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# INTERACTION BETWEEN PHYSICAL PAIN AND NEGATIVE AFFECT IN PREDICTING LEVELS OF SALIVARY INFLAMMATION IN YOUNG ADULTS

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## ABSTRACT

The relationship between inflammation and self-reported pain symptomology has been examined in past research, which has established that a positive relationship exists between these variables. Negative affect may moderate such a relationship because it may act as a psychological stressor similarly to pain; however, the degree to which negative affect may moderate this association is unclear. The primary goals of the present research were to examine associations between salivary inflammatory markers of C-reactive protein (CRP) and Interleukin 6 (IL-6) and both acute pain intensity as well as perceived interference from pain, and whether negative affect significantly moderated these associations. Data from a larger study were used to examine 137 young adults with and without chronic pain via a 14-day daily diary protocol that included a morning and evening survey of affect and current pain intensity. Analyses controlled for gender, age, and body mass index (BMI). Results indicated that lower levels of acute pain intensity were associated with significantly elevated levels of salivary CRP ($p<.05$) but were not significantly associated with IL-6. Negative affect moderated the association between pain interference and IL-6, such that pain interference was significantly associated with elevated levels of IL-6 among those with higher negative affect ($p<.05$). Conversely, no significant moderation by negative affect on associations between acute pain intensity and either of the salivary markers of inflammation was observed. These findings suggest that measures of subjective pain and greater negative affect may sometimes interact such that the combination of high reported interference from pain and high negative affect may potentially signal risk of having elevated salivary inflammatory markers. Such findings may have important implications among young adults as an early indication of disease risk. The potential use of affect and perceptions of pain interference as screening tools will be discussed.

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## Introduction

Individuals with chronic pain conditions are much more likely to have higher levels of inflammation than others (Ji, Nackley, Huh, Terrando, & Maixner, 2019; Ji, Chamessian, & Zhang, 2017; Paley & Johnson, 2016), and some relatively recent research suggests that even among those without chronic pain, higher levels of reported pain are linked with higher circulating levels of inflammatory biomarkers (DeVon, Piano, Rosenfeld, & Hoppensteadt, 2014). Recent studies have also linked greater reported negative affect with elevated levels of peripheral inflammation (Graham-Engeland et al., 2018; Slavish et al., 2019; Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Even low-grade chronic elevations in peripheral inflammation appear to put individuals at risk for future impairment and disease (Hotamisligil, 2006; Wärnberg, Cunningham, Romeo, & Marcos, 2010); among young adults, chronically high levels of inflammation is a risk factor for worse health trajectories in later life, particularly type 2 diabetes mellitus (DeBoer, 2013) and coronary heart disease (Golia et al., 2014). For this reason, it would be valuable to discover whether easily reported behavioral phenomenon (e.g., negative affect and perceived pain) are associated with higher levels of inflammation.

Several prior studies have found evidence of an association between pain and negative affect (Gaskin, Greene, Robinson, & Geisser, 1992; Wade, Price, Hammer, Schwartz, & Hart, 1990; Huyser & Parker, 1999; Graham-Engeland, Zawadzki, Slavish, & Smyth, 2016), and negative affect can exacerbate perceptions of pain and stress (Edwards, Dworkin, Sullivan, Turk, & Wasan, 2016). Greater negative affect may potentially function synergistically with physical pain to predict elevated inflammation throughout the body. The present study focuses on the extent to which reported pain and negative affect may interact to predict levels of salivary CRP and IL-6 in a sample of young adults, some of whom have self-reported chronic pain.

Negative affect, a component of individual distress (Watson, 1988), can be captured in multiple ways and can be viewed as a reaction to stress that encapsulates the appraisal process. For example, negative affect can be assessed (as it was in this study) with momentary feelings of negativity (using multiple reports per day of high arousal feelings, including scared, upset, and nervous) that are then averaged across two weeks to create a representation of characteristic negative affect. With regard to pain, it is important to look at the impact of both acute pain intensity, which is a measure of the perceived severity of an individual's pain, and pain interference, which is a measure of the degree to which an individual perceives that their pain is disrupting their daily life activities (Amtmann et al, 2010). Though these key concepts are self-reports, all are important psychological/behavioral measures that may have serious biological consequences.

