

Pilot Study of Inflammatory Responses Following a Negative Imaginal Focus in Persons With Chronic Pain: Analysis by Sex/Gender

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ABSTRACT

Background: Recent evidence suggests that differential stress and immune responses may play a role in the sex/gender disparity for pain. Pain pathology and psychological stress are both associated with elevated levels of proinflammatory cytokines.

Objective: This pilot study tested a negative imaginal focus to assess whether it would elicit a proinflammatory cytokine response and whether responses would vary by sex/gender.

Methods: Adults with chronic musculoskeletal pain were recruited from an outpatient, multidisciplinary pain clinic in Portland, Oregon, between 2007 and 2008. All participants underwent a psychologist-guided 10-minute focus on the negative aspects of their pain condition and the imagined worsening of their pain; no control group was used. Serum collected at baseline and postfocus (1, 2, and 2.5 hours) was assayed for interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α). Cortisol was assayed at each time point and at 15 minutes postfocus.

Results: Thirty-six outpatients (aged 26–62 years; 23 women, 13 men) participated in the study. Compared with men, women displayed greater negative emotional expression during the experiment, and this in turn mediated their IL-6 inflammatory responses. Relative to men, the IL-6 response trajectory was delayed for women. The IL-6 and TNF- α findings suggest women's maximal cytokine responses were not captured by the final time point.

Conclusions: This pilot study provides preliminary evidence that women with chronic pain may experience increased and delayed inflammatory responses following negative emotional expression induced by thinking negatively about their pain condition. The findings have implications for pain catastrophizing research. This early-phase research suggests that the timing and duration of the cytokine response are critical factors to consider in future pain research. (*Gen Med.* 2010;7:247–260) © 2010 Excerpta Medica Inc.

Key words: pain, catastrophizing, inflammation, IL-6, gender differences, sex differences.

INTRODUCTION

Among the factors known to strongly influence pain experience and outcomes are pain catastrophizing and female sex/gender. The relatedness of these factors is unclear. Pain catastrophizing has been associated with increases in the proinflammatory cytokine interleukin-6 (IL-6),¹ thereby suggesting a possible mechanism to link pain catastrophizing to pain pathophysiology. Sex/gender differences in cytokine response to pain catastrophizing have not been fully described; however, a female predilection for increased inflammatory responses would support a heuristic biochemical model of catastrophizing in women with pain.

Pain catastrophizing is a cascade of negative cognition and distressing emotion related to pain.² It comprises 3 factors: rumination, magnification of pain, and helplessness regarding pain. It predicts increased pain intensity^{3,4} and a greater utilization of medical services over time,⁵ and is associated with increased disability.⁴ Importantly, pain catastrophizing is also associated with poor response to opioids,⁵ and it is implicated in the development of chronic pain.^{6,7}

Pain catastrophizing is typically measured as a trait variable. For instance, the pain catastrophizing scale instructs people to consider how they generally think and feel when they are in pain, while they are completing the questionnaire.² In experimental studies, it is also important to assess state catastrophizing, that is, the extent to which one has engaged in catastrophizing in vivo, during the course of the experiment.⁸

A literature search was conducted using the PubMed database and the terms *catastrophizing*, *cytokine*, and *IL-6* for all years available. The literature search revealed that Edwards et al¹ conducted the only study to have examined pain catastrophizing and cytokine response. In a sample of 42 healthy adults, IL-6 was measured at baseline and 1 hour after acute pain stimulus. The significant IL-6 increases that were found in people reporting greater catastrophizing during the pain stimulus ($P < 0.01$) serve as preliminary evidence that pain pathways may be affected by catastrophizing.

Indeed, proinflammatory cytokines are implicated in pain processes, and IL-6 and tumor necro-

sis factor- α (TNF- α) are the cytokines most studied in pain research. Circulating levels of IL-6^{9,10} and TNF- α ^{11,12} predict pain levels, are associated with hyperalgesia,^{13,14} and are linked to the development and progression of chronic pain.^{13,15,16} Both of these cytokines oppose opioid analgesia.¹⁷ IL-6 is of particular interest because acute stress elicits increases in IL-6.¹⁸

Pain catastrophizing and cytokine response have not been tested in a clinical sample, nor has the relationship been tested without a pain stimulus. The study by Edwards et al¹ found no significant gender difference in IL-6 response at 60 minutes. However, cytokine responses may not optimize until 90 minutes poststressor¹⁸; therefore, sex/gender differences may emerge later.

The present study was designed to test elements central to pain catastrophizing in a clinical sample, by using a negative imaginal focus that mimicked in vivo pain catastrophizing. During the 10-minute induction, participants focused on the negative aspects of their pain condition and imagined their pain worsening; no pain stimulus was used. We hypothesized that (1) the negative focus would elicit an IL-6 response, and we aimed to characterize the response; (2) there would be a sex/gender difference in IL-6 response, and that increases in IL-6 would be mediated by participant response to the negative focus; and (3) there would be a TNF- α response, and we aimed to characterize its sex/gender effects. We expected no cortisol increases, as they are found following novel stressors¹⁹; the negative focus in the present study involved a familiar exposure to one's own negative thoughts.

