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Individuals with both higher recent negative affect and physical pain have higher levels of C-reactive protein

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ABSTRACT

Conceptualizing physical pain and negative affect as potentially interactive, we hypothesized that higher levels of peripheral inflammatory markers would be observed consistently only among individuals with both higher negative affect and pain symptomatology. Participants were generally healthy midlife adults from the Bronx, NY ($N = 212$, $M_{age} = 46.77$; 60.8% Black, 25.5% Hispanic/Latina/o) recruited as part of a larger study. Key measures were: reported pain intensity and pain interference at baseline, recent negative affect averaged from self-reports 5x/day for 7 days, and peripheral inflammatory markers (C-reactive protein [CRP] and a composite cytokine measure based on seven cytokines). Controlling for age, BMI, gender, and education, recent negative affect significantly interacted with both pain variables to explain variance in CRP, with higher CRP levels observed only in individuals with both higher negative affect and either higher pain intensity or pain interference. These findings contribute to an emerging literature suggesting that negative affect, pain, and inflammation are related in important and complex ways.

Higher levels of peripheral inflammatory markers – including C-reactive protein (CRP) and inflammatory cytokines (e.g., interleukin [IL]-6) – predict development and progression of multiple diseases, as well as frailty and disability (Furman et al., 2019; Kiecolt-Glaser et al., 2003; Leonardi et al., 2018; Stoner et al., 2013). Although inflammation is strongly linked mechanistically with pain in multiple ways and across numerous conditions (Omoigui, 2007; Raoof et al., 2018; Taneja et al., 2017), findings linking measures of pain symptomatology with levels of peripheral inflammatory markers have primarily been 1) among individuals with specific health conditions, and 2) inconsistent across studies (DeVon et al., 2014; van den Berg et al., 2018). Particularly in general populations (i.e., those not characterized by chronic pain), pain is not always associated with inflammatory levels (DeVon et al., 2014; Eslami et al., 2017). Null associations between pain and inflammation are somewhat surprising, not only because of known mechanistic connections between pain and inflammation but also because physical pain is broadly conceptualized as stressful and is strongly linked with negative affect (Gatchel et al., 2007; Graham-Engeland et al., 2016; Lumley et al., 2011; Mathur et al., 2018), and because chronic psychological stress and negative affect have been associated with higher levels of peripheral inflammatory markers (Glaser and Kiecolt-Glaser, 2005; Graham-Engeland et al., 2018, 2019; Kiecolt-Glaser et al., 2002; Miller et al., 2011; Sturgeon et al., 2016). These findings, along with stress and health theory, lead us to hypothesize that pain and negative affect may have interactive effects in a general, midlife population. Such interactive effects may help explain the inconsistent associations reported between pain symptomatology and inflammation, and may also elucidate strategies to determine which individuals are at greatest risk of persistent

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inflammation. The goal of the present research was to examine whether negative affect and two important aspects of pain symptomatology – pain intensity and pain interference (the extent to which individuals perceive pain-related disability) – interact to explain variation in systemic inflammation, with the expectation that individuals reporting both higher pain symptomatology and negative affect would exhibit higher levels of peripheral inflammatory markers compared to all others.

Laboratory and surgical studies involving manipulation of pain (Beilin et al., 2003; Cruz-Almeida et al., 2012; Geiss et al., 1997) and inflammation (Andreasson et al., 2019; Wegner et al., 2014) suggest that proinflammatory cytokines and pain have interactive reciprocal effects, involving a number of well-characterized mechanisms (involving both peripheral mediators and central nervous system sensitization) (Baral et al., 2019; Taneja et al., 2017; Taves et al., 2013; Watkins et al., 1995). Although inflammation contributes to adaptive processes in the early stages of acute injury (Engeland and Marucha, 2009; Muire et al., 2020; Walters, 2019), long-lasting peripheral inflammation is linked with poorer health and chronic pain (Baral et al., 2019). Clearly some chronic pain conditions are more intimately related to inflammation, but there appears to be some involvement of inflammation in a number of specific pain states (Raoof et al., 2018; Watkins et al., 1995), with some researchers theorizing that the majority of pain states involve inflammation in some way (Omoigui, 2007). It is thus unsurprising that individuals with chronic pain conditions tend to have higher inflammation than those who do not (DeVon et al., 2014). However, the association between acute or recent pain symptomatology and peripheral inflammatory markers is not always apparent when examined cross-sectionally. In a review of studies that reported associations between pain and inflammation among individuals with an inflammatory-related chronic pain condition, only about half the reviewed studies reported a significant association between pain symptomatology and inflammatory markers (DeVon et al., 2014). In another review, severity of pain among patients with chronic low back pain was linked with IL-6 and CRP across studies, but not consistently with other markers, such as tumor necrosis factor (TNF)-α (van den Berg et al., 2018). One study found that higher levels of pain were associated with higher levels of CRP among older adults who were caregivers of a spouse with dementia, but not among controls matched on age and socioeconomic status (Graham et al., 2006), suggesting the possibility that stress strengthens the association between pain and inflammation. The present research builds from this finding to test the premise that negative affect [which is often an indication that an individual is reacting negatively to a stressor (for reviews, see Graham-Engeland et al., 2019; Lacourt et al., 2015)] may help account for discrepancies in the association between pain and inflammation.

