Autonomic dysfunction in fibromyalgia

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Abstract:

Autonomic nervous system dysfunction characterized by sympathetic hyperactivity at rest and sympathetic hypo- reactivity to physical stressors has been demonstrated in individuals with fibromyalgia. However, little is known regarding the autonomic responses to mental stressors. This study investigated autonomic and subjective responses in 10 female fibromyalgia subjects and 10 age-matched female controls during a physical (active standing) and mental challenge (Stroop word task).

There were no group differences in autonomic measures at baseline. However, fibromyalgia subjects were more anxious, fatigued and in more pain. During the active stand task, fibromyalgia subjects had less increase in sympathetic and less decrease in parasympathetic modulations of heart rate compared to controls. However, during the cognitive challenge group differences were not observed in either autonomic or subjective responses. These results confirm previous findings that individuals with fibromyalgia are less reactive to physical challenges than controls, but also suggest that they do not respond differently during mental challenges.

Résumé de communication:

Un dysfonctionnement du système nerveux autonome caractérisé par hyperactivité sympathique au repos et hypo-réactivité en réponse aux facteurs de force physiques a été démontré dans les individus avec la Fibromyalgie. Cependant, peu est connu concernant leurs réponses autonomes aux facteurs de force mentaux. Cette étude a investigué les réponses autonomes et sympathiques dans 10 sujets femelles avec la Fibromyalgie et 10 sujets sains d'âge comparable pendant un défi physique (position debout) et mental (tâche de mot de Stroop).

Il n'y avait aucune différence de groupe dans les mesures autonomes à la ligne de base. Cependant, les sujets avec la Fibromyalgie étaient plus inquiets, fatigués et en plus de douleur. Pendant le défi physique, les sujets avec la Fibromyalgie ont eu moins d'augmentation sympathique et moins de diminution parasympathique de modulation de rythme cardiaque comparé aux sujets sains. Cependant, pendant le défi cognitif on n'a pas observé une différence dans les réponses autonomes ou subjectives. Ces résultats confirment des conclusions précédents que les individus avec la Fibromyalgie sont moins réactifs aux défis physiques que des sujets sains, mais suggèrent également qu'ils ne répondent pas différemment pendant des défis mentaux.

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Introduction

Introduction and Statement of the Problem

Fibromyalgia is a chronic pain disorder characterized by widespread musculoskeletal pain, hypersensitivity to palpation in at least 11 out of 18 defined tender points, fatigue and sleep disturbance (Wolf et al., 1990). Fibromyalgia is often associated with irritable bowel syndrome, chronic fatigue syndrome, headache, dizziness, Raynaud's phenomena and psychiatric illness (Mease, 2005). It is estimated that fibromyalgia affects 3.4% of women and 0.5% of men (Wolfe et al., 1995). Many patients with fibromyalgia suffer significant disability and reduced quality of life. The diagnosis and treatment of fibromyalgia poses a challenge to researchers and clinicians. Despite significant progress in our understanding of this disorder the etiology of fibromyalgia remains unclear.

Many patients report the onset of fibromyalgia symptoms following physical or emotional stress (Clauw and Chrousos, 1997) and that current stressful experiences often exacerbate their symptoms (Okifuji and Turk, 2002). In addition, many of the co morbidities associated with fibromyalgia are also considered 'stress related' (Hudson et al., 1992). The co-morbidities with other stress related disorders and the close association of fibromyalgia symptoms and periods of stress have led to the suggestion that stress may play a role in the development and perpetuation of fibromyalgia.

Stress can be defined as a potential threat to an organism's homeostasis caused by a physical assault or psychosocial burden (Van et al.,2005). These

stressors cause activation of a set of neuronal, hormonal and behavioural responses collectively known as the *stress response*.

The autonomic nervous system (ANS) is one of the two main systems responsible for coordinating the stress response. The role stress and the ANS plays in the pathogenesis of fibromyalgia is evident from both a psychosocial and neurobiological perspective. ANS dysfunction has been demonstrated in patients with fibromyalgia. Fibromyalgia subjects display sympathetic hyperactivity at rest and sympathetic hypo-reactivity to physical and orthostatic challenges, with the most consistent results demonstrated by heart rate variability analysis. However, little is known regarding autonomic responses of individuals with fibromyalgia to a **cognitive** challenge.

This study investigated autonomic responses of heart rate, heart rate variability, galvanic skin responses and subjective reports of anxiety, stress, fatigue and pain in fibromyalgia subjects and controls exposed to an orthostatic and cognitive challenge.

Background Information

Clinical Presentation

Fibromyalgia is a complex disorder that involves an interaction of physical and psychological symptoms. Although chronic pain is the cardinal feature, fibromyalgia is commonly associated with symptoms such as sleep disturbance, fatigue, headache, anxiety, dizziness and irritable bowel syndrome. As many as 80% of patients with fibromyalgia also fulfill criteria for chronic fatigue syndrome, up to 80% have headaches, and up to 60% have irritable bowel (Aaron and Buchwald, 2001). It has been suggested many of these symptoms can be

attributed to an aberrant autonomic function (Martinez-Lavin and Hermosillo, 2000) and reflect a possible dysfunction in the stress response (Adler and Geenen, 2005).

Fibromyalgia patients report symptom exacerbation during periods of high stress. One report reveals that 65% of fibromyalgia patients felt stress was an aggravating factor of their symptoms (Okifuji and Turk, 2002). It is also believed that stressful life events may trigger the onset of fibromyalgia. Investigations reveal adverse/stressful life events such as physical trauma and illness (Al-Allaf et al., 2002), work related stress (Kivimaki et al., 2004) and severe emotional distress often precedes the onset of fibromyalgia symptoms (Anderberg et al., 2000). Exposure to adverse early life events may also increase an individual's susceptibility to stress later in life, rendering the individual prone to stress related disorders such as fibromyalgia (Van and Egle, 2004). Retrospective studies reveal a higher prevalence of childhood trauma in fibromyalgia patients compared to controls (Anderberg et al., 2000;Van et al., 2001;Boisset-Pioro et al., 1995) and rheumatoid arthritis patients (Boisset-Pioro et al., 1995;Poyhia et al., 2001). Furthermore, a history of childhood trauma in fibromyalgia patients is associated with significantly greater health care usage, increased pain reports and functional disability (Taylor et al., 1995;Alexander et al., 1998). At this time it is difficult to determine how adverse life events contribute to the pathology of fibromyalgia and whether or not adverse life events during childhood or adulthood affect autonomic responses to stress later in life. There is evidence suggesting that adult survivors of childhood abuse who have symptoms of post traumatic stress disorder and/or depression demonstrate autonomic hyperactivity to experimentally induced

stressors such as startle (Metzger et al., 1999) and psychosocial stress (Heim et al., 2000).

The clinical presentation and the high correlation of stressful life events with fibromyalgia symptoms strongly suggest stress may be involved in the pathology. However, examination of the neurobiological mechanisms of the stress response is necessary in order to further understand the relationship.

Neurobiology of the stress response: Autonomic Nervous System

An adequate neurohormonal stress response is mainly processed by two interconnected systems, the locus- coeruleus- norepinephrine axis and the hypothalamic pituitary adrenal axis (HPA).

Acute stress results in activation of the sympathetic nervous system causing a general state of arousal characterized by increased heart rate, respiration rate, blood pressure and blood flow to the muscles (Chrousos and Gold, 1992). Investigations involving heart rate variability analysis, muscle sympathetic nerve activity, skin electrical conductance and cutaneous blood flow are commonly used to examine autonomic nervous system function (Low, 2004a). ANS dysfunction in fibromyalgia has been demonstrated using a variety of these methods.

Autonomic Function in Fibromyalgia: Rest

Previous investigations suggest individuals with fibromyalgia have a greater sympathetic activity at rest i.e., have a higher resting heart rate, greater influence of sympathetic modulations of heart rate and a greater skin conductance.

Heart Rate and Heart Rate Variability

Heart rate variability analysis (HRV) is a non invasive technique used to quantify fluctuations in timing between heart beats. Variability in the timing of RR intervals is a naturally occurring physiological process and reflects the balance of the parasympathetic/sympathetic nervous system. Through frequency domain analysis this variability can be divided into at least two frequency components. High frequency (HF), which is thought to be mediated by respiration and reflect parasympathetic influence and low frequency band (LF) which is thought to reflect both parasympathetic and sympathetic modulations. The ratio of LF/HF is used to assess balance between sympathetic and parasympathetic tone, as a greater ratio of LF/HF is indicative of higher sympathetic tone (Task Force of the European Society of Cardiology, 1996).

Investigations involving HRV frequency domain analysis reveal that fibromyalgia subjects at rest have a greater LF and LF/HF component, a measure of predominately sympathetic modulations (Cohen et al., 2000;Cohen et al., 2001;Furlan et al., 2005;Martinez-Lavin et al., 1998) and less HF component, a measure of parasympathetic modulations compared to controls (Cohen et al., 2000;Cohen et al., 2001;Furlan et al., 2005;Martinez-Lavin et al., 1998). The suggestion of sympathetic hyperactivity at rest is further supported by the higher resting heart rate in fibromyalgia subjects compared to controls (Cohen et al., 2000;Cohen et al., 2001;Furlan et al., 2005). However, there are also investigations which found no significant differences compared to controls in resting heart rate (Elam et al., 1992;Kadetoff and Kosek, 2006;Lund et al.,

2003;Martinez-Lavin et al., 1998;Stein et al., 2004;Thieme and Turk, 2005) or HRV (Martinez-Lavin et al., 1997).

Galvanic Skin Responses: GSR

The sympathetic skin response measures changes in the electrical properties of the skin due to sweat gland activity. Activation of the sympathetic nervous system causes filling of the sweat glands resulting in phasic changes in conductance. These changes in skin conductance, often referred to as galvanic skin responses (GSR) can be recorded and thus provide an index of sympathetic nervous system activity. Thus, an increase in GSR amplitude is reflective of an increase in sympathetic nervous system activity (Critchley, 2002). Only a few studies have investigated resting skin conductance in fibromyalgia subjects, and results have been mixed. Qiao et al., (1991) demonstrated greater skin conductance at rest in fibromyalgia while a recent study by (Thieme and Turk, 2005) revealed no significant difference.

The above investigations suggest that when compared to controls, individuals with fibromyalgia exhibit sympathetic hyperactivity at rest, yet the most consistent results are revealed through HRV analysis.

Autonomic Function : Physical Challenge

In addition to evidence suggesting autonomic dysfunction in fibromyalgia at rest, differences in autonomic responses to challenge have also been demonstrated. When faced with physical and/or orthostatic challenge individuals with fibromyalgia are *less* responsive and exhibit sympathetic hypo-reactivity.

HRV: Challenge

Neurally mediated syncope is an acute cardiovascular reaction produced by sudden change in autonomic nervous system activity where the normal pattern of autonomic outflow (increased sympathetic, decreased parasympathetic) which maintains blood pressure in the standing position is transiently reversed. In neurally mediated syncope, there is an increase in parasympathetic activity producing bradycardia as opposed to the normal response of an increased sympathetic activity (Kaufmann and Bhattacharya, 2002). The head up passive tilt test is often used to investigate unexplained syncope and to quantify sympathovagal balance. The procedure involves using a tilt table to passively alter an individual's position from supine to a 60 degree angle. In normal healthy individuals passive tilt causes an increase in LF component of HRV and a decrease in HF component of HRV. This is thought to reflect a reduction in vagal and an increase in sympathetic modulation of heart rate (Kaufmann and Bhattacharya, 2002;Low, 2004b;Wieling and Van Lieshout, 1993). A recent study by Furlan et al., (2005) found in response to passive tilt test fibromyalgia subjects had less decrease in HF band, less increase in LF band and a significantly greater number of syncope or presyncope symptoms compared to controls. Active standing is another method of inducing orthostatic challenge where similar responses of an increase in the LF component and a decrease in HF are exhibited in controls (Cohen et al., 2001). Again, individuals with fibromyalgia were found to have *less* increase in LF band and *less* decrease in HF during active standing (Martinez-Lavin et al., 1997). *HRV analysis has demonstrated that individuals with fibromyalgia have less increase in LF band and less decrease in HF during*

orthostatic challenge when compared to controls, indicative of a blunted sympathetic response.

Heart Rate: Challenge

In healthy individuals, heart rate increases during orthostatic/physical challenges such as active standing, passive tilt and physical exercise including isometric and aerobic exercise. Individuals with fibromyalgia show *less i*ncrease in heart rate to physical tasks such as active stand (Cohen et al., 2001), passive tilt (Bou-Holaigah et al., 1997) and bicycle ergometry (Lund et al., 2003;van Denderen et al., 1992). However, there are a few studies which reveal no significant differences in heart rate responses to passive tilt (Furlan et al.,2005), active stand (Martinez Lavin et al., 1997) and isometric exercise (Elam et al.,1992). On the other-hand, one recent investigation revealed that fibromyalgia subjects had a greater heart rate increase after isometric contraction at the point of exhaustion but no significant difference at two minutes of contraction (Kadetoff and Kosek, 2006). *In summary, cardiovascular evaluation utilizing HRV has demonstrated that fibromyalgia subjects when faced with an orthostatic or physical challenge have a blunted sympathetic response; however heart rate responses to challenge are less consistent.*

Galvanic Skin Response (GSR): Challenge

Challenge to the sympathetic nervous system causes an increased state of arousal resulting in an increase in sweat gland activity and thus a subsequent increase in skin conductance, i.e. an increase in GSR. Sympathetic skin responses can be triggered by stimuli which activate the sympathetic nervous system such as

an inspiratory gasp, electrical stimulation, a cough or a loud noise (Critchley, 2002). Only a few studies have examined skin conductance responses to physical/physiological challenge in fibromyalgia. Qiao et al., (1991) measured sympathetic skin responses to startling loud acoustic stimulation and to cold pressor test in controls and fibromyalgia subjects. During the cold pressor test skin conductance increased significantly *more* in fibromyalgia subjects than in the controls, yet they found no significant difference in skin conductance responses during the acoustic stimulation. Care should be taken when interpreting these results as although the cold pressor test effectively and reliably activates the sympathetic nervous system, it is also a painful stimuli. Since it is well established that individuals with fibromyalgia are hypersensitive to a variety of noxious and innocuous stimuli (Petzke et al., 2003;Lutgendorf et al., 2004a) it is difficult to interpret these findings as it is likely that different mechanisms of sympathetic activation are involved.

Overall, the above investigations involving HRV analysis suggest that individuals with fibromyalgia show less sympathetic stress response when faced with a physical and orthostatic challenge. However, heart rate and GSR responses are less consistent.

Autonomic Function: Cognitive Challenge

In healthy individuals laboratory mental stress tasks such as Stroop color word task, mental arithmetic and public speaking have been shown to reliably induce a physiological state of arousal characterized by increased heart rate (Becker et al., 1996b;Hoshikawa and Yamamoto, 1997;Jain et al.,

2001;Lautenbacher et al., 1994;Lutgendorf et al., 2004a;Morell et al., 1988;Pagani et al., 1991) skin conductance (Flor et al., 1985;Morell et al., 1988;Zotti et al., 1991) and decreased HRV (Hoshikawa and Yamamoto, 1997;Johnsen et al., 2003;Pagani et al., 1991). However, little is known about autonomic responses to cognitive or psychological challenge in fibromyalgia. Investigations involving a math stressor (Elam et al.,1992) and a social conflict stressor (Thieme and Turk, 2005) found no significant differences in heart rate responses between healthy controls and fibromyalgia. However, Theime and Turk, (2005) found that during a math and social conflict stressor fibromyalgia subjects had a significantly *higher* sympathetic skin responses compared to controls.

Rationale for the study, hypothesis and specific aims:

Many theories regarding the etiology of fibromyalgia have been proposed. However there is still much controversy and no single theory is widely accepted. The clinical presentation and psychosocial evidence indicates stress has a role in the onset and maintenance of the disorder. Yet the evidence is largely correlative and relies on retrospective data and patient reports. An underlying mechanism for this relationship has not yet been established and thus it remains unclear how stress contributes to the etiology of fibromyalgia. One possibility underlying the link between fibromyalgia and daily life stressors is a disruption of the stress response in fibromyalgia. Previous investigations involving predominately HRV analysis reveal dysregulation of the ANS characterized by sympathetic hyperactivity at rest with a subsequent sympathetic hypo-reactivity in response to a physiological and orthostatic challenge. Yet a systematic examination of the autonomic responses of fibromyalgia subjects exposed to mental challenge has not yet been completed. The present investigation aims to further our understanding of autonomic responses to challenge in fibromyalgia subjects and controls. Further delineating the underlying pathophysiology in fibromyalgia is imperative. The lack of definitive pathology renders effective treatment difficult leading to a reduction in an individual's quality of life and significant burden on our health care system.

Objectives:

To compare the autonomic responses and subjective ratings of individuals with fibromyalgia and controls subjected to:

a) cognitive challenge

b) orthostatic challenge

Hypothesis:

Published data suggests that fibromyalgia patients when compared to controls have higher sympathetic tone at rest with a subsequent blunted sympathetic response to physical/orthostatic challenge. Therefore we hypothesize

1. Individuals with fibromyalgia compared to controls will have higher sympathetic tone at rest and thus will exhibit
    a) higher heart rate
    b) higher galvanic skin response (GSR)
    c) lower HRV: greater LF component, less HF component

2. Individuals with fibromyalgia compared to controls will have reduced sympathetic response to orthostatic and cognitive challenge. Fibromyalgia subjects will have:

    a) less increase in heart rate

    b) less increase in GSR

    c) less increase in LF (sympathetic)

    d) less decrease in HF (parasympathetic)

Methods

Subjects

Ten female fibromyalgia subjects and ten control female subjects between the ages of 35 and 65 years, matched for age and reproductive status were recruited (see Table 1 for subject demographics). The fibromyalgia subjects recruited were previously diagnosed by a physician based on the presence of widespread pain for at least six months and pain on palpation in a minimum of 11 of 18 tender points, as defined by the ARC-90 (Wolfe et al 1990). Potential subjects were excluded if they presented with any of the following conditions:

1) Any chronic pain condition other than fibromyalgia (e.g., arthritis, neuropathies, sciatica)

2) Neurological disease, cardiovascular disease or autonomic disease

3) Pregnancy or breastfeeding

4) Habitual consumers of alcohol or recreational drugs

5) Major Depressive Disorder and Anxiety Disorder

6) Individuals using cardiac medications or medications affecting the ANS including anti- hypertensives and anti depressants

General Procedure

Fibromyalgia and control subjects were given two stress tasks, an orthostatic and cognitive challenge. Autonomic measures of heart rate, GSR and HRV were recorded throughout the experimental session. Using visual analogue scales (VAS), self report ratings of Anxiety, Stress, Fatigue and Pain were taken immediately after each task. After obtaining informed consent, general

instructions were given and the autonomic measurement devices were attached to the subject. Recordings began with a baseline period of rest followed by an orthostatic challenge, the congruent word task and the Stroop colour word challenge. Each of these tasks were separated by a rest period in which the subject was instructed to close her eyes and relax while the lights were turned off and ocean sounds were played. The experiment concluded with a tender point examination and administration of the fibromyalgia impact questionnaire. See Figure 1 for graphical depiction of the experimental design.

Baseline:

Subjects were seated in an upright chair and instructed to sit quietly and to keep movement to a minimum for a period of 5 minutes.

Orthostatic Challenge: Active Stand

Active standing, a task frequently utilized to induce orthostatic stress, was employed. A normal response to active standing has been described by (Wieling et al., 1985) in three stages. During the *initial response phase* there is an abrupt increase in heart rate resulting from a rapid inhibition of cardiac vagal tone. This inhibition is initially due to the exercise reflex (primary peak) and then due to diminished activation of arterial baroreceptors caused by a temporary fall of arterial pressure as blood pools in the legs (secondary peak). The second phase (1-2 min), *the early circulatory stabilization,* involves a sympathetic mediated heart rate increase. Heart rate stabilizes at the final stage of orthostasis, *prolonged orthostasis* (>5 min). In healthy adults heart rate variability analysis has demonstrated a significant increase in LF component and decrease in HF during active standing (Low, 2004; Matsushima et al., 2004).

