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Physiologic Effects of Stress in Gulf War Veterans

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13. ABSTRACT
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The cause of Gulf War illnesses (GWI) remains unclear. There appears to be a constellation of symptoms and syndromes that occur in a higher rate in deployed than non-deployed individuals. Although the precise reason for GWI remains unknown, symptoms nearly identical to GWI are noted in several syndromes that occur at a high rate in the general population, including fibromyalgia, chronic fatigue syndrome, somatoform disorder, and multiple chemical sensitivity.

A significant problem encountered when examining potential pathogenic mechanisms for GWI is demonstrated by a series of articles which appeared recently in the JAMA. In this issue were population-based studies which demonstrated that individuals who were deployed to the Gulf War were more likely to display the above-noted syndromes and symptoms, and physiologic studies performed on small numbers of highly selected subjects which demonstrated some abnormalities in neural function. However, there is no way to determine if the abnormalities noted in these few individuals who had physiologic studies are generalizable to the entire population, or to verify the validity of self-report data.

In the present study, we propose to eliminate this problem. This proposal describes a collaborative effort between investigators performing a population-based study of this illness, and another group studying pathophysiologic responses to stress which could be responsible for this symptom complex. Using this methodology, a representative sampling of symptomatic and asymptomatic PGW veterans will be selected from a large database and recruited to come to Georgetown to have a battery of physiologic studies performed to assess function of the human "stress response", including the autonomic nervous system, hypothalamic-pituitary-adrenal axis, and nociceptive function. We feel that this unique method of selecting appropriate subjects and controls, and of obtaining valid self-report data, will significantly enhance both studies.

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INTRODUCTION

In 1990 and 1991, the U.S. deployed approximately 700,000 troops to the Persian Gulf (PG) to liberate Kuwait from Iraqi occupation. Fortunately, there were relatively few combat and non-combat related injuries and diseases during this conflict in comparison with previous military campaigns. However, a significant number of veterans developed a constellation of symptoms and syndromes which have defied explanation, and have been termed Persian Gulf War Illnesses (PGWI). Several expert panels have been convened to examine the potential causes for these illnesses. There is agreement that this is not a single illness due to a single cause, and that most potential environmental exposures are unlikely to have contributed to this illness\(^1,2\). The consensus of these reports is that physical, emotional, and immune stressors are capable of causing these types of non-specific symptoms, and that this is an area requiring further investigation.

There are several reasons for the conclusion that "stress" may be responsible for many of the symptoms seen in PGW veterans. Similar illnesses have been noted after nearly every major conflict, although these syndromes have had different names and attributions (e.g. Post Traumatic Stress Disorder [PTSD] and Agent Orange after the Vietnam conflict, "shell shock" after World War I, etc.)\(^3,4\). More importantly, similar stress-related disorders occur commonly in the general population, with the currently preferred semantic terms being fibromyalgia (FM), chronic fatigue syndrome (CFS), somatoform disorder, and multiple chemical sensitivity (MCS)\(^5,6,8\). Therefore, it is plausible that the majority of the symptoms experienced by individuals with PGWI fall within a continuum of conditions which are best exemplified by FM and CFS, and were caused by exposure to a variety of stressors in conjunction with deployment to the PG.

The pathogenesis of symptoms in illnesses such as FM, CFS and PGWI is controversial. Some contend that these are primarily psychiatric conditions\(^10,11\). We have been extensively involved in the study of FM and CFS, and although there are clearly psychological co-morbidities with these illnesses, there are also substantial data suggesting that there is a physiologic basis for the symptoms these individuals experience. We have been particularly involved in the study of neurohormonal dysregulation of the human stress response that is common to FM and CFS.

The purpose of this proposal is to intensively study the activity of the biological stress response in individuals with PGWI, to determine if such persons display the same types of "blunting" of stress response function as is noted in FM and CFS. This proposal will "link" two DOD-funded groups to offer a unique manner of examining PGWI, integrating the best features of population-based studies, and intensive clinical evaluations. The Klemm Analysis Group has funding to assess the symptoms of 20,000 Persian Gulf and Persian Gulf-era women veterans (The Persian Gulf Women's Health Linkage Study - PGWHLS). The proposed study would recruit a representative sample of these Persian Gulf women with unexplained illness, and a control group of these Persian Gulf women veterans who are asymptomatic but are matched for location of deployment, age, and race. These individuals will be brought to the Clinical
Research Center at Georgetown, and will undergo a battery of tests examining function of the human stress response, most of which have been shown to be abnormal in individuals with FM and CFS. In addition, both groups will undergo a comprehensive clinical and psychological evaluation. This joint effort will significantly strengthen the results of both studies, enabling us to validate the results of self-report data on a representative subset of the large cohort, and to perform sophisticated physiologic testing on an unbiased and better matched sampling of cases and controls.

HYPOTHESES, TECHNICAL OBJECTIVES, and SPECIFIC AIMS:

1) To perform an extensive clinical and psychological evaluation on a representative sampling of symptomatic and asymptomatic individuals who were deployed to the GW. This will allow us to validate and expand on self-report data collected in the large cohort, as well as to identify risk factors (e.g., differences in pre-morbid psychological status) which may be predictive of developing these unexplained symptoms.

2) To determine if PGWI subjects display evidence of a low central corticotropin releasing hormone (CRH) state. CRH is agreed to be the central neurohormone controlling the overall activity of the biological stress response. Illnesses such as FM and CFS have been hypothesized to be due to a low CRH state, although central CRH cannot be directly measured in humans. Thus, to obtain inferential data regarding central CRH levels, we will infuse subjects and controls with interleukin-6 (IL-6), which in animals and humans has been shown to lead to central CRH release. We have shown that FM subjects have a markedly exaggerated ACTH response to IL-6, and will determine if this same finding is noted in PGWI subjects. This finding of hypothalamic hypersensitivity to a stimulus of CRH release is consistent with what would be expected in a chronically low CRH state (or a comparable abnormality in hypothalamic secretagogues). We hypothesize that we will find a low baseline CRH state in PGWI subjects, which would be significant because the direct central effects of low CRH would be to cause diffuse or regional pain (e.g. myalgias, arthalgias, headache) and fatigue.

3) To determine if PGWI subjects display evidence of impaired activation of both the adrenomedullary and sympathoneural components of the sympathetic nervous system. There are data suggesting that individuals with FM and CFS display blunted sympathetic activity in response to stressors. We will examine this same response in PGWI subjects, to determine if these individuals display similarly blunted sympathetic function, normal function, or a different abnormality in sympathetic function (e.g., enhanced function, as is seen in PTSD). Dysautonomia is believed to be important in the pathogenesis of FM and CFS, as well as entities (i.e. migraine headache and irritable bowel syndrome) that are seen commonly in PGW veterans.

4) To determine if PGWI subjects have evidence of decreased peripheral responsiveness to catecholamines. We present preliminary data demonstrating that individuals with FM not only display decreased release of catecholamines, but also decreased responsiveness. If we confirm this same finding in PGWI subjects, the
combined effects of decreased release of catecholamines in response to stressors, and
decreased responsiveness at the tissue level, would be expected to have a more
profound physiologic effect than either one or the other.

5) To determine if significant abnormalities of one or more of these
various components of the stress response can be noted in most persons with
PGWI, and that the nature of the abnormality can predict the predominant clinical
symptoms. None of the above Specific Aims are worthwhile if we do not determine the
clinical significance of these abnormalities in stress response function. Although
illnesses such as FM and CFS are sometimes described as discrete and unique, in
reality this is a large and heterogeneous aggregation of symptoms that are described
using a variety of semantic terms. It is likely that there are many concurrent
pathophysiologic processes responsible for this heterogeneity in symptom expression.
To demonstrate clinical subsets of subjects that can be identified by their
pathophysiologic characteristics, we will concurrently study the biological stress
response, psychological status, physical status, and symptom reporting. We will
perform several types of analyses to determine which physiologic and psychologic
abnormalities predict which clinical features.