SUBJECTS AND METHODS

This was a prospectively designed, pre-post analysis study conducted at a university research facility.

Subjects

Participants were recruited from flyers placed in an outpatient, multidisciplinary, university pain clinic at Oregon Health & Science University, Portland, Oregon, between 2007 and 2008. Fifty-five persons responded to the study advertisement. Thirteen candidates were excluded, owing

to either an inflammatory pain diagnosis (eg, systemic lupus erythematosus, rheumatoid arthritis) or a fibromyalgia diagnosis. All eligible persons were evaluated by a pain physician at the pain clinic, and standard evaluation practice included assessment of multiple pain complaints and assessment for fibromyalgia. Thus, we were assured that our exclusionary criterion of fibromyalgia diagnosis was satisfied. Other exclusionary criteria included active or recent infection or virus, blood/injection/needle phobia, active suicidality, psychosis, current substance abuse, limited venous access, active corticosteroid regimen, and pregnancy. Medical diagnoses were confirmed by chart review. All persons who met enrollment criteria met with the principal investigator to verify eligibility, and written informed consent was obtained. The study was approved as ethical by the Oregon Health & Science University Institutional Human Subjects Review Board.

Forty-two persons were enrolled; 2 did not arrive at the experiment site on their scheduled day and chose to drop out of the study. The study participation rate was 95%. Forty persons completed the study; however, 4 individuals were excluded from the final analysis because of nursing errors in serum protocol ($n = 1$), a missed exclusionary diagnosis ($n = 1$), and unreliable, extreme outlying assay values ($n = 2$).

Study Day Measures

The following measures were included to evaluate whether level of depressive symptoms, trait pain catastrophizing tendencies, and current pain intensity level would influence the experimental findings.

Center for Epidemiological Studies Depression Scale

The 20-item Center for Epidemiological Studies Depression Scale (CES-D)²⁰ is widely used to determine level of depressive symptoms for research purposes. The response to each question is quantified on a 4-point scale (0–3), and scores are summed for a total score (0–60). A cut score of 27 is reliable and valid for determining clinically significant depressive symptoms in persons with chronic pain.²¹

Pain Catastrophizing Scale

The 13-item Pain Catastrophizing Scale (PCS)² is reliable, valid, and widely used to determine pain catastrophizing tendencies in both research and clinical settings.²² Responses to questions are quantified on a 5-point scale (0–4), and are summed for a total score of 0 to 52. In addition, the PCS also has 3 subscales (rumination, magnification, and helplessness). This measure was given once to participants, at baseline. The total score was used to determine trait pain catastrophizing tendencies in the daily lives of participants.

Current Pain Intensity

Participants were asked, “How much pain are you feeling right now?” Pain intensity was reported verbally using the 11-point numerical rating scale (0 = no pain, 10 = worst pain imaginable), which is reliable and valid for measuring pain intensity.^{23,24} Pain intensity was also measured at each prospective time point.

Negative Imaginal Focus

Once seated in a private room, participants were encouraged to close their eyes to deemphasize the social aspect of having the investigator present and to facilitate an imaginal focus. The 10-minute induction was guided by the same investigator. For the duration of the focus, participants were asked to think about their pain (rumination), to imagine their pain worsening (magnification) in the near future, and to imagine having little or no control over the pain and resulting circumstances (helplessness). Participants were asked to describe their emotional experience associated with this focus on worsening pain, and to discuss areas of their lives that would be most affected by worsening pain. Participants were also asked to describe their vision of “worst-case scenario” as it pertained to worsening, uncontrolled pain.

Immediately following the induction, participants rated the intensity of distress they experienced during the induction (0 = no distress, 10 = extreme distress). The intensity of negative emotional expression was rated by the investigator performing the induction (0 = no negative emotional expression, 10 = extreme negative emo-

tional expression); **Table I** provides information and examples of the rating procedure. Negative emotional expression ratings were generated from observed negative affective expression and negative emotional content of participant descriptions; evident emotional distress was rated highest. Sadness expressed in vocal tone and in thematic content was rated higher. Descriptions that involved statements such as “I would feel completely hopeless” or “I would want to kill myself if my pain increased and I was unable to care for myself” were rated higher. Participants were rated lower on the negative emotional expression scale if negative affective expression was absent or mild; generally in these cases, participant descriptions are limited to adjectives such as “frustrating” or “difficult.” The induction was timed and stopped after the 10-minute mark.

Participants remained in the private room and rested alone for 2.5 hours while physiological data were gathered at the designated time points along with current pain ratings. Participants were not instructed to focus their thoughts in any particular manner. They were offered neutral reading material (eg, a nature or home decorating maga-

zine) to occupy their time during the rest period. To minimize external emotional influence, outside reading material and cell phone calls were not allowed during the study. Participants were offered pillows, blankets, water, and uncaffeinated tea by the nursing staff throughout the study.

Physiological Measurements

Blood pressure, heart rate, and pain levels (0–10) were measured at baseline, immediately postinduction, and at 15 minutes, 1 hour, 2 hours, and 2.5 hours postinduction. Ten milliliters of whole blood were collected at each designated time point. Serum was assayed for IL-6, TNF- α , and cortisol for the following time points: baseline, 1 hour, 2 hours, and 2.5 hours postinduction (cortisol was also assayed at the 15-minute time point). Unused serum was stored in case reanalysis was necessary.