During the orthostatic challenge subjects were asked to quickly change postures from supine to standing and then return to supine position. The subject began in the supine position for 5 minutes, after which VAS scores were taken and the subject was then passively moved to the seated position by an adjustable chair. The subject was then asked to stand as quickly as possible and to remain still and not to speak. After the 5 minute stand VAS scores were recorded and the subject was asked to sit down. The chair was adjusted to the supine position where the subjects remained for another 5 minutes and VAS scores were then recorded. Autonomic recordings began once the subject was in the appropriate position and thus did not include position changes or VAS reports.

Cognitive Challenge

Cognitive stress was induced by a well established stress task, the Stroop colour word discrimination task (Becker et al., 1996a;Lutgendorf et al., 2004b;Renaud and Blondin, 1997;Stroop, 1935). In the Stroop task the participant is presented with a series of words which are names of colours. The words are written in various colours, however the name of the colour word does not correspond to the actual colour in which the word is written. In this experiment, power point slides were projected onto a screen directly in front of the subject. Each slide contained a series of five colour words written on a black background. The colour words included BLUE YELLOW RED ORANGE PURPLE GREEN and were presented at random. Transition between slides ranged from 3-5 seconds and the total Stroop presentation was 5 minutes. The subjects were asked to name the colour of the word not just read the word. Subjects were informed that their performance would be monitored and encouraged to name the colours as quickly

and as accurately as possible. At two and four minutes of the presentation the subjects were told they are "making too many mistakes and need to answer more quickly and accurately". The number of correct responses was recorded manually by the experimenter.

To control for speech effects and to ensure the Stroop task was indeed an effective stressor, a control condition was also presented. A congruent word task was introduced, where the name of the color word corresponds to the actual colour of the word. The congruent word presentation was in the exact same format (number of words per slide and timing of the slides) as the Stroop presentation and included the same colour words.

Dependent Measures

Autonomic Measures:

Autonomic measures of skin conductance, heart rate and HRV were recorded throughout the experimental session using the ProComp Infiniti (Thought Technology, Montreal, Canada). Three unigel electrodes which are connected to a preamplified electrocardiogram (EKG) sensor were attached to the participant's chest in order to assess heart rate in beats per minute (sampling rate of 2048 Hz). The positive electrode was placed just below the xyphoid process, the ground electrode on the second left intercostal space anteriorly and the negative electrode on the corresponding right side (see Figure 2a). Two circular Ag/AgCl electrodes (1cm diameter), positioned on the distal phalanx of the index and middle finger of the right hand, recorded skin conductance amplitude, galvanic skin response (GSR), in microsiemens (μS) (sampling rate of 32 Hz). Heart rate variability measures were calculated by the Biograph Infiniti software

package (Thought Technology, Montreal, Quebec). A single normal cycle of the EKG signal represents the successive atrial and ventricular depolarisation/repolarisation which occurs with every heart beat. These can be approximately associated with the peaks and troughs of the EKG waveform labelled P,Q,R,S and T. The RR-interval is the time between successive R-peaks, the inverse of this time interval gives the instantaneous heart rate. Heart rate variability refers to beat to beat alterations in heart rate and is found by calculating the variability of a series of RR-intervals (see Figure 2b). For spectral analysis the Biograph Infiniti software uses a fast Fourier transformation to convert the overall variance of the EKG signal into its frequency components. The three main frequency components were divided according to the recommendations of the Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology: very low frequency (VLF < 0.04 Hz), low frequency (LF: 0.04-.15Hz) and high frequency (HF: 0.15-.4 Hz) domain measures. The frequency domain components are expressed as normalized units (% of total power).

Recordings were divided into the following 5 minute conditions: see Figure 1

1. Baseline
2. Supine 1
3. Stand — Orthostatic Challenge
4. Supine 2
5. Rest Period 1
6. Congruent Words
7. Rest Period 2 — Cognitive Challenge
8. Stroop Words
9. Rest Period 3

Physiological recordings began at the start of each task and were paused immediately after completion, such that the recordings did not include the VAS ratings or position changes. An average value for each of the nine 5-minute tasks was provided and used in the statistical analysis.

Visual Analogue Scales:

Immediately after each task the subject was asked to rate their Anxiety, Stress, Fatigue and Pain using VAS. The 200 mm anxiety scale is anchored with -100 (extremely anxious) and 100 (extremely calm) with a midpoint of 0 labelled neutral. Subjective reports of Stress, Fatigue and Pain were assessed on separate scales each anchored at 0 (not at all) and 100 (extremely). Subjects were presented with the scales and asked to give a response verbally. See Figure 3 for example of VAS used.

At the end of the experimental session a *tender point exam*, as defined by the ACR-90 (Wolfe et al., 1990), was conducted (see Appendix A). The participant was asked to use a VAS to rate the pain of each tender point after the application of 4 kg of pressure applied using the experimenter's thumb. After the tender point exam, the Fibromyalgia Impact Questionnaire (Burckhardt et al., 1991) was administered to measure fibromyalgia symptom severity on a variety of domains such as physical function, mood and fatigue.

Statistical Analysis:

Statistical analyses were performed with Statistica 6.0 (StatSoft, Inc, OK, USA), using a significance level of $p<0.05$ for all analyses unless corrections for multiple comparisons were necessary. The data was assessed for normality using

the Shapiro Wilk W Test. A significant W statistic indicates that the hypothesis of a normal distribution should be rejected, i.e .a significant W indicates skewed data. If the data were not normally distributed than non-parametric statistical tests were used. If there was no equivalent non-parametric test a natural logarithmic transformation was performed. See Appendix B for Shapiro Wilk W Test results indicating the frequency distribution and type of statistical test chosen.

Frequency Distributions- Are the data normally distributed?

Autonomic measures of heart rate and HRV (LF ,HF and LF/HF) were normally distributed; therefore parametric statistical tests were used. GSR was found to be skewed and thus a logarithmic transformation was performed in order to normalize the distribution and then parametric tests were used. Subjective measures were highly skewed as well and thus non-parametric statistical tests were chosen (see Appendix B).

Baseline Differences

Group differences in autonomic measures of heart rate, log of GSR, LF, HF and LF/HF at baseline were analyzed using independent t tests. Subjective reports of anxiety, stress, fatigue and pain at baseline were analyzed with the Mann Whitney test, a non-parametric alternative.

Stress Effect

In order to determine if the stress condition induced the desired heightened state of arousal, within group changes were analyzed. Within group changes of heart rate, log of GSR, LF, HF were analyzed with separate ANOVAs with GROUP (fibromyalgia vs control) and CONDITION (Pre rest, Stress, Recovery) as the within subject factor. Separate ANOVAs were performed for the orthostatic

challenge and cognitive challenge. The *Tukeys* HSD was used as pairwise comparisons as appropriate. Since it was determined that the subjective data (anxiety, stress, fatigue and pain ratings) were not normally distributed a Friedman's ANOVA, a non- parametric alternative to a one factor within subject design was used. Separate Friedman's ANOVA were performed for fibromyalgia and control subjects with CONDITION (Pre Rest, Stress, Recovery) as the within subject factor. Wilcoxin Pairwise Comparisons were used as appropriate and the alpha level was corrected for multiple comparisons. For the physical challenge Wilcoxin pairwise comparisons an alpha level of 0.025 was used (2 comparisons: supine 1 vs stand, stand vs supine 2). For the cognitive challenge Wilcoxin pairwise comparisons an alpha level of 0.0125 was used (4 comparisons: rest 1 vs congruent, congruent vs rest 2, rest 2 vs stroop, stroop vs rest 3).

Group Differences: Fibromyalgia vs Controls

The autonomic and subjective responses to the physical and cognitive challenge were compared between control and fibromyalgia subjects. Autonomic responses are expressed as a percent change (%Δ) which was calculated by the formula:

$$\frac{\text{stress condition- preceeding rest}}{\text{preceeding rest}} \times 100$$

Subjective responses are expressed as a change score (Δ) which was calculated by stress- pre rest. These changes scores were not normally distributed therefore non parametric statistical tests were chosen. See Appendix C for assessment of normality. Differences in autonomic and subjective responses between fibromyalgia subjects and controls were analyzed by the Mann Whitney

test, a non parametric alternative to the independent t test. For the physical challenge two comparisons were made:

1. Stand Task (change from supine 1 to stand)
2. Stand recovery (change from stand to supine 2)

For the cognitive challenge two comparisons were made:

1. Stroop Words (change from rest 2 to Stroop)
2. Stroop Words Recovery (Stroop to rest 3)

Stroop as a Stressor: Stroop vs Congruent

In order to ensure the Stroop task was an adequate stressor the autonomic responses (%Δ) and subjective reports (Δ score) during the Stroop condition were compared to the congruent condition using the Wilcoxin test, a non parametric equivalent to a related samples t test.

Correlation between Autonomic and Subjective Measures:

As subjective data was not normally distributed Spearman's Ranked Correlation coefficents were used to address the relationship of autonomic responses and subjective reports.

Results:

Baseline:

There were no significant differences between control and fibromyalgia subjects at baseline for heart rate, log of GSR, LF, HF or LF/HF (see Table 2a). Subjectively, fibromyalgia subjects reported feeling significantly less calm, more fatigued and in more pain than control subjects. Subjective reports of stress at baseline did not differ significantly between groups (see Table 2b).

Physical Challenge: Stand Task

a. *Autonomic Measures*

Heart Rate:

Stress Effect:

Figure 4 shows heart rate for healthy control and fibromyalgia subjects during the stand task. Both control and fibromyalgia subjects had a significant increase in heart rate in the stand position compared to supine. A 2x3 ANOVAs with GROUP (fibromyalgia vs control) as the between subject factor and CONDITION (Supine 1, Stand, Supine 2) as the within subject factor were performed on the heart rate data. There was no significant effect of group $F(1,18)= 0.95$ $p=.34$. However, there was a significant main effect of condition: $F(2,36)= 36.3$, $p<0.0001$ but no significant interactions $F(2,36)= .855$ $p=.43$. Tukey's post hoc test revealed that BOTH groups had a significant increase in heart rate in the stand position compared to supine (see Figure 4).

Group Differences: Δ% Heart Rate

Fibromyalgia and control subjects had similar magnitude of heart rate change during the stand task (see Table 3). There were no significant differences in the % change (%Δ) in heart rate between fibromyalgia and control subjects during the **stand task** (supine 1 to stand) or during the **recovery** (stand to supine 2) (see Table 3).

GSR

Stress Effect:

Figure 5 shows the log of GSR for healthy control and fibromyalgia subjects during the stand task. Both control and fibromyalgia subjects had a significant increase in log of GSR in the stand position compared to supine. A 2x3 ANOVAs with GROUP (fibromyalgia vs control) as the between subject factor and CONDITION (Supine 1, Stand, Supine 2) as the within subject factor were performed on the log of GSR data. The ANOVAs revealed a non significant effect of group: $F(1,18)=0.10$, $p= 0.75$, a significant main effect of condition: $F(2,36)=20.0$ $p<0.0001$ and no significant interactions: $F(2,36)=0.74$, $p=.48$. Tukey's post hoc test revealed that BOTH healthy control and fibromyalgia subjects had a significant increase in log of GSR in the stand position compared to supine (see Figure5).

Group Differences: Fibromyalgia vs Controls %ΔGSR

Fibromyalgia and control subjects had a similar magnitude of log of GSR change during the stand task (see Table 3). There were no significant differences in the % change in log of GSR between fibromyalgia and control subjects during

the **stand** task (supine 1 to stand) or during the **recovery** (stand to supine 2) (see Table 3).

Heart Rate Variability:

Stress Effect:

A 2x3 ANOVA with GROUP (fibromyalgia vs control) as the between subject factor and CONDITION (Supine 1, Stand, Supine 2) as the within subject factor was used to assess changes in HRV parameters during the stand task. Only control subjects had a significant modulation in HRV parameters during the stand task.

LF:

Figure 6 shows the LF for healthy control and fibromyalgia subjects during the stand task. The LF power, a measure of parasympathetic and sympathetic modulations of heart rate, increased significantly during the stand only in control subjects. There was no significant effect of GROUP $F(1,18)=1.8$, $p= 0.2$. However, there was a significant effect of CONDITION $F(2,36)= 8.0$, $p<0.01$ and a significant interaction $F(2,36)=4.7$ $p<0.05$. Post Hoc analysis revealed that only controls had a significant increase in LF in the stand position compared to supine (see Figure 6).

HF:

Figure 7 shows the HF for healthy controls and fibromyalgia subjects during the stand task. Similarly, the HF power, a measure of parasympathetic modulations of heart rate, decreased significantly during the stand, only in control subjects. There was no significant effect of GROUP $F(1,18)=1.8$, $p=0.20$. Yet there was a significant effect of CONDITION $F(2,36)=11.0$, $p<0.001$ and a

significant interaction $F(2,36)=5.22, p<0.01$. Post hoc tests revealed that only controls had a significant decrease in HF during the stand position (see Figure 7).

LF/HF

Figure 8 shows the LF/HF for healthy controls and fibromyalgia subjects during the stand task. The LF/HF power, a measure of sympathetic tone increased significantly during the stand task only in control subjects. The ANOVA revealed a non significant effect of Group $F(1,18)==0.35$, $p=0.56$, a significant effect of condition $F(2,36)=7.9$, $p<0.001$ and a significant interaction $F(2,36)=4.7$, $p<0.02$. Post hoc tests revealed that only controls had a significant increase in LF/HF in the stand position compared to supine (see Figure 8).

Group Differences: Fibromyalgia vs Controls %Δ

HRV analysis revealed that fibromyalgia subjects compared to control subjects were hypo-responsive to the orthostatic challenge. Controls had a significantly greater %Δ (greater increase) in LF and LF/HF compared to fibromyalgia subjects during the **stand task** (supine 1 to stand). Accordingly, the %Δ (decrease) in HF was significantly greater in controls (see Table 3). There were no significant differences between fibromyalgia and control subjects in %Δ HF during the **recovery** (stand to supine 2). However, control subjects had a significantly greater %Δ (decrease) in LF and LF/HF during the recovery (see Table 3).

Stand Task

b. Subjective Measures:

The Shapiro Wilkes test revealed that the subjective reports of anxiety, stress fatigue and pain were not normally distributed; therefore non -parametric alternative statistical tests were chosen.

Anxiety

Within Group Comparison:

In order to assess changes in anxiety ratings during the stand task separate Friedman's ANOVA for each subject group were performed with CONDITION (Supine 1, Stand, Supine 2) as the within subject factor. Figure 9 shows the anxiety ratings of control and fibromyalgia subjects during the stand task. During the stand task only control subjects had a significant increase in anxiety. There was a significant effect of condition on anxiety ratings for controls $S=(10,2)=11.3$, $p<0.01$ but not in individuals with fibromyalgia $S(10,2)= 5.2$, $p=0.07$. Wilcoxin pairwise comparisons revealed that controls reported a significant increase in anxiety during the stand task compared to the supine 1. An alpha level of 0.025 was used to correct for multiple comparisons (2 comparisons: Supine 1 vs Stand & Stand vs Supine 2) (see Figure 9).

Between Group Comparison:

There were no significant differences between individuals with fibromyalgia and controls in anxiety (Δ) compared to controls during the **stand**

task (supine 1 to stand) or during the **recovery period** (stand to supine 2) (see Table 4).

Stress

Within Group Comparison:

Figure 10 shows subjective reports of stress for control and fibromyalgia subjects during the stand task. Separate Friedman's ANOVA for each subject group with CONDITION (Supine 1, Stand, Supine 2) as the within subject factor were used to assess changes in stress ratings during the stand task. There was no significant effect of condition on stress ratings for controls S=(10,2)=5.3, p=0.07 and in individuals with fibromyalgia S(10,2)= 3.7, p=0.16 (see Figure 10).

Between Group Comparison:

There were no significant differences between control and fibromyalgia subjects for Δ stress ratings during the **stand task** (supine 1 to stand) or during the **recovery** (stand to supine 2) (see Table 4).

Fatigue

Within Group Comparison:

Figure 11 shows subjective reports of fatigue for healthy control and fibromyalgia subjects during the stand task. Separate Friedman's ANOVA for each subject group with CONDITION (Supine 1, Stand, Supine 2) as the within subject factor were used to assess changes in fatigue ratings during the stand task. There was a significant effect of condition on fatigue ratings for controls S=(10,2)=14.3, p<0.0001 but no significant effect of condition in individuals with fibromyalgia S(10,2)= 3.9, p=0.14. Wilcoxin pairwise comparisons revealed that healthy controls reported a significant increase in fatigue during the stand task

compared to the supine 1. An alpha level of 0.025 was used to correct for multiple comparisons (2 comparisons: Supine 1 vs Stand, Stand vs Supine 2) (see figure 11).

Between Group Comparison:

There were no significant differences between control and fibromyalgia subjects for Δ fatigue ratings during the **stand task** (supine 1 to stand) or during the **recovery** (stand to supine 2) (see Table 4).

Pain

Within Group Comparison:

Separate Friedman's ANOVA for each subject group with CONDITION (Supine 1, Stand, Supine 2) as the within subject factor were used to assess changes in pain ratings during the stand task. Figure 12 shows the pain ratings for healthy control and fibromyalgia subjects during the stand task. There was no significant effect of condition on pain ratings in controls: S=(10,2)=6.0, p=0.06 or in individuals with FM S(10,2)= 0.93., p=0.63 (see Figure 12).

Between Group Comparison:

There were no significant differences between fibromyalgia and controls for Δ pain ratings during the **stand task** (supine 1 to stand) or during the **recovery** (stand to supine 2) (see Table 4).

Cognitive Challenge

a. Autonomic Measures

Heart Rate

Stress Effect:

Figure 13 shows heart rate for healthy control and fibromyalgia subjects during the cognitive challenge. A 2x5 ANOVAs with GROUP (fibromyalgia vs control) as the between subject factor and CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were performed on the heart rate data. The ANOVA revealed a non significant effect of group: $F(1,18)=0.02$, $p=0.88$, a significant main effect of condition: $F(4,72)= 14.93$, $p<0.0001$ and no significant interaction $F(4,72)=0.09$, $p= 0.99$. Tukeys post hoc test revealed that both controls and fibromyalgia subjects had a significant increase in heart rate during the Stroop condition compared to Rest 2, but there was no significant increase in heart rate from Rest 1 to congruent in either group (see Figure 13).

Group Differences: Fibromyalgia vs Controls %Δ Heart Rate

Fibromyalgia and control subjects had similar magnitude of change in heart rate during the Stroop task (see Table 5). There were no significant differences in the % change in heart rate between fibromyalgia and control subjects during the **Stroop task** (rest 2 to Stroop) or during the **Stroop recovery** period (Stroop to rest 3) (see Table 5).

Galvanic Skin Response

Stress Effect:

Figure 14 shows log of GSR for healthy control and fibromyalgia subjects during the cognitive challenge. A 2x5 ANOVAs with GROUP (fibromyalgia vs controls) as the between subject factor and CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were performed on the log of GSR data. The ANOVA revealed a non significant effect of group: $F(1,18)=1.5$, $p=0.24$, a significant effect of condition: $F(4,72)=24.7$, $p<0.0001$ and no significant interaction $F(4,72)=1.1$, $p= 0.36$. Tukeys post hoc test revealed that both control and fibromyalgia subjects had a significant increase in GSR during the Stroop condition compared to Rest 2. As well, controls also had significant increase in GSR from Rest 1 to Congruent (see Figure 14).