In recent years, the association between affect and health has been increasingly investigated. Differential results have come from studies that have assessed chronic negative affect (or negative affect that is relatively consistent and typical of one's character), as compared to state negative affect (or negative affective states that vary from moment to moment, such as during times of acute stress or other variability). Longitudinal work has suggested that state negative affect can be a more accurate predictor of symptomology than chronic negative affect (Brown & Moskowitz, 1997). However, in general the extant literature suggests that chronic negative affect likely promotes greater reporting of a variety of physiological symptoms, including fatigue and pain (Van Diest et al., 2005), compared to more transient states. Previous research has shown that greater chronic negative affect is linked with several diseases including cardiovascular disease (Kubzansky, Cole, Kawachi, Vokonas, & Sparrow, 2006; Donker, 2000), diabetes (Carnethon, Kinder, Fair, Stafford, & Fortmann, 2003), cancer (Reiche, Morimoto, &

Nunes, 2006), the common cold (Cohen et al., 1998), and arthritis (Huyser & Parker, 1999). Negative associations between chronic negative affect and health conditions have held even when controlling for risk factors (Consedine, Magai, Cohen, & Gillespie, 2002). This is critical because other work has linked chronic negative affect with health behaviors such as greater likelihood of smoking (Kassel, Stroud, & Paronis, 2003), poor exercise (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002), greater likelihood of drug and alcohol use (Wills, Sandy, Shinar, & Yaeger, 1999), and poor diet, including consumption of large portions (Arnow, Kenardy, & Agras, 1995) and more consumption of foods with high sugar and fat content (Dube, LeBel, & Lu, 2005).

Previous literature suggests that negative affect may potentially contribute to greater inflammation, which when chronically elevated may ultimately lead to disease, as described above. Negative affect, similarly to pain and the experience of psychological stressors, seems to affect health both directly and indirectly. As mentioned above, negative affect may promote poor health behaviors that eventually lead to morbidity (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Alternatively, through its connections with stress (e.g., both as a result of stress and by promoting additional stress) negative affect is linked with the activation and alteration of important biological systems, such as the hypothalamic-pituitary-adrenal (HPA) axis and the immune, endocrine, and cardiovascular systems (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Specifically, negative emotions and related psychological stress may promote heightened release of pituitary and adrenal hormones such as catecholamines and cortisol, which can induce immune responses (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). Additionally, negative emotions may directly increase production of proinflammatory cytokines, such as IL-6, and also may indirectly promote release of proinflammatory cytokines. Chronically high levels of

proinflammatory cytokines can result, leading to the type of chronic inflammation that is linked with delayed healing, frailty, and disease (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002).

Findings from previous research have provided evidence that recent negative affect assessments aggregated over a week predicted peripheral inflammation based on an aggregated measure of multiple cytokines determined from peripheral blood (Graham-Engeland et al., 2018). Correspondingly, related research has shown that negative affect can stimulate the release of proinflammatory cytokines similarly to an infection or injury, which over time may aid in the dysregulation of the immune system, leading to a variety of negative health consequences (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). However, literature focusing on negative affect as a predictor of blood-derived inflammatory markers (IL-6 and CRP) is inconsistent, differing sometimes depending on measurement, such as retrospective versus momentary measures of negative affect (Graham-Engeland et al., 2018). Specifically, although one study discussed above found that aggregated momentary measures of negative affect were more closely related to increases in inflammatory cytokines, neither recalled nor momentary negative affect were associated with CRP (Graham-Engeland et al., 2018). This type of inconsistency in previous literature shows a need for future work to provide clarity, but some significant findings provide promise of an association between negative affect and inflammation, thus supporting the goals of the current study.

