. At the 6-month visit, the Nurse Coordinator will complete the end of study procedures and ask the participant to return any unused medications to the Miami VA via mail.

During the consent procedure, subjects will be informed that genetic testing will be performed and that samples of blood will be stored at the Miami VAMC research laboratory for later analyses. We will notify subjects that names and addresses will be kept and can be linked to their identification numbers for later contact and to allow subjects access to laboratory data that may be useful in their clinical care. On completion of the study, samples and clinical data will be de-identified and biorepository samples to be used in future studies requiring informed consent will include genetic testing. Informed consent documents will be submitted to the individual VAMC IRB for approval and oversight. After giving informed consent, subjects will be scheduled for clinical assessment at the participating VAMC or via telephone interview with the Miami VA research team using the virtual platform. Those meeting the study requirements will then be randomized in a 1:1 manner to the treatment or control (placebo) condition; randomization will be stratified by study center and gender. The CSP Pharmacy Center will use an electronic randomization and drug assignment system similar to the system used for most CSP studies, based on the program developed by this study's statistician. The research pharmacy will work with the local study coordinators to dispense study drug and placebos. In order to reduce variation in the data, all laboratory testing will be done at the Miami VAMC. Samples will be sent via overnight express. For the virtual platform, samples will be drawn and packed by phlebotomists at Quest Laboratories in the participant's local area and shipped via overnight express for analysis at the Miami VAMC.

Gulf War Registry recruitment

It is critical that the recruitment projected in this study be on timeline, in order to complete the study in the 3 year timeframe. Prior clinical trials studies have suffered from difficulties with recruitment, often because the per site recruitment targets were ambitious, access to the study population limited, or studies were understaffed. For that reason, we have paid attention to all of these issues, performing this study across 4 sites with established track records of successful recruitment. In addition, we have included a national recruiter working from the national and regional registry, contacting potential subjects and performing scripted screening interviews to refer to the study. A similar strategy was recently employed by the Synergy trial, fully recruiting and completing a similarly powered (number of subjects)/number of sites study in just 18 months. Thus, the study population will be drawn from established GWI populations from 4 VAs with a track record of successful recruitment for clinical studies.

The PI, Dr. Klimas, will seek a DUA to access the national Gulf War Registry list. After gaining access, the Registry Recruiter will mail an introductory letter to the GW veteran. Upon response, the Registry Recruiter will call the veteran by telephone to discuss the study eligibility and then will mail eligible veterans a study packet containing information about the study and directions to the study site. The Registry Recruiter will coordinate scheduling an appointment and travel arrangements, if necessary. After reporting to the clinic site, veterans will be asked for consent to participate, after being fully informed about the study, including risks and human subjects' protections.

Screening and assessment procedures

Prior to enrollment in the study, subjects will undergo a screening visit. Subjects will be consented and screened for exclusion criteria and symptoms. For the virtual platform, the Nurse Coordinator will conduct the screening via telephone.

Procedure if Psychiatric Problem is Discovered During Assessment

1. If the Research Coordinator or Principal Investigator assesses a risk to the participant, she will walk with the participant to the Miami VA ER for evaluation of suicidal ideation. The research team will follow the plan submitted with the original amendment (see attached). If the Research Coordinator or PI assesses a risk during the telephone interview on the virtual platform, the call will be transferred to the VA Suicide Prevention hotline according to procedures outlined in the Psychological Safety Plan.
2. In the case where a participant displays a psychiatric problem that is known to his or her clinician as noted in CPRS, then the research coordinator will place a note in the study file specifying that the patient was excluded from this research study.
3. If the test results indicate a psychiatric problem that is not noted in CPRS, the research coordinator will assess the urgency of the situation while the potential participant is in the clinic.
    a. If the patient's behavior/mental health status is deemed to be emergent or urgent, the research coordinator will walk the patient to the ER at the Miami VA for immediate treatment and will stay with the patient until services are rendered. The Miami VA ER and/or After-Hours Mental Health Staff will conduct an assessment and management of distressed veterans who need assistance during the assessment or call after hours.
    b. If the psychiatric problem is non-urgent, the research coordinator will make a note in the research study file and will place a note in CPRS indicating a referral for follow-up with the patient's primary care physician at the VA. If the patient does not have a primary care physician at the VA, the research team member will arrange for one to be assigned. The Research Coordinator will be responsible to follow up with the patient in order to provide continuity of care as needed and to confirm that the patient follows up with the primary care physician.

Screening labs

Screening labs as outlined will be shipped to the Miami lab. This will include a history and physical exam; blood tests directed at detecting conditions, which would explain the severity of the illness and thus be exclusionary (e.g. untreated hypothyroidism, severe liver disease, anemia etc.). Additional blood tests that are being evaluated to better understand the illness or develop the data needed to prove their utility as surrogates for severity of illness. Blood volume at any one visit will not exceed 120 ml (8 tablespoons). Risk of blood draw includes local bruising and vasovagal responses to phlebotomy (fainting). In the on-site initiation visit as well as the week 8,16, and 24 week visits, the subject will have a brief physical exam emphasizing vital signs, autonomic function (lying and 10 minute stand BP and P), and trigger point assessment. For the virtual platform, the subject will not have this brief physical exam.

For the virtual platform, the Miami VA research team will ship collection boxes directly to the participant. Laboratory tests will be combined for the screening and baseline visits. During the screening visit, (after signed consent is received), the tests will include all baseline tests and the exclusion tests. Samples will be drawn and packed by phlebotomists at Quest Laboratories in the participant's local area and shipped via overnight express to Miami VA.

The subject will be given a web link to the REDCap platform of self-administered questionnaires to be completed within 48 hours prior to the drug initiation visit. Scoring will follow standardized scales.

During the screening visit, the subject will be taught the proper technique for using the activity monitor, assign and practice the web login for the REDCap based platform by performing the core assessments on the computer in the office, assisted by research staff as needed, and perform the studies that require a face-to-face interaction (SCID). The SCID will be performed by telephone for the virtual platform. They also complete the following standardized scales either at home on a weblink within 4 days of each scheduled assessment or on site (cognitive testing is always on site). All subjects wear an activity monitor from the first assessment throughout the 6-month period, which links in any wireless environment to update the activity / sleep data files. For the virtual platform, all assessments will be completed via the telephone interview format or via online surveys in the REDCap platform. The research staff will mail the Fitbit directly to the subject's home, after the screening interview and satisfactory completion of the laboratory tests and assessment forms that confirm eligibility.

Medication review: review for exclusionary meds, document all medications and over the counter supplements.

Screening blood draw: TSH, ANA, RF, CMP, CBC, ESR, HIV serology, Hep and Hep C, CRP, Hemoglobin A1c for screening purposes. Females will also have a pregnancy test. The focus is on exclusionary conditions. A sample also will be drawn for the biorepository.

Informed Consent for Screening

The research staff members who conduct the screening will administer informed consent prior to the initiation of testing. An introductory informed consent script that informs study participants about the study, their rights, the randomization of groups to receive the study medication or the placebo, study-related laboratory tests, cognitive test procedures, and the surveys on the REDCap database. This document will be read to participants before beginning the screening tests. A separate document will be recorded for each potential participant. After guiding the potential participant through the informed consent topics, the interviewer will ask the participant to sign the written consent and will give a copy to the participant. For the virtual platform, Miami VA research staff will mail 2 copies of the informed consent to the prospective participant. Once the signed copy is received, the Miami VA research staff will contact the participant to initiate study procedures. A consent form will be mailed to a potential participant only if he/she is interested in the study. If the individual does not return a signed consent form to the Coordinator, the individual will demonstrate that he/she has opted to not participate in the study.