Group Differencs: Fibromyalgia vs Controls %ΔGSR

Fibromyalgia and control subjects had similar magnitude of GSR change during the Stroop task (see Table 5). There were no significant group differences in the % change in log of GSR between fibromyalgia and control subjects during the **Stroop task** (rest 2 to Stroop) or during the **Stroop recovery** (Stroop to rest 3) (see Table 5).

Heart Rate Variability

Stress Effect:

Figure 15, 16 and 17 shows LF, HF and LF/HF respectively for healthy control and fibromyalgia subjects during the cognitive challenge. Neither control nor fibromyalgia subjects had significant changes in heart rate variability parameters during the Stroop task.

LF:

A 2x5 ANOVAs with GROUP (Fibromyalgia vs control) as the between subject factor and CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were performed on the LF data. The ANOVA revealed a non significant effect of group $F(1,18)=0.03$, $p=0.86$ and effect of condition $F(4,72)= 2.1$, $p=0.08$ as well as a non significant interaction $F(4,72)=1.8$, $p=0.15$ (see Figure 15). Post Hoc tests were not performed as there were no significant main effects or interactions.

HF:

A 2x5 ANOVAs with GROUP (Fibromyalgia vs controls) as the between subject factor and CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor was performed. The ANOVA revealed a non significant effect of group $F(1,18)= 0.002$, $p=0.96$, a non significant effect of condition $F(4,72)= 1.2$, $p=0.32$ and a non significant interaction $F(4,72)=1.33$ $p=0.27$ (see Figure 16). Post Hoc tests were not performed as there were no significant main effects or interactions.

LF/HF:

A 2x5 ANOVAs with GROUP (Fibromyalgia vs controls) as the between subject factor and CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor was performed. The ANOVA revealed a non significant effect of group $F(1,18)= 0.03$, $p=0.86$, a non significant effect of condition $F(4,72)= 1.3$, $p=0.29$ and a non significant interaction $F(4,72)=1.9$, $p=0.13$ (see Figure 17). Post Hoc tests were not performed as there were no significant main effects or interactions.

Group Differences: Fibromyalgia vs Controls %ΔHRV

Control and fibromyalgia subjects had similar magnitude of %Δ in HRV parameters. There were no significant differences in the %Δ in LF, HF or LF/HF between fibromyalgia and control subjects during the **Stroop task** (rest 2 to Stroop) or during the **Stroop recovery period** (Stroop to rest 3) (see Table 5).

Cognitive Challenge

Subjective Measures

The Shapiro Wilkes test revealed that subjective reports of anxiety, stress, fatigue and pain data were not normally distributed therefore non parametric statistic tests were chosen.

Anxiety

Within Group Comparison:

Figure 18 shows anxiety ratings for healthy control and fibromyalgia subjects during the cognitive challenge. Both control and fibromyalgia subjects had a significant increase in anxiety during the Stroop task. Separate Friedman's

ANOVA for each subject group with CONDITION (Rest 1, Congruent,Rest 2, Stroop, Rest 3) as the within subject factor were used to assess changes in anxiety ratings during the cognitive challenge. The ANOVA revealed a significant effect of condition on anxiety ratings for controls S=(10,4)=21.67 p<.001 and individuals with fibromyalgia S=(10,4)= 19.07 p<0.01. Wilcoxin pairwise comparisons revealed that both groups reported a significant increase in anxiety during the Stroop task compared to the rest period. There was no significant increase in anxiety during the congruent task. An alpha level of 0.0125 was used in order to correct for multiple comparisons (4 comparisons: Rest 1 vs Congruent, Congruent vs Rest 2, Rest 2 vs Stroop, Stroop vs Rest 3) (see Figure 18).

Between Group Comparison:

Fibromyalgia subjects showed less increase in anxiety compared to controls during the **Stroop task** (rest 2 to Stroop) and during the **Stroop recovery period** (Stroop to rest 3) but the difference did not reach significance (see Table 6).

Stress

Within Group Comparison:

Figure 19 shows subjective stress ratings for healthy control and fibromyalgia subjects during the cognitive challenge. Both control and fibromyalgia subjects reported a significant increase in stress during the Stroop task. For each group separate Friedman's ANOVAs with CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were used to assess changes in subjective reports of stress during the Stroop task. There was a significant effect of condition on stress ratings for controls S=(10,4)=24.1

$p<0.0001$ and individuals with fibromyalgia $S(10,4)= 26.5$ $p<0.0001$. Wilcoxin pairwise comparisons revealed that both control and fibromyalgia subjects reported a significant increase in subjective stress report during the Stroop task compared to the rest period. There was no significant increase in stress during the congruent word task. An alpha level of 0.0125 was used in order to correct for multiple comparisons (4 comparisons: Rest 1 vs Congruent, Congruent vs Rest 2, Rest 2 vs Stroop, Stroop vs Rest 3) (see Figure 19).

Between Group Comparison:

There were no significant differences between control and fibromyalgia subjects in Δ stress ratings during the **Stroop task** (rest 2 to Stroop) or during the **Stroop recovery period** (Stroop to rest 3) (see Table 6)

Fatigue

Within Group Comparison:

Figure 20 shows fatigue ratings for healthy control and fibromyalgia subjects during the cognitive challenge. Separate Friedman's ANOVA for each subject group with CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were used to assess changes in fatigue ratings during the Stroop task for each group. There was a significant effect of condition on fatigue ratings for controls $S(10,4)=14.2$, $p<0.01$ but not for individuals with fibromyalgia $S(10,4)= 9.4$ $p=0.07$. Wilcoxin pairwise comparisons revealed that there was a tendency for controls to report an increase in fatigue during the Stroop task compared to the rest 2 but this difference did not reach significance. An alpha level of 0.0125 was used in order to correct for multiple comparisons (4

comparisons: Rest 1 vs Congruent, Congruent vs Rest 2, Rest 2 vs Stroop, Stroop vs Rest 3) (see Figure 21).

Between Group Comparison:

There were no significant group differences in Δ fatigue ratings during the **Stroop task** (rest 2 to Stroop) or during the **Stroop recovery period** (Stroop to rest 3) (see Table 6).

Pain

Within Group Comparison:

Figure 21 shows pain ratings for healthy controls and fibromyalgia subjects during the cognitive challenge. Separate Friedman's ANOVA for each subject group with CONDITION (Rest 1, Congruent, Rest 2, Stroop, Rest 3) as the within subject factor were used to assess changes in pain ratings during the Stroop task for each group. There was no significant effect of condition on pain ratings for control $S=(10,4)=2.4$, $p=0.66$ or for individuals with fibromyalgia $S(10,2)= 7.9$ $p=0.09$.

Between Group Comparison:

Fibromyalgia subjects had a significantly greater Δ pain ratings during the **Stroop task** (rest 2 to Stroop) compared to controls. However, there were no significant differences in Δ pain ratings during the **Stroop recovery period** (Stroop to rest 3) (see Table 6).

Stroop as a Stressor:

In order to assess the effectiveness of the Stroop stressor, the autonomic (Δ% scores) and subjective responses (Δ scores) during the Stroop task were compared to the autonomic and subjective responses during congruent task. The

Stroop condition resulted in a significantly greater increase in heart rate, anxiety and subjective stress report than the congruent word task (see Table 7,8,9,10). In healthy controls the %Δ in heart rate from rest 2 to Stroop was significantly greater than the %Δ in heart rate from rest 1 to congruent words (see Table 7). Similarly, the Δ anxiety and the Δ subjective stress from rest 2 to Stroop was significantly greater than the change from rest 1 to congruent words (see Table 8). In fibromyalgia subjects similar effects were seen in anxiety and stress ratings (see Table 10), however there was only a trend for greater heart rate increase during the Stroop compared to congruent word task (see Table 9). There was no significant difference in %Δ in GSR or %Δ in HRV parameters between Stroop and congruent word conditions for either group.

Stroop Performance

. Performance on the stroop task was assessed by recording the number of correct responses. There were no significant differences in number of correct responses during the stroop task between controls and fibromyalgia subject [Controls: 230.6 (+/- 12.7) Fibromyalgia: 241.44 (+/- 10.9),$t(15)= -0.65$, $p=0.53$].

Relation of autonomic responses and subjective reports

Correlations between autonomic measures and subjective reports are summarized in table 11. In healthy controls all autonomic measures of heart rate, log of GSR, and HRV were significantly correlated with subjective reports of anxiety, stress, and fatigue. In fibromyalgia subjects only log of GSR was significantly correlated with subjective reports.

Discussion:

Summary of Main Findings

There were no significant differences between control and fibromyalgia subjects at baseline for autonomic measures of heart rate, GSR and HRV parameters. However, fibromyalgia subjects were significantly less calm, more fatigued and in more pain than controls at baseline. Fibromyalgia and control subjects had similar heart rate and GSR responses to the stand task. HRV analysis demonstrated that fibromyalgia subjects were hypo-responsive during the stand task as they had less %Δ in LF, HF and LF/HF during the stand compared to controls. There were no significant group differences in subjective responses of anxiety, stress, fatigue and pain during the stand task. Both groups showed an increased state of arousal during the Stroop task compared to the rest period and congruent word task as determined by heart rate responses and reports of anxiety and stress. However, fibromyalgia and control subjects responded similarly to the cognitive challenge, as there were no significant differences between fibromyalgia and control subjects in autonomic and subjective responses (Δ scores).

Physical Challenge

Consistent with other studies the results of this investigation demonstrate that individuals with fibromyalgia show *less* changes in HRV parameters (sympathetic hypo-reactivity) in response to orthostatic challenge. A normal response to active standing has been described by (Wieling et al., 1985) in three stages. During the *initial response phase* there is an abrupt increase in heart rate resulting from a rapid inhibition of cardiac vagal tone. This inhibition is initially due to the exercise reflex (primary peak) and then due to diminished activation of

arterial baroreceptors caused by a temporary fall of arterial pressure as blood pools in the legs (secondary peak). The second phase (1-2 min), *the early circulatory stabilization,* involves a sympathetic mediated heart rate increase. Heart rate stabilizes at the final stage of orthostasis, *prolonged orthostasis* (>5 min). In healthy adults heart rate variability analysis has demonstrated a significant increase in LF component and decrease in HF during active standing (Hilz and Dutsch, 2006;Low, 2004b;Matsushima et al., 2004). Therefore, an increase in heart rate, an increase in LF component and a decrease in HF component would be a normal response to active standing, indicating increased sympathetic activity.

In the present study both control and fibromyalgia subjects had a significant increase in heart rate and GSR during the stand task. However, only controls had significant changes in HRV parameters. Accordingly, fibromyalgia subjects had significantly *less* % increase in LF and LF/HF and *less* % decrease in HF during the stand task compared to controls. Since LF and LF/HF are reflective of sympathetic modulations of heart rate and HF is reflective of parasympathetic modulations of heart rate these results are indicative of sympathetic hypo-reactivity in fibromyalgia subjects. Martinez- Lavin et al., (1997) demonstrated that controls had an increase in LF power when changing from supine to stand while fibromyalgia subjects had a decrease in LF power. Cohen et al., (2001) found male fibromyalgia subjects had significantly less increase in LF power and significantly less decrease in HF power compared to control male subjects. Taken together, these studies suggest individuals with fibromyalgia have less sympathetic responses to an active standing task. Furlan et al., (2005)

demonstrated similar finds of hypo-responsiveness in HRV parameters during a passive tilt test. The present results further support previous investigations involving HRV, demonstrating sympathetic- hypo reactivity to orthostatic challenge in fibromyalgia subjects.

Mixed results have been reported with respect to heart rate, some studies have shown when compared to controls, fibromyalgia subjects have less change in heart rate (Cohen et al.,2001; Van Denderen et al.,1991; Lund et al., 2003) while others have shown no significant differences in response to physical challenge (Martinez Lavin et al.,1997; Furlan et al.,2005; Elam et al.,1992). The present study reveals a tendency for individuals with fibromyalgia to have less increase in heart rate compared to controls during the stand task, however, this difference did not reach significance. Again, the present results are consistent with the literature.

Similarly, the mean change in GSR during the physical challenge was less in fibromyalgia than in control subjects but the difference was not significant. Little is known regarding skin conductance responses in fibromyalgia during a physiological challenge. Qiao et al., (1991) demonstrated that compared to controls, fibromyalgia subjects had a greater sympathetic skin response to a cold pressor test yet there was no differential response to loud acoustic stimulation. However, these results are confounded and difficult to interpret as the cold pressor test, although a physiological stressor, also induces pain. Since individuals with fibromyalgia are hypersensitive to a variety of noxious stimuli (Petzke et al.,2003;Lautenbacher et al.,1994) it is likely that different mechanisms of sympathetic activation are involved.

Previous literature has demonstrated that individuals with fibromyalgia exhibit hypo-reactivity to a variety of physical and orthostatic stressors, as determined by ANS function (Cohen et al.,2001; Martinez-Lavin et al.,1997; Furlan et al., 2005) and cortisol production (Crofford et al., 2004;Kirnap et al., 2001). However, it is not known whether this tendency extends to subjective responses to stress, i.e., whether or not individuals with fibromyalgia perceive the stressors differently than control subjects. In the present investigation there was a tendency for individuals with fibromyalgia to have less of an increase in anxiety ratings during the stand task than do controls, however this difference did not reach significance. This tendency can in part be accounted for by the higher baseline anxiety ratings in fibromyalgia subjects in the supine position. There is also a high prevalence of psychological co-morbidites in fibromyalgia (Thieme et al., 2004) and thus the tendency for less increase in anxiety could also be explained by mood and affective disturbance.

Cognitive Challenge

In the present study there were no significant group differences in autonomic or subjective responses to the Stroop task. Fibromyalgia and control subjects had similar heart rate, GSR, HRV responses as well subjective reports of anxiety, stress, fatigue and pain during the cognitive challenge. Neither of the two previous studies evaluating autonomic responses to a cognitive challenge found significant group differences in heart rate responses (Elam et al., 1992;Thieme and Turk, 2005). However, Thieme & Turk (2005) found that individuals with fibromyalgia had a greater GSR amplitude (hyper- responsive) to math and social conflict. Interestingly, the HYPER-responsivity of GSR in fibromyalgia to

cognitive challenge is in contrast to the HYPO- responsivity of other autonomic measures such as heart rate and HRV responses to physical challenge. Although we did not confirm a significant finding of hyper -responsivity of GSR to cognitive challenge, it is noteworthy that in our study it is *only* the GSR responses to the cognitive challenge for which fibromyalgia subjects show a tendency towards HYPER reactivity, not HYPO- reactivity. The mean % change in GSR was higher in fibromyalgia, yet the difference was non significant. It is likely that the great inter-individual variability in both groups partly accounts for the non significant difference. It is difficult to determine why GSR to cognitive challenge in fibromyalgia subjects had a tendency toward increased responsiveness as opposed to the general trend of decreased responsiveness observed with other autonomic measures to physical challenge. However, it is noteworthy that sympathetic activity involves almost exclusively the neurotransmitter noradrenaline, a quick acting transmitter. Sweat gland activity is the one exception, as it involves the neurotransmitter acetylcholine, a slow acting transmitter typically involved in parasympathetic activity. Although it is difficult to extract the exact mechanism involved in the increased reactivity in GSR responses, consideration of the differences in neurotransmitters may be of importance.

We did not observe a significant modulation in HRV parameters in either controls or fibromyalgia subjects during the Stroop task. If the Stroop task did act as a stressor resulting in a sympathetic response one would expect an increase in LF power and LF/HF power, the measure of sympathetic modulations of heart rate and a decrease in HF power, a measure of parasympathetic modulations.

However, there is conflicting evidence regarding HRV responses during the Stroop task in healthy controls. Jain et al., (2001) examined controls during an eight minute Stroop task and found subjects had a significant increase in LF and significant decrease in HF compared to rest period. However, they did not compare changes in HRV to a control condition, such as a congruent word task. Hoshikawa and Yamamoto (2000) demonstrated a decrease in HRV as measured by time domain analysis but no significant differences in the frequency domain analysis, i.e, no significant changes in LF or HF frequency power during the Stroop. On the other hand, Pagani et al., (1991) revealed a significant reduction in HF but no significant change in LF. In our study, we did not observe any significant changes in the % of power of LF or HF during the Stroop task for either group. It is important to note that these values are expressed as a percentage of total power (normalized unit) where the very low frequency, low frequency and high frequency make up the total spectrum power density. It is possible that if there were changes in the absolute values of the power components in such a way that both LF and HF components of power changed similarly then differences in the change of % of power values would not be observed.

It has been suggested that different types of social/cognitive/psychological stressors have different mechanisms of stress response activation. For instance, a review by Kajantie and Phillips (2005) suggested that psychological stressors involving ego such as public speech or math stressor with harassing comments launches a stress response predominately involving the hypothalamic- pituitary-adrenal axis, while stressors such as Stroop and math with out harassing comments involves predominately the sympatho-adrenal axis. Thus, it may be

difficult to draw accurate comparisons between studies involving different types of psychological stressors and to extend any findings found during one type of stressor. The present study involved a Stroop word task, which has been suggested to involve predominately the sympatho-adrenal axis. Yet, the subjects also received harassing comments from the experimenter, which is known to involve predominately the hypothalamic- pituitary- adrenal axis. Although the two systems work in a coordinated manner hypothalamic-pituitary –adrenal a responses, i.e. cortisol production may have been a more sensitive measure to detect differential responses between control and fibromyalgia subjects. There is also a large body of literature describing HPA axis dysfunction, specifically hypo-cortisol production in individuals with fibromyalgia (Crofford et al., 2004;Kirnap et al., 2001). In the future, perhaps adjusting the task difficulty or manipulating the presence of harassing comments while employing a variety of psychological/cognitive stressors could be more effective at teasing out differences in autonomic responses to cognitive stress between fibromyalgia and control subjects.

There is evidence that individuals with fibromyalgia often have cognitive disturbances, such as memory and concentration difficulties (Suhr, 2003;Landro et al., 1997). The Stroop task requires the inhibition of an automatic cognitive activity, word reading, and thus demands considerable mental effort to suppress these strong habitual responses (Stroop, 1935). In the present investigation performance during the Stroop task was similar in control and fibromyalgia subjects.

Stroop as a Stressor

We indeed confirmed that the Stroop task was an effective stressor as the % increase in heart rate was significantly higher in the Stroop condition compared to the congruent word task and the preceding rest period. Furthermore, the subjective ratings of anxiety and stress were also significantly higher in the Stroop condition compared to the congruent word task and the preceding rest period. On the other hand, there were no significant differences in the %Δ in GSR during the Stroop task compared to the congruent word task. GSR is a highly sensitive measure such that inspiratory gasp, cough, sneeze and talking can evoke a GSR response (Critchley, 2002). Hence, GSR differences due to actual stress could have been masked if the verbal output during the Stroop and congruent word task was similar. This could partly explain the reason for non significant differences in GSR responses between the Stroop and congruent word task. Although we were able to record the number of correct responses during the Stroop task we did not record total number of responses and thus do not have a measure of total verbal output during the Stroop compared to the congruent word task. Hence, we can not draw such conclusions.

Stroop as an attention task

The Stroop task is a well established laboratory stressor; however it is also commonly used as an attention task (Roelofs et al., 2002). It has been shown that attentional tasks can modulate pain perception, as directing one's attention away from the pain can lead to a reduction in perceived pain (Villemure et al., 2003). Interestingly, in the present study individuals with fibromyalgia rated their pain less during the Stroop condition compared to the previous rest period, suggesting

that the Stroop task did act as a distraction from their ongoing pain. Yet this difference did not reach significance.

Baseline Differences- sympathetic hyperactivity?