Procedure

Study times were standardized to 11:00 AM to control for circadian effects for IL-6, TNF- α , and cortisol. Cortisol typically peaks between 6:00 AM and 8:00 AM and gradually declines over the course of the day.²⁵ Similarly, TNF- α and IL-6 have been

Table I. Examples of negative emotional expression rating.

Sex/ Gender	Affect	Descriptions of Imagined Life With Increased Pain	Negative Emotional Expression Rating*
Female	Tearful, sad, depressed	I would have nothing to give. It's really scary. Very scary. I would want to kill myself if it got worse.	10
Female	Sobbed, sad, depressed	I can't imagine living that way; it feels terrifying. I wouldn't want to live.	10
Female	Expressively sad, depressed	I'd be in a black hole. I wouldn't want to live. I would withdraw; it would be miserable.	8
Female	Expressively sad	It's very scary. I'd feel hopeless. I don't know what I would do.	8
Female	Sad	I would withdraw and feel depressed. It would be frustrating. I would think of acceptance.	7
Male	Stoic	It would be depressing. I can imagine being alone and destitute. It feels poor.	6
Male	Stoic	It's difficult to imagine. It would be lonely. It would be hard. I would be angry.	6
Male	Stoic	It would be frustrating. I would get moody, irritable. It makes me want to prevent it.	5

*0 = no negative emotional expression, 10 = extreme negative emotional expression.

found to have peaks at 7:00 AM and 9:00 AM, respectively, and nadirs at noon.²⁶ Therefore, under normal circumstances, we would expect IL-6, TNF- α , and cortisol to be decreasing during our chosen experiment time. Participants ate breakfast and took their prescribed and over-the-counter medications as usual. Prestudy caffeine consumption was allowed, but participants were asked to minimize their consumption of caffeinated beverages the morning of their study. Following arrival at the study site, no caffeine or solid food was consumed until the study was completed. Height and weight were measured to calculate body mass index (BMI; kg/m²). A topical anesthetic was offered to minimize acute pain before placing an indwelling peripheral intravenous catheter (22-gauge or 20-gauge) in the arm or hand. Participants rested for 25 minutes prior to the baseline blood draw. Ten milliliters of whole blood were drawn into a 10-mL Vacutainer (Becton, Dickinson and Company, Franklin Lakes, New Jersey) at baseline (immediately prior to the negative imaginal focus). Serum was frozen at -80°C until the time of assay.

The final blood draw at 2.5 hours after the negative imaginal focus marked the end of the study. The investigator debriefed subjects and assessed their affective state and subjective level of distress. Only one person was in a continued negative state at the end of the experiment; this participant received 45 minutes of counseling and reported feeling less distress. Participants were called the next day to assess for any adverse events, including a potential increase in negative mood status.

Serum Analysis

Cytokine levels were analyzed in duplicate by Assaygate, Inc. (Ijamsville, Maryland) with BioPlex microparticles (Bio-Rad Laboratories, Inc., Hercules, California). Cortisol was analyzed using the Immulite system (Siemens Healthcare Diagnostics Inc., Deerfield, Illinois), an automated chemiluminescent assay.

Statistical Analysis

We computed means (SDs) and ranges for baseline variables (age, BMI, pain intensity, opioid use, CES-D,

and PCS); sex/gender differences were compared with 2-sided *t* tests. Means (SDs) for all outcome variables were computed for each time point. A 2-sided *t* test was used to assess the change in cortisol (baseline to 15 minutes postinduction). The *P* values reported are all 2-sided, and 0.05 was used as a criterion for statistical significance. All analyses were performed with Stata 9.1 (StataCorp LP, College Station, Texas).

Analysis of Cytokine and Cortisol Responses

The analysis of the time trajectories of the basic outcomes (cytokine and cortisol levels) was fashioned to account for between-person differences. If individuals have different rates of response and achieve their maximal responses at different times, then conventional analysis of point-in-time results is unlikely to uncover the actual pattern. Therefore, we estimated for each person (and each outcome measure) the value of the maximal response (whether positive or negative from baseline), the time of the maximal response, and the terminal slope of the response curve at the end of the experiment (2.5 hours). This was accomplished by fitting a quadratic in time—of the form $y = \beta_1 t + \beta_2 t^2 + e$, where *y* represents the change from baseline—to each outcome trajectory for each participant separately. From the fitted equations, the maximal responses, maximal response times, and slopes at 2.5 hours can be computed directly. These values were then used in a simultaneous regression (the seemingly unrelated regression procedure of Stata) on the explanatory factors, gender, and negative emotional expression. Furthermore, we included negative emotional expression in the simultaneous regression as an outcome, with sex/gender as an explanatory factor. This permitted us to estimate the direct effects of gender on negative emotional expression, the direct effects of sex/gender on the 3 trajectory-based outcomes, and the indirect effects of sex/gender on trajectory-based outcomes with negative emotional expression as a mediating variable. In some analyses we included further explanatory factors (age, CES-D, PCS, BMI, and baseline pain intensity).