The premise that negative affect may help account for discrepancies in the association between pain and peripheral inflammation is further supported by strong bi-directional connections between pain and negative affect. Not only does pain contribute to negative affect, but negative affect can heighten pain states through a variety of mechanisms – including pain catastrophizing and associated behaviors (e.g., hypervigilance to pain, avoidance) and physiological pathways including central nervous system sensitization (Graham-Engeland et al., 2016; Lumley et al., 2011; Sullivan et al., 2001). These bi-directional pathways are a cornerstone of the biopsychosocial model of pain, which acknowledges that pain becomes exacerbated and particularly problematic (i.e., more likely to transition from acute to chronic pain and to be linked with disability) in the presence of stress or affective disturbance (Edwards et al., 2016; Gatchel et al., 2007). Supporting a role for negative affect, substantial personal and social stress develops when pain results in activity limitations and interference in role function, (Dueñas et al., 2016); further, the construct of pain interference inherently involves a psychological component related to the negative evaluation of one's abilities in daily life (White et al., 2014). This highlights the importance of examining both pain intensity and pain interference as factors that may interact with negative affect, which is in keeping with recommendations that pain symptomatology is best captured by both pain intensity and its consequences – including perceptions of interference with life activities (Darnall and Sullivan, 2018; Farrar, 2019; Thomas et al., 2016).

Although several studies have examined the association between inflammation and pain controlling for negative affect (e.g., Karshikoff et al., 2015; Schrepf et al., 2015), or the association between negative affect and inflammation controlling for pain (e.g., Slavish et al., 2020), only a few studies have reported relevant findings that are truly suggestive of interactions between affect, pain, and inflammation. For example, a few studies with samples of individuals with chronic pain conditions have provided evidence that manipulation of negative mood seems to relate to greater peripheral inflammation (Graham-Engeland et al., 2019) or imaging biomarkers related to neuroinflammation (Albrecht et al., 2021); importantly, however, these studies have not directly observed interactions with pain specifically, such that interactions with all three variables were not clearly observed. Another study found lower pain tolerance among women who evidenced both greater negative affect and elevated IL-6 levels in response to a vaccine, relative to a control group (Lacourt et al., 2015). Lastly, in healthy male participants, induced systemic inflammation (in response to lipopolysaccharide injection) led to a decreased pain threshold, which appeared strengthened at times when negative mood was increased (Wegner et al., 2014). Given these limited findings and because both negative affect and pain may induce similar physiological changes that are related to inflammation (e.g., central nervous system sensitization) as reviewed above, we hypothesized that negative affect may strengthen associations between peripheral inflammation and pain intensity or pain interference. No study to our knowledge has examined this specific question, which is one that we believe is important to help clarify inconsistencies in the literature and to better determine risk factors for high inflammation in a general population.

In this study, we examined whether pain intensity and pain interference interacted with negative affect to explain variance in the levels of two peripheral inflammatory markers (CRP and a composite inflammatory cytokine measure). This was conducted in a socioeconomically and ethnically diverse sample of midlife adults who were systematically recruited to be representative of a community in the Bronx, NY. We neither selected for nor excluded participants on the basis of pain disorders, generating a sample with considerable variability in pain experience. As this sample is not characterized by an inflammatory pain condition, we did not expect significant main effects between these pain measures and our peripheral inflammatory markers. In previous work with these data (which did not explore associations with pain), there was a significant positive association between recent negative affect and inflammatory markers (Graham-Engeland et al., 2018). Thus, as the main effects of negative affect on inflammation have already been reported, we do not focus on these main effects here and instead focus on the potential for an interaction between negative affect and pain in predicting levels of inflammation.

The primary analyses in the present paper also focus on recent negative affect, as opposed to negative affect retrospectively recalled over a longer period of time. Both measures of negative affect are likely linked with pain symptomatology, based both on theory as well as empirical findings (Gaskin et al., 1992; Graham-Engeland et al., 2016). However, in our own work linking negative affect and inflammation, we found that negative affect derived from ecological momentary assessments [EMA] one week prior to a blood draw was linked with inflammatory markers, whereas negative affect recalled across one month in that same study was not (Graham-Engeland et al., 2018). Multiple other studies have also found that measures of recent psychosocial stress and emotions derived from EMA better predict physiological markers indicative of disease risk compared to retrospective measures (for review, see Conner and Barrett, 2012), perhaps in part because they reduce self-report bias and the difficulty of recalling emotion over a longer time

period (Moskowitz and Young, 2006; Shiffman et al., 2008; Smyth and Stone, 2003). Retrospective assessments may better capture broad, global self-perceptions, whereas measures of recent affect derived from aggregated momentary reports (of how individuals feel in the moment) may better reflect experienced emotion during the sampling period (Shiffman et al., 2008). Our main hypothesis for the present work was thus that individuals reporting both pain symptomatology (i.e., higher pain intensity and/or greater pain interference) and higher recent negative affect would evidence the highest levels of both CRP and the cytokine composite measure. Given mixed findings in the literature linking pain symptomatology and peripheral inflammatory markers among community samples that were not recruited on the basis of pain or inflammatory conditions (and in which neither pain nor inflammation were manipulated), we examined associations between pain symptomatology and inflammatory markers on an exploratory basis.