After the informed consent process, it will be verified and documented whether the participant meets the inclusion/exclusion criteria for the study. The interview will terminate if it is determined that the participant is ineligible. Only once informed consent has been obtained from the study subject will the subject be considered to be enrolled in the study. The list of subjects will reside on the respective VA medical center network drive and will be secured in compliance with all VA confidentiality and information security requirements in the investigator's file for each study. For the virtual platform, no data, except that which is necessary for scheduling will be collected prior to receiving the signed informed consent.at the Miami VA. This information will include that the prospective participant has a primary care clinician and receives services at a specific VA medical center. The Coordinator will document this information during the prescreen telephone contact and will not mail an informed consent to a veteran who does not have a primary care clinician. All participants will be required to be enrolled at the VA, regardless of whether they are recruited to participate in on-site visits or in telephone visits using the virtual platform. Should the veteran experience psychological distress during the study visits, he or she will be referred for services at the VA medical center.

Enrollment
Potential participants will be considered to be enrolled in the study upon meeting eligibility criteria, completion of written informed consent, satisfactory completion of screening laboratory tests. No data, except that which is necessary for scheduling will be collected prior to informed consent.

Laboratory Specimens and Evaluations
During the course of this study, clinical laboratory specimens will be collected for research purposes. In order to determine eligibility for study participation, the subject will be consented and a screening lab test will be performed to make a diagnostic determination regarding GWI status and to evaluate the presence or absence of exclusionary medical conditions. For the virtual platform, the Miami VA research team will ship collection boxes directly to the participant. Samples will be drawn and packed by phlebotomists at Quest Laboratories in the participant's local area and shipped via overnight express for analysis at the Miami VAMC. The health assessment also will consist of a medical history, physical examination, and laboratory testing listed in Table 1.

Protocol for Blood Samples. At each of the clinical sites, blood samples will be obtained from each subject for the tests shown in Table 1 as well as the study sample storage for batched assays.

Appropriate samples for CBC, CMP, and U/A are sent to the Miami Lab. Whole blood samples are collected from the patients, according to the outline shown in Table 3. The blood samples are to be collected in afternoon and shipped by overnight carrier to the lab at the Miami VA Medical Center. Salivary samples will be collected 4 times over 24 hours and stored frozen at the sites, to be shipped on dry ice in batches to Miami VA.

Table 3. Blood Samples each onsite visit

	Serum	Plasma	PBMC
Tubes Total: 64 ml	**one 10 ml red top (siliconized)**	**two 7 ml purple top EDTA**	**four10 ml green tops (sodium**

Types of samples and storage requirements
1. Peripheral blood mononuclear cells are separated from blood and cryopreserved according required protocol. Viability and cell count is determined and aliquots are placed in freezer vials and stored in liquid nitrogen or at -80^{0}C as required by protocol.
2. Plasma is separated from the cellular component of blood by centrifugation and stored in required aliquots at -80^{0}C.
3. Serum is separated from the clot by centrifugation and stored in required aliquots at -40^{0}C.

Monitoring of freezers All mechanical freezers are connected to emergency outlets. If a freezer goes down, an alarm goes off in the building engineering section and lab personnel are immediately notified. In addition, the lab personnel check the interior temperature each day and record it on a chart attached to the freezer door. If the building power is disrupted, the freezers are automatically connected to the emergency generator system. Liquid nitrogen freezers are checked each day. The level of liquid nitrogen is measured and adjusted if needed. A liquid nitrogen storage tank is available in the freezer room. The storage tank is replaced each month.

Laboratory Measurements (Measured at T0, 16w, and T24 w)
1. **CoQ10 in plasma:** The method of Tang and Miles (2012) will be used to measure reduced (ubiquinol) and oxidized (ubiquinone) CoQ10. Plasma is extracted with 1-propanol. After centrifugation, the 1-propanol supernatant is directly injected into HPLC and monitored at a dual-

electrode. This method meets regulatory requirements for clinical laboratories, and has been proven reliable for analysis of clinical and research samples for clinical trials. CoQ10 screening will take place at the 8 week visit.

2. **Hormone studies: Hypothalamic-pituitary-thyroid (HPT) axis.** Thyroid status is assessed using a chemiluminescent method (TSH, free T3, free T4), Hypothalamic Pituitary Gonadal axis will be measured using ELISA methods measuring testosterone (free), FSH, LH and estrogen. Cortisol will be measured using a 24 hour salivary collection with 4 time points to assess circadian rhythm. The measures will be evaluated at baseline and to evaluate response to treatment.

3. **Safety and screening labs**: CBC, CMP and urinalysis will be performed at every time point as a safety screen: HIV, Hep B, C, Rheumatoid factor, Antinuclear antibody, CBC, CRP, and HbA1c, and LFTS will be drawn at screening to rule out exclusionary illnesses.

Laboratories Performing Evaluations

The clinical laboratories under the direction of Dr. Mary Ann Fletcher and Dr. Nancy Klimas at the Miami VA will be used for medical laboratory testing. Local VA laboratories will pack, and ship blood and saliva tests and will discard any residual samples. All research specimens will be processed at the Miami VA.

Laboratory Storage

Biological samples and laboratory data will be stored at the Miami VA Healthcare System, (Miami, FL) and related clinical data will be stored on a secure server. All samples will be processed and maintained according to documented SOPs that follow generally accepted good laboratory practices in order to assure sample quality, integrity and long-term stability.

All clinical data for consenting subjects will be stored indefinitely under conditions protecting PHI. In the event that a subject withdraws consent, Dr. Klimas will be informed of their Subject ID and their clinical information will be expunged from the database, and biological samples will be destroyed.

<u>Data Analysis Plan</u>

Statistical considerations

General Statistical Approach: Patients will be randomized in a 1:1 manner to Coq10 or placebo; randomization will be stratified by study center and gender. This study includes a comprehensive survey of patient status using a broad set of clinical assessments and laboratory assays designed to assess the response to treatment Unless otherwise specified, two-sided $P < 0.05$ will be considered statistically significant. Statistical analyses will be carried out using SAS Version 9.4 or higher.

a) Baseline: Differences in continuous baseline variables will be compared by a two-sample t test. The Mann-Whitney U test will be used in the case of non-normal distribution. For baseline categorical variables, Fisher's exact test will be used.