The study of the physiologic and psychologic responses to stress is very difficult
and fraught with pitfalls. The above-noted biologic studies will be performed by a multi-
disciplinary team of investigators with established expertise in FM and CFS, as well as
in the measure of neuroendocrine and autonomic function. This study combines state-
of-the-art knowledge of the abnormalities in stress response function that are seen in
illnesses such as FM and CFS, and will apply these same methodologies to individuals
with PGWI. There are three potential conclusions from this work; stress response
function in the PGWI subjects is: 1) blunted as in FM/CFS, 2) normal, or 3) abnormal,
but different than FM/CFS. Regardless of which is found, we feel that this information
will shed significant insight into the pathophysiology of PGWI and similar post-war
syndromes, and may also shed novel insights into the prevention or treatment of this
symptom complex.

BACKGROUND (cont). Overview of the human stress response. The human
stress system has been the subject of intense study. Historically, Cannon and Selye
have been particularly instrumental in shaping our ideas regarding the essence of this
response. Cannon suggested that "homeostasis" was physiologically achieved by the
activation of compensatory and competing systems within the body, although generally
avoiding reference to "stress" 15. Selye was the first to popularize the notion that
"stress" was a scientifically credible concept, and began to establish the relationship
between abnormalities in the stress response, and the development of disease 16

With these foundations, continued research has shown that this system is
considerably more complex than originally proposed. Although Selye suggested
nonspecificity of the stress response, it is now clear that different types of stressors
elicit markedly different biological responses 17-19. The pattern of biological response
depends on both properties of the stressor (e.g., type and intensity), as well as
characteristics of the host (e.g., psychological status, novelty and ability to cope with
the stressor). We now also recognize that the control of this system is more complicated than originally postulated, with central and peripheral interrelationships between synergistic and competing systems.

**Principal components of the stress response.** The major components of the stress response include the neural and adrenomedullary components of the sympathetic nervous system, the hypothalamic pituitary adrenal (HPA) axis, and the parasympathetic nervous system. Other less prominent components which will not be discussed in this proposal include the vasopressin, renin-angiotensin, and endogenous opioid systems.

The sympathoneural system consists of nerve networks which begin in the locus ceruleus and other cell groups in the medulla and pons, and innervate a number of tissues: blood vessels, heart, reticuloendothelial organs, and salivary and sweat glands. The primary neurotransmitter released in this system is norepinephrine. Examples of stressors which lead to a prominent sympathoneural response include exercise and orthostasis. The adrenomedullary component of the sympathetic response consists of the cells in the adrenal medulla which release (primarily) epinephrine in response to preganglionic spinal input. The functions of epinephrine have been well-characterized, and include increased heart rate and cardiac contractility, bronchodilation, relaxation of visceral smooth muscle, shunting of blood flow to muscles, and stimulation of the reticular activating system. Activities which elicit prominent adrenomedullary responses include hypovolemia, hypoglycemia, and both pain and emotional distress.

The HPA axis exerts the primary control of the release on glucocorticoids from the adrenal cortex. This response begins with corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) release in the hypothalamus, eliciting corticotropin (ACTH) release from the pituitary, which acts on the adrenal glands. The acute and chronic effects of both steroid deficiency and excess are well described. Stressors which have a prominent HPA effect are similar to those that accompany sympathetic adrenomedullary activity.

The parasympathetic nervous system (PNS) can augment the effects of the sympathetic nervous system by withdrawing input, or attenuate sympathetic responses with increased output. This system also controls many vegetative functions. The principal neurotransmitter in this system is acetylcholine. Although the PNS has close interrelationships with the sympathoneural response, the adrenomedullary sympathetic system appears to function fairly autonomously from the PNS.

**Central control of the stress systems.** The central control of the stress response is quite complex (see figure at right). Current evidence supports a pivotal role of CRH in coordinating the stereotypical responses to stressors, as noted. Many factors can raise or lower CRH and thus effect the overall stress response, as are noted in the figure. In addition to the general level overall activity of this system, however, there is "fine tuning" of each of the effector systems that can occur by a number of direct and feedback mechanisms. Furthermore, other compounds such as AVP act in synergy with CRH to lead to these changes.
**Catecholamine systems.** Three primary catecholamine systems operate in the autonomic nervous system. Norepinephrine is the primary neurotransmitter of the sympathoneural component, epinephrine is the primary effector hormone for the adrenomedullary system, and the DOPA-dopamine system involves several compounds largely involved in sodium balance. All of these catecholamines have both central and peripheral effects.

Peripheral responsiveness to catecholamines can change rapidly in response to several factors. This may involve changes in receptor number, activity, or both. The majority of the data on this phenomenon concerns changes in β receptor number. Both glucocorticoids and thyroid hormone stimulate the synthesis of β receptors, and deficiencies in these hormones will lead to lower receptor density. In situations of prolonged catecholamine excess, such as congestive heart failure, β receptor density is diminished, and in the converse situation (chronic β-blocker use) β receptor density is increased. When β receptor density changes, the effects on different tissues (e.g., lymphocytes and myocardium) generally occur in parallel. The data are less clear regarding changes in α receptor density, or changes in function of α or β receptors, as can occur when there is uncoupling of activation of adenyl cyclase.

**Interactions between glucocorticoids and catecholamines.** The central and peripheral interactions between the two principal stress effector hormone systems, glucocorticoids and catecholamines, are particularly complex. Many of the same stressors that lead to adrenomedullary stimulation also lead to HPA axis stimulation, and glucocorticoid release. These systems also share reciprocating feedback systems, since exogenous administration of corticosteroids leads to a decrease in both neural and adrenomedullary sympathetic activity, and the administration of catecholamines inhibits HPA axis activity. At the tissue level, in most instances these compounds work synergistically. In fact, glucocorticoids have a number of permissive effects on catecholamine function. Glucocorticoids block the extraneuronal uptake of catecholamines, thus increasing activity. Corticosteroids are also required for the normal peripheral responses of binding to β-adrenergic receptors, and for the synthesis of catecholamines.

**Overview of FM, CFS, and related conditions.** Although FM is defined on the basis of chronic widespread pain, and CFS is defined on the basis of chronic fatigue and a requisite number of minor symptoms, there is general agreement that there is considerable overlap between these disorders, with at least half of the individuals who meet criteria for one of these diagnoses also having the other. There is also considerable overlap between these two disorders and other systemic syndromes (e.g. somatoform disorders and multiple chemical sensitivity), and numerous organ-specific conditions such as irritable bowel syndrome, migraine and tension headaches, and a number of regional pain syndromes. Several studies have shown that the symptoms experienced by PGW veterans with unexplained illnesses are very similar to those encompassed within this spectrum of illness, especially when this is viewed in entirety. Within this spectrum, FM and CFS are the best studied from a physiologic standpoint.
The stress response in FM and related conditions: general overview. The accumulated information suggests that the biological stress response is blunted in the chronic phase of FM and CFS. The observed changes are consistent with a low central CRH state, although there are many other plausible explanations. The expected biological consequences of a low central CRH state are similar to those seen in FM/CFS, and the opposite of that seen in acute stress: hypoarousal or fatigue, diffusely increased peripheral and visceral nociception, and decreased sympathetic tone.

Abnormalities in the hypothalamic pituitary axes in FM and CFS. There have been abnormalities noted in most of the hypothalamic-pituitary axes in FM and CFS, and when viewed in aggregate these findings suggest a low central CRH state, and reduced responsiveness of the adrenal glands to ACTH. In CFS there is a blunting of the HPA axis, including low 24 hour free cortisol excretion, increased adrenocortical sensitivity to ACTH, and attenuated ACTH responses to CRH. These abnormalities are consistent with a tertiary (hypothalamic) adrenal insufficiency. Neuroendocrine studies in FM have yielded similar results, with a relative hyporesponsiveness of the adrenal glands (decreased production of cortisol in response to CRH or ACTH), low 24 hour urine free cortisol, but an exaggerated pituitary response to CRH. Although these data appear to indicate a primary rather than tertiary (hypothalamic) adrenal insufficiency as noted in CFS, the aggregate data in FM indicate hypothalamic CRH hyposcretion as well. For example, the normal circadian rhythmicity is disturbed in this condition, indicated by a deficient morning cortisol surge, and supranormal cortisol values are noted during other times of the day.

Further evidence of a hypothalamic defect of the stress response in FM is found in data examining the response to various stressors in FM. Van Denderen exercised ten FM subjects and controls, and cortisol levels paradoxically fell rather than rose in response to physical exertion. Adler and colleagues found that FM subjects demonstrated statistically significant decreases in baseline ACTH, and an attenuated increase in ACTH in response to hypoglycemia.

