Technical Abstract: W81XWH-08-GWIRP-IIRA
Mechanisms of Mitochondrial Defects in Gulf War Syndrome

**Background:** Gulf War syndrome (GWS) is associated with increased incidences of amyotrophic lateral sclerosis, pain syndromes, muscle complaints that include fatigue and myalgias, as well as other neurological symptoms. Approximately 100,000 individuals have medical complaints consistent with GWS. Clinical manifestations are similar to those identified in Chronic Fatigue Syndrome (CFS). Mitochondrial defects are identified pathologically, metabolically, and genetically in some patients with CFS. GWS has significant evidence for mitochondrial dysfunction with abnormalities in exercise physiology, abnormalities in mitochondrial morphology, biochemical defects in mitochondrial function, abnormalities in free radical generation affecting mitochondrial integrity, gene expression in genes affecting mitochondrial function, and mtDNA mutations (inherited, somatic, and sporadic during embryogenesis). Gene expression abnormalities in CFS show abnormalities in genes that are related to mitochondrial function. Hence, investigation of mitochondrial dysfunction in GWS is a priority.

**Objective/Hypothesis:** The carrier rate of mitochondrial DNA (mtDNA) mutations in the general population is 1 in 200 individuals, making mitochondrial dysfunction a potentially important mechanism in GWS. Mitochondrial dysfunction is an important in the pathogenesis of GWS through the interaction of inherited and acquired nuclear DNA and/or mtDNA coded mitochondrial genes with environment exposures (pre-Gulf War exposures plus Gulf War associated exposures). Mitochondrial mechanisms of disease could unify many of the diverse variables associated with GWS and lead to rational treatment strategies for these patients. We have identified significant mitochondrial defects in CFS/fibromyalgia patients as well as a significant Complex V defect in a patient diagnosed with GWS.

**Specific Aim I:** After informed consent, blood samples and a skin biopsy will be obtained from 50 individuals diagnosed with GWS who have myalgia and fatigue patients.

**Specific Aim II:** Characterize mitochondrial cellular energetics in GWS patients relative to age and gender matched controls in blood and skin cells (fibroblasts) using the following approaches: (1) high resolution respirometry of intact cells, (2) quantitative analysis of individual mitochondrial proteins (denatured, Western blot), (3) analysis of intact OXPHOS enzyme complexes and supercomplexes (non-denatured, Blue Native and Clear Native gels), (4) in gel enzyme activity assessment of intact OXPHOS enzyme complexes and supercomplexes (Clear Native gel, in-gel activity measurements), (5) mtDNA copy number quantitation to assess for defects in regulating mtDNA replication, and (6) cellular coenzyme Q10 quantitation (endogenous synthesis is impaired in certain types of mitochondrial dysfunction).

**Specific Aim III:** Assess the mitochondrial DNA (mtDNA) from each patient with GWS for mtDNA mutations by whole genome sequencing of leukocyte and skin cell mtDNA. Based on the findings from Specific Aim II, selected nuclear coded OXPHOS genes will be sequenced to assess for mutations that increase susceptibility to GWS.

**Study Design:** Our laboratory has two decades experience with mitochondrial research and a large bank of over 25,000 patient samples (muscle, fibroblasts, EBV transformed lymphocytes, cerebrospinal fluid) that will allow statistical analysis of the data relative to gender/age matched control. In this prospective study, mitochondrial abnormalities in GWS cells are identified by comparison with tissue/cells from individuals with mutations known to cause mitochondrial dysfunction and controls.

**Impact:** Mitochondrial mechanisms of disease could unify many of the diverse variables associated with GWS and lead to rational treatment strategies for these patients. Once the pathogenesis of GWS is firmly linked to mitochondrial dysfunction, we plan to initiate clinical trials for GWS as we are currently developing for patients with mitochondrial disease.
Gulf War syndrome (GWS) is associated with increased incidences of amyotrophic lateral sclerosis, pain syndromes, muscle complaints that include fatigue and myalgias, as well as other neurological symptoms. Approximately 100,000 individuals of the 700,000 veterans deployed in 1990-1991 Gulf War have medical complaints consistent with GWS. Clinical manifestations are similar to those identified in Chronic Fatigue Syndrome (CFS). Abnormalities in the part of the cell known as mitochondria have been delineated in GWS and CFS. We propose that GWS is determined by a complex interaction of variables that impair mitochondrial function that include genetic susceptibility, pre-Gulf War exposures, Gulf War associated exposures, and aging. This study will be the first comprehensive investigation of mitochondrial function in GWS. Our objective is to establish the cause for symptoms in affected veterans, develop testing that can more easily identify GWS, and ultimately develop treatment protocols for GWS.

The mitochondria have many functions that include changing the foods we eat into a usable form of energy known as ATP (adenosine triphosphate). All the cells of our body use this energy to run the biochemical reactions that allow our cells to function properly. Hence, the mitochondria act as tiny power plants inside of every cell. These power plants also utilize about 95% of the oxygen that we breathe. When the energy (ATP) is produced at normal levels, the cells function normally. When the energy (ATP) is reduced, the cells develop a variety of problems (analogous to a city during a brown out where energy dependent functions begin to fail). The central nervous system and muscle are often affected. These diseases can have their onset at any age and can even be triggered by exposure to certain chemicals and drug exposures. Multiple lines of evidence from the literature and from patients studied in our laboratory suggest that the mitochondria are not functioning properly in GWS and in CFS. Hence, detailed investigation of mitochondrial dysfunction in GWS is a priority.

Over the last two decades, our group has been dedicated to working with patients with mitochondrial defects. Over the years, we described many inherited mutations and biochemical defects that impair mitochondrial function. Our proposal is unique in that we integrate a variety of specialized laboratory techniques that characterize mitochondrial function into a comprehensive investigation. Our proposal is designed to characterize mitochondrial function in 50 veterans with GWS using blood and skin cells. We will be investigating the skin and blood cells by characterizing precisely how mitochondria are working through detailed investigation of mitochondrial enzyme function, of mitochondria within living cells, of mitochondrial proteins, and of mitochondrial genes.

Mitochondrial mechanisms of disease could unify many of the diverse variables associated with GWS and lead to rational treatment strategies for these patients. Once the pathogenesis of GWS is firmly linked to mitochondrial dysfunction, we plan to initiate clinical trials for GWS as we are currently developing for patients with mitochondrial disease.