RESULTS

The final sample comprised 36 individuals (23 women, 13 men), aged 26 to 62 years, who were under-

going treatment for chronic musculoskeletal pain (**Table II**). The sample was 94.4% white ($n = 34$) and 5.6% Native American ($n = 2$), with a mean age of 47.5 years. The primary pain types were lumbosacral (17; 47.2%), cervical neck (9; 25.0%), shoulder (4; 11.1%), lower extremity and foot (3; 8.3%), and myofascial (3; 8.3%). Thirty participants (83.3%) had pain at ≥ 2 sites (eg, lumbosacral and cervical neck). Chart review and clinical interview indicated that 28 participants (16 women, 12 men; 77.8%) were taking prescribed opioid medications, and 27 participants (75.0%) were regularly taking anti-inflammatory medications. Nine participants (7 women, 2 men; 25.0%) took anti-inflammatory medications on the morning of their experiment day. Ten persons (6 women, 4 men; 27.8%) were smokers. Regarding menstrual status, 6 women were premenopausal, 8 women were postmenopausal, and 9 women had an undetermined status; however, the age range for these undesignated women was 49 to 57 years, strongly suggesting that their menstrual status was postmenopausal.

Baseline Characteristics

Men were significantly more likely than women to be taking opioid medication ($P = 0.01$). **Table II** presents other baseline characteristics by sex/gender. Results showed no significant sex/gender differences for any of the measurements reported here (age,

BMI, pain intensity, CES-D, and PCS). Given the lack of differences for the aforementioned variables, here we report the following means for the full sample. The mean level of depressive symptoms on the CES-D scale was 24.1, with 39% of participants meeting or exceeding the clinical cut score of 27. The mean score for the PCS was 25.4. For all modeling reported in the following paragraphs, the additional explanatory factors (age, BMI, baseline pain intensity, CES-D, and PCS) were not found to be significantly related to cytokine responses.

In terms of the negative imaginal focus, although each person's description was different, predictable themes of heightened distress, feelings of helplessness, and depressed mood emerged. As people imagined their pain worsening in the near future, participants predominantly described an expected decline in function. In terms of emotional responses, fear of losing primary relationships or employment was described by some participants. Eight participants (all women) cried during the induction; ratings for these participants were 9 to 10 on the negative emotional expression rating scale. Three of these women sobbed for several of the 10 minutes, and they were rated as 10 on the negative emotional expression rating scale. At next-day telephone follow-up, none of the participants reported any adverse effects related to their participation in the study.

Table II. Baseline characteristics by sex/gender in adults with chronic musculoskeletal pain.

Characteristic	Women (n = 23)		Men (n = 13)		P
	Mean (SD)	Range	Mean (SD)	Range	
Age, y	47.0 (7.7)	25.9–62.4	47.8 (8.0)	36.1–58.7	0.79
BMI, kg/m ²	33.1 (7.7)	17.2–46.5	31.0 (5.2)	23.9–40.7	0.41
Current pain intensity*	4.3 (1.9)	1.0–8.0	4.0 (1.6)	2.0–7.5	0.56
Depressive symptoms [†]	21.6 (11.4)	6.0–46.0	28.7 (11.1)	17.0–50.0	0.09
Pain catastrophizing [‡]	23.7 (11.6)	6.0–46.0	27.0 (10.2)	11.0–39.0	0.40

BMI = body mass index.

*Scored on a 0 to 10 scale, 0 = no pain and 10 = worst pain imaginable.

[†]Scored using the 20-item Center for Epidemiological Studies Depression Scale.²¹ Responses to each question are quantified on a 4-point scale (0–3), and scores are summed for a total score of 0 to 60.

[‡]Scored using the 13-item Pain Catastrophizing Scale.² Responses to each question are quantified on a 5-point scale (0–4; summed score ranges from 0–52).

The mean (SD) for negative emotional expression observed during the experiment was 8.42 (1.40) for women and 6.37 (1.14) for men. **Table III** reports the means of the cytokines and cortisol by time point and sex/gender. As mentioned previously and explained further in the discussion, we did not pursue analyses of the results by time point. Instead, we computed the outcomes shown in **Figure 1** based on time trajectories.

Although the change in cortisol from baseline to 15 minutes postinduction was significant ($P = 0.025$), the direction of the change was negative, per normal diurnal sloping. We found no positive cortisol response to the induction, nor did we find any significant sex/gender effect on cortisol change. Furthermore, we found no significant relationship between cortisol and cytokine responses.

Primary Outcomes (Cytokine Responses)

Interleukin-6

Table IV provides the regressions for IL-6 (β refers to the regression coefficient). In a simultaneous regression on negative emotional expression alone, we found a positive relationship with the maximal response that was not statistically significant, a significant positive relationship with terminal slope ($\beta = 0.53$; $P = 0.015$), and no significant effect on the time of maximal response. With respect to gender differences, women had significantly higher levels of negative emotional expression than did

men ($P < 0.001$). Sex/gender had a marginal positive effect on the time of maximal response.