Previous reports suggest that individuals with fibromyalgia display sympathetic hyperactivity at rest although results amongst studies are often inconsistent. Investigations involving heart rate have demonstrated individuals with fibromyalgia have higher resting heart rates (Cohen et al., 2000;Cohen et al., 2001;Furlan et al., 2005) compared to healthy controls but a fair number of studies have also demonstrated no significant differences in resting heart rate (Elam et al., 1992;Kadetoff and Kosek, 2006;Lund et al., 2003;Martinez-Lavin et al., 1998;Thieme and Turk, 2005). Investigations involving HRV analysis reveal more consistent results, demonstrating that individuals with fibromyalgia have a greater LF component of power (sympathetic and parasympathetic modulations) and less HF component (parasympathetic modulations) than controls at rest (Cohen et al., 2000;Cohen et al., 2001;Furlan et al., 2005;Stein et al., 2004). Martinez- Lavin et al (1998) demonstrated that fibromyalgia subjects have a greater LF band component at night compared to controls. The authors suggested that this sympathetic hyperactivity at night could be involved with the sleep disturbances experienced in fibromyalgia. Less is known regarding skin conductance (GSR), one study revealed no significant difference in resting GSR (Thieme and Turke, 2005) while another study described a trend for higher resting GSR (Qiao et al.,1991). Taken together, these studies suggest sympathetic hyperactivity at rest. However, in the present investigation we did not find any significant baseline differences in GSR, heart rate, or HRV parameters.

It is important to consider the level of physical fitness of an individual, as individuals who have a high level of physical fitness have greater parasympathetic activity and lower resting heart rate compared to individuals in poor physical condition (Goldsmith et al., 2000). Therefore, since individuals with fibromyalgia suffer significant disability and reduced mobility (Bennett, 1996), the sympathetic hyperactivity at rest could be explained by poor physical fitness. In our study we did not find any baseline differences in heart rate, GSR or HRV parameters. The discrepancy between our study and those that found sympathetic hyperactivity at rest could be related to differences in physical fitness and disability. Since degree of disability is often related to disease severity, it is possible that the discrepancy in baseline autonomic tone is a reflection of differences in patient populations. There were, however, differences in baseline levels of anxiety, fatigue and pain. Again this is consistent with the clinical picture of fibromyalgia and the diagnostic criteria of widespread musculoskeletal pain and fatigue (Wolfe et al.,1990) and reports of anxiety disorders (Thieme et al., 2004).

Relationship between autonomic measures and subjective reports

Sympathetic activation can be considered closely linked to emotion (Critchley, 2002). The present investigation revealed that all autonomic measures of control subjects were significantly correlated with subjective reports. However, only GSR was significantly correlated with subjective reports in fibromyalgia subjects. A study by (Brosschot and Aarsse, 2001) revealed that fibromyalgia patients showed a higher level of affective-autonomic response dissociation when shown emotional movie excerpts. Specifically, fibromyalgia subjects showed higher heart rate response to the movie excerpts while their affective responses

were similar to controls. The present investigation confirms that there may be an affective- autonomic response dissociation in individuals with fibromyalgia.

Differences between cognitive and physical challenge

In the present investigation, HRV analysis demonstrated individuals with fibromyalgia exhibit sympathetic hypo-reactivity to physical challenge, but the same effect was not observed with a cognitive challenge, as fibromyalgia subjects behaved similarly during the cognitive challenge. During the physical challenge of active standing, there is a rapid movement of blood from the thorax to the lower legs resulting in a decrease in venous return and cardiac output. This triggers compensatory mechanisms which decrease vagal tone and increase sympathetic tone, resulting in an increased heart rate (Wieling et al., 1985). This mechanism of sympathetic nervous system activation is different from the stress response resulting from the cognitive challenge. Although the neural circuitry involved in the sympathetic nervous system processing of psychological stress has yet to be fully delineated, the HPA response to psychological stress has been more deeply studied. The HPA response to cognitive/psychological stress is thought to involve the sensory cortex as the point of initiation (McDougall et al., 2005). The stress response is then continually processed and integrated in areas such as the prefrontal cortex, hippocomapus and amygdaloid regions. At each of these levels of integration there are many direct and indirect connections with the sympathetic nervous system and thus allow for modulation of the sympathetic nervous system (McDougall et al., 2005). Therefore taking into account that the sympathetic nervous system activation in response to a physical stressor is initiated from a "peripheral" trigger while sympathetic nervous system activation in response to a

cognitive stressor is likely "centrally processed" it is reasonable that two different response patterns were observed in our study.

In the past fibromyalgia was considered to be a psychosomatic disorder, since identifying a physiological basis for the pathology was difficult. Hence, the pain was often considered to be "in the patient's head" and a distortion in patient reporting (Gracely et al., 2003). Importantly, the present investigation demonstrated a physiological difference in the sympathetic stress response to a *physical stress,* not a *cognitive/psychological stress* thus further discrediting the notion of a predominately psychological basis of fibromyalgia.

Stress, Pain and the Sympathetic Nervous System

Clinical and neurobiological evidence suggests a dysfunction in the stress response in individuals with fibromyalgia. However, at this time it is difficult to understand the link between alterations in the stress response and clinical pain. The role stress plays in pain perception is still unclear, however a better understanding of this relationship is being revealed through the animal literature. There is the concept of stress induced analgesia whereby extreme acute stress results in analgesia, while chronic stress can lead to hyperalgesia (Gamaro et al., 1998;King et al., 2003;Quintero et al., 2000). However, this relationship may be more complicated as upon closer examination the outcome of analgesia or hyperalgesia is often dependent on whether the pain test is testing reflex responses such as the tail flick test or operant behaviour such as escape behaviours (King et al., 2003). Humans studies indicate that acute experimental stressors can induce both analgesia (Logan et al., 2003;Rhudy and Meagher, 2003) and hyperalgesia (Caceres and Burns, 1997;Levine et al., 1993) in healthy adults. The variety of

responses appear to be largely dependent on gender, subjective response of perceived stress, physiological reactivity and whether the pain test evaluated pain thresholds or intensity ratings (Rhudy and Meagher, 2003;Caceres and Burns, 1997). Despite anecdotal evidence suggesting that stress influences pain perception in chronic pain, little is known about how experimental stress influences clinical or experimental pain perception in chronic pain patients.

Autonomic Nervous System Dysfunction: Can it explain the symptoms of Fibromyalgia

Although chronic widespread pain is the cardinal feature of fibromyalgia a wide range of symptoms including irritable bowel, chronic fatigue, sleep disorders, depression, anxiety, Reynauds phenomena and orthostatic intolerance are also associated with the disorder. Martinez-Lavin and Hermosillo (2000) suggested that many of these symptoms can in part be explained by autonomic nervous system dysfunction, specifically sympathetic hyperactivity at rest and hypo-reactivity to stress. He suggested that this phenomena agrees with the principle of chronic hyper-stimulation of beta adrenergic receptors which leads to receptor desensitization and down regulation. He also suggests that the sympathetic hyperactivity may also explain symptoms of irritable bowel, sleep disturbance and anxiety. For instance, bowel and bladder function is under control of the autonomic nervous system such that activation of the sympathetic nervous system causes vasoconstriction in the gut organs. Although, a complete understanding of the underlying pathology in irritable bowel syndrome is not fully understood it is believed that the disorder is a result of a dysregulation of the autonomic nervous system involving increased sympathetic and decreased

parasympathetic activity (Aggarwal et al., 1994). Other symptoms such as Raynaud's phenomena, where there is abnormal vasoconstriction in the distal digits of hands, are also associated symptoms of fibromyalgia which are known to be caused in part by ANS dysregulation. It has also been suggested that the inability to respond to different stressors could explain the constant fatigue observed in fibromyalgia patients (Martinez-Lavin and Hermosillo, 2000). In addition to these visceral symptoms, there is a high prevalence of affective disorders such as depression and anxiety in fibromyalgia (Thieme et al., 2004). A reduction in HRV has been demonstrated in individuals with depression and anxiety disorders. In particular, investigations revealed that individuals with anxiety disorders such as panic disorder are hypo- reactive to stressors such as the startle (Gorman and Sloan, 2000b) and an active stand task (Yeragani et al., 1998). Although the present study did not find sympathetic hyperactivity at rest, it did reveal autonomic dysfunction in response to a physical challenge. Taking together the underlying pathology of many of the associated symptoms and the results of investigations demonstrating autonomic nervous system dysfunction it is possible that these symptoms may in part be due to ANS dysfunction. However, it is difficult to determine whether the chronic pain in fibromyalgia led to ANS dysfunction or the ANS dysfunction led to the chronic pain.

Role of other factors and limitations of the study:

There are many factors which influence the ANS function and subsequent ANS responses to both physical and cognitive stress. It is well recognized that fibromyalgia patients have a high prevalence of affective disorders such as depression and anxiety (Thieme et al., 2004). Involvement of the autonomic nervous system, specifically a reduction in HRV has been demonstrated in individuals with depression and anxiety (Gorman and Sloan, 2000a). Yeragani et al., (1997) evaluated HRV in individuals with panic disorder during an active stand task and found that the panic disorder patients had less LF and greater HF component of power than controls. The same dysregulation was observed in the present study as fibromyalgia subjects had less increase in LF and less decrease in HF during the stand task compared to control subjects. However, depression and anxiety disorders did not likely play a role as subjects were screened and excluded for major depressive and anxiety disorders. Hormonal status, in particular estrogen exposure, has also been implicated in affecting sympatho-adrenal responses to stress. A recent review by (Kajantie and Phillips, 2006) examining gender differences in ANS stress responses reported that between puberty until menopause women have less sympathetic responses to physical and psychological stress compared to males. However after menopause, women have increased sympathetic responses compared to males and pre-menopausal woman. Yet, if the women are given hormonal replacement therapy sympathetic responses are subsequently reduced. It is thus possible that pre-menopausal women would be hypo-responsive to stress compared to post- menopausal women. In the present study we controlled for hormonal status and prevalence of hormone replacement

therapy and thus this is unlikely to have affected our results. Obesity has also been associated with alterations in autonomic nervous system function, in particular an increase in cardiac sympathetic drive (Snitker et al., 2000). In our study we did not control for body weight and it is thus possible that this could have affected autonomic function.

Aside from subject characteristics and associated pathology there are also a number of statistical and methodological considerations. Firstly, the sample size is small, as there were 10 fibromyalgia subjects and 10 control subjects. If a larger sample size would have been employed some of the differences observed may have reached significance. Furthermore, there was significant inter-individual variability amongst the dependent variables even in healthy controls, therefore making it difficult to obtain significant results. Thus, increasing the sample size would increase the statistical power. The main finding of the present study is confirmation of hypo- reactivity to orthostatic challenge in individuals with fibromyalgia by HRV analysis. There is a technique which assesses HRV on the basis of fluctuations in blood pressure and thus provides an index of arterial baroreflex control of heart rate (Hilz and Dutsch, 2006). However, blood pressure was not monitored in the present study. During active standing blood pools in the legs causing a drop in blood pressure and thus activating the baroreceptor reflex to re-stabilize pressure. Thus the alterations observed in the HRV analysis could be a result of diminished baroreceptor sensitivity. A study by (Bou-Holaigah et al., 1997) did in fact find the fibromyalgia subjects had less increase in blood pressure during orthostatic challenge. If the autonomic dysfunction is indeed related to

alterations in baroreflex sensitivity this may explain the lack of group differences during the cognitive challenge.

Summary:

Fibromyalgia is a chronic pain disordered characterized by widespread musculoskeletal pain and tenderness in 11 out of 18 tender points. Many theories regarding the underlying pathology have been suggested, however the etiology remains unknown. Stress has been suggested as contributing to the etiology yet the data is largely correlative and relies on patient reports. Dysfunction of the ANS, one of the two main systems involved in coordinating the stress response has been demonstrated in individuals with fibromyalgia. Investigations involving HRV analysis reveal that fibromyalgia subjects compared to controls display sympathetic hyperactivity at rest but are less responsive to physical and orthostatic challenge. However, little is known about how individuals with fibromyalgia react to a cognitive or psychological challenge. The present study investigated autonomic responses of heart rate, GSR, and HRV as well as subjective reports of anxiety, stress, fatigue and pain in 10 female fibromyalgia subjects and 10 age matched healthy controls during an orthostatic challenge (active stand) and a cognitive challenge (Stroop word task). This study revealed that fibromyalgia subjects had significantly less changes in HRV parameters during the stand task compared to controls. Yet, during the Stroop task fibromyalgia and control subjects had similar autonomic and subjective responses. These results confirm previous findings that individuals with fibromyalgia are less reactive to physical challenges than controls, but also suggest that they do not respond differently during mental challenges.

Conclusion

Fibromyalgia is a complex disorder that involves multiple dimensions of physical and psychological function. Although advances in our understanding of the disorder have been made the complex clinical presentation and lack of readily definable pathology still leaves us with many questions. The co morbidities with 'stress related' disorders and the high correlation of adverse life events and fibromyalgia symptoms strongly suggest that stress contributes to the pathology. Recent developments in psychophysiological research support this relationship and demonstrate that fibromyalgia may be related to a dysfunction in the stress response. Dysfunction of the two main systems involved in the stress response, the hypo thalamic pituitary adrenal axis and the sympathetic nervous system have been demonstrated in individuals with fibromyalgia. Specifically, with regards to autonomic function reports reveal that fibromyalgia subjects display sympathetic hyperactivity at rest and exhibit a blunted sympathetic response to orthostatic and physical challenge, with the most consistent results demonstrated using HRV analysis. Despite trends emerging in the data there are still missing pieces of the puzzle. A systematic examination of autonomic responses to cognitive challenge in fibromyalgia has not yet been completed. The present investigation attempts to enhance our understanding of the autonomic nervous system dysfunction in fibromyalgia subjects by examining responses to cognitive and orthostatic challenge.

The present investigation did not reveal any baseline differences in autonomic function between individuals with fibromyalgia and controls. Yet, at baseline subjective reports of anxiety, fatigue and pain were significantly higher

in fibromyalgia subjects than controls. These findings support the diagnostic criteria for fibromyalgia of the American College of Rheumatology and the numerous reports indicating individuals with fibromyalgia are more anxious, fatigued and in more pain than controls.

The present investigation confirms the sympathetic hypo-reactivity to an orthostatic challenge, an active stand task, but also demonstrates that fibromyalgia and control subjects respond similarly to a cognitive challenge, the Stroop task. During the stand task, HRV analysis revealed that fibromyalgia subjects had less increase in sympathetic tone and less decrease in parasympathetic tone compared to controls. On the other hand, during the cognitive challenge, autonomic responses of heart rate, GSR and HRV parameters as well as subjective reports of anxiety, stress, fatigue and pain were similar between groups. In summary, the present study demonstrates that fibromyalgia subjects reacted differently to a physical challenge (less sympathetic response) as demonstrated by HRV analysis but not a cognitive challenge. In the past, due to a lack of readily definable pathology, fibromyalgia was often considered a psychosomatic disorder and a distortion in patient reporting. Thus, the finding that fibromyalgia subjects react differently *physiologically* to a *physical* but not a cognitive challenge further discredits the notion of a purely psychological basis of the disorder.

At this time it is difficult to make a direct link between a dysregulation of the stress response, autonomic nervous system dysfunction and the clinical presentation of fibromyalgia. A more complete understanding of the role of stress and the sympathetic nervous system in pain perception is necessary. Investigating the role stress plays in contributing to the etiology of fibromyalgia provides a

method to examine the disorder from a bio-psychological perspective. This multi-disciplinary approach is suiting as the patient history and clinical presentation of fibromyalgia involves an interaction of psychosocial and neurobiological factors. A further understanding of the etiology of fibromyalgia will enable advances in the identification and management of fibromyalgia and ultimately help reduce the burden of illness.

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Table 1: Subject Demographics

Demographic Data expressed as mean ±standard deviation

Comparisons between fibromyalgia and control subjects were made by independent t- tests

	Controls	**Fibromyalgia**	**p**
Age	50.5 (± 9.4)	52.7 (± 9.4)	p= 0.58
Tenderpoint Exam	1.8 (± 2.0)	14.5 (± 2.5)	p<0.0001
Fibromyalgia Impact Questionnaire	8.8 (± 8.1)	51.8 (± 18.5)	p<0.0001
Duration of Illness	----------	8.2 (±3.3) years	p<0.0001

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Demographic Data expressed as mean ±standard deviation

Comparisons between fibromyalgia and control subjects were made by independent t- tests

Table 2a: Autonomic Measures for fibromyalgia and control subjects at baseline

		Fibromyalgia	Controls	t,p
Heart Rate (beats per minute)		72.5 (± 3.0)	68.3 (±3.0)	-0.96, p=0.34
HRV (%)	LF	37.5% (±2.4)	40.0% (±2.7)	0.7 p=0.49
	HF	41.3% (±2.7)	40.0 % (±3.3)	-0.3, p= 0.77
	LF/HF	0.98% (± 0.13)	1.1% (±0.16)	0.64 p=0.53
Log of GSR		0.14 (± 0.11)	0.26 (± 0.07)	0.86, p=0.41

Baseline Values are expressed as mean ± standard error
Differences in autonomic measures at baseline were compared with independent t- tests

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 2b: Subjective reports for fibromyalgia and control subjects at baseline

	Fibromyalgia	Controls	U,p
Anxiety	46.0 (± 10.9)	80.0 (±3.9)	**14.0 p<0.01**
Stress	3.9 (±2.4)	1.5 (±1.1)	48.0 p=0.88
Fatigue	18.0 (±7.7)	1.5 (±1.1)	**27.0, p <0.05**
Pain	18.5 (± 7.8)	0.0	**25.0 p<0.05**

Baseline Values are expressed as mean VAS ± standard error
Baseline Differences were compared with the Mann-Whitney test

Table 3: Autonomic responses to physical challenge in fibromyalgia (FM) and healthy control (HC) subjects

		Physical Challenge (Stand test)			Physical Challenge (Stand Recovery)		
		FM	**HC**	**U, p**	**FM**	**HC**	**U, p**
ΔHRV (%)	ΔLF	**8.8 (±8.2)**	**40.7 (±8.2)**	**15.0, p<0.01**	**5.6 (±14.3)**	**-27.2 (±5.0)**	**24.0, p=0.05**
	ΔHF	**- 0.05 (±13)**	**- 30.6 (±5.0)**	**18.0, p<0.05**	13.6 (±10.1)	61.1 (±19.6)	28.0, p=0.1
	ΔLF/HF	**26.2 (±17.9)**	**125.0 (±36.1)**	**16.0, P<0.01**	**15.5 (±30.2)**	**-47.2 (±7.9)**	**20.0, p=0.02**
Δ% HR		12.7 (±4.3)	21.8 (±4.3)	33.0 p=0.20	-14.1 (±3.8)	-19.1 (±3.0)	44.0 p=0.65
Δ%logGSR		53.1 (±20.5)	80.9 (±19.3)	38.0 p=0.36	-27.4 (±12.7)	-29.7 (±8.6)	38.0, p= 0.36

Autonomic Responses are expressed as a percent change (Δ %) +/- standard error

Δ % for Stand is calculated by $\frac{\text{Stand - Supine 1}}{\text{Supine 1}} \times 100$

Δ % for Stand Recovery is calculated by $\frac{\text{Supine 2 - Stand}}{\text{Stand}} \times 100$

Group Differences were assessed by Mann Whitney 2 sample test

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 4: Subjective responses to physical challenge in fibromyalgia (FM) and healthy control (HC)

	Physical Challenge (Stand test)			Physical Challenge (Stand Recovery)		
	FM	**HC**	**U, p**	**FM**	**HC**	**U, p**
ΔAnxiety	-25.5 (±15)	-54.4 (±13)	30.0 p=0.13	28.5 (±8.7)	46.2 (± 14.1)	41.5, p=0.5
ΔStress	7.4 (±6.4)	2.4 (± 1.9)	32.0 p=0.17	-16.3 (±6.1)	-3.3 (±1.4)	38.5, p=0.38
ΔFatigue	14.5 (±6.3)	14.1 (±5.1)	48.0 p=0.88	-12.5 (±6.1)	-14.6 (± 5.0)	44.5, p=0.68
ΔPain	4.0 (±6.9)	-1.9 (±1.1)	40.0 p=0.41	-1.5 (± 6.9)	0.0 (±1.1)	50.0, p=1.0