In addition to linkages between negative affect and inflammation, pain has also been associated with inflammation. Both chronic and acute pain can act similarly to psychological stressors (Crofford, 2015), activating endocrine response systems including both the hypothalamic-pituitary-adrenocortical (HPA)-axis and the sympathetic-adrenal-medullary (SAM)-system, which produce important inflammatory responses (Cohen, Janicki-Deverts, &

Miller, 2007). Conversely, inflammatory markers, including IL-6, may also play a role in triggering pain symptomology via indirect stimulation of the sympathetic nervous system (Zhang & An, 2007). One study found that chronic pain was indirectly related to abnormal sympathetic sprouting in the dorsal root ganglion nerve via IL-6, which in turn promoted greater production of proinflammatory cytokines and produced the sensation of pain in the absence of peripheral nerve injury (Zhang & An, 2007). Proinflammatory cytokines are necessary during times of acute, peripheral injury or infection in order to stimulate and regulate the appropriate immune response (Heinrich et al., 2003). In doing so, they trigger the acute phase response by stimulating release of acute phase proteins, such as CRP, from the liver in order to activate a systemic, complementary response to fight the injury/infection (Mortensen, 2001). Although helpful during times of acute stress, however, inflammatory functions can contribute to pain related to swelling. Moreover, if inflammatory responses persist over time, a variety of chronic conditions may arise including weakened immune systems, cardiovascular disease, and depression (Cohen, Janicki-Deverts, & Miller, 2007), all of which may also contribute to pain-related conditions.

Peripheral blood markers of inflammation, including IL-6 and CRP, can function as robust indicators of disease risk and mortality among healthy young and older age adults (Kalhan et al., 2010; Reuben et al., 2002). For example, one specific study found that systemic inflammation in young adults, as indicated by chronically elevated fibrinogen and CRP levels, was positively associated with poor lung function and lung diseases (Kalhan et al., 2010). Additionally, a study conducted on a sample of high-functioning elderly people found that older adults with several markers of inflammation, including both CRP and IL-6, had increased mortality risk (Reuben et al., 2002). More recently, there is increasing interest using less invasive methods to collect biomarkers of inflammation through salivary samples (Slavish, Graham-

Engeland, Smyth, & Engeland, 2015). The use of salivary samples to assess inflammation allows for easier collection in ecological settings at various times throughout the day compared to serum inflammatory markers (Slavish, Graham-Engeland, Smyth, & Engeland, 2014). However, evidence of the validity and reliability of salivary biomarkers in relation to serum biomarkers remains relatively unclear. Elevated levels of salivary biomarkers (such as CRP) have been shown to be fairly reliably correlated with heightened serum inflammatory markers, but it remains unclear the extent to which salivary inflammation maps onto peripheral levels of inflammation (for review, see Slavish, Graham-Engeland, Smyth, & Engeland, 2015 and Szabo, Slavish, & Graham-Engeland, under review). There are some confounding variables that may affect salivary measures of biomarkers, such as oral infections which may raise salivary cytokine levels and therefore inaccurately represent serum inflammation (Rathnayake et al., 2013). Nonetheless, a recent review study provided preliminary evidence for the utility of salivary inflammatory markers as a valid indication of systemic inflammation, particularly when an individual experiences acute stress (Engeland, Bosch, & Rohleder, 2019). Salivary collection of biomarkers remains a promising method to measure inflammation due to its lesser invasiveness, affordability, and ease of extensive collections in comparison to drawing blood (Slavish, Graham-Engeland, Smyth, & Engeland, 2015).

