Preliminary Evidence of Increased Pain and Elevated Cytokines in Fibromyalgia Patients with Defective Growth Hormone Response to Exercise

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Abstract: Mounting evidence suggests fibromyalgia (FM) symptoms are influenced by dysfunction of the hypothalamic-pituitary-hormonal axes (HPHA) and the immune response system. The predominant FM symptoms of widespread pain, fatigue, sleep disturbance, depression, stiffness and exercise intolerance are related to abnormal levels of growth hormone (GH) and are reminiscent of "sickness behavior"; a syndrome initiated by the production of pro-inflammatory cytokines in response to various stressors. Cognizant of the reciprocal relationship between HPHA activity and the immune response system, we hypothesized that serum cytokine levels and FM symptom severity would be higher in FM patients with defective growth hormone response to exhaustive exercise compared to those without. Outpatients with FM (n = 165) underwent a Modified Balke Treadmill Protocol and GH response to exhaustive exercise was measured in peripheral blood samples. Levels of IL-1α, IL-1β, IL-1RA, IL-6, IL-8, IL-10, and TNF-α were measured from stored serum on a subset of 24 participants (12 with and 12 without normal GH response to exhaustive exercise). FM symptom severity was assessed using the Fibromyalgia Impact Questionnaire (FIQ), number of tender points and cumulative myalgic scores. GH dysfunction was associated with increased pain scores on the FIQ (p = 0.024), a greater number of tender points (p = 0.014), higher myalgic scores (p = 0.001) and higher pre-exercise levels of inflammatory cytokines IL-1α (p = 0.021), IL-6 (p = 0.012), and IL-8 (p = 0.004). These results suggest that a defective growth hormone response to exercise may be associated with increased levels of blood cytokines and pain severity in FM patients.

Keywords: Fibromyalgia, cytokines, hypothalamic-pituitary-growth hormone axis, hypothalamic-pituitary-adrenal axis, immune response system, cortisol.

Fibromyalgia (FM) is currently viewed as a common pain syndrome related to dysfunction of central nociceptive sensory processing [1, 2]. A similar neurosensory dysfunction probably accounts for the common association of FM with many other painful syndromes [3]. However, several of the other symptoms experienced by FM patients, such as mood disorders, fatigue, specific sleep disturbances, stiffness and post-exertional pain cannot be fully explained by the abnormalities of central pain processing. This has led researchers to consider dysfunction of the stress axis, both the autonomic and humeral components, as possibly being relevant to some FM symptoms [4, 5]. While, overall, there are currently no robust biomarkers for FM except for experimental pain testing [6], there is emerging evidence that some patients with FM [7] or widespread pain [8] have a reduced resiliency of the hypothalamic-pituitary-hormonal axes (HPHA) with regard to both the secretion of cortisol and growth hormone (GH) [9]. Previous work from our

group has reported hypothalamic-pituitary-growth-hormone (HPGH) axis with low plasma IGF-1 levels – a measure of 24-hour GH secretion – in about one third of persons with FM [10]. Further experiments have shown that the reduced GH secretion in FM patients is a result of increased hypothalamic somatostatin tone, which inhibits GH secretion [11]. In addition, depression is associated with increased secretion of corticotropin-releasing hormone (CRH), which in turn stimulates the secretion of somatostatin and cortisol. The HPHA and the immune response system are comprised of multiple homeostatic systems with negative and positive feedback mechanisms for CRH, cortisol, somatostatin, GH, and cytokine release. The balance can be affected uniquely and synergistically by each of the stress, growth and reproductive hormonal axes and cytokines. A visual schematic of the acute and chronic stress response and the relationships between these key hormones is depicted in Fig. (**1**).

It has been noted that some FM symptoms are reminiscent of "sickness behavior", a syndrome of pain, fatigue, reduced activity, impaired cognition and depressed mood caused by activation of the immune response system

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Fig. (1). Effects of chronic stress on biochemical markers of the HPA and HPGH axes and immune response system. Acute and chronic stressors stimulate the release of corticotropin-releasing hormone (CRH) from the hypothalamus which in turn stimulates the pituitary to release adrenocorticotropic hormone (ACTH). ACTH travels thru the circulatory system to the adrenal glands and stimulates the release of cortisol. Stressors also stimulate the release of IL-1β in the brain which initiates the release of TNF-α. IL-1β directly activates the HPA axis, culminating in the release of cortisol, which stimulates the production of IL-6. Cortisol also affects the growth and reproductive axes (solid lines indicate activation while dashed lines indicate inhibition).

and characterized by increased production of inflammatory cytokines and chemokines [12, 13]. Sickness behavior is usually described as the integrated set of behavioral changes that develop as a result of infection [14]. However, it is now apparent that activation of the immune response system is not limited to infection, but can also occur in chronic stress, obesity, depression, exhaustive exercise and in overtraining syndrome [15-19]. Moreover, there is good evidence to implicate the interaction of inflammatory cytokines with glial cells as a trigger for the development of sickness behavior [20].