Subjective responses are expressed as a change score (Δ) +/- standard error
Δ for Stand is calculated by Stand – Supine 1
Δ for Stand Recovery is calculated by Supine 2 – Stand

Group Differences were assessed by Mann Whitney 2 sample test

Table 5: Autonomic responses to mental challenge in fibromyalgia (FM) and healthy control (HC)

		Mental Challenge (Stroop test)			Mental Challenge (Stroop Recovery)		
		FM	**HC**	**U, p**	**FM**	**HC**	**U, p**
ΔHRV (%)	ΔLF	11.5 (±7.3)	13.1 (±7.6)	48.0 p=0.88	2.5 (±6.4)	-1.5 (±5.7)	48.0 p=0.88
	ΔHF	- 3.2 (±5.8)	-5.2 (±5.4)	49.0 p=0.08	-1.1 (± 6.3)	2.8 (±5.8)	44.0 p=0.45
	ΔLF/HF	22.2 (±13.5	27.4 (±10.8	49.0, p= 0.08	10.1 (±13.2)	0.9 (±10.8)	45.0, p=.71
ΔHR (%)		9.6 (±3.9)	9.9 (±2.1)	39.0 p=0.41	-7.4 (± 2.5)	-7.7 (±2.2)	46.0 p=0.76
Δlog GSR (%)		129.3 (±73)	47.0 (±12)	48.0, p=0.88	-11.3 (±12.0)	-13.1 (±5.8)	50.0, p=1.0

Autonomic Responses are expressed as a percent change (Δ %) +/- standard error

Δ % for Stroop is calculated by $\frac{\text{Stroop - Rest 2}}{\text{Rest 2}} \times 100$

Δ % for Stroop Recovery is calculated by $\frac{\text{Rest 3 - Stroop}}{\text{Stroop}} \times 100$

Group Differences were assessed by Mann Whitney 2 sample test

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 6: Subjective Responses to mental challenge in fibromyalgia (FM) and healthy control (HC) subjects

	Mental Challenge (Stroop test)			Mental Challenge (Stroop recovery)		
	FM	**HC**	**U, p**	**FM**	**HC**	**U, p**
ΔAnxiety	-68.8 (±20)	-120.8 (±22)	28.5 p=0.1	61.5 (±14.1)	111.5 (±19.6)	26.5, p=0.08
ΔStress	50.8 (±9.2)	57.4 (±12)	43.0 p=0.6	-46.7 (± 6.7)	-58.1 (±11.6)	36.5, p=0.31
ΔFatigue	14.9 (±5.1)	29.3 (±11)	43.0 p=0.6	-24.5 (±9.4)	-30.0 (±9.8)	47.5, p=0.85
ΔPain	-13.6 (±5.7)	5.0 (±5.0)	**18.0 p=0.02**	5.0 (±5.0)	-13.6 (± 5.7)	31.5, p=0.16

Subjective responses are expressed as a change score (Δ) +/- standard error
Δ for Stroop is calculated by Stroop – Rest 2
Δ for Stroop Recovery is calculated by Rest 3- Stroop

Group Differences were assessed by Mann Whitney 2 sample test for both the physical and mental challenge condition.

Table 7: Comparison of autonomic responses during the Stroop and congruent word task for control subjects

		Stroop	Congruent	W,p
ΔHRV (%)	ΔLF	13.1 (±7.6)	17.2 (±-7.7)	W=25.0 p=0.80
	ΔHF	-5.2 (±5.3)	-7.5 (±3.4)	W=25.0 p=0.8
	ΔLF/HF	27.4 (±16.4)	30.7 (± 13.6)	W=27.0 p= 0.96
ΔHR (%)		9.8 (±2.1)	2.5 (±1.8)	W=4.0 **P<0.02**
ΔlogGSR (%)		47.9 (±11.6)	92.4 (±24.3)	W=17.0 p=0.28

Autonomic Responses are expressed as a percent change (Δ %) +/- standard error

Δ % for Stroop is calculated by $\frac{\text{Stroop - Rest 2}}{\text{Rest 2}} \times 100$

Δ % for Congruent is calculated by $\frac{\text{Congruent – Rest 1}}{\text{Rest 1}} \times 100$

Comparison of Stroop vs Congruent condition was assessed by Wilcoxin Pairwise Comparison.

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 8: Comparison of subjective responses during the Stroop and congruent word task for controls

	Stroop	Congruent	W,p
ΔAnxiety	-120.8 (±21.8)	-50.7 (±14.9)	W=0.0 **P<0.005**
ΔStress	57.4 (±11.6)	10.9 (±5.4)	W=0.0 **P<0.005**
ΔFatigue	29.3 (± 10.5)	12.6 (±8.1)	W=3.0 p=0.06
ΔPain	----------	----------	----------

Subjective responses are expressed as a change score (Δ) +/- standard error
Δ Stroop is calculated by Stroop – Rest 2
Δ Congruent is calculated by Congruent – Rest 1

Comparison of Stroop vs Congruent condition was assessed by Wilcoxin Pairwise Comparison.

Table 9: Comparison of autonomic responses during the Stroop and congruent word task for fibromyalgia subjects

		Stroop	Congruent	W,p
ΔHRV (%)	ΔLF	12.0 (±7.2)	-4.7 (±-6.1)	W=14.0 p=0.17
	ΔHF	-3.2 (± 5.8)	10.4 (±7.1)	W=16.0 p=0.24
	ΔLF/HF	22.2 (±13.5)	-8.7 (±10.3)	W=13.0 p= 0.14
ΔHR (%)		9.6 (±3.9)	4.7 (±2.0)	W=11.0. **P<0.09**
ΔlogGSR (%)		129.0 (±73.0)	49.6 (±18.1)	W=20.0 p=0.77

Autonomic Responses are expressed as a percent change (Δ %) +/- standard error

Δ % for Stroop is calculated by $\frac{\text{Stroop - Rest 2}}{\text{Rest 2}} \times 100$

Δ % for Congruent is calculated by $\frac{\text{Congruent – Rest 1}}{\text{Rest 1}} \times 100$

Comparison of Stroop vs Congruent condition was assessed by Wilcoxin Pairwise Comparison.

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 10: Comparison of subjective responses during the Stroop and congruent word task for fibromyalgia subjects

	Stroop	Congruent	W,p
ΔAnxiety	-68.8 (±-19.6)	-19.5 (±11.2)	W=8.0 **P<0.05**
ΔStress	50.8 (±9.2)	12.8 (±4.2)	W=2.7 **P<0.008**
ΔFatigue	14.8 (±5.1)	8.7 (±8.2)	W=16.0 p=0.44
ΔPain	-13.6 (±5.7)	-4.1 (±5.0)	W=16.5 P=0.5

Subjective responses are expressed as a change score (Δ) +/- standard error
Δ Stroop is calculated by Stroop – Rest 2
Δ Congruent is calculated by Congruent – Rest 1

Comparison of Stroop vs Congruent condition was assessed by Wilcoxin Pairwise Comparison.

Table 11a: Spearman Rank Order Correlations of autonomic and subjective measures in controls during physical and cognitive challenge

	Anxiety		Stress		Fatigue		Pain	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
HR	**-0.75**	**p<0.05**	**0.82**	**p<0.01**	**0.83**	**p<0.01**	0.23	p=0.55
Log of GSR	**-0.86**	**p<0.01**	**0.67**	**p<0.05**	**0.72**	**p<0.05**	0.38	p=0.32
LF	**-0.73**	**p<0.05**	**0.8**	**p<0.01**	**0.80**	**p<0.01**	0.23	p=0.55
HF	**0.72**	**p<0.05**	**-0.87**	**p<0.01**	**-0.87**	**p<0.01**	-0.17	p=0.65
LF/HF	-0.6	p=0.09	**0.8**	**p<0.01**	**0.78**	**p<0.05**	0.07	p=0.85

Results are expressed as correlation coefficients. Significant correlations are marked in bold

HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Table 11b: Spearman Rank Order Correlations of autonomic and subjective measures in fibromyalgia subjects during physical and cognitive challenge

	Anxiety		Stress		Fatigue		Pain	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
HR	-0.39	p=0.29	0.3	p=0.43	0.03	p=0.93	-0.22	p=0.56
Log of GSR	**-0.79**	**p<0.01**	**0.73**	**p<0.05**	**0.82**	**p<0.05**	-0.18	p=0.64
LF	-0.38	p=0.31	0.43	p=0.24	0.1	p=0.80	0.35	p=0.36
HF	0.28	p=0.47	-0.25	p=0.52	0.08	p=0.83	-0.27	p=0.49
LF/HF	-0.26	p=0.50	0.3	p=0.43	-0.15	p=0.70	0.42	p=0.26

Results are expressed as correlation coefficients. Significant correlations are marked in bold
HRV: Heart Rate Variability
LF: Low Frequency
HF: High Frequency

HR: Heart Rate
GSR: Galvanic Skin Response

Figure 1: Experimental Protocol

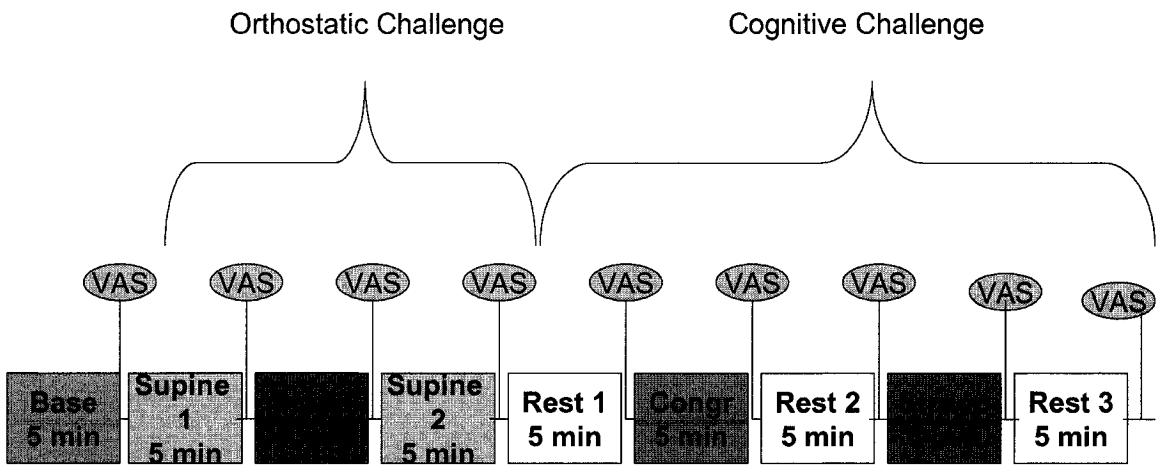


Figure 1: Experimental Protocol
The order of task presentation was constant for all subjects. Following the last VAS recording subjects were given the fibromyalgia impact questionnaire and a tender point exam was completed

Figure 2a: Electrode Placement

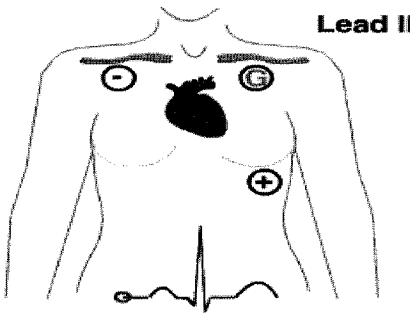


Figure 2a: Negative Electrode placed on right anterior second intercostal space. Ground electrode placed on corresponding spot on left side. Positive electrode placed slightly left of just below the xyphoid process

Figure 2b: Graphical Depiction of a normal cycle of an electrocardiogram

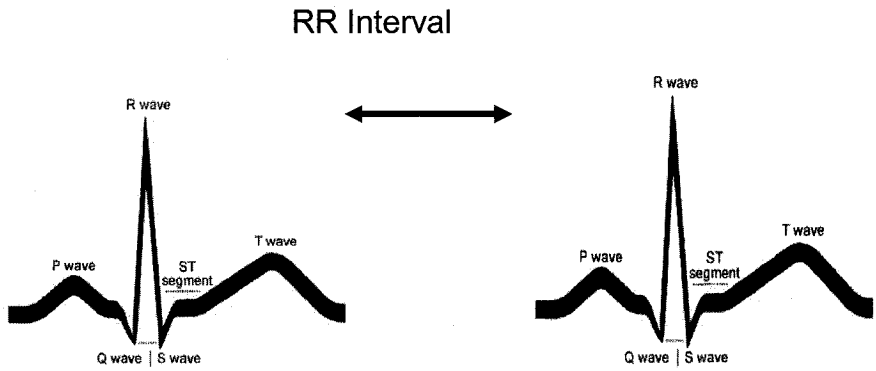


Figure 2b:

The RR Interval is the time between successive R- peaks; the inverse of this time interval gives the instantaneous heart rate. Heart rate variability is found by calculating the variability of a series of RR interval

Figure 3: Visual Analogue Scales

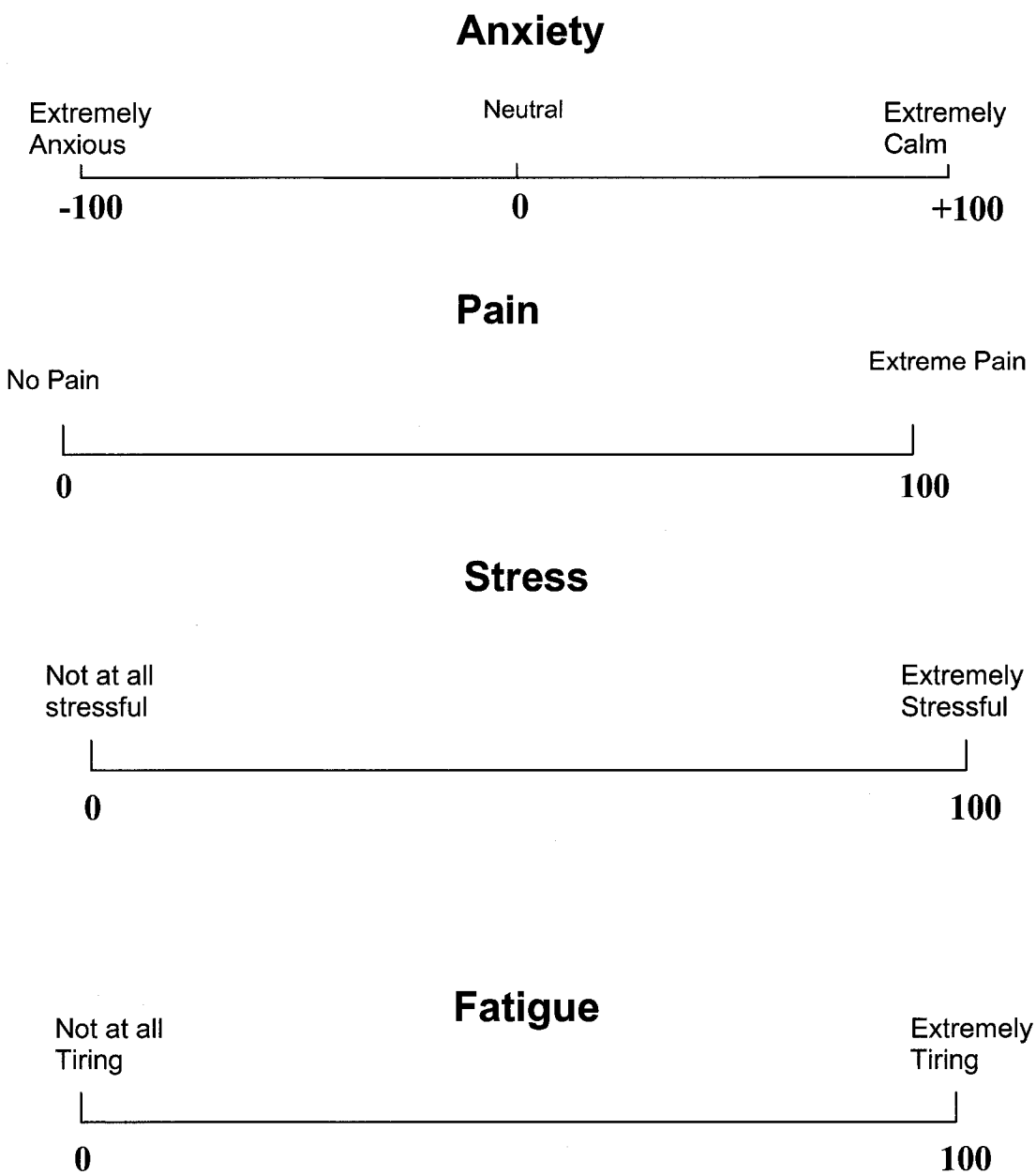
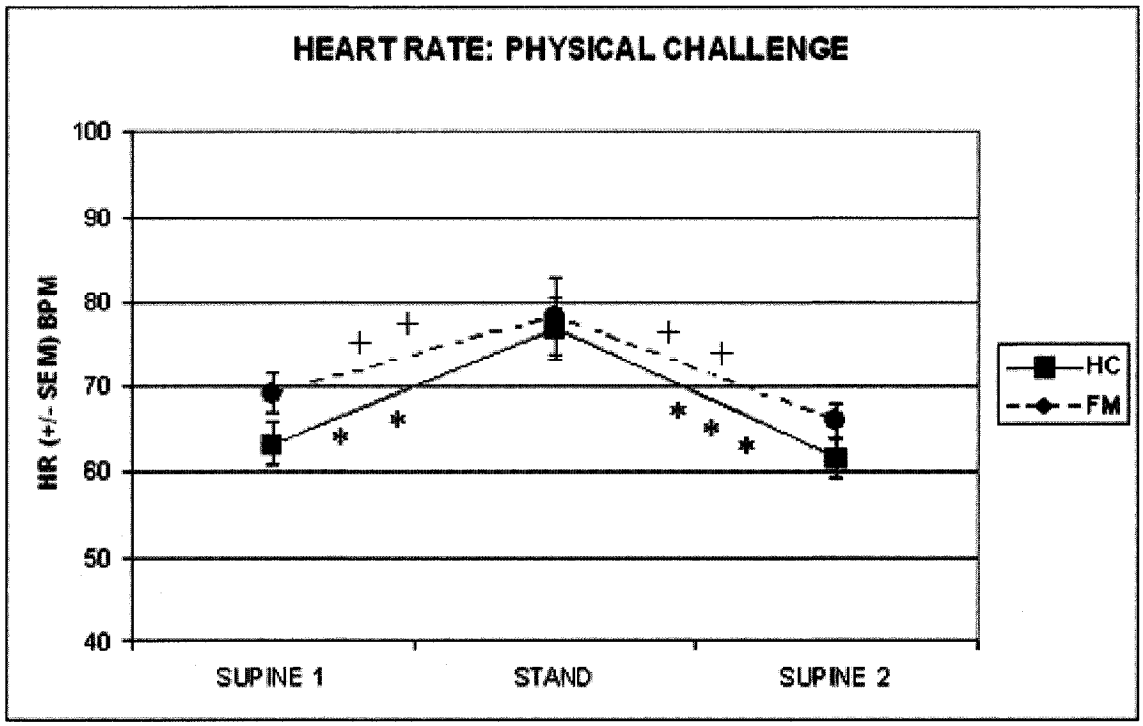


Figure 4: Heart rate (HR) of fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge

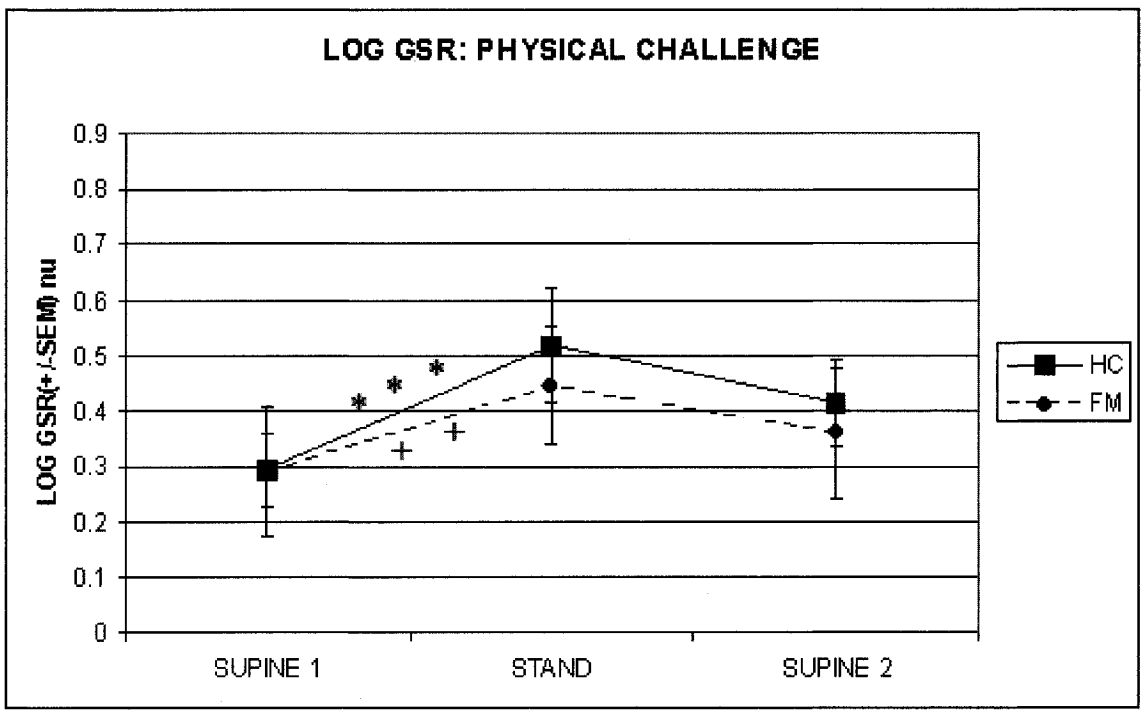


Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

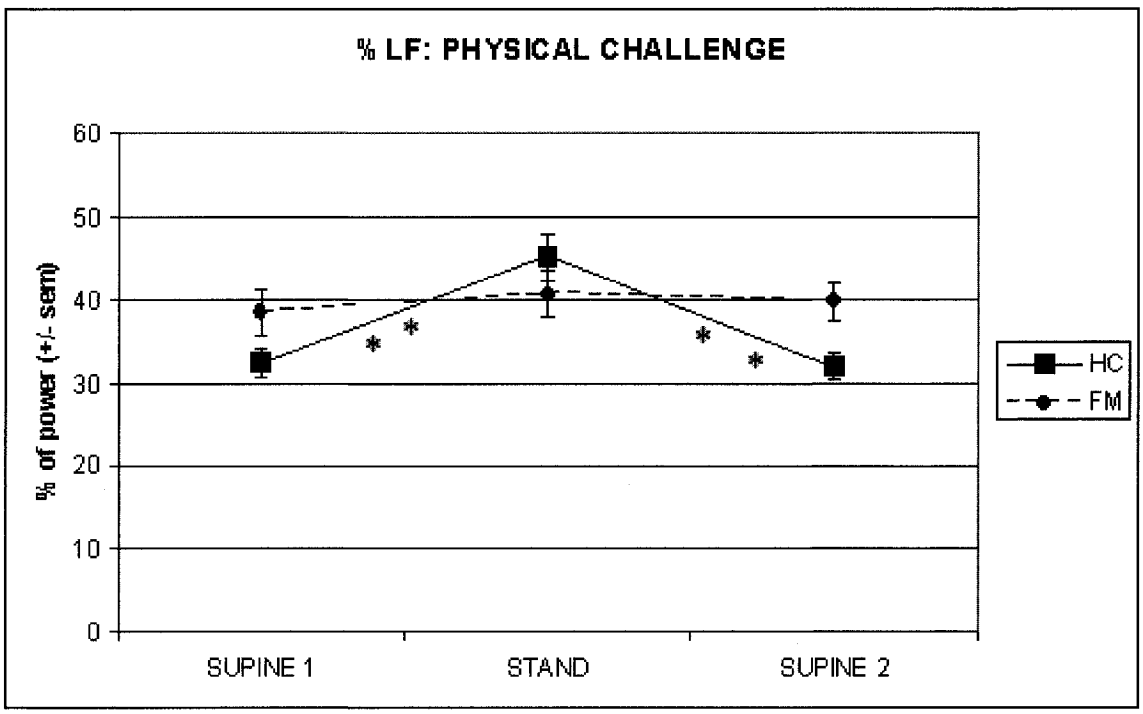
Figure 5: Log GSR of fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$	**FM:** + $p<0.05$
** $p<0.01$	++ $p<0.0$
*** $p<0.001$	+++ $p<0.001$

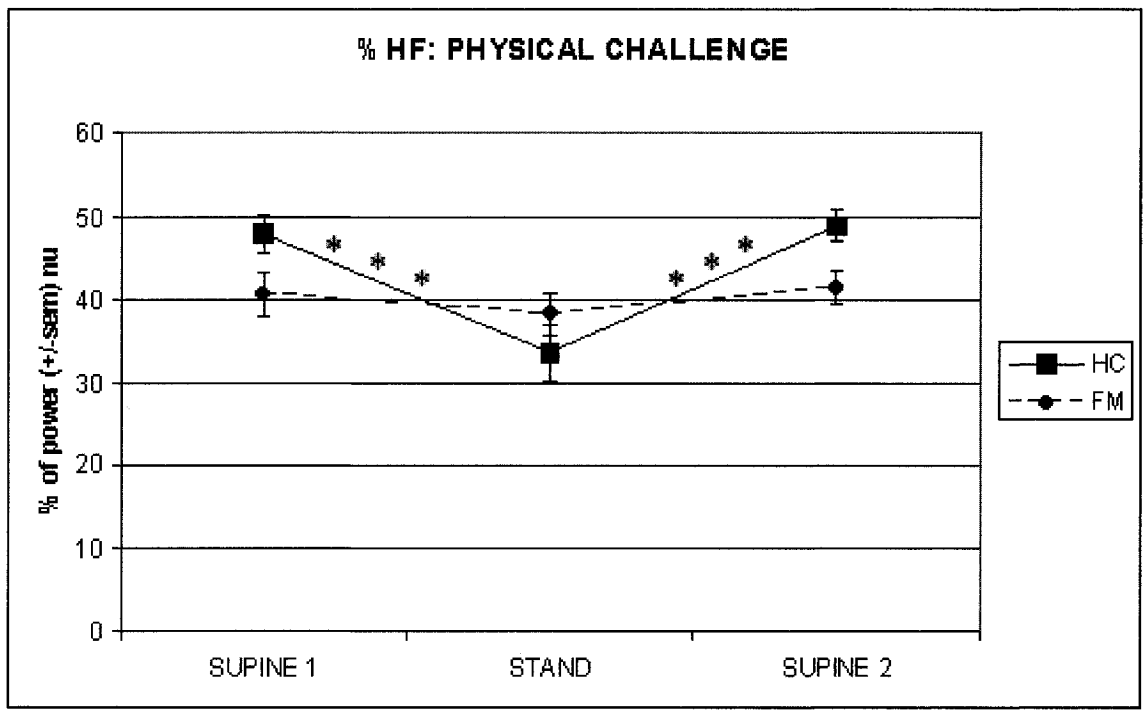
Figure 6: % of LF power in fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$	**FM:** + $p<0.05$
** $p<0.01$	++ $p<0.01$
*** $p<0.001$	+++ $p<0.001$

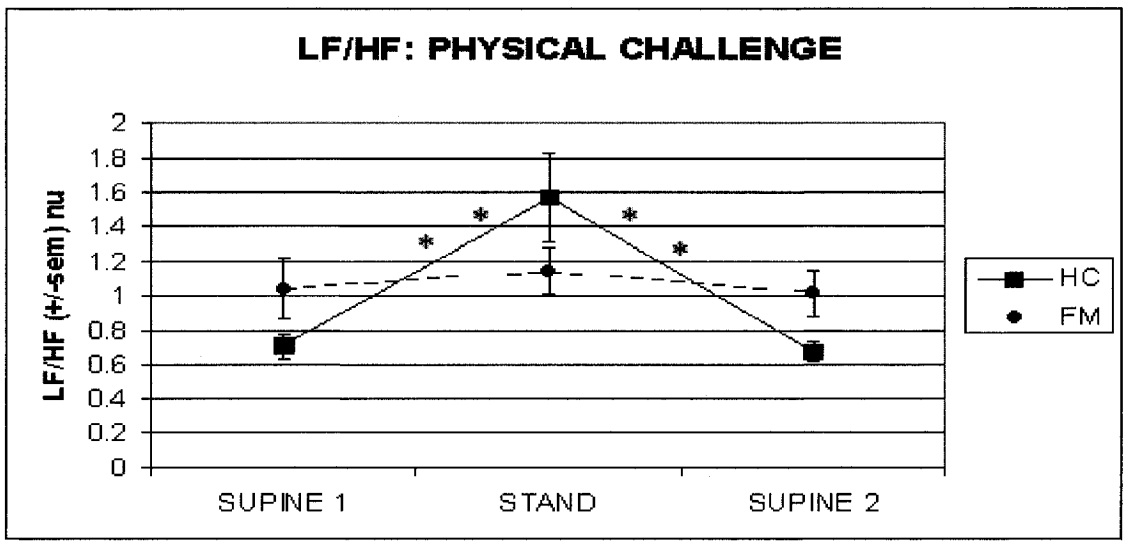
Figure 7: % of HF power in fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$	**FM:** + $p<0.05$
** $p<0.01$	++ $p<0.01$
*** $p<0.001$	+++ $p<0.001$

Figure 8: % of LF/HF power in fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge

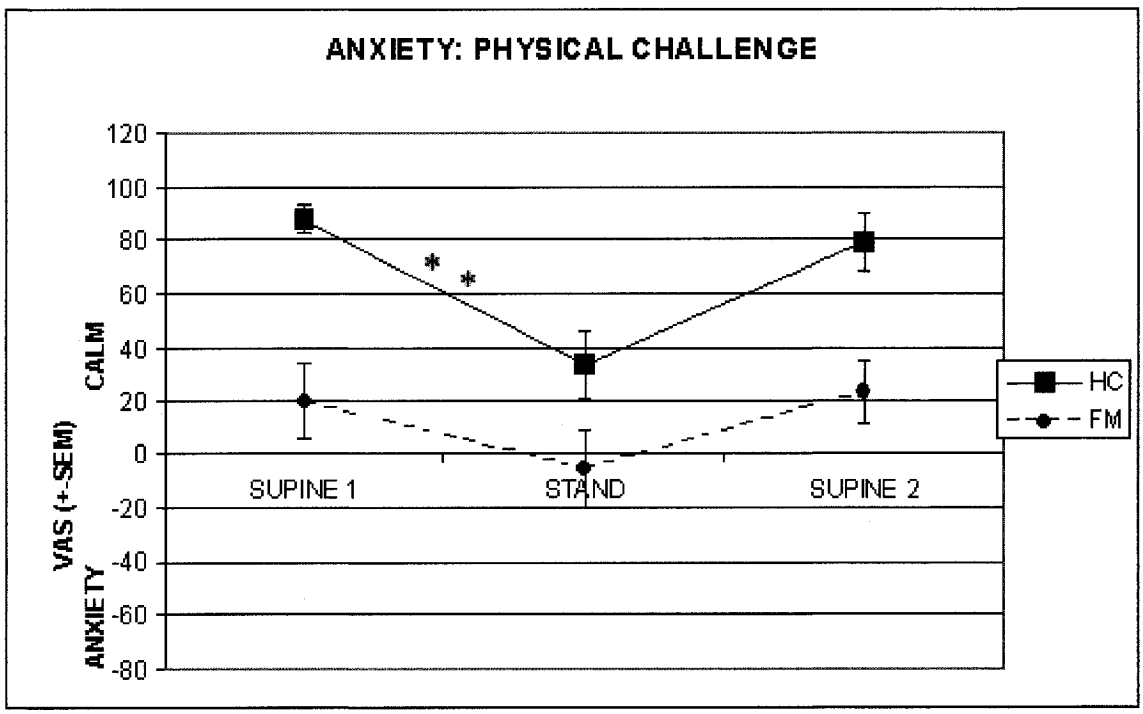


Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 9: Anxiety ratings of fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge

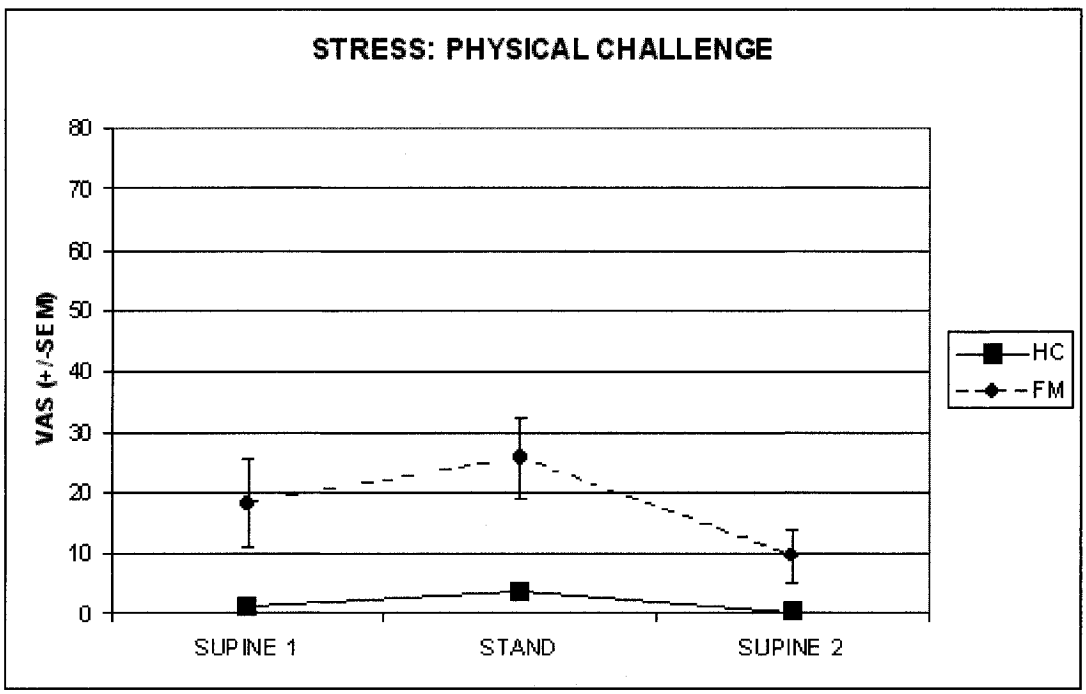


Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Physical challenge alpha= 0.025

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 10: Subjective stress reports in fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge

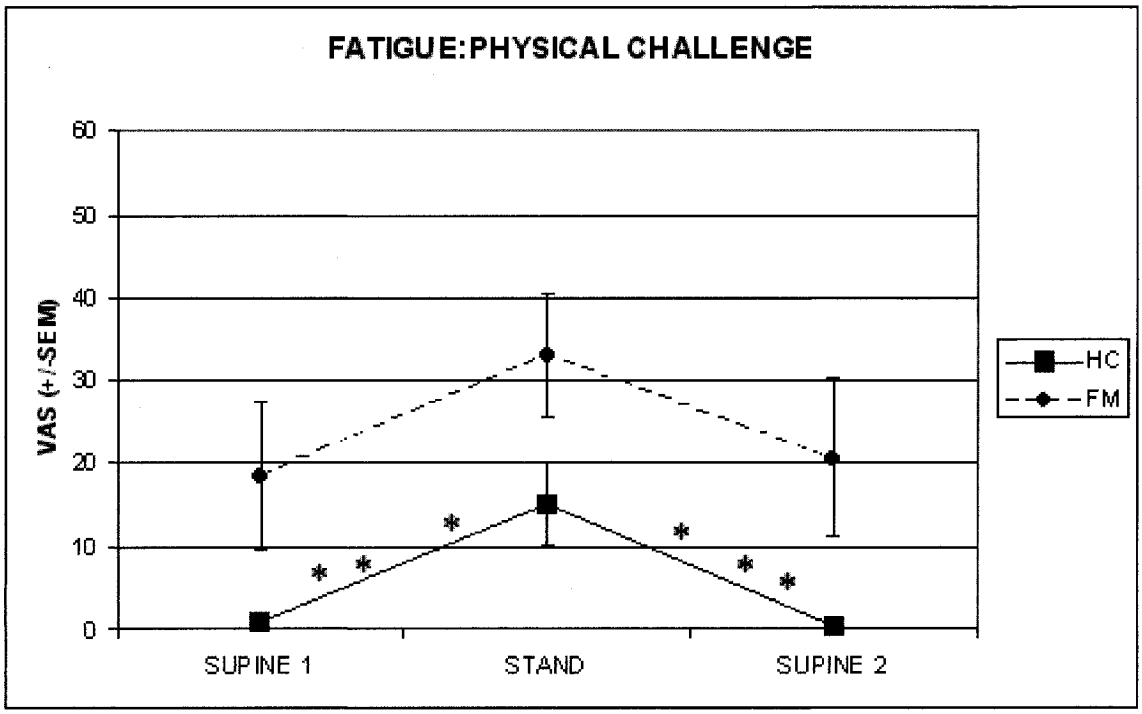


Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Physical challenge alpha= 0.025

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 11: Fatigue Ratings of fibromyalgia (FM) and healthy control (HC) subjects during the physical challenge

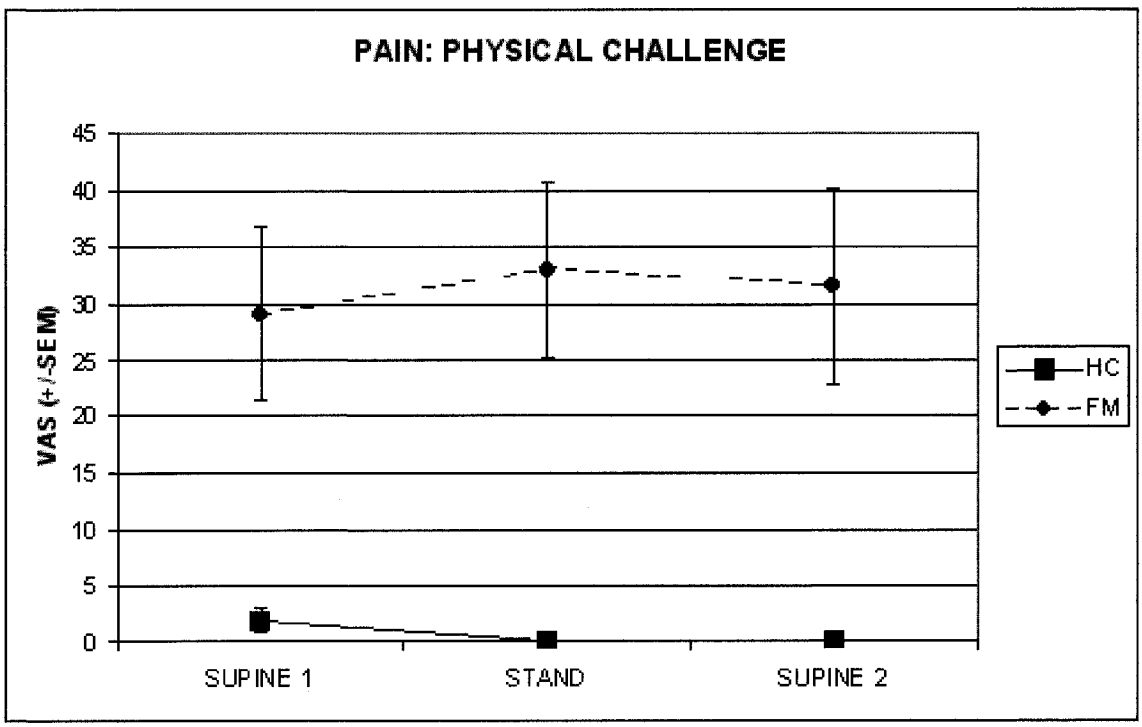


Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Physical challenge alpha= 0.025