1. Method

1.1. Overview and participants

The data used for the present research were drawn from the first wave of The Effects of Stress on Cognitive Aging, Physiology, and Emotions (ESCAPE) study. This project used systematic probability sampling to recruit participants from a housing cooperative in the Bronx, New York (for more information, see Scott et al., 2015). All participants gave informed consent in accordance with IRB approval obtained for this study. Based on inclusion criteria, participants were between the ages of 25–65, ambulatory, fluent in English, and free of visual impairment. Of the 266 participants, 235 completed surveys that included pain assessments, momentary assessments of negative affect, as well as blood draws to measure inflammation. Of these, eight were missing values for at least one of the primary study variables (two missing one or both pain variables and six missing inflammatory markers), and eight more were missing information related to body mass index (BMI), which (along with gender and age) was considered an important covariate on an *a priori* basis (O'Connor et al., 2009). Five additional participants who were using immunosuppressants were excluded. Adequate compliance with the ecological momentary protocol for this analysis was defined as at least 80% of assessments completed (McCabe et al., 2011), which resulted in two additional participants being excluded. Demographic characteristics of the final analytical sample of 212 participants are described below. The final sample had higher recent negative affect than the excluded sample ($p = .04$) and was marginally more likely to have a college degree ($p = .06$). The final sample evidenced no significant differences from the larger sample ($N = 266$) in pain measures (*p*s=.91 and *p*=.89 for intensity and interference respectively), trait negative affect ($p = .24$), neuroticism ($p = .39$) inflammatory markers ($p = .51$ for CRP and ranging from 0.29 to 0.79 for individual cytokines), BMI ($p = .69$), age ($p = .27$), gender ($p = .84$), Black race ($p = .14$), or Hispanic/Latina/o ethnicity ($p = .64$).

1.2. Procedures

A baseline survey assessed demographic information and measures of pain intensity and pain interference, which are described in detail below. Approximately one week later, this was followed by an ecological momentary assessment (EMA) phase, during which participants were "beeped" five times a day at quasi-random intervals for 14 consecutive days for smart-phone based assessments that included negative affect (for more information, see Graham-Engeland et al., 2018). A fasting blood draw at the end of the EMA period (median = 3 days later, for details see Graham-Engeland et al., 2018) took place in the morning hours (between 7:00am and 11:00am). At the time of the blood draw, participants were screened for any signs of acute illness (e.g., temperature above 100.4 °F) and rescheduled if needed.

1.3. Measures

Inflammatory biomarkers. We used the same set of eight inflammatory biomarkers that has been used in prior work with the ESCAPE study (i.e., biomarkers that have been linked previously with either stress, affect, and/or pain): CRP (a broad marker of systemic inflammation), the proinflammatory cytokines IL-1β, IL-6, TNF-α, interferon-γ (IFN-γ), and IL-8; and the anti-inflammatory markers IL-4 and IL-10 (Scott et al., 2015; Stoner et al., 2013). The blood samples were collected in EDTA coated tubes for CRP analysis and sodium heparin coated tubes for cytokine analysis. EDTA samples were kept on ice prior to centrifugation (3000g × 15 min), and heparin samples were maintained at room temperature. All assays were performed in duplicate. Cytokines were measured from blood plasma using multiplex bead arrays on a Luminex platform (MagPix), using kits from Thermo Fisher Scientific, Waltham, MA); for these assays, the minimum detection limit ranged from 0.02 to 2.77 pg/mL, and the inter-assay CVs ranged from 7.0 to 9.8%. High sensitivity CRP was measured from blood plasma using ELISA (Cayman Chemical, Ann Arbor MI); the minimum detection limit was 46.9 pg/mL, the intra-assay coefficients of variation (CVs) ranged from 1.9 to 7.0%, and the inter-assay CV was 9.84%.

Inflammatory markers were transformed using a log (x + 1) formula prior to analyses to help correct for non-normal distribution, and the cytokines were winsorized (based on 3 SD) to minimize the influence of outliers on analyses without unnecessarily eliminating participants (Graham-Engeland et al., 2018; Knight et al., 2020). In previous work, a factor analysis determined that all cytokines grouped together as a reliable single factor, with CRP clearly forming a separate factor (for more details, see Graham-Engeland et al., 2018); a two-factor solution for the cytokines (such as with inflammatory and anti-inflammatory cytokines in separate factors) did not fit our data. Conceptually, it makes sense that peripheral inflammatory and anti-inflammatory cytokines often correlate positively with each other in cross-section, because anti-inflammatory cytokines rise to buffer (or limit) inflammation, a phenomenon which has been often observed in other work (Dorn et al., 2016; Pripp and Stanišić, 2014). To minimize Type 1 error due to multiple comparisons, we used this composite cytokine measure (which was computed based on z-scored cytokine values that were then averaged) in all primary analyses. However, because individual cytokines may be of particular interest to readers, the results of regression analyses predicting individual cytokines are presented as well.

Pain. Recent pain intensity and pain interference were assessed using the widely used and validated PROMIS Pain Intensity and Interference scales (Amtmann et al., 2010; Cella et al., 2010, 2019). For pain intensity, three items measured current pain intensity, average pain intensity over the past week and worst pain intensity over the last week, each on a scale of 3–15, where a score of three indicated no pain and higher scores indicated greater pain. For recent pain interference, four items measured how much pain interfered with daily and social activities, work, and household chores in the past week; items were scored on a scale of 4–20, where a score of four indicated no pain interference and higher scores indicated greater interference. Participants answered all items for both scales. The pain severity and pain interference scales demonstrated high internal consistency in the present sample, α′s = 0.89 and 0.96, respectively.