b) Evaluating the efficacy of CoQ10 in GWI: The primary analysis is to compare treatment groups on change from baseline SF-36 physical function score over time (6 monthly time points from Week 4 to Week 24) in a repeated measures setting using a final two-sided 0.049 level of significance (a 0.049 level is being used at the end of the study instead of 0.05 to account for the interim analysis further described below). This will be carried out using a mixed model (MM) analysis of variance (ANOVA) with effects for treatment, time, baseline physical function score and the randomization strata of study center and gender. The within-patient correlation structure will be assumed to be unstructured. If this analysis shows a significant treatment difference across time or a significant treatment-by-time interaction, comparisons across treatment conditions also will be carried out separately at each time point using a Bonferroni alpha-adjustment (significance level at each time point = 0.049 divided by 6, the number of planned post-baseline time points) to account for the multiple comparisons. Data will be analyzed as an intention-to-treat model; i.e., all

randomized subjects will be included using available data (i.e., a patient does not have to provide data through the end of the study to be included in the analysis; this is the strength of the MM ANOVA approach). A secondary supportive analyses will be carried out where missing physical function data at each time point are first imputed via monotone multiple linear regression imputation, under the assumption the missing data mechanism is missing at random prior to carrying out the mixed model analysis. The imputation regression model at each time point will include randomized treatment, measures of the primary outcome at earlier time points and baseline covariates that will be specified in the final detailed statistical plan. Fifty imputed datasets will be created, and the MM ANOVA will be run on each data set; the 50 MM ANOVA results will then be combined across the data sets using PROC MIANALYZE in SAS to obtain one overall assessment of treatment difference on the imputed data.

c) Sample size justification and power analysis: In this study population, we rely on the literature of Phase III studies of other inflammatory states to determine assumptions required for sample size calculations. Our primary clinical outcome variable is physical function of the SF36 measured over time (approximately monthly from week 4 through week 24; i.e., 6 measurements over time). Our current data suggests a coefficient of variation (CV) of approximately 0.40 for physical function at a given time point. Assuming this and assuming a relative increase in SF36 of at least 21% for Coq10 over placebo at each time point, then there is a 0.525 treatment effect size at each time point. Under this effect size, power is at least 95% to detect a treatment difference over time using an evaluable group sample size of n=90 subjects at a two-sided 0.049 level of significance regardless of the within- patient correlation between time points. To account for approximately 10% attrition, 100 subjects per group will be randomized. Such an evaluable sample size will also allow at least 80% power to detect a relative increase t of 21% separately at each time point after using a Bonferroni multiple comparison adjustment across the six time points.

d) Subgroup analysis on the primary endpoint: Treatments will be compared within each gender. The purpose of this analysis is not to show a statistically significant Coq10 effect on the primary endpoint within each gender (due to potential lack of power; e.g., if there are 25 evaluable women in each treatment group, power ranges from approximately 50% to 95% depending on the within-patient correlation between time points under the effect size assumptions used in the primary power calculation), but rather to investigate consistency of treatment difference across genders. In addition, treatment-by-gender interaction will be assessed in a mixed model repeated measures model that includes the main effects in the primary analysis and a term for treatment-by-gender interaction; an interaction p-value <0.15 will be considered as possibility indicating lack of consistency in treatment effect across gender. Similar analyses will be conducted to assess consistency of results across study centers.

e) Other clinical efficacy end points will look at each of the self-report assessment measures across the domains of fatigue, pain, sleep, and the SF36 subscales of function and severity. Objective measures include laboratory data that could reasonably be considered a surrogate for illness severity (e.g., measures of immune activation, inflammation, cell function, and NPY), a focus of several of our prior publications (Fletcher 2002, 2009, 2010; Maher 2007; Broderick 2009, 2010). Other objective measures include cognitive assessments and the activity monitor data which will be measured at each assessment point at the beginning of the study and on the 24th week of treatment. These will all be analyzed in a similar manner as the primary endpoint. We will also look at the effect of duration of study drug exposure on a case by case basis if there are individuals who fail to complete 24 weeks of study drug, and will also look at the change in CoQ10 levels as a predictor of response. These endpoints are considered exploratory and no adjustment of significance level for the multiple comparisons will be applied.

f) Safety Endpoints: The primary safety variable is the incidence of treatment emergent adverse events (TEAEs). A TEAE is an event that emerges, or a pre-existing event that worsens, any time after initiation of first study treatment. Adverse events will be coded by using the most recent released version of MedDRA at the time of study start. The incidence of TEAEs will be presented overall and by MedDRA system organ class and preferred term. TEAEs will also be presented by

severity (mild, moderate, or severe). Patients experiencing more than one occurrence of the same preferred term or system organ class will be categorized under the maximum severity experienced for that preferred term or system organ class. Similar analyses of TEAEs will be conducted by relationship to drug. Patients experiencing more than one occurrence of the same preferred term or system organ class will be categorized under the most related category experienced for that preferred term or system organ class. This will be repeated for serious TEAEs. Descriptive statistics (sample size, mean and median, quartiles, minimum and maximum) of laboratory values will be presented for each visit in which they were collected. Similarly, descriptive statistics from the change from baseline to each visit will be presented. Box plots of the changes from baseline will be presented across visits.

g) Evaluation of blinding: Each subject and investigator will be asked at the end of their follow-up to guess whether they believe the subject was randomized to active treatment or sham group. Frequencies of correct and incorrect responses will be provided separately for subjects and for investigators.

Data and Safety Monitoring Plan

Risk management and Emergency Response:

This study has been assigned for administrative and scientific oversight to the Clinical Services Research and Development Data Monitoring Committee (DMC) to Dr. Domenic Reda and his staff based at the Hines VAMC. They will monitor the study for safety, efficacy, and progress. Study participants will be asked to self-monitor and report to study personnel any episodes of symptoms. Adverse reactions will be reported to the Project Manager (Ms. Cohen) who will communicate with Dr. Klimas, the local IRB, and the DMC. While other trials have not seen serious adverse reactions, standard protocols are in place, with moderate to severe reactions reported to both the IRB and the DMC All unanticipated problems involving risk to subjects or others, serious adverse events, and all subject deaths associated with the protocol will be reported. Comments will be recorded on the outcomes of the event or problem. In the case of a serious adverse event, an assessment will be made on the relationship to the participation in the study. All reports determined by the investigators or DMC to be possibly or definitely related to study participation and reports of events resulting in serious adverse events will be promptly forwarded to the VACO ORD.

Should a systemic allergic reaction occur, subjects are advised to call 911, and take an antihistamine while awaiting response. The most common complaint with ubiquinol is GI upset and diarrhea. This is handled by taking the medication with food and if necessary with dose adjustment to 50%. All of the VA medical centers have a VA Emergency room that is available 24/7 if for some reason the study physician or back up is not available.

Veterans are the study population and are cared for by the VA health care system, which will assume the costs of study related injuries. Women of childbearing years are asked to take measures to avoid pregnancy during the study. Barrier methods or better are sufficient.

Psychological Safety Plan

We aim to ensure the safety of psychological study participants. Specific objectives of this safety plan are to ensure that:

1. Participants requesting assistance receive prompt response from mental health professionals who are knowledgeable safety risk assessment
2. The study team responds when participant's affect or behavior raises concerns (e.g., expressions of extreme distress), even if the participant him or herself does not request help.

The study team will check the on-line questionnaire HAM-D Question #3 regarding suicidality and will alert Dr. Klimas. Dr. Klimas will call the participant by telephone to assess the situation. If necessary, she will transfer the participant to the national VA Suicide Prevention Hotline. She will give the veteran the toll free number for the national VA Suicide Prevention Hotline (1-800-273-TALK (8255)) so that veterans, regardless of proximity to the Miami VA Healthcare System, can receive assistance in case of emergency. The hotline directs veterans to a VA professional who can immediately address their crisis situation. The local Suicide Prevention Coordinator then provides follow-up and assurance that these veterans in crisis receive on-going care. The study team will record and report to the IRB any incidents they are made aware of in which a participant calls the suicide prevention hotline. During the on-site assessment, if the Research Coordinator or Principal Investigator assesses a risk to the participant, she will walk with the participant to the Miami VA ER.