Elevated levels of pro-inflammatory cytokines have been reported in diverse circumstances including infections, obesity (especially visceral obesity) [21], excessive exercise (*e.g.* marathoners) [22, 23], PTSD [24], type-2 diabetes [25], and depression [26]. Although fibromyalgia is generally considered to be a non-inflammatory disorder, recent research has demonstrated the role of spinal neuroimmune responses as contributing to persistent pain [14]. Cytokines and growth factors have been strongly implicated in the generation of pathological pain states throughout the nervous system; in particular, the pro-inflammatory cytokines interlukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) are up regulated both locally and in the spinal cord in persistent pain [27-30]. In addition, serum levels of IL-8 have been reported to be increased in five FM studies [31-35]; although two studies failed to confirm these reports [36, 37].

Despite the importance of IL-1β, IL-8 and TNF-α in the development and maintenance of inflammatory pain and in central sensitization, the role that these cytokines play in FM symptomatology has been largely unexplored. Cognizant of the observation that some FM patients have an impaired GH response to the stress of exhaustive exercise [38, 39] and recognizing the reciprocal relationship between the immune response system and HPHA activity [40-42], we hypothesized that serum levels of inflammatory cytokines and chemokines would be elevated in FM patients with a defective GH response to exhaustive exercise. Additionally, since activation of the immune response system triggers sickness behavior, we proposed that FM symptom severity would be higher in FM patients with HPHA dysfunction.

MATERIALS AND METHODOLOGY

Protocol

Approval of the protocol, as described elsewhere [38], was obtained from the university's Investigational Review Board and all participants gave written informed consent before any procedures were performed. Participants were selected from a parent study of 165 FM participants who previously underwent the stress of exhaustive exercise using the modified Balke Treadmill protocol. Inclusion and exclusion criteria as previously described [38] included participants who were 18-60 years of age and diagnosed with FM as per the 1990 American College of Rheumatology (ACR) criteria [43].

Modified Balke Treadmill Exercise Protocol [44]

This protocol was used to induce an acute state of stress. Participants who had fasted overnight were exercised on a

treadmill with a fixed gradient to the point of volitional exhaustion (VO$_2$ Max), which was defined when either of 2 goals was reached: 1) respiratory rate index greater than 1.1 for at least 30 seconds, indicative of anaerobic metabolism, or 2) exhaustion (Borg) scale value of 10 (maximum perceived effort). Electrocardiography and blood pressure monitoring were performed every 5 minutes. All testing was performed between 10:00 AM and 2:00 PM. Since the magnitude of the GH response to exercise is related to the intensity and the duration of exercise, the amount of work performed by each participant during the test was calculated using the following equation: workload = (distance/time) x treadmill grade x weight.

Blood Collection & Storage

Peripheral blood was collected through an indwelling intravenous catheter that was inserted at least 60 min prior to the pre-exercise blood draw. Samples were drawn immediately before exercise (pre-exercise), immediately after exercise (post-exercise), and 1 hour after the end of exercise (1-hr. post-exercise). The blood was allowed to clot and the resulting serum was stored at -70C prior to running batched analyses.

Insulin-like Glucose Factor-1, Growth Hormone & Cortisol

Twenty-four hour GH function was measured by proxy *via* insulin-like glucose factor-1 (IGF-1), a long-term marker of pulsatile GH secretion. Pre-exercise serum samples were analyzed by immunoradiometric assay (IRMA, Diagnostic Systems Laboratories, Webster, TX). Sensitivity of the assay is 0.80 ng/dL. The mean intra-assay coefficients of variation (CV) were 2.6% and mean inter-assay CV was 4.5%. Data on IGF-1 was categorized as 0 = low for age and 1 = expected for age. Growth hormone was measured by a non-competitive chemiluminescence assay (Diagnostic Products, Los Angeles, CA) from all three time points. Sensitivity of this assay is 0.01 ng/mL; the mean intra-assay CV is 6.0% and the mean inter-assay CV is 5.8%. Growth hormone response was calculated using the following equation: $GH^{Post-exercise}$-$GH^{Pre-exercise}$. Normal GH response to exercise to VO$_2$ Max is $\geq$ 5ng/mL. Cortisol was measured in duplicate by immunoassay (R&D Systems) according to the manufacturer's protocol. Sensitivity of this assay is 0.01 pg/mL. The inter-assay CV was below 10.5% and the intra-assay CV was below 13.4%. The normal resting level range of cortisol is 300-2500 pg/mL.