Because of the complexity and plasticity of the HPA axis, it is more appropriate to emphasize the similarities between the HPA axis in FM and CFS than to accentuate minor differences: these conditions are both characterized by an underactive and blunted "stress response" at the tissue level. Similar changes in the HPA axis are seen in post-traumatic stress disorder, atypical depression, and seasonal affective disorder. These changes are opposite to those seen in melancholic depression, which is characterized by chronically increased stress system activity. The reason for these opposite changes in the function of the stress response in clinically similar disorders is not understood. Some have hypothesized that any disturbances in stress system activity, be it increased or decreased, can upset homeostasis, and, thus, impair performance. Alternatively, subtypes of major depression may have biologically disparate causes. A closer examination may reveal that only certain subtypes of depression are associated with FM and CFS. Finally, it is possible that the HPA abnormalities are surrogates for other neurochemical changes that lead to the pain and fatigue seen in these conditions.
Other hypothalamic-pituitary axes. Insulin-like growth factor (IGF-1) has been shown to be low in individuals with FM. This finding is not specific for FM, in that it is also low in a number of other rheumatic diseases such as osteoarthritis and rheumatoid arthritis. The defect in IGF-1 synthesis in FM has been demonstrated to likely be due to a defective hypothalamic response. This is expected, since although acute stress typically leads to an elevation in growth hormone (and therefore IGF-1), chronic stress leads to suppression of growth hormone and IGF-1 secretion. Also, individuals with FM display blunted secretion of thyrotropin and thyroid hormones in response to thyroid releasing hormone (TRH), once again suggestive of a blunted stress response.

Abnormalities in autonomic function in FM and CFS. There have been several studies suggesting that autonomic function is abnormal in FM and CFS. Elam and colleagues studied muscle sympathetic activity and found it to be reduced at baseline in FM. Qiao demonstrated that FM subjects display decreased microcirculatory vasoconstrictor response to both cold and auditory stimulation, and a high baseline skin conductance, both suggesting either diminished sympathetic or elevated cholinergic tone. Bennett and colleagues found that FM subjects had a higher than expected rate of a positive Nielson test (cold induced increased in finger systolic blood pressure), and displayed an increased density of α2 receptors on platelets. The notion that central sympathetic input is diminished in FM is also supported by data showing that the principal metabolite of norepinephrine (3-methoxy-4-hydroxyphenethyline), is low in the CSF of FM subjects (the metabolite of norepinephrine is measured because the parent compound is undetectable in the CSF).

Perhaps the most consistent finding regarding autonomic function is that FM subjects have an impaired catecholamine response to a variety of different stressors; in different studies exercise, muscle contraction, and noise led to sympathetic responses which were diminished when compared to control groups. In the study noted by van Denderen above, submaximal exercise, which induces primarily a sympathoneural response, led to an attenuated norepinephrine response. In the Adler study, hypoglycemia, which is primarily a stimulus for adrenomedullary activation, elicited an attenuated rise in epinephrine in the FM subjects. Martinez-Lavin performed tilt table testing in 19 subjects with FM, and found a decrease in the rise of the low frequency component of heart rate variability, and interpreted this as indicative of a diminished sympathetic response (for further discussion of interpretation of heart rate variability analyses, see Preliminary Data). Vaeroy and colleagues noted that FM subjects displayed a diminished vasoconstrictory response to a cold pressor test; this stressor elicits primarily a sympathoneural response. Although the autonomic nervous system has not been as extensively studied in CFS, these subjects have been noted to experience a high prevalence of neurally mediated hypotension on tilt table testing, which in turn is felt to be related to autonomic dysfunction.

Summary: The background information we have presented supports the fact that there is blunting of the human stress response in subjects with FM and CFS. The
aggregate data suggest abnormalities in both the hypothalamic pituitary axes and the adrenomedullary and sympatheural components of the sympathetic nervous system. However, the physiologic significance of these changes is not clear. In any of these studies, only a minority of subjects display abnormal HPA or sympathetic function. Although there are differences in group means in many of these parameters, there is considerable overlap among the individual values for the FM and control groups.

**PRELIMINARY DATA:** We have performed a series of studies which have attempted to better delineate HPA and autonomic function in persons with FM and CFS. We feel that these preliminary data, along with the background information presented, support the notion that there is blunting of stress response function in FM and CFS, and justify examining these same physiologic properties in PGWI. Furthermore, the Preliminary Data show larger differences in stress responses function between FM/CFS subjects and controls than previously noted, increasing the likelihood that these changes are physiologically significant.

**Autonomic function in FM/CFS. Tilt table testing.** We performed tilt-table testing on 34 subjects who met criteria for both FM and CFS, and 22 age- and gender-matched healthy controls (HC). To reduce the likelihood that changes seen in the FM subjects occurred as a result of deconditioning or medications, the subjects were all ambulatory, performed some type of regular exercise, and had been off all medications except non-narcotic analgesics for at least two weeks. This is an important difference from the studies of Bou-Holaigah et. al., where many subjects were taking medications, and there was no attempt to control for fitness level.

A standard tilt table protocol was used, consisting of initially lying the subject flat, placing an intravenous catheter, connecting an automated blood pressure and ECG recorder, and monitoring these parameters for 15 minutes. At the end of this period, the subject was tilted head-up to 60°. Vital signs (VS) were recorded each minute for 30 minutes, the subject was then returned to the supine position, and VS were recorded for 5 additional minutes. The baseline pulse (P) and blood pressure (SBP/DBP) were not statistically different in the two groups (FM P=79, BP 118/75; HC P=73, BP 123/79). Eleven of the FM subjects (32%) and two of the HC (9%) had a positive test, defined as syncope or pre-syncope associated with hypotension. In all instances the syncopal episode occurred at least 5 minutes after tilting, and had characteristics suggestive of neurally mediated hypotension rather than orthostatic hypotension. No clinical variables (including presence of certain symptoms, pain, or fatigue level) predicted which FM subjects had a positive test, except that the baseline BP displayed a trend towards being lower in those with positive tests (110/70 vs. 117/75, p=0.13, 0.10).

These data add to the evidence that some individuals with FM and CFS display episodic autonomic dysfunction, but suggest that this occurs in a minority of subjects who are medication-free, ambulatory, and not preferentially selected on a basis of previous syncopal symptoms.

**Heart rate variability (HRV).** The purpose of this study was to use ambulatory Holter monitoring to assess autonomic function in subjects with FM and CFS. Detailed
analysis of heart rate variation is a relatively new technique for measuring autonomic activity. With continuous ambulatory Holter monitoring, a recording of consecutive R-R intervals can be analyzed using automated methods to determine the amplitude at different points in the spectrum. Discrete portions of the spectrum have been shown to roughly correspond to different central influences: the low frequency (LF) band [0.025 - 0.10 Hz] to sympathoneural and neurohumoral factors, the mid-frequency (MF) band [0.10 - 0.20 Hz] to both sympathetic and parasympathetic pathways, and the high frequency (HF) band [0.20 - 0.30 Hz] to parasympathetic pathways.

Thirty-two subjects meeting criteria for both FM and CFS, who were medication free for at least two weeks and who were not chosen because of specific symptomatology, had 24 hour ambulatory Holter recordings. These results were compared to those of 16 age- and gender-matched controls. Holter data were analyzed using Spacelabs software.

The CFS/FM subjects displayed diminished amplitudes in all areas of the spectra, which reached statistical significance in the LF band (797msec² +/- 81 [SE] vs. 1563 +/- 300, p=0.025), but not in the MF (281 +/- 50 vs. 506 +/- 135, p=0.13) or HF bands (113 +/- 24 vs. 249 +/- 62, p=0.054). The LF (1146 +/- 252) and HF (343 +/- 91) values for ten GWI subjects fell between those of patients and controls. These data add to the evidence that individuals with FM, CFS, and subsets of PGWI display dysautonomia. The low LF recording suggests sympathetic hypofunction, although there is not unanimity regarding the precise determinants of the LF band (see discussion in Methods). The HF component is felt to be an accurate measure of cardiac parasympathetic activity, and these data suggest a trend towards this likewise being diminished in

Ambulatory Monitoring to Measure Autonomic Response to Stressors. Autonomic responses to standard stressors can also be determined from portions of a Holter tape. Using event markers to determine the exact point in time at which a stressor is applied, the frequency domain indices LF and HF can be measured in the periods before and immediately a stressor. A decline in HF power reflects a clearcut decrease in vagal modulation of heart rate, but the interpretation of a change in LF is less clear cut. However, most investigators believe that changes in the LF/HF ratio associated with a stressful challenge accurately reflect changes in the sympathovagal balance, with an increase reflecting a change toward more sympathetic predominance and vice versa. This pilot study was performed to determine HRV of FM and controls in response to stressors designed to assess specific physiological systems. HF-HRV and LF-HRV of FMS patients and matched controls were analyzed in response to a stressor that had a predominant adrenomedullary action (dolorimeter pain testing) and to a stressor with a predominant autonomic action (COGSCREEN computerized cognitive challenge). Although statistical significance was not possible with this sample size, clear linear trends were detectable.