There is relatively little research on the association between pain, negative affect, and inflammation using salivary measures of biomarkers. In one study, recent and persistent negative affect coinciding with PTSD in a sample of women was found to be positively correlated with salivary levels of IL-6 (Newton, Fernandez-Botran, Miller, & Burns, 2014). There still remains a gap in the literature, however, regarding the interaction of pain and negative affect in predicting salivary inflammatory markers. Considering that pain perception and negative affect can both be

construed as either psychological stressors themselves or as markers of psychological stress (Cohen, Janicki-Deverts, & Miller, 2007; Crofford, 2015), it is possible that a moderation effect of negative affect on pain exists, with the two variables synergistically interacting to predict elevated inflammation to a greater degree than either variable can predict on its own.

The present study focused specifically on exploring the moderation effect of negative affect in explaining an association between pain and measures of salivary inflammation. Data included self-reported measures of pain (aggregated measures of acute pain intensity and pain interference assessed twice daily during a 14-day period), self-reported measures of negative affect (total negative affect score aggregated over a 14-day period), and salivary inflammatory markers IL-6 and CRP collected among a sample of relatively healthy young adults both with and without chronic pain. Based on previous literature described above linking both pain and negative affect with inflammation, and more recently linking pain and negative affect, it was hypothesized that greater average reporting of negative affect would interact with greater average reporting of pain to be associated with elevated levels of salivary IL-6 and CRP. It was also hypothesized that increases in both pain measures and negative affect would independently be associated with elevated inflammatory markers, consistent with previous literature findings mentioned above.

# Methods

## Participant Characteristics

College students from a large university in central Pennsylvania were recruited in undergraduate classes and through campus list services, posters, and online research volunteer forums as part of a larger study investigating connections between pain, inflammation, daily stress, and health. All procedures were approved by the Institutional Review Board at the associated university and consent was obtained from all participants. Eligibility for the study included undergraduate enrollment at the university, being of age 18 or older, having good subjective oral health (i.e., no gum disease, regular teeth brushing, etc.), not having recent dental cleanings in the seven days prior to participation in the study, and not having any recent acute pain from an injury or dental procedure within the past month. Participants were recruited into one of two groups: those with chronic back pain and those without ongoing chronic pain. In the current study, chronic back pain was defined as back pain persisting for three months or longer, or intermittent back pain persisting six months or longer, resulting from any cause.

Of the 139 respondents, two participants were excluded due to failure of proper enrollment in the study because of either a missed visit or lack of questionnaire responses. The final sample included in the analyses consisted of 137 young adults with mean age 20.58 ± 1.83 years. Of those in the final sample, 41.6% were female, 74.5% were White, and average BMI was 23.02 (*SD*=4.13). Of the final participants, 58 (42.3%) reported having chronic back pain.

## Procedure

Data used in the present study were previously collected for the Pain, Affect, Stress, and Sociality (PASS-2) study, conducted by the Stress and Health Lab at the Pennsylvania State University. Demographic characteristics and health behaviors of participants were first assessed

in an online survey at baseline. Participants then attended an in-person training session where informed consent was acquired from all participants and instructions were given on completing the daily diary during the following two weeks. The 14-day daily diary portion, which included a morning and an evening survey, began the morning following the in-person training session. The morning survey was available from 5 a.m. until 12 p.m., and the evening survey was available from 6 p.m. until 3 a.m. each day. Surveys were distributed via a link to participants' emails. Participants were instructed to complete the morning survey within 30 minutes of waking and the evening survey within 30 minutes of going to bed, in order to reduce recall bias. Both surveys assessed recent negative affect, recent pain interference, and current pain intensity. Saliva samples were collected between 1 and 5 p.m. via passive drool during the initial training session and again approximately two weeks later after completion of the daily diary portion of the study, though only data from the second collection was used in this analysis. All procedures were performed in accordance with the ethical standards of the institutional research committee.