Recent negative affect. During the two-week EMA phase, participants were asked to indicate how much they currently felt *tense/anxious, depressed/blue, angry/hostile, unhappy,* and *frustrated*, using sliding scales from "not at all" to "extremely" (which equated to a scale from 0 to 100). Negative affect items were averaged to form a score for each report, with recent negative affect (person average) then computed across all EMAs in concordance with prior published work with this sample. Negative affect obtained from the seven days closest to the blood draw (i.e., week 2) was used in primary analyses, because prior work with these data established that week 2 negative affect was associated with the inflammatory cytokine composite (but not CRP), whereas negative affect from week 1 was

not (Graham-Engeland et al., 2018). On an exploratory basis, the primary analyses were also run using negative affect obtained from the full two weeks. Average recent negative affect score reliability, based on the intraclass correlation and corrected for the average number of EMA surveys completed per person,[1] revealed excellent reliability across weeks one and two combined (0.98) and for week two only (0.97).

Recalled negative affect. Participants also completed a retrospective adjective checklist at baseline that included items from the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) as well as other emotion items. This measure asked participants how often they recalled feeling each emotion listed over the past month on a scale from 1 (not at all) to 7 (extremely). Recalled negative affect was computed from 10 items (irritable, sad, tense, bored, stressed, depressed, nervous, sluggish, upset, and disappointed) and evidenced good internal consistency (Cronbach's alpha = .90).

Covariates. Age, gender, and BMI were used as covariates in all analyses because they are commonly associated with inflammation (O'Connor et al., 2009). Sociodemographic and behavioral factors such as Black race, Hispanic/Latina/o ethnicity, income, marital status, education, and tobacco use were considered as additional covariates based on whether they were associated with the cytokine composite or CRP. Of these, only having a college degree was associated with either inflammatory marker (it was associated with lower levels of both the cytokine composite and with CRP) and was thus included as a covariate. We did not control for health conditions in our primary set of analyses, as doing so seemed counter to our desire to investigate connections in a broad sample that is diverse with regard to health; however, as a sensitivity analysis we added the number of health conditions (summed from a list of 26 conditions, such as high blood pressure and kidney problems) to create a morbidity index and used it as a covariate in a separate model. As an additional sensitivity analysis we tested neuroticism as a separate covariate because neuroticism is often linked with greater report of both negative affect (momentary affect as well as retrospectively recalled affect) and pain (Affleck et al., 1992; Raselli and Broderick, 2007) and could explain not just a relationship between such factors but also help account for their interaction. Neuroticism was measured using a 24-item International Personality Item Pool (IPIP) scale (Johnson, 2014; α = 0.88).

1.4. Statistical analyses

Statistical analyses were conducted using R (version 4.0.3) and R Studio (version 1.2.5033). For all analyses, gender was coded as female = 0, male = 1 (no participants reported other gender identifications), and education was coded as college graduate = 1 or not = 0. We first examined bivariate correlations, and then used the 'lm' function to generate linear regression parameter estimates with all continuous variables centered on the sample average. To adjust for heteroscedasticity (Long and Ervin, 2010), robust standard errors, confidence intervals, and significance tests were estimated using the 'sandwich' package using the vcov option 'vcovHC' and the default type option (i.e., HC3) (Zeileis, 2004, 2006). Because exploring potential interactions between negative affect and either pain variable was part of our primary interest, all regression models included an interaction term (negative affect * intensity or interference) in addition to including the main effects of negative affect and pain intensity/interference. For each significant interaction, we estimated simple slopes comparing differences across levels of the pain variable (i.e., simple slopes) at high [+1 SD] and low [−1 SD] levels of negative affect.

[1] See Hox, Moerbeek, and van de Schoot (2017), Chapter 10, for more information on the appropriateness and calculation of the ICC and score-level reliability for intensive repeated measures data.

2. Results

Preliminary analyses. Participant demographic characteristics are shown in Table 1. Bivariate associations were examined between key study variables and are presented in Table 2. As expected, higher pain intensity ($M = 6.62$, $SD = 3.02$) was correlated with greater pain interference ($M = 7.42$, $SD = 4.37$), $r = 0.77$, $p < .0001$, and (as previously reported) higher recent negative affect (averaged from EMA in week 2) was correlated with the higher recalled negative affect variable that was used in exploratory analyses ($r = 0.29$, $p < .001$). Recent negative affect (week 2 $M = 24.16$, $SD = 17.56$) was not significantly correlated with either pain variable ($rs = 0.05$ for both pain intensity and pain interference). In contrast, recalled negative affect was correlated with both pain intensity ($r = 0.15$, $p < .05$) and interference ($r = 0.19$, $p < .01$). Neither pain intensity nor pain interference was significantly correlated with either inflammatory variable, although greater pain interference was marginally correlated with higher CRP ($r = 0.13$, $p = .063$). In keeping with prior report of an association between recent negative affect and inflammatory cytokines (Graham-Engeland et al., 2018), recent negative affect was significantly correlated with the cytokine composite ($r = 0.14$, $p < .05$). Neuroticism was significantly correlated with recent negative affect ($r = 0.31$, $p < .001$) and recalled negative affect ($r = 0.57$, $p < .001$) but not with either pain variable or inflammatory marker.

Given that this was a sample of community dwelling midlife adults not recruited on the basis of health or specific health condition, health was quite variable among participants. As shown in Table 1, the average number of health conditions was 3.37, with considerable variability ($SD = 2.57$). The health conditions included conditions known to be linked with pain, including osteoarthritis/fibromyalgia ($n = 40$), rheumatoid arthritis, multiple sclerosis, lupus, or other autoimmune problems ($n = 11$), irritable bowel syndrome, stomach ulcers, or serious issues with stomach/bowels ($n = 24$), frequent headaches, including migraine ($n = 50$), and diabetes ($n = 26$). Other endorsed health conditions included allergies or hay fever ($n = 110$), high blood pressure ($n = 61$), asthma or other serious respiratory problems ($n = 39$), thyroid issues (n = 13), liver problems ($n = 9$), coronary heart/artery disease ($n = 6$), and kidney problems ($n = 6$).