HRV was assessed and analyzed as described above. The FM patients displayed blunting in the LF band (480msec² +/- 77 [SE] vs. 1601 +/- 663, p=0.11), but
less blunting in the HF bands (48 +/- 14 vs. 1320 +/- 1033, p=0.25). Five FM patients and 9 controls participated in the pain challenge. Reversed findings were noted with this challenge. FM patients displayed minor blunting in the LF band (1260 +/- 618 [SE] vs. 2102 +/- 500, p=0.26), but significant blunting in the HF bands (107 +/- 33 vs. 1265 +/- 313, p=0.006).

These data add to the evidence that individuals with this spectrum of illness display dysautonomia. These data also add support to the data that sympathetic tone may be diminished, but there is not unanimity regarding the precise determinants of the LF band.

Responsiveness to intravenous isoproterenol infusion. This pilot study was performed to determine whether FM subjects display a change in the peripheral sensitivity to catecholamines. Among the best ways to accomplish this is to infuse fixed dosages of sympathomimetic drugs, and assess the cardiovascular response. Past data have suggested that this can be done safely, and that catecholamine sensitivity varies somewhat in the general population, but is not affected by gender, weight, or race. We chose to use isoproterenol because this commercially-available compound is primarily a beta agonist, and peripheral beta receptor sensitivity is known to change in response to many different stimuli (see Background).

In this protocol, an intravenous catheter is placed, and the individual lies supine for 15 minutes. 50 ml syringes containing varying dosages of isoproterenol in D5W are then administered IV push, followed by a 3 ml D5W flush. The peak heart rate is noted, and a 6-lead is repeated with a different dose of isoproterenol or placebo (the heart rate returns to baseline in approximately 3 - 4 minutes). A total of seven doses of isoproterenol are given (0.2, 0.4, 0.6, 1, 2, 4, and 6 μg), with three placebo injections randomly interspersed.

The mean increase in heart rate over baseline that occurs with the three placebo injections is first calculated (usually approximately 5 beats/min). This value is subtracted from the difference between the baseline and maximum post-treatment heart rate (which usually occurs approximately 45 seconds after completing the injection) for each dose of isoproterenol. Dose response curves are then calculated for each subject, and the mean dose of isoproterenol necessary to raise the heart rate by 25 beats/min (CD 25) is calculated.

The CD25 was significantly higher in the 18 FM subjects than in 14 gender-matched controls (3.9 μg for FM vs. 1.2 μg for controls) (see figure at right.)

This fairly dramatic decrease in β-receptor responsiveness in FM suggests that there is a decreased response to β-receptor occupation in this illness. The combination of the previously-noted attenuated catecholamine release, as well as decreased catecholamine activity at the tissue level, would cause more profound functional, physiologic disturbances than either alone. These data also point out the importance of studies of more than one effector arm of the autonomic nervous system.

The HPA axis in FM: further evidence of a low CRH state. We have presented background information supporting, but not confirming, a low central CRH state. It is important to establish if this is true, because low CRH could directly lead to diffuse pain, fatigue, and other symptoms seen in this spectrum, independent of the plethora of indirect
effects of such a deficiency. One way to test this is to infuse individuals with IL-6, a cytokine which is known to act centrally to release CRH and AVP. If there were low chronic hypothalamic CRH release and normal pituitary function, it would be anticipated that stressors such as IL-6 that release CRH would cause an accentuated increase in ACTH release.

We have previously characterized the responses to IL-6 infusion in both normals and various diseased cohorts. This test is well tolerated, and will lead to stimulation of both the HPA axis and the sympathetic nervous system. In this particular pilot study we studied six women who met ACR criteria for the diagnosis of FM, and compared the results to ten normal women volunteers. All subjects were given 3.0 μg/kg rhIL-6 subcutaneously (Sandoz Pharmaceuticals, New Jersey, NJ). This dose was chosen because of pilot data, in Appendix A, showing that this dosage was well-tolerated and lead to an optimum ACTH and cortisol response. The mean rise in ACTH, and the peak ACTH level, after an IL-6 infusion was significantly greater in the subjects (Δ ACTH 190.2 +/- 41.2 vs. 92.3 +/- 14.3 pg/ml, p<.02; peak ACTH 203 +/- 39.6 vs. 105.0 +/- 14.7 pg/ml, p<.02). (See figure at right.)

Despite this greater increase in ACTH, cortisol responses were nearly identical in the two groups (Δ cortisol 22.7 vs. 22.1). There was no difference in basal level of ACTH between groups. Basal cortisol levels tended towards lower in the FM group (8.7 +/- 1.3 vs. 12.7 +/- 1.7 mcg/ml, p=.13). These data support a low central CRH state, and validate the use of IL-6 as a direct stimulant of CRH release. Once again, these data suggest chronic hyposecretion of CRH (or perhaps AVP, the other major hypothalamic secretagogue), as well as a subtle degree of adrenal hypofunction.

**MILITARY SIGNIFICANCE:** There is no study which can be done on PGWI that will definitively address the issue of whether stress caused these illnesses. However, at this point, we believe that the study described will give considerable insights into this problem, and will lead to further studies which can more definitively address this question. As noted previously, there can be essentially three conclusions to this study: stress response function in PGWI subjects is the same as in FM/CFS, normal, or abnormal but different than FM/CFS. If we demonstrate that stress response function is the same as FM/CFS, this would add considerable credence to the hypothesis that PGWI and other post-war syndromes fall within the continuum encompassed by FM, CFS, and related disorders. Although this would not exclude a toxic or environmental exposure as the cause of this symptom complex, it would decrease the likelihood that this is the case. If, on the other hand, we show that stress response function is normal in PGWI subjects, it would seem less likely that this illness is caused by exposure to stressors, and would suggest that this is a different illness than FM/CFS. Finally, if we identify a unique abnormality in stress response function, this would be of obvious import, since this would lead to re-examination of the pathophysiology of this illness.

We expect that we will show that PGWI is physiologically the same disorder as FM/CFS. If we demonstrate this, we will need to begin to explore why and how these changes occur. This will likely involve longitudinal studies that examine the biological and
psychological responses to stress in healthy individuals, who are then followed prospectively as they are exposed to stressors (e.g., in soldiers going into combat). This will ultimately allow us to determine which types of baseline abnormalities (clinical or physiologic) predict the development of this spectrum of illness, and identify the biological sequence as an individual converts from healthy to ill. We feel that this is likely to be an area that the military is going to need to intensively explore if we are to prevent this type of epidemic of illness after future conflicts.

**METHODS:** We believe that the Background and Preliminary Data support the thesis that FM and CFS are characterized by a number of disturbances in human stress response function. We have shown data to support a low central CRH state, adrenal hyporesponsiveness to ACTH, diminished activity of both the sympathoneural and adrenomedullary components of the sympathetic nervous system, and decreased responsiveness to catecholamines at the tissue level. The planned study will examine whether PGWI subjects display these same abnormalities, and explore the clinical significance of these findings.

**Subject selection.** A unique aspect of this study is the subject selection. Nearly all physiologic studies of PGWI to date have been on highly selected cohorts that present to tertiary care centers for testing. It is likely that this group of individuals who are seeking health care is not representative of the entire PGWI population. In the study of FM and CFS, as with many other illnesses, subjects who seek health care have a significantly higher rate of psychologic and non-psychologic co-morbidities, and of disability, than those with the same illness who are identified in the population. These types of bias limit the generalizability of any physiologic findings noted, and make it difficult to render inferences on potential causes of these illnesses.