**Measures**

**Acute Pain Intensity.** The West Haven-Yale Multidimensional Pain Inventory (MPI) Pain Severity subscale was used to assess the participants' current pain intensity and suffering (Riley et al., 1999). The MPI was developed specifically for chronic pain patients to assess the impact that chronic pain has on patients' lives (Kerns, Turk, & Rudy, 1985). The inventory consists of twelve scales among three parts: the impact of pain on patients' lives, responses received by the patient upon communication of their pain to others, and the extent to which the patient engages in typical activities of daily living (Kerns, Turk, & Rudy, 1985). Items in the pain severity subscale, like in all of the subscales, are measured on a 7-point scale ranging from 0-6, with (0) indicating "no pain" and (6) indicating "extreme pain". Participants' responses from

the two surveys each day during the 14-day period of daily dairy were averaged into one score for data analyses, with a higher score again indicating more severe pain (Kerns, Turk, & Rudy, 1985).

**Pain Interference.** Participants reported their recent pain interference in both the morning and evening surveys based on the Pain-Related Life Interference subscale of the MPI (Riley et al., 1999). This variable was measured to gain a sense of how the subjects' pain was interfering in several aspects of their life, including: family and partner functioning; school, work, and related activities; and friend relationships and recreational activities (Kerns, Turk, & Rudy, 1985). The Pain Interference subscale includes nine items to asses interference in each distinct area of life ranging from social activities to family interaction, all measured on a 7-point scale similar to the scale mentioned above, ranging from 0-6, with (0) indicating "no interference" and (6) indicating "extreme interference". Participants' responses over the 14-day daily diary portion of the study were again averaged into one final score, representative of the impact of their chronic pain (or lack thereof) in their daily lives.

**Recent Negative Affect.** Morning and evening surveys assessed recent negative affect using the 10-item composite measures of the Positive and Negative Affect Schedule (PANAS) (Watson, Clark, & Tellegen, 1988). Participants reported the degree to which they felt each of the high arousal negative affect items (scared, afraid, upset, distressed, jittery, nervous, ashamed, guilty, irritable, hostile) over the past two hours on a 5-point scale ranging from 1-5, with (1) indicating "very slightly or not at all" and (5) indicating "extremely". After completion of the study, participants' negative affect scores over the 14-day daily dairy portion were averaged to create a negative affect scale with total scores on each scale ranging from 10-50. Higher total scores represented greater levels of recent morning or evening negative affect (Watson, 1988).

**Salivary IL-6 and CRP.** Saliva samples consisting of 5mL or more were immediately refrigerated at -20°C, then centrifuged for 15 minutes, aliquoted into cryovials, and stored at -80°C. To collect the salivary IL-6 and CRP data, samples were assayed using enzyme linked immunosorbent assay kits (Salimetrics LLC, State College, PA, USA). For participants whose assayed saliva samples had IL-6 levels below detectable limits, a value of 0.0000001 pg/mL was imputed to indicate very small IL-6 values that were still considered meaningful. All saliva samples assayed for CRP were within detectable ranges. To resolve skewness and kurtosis of the non-normal distributions of IL-6 and CRP, all values were log-10 transformed for analyses (Slavish et al., 2019).

## Statistical Analyses

Data analyses were performed using the statistical software SPSS version 25. Bivariate correlations were run for all study variables, and t-tests were run to examine group differences. Hierarchical linear regression was utilized to determine the primary moderation effect of negative affect on the association between key pain variables (acute pain intensity and pain interference) and salivary inflammatory markers IL-6 and CRP. Linear regression was also used to examine the direct, independent effects of both pain variables and of negative affect on inflammation. All variables were mean centered to allow for interpretation of typical levels of pain and negative affect across the sample. Chronic pain status was analyzed as a potential secondary moderator; however, moderation of chronic pain on the association between all main predictor variables (acute pain intensity, pain interference, and negative affect) and both salivary inflammatory markers (IL-6 and CRP) were not statistically significant. Thus, for the purposes of the present project, the entire sample was considered together for all analyses. Covariates (i.e., gender, age, and BMI) were controlled for in all analyses in correspondence with previous

research that has revealed confounding effects of gender, age, and BMI on inflammatory markers (O'Connor et al., 2009; Spencer et al., 2010).