Interaction models. We first examined the association between pain variables and inflammatory markers on an exploratory basis. In keeping with the observed non-significant bivariate correlations, in regression models with covariates there were no main effects of either pain variable on either inflammatory variable ($\beta s = -0.01$ to 0.06, $ps > .05$). Contrary to expectations, recent negative affect did not significantly interact with either pain variable to predict the cytokine composite (NA*intensity: $\beta = 0.04$, $p = .54$, 95%CI = −0.10,0.19; NA*interference: $\beta = -0.01$, $p = .89$,

Table 1
Sample demographics and characteristics (N = 212).

	Mean (SD) or N (%)
Age, years	46.83 (10.86)
Gender	137 women (64.62%)
Black, non-Hispanic	129 (60.84%)
White, non-Hispanic	19 (8.96%)
Hispanic/Latina/o, White	39 (18.40%)
Hispanic/Latina/o, Black	15 (7.08%)
Other race (including Asian, American Indian, Alaska Native)	10 (4.72%)
College graduate	98 (46.23%)
Household income < $40,000[b]	88 (45.36%)
Employed	110 (52.38%)
Married/cohabitating as if married	86 (40.57%)
Body Mass Index (BMI)	32.04 (8.22)
Number of chronic health conditions	3.37 (2.57)

Notes. [a] = Information about income was missing for 16 participants; the percentage provided is of those who reported information.

Table 2
Bivariate correlations between (and means and standard deviations of) key variables used in primary analyses.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Age	–													
2. Female gender	.04	–												
3. BMI[a]	-.01	-.12†	–											
4. Education [a]	.03	-.11	**-.19**	–										
5. Black race	.05	.01	-.07	-.10	–									
6. Hispanic ethnicity[a]	-.05	-.03	-.11	-.11	**.73**	–								
7. Conditions[a]	**.40**	**-.15**	**.15**	.02	.06	.08	–							
8. Pain intensity	**.15**	-.05	.05	-.05	-.01	-.01	**.36**	–						
9. Pain interference	.13†	-.02	.12†	-.07	.03	.07	**.41**	**.77**	–					
10. Recent NA	-.05	-.02	-.06	-.03	.06	.07	.05	.05	.05	–				
11. Recalled NA	-.10	-.10	.00	-.01	.03	-.07	**.16**	**.15**	**.19**	**.29**	–			
12. Neuroticism	-.01	-.09	.13†	-.10	.04	-.04	.11	.09	.08	**.31**	**.57**	–		
13. CRP (lg)[a]	-.03	.03	**.52**	**-.18**	.07	-.03	.10	.06	.13†	.04	.06	.02	–	
14. Composite (lg)[a]	.04	.13†	-.09	**-.14**	.05	.08	-.03	.06	.00	**.14**	-.05	-.03	-.02	–
Mean	46.83	0.35	32.04	0.46	0.39	0.25	3.37	6.62	7.42	24.16	35.63	2.51	0.16	−0.03
SD	*10.86*	*0.48*	*8.22*	*0.50*	*0.49*	*0.44*	*2.57*	*3.02*	*4.37*	*17.56*	*12.89*	*0.64*	*0.17*	*0.63*

Notes. Significant values at $p < .05$ or greater are bolded. † = marginal significance at $p < .10$.
[a] BMI = body mass index. Education = college/university completion. Hispanic ethnicity = Hispanic/Latina/o ethnicity. Conditions = number of health conditions. CRP (lg) = C-reactive protein (logged; mean values provided above); Composite (lg) = cytokine composite (logged; mean values of individual cytokines provided above). Non-continuous data (gender, education, race, and ethnicity) were coded as 1 or 0; demographic characteristics are provided in Table 1.

95%CI = −0.15,0.13). As shown in Table 3, results with individual cytokines were generally consistent and in the same direction as primary findings (that is, not significantly associated with key study variables in regression analyses examining main effects or interactions models). However, in keeping with expectations, recent negative affect interacted with both pain intensity and pain interference to predict CRP (NA*intensity interaction: $\beta = 0.14$, $p = .03$, 95%CI = 0.01,0.26; NA*interference interaction: $\beta = 0.20$, $p = .01$, 95%CI = 0.05,0.35; individual inflammatory markers analyses also presented in Table 3). As shown in Fig. 1 and as elaborated in Table 4, the association between both pain variables and CRP was only significant for those with higher recent negative affect, such that a significant positive association between pain symptomatology (both intensity and interference) and CRP was observed only among those with higher recent negative affect.

Exploratory and sensitivity analyses. On an exploratory basis, we ran the analyses described above using recalled affect instead of recent (EMA-derived) negative affect. Recalled affect did not significantly interact with either of the pain variables in predicting the cytokine composite (recalled NA*intensity interaction: $\beta = 0.04$, $p = .60$; recalled NA*interference: $\beta = 0.07$, $p = .34$) or CRP (recalled NA*intensity interaction: $\beta = 0.06$, $p = .27$; recalled NA*interference: $\beta = 0.05$, $p = .50$). When using recent negative affect from EMA across the full two weeks, findings were effectively the same as with primary analyses (which used recent negative affect from the week closest to the blood draw); that is, recent NA interacted significantly with both pain intensity and pain interference to predict levels of CRP (but not the cytokine composite) when using either recent negative affect variable derived from EMA. We also ran two sensitivity analyses to examine the boundaries of our primary findings: We ran our primary models adjusting for number of health conditions, and, separately, adjusting for neuroticism. The inclusion of neither health conditions nor neuroticism meaningfully changed results. Lastly, as detectability of some cytokines was low (as shown in Table 3; particularly IL-4 and IL-1β, the latter also having low variability), we additionally ran our analyses using the composite variable without IL-1β and IL-4; results were not meaningfully changed when either or both of these cytokines were excluded.