Tumor Necrosis Factor- α

Regression results presented in **Table IV** show that women had a marginally significant higher maximal response when adjusting for negative emotional expression. Negative emotional expression tended to produce larger terminal slopes (and therefore potential maximal responses outside the 2.5-hour experiment), but this was evidently insufficient to give an overall sex/gender effect (**Figure 1**). The indication is, therefore, that any elevated TNF- α response is related to sex/gender in some way that does not involve negative emotional expression.

Figures 2 and **3** depict the relationship between the slope of the cytokines and negative emotional expression by sex/gender. These figures were included to illustrate that the correlations between the fitted curves and the raw data were very good, and thus strengthen the interpretation of the terminal slopes for both cytokines.

The influence of smoking status and menopausal status was examined in models that controlled for sex/gender; neither variable was found to significantly affect the fundamental outcomes of cytokine responsiveness.

DISCUSSION

In summary, preliminary evidence from this pilot study suggested an increased terminal slope for

Table III. Means (SDs) for outcome variables by time point and sex/gender.

Variable	Time (h)				
	0	0.25	1	2	2.5
IL-6, pg/mL					
Female	4.3 (7.8)	–	4.6 (8.5)	5.2 (9.1)	6.0 (10.1)
Male	8.8 (18.3)	–	10.3 (19.4)	10.4 (19.2)	10.3 (18.7)
TNF- α , pg/mL					
Female	3.1 (2.2)	–	3.4 (2.7)	3.0 (1.8)	3.1 (2.1)
Male	3.1 (1.7)	–	3.1 (1.6)	2.6 (1.0)	2.6 (1.1)
Cortisol, ng/mL					
Female	91.9 (73.4)	73.1 (64.5)	58.3 (41.8)	55.7 (31.7)	74.9 (58.2)
Male	70.1 (17.5)	58.2 (17.7)	51.6 (20.4)	68.1 (36.5)	56.2 (27.2)

IL-6 = interleukin-6; TNF- α = tumor necrosis factor- α .

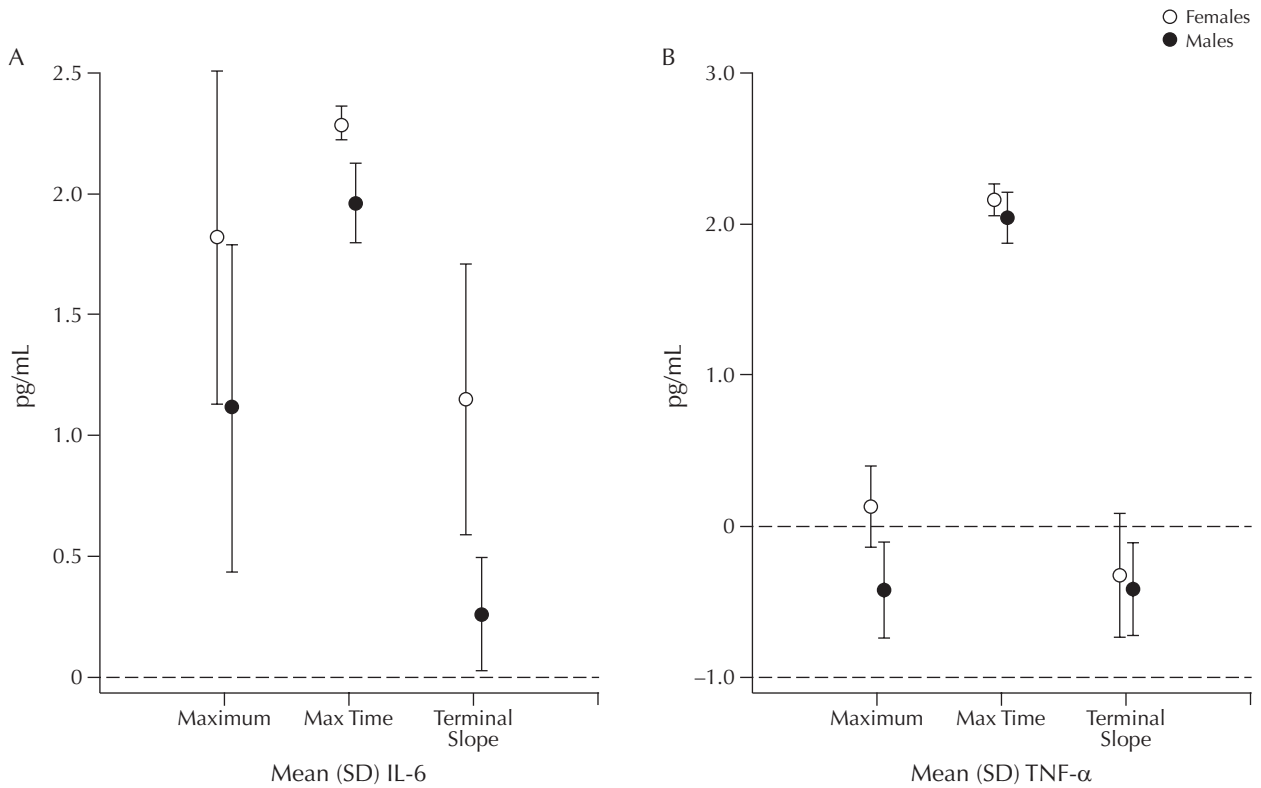


Figure 1. Maximal response, time of maximal response, and terminal slope by sex/gender for (A) interleukin-6 (IL-6) and (B) tumor necrosis factor- α (TNF- α).