Inflammatory Markers

Pre-exercise serum levels of IL-1α, IL-1β, IL1RA, IL-6, IL-8, IL-10, and TNF-α were measured using a bead-based immunoflourescence assay (Luminex Inc., Austin, TX). Multiple cytokine analysis kits (LINCOplex kits) were obtained from Linco Research Inc. (St. Charles, MO). Millipore multiscreen 96 well filter plates (Bedford, MA) were used for all multiplex cytokine kits. Assays were run in duplicate according to the manufacturers' protocol. Data were collected and analyzed using the Luminex-100 system Version IS (Luminex, Austin, TX). A four-parameter regression formula was used to calculate the sample concentrations from the standard curves.

Instruments and Other Testing

Participants completed investigator-conducted evaluations and self-report instruments chosen specifically for their psychometric properties and appropriateness for use in an FM population.

Demographic Data Form

The Demographic Data Form, an investigator-designed questionnaire, was used to obtain demographic information including age, gender, disability status, marital status, estrogen status, and the year FM was diagnosed by a health care professional.

Revised Beck Depression Inventory (BDI-R)

The BDI is a well-known, 21-item scale that measures mood and behaviors characteristic of depression [45]. The BDI has been adapted for use with FM patients by removing from the total score 3 items that are characteristic of all FM patients (fatigue, sleep difficulties, and effort required to get things done), and which, therefore, do not correlate well with major depressive disorder. This revision, the BDI-R, was selected because it has better accuracy, sensitivity, and specificity in an FM population than the original [46].

Fibromyalgia Impact Questionnaire (FIQ)

Fibromyalgia symptoms were measured with the FIQ; a 10-item instrument that uses a four-point scale to measure physical functioning and a 0–100 mm Visual Analog Scale (VAS) to measure symptoms of pain (0 = no pain and 10 = very severe pain), fatigue (0 = no tiredness and 10 = extreme tiredness), sleep (0 = awakening un-refreshed and 10 = awakening well-rested), stiffness (0 = no stiffness and 10 = very stiff), anxiety (0 = no anxiety and 10 = very anxious), and depression (0 = no depression and 10 = very depressed), along with levels of disability and overall well-being during the previous week. Total FIQ scores range from 0–100, with higher values indicating a more negative impact of FM [47].

Pain Assessments

A combination of three pain measures was used: the FIQ pain VAS subscale, the number of tender points, and the Cumulative Myalgic Score (CMS). The 100 mm VAS for pain rated the participant's perception of pain intensity over the previous 7 days, with anchors at 0 (no pain) and 10 (very severe pain). The CMS is an 18-item scale that diagnoses FM as per the 1990 American College of Rheumatology (ACR) criteria plus rates the amount of pain associated with 4-kilograms of pressure applied to 18 tender points commonly found in FM [43]. Pain severity was measured by having the participants rate their pain level on a 0-3 scale (0 = no pain, 1 = some pain, 2 = ouch, 3 = moves away) when 4 kg of pressure was applied at each site. Higher scores indicate more pain, with a total possible score range of 0 to 54. The number of non-zero scores out of 18 determined the total tender point count. A single examiner performed all tender point evaluations.

Flannigan's Quality of Life Scale (QOLS)

The QOLS, used to measure broad dimensions of overall quality of life, is a 16-item Likert-type scale that assesses

multiple areas of well-being and life satisfaction [48]. The concept of QOL is measured on a continuum where 1 = terrible and 7 = delighted. The possible range of scores is 16 to 112, with higher scores indicating better well-being and quality of life. It has been validated in an FM sample with an internal consistency reliability alpha equaling .82 to .88 and test-retest reliability of .84 [49, 50].

Body Mass Index (BMI)

Body weight and height were measured in kilograms and meters using a calibrated standing model scale (Detecto). Body mass index (BMI) was calculated using a standard formula of weight in kilograms divided by height in meters squared. Body composition (lean body mass and fat body mass) and percentage of body fat were derived from 7-point skin-fold caliperimetry (chest, axilla, triceps, sub scapula, abdomen, suprailiac, thigh) with a 2 prong spring-loaded Harpenden caliper per standardized anthropomorphic guidelines [51].