# Results

## Descriptive Statistics

Means and standard deviations of the main variables and covariates involved in this study are presented in Table 1. The mean score of the sample on the PANAS scale was 19.63 (*SD*=7.45), and participants' responses ranged from 10-44. The sample's mean score on the MPI Pain Interference subscale was 1.00 (*SD*=0.84), while the sample's mean score on the MPI Pain Intensity subscale was 1.16 (*SD*=1.33).

When comparing the chronic and non-chronic pain groups, there was a significant difference in gender between the groups ($p<.005$). The chronic pain group was largely composed of males (72.4% male), whereas the non-chronic pain group had a roughly equal split of males and females (48.1% and 51.9%, respectively). The groups did not differ significantly with regards to demographic characteristics of age or BMI. Again, these groups were merged for the present main analyses.

### Correlations and T-Tests

Table 2 presents bivariate correlations for all main study variables and covariates. Of relevance to key analyses, there was a significant positive association between acute pain intensity and pain interference ($p<.01$), and there was also a significant positive association between IL-6 and CRP ($p<.05$). In addition, greater acute pain intensity and greater pain interference were significantly correlated with greater negative affect, with $p<.01$ and $p<.05$, respectively. There was no significant correlation between acute pain intensity and IL-6, but greater acute pain intensity was correlated with lower levels of CRP ($p<.05$), which shows a relationship in the opposite direction than expected. No significant correlations were detected between pain interference and either IL-6 or CRP.

With regard to covariates, a group difference was detected in acute pain intensity by gender ($t$=3.280, $p$<.01), indicating that men were more likely to report higher pain intensity in the present study. A group difference was also detected in BMI by gender ($t$=2.763, $p$<.01), indicating that women in the study typically had greater BMI. No group differences were found in pain interference or negative affect by gender. Finally, there was a positive association between age and BMI ($p$<.05), with older participants typically having greater BMI.

**Linear Regressions**

Table 3 presents linear regression data for interaction effects and for all main effects. As expected, a significant moderation effect was detected between pain interference and negative affect on IL-6 ($p$<.05) such that for those with greater negative affect, the association between pain interference and IL-6 was stronger, as seen in Figure 1. However, no significant moderation effects were detected between pain interference and negative affect on CRP. No significant moderation effects were found between acute pain intensity and negative affect on IL-6 or CRP. A significant main effect of acute pain intensity on CRP was found ($p$<.05), but it was an inverse association that was the opposite direction than expected. No significant effects were found between acute pain intensity and IL-6, between pain interference and either inflammatory marker, or between negative affect and either inflammatory marker. Supplementary analyses showed no moderation effects of chronic pain status on either pain variable in the association between pain and inflammation, as previously mentioned.

## Discussion

The present research focused on whether reports of physical pain (pain intensity and pain interference) and negative affect interacted to predict levels of salivary inflammatory markers, and ultimately how this interaction may play a role in exacerbating disease risk related to chronic elevated inflammation. This research was conducted with a sample of young adults who were recruited into one of two groups, those having self-reported chronic pain or those with no reported ongoing pain, although chronic pain was not found to be a significant moderator and thus all participants were considered together. We expected that negative affect would interact with the pain variables, such that the highest levels of inflammation would be observed among those with both high negative affect and high pain intensity and/or reported interference from pain. We also predicted that those with high pain intensity, pain interference, and/or negative affect would have higher levels of salivary inflammatory markers CRP and IL-6 compared to those with no reported pain or lower negative affect, but to a lesser degree than those with the combination of pain with negative affect.