Because the study is based at the Miami VA, the study team will take additional measures to inform the Miami VA mental health after-hours and ER staff about this study. Specifically, we will distribute an information letter describing the study and listing the contact information for the study PI and Research Coordinator.

Risk Assessment Personnel

Professionals assessing potential risk in participants include Research Coordinator, Principal Investigator, VA Suicide Prevention Hotline staff, Miami VA ER and after-hours mental health staff. The Research Coordinator and Principal Investigator will assess risk in participants during administration of the structured clinical interview, completion of questionnaires or when participants request that a member of the study team contact them during regular business hours because of distress. The Principal Investigator will assess risk in participants in situations where the Research Coordinator identifies a potential need for intervention. The VA Suicide Prevention Hotline will conduct assessments among veterans who call the hotline. The Miami VA ER and After-Hours Mental Health Staff will conduct assessment and management of distressed veterans who call after hours. The Research Coordinator will be responsible for managing communication among these professionals to provide continuity of care as needed and report any adverse events among participants to the IRB.

Safety

Coenzyme Q10, (ubiquinol) is widely used as an overly the counter supplement and is considered to be safe by the FDA, with rare reports of side effects. We will be monitoring for symptoms that suggest allergic reactions (rash, stomach upset) or any other medication related side effect. It is hoped that the medications will improve your energy levels. Should this happen, there is a possibility that it will interfere with sleep. If this occurs, we will assess the benefit of reducing the dose. Dosing schedules will be reviewed at the investigators meeting before study initiation, and standard operating procedures to handle any perceived drug intolerance such as GI side effects will be addressed by the clinicians. Typically, a dose modification, reducing the dose by half for a week then increasing back to the target range over 2 weeks can be employed to determine if the presumptive associated side effect recurs. In the final analysis, we will review blood levels to determine if drug levels predict response or adverse reaction.

Adverse reactions of drug intolerance will be categorized by severity, body system, and number of events per individual studied. Serious reactions will be reported promptly to the IRB and CSR&D Data Monitoring Committee (DMC). Study personnel, including the PI, who also is the Director of the GWI clinic, will be available by telephone 24/7 and in clinic during routine hours. The Miami VAMC Emergency Department will serve as back up should a serious adverse reaction occur when personnel are not available.

Risks/ Benefits Assessments for Ubiquinol
Both ubiquinone and ubiquinol have been used extensively in clinical trials and in clinical settings. Whether efficacious or not, one remarkable observation is the reassuring safety profile of both preparations. Ubiquinone has been used in dosages up to 4,500 mg per day in frail populations (Parkinson's) and has seen positive results at the dosages proposed in this study in congestive heart failure and in illnesses known to be secondary to mitochondrial disorders (reviewed in Kern 2011, Kizhahkkedath 2013).

The most common adverse effects are gastrointestinal in nature and can include nausea, epigastric pain, diarrhea, heartburn, and appetite suppression. However, the prevalence of these adverse effects was less than 1% in reported studies (Jelin, 1999). Gastrointestinal effects of CoQ10 may be lessened with a dosage reduction or may subside with continued therapy. Asymptomatic elevations in serum lactate dehydrogenase and hepatic enzymes were observed and may occur with oral dosages of CoQ10 in excess of 300 mg/day; however, cases of serious hepatotoxicity have not been reported (Greenberg, 1990; Micromedix, 2014). If female veterans are pregnant, are breastfeeding, or become pregnant, ubiquinol might involve risks to the embryo or fetus, which are currently unforeseeable. For this reason, you will be required to take a pregnancy test in the beginning of the study and use adequate protection to prevent pregnancy. There is the possibility that ubiquinol may interfere with sleep and may lower blood pressure. For participants who commonly have very low blood pressure, they should check blood pressure carefully. For the virtual platform, if the clinician learns that the potential participant has a history of low blood pressure, then the participant will be excluded from the virtual study option. In congestive heart failure studies, clinical relapse was noted on withdrawal of CoQ10, whereas reinstatement of therapy resulted in improvement (Mortenson, 1990; Mortenson, 1985). We selected a formulation that has been used in prior clinical trials in other disease states and have safety data, bioavailability data as well as efficacy data published (Chandran et al., 2012; Sanmukhani et al., 2014; Kizhakkedath et al., 2013; Kern, 2011). Based on the literature review of treatment responses in other illnesses and Dr. Golomb' s experience, and conversations with Kaneka Nutrients (the primary source of ubiquinol in the USA) we will use ubiquinol 200 mg (the equivalent of ubiquinone 600mg) after a 2 month "loading" of 400 mg to correct deficiency states. It will be given as a morning dose, with food, to avoid evening side effects of sleeplessness and to optimize its bioavailability.

Adequacy of Protection Against Risk
Minimal risk is expected to be incurred by research participants, according to the safety records of the selected compound, an oil-based Ubiquinol soft gel. Ubiquinol has established safety profiles at doses that exceed the proposed treatment range and efficacy profiles established in other diseases. The blood draws in the study pose the risk of fainting from vaso-vagal reactions, local bruising, and discomfort. Minimization of risk includes having blood drawn only by experienced Registered Nurse or MD personnel.

Although the risks to the study participants are no greater than daily activity, the cognitive tests may cause discomfort. However, interviewers will be instructed and trained to identify potential problems and to tailor the testing as appropriate so as to minimize that risk. Throughout the testing, the research staff will remind participants of the goal of the testing, allowing short breaks when necessary. For the survey component, the risks are no greater than daily activity. Psychological risk associated with self-disclosure is handled by having interviewers who are trained psychologically in order to provide appropriate interaction and counseling. If any adverse reactions occur during the interview, Dr. Klimas will be available. For this study, there is a risk of loss of confidentiality. For the survey component, the data that will be accessed through the REDCap platform will be de-identified, with the identifiers remaining embedded in the database system. Unique participant ID numbers will be assigned to survey data and will be inputted into the database on a secure, password-protected,

encrypted computer and entered into a secure database.

Potential benefits:
It is our hope the subjects in the proposed clinical interventions will see clinical benefit. As this is the first time these treatments have been used in so large a GWI population, it is not assumed, and the duration of benefit is also not predictable. Subjects in both arms of the study have the benefit of a thorough evaluation, careful management, access to lab results not normally performed in the course of routine care (cytokines, immune studies and genomic studies), and will gain a better understanding of their illness. Ubiquinol is also associated with a reduction of the oxidized form of cholesterol (LDL) which will be measured, another potential benefit. in the experience of our team study volunteers have a sense of satisfaction in helping others through their participation, an intangible, but a benefit.

Monitoring Plan:
A data safety and monitoring plan will be implemented to ensure that there are no changes in the benefit/risk ratio during the study and that confidentiality of research data is maintained. The Principal Investigator, Co-Investigators, and research staff will be responsible for data safety and monitoring. All data obtained in the course of the study will be confidential. All participants will be informed of their confidentiality rights at the time informed consent is administered. Data collection and management will be conducted in strict accordance with the policies and procedures set forth by the VA Research & Development Office Data Security and Privacy Policy.