Statistical Analysis

All data analyses were conducted using the Statistical Programs for Social Sciences (SPSS), version 15.0. All analyses of significance ($p \leq 0.05$) used a 2-tail distribution. Group comparisons were preformed for nominal data using the chi-square test or Fisher's exact test for small expected numbers. Student's t-tests were used to compare growth hormone response to exhaustive exercise, FM symptom severity, and biochemical markers between groups. When the assumption of equal variances was violated, a Welch's test was used. Analysis of covariance (ANCOVA) was conducted to measure the effect of workload [workload = (distance/time) x treadmill grade x weight], BMI, and percentage of body fat on GH response to exhaustive exercise between groups with the independent variable having 2 levels (GH responders versus non-responders) and the dependent variable being GH response ($GH^{Post-exercise}$-$GH^{Pre-exercise}$). Similarly, ANCOVA was used to control for BMI and percentage of body fat in the between group analyses of immune response system markers. Pearson's correlation coefficients were computed among biochemical markers and FM symptoms specific to our a priori hypotheses that were found to differ significantly between groups. While correlational analyses were intended to be exploratory, we computed adjusted Bonferroni p values (.05/14 = 0.004) to allow the reader to further interpret the statistical rigor of the correlations.

RESULTS

Demographic Characteristics

The sample consisted of 24 non-Hispanic Caucasian females between the ages of 28 and 60. Interestingly, only 12 of the 165 participants from the parent study mounted a normal GH response to exhaustive exercise ($\geq$ 5ng/dL increase during exercise), and thus restricted the sample size to 12 participants per group. For the comparison group, 12 female participants who did not mount a GH response to exhaustive exercise (0 ng/dL increase during exercise) were randomly selected from the remaining 153 participants. No significant differences on demographic features were found between the 141 participants from the parent group and the 24 participants selected for the secondary analysis group (p =

.214 to 1.0). Sera from only seven of the 12 participants with HPHA dysfunction were in sufficient quantity and quality to perform immune response system biomarker analysis. The five participants without sufficient sample to analyze did not significantly differ on demographic characteristics from the seven responders whose sera were analyzed (p = 0.140 to 1.0). Menopause was more prominent in those with HPHA dysfunction. While groups did not statistically differ on receiving disability wages, only 33.3% of participants with HPHA dysfunction were able to work outside of the home compared to 85.7% of those with normal HPHA function (p = 0.029). No significant differences existed between the two groups of participants with and without HPHA dysfunction on gender, age, ethnicity, race, marital status, education or estrogen status (Table **1**).

Clinical Features

HPHA dysfunction was associated with being diagnosed with FM approximately three times longer than those participants with normal HPHA function. Specifically, whereas GH responders had been diagnosed with FM for an average of 3.2 years, the mean time since diagnosis in non-responders was 9.4 years (Table **2**). HPHA dysfunction was also associated with increased pain on the FIQ VAS, increased number of tender-points and higher cumulative myalgic scores, a higher BMI and an increased percentage of body fat (p = 0.047). However, no significant differences were found between the two groups in levels of physical impairment, depression, stiffness, fatigue, sleep quality, overall quality of life or total impact of FM on daily activities. Groups did not differ on serum IGF-1 levels or IGF-1 status (normal versus abnormal for age).

Response to Exhaustive Exercise Testing

Although all participants were classified as sedentary (engaging in planned exercise for less than 30 minutes a week during the three months prior to the treadmill test), GH non-responders were significantly less physically fit than GH-responders based on VO_2 Max (Table **3**). As expected, the workload achieved during the treadmill test was significantly lower in GH non-responders versus responders (p = 0.001). An ANCOVA was preformed to rule out the potential effect of the amount of work performed during the test and percent of body fat content on GH response to exercise. A significant difference in GH response was maintained between the two groups even after controlling for workload (p < 0.001), percentage of body fat (p = 0.001), and both simultaneously (p = 0.006).

Biochemical Markers

Serum IGF-1 levels did not differ between groups (Table **4**). Although only marginally significant, pre-exercise cortisol levels tended to be lower in GH non-responders than responders. Non-responders had significantly higher pre-exercise levels of inflammatory cytokines IL-1α, IL-6, and the chemokine IL-8, but not IL-1β, IL-1RA, IL-10, or TNF-α. Since adipose tissue mass can influence the levels of systemic immune response system biomarkers, between group levels were compared while controlling for percentage of body fat. The use of percentage of body fat as a covariate did not influence the observed group differences in IL1-α (p = 0.034), IL-6 (p = 0.021) nor IL-8 (p = 0.006).