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 12: Pain ratings of fibromyalgia(FM) and healthy control (HC) subjects during the physical challenge

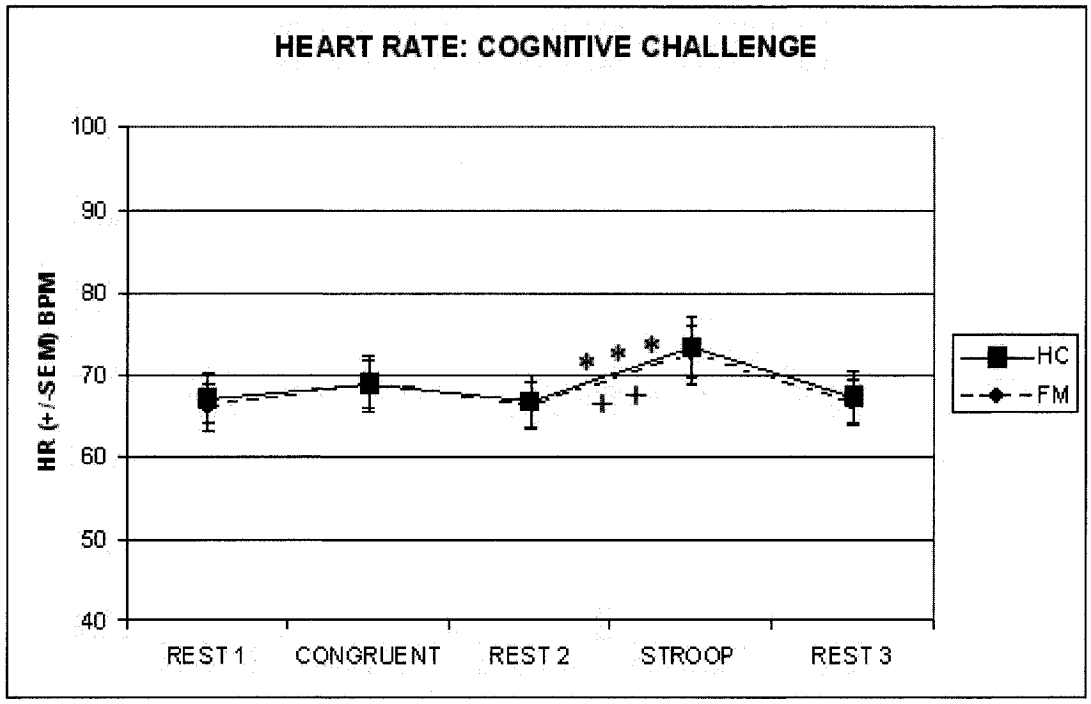


Within Group Comparisons analyzed by Wilcoxin test.
Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Physical challenge alpha= 0.025

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 13: Heart rate of fibromyalgia (FM) and healthy control subjects (HC) during the cognitive challenge

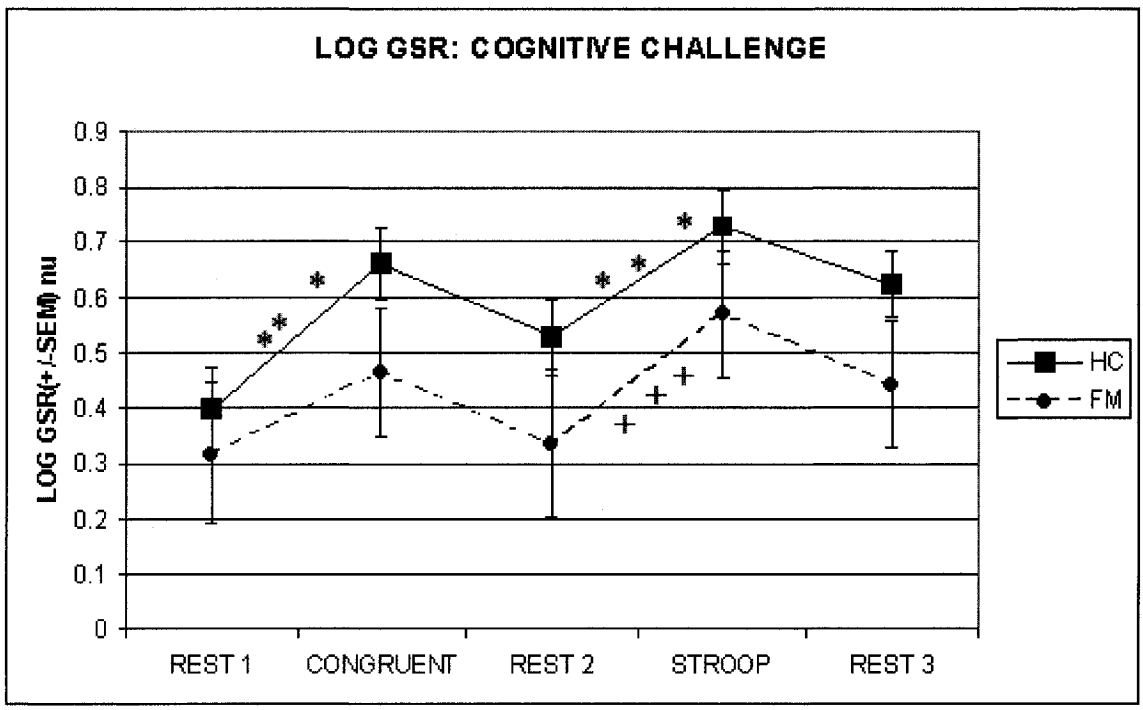


Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

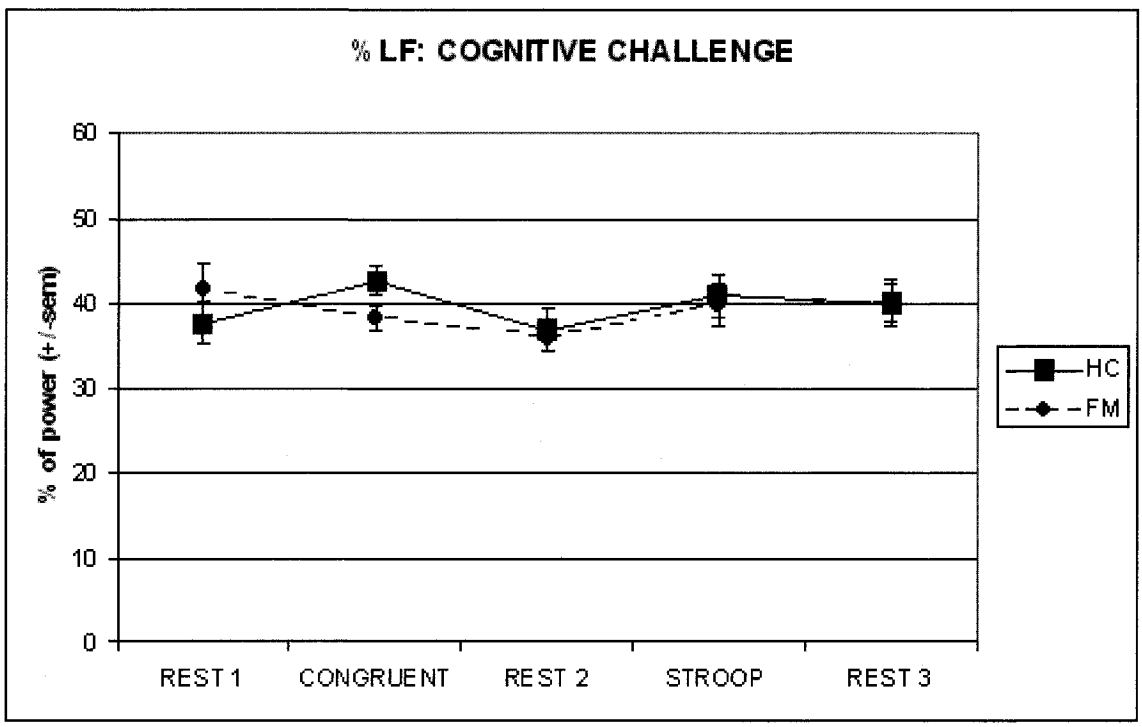
Figure 14: Log GSR of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$	**FM:** + $p<0.05$
** $p<0.01$	++ $p<0.0$
*** $p<0.001$	+++ $p<0.001$

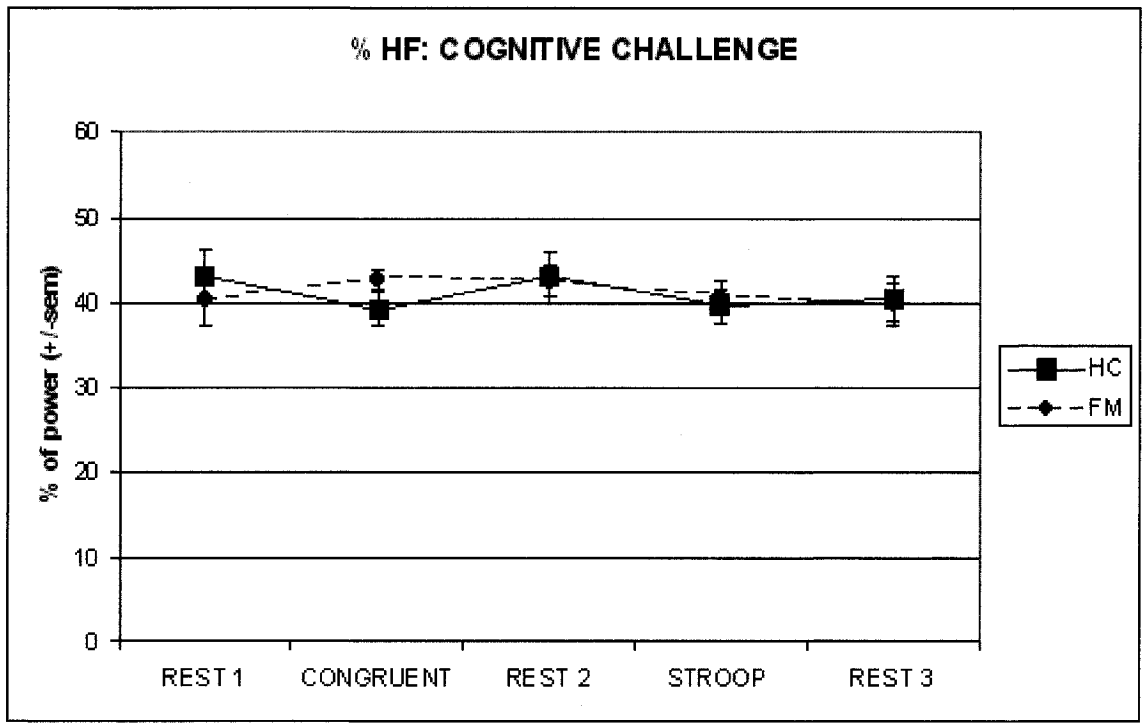
Figure 15: % of LF power of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * p<0.05	**FM:** + p<0.05
** p<0.01	++ p<0.0
*** p<0.001	+++ p<0.001

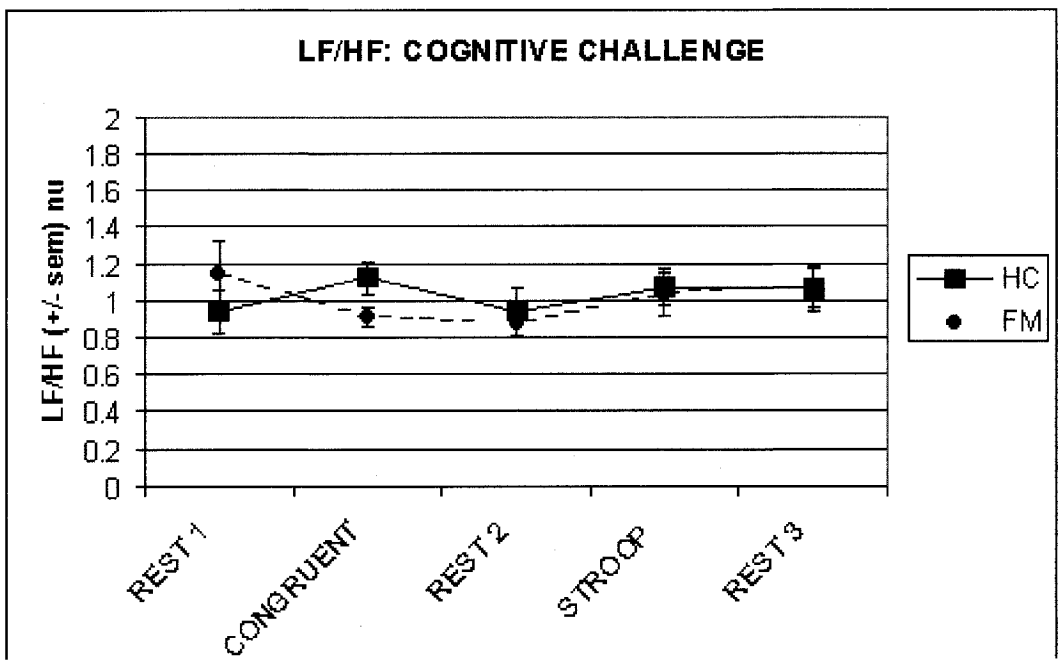
Figure 16: % of HF power of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * p<0.05	**FM:** + p<0.05
** p<0.01	++ p<0.0
*** p<0.001	+++ p<0.001

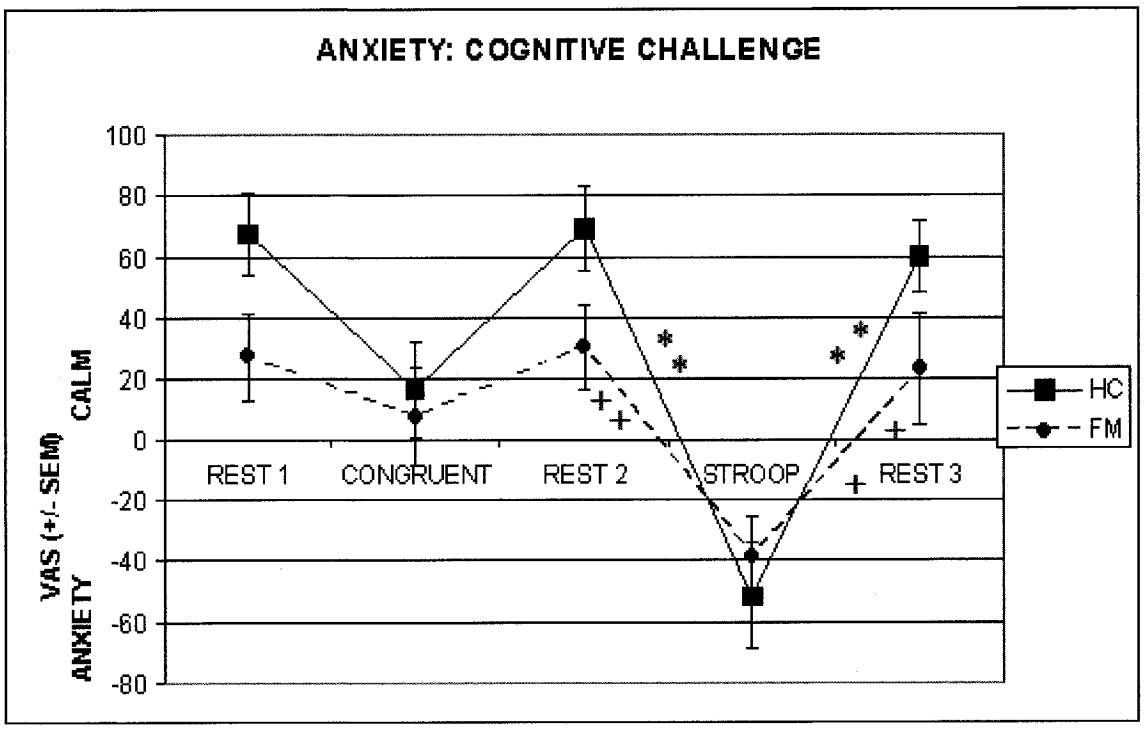
Figure 17: % of LF/HF power of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge



Within Group Comparisons analyzed by Tukeys post hoc test. Significant within group comparisons are marked.

HC: * $p<0.05$	**FM:** + $p<0.05$
** $p<0.01$	++ $p<0.0$
*** $p<0.001$	+++ $p<0.001$

Figure 18: Anxiety ratings of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge

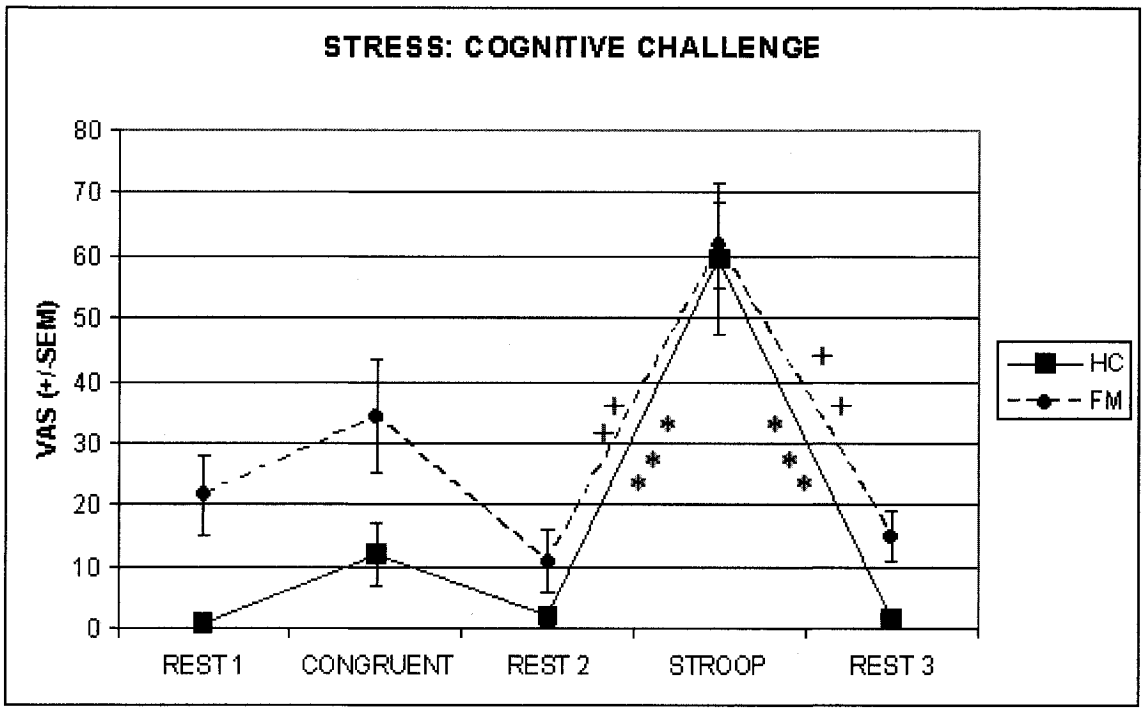


Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Cognitive Challenge alpha= 0.01

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 19: Subjective stress reports in fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge



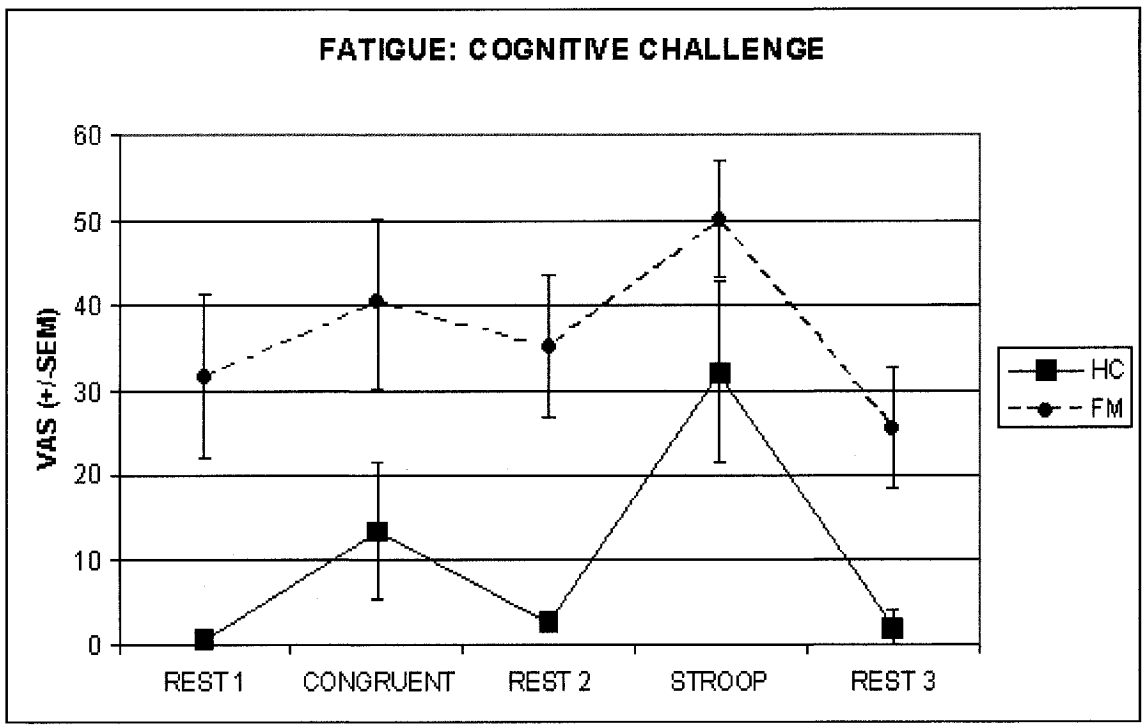
Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked.