Klemm Analysis Group will use self-reported symptoms of the 20,000 PGW women participating in PGWHLs to select a representative group of subjects and controls. Subjects with PGWI will be defined as those women veterans who report the onset of two of three of the following symptoms beginning after the Gulf War that have been present six months or more: 1) fatigue severe enough to limit daily activities, 2) pain in two or more regions of the body, and 3) memory difficulties. This definition for unexplained Gulf War Illnesses is identical to that used for the VA/DoD Cooperative trials studying the treatment of these conditions, as well as the definition arrived at by factor analysis in the recent studies published in JAMA regarding Gulf War illnesses. It has become increasingly clear that this spectrum of illness at present can only be defined on the basis of self-report of symptoms, and that these three groups of symptoms (fatigue, pain, and memory complaints) represent the most common complaints seen within this spectrum of "Chronic Multisymptom Illnesses." Other symptoms and illnesses will be recorded, but the presence of two of these three symptoms will define a "case" for purposes of this study.

A stratified random sample of 80 non-excluded (see below) women classified as having PGWI will be selected. The strata will be defined by (1) a geographic classification
(i.e., units located in the same geographic area), (2) age group, and (3) race category. The sample of 80 PGW women will be allocated to the strata using proportional allocation. This method gives each woman in the eligible population an equal chance of being selected for the sample, and is superior to a straight simple random sampling procedure. With this procedure, the sample size for each stratum will be proportional to the total number of PGW in the stratum.

Because the women ineligible for the study cannot be identified prior to sampling, and to allow for noncooperation, we will randomly select at least five times as many women in each stratum as the target sample size for the stratum. The women selected will be placed in random order, based on a random number assigned to each selection using a random number generator. We will begin recruiting from the list of selections in the random order they are listed, and will stop when the number recruited equals the allocated sample size.

Exclusion criteria for all study participants will include:

1) subjects must not consume any antidepressant, tricyclic compound, benzodiazepine, anti-inflammatory, glucocorticoid, or antipsychotic medication for two weeks prior to study (these drugs could interfere with testing being performed),

2) individuals with disorders known to affect the HPA axis or autonomic function will be excluded (cigarette smoking, greater than the equivalent of two cups of coffee caffeine use, current substance abuse, a weight of 30% greater or 20% less than predicted, hypertension, diabetes, known coronary artery disease).  

3) males, as they are not a part of the Klemm Persian Gulf Women's Health Linkage Study, will not be eligible for participation.

4) subjects who are pregnant will not be eligible for participation.

5) persons over the age of 60

The exclusion criteria were all chosen because these factors affect either HPA or autonomic function (or both). If the investigators find that the exclusion criteria are too stringent to recruit an adequate sample size, they will modify the allowed drugs, and/or eliminate the smoking or caffeine exclusions, and consider these as co-variates.

There are two confounding factors that affect either HPA or autonomic function that will not be addressed in subject selection: psychiatric co-morbidities and aerobic fitness level. The investigators felt that these are inherent components to this spectrum of illness, and that excluding individuals with these features would undermine the validity of this study. These factors will be dealt with by having an adequate PGWI sample to do sub-group analysis (e.g., PGWI with vs. without depression), and to carefully measure these variables so they can be examined as co-variates.

Only Klemm staff will know the specific reasons an individual was excluded from the study; her study status (i.e., subject or control) and self-reported information. Both subjects and controls will be compensated $250 for participating in the study; Klemm will process the payments to the participants. The Klemm staff will be responsible for all recruitment of subjects and controls, and will arrange travel to and from Georgetown.
Although we are only planning to recruit 80 PGWI subjects in this manner, we may increase this number as high as 120 if we find that we are having difficulty recruiting subjects for the previously-funded Clauw proposal.

**Control selection.** A stratified random sample of 40 control cases will also be selected for the study. The use of the same strata (except severity of PGWI) will allow us to match the experimental and control samples on geographic location, age, and race. Matching on geographic location will control for the possibility that all subjects who were in certain geographic areas (or groups of units) and received similar exposure might develop the same changes in stress response function, with only some being symptomatic.

PGW women participating in Klemm's PGWHLS who had none of the symptoms forming the definition of PGWI will be identified as potential controls. In order to match the stratum characteristics of the experimental and control groups, the sample size for each of the control group strata will be half of the sample size assigned to the corresponding experimental group strata. (The corresponding experimental group strata will be all of those that have the same stratum definitions for the geography, age, and race variables.)

As with the experimental sample, we will select at least five times as many PGW women from each stratum as is designated for the study. We will then randomize the order of the selections, and then recruit in the random order of the list until the target number of cooperators is obtained. The matching of the two samples in terms of the stratum allocations will optimize the comparison between the experimental and control groups.

In addition to these 40 controls, we will compare the results of biological stress response data to a group of at least 20 FM subjects who will be studied using the same protocol; this is being done as a part of other funded studies so no additional monies are requested.

**Methods.** Eligible subjects and controls will give informed consent and be scheduled for admission to the Georgetown University Medical Center Clinical Research Center (CRC). Premenopausal females subjects will only be studied in the follicular phase of the menstrual cycle. All subjects will be sent instructions to follow a high salt diet for at least three days prior to admission, to reduce any effects that negative sodium balance have on stress system function. A "spot" urine value will be assessed for each subject in the study, so that data can be analyzed to determine if abnormalities in sodium balance are playing any role. Subjects will also bring a 24 hour urine collection which they had begun the previous day, to be used for a baseline 24 hour urinary free cortisol value.

Testing will occur throughout the two-day period in the CRC, which is kept at a constant temperature of approximately 72°F. The schedule of testing is listed below, and the sequence will be identical for subjects and controls. The Research Assistant will be responsible for scheduling subjects for the study and will be the only one to know the clinical status of the subjects. All testing will be blinded. Prior to initiating any protocol procedures, each subject shall initial and date each page, except for the final page, of the informed consent to indicate that they have read and understand what is written before them. The last page is reserved for subject, witness, and investigator signatures indicating that informed consent has been given, and that the subject is
willing to participate in the investigation. Once consent has been provided, and within 48 hours of IL-6 infusion, a serum pregnancy test will be performed on all women of child-bearing potential. Kim Groner, RN, CANP (202-784-4888) is the study coordinator for the current investigation. If at any time the procedures to be followed in this protocol or the informed consent are modified or terminated, all appropriate regulatory bodies will be notified in writing.

An adverse event temporally related to participation in the study should be documented whether or not considered to be related to the test article. This definition includes intercurrent illnesses and injuries, and exacerbations of pre-existing conditions. In case of such event, the following will be included in all IND safety reports: Subjects identification number and initials; associate investigator's name and name of the MTF; subject's date of birth, gender, and ethnicity; test article and dates of administration; signs/symptoms and severity; date of onset; date of resolution or death; relationship to study drug; action taken concomitant medication(s); including dose, route and duration of treatment, and date of last dose.

Serious and unexpected adverse experiences will be immediately reported by telephone to the USAMRMC Deputy Chief of Staff for Regulatory Compliance and Quality, Human Use Review and Regulatory Affairs Division (HURRAD), (301-619-7803). A written report will follow the initial telephone call within three working days. Address the written report to the the U.S. Army Medical Research and Material Command, ATTN: MCMR-RCQ-HR, 504 Scott Street, Fort Detrick, Maryland, 21702-5012.

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<td>Cortisol, ACTH, catecholamines drawn</td>
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<td>8:30am</td>
<td>Holter monitor placed</td>
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<td>Dolorimeter/tender point exam</td>
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<td>10:00am</td>
<td>Isoproterenol Test</td>
<td>9:30am</td>
<td>Computerized cognitive testing</td>
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<tr>
<td>11:30am</td>
<td>Tilt table testing</td>
<td>11:00am</td>
<td>IL-6 infusion</td>
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| AFTERNOON | 12:30pm: Lunch  
2:00pm: Psychological interview | Lunch  
Remove Holter monitor  
Discharge |

The choice of stressors to be used in this battery, and the timing of these tests in relation to one another, was carefully considered. The choice of IL-6 was simple; there are no inherent differences in how subjects and controls should interpret or react to this stressor, and the compound is well-tolerated. Our choice of sympathoneural stressors were orthostasis (tilt-table) and cognitive testing\(^{18}\). We carefully contemplated the pros and cons of sub-maximal exercise as an alternative to tilt-table testing, but ultimately decided on tilting because of the difficulties encountered in exercising many subjects with this type of illness. Pain was chosen because this is a good adrenomedullary stressor, and because this testing was to be done anyway. An important consideration was whether the effects of certain stressors may engender an inherently different response in subjects and controls. Some stressors might lead to an increased response from the subjects because they would be perceived as more aversive, whereas the subjects may display an attenuated response to other stressors because the subjects cannot be stressed to the same level (e.g., exercise). For this battery, we chose two stressors which we realized may be differentially interpreted by subjects (pain and cognitive challenge), and two that should not (IL-6 and orthostasis). Where differential interpretation might occur, it was important that the stressor would be over-interpreted by the subjects, thus bias results towards disproving our hypotheses.