Table 1.   Demographic Characteristics of Sample

Variable	Group		
	Responders (n =7)	**Non-Responders (n = 12)**	**Pearson's χ^2 or Fisher's Exact Test (2-Tailed)**
Gender (Female)	7	12	NS
Ethnicity			NS
▪ White	7	12	
Age Mean ± SD	46.4 ± 3	51.5 ± 2.5	.376
Marital Status			.466
▪ Married or living together	6	8	
▪ Divorced or single	1	4	
Education			.10
▪ Some college/ trade school	3	4	
▪ College degree or higher	4	8	
Employed outside the home			.029[*]
▪ Full time	5	3	
▪ Part Time	1	0	
▪ Not employed	1	9	
Receiving Disability Payments			1.0
▪ Yes	0	1	
▪ No	7	11	
Estrogen Status			.147
▪ Positive (Natural Production or ERT)	6	5	
▪ Negative (No menses ≥1 yr. or no ERT)	1	7	
Menopause Status			.021[*]
▪ Pre-menopausal	4	3	
▪ Peri-menopausal	2	0	
▪ Post-menopausal	1	9	

Responders = GH response to exercise of ≥ 5 ng/mL. Non-responders = GH response to exercise of 0 ng/mL. SD = Standard Deviation. χ^2 =Pearson's Chi Square. ERT = Estrogen Replacement Therapy. * p ≤ 0.05.

Correlations

Correlations were preformed on demographic characteristics, clinical features and biochemical markers that were found to differ significantly between groups. As no differences existed between groups on fatigue, sleep, depression, stiffness, IL-1β, IL-1RA, IL-10 and TNF-α, correlational analyses were not performed on these variables. Correlational analyses presented in Table **5** show that 43 correlations were statistically significant at a 0.05 alpha level and were greater than or equal to r = 0.464. When the Bonferroni-method for correction of multiple comparisons was applied (0.05/14 = 0.004), 20 correlations remained significant. As this study was exploratory in nature, the authors leave the interpretation of importance of the 23 correlations that did not remain significant at the adjusted alpha value to the reader.

The number of years diagnosed with FM was positively correlated with BMI (p = 0.041) and number of tender points (p = 0.039), but was not significant at an adjusted alpha of 0.004.

Body mass index correlated positively with percent of body fat (p < 0.001) and pain as measured by the FIQ VAS (p < 0.001). It inversely correlated with physical endurance as measured by the number of seconds on the treadmill (p = 0.045), physical fitness as measured by VO_2 Max (p = 0.005), serum GH levels immediately following exhaustive exercise (p = 0.004), GH levels 1-hour post-exercise (p = 0.003) and GH response (p = 0.006). Only percentage of body fat, pain, and GH levels at 1-hour post-exercise remained significant at the adjusted alpha level of 0.004.

Percentage of body fat was inversely related to physical endurance (p = 0.038), physical fitness (p = 0.002), GH post-exercise (p = 0.023), GH 1-hour post-exercise (p = 0.008), and GH response (p = 0.027). Only physical fitness and GH levels at 1-hour post-exercise remained significant at the adjusted alpha level of .004.

Table 2.   Clinical Features of Sample

Variable	Group		Independent t-Test (2-Tailed)
	Responders (n = 7) ± SD	Non-Responders (n = 12) ± SD	
Age (Years)	46.4 ± 8.0	51.5 ± 8.7	0.785
Years diagnosed with FM	3.2 ± 3.0	9.4 ± 5.7	0.008[**]
BDI-R Score	9.1 ± 6.3	8.3 ± 7.9	0.816
FIQ Depression VAS Score	3.43 ± 3.0	3.42 ± 3.0	0.993
FIQ Pain VAS Score	4.1 ± 2.1	6.5 ± 1.9	0.024[*]
FIQ Fatigue VAS Score	6.6 ± 2.7	7.4 ± 1.6	0.395
FIQ Sleep VAS Score	6.7 ± 3.9	8.1 ± 1.5	0.398
FIQ Stiffness VAS Score	5.9 ± 2.7	7.7 ± 2.1	0.123
FIQ Physical Impairment Subscale	2.9 ± 1.9	4.1 ± 2.8	0.340
Total FIQ Score	47.4 ± 19.9	59.0 ± 17.8	0.206
Quality Of Life Scale Score	68.7 ± 11.5	74.3 ± 15.5	0.425
Number of Tender Points	15.9 ± 1.8	17.6 ± 1.0	0.014[*]
Cumulative Myalgic Score	28.4 ± 6.8	40.5 ± 5.8	0.001[***]
Body Mass Index	27.3 ± 6.1	33.3 ± 4.5	0.025[*]
Percent Fat Mass	24.5 ± 9.7	34.4 ± 9.4	0.047[*]
Percent Lean Muscle Mass	51.7 ± 5.8	57.3 ± 5.5	0.054
IGF-1 Status (normal *vs* abnormal for age)	3 *vs* 4	6 *vs* 6	1.0

Responders = GH response to exercise of ≥ 5 ng/mL. Non-responders = GH response to exercise of 0 ng/mL. SD = Standard Deviation. BDI-R = Beck Depression Inventory-Revised. FIQ = Fibromyalgia Impact Questionnaire. VAS = Visual Analogue Scale. IGF-1 = Insulin-like Growth Factor-1. * $p \leq 0.05$. ** $p \leq 0.01$. *** $p \leq 0.001$.