Overall, analyses looking at the moderation of the pain variables and negative affect in predicting salivary inflammation levels were mixed. There was not a significant interaction between pain interference and negative affect when predicting salivary CRP. However, as predicted, negative affect significantly interacted with pain interference when predicting salivary IL-6. These results suggest that negative affect may have a synergistic effect on IL-6 when in combination with perceived interference from pain. No evidence was found to support predicted interactions between acute pain intensity and negative affect on either IL-6 or CRP. This could suggest that although greater negative affect may heighten acute pain intensity, they may not interact to predict inflammation. It is also possible that unmeasured or untested variables may

explain relationships between acute pain intensity, negative affect, and inflammation in an unknown way.

Another potential explanation exists for our finding that pain interference interacted with negative affect to predict pain whereas pain intensity did not. Pain intensity does not as clearly incorporate a mood component, whereas pain interference does. Pain interference and acute pain intensity were both positively correlated with negative affect, meaning that as pain and its consequences become more intense, an individual tends to experience more negative affect. As described earlier, negative affect can be defined as a factor of individual distress, encompassing mood states such as nervous, anxious, angry, and guilty (Watson, 1988). Negative affect has been shown to be consistently correlated with various somatic health complaints, but less often related to any objective physical symptoms of illness (Van Diest et al., 2005). Pain interference may better capture the emotional component of pain, such that those who report both interference and negative affect may be those who are coping less well with stress and/or discomfort.

The current study also has a significant implication with regards to the interaction of negative affect and pain interference in predicting salivary inflammation, which growing evidence suggests may be an important health indicator for people both with and without chronic pain. Results showed that moderation of negative affect on pain interference is positively associated with elevated salivary IL-6, suggesting that the combined experience of negative emotions and pain in diminishing daily activities of life is associated with salivary inflammation and perhaps peripheral levels of inflammation in the body. Although IL-6 is beneficial in regards to acute tissue injury or acute infection, it can become harmful if levels remain high over a long period of time (Tanaka, Narazaki & Kishimoto, 2014). It may be useful for future research to

target the subset of the population who experience negative affect and pain interference, perhaps particularly those with chronic pain; identifying such individuals – even those who are relatively young – may help determine individuals for whom interventions to minimize their risk of inflammation-related disease are warranted.

With regards to the remaining findings, it is important to note that no variable studied solely was indicative of increased inflammatory markers. This may indicate that individuals who suffer from only pain interference, acute pain intensity, or negative affect may be appropriately coping with the pain-related stress they experience. However, as discussed above, findings suggest that the interaction of pain and negative affect may have significant consequences on inflammation, at least for salivary IL-6. Thus, it is possible that monitoring individuals who solely report pain or negative affect more closely would be helpful in preventing the future interaction of negative affect and pain interference, which would put them at risk of heightened inflammation and inflammation-related disease, as mentioned above.

Results of the main effects did not indicate strong prediction of salivary inflammation by pain interference, pain intensity, or negative affect when these variables were considered alone. No significant association was detected between pain interference and IL-6 or CRP, suggesting that pain interference may not be a significant contributor of heightened inflammation throughout the body, particularly among young adults. Pain interference refers to the degree to which pain disrupts normal daily activities (Amtmann et al., 2010), which may be more likely to predict mood than physiological health. Additionally, no significant association was found between acute pain intensity and IL-6, although a significant negative association with CRP was detected, which was the opposite trend expected (DeVon, Piano, Rosenfeld, & Hoppensteadt, 2014). These null findings, however, are not too surprising due to the deficiency of literature

linking pain and inflammation in individuals without chronic pain. Finally, with regards to the associations between negative affect and inflammation, no significant results were found for either IL-6 or CRP; these results contribute to the mixed findings of previous literature regarding the association between negative affect and inflammation.