The issue of how one test might affect another was also considered. The investigators decided that the IL-6 infusion was the only test we could not give before another test, because the biologic effects were likely to persist for several days. Thus, it is scheduled last. It is conceivable that any of the other tests - tilt table testing, cognitive or pain testing - could have subtle effects on the results of subsequent testing. However, the investigators ultimately decided that the proposed laboratory stressors were quite modest, and less than or equal to what an average individual would experience in a two-day period. Furthermore, a two day protocol which incorporates several mild stressors is arguably a better approximation of how the stress system operates in real life than if any single test were performed in total isolation.

**Blood sample collection.** Serum and plasma will be collected utilizing standard venipuncture techniques. For each stressor, baseline studies will be drawn via an indwelling catheter, after the subject has been resting supine for at least 20 minutes. Post-stressor serum and plasma will be drawn at 5, 10, 15, and 30 minutes after the stressor is completed. The exception is the IL-6 infusion, where our pilot data suggests that the peak increases in ACTH and cortisol occur at 60 and 90 minutes, so these time points will be utilized. Once blood is collected, it will be placed on ice immediately and kept dark, and will then be centrifuged within two hours. Sera/plasma will be distributed into several aliquots and stored at -70°C in two different freezers for later batch analysis. We will also save buffy coat white blood cells for potential later use, either for cell receptor or
DNA analysis.

**Autonomic function. Overview.** It is difficult to comprehensively assess autonomic function in humans. A battery of tests is required to evaluate both the sympathoneural and adrenomedullary components of sympathetic tone, parasympathetic tone, and responsiveness of the effector organ. For this study, we have chosen the plasma level of epinephrine to reflect adrenomedullary influences, the level of norepinephrine to indicate sympathoneural influences, and the HF component of heart rate variability analysis to indicate the parasympathetic component of autonomic tone. The relative advantages and disadvantages of some of these tests is briefly discussed, to provide a frame of reference for why this battery was chosen.

The measure of plasma and/or urinary catecholamines can provide some useful information, but these data must be interpreted with caution. The most popular clinical approach for assessing overall sympathetic activity is to consider plasma epinephrine a surrogate for adrenomedullary function, and norepinephrine a surrogate for sympathoneural function. There is general agreement that plasma measures of epinephrine accurately assess adrenomedullary function, but there are several caveats in interpreting norepinephrine levels as a surrogate for sympathoneural activity. The plasma level of norepinephrine is related to both the rate of release from nerve terminals, and the clearance of the substance. However, most of the norepinephrine released by nerve terminals does not reach the plasma since it is taken up into the nerve terminal. Thus any factors which affect this reuptake (e.g., concurrent drugs, differences in enzyme activity) will affect this determination. Also, under stress some norepinephrine is released from the adrenal medulla, so in these circumstances plasma norepinephrine cannot be considered to be solely due to spillover from nerve terminal release. The plasma norepinephrine concentration is also partially dependent on the area where venous sampling is taking place, since it reflects regional sympathoneural activity in that area of the body (e.g., the forearm). Finally, a number of other factors such as room temperature, posture, and diet (total caloric, salt, and caffeine intake, and smoking) can affect plasma catecholamine levels. These factors have been controlled for in this study.

In this battery, the plasma levels of norepinephrine and epinephrine before and after exposure to stressors (tilt, IL-6 infusion, pain, and cognitive stressor), as well as LF - HRV data over these same time frames, will be used to estimate the sympathoneural and adrenomedullary components of autonomic tone. The peak change in each of these values will be calculated in response to each of the four stressors for both subjects and controls, to test the hypothesis associated with Specific Aim #3. These analyses will be performed by HPLC with electrochemical detection by Dr. Goldstein, who oversees the performance of these assays for the NIH, using techniques previously described.

Sympathetic nerve activity can be directly measured in the skin or an extremity. Since such studies are technically difficult, and several studies have demonstrated a close correlation between arm sympathetic nerve activity and antecubital plasma norepinephrine levels, both at baseline and with response to stressors, we will use norepinephrine values as the surrogate measure of sympathoneural activity.
Spectral analysis of heart rate variability can give information on the central contributions to autonomic tone. Heart rate variability monitoring has been demonstrated to be an accurate means of assessing the parasympathetic component of autonomic tone (HF component), and gives some indication of sympathetic influences (LF component). This can be performed by temporal or spectral analysis over an entire 24 hour period, or over short intervals of time, to determine how the autonomic nervous system functions in response to specific stimuli. **Subjects** will wear a standard ambulatory ECG recorder for the period of the study. Data will be analyzed using a dedicated Marquette series 8000 analyzer with specialized software for Heart Rate Variability. A diary will be kept so that we can later analyze how the **subjects**, and controls, respond to each of these stimuli. In addition, this Holter monitor allows the "marking" of "events" (e.g. the onset of tilt table testing, IL-6 infusion) on the tape, so that the autonomic responses to these specific stressors can be analyzed. To determine the LF and HF components, we will use the guidelines recently suggested by an international conference. These analyses will be performed by Dr. Barbey. These data will be used for secondary data analyses; the HF component of HRV for 24 hours, or during stressful events, will be considered to be the primary measure of parasympathetic tone, and the LF component will be analyzed and compared to catecholamine data to determine how accurately it predicts either component of sympathetic tone.

**Tilt table testing.** Tilt table testing is being used primarily as an orthostatic stressor in this study. We will perform concurrent measures of vital signs, catecholamine levels, LF and HF determinations of heart rate variability during the procedure. The 5 and 15 minute time points are when the most dramatic changes in catecholamines occur. This testing will be done as described in the Preliminary Data.

**Isoproterenol infusion.** The isoproterenol infusion will be also performed as described in the Preliminary Data. The only exception is that we will also record blood pressure at all time points, as well as pulse and EKG data. The CD 25 of the PGWI group will be compared to the same value for the controls, to test the hypothesis associated with Specific Aim #4.

**Neuroendocrine studies.** There are several methods which can be utilized to further examine the integrity of the HPA axes. We have chosen to look at the response of the HPA axis to standardized stressors, and in response to an infusion of IL-6, which stimulates hypothalamic secretagogue release. Therefore, we have designed this study to look at the level of ACTH and cortisol at given points in time, in response to several stressors, to determine both the basal level of these hormones and the capacity to change levels in response to a physiologic stimulus. We will have collected this same data taken under identical conditions on both PGWI **subjects** and controls, at both baseline and after stressors. Plasma ACTH and cortisol values will be obtained at baseline and 15 minutes and 30 minutes after exposure to each stressor. The assays for ACTH and cortisol (plasma and 24 hour free urinary) will be performed by Dr. Chrousos via a contract with Corning-Hazelton labs, using RIA methods previously described. The peak change in ACTH in response to **3.0 μg/kg dose** of IL-6 infusion will be used to test the hypothesis associated with Specific Aim #2, and these values in response to other stressors will be
used for secondary analysis of data. Records will be kept regarding IL-6 infusion to include the subject's initials, dose of medication infused, and the amount of medication administered. If there is remaining medication, it will be disposed of in a manner consistent with the manufacturer's and/or the FDA's recommendations. Because IL-6 (BB-IND-5419) is an investigational drug, we will keep all of the records pertaining to its use in this particular study for two years after the IND is approved, or for two years after the IND is withdrawn. All of the study data will be kept locked in the research assistant's office; regulatory documents will be kept in the principle investigator's office.