Table 3.   Growth Hormone Response to Exhaustive Exercise

Variable	Group		Independent t-Test (2-Tailed)
	Responders (n = 7) ± SD	Non-Responders (n = 12) ± SD	
Growth Hormone (ng/mL)			
▪ Pre-exercise	0.6 ± 0.5	1.9 ± 3.6	0.251
▪ Post-exercise	12.2 ± 7.6	0.7 ± 1.5	0.007[*]
▪ 1-hr. Post-exercise	1.0 ± 0.8	0.2 ± 0.2	0.048[*]
▪ Response	11.6 ± 7.3	0	0.006[*]
Physical Fitness-VO$_2$ Max (Units)	24.6 ± 3.9	18.2 ± 3.0	0.001[***]
Physical Endurance (Seconds on TM)	988.3 ± 200.4	446.3 ± 226.5	< 0.001[***]
Workload (kJ)	74.2 ± 13.7	41.2 ± 23.6	0.001[***]

Responders = GH response to exercise of ≥ 5 ng/mL. Non-responders = GH response to exercise of 0 ng/mL. SD = Standard Deviation. VO$_2$ Max = Volitional Exhaustion. TM = Treadmill. Response = GH[Post exercise]-GH[Pre-exercise]. * $p \leq 0.05$. *** $p \leq 0.001$.

Pain, as measured by the FIQ VAS, correlated strongly with BMI ($p < 0.001$), but not percentage of body fat ($p = 0.271$). The more pain one had, the less physical endurance they had ($p = 0.002$) and the less physically fit they were ($p = 0.010$). Pain as measured by the number of tender points strongly correlated with CMS scores ($p > 0.001$) and moderately with number of years diagnosed with FM ($p = 0.039$). The number of tender points inversely correlated with physical endurance ($p = 0.034$), physical fitness ($p = 0.008$), GH levels immediately post-exercise ($p = 0.011$), and GH response ($p = 0.019$).

Pain as measured by CMS scores positively correlated with number of years diagnosed with FM ($p = 0.032$) and number of tender points ($p < 0.001$). CMS scores inversely correlated with physical endurance ($p = 0.008$), physical fitness ($p = 0.003$), GH levels immediately post-exercise ($p = 0.001$) and GH response ($p = 0.002$).

Physical endurance inversely correlated with all three pain measures: FIQ VAS scores ($p = 0.002$); CMS scores ($p = 0.008$); and number of tender points ($p = 0.034$). Physical endurance was also inversely correlated with IL-1A ($p = 0.043$), IL-6 ($p = 0.045$), IL-8 ($p = 0.032$), serum GH levels immediately following exhaustive exercise ($p = 0.003$) and

Table 4.   Biochemical Marker Differences between Groups

Variable	Group		Independent t-Test (2-Tailed)
	Responders (n = 7) ± SD	Non-Responders (n = 12) ± SD	
IGF-1 (ng/dL)	159.1 ± 54.7	138.9 ± 48.2	0.412
Cortisol (pg/mL)	0.513 ± 0.181	0.346 ± 0.198	0.085
IL-1α (pg/mL)	234.3 ± 170.2	597.7 ± 435.5	0.021[**]
IL-1β	4.5 ± 9.3	5.5 ± 13.2	0.870
IL-1RA	145.9 ± 158.5	510.7 ± 635.7	0.158
IL-6	96.2 ± 83.8	257.7 ± 164.9	0.012[*]
IL-8	17.0 ± 14.5	51.7 ± 30.3	0.004[**]
IL-10	1.0 ± 2.7	12.9 ± 30.4	0.323
TNF-α	5.8 ± 7.5	9.2 ± 13.3	0.540

Responders = GH response to exercise of ≥ 5 ng/mL. Non-responders = GH response to exercise of 0 ng/mL. SD = Standard Deviation. * p ≤ 0.05. ** p ≤ 0.01.

at 1-hour post-exercise (p = 0.001), and GH response (p = 0.005).