3. Discussion

Very little research has directly examined interactive effects of negative affect and pain symptomatology in predicting inflammation, despite research supporting the premise that negative affect and both pain intensity and pain interference might interact. The primary goal of the present research was to determine whether pain intensity and pain interference interacted with recent negative affect to explain variance in levels of two measures of inflammatory state: CRP and a composite measure comprised of seven cytokines (IL-1β, IL-6, TNF-α, IFN-γ, IL-8, IL-4, IL-10). This was examined in a sample of generally healthy midlife adults who were recruited as part of a larger study, and who were diverse with regard to pain, health conditions, and demographic factors. After

Table 3
Standardized parameter estimates from 18 individual models regressing CRP, the cytokine composite, or each individual cytokine onto each pain variable and each pain variable's interaction with recent negative affect (NA).

		Main effect of pain intensity		Interactive effect of NA and pain intensity		Main effect of pain interference		Interactive effect of NA and pain interference	
	Raw Mean (*SD*); *range (min-max)*	β	95% CI	β	95% CI	β	95% CI	β	95% CI
CRP	6.14 (*9.48*); *.003-57.*93 mg/L	0.02	−0.09, 0.13	**0.14***	**0.01, 0.26***	0.06	−0.07, 0.19	**0.20****	**0.05, 0.35****
Composite	-[a]	0.04	−0.10, 0.19	0.06	−0.09, 0.21	−0.01	−0.15, 0.13	0.07	−0.10, 0.25
IL-1β	0.01 (*0.03*); *0-0.19* pg/mL	0.09	−0.05, 0.23	0.13†	−0.004, 0.27†	0.06	−0.09, 0.21	0.12	−0.03, 0.27
IL-4	1.03 (*7.17*); *0-101.32* pg/mL	0.02	−0.13, 0.17	0.02	−0.13, 0.16	−0.05	−0.19, 0.09	0.03	−0.12, 0.19
IL-6	0.19 (*1.19*); *0-16.5* pg/mL	0.07	−0.06, 0.20	0.08	−0.07, 0.24	0.06	−0.10, 0.22	0.10	−0.09, 0.29
IL-8	0.55 (*1.21*); *0-15.96* pg/mL	0.04	−0.08, 0.16	0.13†	−0.01, 0.26†	0.03	−0.10, 0.16	0.09	−0.07, 0.25
IL-10	0.41 (*1.67*); *0-22.10* pg/mL	0.01	−0.14, 0.16	0.03	−0.12, 0.19	−0.04	−0.19, 0.11	0.04	−0.12, 0.21
TNF-α	0.18 (*0.84*); *0-9.76* pg/mL	0.05	−0.11, 0.21	0.00	−0.15, 0.16	−0.02	−0.18, 0.15	0.03	−0.14, 0.20
IFN-γ	0.16 (*0.78*); *0-9.76* pg/mL	−0.04	−0.17, 0.09	0.01	−0.13, 0.15	−0.11†	−0.23, 0.01†	0.04	−0.12, 0.19

Notes. CI = confidence interval; NA = recent negative affect. Statistically significant estimates and CIs are bolded.
**$p < .01$, *$p < .05$, †$p < .10$.
[a] Given how the composite was created, raw means would not be meaningful; means for each component are provided separately.