All study subjects will be assigned a study ID number by the research staff at their respective VA medical center at the time of enrollment in the study. Subjects will be identified by that number during the course of the study and data analysis. REDCap survey data will be entered as coded numerical values linked only to that subject's study ID number. Windows integrated security will be used for all computer access by the research staff. At each VA medical center, all PCs, laptops, workstations and remote devices will be secured with a password-protected screensaver, wherever possible, and set to deactivate after being left unattended for 15 minutes or more, or by logging-off when the equipment will be unattended for an extended period. User and role permissions will be defined at the computer, file, directory, server and database level to ensure data security. All research records will be maintained in accordance with the Veterans Health Administration (VHA) Records Control Schedule. Paper records will be disposed of using methods deemed appropriate by the respective VA medical center Privacy Officer, and all electronic data will be sanitized using methods rendered appropriate by the VA ISO.

The project team will meet weekly until the analysis commences to discuss the study (e.g., study goals, progress, modifications, documentation, recruitment, retention, and data analysis), and address any issues or concerns at the time. The discussion will include issues pertaining to data integrity, the assurance of patient confidentiality, and any needed changes will be instituted immediately to assure optimal subject protection. Breaches of confidentiality will be brought to the attention of the appropriate persons immediately as will any proposed changes to the data safety and monitoring plan.

These meetings will be overseen by the site PI at each medical center. Minutes will be kept for these meetings and will be on file. In addition, the site PI and Dr. Klimas may review study documentation and/or consent forms to ensure that subject's confidentiality is maintained. Any instances of protocol deviations, breaches of confidentiality, adverse events or other problems will be reported adverse effects will be reported immediately using the standard forms and/or procedures set forth by the IRB. Each VA site has an interim reporting requirement of all moderate to severe events to alert the site PI, with appropriate tracking logs and forms. All sites will be asked to share the VA Tracking Log for

Reportable and Non-Reportable Events and to follow the report of protocol deviations, violations, and/or Noncompliance with Dr. Klimas and the Project Manager, Devra Cohen, at the time of their scheduled IRB Continuing Review. In addition, all site PIs will report unanticipated serious adverse events (U-SAE), unanticipated problems (UAP) involving risks to participants and others to Dr. Klimas and Ms. Cohen in a timely manner. Research coordinators may review study documentation to ensure that subject's confidentiality is maintained.

Any paper notes on the forms will be collected and stored in a locked file cabinet in an office within the respective VA medical centers and staff will be responsible for their respondent confidentiality. The input from all individual surveys will be consolidated into an aggregated summary and no specific comments in the surveys will be attributed to an individual. Electronic data from the surveys will be kept on a secure, password-protected, encrypted VA computer and entered into a secure database behind VA firewalls. Unique participant ID numbers will be assigned to the survey data and will be inputted into the database. A crosswalk linking individual information to unique ID will be kept in a separate location in a password-protected file on a VA secure server, to which only investigators and analysts assigned to this project and listed on the research staff form will have access. A study investigator will review all files for consistency, accuracy and completeness and de- identification. Physical and electronic records will be kept according to the respective VA medical center's guidelines.

Data Handling

Confidentiality and sharing of data from human subjects: All database functionality will be designed in strict compliance with Health Insurance Portability and Accountability Act (HIPAA) standards in the U.S. and the Privacy of Information Act in Canada. All data collected about participants will be treated as highly confidential. To protect study subjects from confidentiality risks, the data will be entered and stored on REDCap/ MySQL database hosted at Boston University servers with a comprehensive software security system to limit access of data to authorized personnel only. Access to specific data will be defined in each research personnel's user profile and established based on that individual's role in the project. All paper records containing identifying information (including the subject contact list and linking codes) will be kept in locked files in locked offices at the site VA. The study identifier (ID) number will be included on all records; these identification methods are consistent with HIPAA guidelines. Only de-identified data including genetic/genomic data will be sent to the Computation Science Core team. Current or future results from procedures described in this proposal could provide information about participant susceptibility to certain ailments or conditions. Should any results of immunological or standard screening tests performed in this study demonstrate clinically relevant abnormalities, that information will be communicated to the participant's attending physician in accordance with local laws and the IRB medical ethics approvals in effect. Genetic/ genomic data will not be shared beyond authorized members of this research consortium. Results of the study will be shared with the participants but will be reported in such a manner that individual participants cannot be identified.

Data infrastructure

The core component of the project's data management and data storage infrastructure is the Research Electronic Data Capture (REDCap) environment, an existing web- based relational database developed specifically by Vanderbilt University for clinical research and available to the general research community. REDCap is a secure, web-based application for building and managing online databases that are based on PHP webserver + JavaScript programming languages and uses a MySQL database engine for data storage and manipulation. The standard REDCap MySQL database is built around five components: i) a metadata table describing the database configuration (data field types and naming used for automatic creation of separate data storage table); ii) a data transaction log used to store all information about data changes and exports; iii) a document storage table used to store consent forms, and analysis code as well as analysis results

exported from applications such as SAS, SPSS, R, MatLab, Stata, Excel, and others; iv) a user privileges table containing details of access rights and expiration settings; and v) a set of flat data tables used to store all collected data (typically one record [row]) per sample with all associated data fields stored in columns). REDCap accomplishes key functions through the use of a single study metadata table referenced by presentation-level operational modules. This abstract meta-data programming model allows research teams to autonomously develop study-related databases in an efficient manner as an easily maintainable resource for multiple concurrent studies [Nadkarni et al., 2000]. The REDCap initiative is driven by a user consortium composed of 396 active institutional partners in 46 countries. Use of this environment is fully supported by a dedicated team of database architects located at Vanderbilt University. NSU is a full member of this software consortium.

Data Identifiers

Each study participant will be assigned a unique code number from which they can be identified. Personal identifiers such as name, address, and date of birth will not be used on any study collection sheets except for the study contact sheet. This identifiable information is collected for patient tracking and safety purposes. The electronic information from the contact sheet will be stored separately from the main study database in a password-protected file at the site on a VA computer behind a VA firewall. Any paper versions of the contact sheet will be stored in locked file cabinets separate from other study data and accessible only by authorized study staff. In study databases, all data will be coded with the unique study identifier for each participant and stored in a de-identified manner.

Data Collection and Quality Assurance

The research coordinators at each study site will be responsible for managing the local study data. After a patient consents to participate in the study, the site clinical research assistant will create a patient casebook, which will contain the consent forms, all relevant source documents, and any other information pertinent to the study. The completed summary study forms will be uploaded electronically to REDCap for cleaning and processing into an electronic data document allowing for real-time data quality assurance and analysis. This will allow clinical research assistants to enter the source document data directly into a study database and thus manage their patient's study activity, handle data clarifications, and correct patient data in real-time. The data will be entered by site research assistants remotely through electronic case report forms. Extensive data checks, including missing values, out-of-range entries, and consistency between variables, both within and across forms, will be built into the system developed for this project. There will be two levels of checking. The first level will be done at the time the data is entered into the case report form. These checks will automatically appear on the screen at the time of entry. After submission of the form into the database, second-level checks against other data already captured for that patient will be done.