IL-6 for women in response to the negative focus on their pain condition and the imagined worsening of their pain.

As shown in **Table IV**, women had a delayed cytokine response that was found to be mediated only by negative emotional expression during the

focus. Indeed, for women, the slope of IL-6 was increasing at the end of the experiment, and we highlight here that, on average, the maximal response for women was not captured by our final 2.5-hour time point. These findings provide evidence that negative emotional expression delays

Table IV. Adjusted regressions for interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α). Effects are regression coefficients (β); *P* values are 2-sided.

Outcome	Maximal Response		Time of Maximal Response		Slope at End of Experiment		Negative Emotional Expression	
	Effect	<i>P</i>	Effect	<i>P</i>	Effect	<i>P</i>	Effect	<i>P</i>
IL-6 Regression β								
Negative emotional expression	0.65	0.069	-0.00	0.950	0.53	0.015		
Female	-0.56	0.652	0.33	0.077	-0.21	0.815	1.96	<0.001
TNF-α Regression β								
Negative emotional expression	-0.21	0.162	0.00	0.957	0.40	0.048		
Female	0.98	0.052	0.12	0.596	-0.74	0.279	2.06*	<0.001

*Female effect on negative emotional expression differs due to 2 cases of missing data in the IL-6 regression.

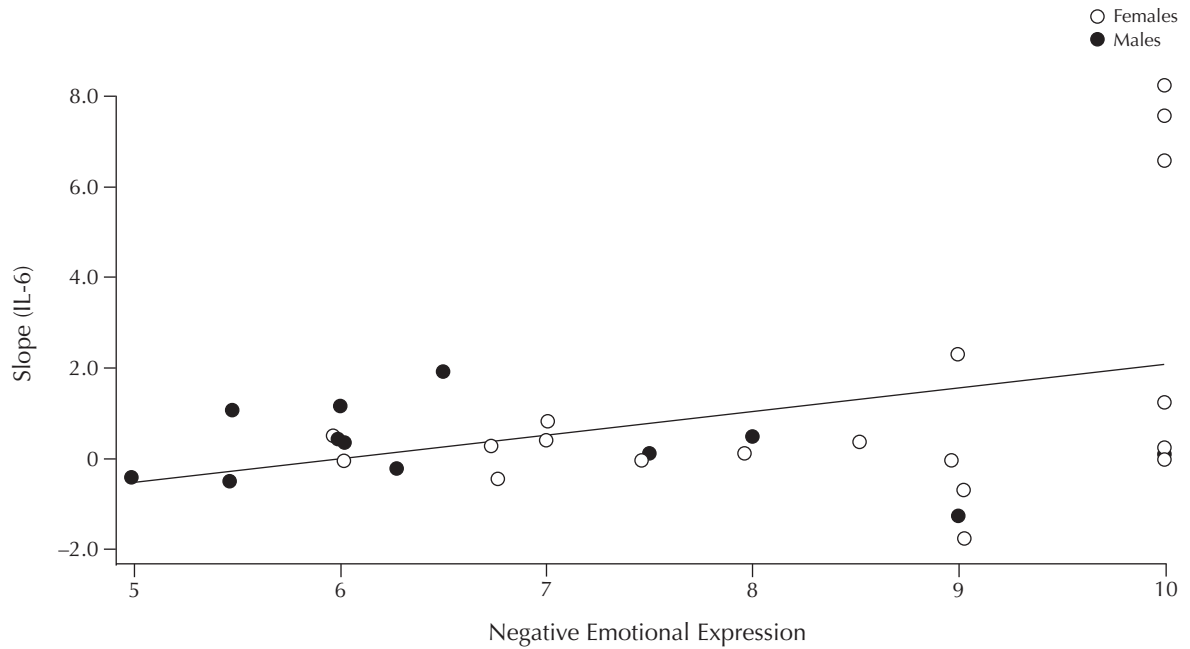


Figure 2. Relationship between the interleukin-6 (IL-6) slope and negative emotional expression by sex/gender.