Alpha level adjusted for multiple comparison: Physical challenge alpha= 0.025; Cognitive Challenge alpha= 0.01

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 20: Fatigue Ratings of fibromyalgia (FM) and healthy control (HC) subjects during the cognitive challenge

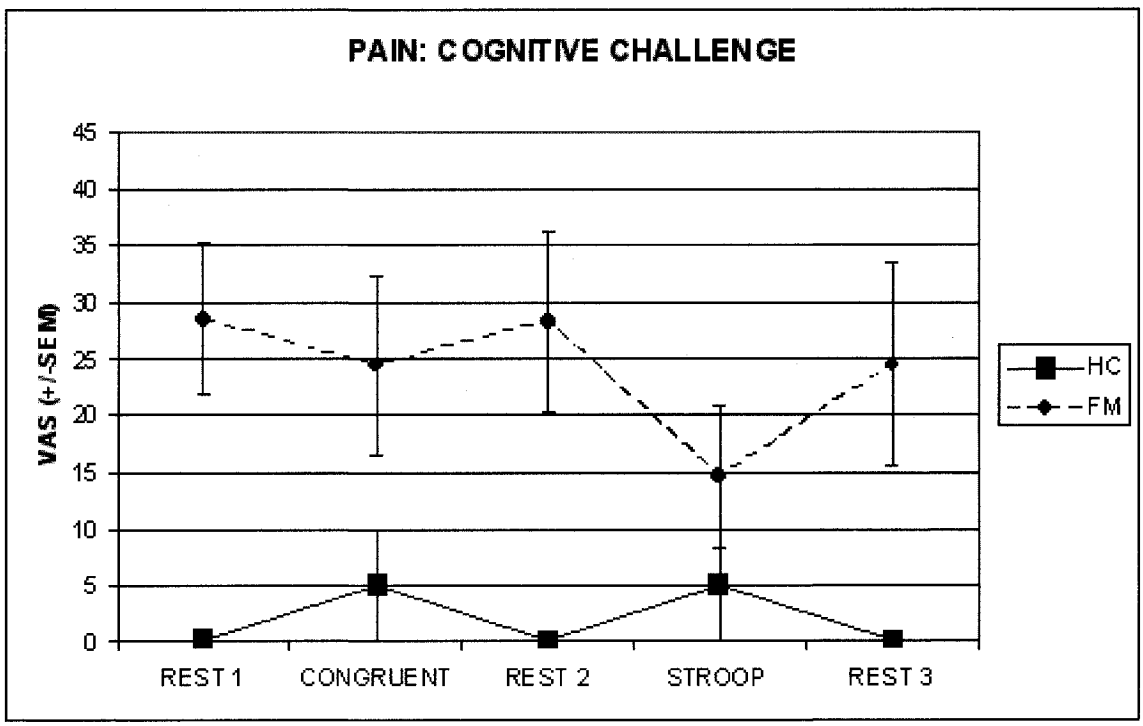


Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison:
Cognitive Challenge alpha= 0.01

HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Figure 21: Pain ratings of fibromyalgia(FM) and healthy control (HC) subjects during the cognitive challenge



Within Group Comparisons analyzed by Wilcoxin test. Significant within group comparisons are marked. Alpha level adjusted for multiple comparison: Cognitive Challenge alpha= 0.01

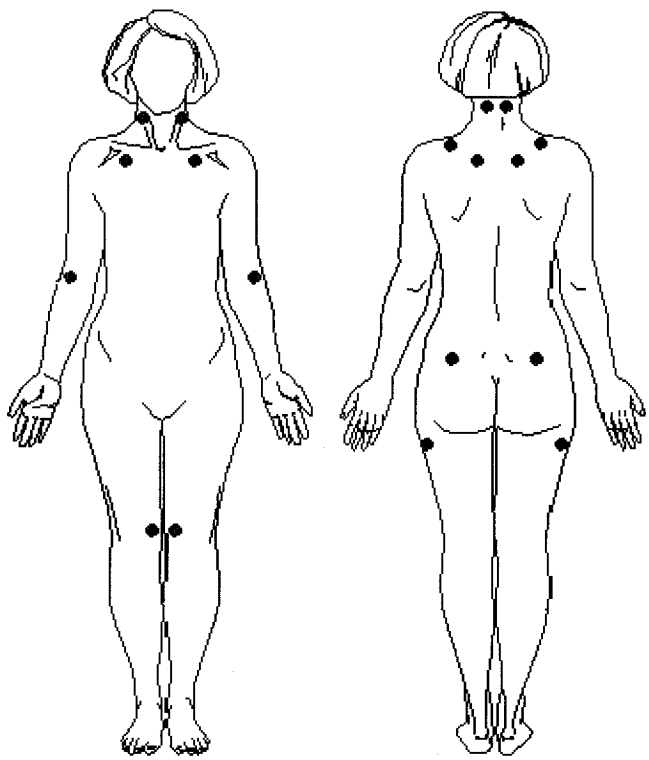
HC: * $p<0.05$
** $p<0.01$
*** $p<0.001$

FM: + $p<0.05$
++ $p<0.01$
+++ $p<0.001$

Appendix A: Tender Point Exam

Tender Point Exam (See Diagram for location of Tender Points)

Tender Point	Pain Rating (0-4)
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	



(1 & 2) Occiput: on both sides (bilateral), at the sub-occipital muscle insertions.
(3 & 4) Low Cervical: bilateral, at the anterior aspects of the inter-transverse spaces.
(5 & 6) Lateral Epicondyle: bilateral, 2 cm distal to the epicondyles
(7 & 8) Knee: bilateral, at the medial fat pad proximal to the joint line.
(9 & 10) Second Rib: bilateral, at the second costochondral junction, just lateral to the junctions on upper surfaces.
(11 & 12) Trapezius: bilateral, at the midpoint of the upper border of the muscle.
(13 & 14) Supraspinatus: bilateral, at origins, above the spine of the scapula (shoulder blade) near the medial border
(15 & 16) Gluteal: bilateral, in upper outer quadrants of buttocks in anterior fold of muscle.
(17 & 18) Greater Trochanter: bilateral, posterior to the trochanteric prominence.

0- no pain
1- mildly painful
2- moderately painful
3- extremely painful

Appendix B: Table of Frequency Distributions

The raw data was assessed for normality using the Shapiro Wilk W test. If the W statistic was significant the data was determined to be skewed and a non parametric statistical tests were chosen.

Heart Rate

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.91	0.31	Y	0.96	0.82	Y
Supine 1	0.96	0.73	Y	0.89	0.18	Y
Stand	0.98	0.96	Y	0.94	0.58	Y
Supine 2	0.93	0.42	Y	0.94	0.53	Y
Rest 1	0.94	0.60	Y	0.95	0.72	Y
Congruent	0.97	0.91	Y	0.95	0.68	Y
Rest 2	0.94	0.59	Y	0.97	0.88	Y
Stroop	0.96	0.75	Y	0.97	0.92	Y
Rest 3	0.86	0.08	Y	0.97	0.88	Y
Statistical Test	Parametric Test: ANOVA					

Table of Frequency Distributions: GSR

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.8	0.02	N	0.67	0.00004	N
Supine 1	0.72	0.002	N	0.88	0.14	Y
Stand	0.93	0.4	Y	0.97	0.91	Y
Supine 2	0.78	0.009	N	0.92	0.39	Y
Rest 1	0.6	0.00006	N	0.94	0.59	Y
Congruent	0.95	0.78	Y	0.87	0.09	Y
Rest 2	0.86	0.08	Y	0.89	0.16	Y
Stroop	0.89	0.2	Y	0.92	0.36	Y
Rest 3	0.95	0.63	Y	0.9	0.23	Y
Statistical Test	LOG TRANSFORMATION					

Table of Frequency Distributions: LF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.91	0.25	Y	0.93	0.42	Y
Supine 1	0.96	0.85	Y	0.89	0.18	Y
Stand	0.99	1.0	Y	0.91	0.29	Y
Supine 2	0.97	0.9	Y	0.92	0.33	Y
Rest 1	0.94	0.5	Y	0.96	0.82	Y
Congruent	0.96	0.77	Y	0.89	0.18	Y
Rest 2	0.96	0.74	Y	0.92	0.34	Y
Stroop	0.94	0.5	Y	0.89	0.18	Y
Rest 3	0.84	0.04	N	0.94	0.53	Y
Statistical Test	Parametric Test : ANOVA					

Table of Frequency Distributions: HF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.94	0.6	Y	0.89	0.16	Y
Supine 1	0.94	0.6	Y	0.91	0.26	Y
Stand	0.96	0.8	Y	0.91	0.32	Y
Supine 2	0.944	0.6	Y	0.88	0.13	Y
Rest 1	0.93	0.4	Y	0.89	0.22	Y
Congruent	0.87	0.1	Y	0.95	0.66	Y
Rest 2	0.9	0.2	Y	0.93	0.47	Y
Stroop	0.88	0.1	Y	0.95	0.68	Y
Rest 3	0.96	0.79	Y	0.96	0.73	Y
Statistical Test	Parametric Test					

Table of Frequency Distributions: LF/HF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.88	0.13	Y	0.9	0.24	Y
Supine 1	0.9	0.2	Y	0.74	0.003	
Stand	0.93	0.41	Y	0.96	0.79	Y
Supine 2	0.9	0.1	Y	0.77	0.07	Y
Rest 1	0.96	0.8	Y	0.91	0.23	Y
Congruent	0.93	0.5	Y	0.95	0.67	Y
Rest 2	0.93	0.4	Y	0.84	0.04	Y
Stroop	0.94	0.51	Y	0.89	0.17	Y
Rest 3	0.97	0.88	Y	0.92	0.33	Y
Statistical Test	Parametric Test					

Table of Frequency Distributions: ANXIETY

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.85	0.07	Y	0.86	0.08	Y
Supine 1	0.77	0.001	N	0.95	0.73	Y
Stand	0.79	0.01	N	0.89	0.17	Y
Supine 2	0.7	0.001	N	0.9	0.24	Y
Rest 1	0.76	0.004	N	0.94	0.55	Y
Congruent	0.81	0.02	N	0.92	0.32	Y
Rest 2	0.71	0.001	N	0.88	0.12	Y
Stroop	0.83	0.04	N	0.98	0.95	Y
Rest 3	0.76	0.005	N	0.92	0.36	Y
Statistical Test	Non- Parametric Test					

Table of Frequency Distributions: STRESS

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.53	0.000009	N	0.54	0.00001	N
Supine 1	0.46	0.000001	N	0.75	0.0032	N
Stand	0.7	0.0012	N	0.83	0.03	N
Supine 2	0.53	0.0000009	N	0.72	0.002	N
Rest 1	0.66	0.0003	N	0.8	0.01	N
Congruent	0.74	0.0026	N	0.91	0.3	Y
Rest 2	0.53	0.000008	N	0.69	0.0007	N
Stroop	0.89	0.17	N	0.89	0.17	Y
Rest 3	0.52	0.000006	N	0.93	0.46	Y
Statistical Test	Non- Parametric Test					

Table of Frequency Distributions: FATIGUE

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	0.53	0.000009	N	0.77	0.0082	N
Supine 1	0.3	0.0000001	N	0.68	0.0005	N
Stand	0.86	0.08	Y	0.92	0.33	Y
Supine 2	0.37	0.0000001	N	0.72	0.0014	N
Rest 1	0.52	0.000006	N	0.89	0.15	Y
Congruent	0.6	0.00006	N	0.89	0.21	Y
Rest 2	0.75	0.0036	N	0.94	0.56	Y
Stroop	0.83	0.04	N	0.94	0.65	Y
Rest 3	0.37	0.0000001	N	0.88	0.13	Y
Statistical Test	Non- Parametric Test					

Table of Frequency Distributions: PAIN

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Base	----	----	---	0.74	0.0027	N
Supine 1	0.65	0.0002	N	0.89	0.2	Y
Stand	----	----	---	0.96	0.79	Y
Supine 2	----	----	---	0.91	0.29	Y
Rest 1	0.37	0.0000001	N	0.91	0.28	Y
Congruent	0.36	0.0000001	N	0.71	0.0012	N
Rest 2	----	----	---	0.86	0.08	Y
Stroop	0.37	0.0000001	N	0.74	0.0021	N
Rest 3	----	----	---	0.8	0.149	Y
Statistical Test	Non Parametric Test					

Appendix C: Table of frequency distributions for the change scores

Autonomic Response (Δ%) and subjective responses (Δ) were assessed for normality using the Shapiro Wilk W test. If the W statistic was significant the data was determined to be skewed and a non parametric statistical tests were chosen.

Table of Frequency Distributions: Δ%HR

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.86	0.07	Y	0.95	0.64	Y
Stand Recovery (stand to supine 2)	0.83	0.03	y	0.93	0.46	Y
Congruent (rest 1 to congruent)	0.96	0.8	Y	0.97	0.89	Y
	0.92	0.35	Y	0.96	0.83	Y
Stroop (rest 2 to stroop)	0.73	0.002	**N**	0.90	0.21	Y
Stroop Recovery (stroop to rest 3)	0.91	0.25	Y	0.92	0.33	Y
Statistical Test	Non Parametric					

Table of Frequency Distributions: Δ% logGSR

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.9	0.25	Y	0.85	0.06	Y
Stand Recovery (stand to supine 2)	0.88	0.12	Y	0.91	0.26	Y
Congruent (rest 1 to congruent)	0.89	0.19	Y	0.99	0.99	Y
	0.96	0.77	Y	0.82	0.03	Y
Stroop (rest 2 to stroop)	0.92	0.34	Y	0.76	0.005	**N**
Stroop Recovery (stroop to rest 3)	0.87	0.09	Y	0.93	0.41	Y
	Non Parametric					

Table of Frequency Distributions: Δ%LF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.91	0.27	Y	0.93	0.44	Y
Stand Recovery (stand to supine 2)	0.96	0.8	Y	0.82	0.02	Y
Congruent (rest 1 to congruent)	0.78	0.0088	**N**	0.88	0.13	Y
	0.91	0.26	Y	0.9	0.2	Y
Stroop (rest 2 to stroop)	0.94	0.5	Y	0.93	0.42	Y
Stroop Recovery (stroop to rest 3)	0.9	0.84	Y	0.77	0.007	N
	Non Parametric					

Table of Frequency Distributions: Δ%HF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.89	0.17	Y	0.7	0.001	N
Stand Recovery (stand to supine 2)	0.87	0.096	Y	0.92	0.4	Y
Congruent (rest 1 to congruent)	0.95	0.69	Y	0.88	0.12	Y
Stroop (rest 2 to stroop)	0.95	0.67	Y	0.88	0.11	Y
Stroop Recovery (stroop to rest 3)	0.96	0.80	Y	0.98	0.94	Y
	Non Parametric					

Table of Frequency Distributions: Δ%LF/HF

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.74	0.003	N	0.93	0.49	Y
Stand Recovery (stand to supine 2)	0.97	0.89	Y	0.7	0.0008	N
Congruent (rest 1 to congruent)	0.85	0.05	Y	0.94	0.6	Y
Stroop (rest 2 to stroop)	0.91	0.27	Y	0.92	0.4	Y
Stroop Recovery (stroop to rest 3)	0.92	0.34	Y	0.89	0.15	Y
	Non Parametric					

Table of Frequency Distributions: Δ ANXIETY

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.89	0.04	N	0.03	0.43	Y
Stand Recovery (stand to supine 2)	0.87	0.11	Y	0.89	0.19	Y
Congruent (rest 1 to congruent)	0.91	0.26	Y	0.89	0.15	Y
	0.87	0.09	Y	0.9	0.23	Y
Stroop (rest 2 to stroop)	0.91	0.29	Y	0.95	0.69	Y
Stroop Recovery (stroop to rest 3)	0.92	0.33	Y	0.92	0.35	Y
Statistical Test	Non Parametric					

Table of Frequency Distributions: Δ STRESS

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.88	0.14	Y	0.88	0.11	Y
Stand Recovery (stand to supine 2)	0.72	0.0014	N	0.83	0.14	Y
Congruent (rest 1 to congruent)	0.79	0.011	Y	0.78	0.0077	N
Stroop (rest 2 to stroop)	0.92	0.32	Y	0.94	0.5	Y
Stroop Recovery (stroop to rest 3)	0.91	0.26	Y	0.94	0.58	Y
Statistical Test	Non Parametric					

Table of Frequency Distributions: Δ FATIGUE

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	0.82	0.0285	Y	0.84	0.046	Y
Stand Recovery (stand to supine 2)	0.84	0.04	Y	0.91	0.23	Y
Congruent (rest 1 to congruent)	0.59	0.00004	N	0.96	0.79	Y
Stroop (rest 2 to stroop)	0.87	0.089	Y	0.94	0.54	Y
Stroop Recovery (stroop to rest 3)	0.86	0.07	Y	0.94	0.54	Y
Statistical Test	Non Parametric					

Table of Frequency Distributions: Δ PAIN

Condition	**Healthy Control**			**Fibromyalgia**		
	W	p	Normal Distribution	W	p	Normal Distribution
Stand (supine 1 to stand)	-----	-----	------	0.93	0.47	Y
Stand Recovery (stand to supine 2)	-----	-----	------	0.86	0.08	Y
Congruent (rest 1 to congruent)	-----	-----	------	0.97	0.9	Y
				0.72	0.0014	N
Stroop (rest 2 to stroop)	-----	-----	------	0.78	0.01	
Stroop Recovery (stroop to rest 3)	-----	-----	------	0.68	0.0005	N
	Non Parametric Test					