Data Confidentiality, Security, and Disposition

The database will not contain information that can directly identify the study subject (such as name, address, etc.). Study participants will be identified by a code number and not their personal identifiers on all study collection sheets except the study contact sheet. The electronic information from the contact sheets is stored separately from the main study database in a password-protected file. Any paper versions of the contact sheets are stored in locked file cabinets separate from other study data and accessible only by authorized study staff. Contact form information will be entered into the electronic system as password-protected files. Study data will be coded with a unique study identifier for each participant and stored in a de-identified manner. Identifiable information will be collected for patient tracking and safety purposes. All private information will be kept on an encrypted, password-protected server to which a small number of people will have access. All staff involved in the research project will have completed the appropriate IRB trainings. Access to the Cross-walk file linking the participant's identifiers and their study data will be restricted to the clinical VA sites. While the study is on-going, the data capture systems will utilize state-of-the-art

technologies in order to protect the data during transmission through web upload. Data will be stored indefinitely after study completion.

References

Alleva R, Tomasetti M, Battino M, et al. The roles of coenzyme Q10 and vitamin E on the peroxidation of human low density lipoprotein subfractions. Proc Natl Acad Sci USA 1995;92:9388- 91.

Baggio E, Gandini R, Plancher AC, et al. Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure. Mol Aspects Med 1994;15(suppl): S287-94.

Bargossi AM, Grossi G, Fiorella PL, et al. Exogenous CoQ10 supplementation prevents plasma ubiquinone reduction induced by HMG-CoA reductase inhibitors. Mol Aspects Med 1994;15(suppl):S187-93.

Bartone PT, Ursano RJ, Wright KM, Ingraham LH. The impact of a military air disaster on the health of assistance workers. A prospective study. J Nerv Ment Dis 1989; 177: 317-28.

Beal MF, Oakes D, Shoulson I, et.al. A randomized clinical trial of high-dosage coenzyme Q10 in early Parkinson disease: no evidence of benefit. JAMA Neurol. 2014 May;71(5):543-52.

Benjamini, Y, Hochberg, Y: Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Statist. Soc. B (1995); 57(1): 289-300.

Benedict, RHB, Schretlen, D, Groninger, L, Dobraski, M, Shpritz, B. Revision of the Brief Visuospatial Memory Test: Studies of normal performance, reliability, and validity. Psychological Assessment, Vol 8(2), Jun 1996, 145-153.

Binukumar BK, Gupta N, Sunkaria A, Kandimalla R, Wani WY, Sharma DR, Bal A, Gill KD. Protective efficacy of coenzyme Q10 against DDVP-induced cognitive impairments and neurodegeneration in rats. Neurotox Res. 2012 May;21(4):345-57.

Broderick G1, Kreitz A, Fuite J, Fletcher MA, Vernon SD, Klimas N. A pilot study of immune network remodeling under challenge in Gulf War Illness. Brain Behav Immun. 2011 Feb;25(2):302-13

Broderick G, Fletcher MA, Gallagher M, Barnes Z, Vernon SD, Klimas NG. Exploring the diagnostic potential of immune biomarker coexpression in Gulf War Illness. Methods Mol Biol. 2012;934:145-64. doi: 10.1007/978-1-62703-071-7_8.

Broderick G1, Ben-Hamo R, Vashishtha S, Efroni S, Nathanson L, Barnes Z, Fletcher MA, Klimas N. Altered immune pathway activity under exercise challenge in Gulf War Illness: an exploratory analysis. Brain Behav Immun. 2013 Feb;28:159-69. doi: 10.1016/j.bbi.2012.11.007. Epub 2012 Nov 29.

Birnbaum Y, Hale SL, Kloner RA. The effect of coenzyme Q10 on infarct size in a rabbit model of ischemia/reperfusion. Cardiovasc Res 1996;32:861-8.

Chopra RK, Goldman R, Sinatra ST, et al. Relative bioavailability of coenzyme Q10 formulations in human subjects. Int J Vitam Nutr Res 1998;68:109-13.

Cordero MD, , Alcocer-Gomez, E,de Miguel, M, et al: Can Coenzyme Q Improve Clinical and Molecular Parameters in Fibromyalgia? Antioxid. Redox Signaling. Antioxid Redox Signal. 2013 Oct 20;19(12):1356-6.

Delis, DC, Kramer, JH, Kaplan, E, Ober, BA. Manual for the California Verbal Learning Test, (2nd ed.)The Psychological Corporation, San Antonio, TX, 2000.

Digiesi V, Cantini F, Brodbeck B. Effect of coenzyme Q10 on essential arterial hypertension. Curr Ther Res 1990;47:841-5.

Digiesi V, Cantini F, Oradei A, et al. Coenzyme Q10 in essential hypertension. Mol Aspects Med 1994;15(suppl):S257-63.

Dumont M, Kipiani K, Yu F, Wille E, Katz M, et al. Coenzyme Q10 decreases amyloid pathology and improves behavior in a transgenic mouse model of Alzheimer's disease. J Alzheimers Dis. 2011;27(1):211-23.

Ernster L, Forsmark-Andrée P. Ubiquinol: an endogenous antioxidant in aerobic organisms. Clin Invest 1993;71:S60-5.

First, MB, Spitzer, RL, Gibbon, M. & Williams, JB, The Structured Clinical Interview for the DSM-IV Axis I Disorders (SCID) 1996.

Folkers K, Drzewoski J, Richardson PC, et al. Bioenergetics in clinical medicine. XVI. Reduction of hypertension in patients by therapy with coenzyme Q10. Res Commun Chem Patl Pharmacol 1981;31:129-40.

Folkers K, Littaru GP, Yamagami T, eds. Biomedical and clinical aspects of coenzyme Q, vol. 6. Amsterdam: Elsevier Science Publishers, 1991:339-48.

Folkers K. Heart Failure is a dominant deficiency of coenzyme Q10 and challenges for future clinical research on CoQ10. Clin Invest 1993;71(suppl 8):S51-4.

Fukuda K, Nisenbaum R, Stewart G, Thompson WW, Robin L, Washko RM, Noah DL, Barrett DH, Randall B, Herwaldt BL, Mawle AC, Reeves WC. (1998) Chronic multisymptom illness affecting Air Force veterans of the Gulf War. JAMA 280: 981-988.

Garrido-Maraver, J, Cordero,MD, Oropesa-Avila, M, et al. Clinical applications of coenzyme Q10. Frontiers in Bioscience 19, 619-633, January, 2014. Ghirlanda B, Oradei A, Manto A, et al. Evidence of plasma CoQ10 reductase inhibitors: a double blind, placebo-controlled study. J Clin Pharmacol 1993;33:226-9.

Golomb BA, Allison M, Koperski S, Koslik HJ, Devaraj S, Ritchie JB. Coenzyme Q10 benefits symptoms in Gulf War veterans: results of a randomized double-blind study, Neural Comput. 2014 Nov;26(11):2594-2651.

Greenberg S, Frishman WH. Co-enzyme Q10: a new drug for cardiovascular disease. J Clin Pharmacol 1990;30:596-608.

Groneberg, DA, Kindermann, B, Althammer,M, et al.. Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. Int J Biochem Cell Biol 2005,37(6), 1208-18.

Hiasa Y, Ishida T, Maeda T, et al. Effects of coenzyme Q10 in patients with stable angina pectoris. In: Folkers K, Yamamura Y, eds. Biomedical and clinical aspects of coenzyme Q, vol. 4. Amsterdam: Elsevier Science, 1984:291-301.