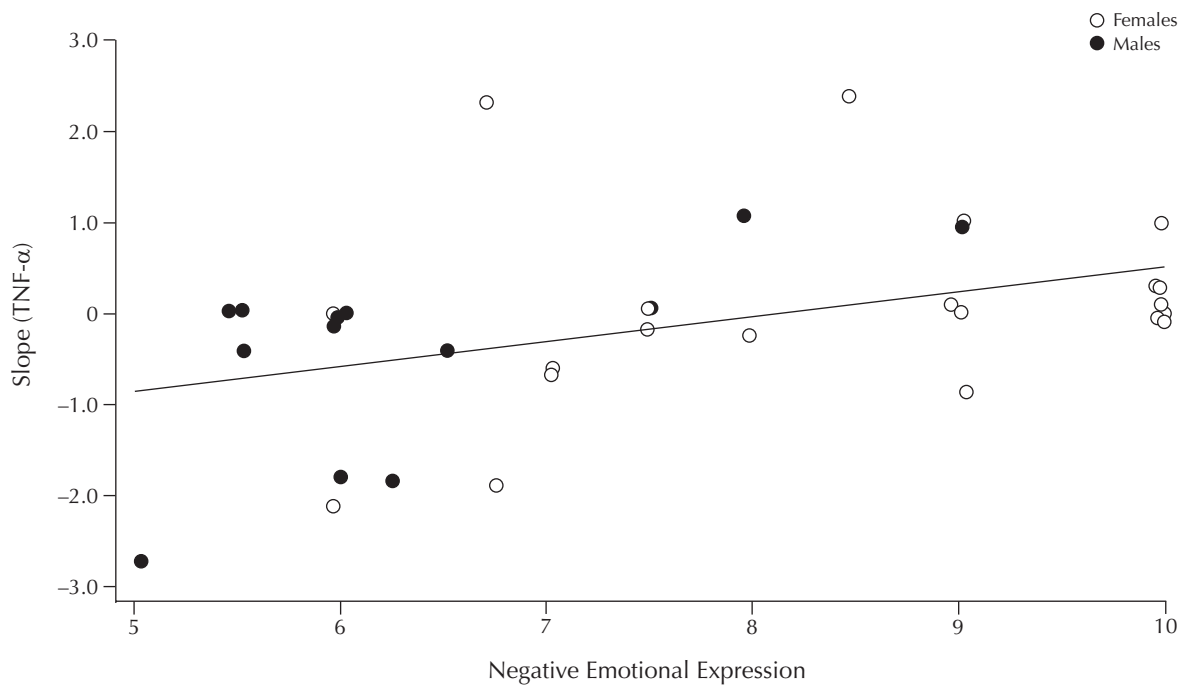


Figure 3. Relationship between tumor necrosis factor- α (TNF- α) slope and negative emotional expression by sex/gender.

maximal IL-6 response (because it increases the chance of values continuing to rise at 2.5 hours) and may increase the maximal response. Women's higher IL-6 terminal slopes were similarly mediated by their higher levels of negative emotional expression. To the extent that there is any delay in time of maximal response, however, there is no evidence that negative emotional expression mediates IL-6 responses.

Although IL-6 increases for women did not reach statistical significance, the response trend suggests that a longer experiment would have found a stronger relationship with the maximal IL-6 response. Future studies may wish to adopt latency measurements of greater duration than the 2.5 hours used here to capture the full cytokine response. Furthermore, the findings suggest a delayed TNF- α response for women, and again, that our final measurement was premature and that perhaps the maximal TNF- α response was not captured. A significant increase for TNF- α was not found, but the data suggest an effect may be emerging for women. A follow-up study with longer latencies is needed to clarify the scope and significance of the response.

One goal of this pilot study was to characterize the cytokine response with respect to sex/gender. We introduced a novel method of analysis for cytokine responses because initial individual-level graphs showed different levels of response and different timings of response for all of our outcome measurements. A conventional analysis, conducted separately at each collection time point, would compare some participants' maximal responses with other participants' intermediate or even minimal changes from baseline. Such an analysis would confound 2 separate aspects of a time trajectory; the maximum reached (maximal response) and the time of the maximal response. Fortunately, we found it possible to fit quadratics to the individual level curves, with high correlations (average of about 0.90 for each outcome measurement). This permitted direct computation of the maximal response and time of maximal response for each individual, for each outcome measure. We also included the slope of the quadratic at the end of the observation period (2.5 hours) because, again, the graphs indicated that a substantial number of

participants appeared to continue to show increasing values, indicating that they had perhaps not yet achieved their maximal responses. We included this feature in the analysis, in part to help guide the decision for a longer observation period in subsequent studies.

Our results suggest that negative emotional expression, sex/gender, and cytokine response appear to be linked, and cytokine response characteristics were most pronounced for women. Importantly, women's maximal response and the slope of the response for IL-6 and TNF- α were mediated by only one factor: negative emotional expression. Women are known to have higher emotional expression than do men.^{27,28} While emotional expression may be beneficial if it promotes problem-focused coping and other mechanisms of adaptive coping,²⁷ negative emotional expression that is linked to rumination and helplessness appears to predict poor adjustment.²⁸ We have presented preliminary evidence regarding the physiological consequences of negative emotional expression for women while acknowledging that further study is needed to confirm sex/gender effects.

Mean values for current pain intensity,²⁹ depressive symptoms (CES-D),³⁰ and trait catastrophizing (PCS)³¹ in the present study were similar to those reported by other studies of outpatient chronic pain samples. Gender differences regarding the level of depressive symptoms did not reach statistical significance, but we note that men's scores for the CES-D were surprisingly higher than women's for this sample. Neither the tendency to catastrophize in one's daily life (as measured by the PCS) nor level of depressive symptoms predicted experimental cytokine response. Consistent with findings from earlier work,¹ these data suggest that proinflammatory increases appear to be related to state variables (ie, negative emotional expression during the experiment) rather than trait variables (level of depression, daily catastrophizing).