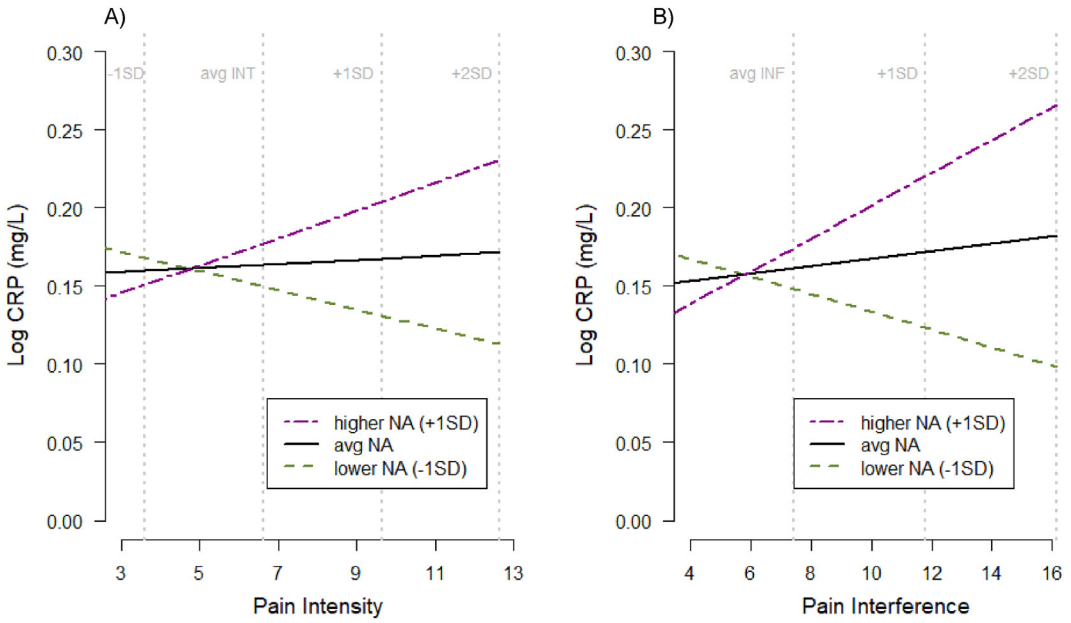

Fig. 1. Interactive effects of levels of recent negative affect ("NA" in the figure) on the association between CRP and recent A) pain intensity and B) pain interference. Analyses were run using continuous data. As shown in 1A there was a positive association between pain intensity and CRP for participants who reported higher levels of recent negative affect (+1 *SD* above the mean, purple, broken line); there was not a significant association between pain intensity and CRP for those who had recent negative affect levels at or below the average of the sample. Similarly, as shown in 1B there was a positive association between pain interference and CRP for participants who reported higher levels of recent negative affect (+1 *SD* above the mean, purple, broken line) and there was not a significant association between pain interference and CRP for those who had negative affect levels at or below the average of the sample. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Simple slopes from linear regression models examining recent negative affect as a moderator of the associations between pain intensity and pain interference with CRP levels.

	β	95% CI
Level of negative affect	*CRP and pain intensity*	
High	**0.16***	**0.003, 0.317***
Average	0.02	−0.09, 0.13
Low	−0.11	−0.28, 0.06
	CRP and pain interference	
High	**0.28***	**0.04, 0.51***
Average	0.06	−0.07, 0.19
Low	−0.15†	−0.33, 0.03†

Note. $N = 212$. CI = confidence interval. Significant values are bolded.
†$p < .10$, *$p < .05$.

covarying for age, gender, BMI, and education, we found that recent negative affect interacted with both pain intensity and pain interference to explain variance in levels of CRP. Specifically, individuals who reported higher recent negative affect and higher levels of either pain intensity or pain interference evidenced higher levels of CRP. These associations were not observed with the cytokine composite (or with individual cytokines). Thus, our premise that negative affect would interact with pain symptomatology to explain variance in inflammatory markers was supported by analyses using CRP as the marker of inflammation but not cytokine levels.

The present finding that recent negative affect interacted with both pain intensity and pain interference to predict levels of CRP among midlife adults is in keeping with the biopsychosocial model of pain, which predicts that the effects of pain are amplified when individuals are experiencing high levels of negative mood or catastrophizing (Edwards et al., 2016; Gatchel et al., 2007). Further, this work builds upon a few studies that have suggested interactive effects between negative affect, pain, and inflammation (e.g., Graham-Engeland et al., 2019; Lacourt et al., 2015). One possibility underlying such findings is that negative affect may function as a signal of stress exposure or difficulties (see, Graham-Engeland et al., 2019; Lacourt et al., 2015; Sin et al., 2015) that promote a positive feedback loop involving higher inflammation (which in turn can promote more negative affect and pain) (Engeland and Graham, 2011). Similarly, pain can also be thought of as an emotional state that motivates individuals to change behavior and which can signal a state of stress (Craig, 2003). Alternatively (or additionally), negative affect may prime the body for inflammatory activity in ways that promote pain sensitivity or pain-related disability, over and beyond stress exposure (Lacourt et al., 2015), perhaps influencing similar mechanisms as physical pain (e.g., central nervous system sensitization).

In our past research with the same sample, most of our analyses testing associations between negative affect and inflammation were null, except for a link between recent negative affect and the cytokine composite (Graham-Engeland et al., 2018); further, we observed no direct significant associations between pain symptomatology and inflammatory markers in the present research. The null associations between pain and inflammatory markers in the present research are consistent with some other findings in general populations (DeVon et al., 2014). However, it is important to note that strong links between pain and inflammation have been established in both experimental research and among individuals with chronic pain conditions (Omoigui, 2007; Raoof et al., 2018). Given that our work was not based on an acute stress paradigm or pain manipulation (nor with a particular population of individuals with an inflammatory chronic pain condition), we tested the direct association between pain and inflammatory markers in this cohort on an exploratory basis. We do not believe that the current study contradicts existing mechanistic research between inflammation and pain. Rather, the present findings are in line with the possibility that although either negative affect or pain experience can confer risk for inflammation in some individuals, those who report higher levels of *both* recent negative affect and pain symptomatology (either pain intensity or interference from pain) may be at particular risk for higher inflammation. In keeping with

calls to better understand the role of affect and stress on pain (Lumley et al., 2011; Zautra and Sturgeon, 2016), a deeper understanding of the interactive roles of affect and stress is needed to clarify the connections between pain and inflammation.

We focused our planned analyses on recent negative affect, largely because our past work with this same cohort established that recent negative affect from the week before the blood draw but not negative affect retrospectively recalled (from the past month) was associated with levels of inflammatory cytokines. In an exploratory analysis, we examined whether recalled negative affect might also interact with pain symptomatology to predict inflammation. Recalled negative affect did not interact with either pain variable to predict levels of the cytokine composite or CRP. This is in keeping with the perspective that temporal dynamics matter when considering how stress and affect relate to inflammatory measures (Graham-Engeland et al., 2018). Affect measures derived from momentary assessments may better capture experienced affect than retrospective measures, which are more likely to reflect self-presentation bias and self-perceptions more broadly (Kahneman and Riis, 2005; Robinson and Clore, 2002). As such, our findings with recent affect may not align with studies that use broader measures related to mood, such as depressive symptomatology or retrospective measures of affect.