Physical fitness positively correlated with physical endurance (p < 0.001), GH levels immediately post-exercise (p < 0.001) and at 1-hour post-exercise (p < 0.001) and GH response (p < 0.001). It inversely correlated with BMI (p = 0.005), percentage of body fat (p= 0.002) and all three pain measures: FIQ VAS scores (p = 0.010); CMS scores (p = 0.003); and number of tender points (p = 0.008).

Serum levels of GH immediately following exercise positively correlated physical endurance (p = 0.003), physical fitness (p = 0.001), GH levels one hour post-exercise (p = 0.007) and GH response (p < 0.001). It inversely correlated with BMI (p = 0.004) and percentage of body fat (p = 0.023), number of tender points (p = 0.011) and CMS scores (p = 0.001).

Serum levels of GH 1-hour post-exercise positively correlated with physical endurance (p = 0.001), physical fitness (p < 0.001), GH levels immediately post-exercise (p = 0.007) and GH response (p = 0.006).

Growth hormone response positively correlated with physical endurance (p = 0.005), physical fitness (p < 0.001), GH levels immediately post-exercise (p < 0.001) and GH 1-hour post-exercise (p = 0.006). It inversely correlated with BMI (p = 0.006), percentage of body fat (p = 0.027), number of tender points (p = 0.019) and CMS scores (p = 0.002).

All of the inflammatory biomarkers directly correlated with each other: IL-1α with IL-6 (p = 0.003) and IL-8 (p = 0.002). Physical endurance negatively correlated with IL-1α (p = 0.043), IL-6 (p = 0.045), and IL-8 (p = 0.032). IL-8 negatively correlated with GH response (p = 0.027). In effect, there were no other significant correlations between

Table 5.   Correlations of Statistically Significant Demographic Characteristics, Clinical Features and Biochemical Markers

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Years Diagnosed with FM	-													
2. BMI	.486*	-												
3. % Body Fat	.488*	*.898******	-											
4. Pain - FIQ VAS	.225	*.952******	.266	-										
5. Pain - Number of Tender points	.489*	.346	.391	.232	-									
6. Pain - CMS	.506*	.429	.361	.389	*.858******	-								
7. Physical Endurance	-.268	-.468*	-.479*	*-.662***	-.488*	-.592**	-							
8. VO₂ Max	-.305	-.621**	*-.668***	-.577**	-.589**	*-.637***	*.813******	-						
9. GH Post-exercise	-.316	-.631**	-.519*	-.412	-.570*	*.697***	*.647***	*.747******	-					
10. GH 1-hr. Post-exercise	-.240	*-.646***	*-.591***	-.444	-.442	-.431	*.714***	*.766******	.599**	-				
11. GH Response	-.310	-.608**	-.507*	-.376	-.534*	-.665**	.620**	*.756******	*.986******	.605**	-			
12. IL-1α	-.024	-.119	-.084	.431	.101	.177	-.469*	-.394	-.348	-.332	-.406	-		
13. IL-6	-.169	-.027	-.009	.403	.094	.186	-.464*	-.349	-.338	-.164	-.392	*.639***	-	
14. IL-8	.028	-.014	.022	.367	.196	.330	-.492*	-.442	-.454	-.184	-.506*	*.669***	*.897******	-

FIQ = Fibromyalgia Impact Questionnaire. TP = Tender points. CMS = Cumulative Myalgic Score. TM = Treadmill. * p ≤ 0.05. ** p ≤ 0.01. *** p ≤ 0.001.
Bolded correlations remained significant at an adjusted p ≤ 0.004.

inflammatory biomarkers and demographic characteristics or other clinical features of FM in this sample.

DISCUSSION

The primary aim of our study was to determine if serum cytokine levels and FM symptom severity would be higher in FM patients with defective GH response to exhaustive exercise compared to those with normal HPHA function. Our data support the idea that, within the context of FM, a defective GH response to the stress of acute exercise is associated with elevated resting levels of some inflammatory cytokines and levels of pain, irrespective of BMI, adiposity, workload, or physical fitness level.

A secondary aim of this study was to explore the relationship between elevated immune response system biomarkers and symptom severity in persons diagnosed with FM with and without concurrent HPHA dysfunction [52, 53]. Five studies in FM have suggested that inflammatory cytokines and chemokines may be associated with FM symptomology [31-35]. Wallace found elevated serum levels of IL-8 in FM and noted that IL-8 increased with the duration of FM symptoms [34]. Subsequent studies reported similar increases in IL-8 in the sera of FM patients and a direct relationship between IL-8 levels and pain severity [54-56]. Analogous to this previous research, we found elevated serum levels of IL-8 in addition to elevated IL-1α and IL-6. While increased levels of TNF-α, and IL-10 have been observed in FM [31, 32, 34, 35, 37], this observation has not been consistent throughout all studies [36, 37] and was not supported by our data.