Hofman-Bang C, Rehnquist N, Swedberg K, et al. Coenzyme Q10 as an adjunctive in the treatment of chronic congestive heart failure. J Cardiac Failure 1995;1:101-7.

Ishiyama T, Morita Y, Toyama S, et al. A clinical study of the effect of coenzyme Q10 on congestive heart failure. Jap Heart J 1976;17:32-42.

Iwamoto Y, Yamaguchi T, Folkers K, et al. Deficiency of coenzyme Q10 in hypertensive rats and reduction of deficiency by treatment with coenzyme Q10. Biochem Biophys Res Commun 1974;58:743-8.

Jason LA, Evans M, Porter N, Brown M, Brown AA, Hunnell J, Friedberg F. The development of a revised Canadian myalgic encephalomyelitis chronic fatigue syndrome case definition. American Journal of Biochemistry and Biotechnology. 2010;6:120–135. doi: 10.3844/ajbbsp.2010.120.135.

Jellin JM, Batz F, Hitchens K. Coenzyme Q10. Pharmacist's letter/prescriber's letter: natural medicines comprehensive database. Stockton, CA: Therapeutic Research Center, 1999:271-3.

Judy WV, Hall JH, Toth PD, et al. Double-blind-double-crossover study of coenzyme Q10 in heart failure. In: Folkers K, Yamamura Y, eds. Biomedical and clinical aspects of coenzyme Q, vol. 5. Amsterdam: Elsevier Science Publishers BV, 1986:315-23

Kamikawa T, Kobayashi A, Yamashita T, et al. Effects of coenzyme Q10 on exercise tolerance in chronic stable angina pectoris. Am J Cardiol 1985;56:247-51.

Kaufmann P, Thompson JL, Levy G, Buchsbaum R, et al. QALS Study Group Phase II trial of CoQ10 for ALS finds insufficient evidence to justify phase III. Ann Neurol. 2009 Aug;66(2):235-44.

Khatta M, Alexander BS, Krichten CM, et al. The effect of coenzyme Q10 in patients with congestive heart failure. Ann Intern Med 2000;132:636-40.

Kishi T, Kishi H, Watanabe T, et al. Bioenergetics in clinical medicine. XI. Studies on coenzyme Q and diabetes mellitus. J Med 1976;7:307-21.

Landbo C, Almdal TP. Interaction between warfarin and coenzyme Q10 [English abstr]. Ugeskr Laeger 1998;160: 3226-7. the GWI field.

Langsjoen PH, Vadhanavikit S, Folkers K. Effective treatment of coenzyme Q10 of patients with chronic myocardial disease. Drugs Exp Clin Res 1985;11(8):577-9.

Langsjoen PH, Folkers K, Lyson K, et al. Effective and safe therapy with coenzyme Q10 for cardiomyopathy. Klin Wochenschr 1988;66:583-90.

Langsjoen PH, Langsjoen PH, Folkers K. Long-term efficacy and safety of coenzyme Q10 therapy for idiopathic dilated cardiomyopathy. Am J Cardiol 1990;65:521-3.

Langsjoen PH, Vadhanavikit S, Folkers K. Response of patients in classes III and IV of cardiomyopathy to therapy in a blind and crossover trial with coenzyme Q10. Proc Natl Acad Sci U S A 1985;82:4240-4.

Langsjoen P, Langsjoen P, Willis R, et al. Treatment of essential hypertension with coenzyme Q10. Mol Aspects Med 1994;15(suppl):S262-72.

Lampertico M, Comis S. Italilan multicenter study on the efficacy and safety of coenzyme Q10 as adjuvant therapy in heart failure. Clin Invest 1993;71(suppl 8):S129-33.

Lee BJ, Tseng YF, Yen CH, Lin PT Effects of coenzyme Q10 supplementation (300 mg/day) on antioxidation and anti-inflammation in coronary artery disease patients during statins therapy: a randomized, placebo-controlled trial. Nutr J. 2013 Nov 6;12(1):142.

Levien TL, Baker DE. Coenzyme Q10. Pharmacist's letter. Stockton, CA: Therapeutic Research Center, 1998;14(2):8-9.

Lucker PW, Wetzelsberger N, Hennings G,et al. Pharmacokinetics of coenzyme ubidecarenone in healthy volunteers. In: Folkers K, Yamamura Y, eds. Biomedical and clinical aspects of coenzyme Q, vol. 4. Amsterdam: Elsevier Science Publishers, 1984:143-51. Markley: HG, CoEnzyme Q10 and riboflavin: the mitochondrial connection. Headache 2002, 52 Suppl 2, 81-7.

Martin SB, Cenini G, Barone E, et al. Coenzyme Q10 and cognition in atorvastatin treated dogs. Neurosci Lett. 2011 Aug 26;501(2):92-5.

Mazzola C, Guffanti EE, Vaccarella A, et al. Non-invasive assessment of coenzyme Q10 in patients with chronic stable effort angina and moderate heart failure. Curr Ther Res 1987;41:923-32.

Mestre T, Ferreira J, Coelho MM, Rosa M, Sampaio C Therapeutic interventions for disease progression in Huntington's disease. Cochrane Database Syst Rev. 2009 JUl8

Micromedex. Healthcare series. Ubidecarenone. Englewood, CO: Micromedex, Inc., 2014.

Morisco C, Trimarco B, Condorelli M. Effect of coenzyme Q10 in patients with congestive

heart
failure: a long term multi-center randomized study. Clin Invest 1993;71:134-6.

Morisco C, Nappi A, Argenziano L, et al. Noninvasive evaluation of cardiac hemodynamics during exercise in patients with chronic heart failure: effects of short-term coenzyme Q10 treatment. Mol Aspects Med 1994;15:155-63.

Mortensen SA, Vadhanavikit S, Muratsu K, et al. Coenzyme Q10: clinical benefits with biochemical correlates suggesting a scientific breakthrough in the management of chronic heart failure. Int J Tissue Reac 1990;XII(3):155-62.

Mortensen SA, Vadhanavikit S, Baandrug U, et al. Long-term coenzyme Q10 therapy: a major advance in the management of resistant myocardial failure. Drugs Exp Clin Res 1985;11:581-93.

Mortensen SA. Perspectives on therapy of cardiovascular diseases with coenzyme Q10 (ubiquinone). Clin Invest 1993;71:116-23.

Mortensen SA, Leth A, Agner E, et al. Dose-related decrease of serum coenzyme Q10 during treatment with HMG-CoA reductase inhibitors. Mol Aspects Med 1997;18(suppl): S137-44.

Murray M. Encyclopedia of nutritional supplements. Rocklin, CA: Prima, 1996:296-308.

Olsen RE, Rudney H. Biosynthesis of ubiquinone. Vitam Horm 1983;40:1-43.

Overvad K, Diamant B, Holm L, et al. Coenzyme Q10 in health and disease. Eur J Clin Nutr 1999;53:764-70.

Peckerman A, Dahl K, Chemitiganti R,et al. Effects of posttraumatic stress disorder on cardiovascular stress responses in Gulf War veterans with fatiguing illness. Auton Neurosci. 2003 Oct 31;108(1-2):63- 72.

Pogessi L, Galanti G, Comeglio M, et al. Effect of coenzyme Q10 in left ventricular function in patients with dilative cardiomyopathy. Curr Ther Res 1991;49:878-86.