Dolorimeter examination. The dolorimeter examination is an accepted method of assessing nociception. In this study this testing will be used as an adrenomedullary stressor (by comparing baseline and post-testing plasma norepinephrine). Dolorimeter data will also be used in Specific Aim #5 to look at the co-variates which influence stress axis function, and in secondary data analysis. In a separate digital exam at the same time, the number of tender points will be also be recorded using standardized techniques, to determine if individuals suffer from FM. This will be performed by the research nurse, who at that point in the protocol will not be aware of whether she is examining a subject or control (since admission scheduling will be done by the research assistant).

Psychological assessment. We will use both interviews and self-report questionnaires to collect information that can be used as co-variates in secondary analyses of data. Since psychological status has an influence on how an individual perceives and reacts to stress, this information is critical. Also, many psychiatric illnesses are known to affect HPA and autonomic function (e.g., major depression, PTSD). The self-report tools chosen used have all been well validated in general populations, as well as in FM and/or CFS. We will measure:

1) Stress. This construct may be among the most important psychological co-variates to analyze thoroughly, since we know that how an individual perceives stress has a profound effect on the biologic response. Two domains of stress will be examined. The first will be current stress or stress perception, which will be measured using the Hassles Scale. This scale measures the daily stress an individual experiences, rather than lifetime. This tool has been well validated, and has been shown to account for variance in symptomatology in FM. Lifetime stressful events (and respondents' subjective reaction to these events) will be measured by the Haeres Life Event Questionnaires.

2) Aerobic fitness. The best self-report measure of aerobic fitness we have identified is the Minnesota Cardiac Fitness questionnaire, which queries the subject regarding aerobic exercise intensity and frequency in both leisure activity and the workplace. This tool has been shown to correlate better than other self-report tools with the concurrently measured level of aerobic fitness in formal exercise studies.

3) Fatigue. In addition to the SF-36 item survey, all participants will complete the Multidimensional Assessment of Fatigue. This is a 14 item survey which measures a number of domains, and has been validated in both CFS and FM.

4) Depression. The Beck Depression Inventory (BDI) The BDI is a 21 item measure
of the severity of current depressive symptoms, and has been extensively validated\textsuperscript{87,88}. This will be used to measure the severity of depression; the SCID will be used to determine whether the individual is depressed. There are other tools which are equally useful in this setting, but one advantage of this tool is that it is simple to score cognitive/affective and somatic/neurovegetative symptoms separately, which is useful in these illnesses\textsuperscript{89}.

5) **Anxiety.** The Beck Anxiety Inventory (BAI) The BAI is a 21 item scale that measures the severity of current anxiety\textsuperscript{90,91}. The BAI measures neuropsychological, subjective, panic, and autonomic symptoms of anxiety. Again, the BAI will measure the severity of anxiety, and the SCID will be used to determine if the individual is suffering from an Anxiety Disorder.

6) **Functional status.** The SF-36 item health survey is a self-report measure of functional health status that has been widely utilized\textsuperscript{92}. Eight domains are be assessed: physical functioning, bodily pain, role limitations due to physical health problems, role limitations due to personal or emotional problems, social functioning, energy/fatigue, and general health perception. We will use the pain, physical function, and energy/fatigue subscales for use in co-variate analysis.

7) **Personality.** The NEO PI-R\textsuperscript{93} is a self-report inventory for the assessment of personality traits based on a five factor model of personality. This testing will be performed to determine if personality type has any influence on HPA or autonomic function, or is a risk factor for the development of illness.

8) **Affective responses to stressors.** An explanation of each of the stressors will be given to each subject in a uniform manner with emphasis on the harmlessness of the procedures. Following this explanation, a brief 5 item pre-test questionnaire will be administered before each stressor to assess subject's anxiety, and attitude toward this test. Following the test, 6 item post-test questionnaire will be given that assesses anxiety, attitude, and coping used during the stressor. Both the pre- and post-test questionnaires have been shown to be significantly associated with pain sensitivity, and to correlate significantly with other standard measures of anxiety\textsuperscript{94}.

All subjects will also have a Structured Clinical Interview for DSM-IV\textsuperscript{95}. The SCID is widely used as the "gold standard" for psychiatric diagnosis in the research setting. The structured clinical interview is used to identify current and lifetime psychiatric disorders such as mood disorders, anxiety disorders, and somatoform disorders. A PTSD module will be included as well. The interview will be administered by Dr. Epstein or a subordinate who has been appropriately trained. These psychological data will be used as co-variates in Specific Aims #1 - 4, and will also be examined in Specific Aim #5. Also, we will examine the effect that a pre-deployment history of psychiatric diagnoses has on any of the outcome variables.

**Cognitive testing (stressor).** A cognitive challenge will be used as one of the sympathoneural stressors, although this type of activity generally also leads to a prominent adrenomedullary response\textsuperscript{96,97}. This stressor may evoke a stronger stress response in subjects than controls, but as with using pain as a stressor, if this were the case the
results would be biased toward finding a greater stress response in subjects than controls (the opposite of what we hypothesize). For this testing, we will use a 50 minute computerized cognitive test (COGSCREEN). COGSCREEN was initially developed for the Federal Aviation Administration to detect subtle changes in the cognitive function of pilots, and focuses on attention, short-term memory, and reaction time. We have decided to use this cognitive challenge because we have considerable normative data in FM subjects. Therefore, we can determine if individuals who score below age- and education-adjusted norms have a different sympathetic response because of accompanying frustration and emotional distress.

**Data analysis and sample size calculations.** All data analysis will be performed by the Klemm Analysis Group, with Dr. Klemm as the lead analyst. The analysis will include correlating self-reported data, medical record information, and clinical evaluations, by particular symptoms and severity of PGWI. In addition, we will perform analysis using all data collected from PGW women veterans responding to Klemm’s PGWHLs.

Data analyses will be conducted to describe the population under study and to explore the relation between clinical and self-reported and medical record data. These include: 1) descriptive analyses of demographic and selected self-reported information for PGWI and non-PGW study participants; 2) assessing non-response bias by comparing demographic, self-reported health conditions, and medical information on women from Klemm’s PGWHLs; and 3) developing an epidemiologic index using the correlation between clinical measures and self-reported and medical record information. We will then use the index to perform index-adjusted comparisons among all women surveyed in Klemm’s PGWHLs. Klemm will utilize neural network methods and data visualization techniques to explore the interactions between and within the clinical measures and the self-reported and medical record data.

**Sample sizes.** The sample size of the study (a total of 80 subjects and 40 controls) was driven by Specific Aim #5. The hypotheses in Specific Aims #2 - #4 could all be adequately tested with smaller sample sizes, since our pilot data indicated significant differences in all of these values with small cohorts. Formal sample size calculations indicate that the hypotheses in Specific Aims #2 - #4 can all be tested with greater than 95% power. These sample sizes will also allow the detection of less than one standard deviation difference between sub-groups of subjects (e.g. depressed vs. non-depressed) and controls.

Selection of an appropriate sample size for Specific Aim #5 was based on the optimization of statistical power for the hypothesis tests and sampling efficiency. From the pool of **20,000** women veterans in the Klemm PGWI study, a sample of 80 cases and 40 controls will be selected from those eligible for inclusion. These samples are large enough to support the determination of differences between cases and controls for the biological/symptomatic correlations and the epidemiological modeling.

For correlation analysis to test Specific Aim #5, specific values of r can be tested against the null hypothesis that \( \rho = 0.0 \) for the cases and controls. With \( n=80 \), measured values of \( r>0.185 \) can be detected at \( \alpha=0.05 \) against the null hypothesis. For \( \rho>0.3 \), the power of this test is 0.86 and the power increases to 0.98 for \( \rho>0.4 \).
Similarly, for the controls (n=40), the proposed sample size is capable of detecting correlations exceeding 0.264 with alpha=0.05, and the test has power equal to 0.83 for \( \rho \geq 0.4 \).

Models for multiple regression in the cases are expected to yield one to there variables with significance, with either individually or collectively. For n=80, a minimum level of \( r^2 \geq 0.15 \) has been selected as the level of interest for variables vs. The null hypothesis of no significant regression model. The power of this test at the selected sample size is 0.9. Therefore, the samples of n=80 for cases and n=40 for controls provides enough power for each statistical test and is clearly an efficient way to sample the PGWI population.