Based on prior observations, researchers have postulated there may be a temporal relationship between increased pro-inflammatory cytokines and FM symptoms [34]. Similarly, our data suggested that cytokine dysregulation is associated with increased pain severity as well as GH dysfunction. Specifically, participants with HPHA dysfunction who reported higher levels of pain had been diagnosed with FM approximately 6 years longer than those with normal GH response to exhaustive exercise. This observation supports the notion that neuroendocrine dysfunction in FM is not a static state, but rather is progressive in nature; a finding reminiscent of Selye's research on adrenal response to chronic stress [57]. Consistent with this idea is our finding that GH non-responders tended to have lower serum cortisol levels than responders.

Correlational analyses revealed significant associations between many variables of interest. Most significantly, participants with poor GH response had higher pain levels, lower physical endurance and poorer fitness levels. In addition, increased pain severity was associated with increased weight (BMI) and greater physical dysfunction (physical endurance and fitness levels). Contrary to the a priori assumptions, our data did not show any correlation between inflammatory markers and pain. It has been suggested in genetic studies that the use of the Bonferroni method of corrections for multiple comparisons may be too stringent of a correction for alpha levels in biochemical correlations, thus significant associations may be missed [58]. This issue needs to be more thoroughly evaluated in FM research and possibly incorporated in future studies. Despite the lack of a statistically significant correlation between pain and inflammatory markers, the previously discussed t-tests provide some preliminary evidence that may link pain and immune system response with HPHA dysfunction in FM.

This exploratory analysis study has a number or limitations that should be considered when interpreting the findings. First, 90% of the sample from the parent study did not mount a normal GH response to exhaustive exercise thus limited the sample size to 12 participants with normal GH function and a comparison group of 12 participants with abnormal GH response. In addition, after exclusion of the five samples that were not of sufficient quality or quantity to be analyzed, the statistical power to detect correlations between groups on biochemical markers and FM symptoms was significantly decreased thus increasing the risk of not detecting meaningful differences between groups. Likewise, the small sample size may have also limited our ability to discern significant associations among biomarkers and FM symptoms. Therefore, it is unclear if these findings would generalize to all persons with FM who exhibit a normal GH response to exercise. Furthermore, our sample was comprised of a homogenous group of women, thus results may not be representative of men and minorities. Although it is not known if the prevalence of abnormal GH response to exercise in FM patients managed in primary care differs from those seen in a tertiary care setting, it is possible and thus needs to be evaluated in future research. While groups did not differ on estrogen status, we are unable to rule out the potential confounding effects of exact estradiol levels on GH response. As the categorization of menopausal status was based on self-report of time from last menses, date of surgically induced menopause, or presence of hormone replacement therapy versus estradiol levels, this measure did not address the other features of menopause such as fatigue, mood, and sleep separate from FM. Therefore it is not known if GH data from this study are related to estradiol levels, a measure that will be critical in follow up studies. Another limitation in this trial is that it did not include healthy controls which could have provided comparison levels for the cytokine profiles.

Our data support the idea that within the context of FM a defective GH response to the stress of acute exercise is associated with elevated resting levels of some inflammatory cytokines and pain level. One potential confounder in our results is the fact that the GH responders were more physically fit than non-responders, as evidenced by the differences in VO$_2$ Max and total work performed during the treadmill test. Thus it is possible that difference in fitness level may have influenced resting cytokine levels, independently of GH status, as was reported in one previous study [53]. Mitigating this explanation for our results is the observation that the resting levels of IL-1α, IL-6 and IL-8 remained significantly elevated in GH non-responders even after controlling for workload or VO$_2$ Max. It is evident that future studies should specifically examine the relationship between inflammatory markers in FM and healthy controls matching for fitness level and the GH response to exercise. Furthermore, un-stimulated cortisol levels may have been insensitive to distinguishing HPA differences between the 2 groups. Future studies may be designed to include the use of the combined dexamethasone suppression/corticotropin-releasing hormone stimulation test, as it is significantly more

sensitive and specific than the unchallenged measurement of cortisol.

CONCLUSIONS

The results reported herein suggest that a defective growth hormone response to exercise may be associated with increased levels of blood cytokines and pain severity in FM. Although this study produced some interesting preliminary findings, cautious interpretation is warranted until these findings can be replicated in a larger, more heterogeneous sample.

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