Proctor SP, Heeren T, White RF, et al. Health status of Persian Gulf War veterans: self-reported symptoms, environmental exposures and the effect of stress. Int J Epidemiol 1998; 27: 1000-10.\

Reeves WC, Lloyd A, Vernon SD, Klimas N, Jason LA, Bleijenberg G, Evengard B, White PD, Nisenbaum R, Unger ER, .Identification of ambiguities in the 1994 chronic fatigue syndrome research case definition and recommendations for resolution.BMC Health Serv Res. 2003 Dec 31;3(1):25

Rossi E, Lombardo A, Testa M, et al. Coenzyme Q10 in ischemic cardiomyopathy. In: Folkers K, Littaru GP, Yamagami T, eds. Biomedical and clinical aspects of coenzyme Q, vol. 6. Amsterdam: Elsevier Science Publishers, 1991:321-6.

Rozen, TD, Oshinsky, ML, Gebeline, CA, et al.: Open label trial of coenzyme Q10 as a migraine preventive. Cephalalgia 2002, 22(2), 137-41.

Sándor PS, Di Clemente L, Coppola G, et al. Efficacy of coenzyme Q10 in migraine prophylaxis: a randomized controlled trial. Neurology. 2005 Feb 22;64(4):713-5.

Sandhir R, Sethi N, Aggarwal A, Khera A. Coenzyme Q10 treatment ameliorates cognitive deficits by modulating mitochondrial functions in surgically induced menopause. Neurochem Int. 2014 Jul;74:16-23.

Sanoobar M, Dehghan P, Khalili M, Azimi A, Seifar F. Coenzyme Q10 as a treatment for fatigue and depression in multiple sclerosis patients: A double blind randomized clinical trial.Nutr Neurosci. 2015 Jan 20.

Schardt F, Welzel D, Scheiss W, et al. Effect of coenzyme Q10 on ischemia-induced ST-segment depression: a double-blind, placebo-controlled crossover study. In: Folkers K, Yamamura Y, eds. Biomedical and clinical aspects of coenzyme Q, vol. 5. Amsterdam: Elsevier Science Publishers BV, 1986:358-94.

Scheirer CJ, Ray WS, Hare N. The analysis of ranked data derived from completely randomized factorial designs. Biometrics 1976; 32: 429-434.

Schmelzer, C, Lindner,I, Rimbach, G, Niklowitz, P, Menke, T, Doring: Functions of coenzyme Q10 in inflammation and gene expression. Biofactors 2008, 32(1-4), 179-83.

Serra G, Lissoni F, Piemonti C, et al. Evaluation of coenzyme Q10 in patients with moderate heart failure and chronic stable effort angina. In: Folkers K, Littaru GP, Yamagami T, eds. Biomedical and clinical aspects of coenzyme Q, vol. 6. Amsterdam: Elsevier Science Publishers, 1991:327-38.

Singh RB, Niaz MA, Rastogi SS, et al. Effect of hydrosoluble coenzyme Q10 on blood pressure and insulin resistance in hypertensive patients with coronary artery disease. J Hum Hypertens 1999;13:203-8.

Spigset O. Reduced effect of warfarin caused by ubidecarenone. Lancet 1994;344:1372-3.
Steele L. Prevalence and patterns of Gulf War illness in Kansas veterans: association of symptoms with characteristics of person, place, and time of military service. Am J Epidemiol. 2000;152:992- 1002.

Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc NatlAcadSci U S A. 2003 Aug 5;100(16):9440-5.

Sullivan S, Krengel M, Proctor S, Devine S, Heeren T, White R. Cognitive Functioning in Treatment- Seeking Gulf War Veterans: Pyridostigmine Bromide Use and PTSD. 2003 J Psychopathol Beh Assess, 25: No. 2.

Tang, PH, Miles, MV. Measurement of oxidized and reduced coenzyme Q in biological fluids, cells, and tissues: an HPLC-EC method. Methods Mol Biol. 2012;837:149-68.

Tomono Y, Hasegawa J, Seki T, et al. Pharmacokinetic study of deuterium-labelled coenzyme Q10 in man. Int J Clin Pharm Ther Toxicol 1986;24:536-42.

Tran, MT, Mitchell, TM, Kennedy, DT, Giles, JT. Role of Coenzyme Q10 in Chronic Heart Failure, Angina, and Hypertension Pharmacotherapy. 2001;21(7).

Trivedi M, Shah J, Hodgson N, Byun HM, Deth R. Morphine induces redox-based changes in global DNA methylation and retrotransposon transcription by inhibition of excitatory amino acid transporter type 3-mediated cysteine uptake. Mol Pharmacol. 2014 May;85(5):747-57.

Yuzuhira T, Takada M, Katayama K. Transport of C14 coenzyme Q10 from the liver to other tissues after intravenous administration to guinea pigs. Biochimica Biophysica Acta 1983;759:286-91.

Watson PS, Scalia GM, Galbraith A, et al. Lack of effect of coenzyme Q on left ventricular function in patients with congestive heart failure. J Am Coll Cardiol 1999;33:1549-52.

Watts GF, Bastelluccio C, Rice-Evans C, et al. Plasma coenzyme Q (ubiquinone) concentrations in patients treated with simvastatin. J Clin Pathol 1993;46:1055-7.

Weber C, Jakobsen TS, Mortensen SA, et al. Antioxidative effect of dietary coenzyme Q10 in human blood plasma. Int J Vitam Nutr Res 1994;64:311-15.

Weber C, Jakobsen TS, Mortensen SA, et al. Effect of dietary coenzyme Q10 as an antioxidant in human plasma. Mol Aspects Med 1994;15(suppl):S9

Weber C, Bysted A, Hølmer H. Intestinal absorption of coenzyme Q10 administered in a meal or as capsules to healthy subjects. Nutr Res 1997;17:941-5.

Weis M, Mortensen SA, Rassing MR, et al. Bioavailability of four oral coenzyme Q10 formulations in healthy volunteers. Mol Aspects Med

Whistler T, Fletcher MA, Lonergan W, Zeng XR, Lin JM, Laperriere A, Vernon SD, Klimas NG.

Impaired immune function in Gulf War Illness. BMC Med Genomics. 2009 Mar 5;2:12. doi: 10.1186/1755-8794-2-12.

Wolfe J, Proctor SP, Davis JD, Borgos MS,Friedman MJ. Health symptoms reported by Persian Gulf War veterans two years after return. Am J Ind Med 1998; 33: 104-13.

Yamagami T, Takagi M, Akagami H, et al. Effect of coenzyme Q10 on essential hypertension: a double-blind controlled study. In: Folkers K, Yamamura Y, eds. Biomedical and clinical aspects of coenzyme Q, vol. 5. Amsterdam: Elsevier Science Publishers BV, 1986:337-43.

Yamagami T, Shibata N, Folkers K. Bioenergetics in clinical medicine: studies on coenzyme Q10 and essential hypertension. Res Commun Chem Pathol Pharmacol 1975;11:273-88.

Yamagami T, Iwamoto Y, Folkers K, et al. Deficiency of activity of succinate dehydrogenase-coenzyme Q10 reductase in leukocytes from patients with essential hypertension. Int J Vitam Nutr Res 1974;44:404-14.