Data analyses. The following hypotheses will be tested (these correspond to Specific Aims #2 - 5, since #1 is descriptive and not a hypothesis):

1) **PGWI subjects display evidence of a low central CRH state.** We will demonstrate that PGWI subjects have a markedly exaggerated ACTH response to an IL-6 infusion, when compared to controls. To demonstrate this, we will compute a t statistic for the difference in ACTH response values between the entire PGWI cohort, and the control group. Pilot data indicate that the difference between FM subjects and normals is much greater than one standard deviation; formal power calculations would therefore state that greater than 90% power to detect a significant difference (\( \alpha=0.05 \)) will be obtained. Because of this large expected difference, we can also expect similar power if a non-parametric test needs to be used because of inhomogeneity of variance.

2) **PGWI subjects display evidence of impaired activity of both the adrenomedullary and sympathoneural components of the autonomic nervous system.** We will compare the catecholamine responses to three stressors in PGWI subjects and controls. For the two sympathoneural stressors (orthostasis, cognitive challenge), the peak change in norepinephrine will be compared in the two groups, and for the adrenomedullary stressor (pain) the peak change in epinephrine will be compared. To demonstrate a statistically significant difference between PGWI subjects and controls, we will compute a t statistic for the difference in catecholamine responsiveness to each of these stressors. Again, pilot data for catecholamine responses to a number of other stressors in FM has demonstrated a greater than one standard deviation difference between FM subjects and normals. Formal power calculations would therefore state that greater than 95% power to detect a significant difference (\( \alpha=0.05 \)) will be obtained with these sample sizes, and a similarly large power is present if a non-parametric test needs to be used.

3) **PGWI subjects have evidence of decreased peripheral responsiveness to catecholamines.** We compare the CD 25 values to an isoproterenol infusion for the 80 subjects and 40 controls. The pilot data suggested a several-fold standard deviation increase in this value in the FM subjects, again giving greater than 95% power at the \( \alpha=0.05 \) level.

4) **Significant abnormalities of one or more of these various components of the stress response can be noted in most persons with PGWI, and the nature of the abnormality can predict the predominant clinical symptoms.** To demonstrate this we will examine the relationship between five primary physiologic variables, and four primary
clinical values, to detect patterns of association among biological and clinical variables. In the design of any such analysis, there would be disagreement regarding the precise measures which should be chosen. The investigators felt that in this instance it was most important that these primary variables be assigned a priori, since the biggest potential danger in this type of analyses is to generate a statistically significant model that is not clinically sensible. The physiologic variables will include peak change in ACTH in response to IL-6 (a surrogate for hypothalamic responsiveness), 24 hour urinary cortisol (a surrogate for the effector arm of the HPA axis), change in norepinephrine with tilting (sympathoneural activity), change in epinephrine with pain testing (adrenomedullary activity), and CD25 to isoproterenol (catecholamine responsiveness). The clinical stratifying features will include the average tender point threshold (degree of tenderness), presence or absence of depression (judged by the SCID), multidimensional assessment of fatigue, and Hassles score (current stress). Using these variables, three different techniques can be used to examine the relationships between these variables.

a) Discriminant function analysis: This method attempts to select from a set of possible predictor variables that combination which most effectively classifies a subject into one of several categories defined on the basis of a single outcome (e.g. high versus low pain threshold). Both biologic and clinical variables can be used for this purpose. 
b) Cluster analysis/factor analysis: This method uses a set of variables to group cases into two or more categories representing similarity in a multivariate framework. Each case is represented by a point in a multi-dimensional space, and those cases which resemble each other will be “closer” according to an appropriate distance measure. By examining the cases which are grouped together in clusters, we attain more insight into the patterns of disease. c) Scaling: In this method correlations among different items are analyzed to form a summary measure or “scale” representing an underlying dimension of the problem, e.g. “Sympathetic responsiveness”. The resulting scales can be studied, for example, to see if they are distributed in a bimodal fashion possibly representing two classes of subjects.

The sample size of 80 subjects projected for this study is consistent with the above analytic methods in that most of our models involve 8 to 10 predictor variables; although exact formulas for the sample size and power do not exist for this situation, it is generally believed that about 10 cases per variable is appropriate for such modeling.

Link with previous DOD study. The data obtained from this study will also be linked with data obtained from another DOD study (DAMD 17-96-1-6042). Both studies employ the similar physiologic stressors and identical order of testing, except the earlier study does not include the IL-6 infusion. Data from the two studies will be analyzed both separately and together to test each hypothesis. The collapsed data set will contain 40 PGWI subjects recruited from the Washington VA Medical Center for the first study (who are approximately 80% male) with the 80 females with PGWI recruited for this study. The collapsed control populations will include 40 healthy normals and 40 symptomatic persons who were not deployed to the Gulf War from the first study (who are age- and gender matched and thus will be 80% male) as well as the 40 asymptomatic female Gulf War veterans who are
recruited for the present study. Therefore, this collapsed data set will contain an adequate number of males to determine whether there are gender differences in these responses, as well as to give a more representative overall population being studied.

PROGRESS / RESULTS

We have not begun recruiting patients for this study due to the difficulty the Klemm Analysis Agency has had in obtaining the appropriate signatures from the Department of Defense. As of this date, this permission has still not been obtained.

Our group has been in weekly contact with the Klemm group to check on their progress (or lack thereof) and to determine whether we could be of assistance in receiving the appropriate approval. In this regard, we have been in contact several times with our contracting officer, and others involved in Gulf War research efforts (e.g., Tim Gerrity of the VA/DoD/HHS Research Working Group) to determine if there was anything that we could do to expedite the approval process. Since this is not our project, though, we have been unable to be of any assistance in this regard.

As of our contact with Dr. Klemm last week, a new contract officer and liaison had been assigned to their project to attempt to secure the necessary approval to commence the project, and she remained optimistic that such approval would be granted. She also remained committed to performing the study, and to recruiting patients for our study.

Our group is extremely frustrated by this lack of progress and has considered several options to circumvent this problem. The most obvious was to use a different subcontractor to recruit these subjects. Unfortunately, though, there is no other group that we are aware of that has access to a similar cohort of subjects. Another option considered was to recruit these subjects ourselves. However, since we do not have access to any military databases, and even if we did would have to go through the same approval process as the Klemm group, this was not viewed as a viable option.

Over the past two years, we have successfully run 119 subjects through a very similar DoD-funded study (DAMD17-95-0010, "Dysregulation of the Stress Response in the Persian Gulf Syndrome). We have had no difficulties with any technical aspect of this project, and so we do not anticipate any such difficulties with the current project. Likewise, we have had no problems with patient attrition. None of the 119 subjects have terminated the study early, and in nearly all the full testing protocol could be performed. The only difficulty with this project has again been in recruiting Gulf War veterans, but we have recently begun to recruit these subjects via different mechanisms than originally described, and will be able to reach our target numbers.

Some of the preliminary data from DAMD17-95-0010 is included in the current proposal, such as the Heart Rate Variability over short time domains. These studies will be added to the analyses originally described, to further augment our ability to examine autonomic responses to stressors. The assays for catecholamines, cortisol, and other
biological tests will be run shortly on the subjects from DAMD17-95-0010, so we do not have comparable data for these parameters. But the pilot data we present and previous studies indicate that each of our stressors should produce differential responses in these measures between PGWI subjects and controls.

Because of our experience with this physiologic testing protocol, we are comfortable that we can run four subjects per week through this study. We can do this using our existing personnel, and beginning July 1, 1999, Georgetown's Clinical Research Center will become an NIH-funded GCRC. After this point in time, we will have additional dedicated GCRC personnel (e.g., nurses, research assistants) that will assist with this study. Given these capabilities, we can theoretically run the 120 subjects through this protocol within one year if necessary. Obviously, though, this again is contingent upon the Klemm group receiving the necessary approval, and their ability to recruit adequate numbers of subjects to participate in our protocol.

REVISED STATEMENT OF WORK

An amended Statement of Work for the next two years is as follows:

Year 2

Recruit and study 20 participants (10 subjects; 10 controls)
Submit yearly report

Year 3

Recruit and study 100 participants (40 subjects; 20 controls)
Conduct data analysis
Develop/submit final report
Develop peer review articles.

If we cannot complete the subject accrual and data analysis by the end of three years, we would request an extension of the study, without additional funding. Most of the funds originally awarded for this study remain unspent (some salaries have been encumbered), so this would be feasible.

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