We have provided preliminary data that suggest women with chronic pain evidence heightened emotional and inflammatory responses to a negative imaginal focus designed to mimic in vivo pain catastrophizing. Other research examining sex/gender dimorphism in pain responsivity supports

the notion of a female diathesis to negative stimuli.^{32,33} In a study examining responses to a pain stimulus in healthy men and women, it was found that men exposed to the pain stimulus experienced a beneficial hypoalgesic effect.³³ To the contrary, women exposed to the pain stimulus experienced heightened pain. The authors concluded that an important female-specific response to acutely painful stimuli may exist. Women with greater reactivity to pain may be less able to activate endogenous pain inhibitory systems, putting them at greater risk of progressing from an acute to a chronic pain state. Therefore, the individual differences in stress reactivity combined with a sex/gender diathesis could play a role in the development of chronic pain conditions.

The present study extends prior work in that a cognitive imaginal stressor was used, rather than a physical stressor. Moreover, the negative imaginal focus was personally relevant; each participant tailored the imagined content of the experiment to reflect personal life circumstances and concerns. The findings suggest that an *in vivo* negative focus may be effective at eliciting a measurable form of emotional distress experienced in varying degrees in women who live with chronic pain. Thus, these findings may provide a model for how real-life cognitive and emotional stress affect persons with pain.

As hypothesized, we did not find an increase in cortisol 15 minutes after the induction. Cortisol decreased during this time frame per normal diurnal patterns. The lack of an association between cortisol and the induction suggests that participants' hypothalamic-pituitary-adrenal axes were habituated to the content of the imaginal focus; in other words, the induction was not a novel stressor for the persons in this sample. The present findings suggest that perhaps the emotional and biochemical cascade triggered by one's own negative thoughts may differ from biochemical cascades triggered following external and physical stress tasks (eg, experimental pain stimulus, public speaking). However, given that this is the first report involving a negative imaginal focus and cytokine responses in a chronic pain sample, further research is needed before this conclusion may be drawn.

We emphasize here that the women in this sample were predominantly menopausal. Given that only 6 women were premenopausal, we were underpowered to explore differences by menstrual status, and this is one limitation of the present study. However, failure to control for menstrual phasing typically results in a blunting, versus an enhancement, of sex/gender differences. Our models that included menopausal status (premenopausal vs postmenopausal) did not yield significant findings for this variable with respect to cytokine responsivity; however, given the small group of premenopausal women, we do not have confidence to determine whether the gender effect found here was hormonally mediated. A study designed and powered to detect such differences could clarify a sex-steroid effect. The strong sex/gender effect found in the present study suggests the possibility that a subset of women with specific characteristics may be particularly susceptible to inflammation induced by negative emotional expression potentially related to catastrophizing or other factors.

The present findings should be interpreted with caution. The female gender of the investigator may have introduced a social bias that prevented males from experiencing and expressing negative emotions. Future studies may consider matching the gender of the investigator to the subject. The small sample size is another limitation, and further studies, balanced by sex, are needed to confirm the reported findings. We highlight here that we did not use an independent rater to determine interrater reliability. However, a second rater would be most advisable when the investigator is not blinded to the results of the experiment, or when the investigator is not blinded to group allocation. In the present study, the investigator was blinded to cytokine results, as the ratings occurred during the experiment and prior to cytokine assay. Additionally, there were no groups in the present study and all participants underwent the same experiment. While we recognize that the study design could be strengthened by having a second rater, such methodology plays a less critical role in the present study for the aforementioned reasons. The current sample was primarily

white, and the findings reported here may vary by ethnicity.

Opioids and anti-inflammatory medications were not controlled in this study, and therefore their influence on the cytokine response of our sample is unknown. However, given that 7 women and 2 men took anti-inflammatory medication on the morning of the study, it is unlikely that the anti-inflammatory medications positively influenced the sex/gender difference in cytokine response. Similarly, men were significantly more likely than women to be taking opioids. Opioids are known to positively influence cytokine levels.³⁴ However, men did not evidence higher levels of basal cytokines or cytokine responses; these findings minimize the likelihood that medications account for the sex/gender differences reported here.

Finally, this pilot study did not make use of a control group. A randomized, controlled experiment would clarify findings from this preliminary work by controlling for the stress effects of the research environment.

CONCLUSIONS

This pilot study extends the understanding of cytokine responses elicited by a negative imaginal focus. Results suggest sex/gender dimorphic responses for IL-6 and TNF- α . Sex/gender differences in IL-6 appear to emerge at 2 hours and grow stronger over time; however, strong significance for differences in maximal cytokine responses may not become evident until after 2.5 hours. Given the pilot nature of the study, the results are preliminary and further research is needed to confirm these findings. However, we highlight the timing and duration of cytokine response as important factors of consideration for future research examining cytokine responses in women. Specifically, future studies examining the inflammatory effects of cognition, emotion, or pain catastrophizing may increase the latency of the final measurement to determine the full duration of the inflammatory response.

Our current understanding of the progression of chronic pain and of inflammatory factors in chronic pain is limited. Future research may focus on whether, over time, repeated acute bursts of

inflammation contribute to higher basal levels of circulating inflammatory factors for women. Gaining a better understanding of the interplay between cognition, emotion, and biology may lead to treatments that attenuate risk factors and temper the progression of chronic pain.

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