It is notable that pain and recent negative affect interacted to explain variance in levels of CRP but not in levels of cytokines. This is in line with past work which showed that the association between pain and CRP (but not between pain and IL-6) was moderated by caregiver status -a well-established chronic stressor (Graham et al., 2006). CRP is an acute phase protein, the production of which is induced by IL-6 (Black, 2003), and for which persistently high levels are a risk factor for cardiovascular disease (Pearson et al., 2003). Although it is an acutely sensitive index of infection, compared to inflammatory cytokines, CRP appears to change less quickly in response to acute *psychological* stress (Marsland et al., 2017) and may be more likely to capture relatively chronic or severe processes related to psychological stress (for reviews, Black, 2003; Graham et al., 2006). Although it is possible that individuals who reported both higher pain symptomatology and negative affect may have been experiencing longer term discomfort or stress than others, this was not possible to determine in the present study, particularly given that retrospective pain was assessed several weeks prior to the blood draw.

3.1. Limitations and future directions

There are important limitations of the present work. As we did not have momentary pain reports in the EMA phase of this study, we could not test the possibility that pain reported closer in time to the blood draw might have related more strongly to the inflammatory markers (including cytokines). Further, we did not have the data to examine specific pain conditions or characteristics of pain such as pain location or duration. This is an important limitation of the present work, as variability in pain condition and such characteristics of pain likely relate to the manner in which negative affect, pain, and inflammation are associated (Raoof et al., 2018). It is important to note that CRP values were relatively high in the present sample; for example, 100 participants having CRP levels >3 mg/L and values ranged from near zero to 57.93 mg/L. These high CRP levels likely reflect our sample of community dwelling midlife adults who were diverse with regard to health status; CRP levels in the present research may also reflect that our sample was comprised of a majority of African-American participants, in whom CRP levels have been observed to be higher than in White participants (Gruenewald et al., 2009; Herd et al., 2012; Ranjit et al., 2007; Ransome et al., 2018). The present sample of midlife adults were living in a metropolitan area (Bronx, NY) and were fairly well-functioning (e.g., ambulatory), despite having an average of >3 chronic health conditions. In keeping with the growing awareness that removing participants with high CRP can inadvertently decrease the ability to examine individuals of interest (Mac Giollabhui et al., 2020), we retained the full sample. Although a strength of this research in terms of generalizability was that our sample was derived from systematic population sampling, these findings may not be generalizable to other populations. It would be valuable to see if results are replicable in other samples, including more uniformly healthy samples as well as among those with specific health conditions.

Another limitation of the present work relates to low detectability of certain cytokines, a common problem in general (non-clinical) samples of adults. For example, 90 samples for IL-4 were below the detection limit of the assay and were thus replaced with zero; for IL-1β, 192 participants had values below the detection limit, and there was particularly low variability in IL-1β. In primary analyses these cytokines were examined as part of the cytokine composite; in sensitivity analyses, we removed IL-4 and IL-1β from the composite and found that results were not meaningfully changed. Nonetheless, it is possible that our null findings with cytokines (both main effects of pain, and the interaction between pain and negative mood) were related to low detectability.

Last and perhaps most importantly, the present research relied on cross-sectional analyses, which precludes determination of temporal or causal connections. Past research suggests that there are bi-directional associations between pain, affect, and inflammatory markers, with each predicting the other and being related in multifaceted ways (Edwards et al., 2016; Engeland and Graham, 2011). As such, it would be valuable for future longitudinal research to examine whether interactions between negative affect and pain predict changes in inflammatory markers over time, in addition to whether interactions between negative affect and inflammation predict changes in pain over time. Pain, pain-related distress, and negative affect are all potential modifiable targets for intervention (Gatchel et al., 2007; Goyal et al., 2014; Lumley and Schubiner, 2019; McRae and Gross, 2020). Future research to unpack nuances in the ways in which affect and pain may interact to predict inflammation may help identify targets for early preventative measures and novel intervention.

3.2. Conclusion

This research adds to a growing literature examining associations between pain symptomatology, negative affect, and inflammatory biomarkers by examining whether recent negative affect and two pain variables interacted to explain variance in levels of CRP and an inflammatory cytokine composite. Importantly, this work was conducted in a diverse sample of midlife adults who were not recruited on the basis of a particular pain disorder. Consistent with our expectations, both pain intensity and perceived interference from pain interacted with recent negative affect to predict levels of CRP, even after accounting for demographic factors and BMI. Specifically, only individuals with higher levels of pain symptomatology and higher recent negative affect evidenced higher levels of CRP. Variance in inflammatory cytokines was not predicted by similar models. The interaction effect on CRP was robust to additional covariates (including health conditions and neuroticism). Taken together with prior research, these preliminary findings suggest that pain symptomatology and recent negative affect may interact to confer or signal risk of low-grade inflammation. It is relatively easy to obtain self-reports of pain symptomatology and recent negative affect, and the present research suggests that there may be value in using such indicators to identify at-risk individuals: Those who report high levels of both pain symptomatology and negative affect may be at particular risk for elevated systemic inflammation and subsequent related health complications.

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Conflicts of interest/competing interests

The authors have no relevant financial or non-financial interests to disclose.

Consent to participate

The institutional review board at the study institution approved the protocol, and informed consent was obtained from all study participants.

Availability of data

The present research utilized data from the Effects of Stress on Cognitive Aging, Physiology, and Emotion (ESCAPE) study; study information, codebooks, and a form to request data access are available from the study Open Science Framework (OSF) site, https://osf.io/4ctdv/files/.

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