An oddity in the findings, as mentioned above, was that acute pain intensity was negatively associated with CRP, which was opposite direction of association expected. It is unclear why this direction of association was observed in the present data, as previous literature has found a positive association between pain and inflammation (Ji, Nackley, Huh, Terrando, & Maixner, 2019; Ji, Chamessian, & Zhang, 2017; Paley & Johnson, 2016), though most prior studies examine blood-derived markers of inflammation as opposed to salivary markers which were examined in the current study. Because CRP is made almost exclusively in the liver via hepatocytes, with no local production in the mouth as there is with IL-6, there may naturally be less CRP in saliva than in blood (Pepys & Hirschfield, 2003). It is also possible that the way in which participants were recruited in the present study, which did not require participants to have a chronic pain diagnosis, resulted in a much healthier overall sample than in past studies of the association between pain and inflammation. In contrast, the majority of previously published studies on this association involved samples with either greater pain or pain from certain conditions (Ji, Nackley, Huh, Terrando, & Maixner, 2019; Paley & Johnson, 2016). This negative relationship, however, should be the focus of future studies in order to better understand the mechanisms that produced this unexpected association.

**Limitations and Future Directions**

The current study possesses several limitations. The sample overall was small (n=121) and fairly homogenous in demographics, with most being White undergraduate students around

the same age (M=20.58 years ± 1.83); clearly, these results cannot be generalized to broader populations. Additional research would be needed to see if these results replicate among a larger, more diverse population. Larger studies may also be able to detect significant findings that are not found within this small sample, which had limited power for moderation analyses and a relatively homogenous sample with somewhat limited range of pain and negative emotion.

In addition, there were several limitations within the type of data collection itself. Firstly, the method of data collection was cross-sectional and non-experimental; as such, the present findings do not enable inferences about causal relationships and directionality. Similarly, pain and negative affect are inherently subjective and thus must involve self-report measures, which poses risk of memory bias or self-presentation bias as with any self-report.

Finally, there is very limited previous work regarding the association between pain or negative affect and salivary biomarkers of inflammation, so understanding in how these associations may differ from associations with blood-based inflammatory markers remains unclear. Recent literature has shown some preliminary evidence of reliable associations between salivary and peripheral levels of IL-6 and CRP (Slavish, Graham-Engeland, Smyth, & Engeland, 2014). However, sole use of salivary biomarkers in this study is classified as a limitation due to the uncertainty in how much salivary inflammation relates to peripheral inflammation.

Even with the limitations discussed above, this research still has value in contributing to the existing literature of the impact pain and negative affect have in predicting inflammation in the body, and sheds light on how they may synergistically interact with one another to further elevate inflammation. Additionally, the current research contributes to the growing literature on the use of salivary inflammatory markers as a less invasive way to collect data from participants to test for inflammation.

Further longitudinal and experimental research is needed, however, to validate that these findings are generalizable to larger populations. Future research is also needed to investigate other related, potential psychological moderators of the association between pain and inflammation, including but not limited to stress, sleep quality, and depression. Future research is also needed to provide a clearer understanding of the correspondence of salivary and blood-based IL-6 and CRP, and how salivary markers of inflammation may predict health outcomes. Such findings may have particularly significant implications in the future development of inflammation screening tests, especially among the population of young adults who are generally perceived to be healthy and therefore are not screened regularly for inflammation (DeBoer, 2013).

**Conclusion**

Although results are preliminary and findings mixed, the present research suggests that there may be some utility of looking at an interaction between reported pain variables and negative affect in determining who may be at risk of chronic low-grade inflammation and subsequent disease. Based on the current research, it seems as though negative affect and pain interference function as synergistic stressors which indicate that a person is not coping well with their pain-related stress and may subsequently suffer greater potential inflammation. Although pain intensity per se did not interact with negative affect to predict salivary inflammation, it is possible that individuals suffering from pain over long periods of time may develop interference from pain and negative mood, potentially posing a risk for heightened inflammation as well. However, these stressors on their own may be insufficient to significantly relate to elevated inflammation. Findings of the current study warrant future research to examine the interaction between negative affect and pain on inflammation among a larger, broader sample. If future

research suggests that there is a connection which predicts exacerbation of inflammation over time, it may be a future target of prevention and intervention programs to reduce risk of chronic inflammation and later chronic disease, particularly among young populations.

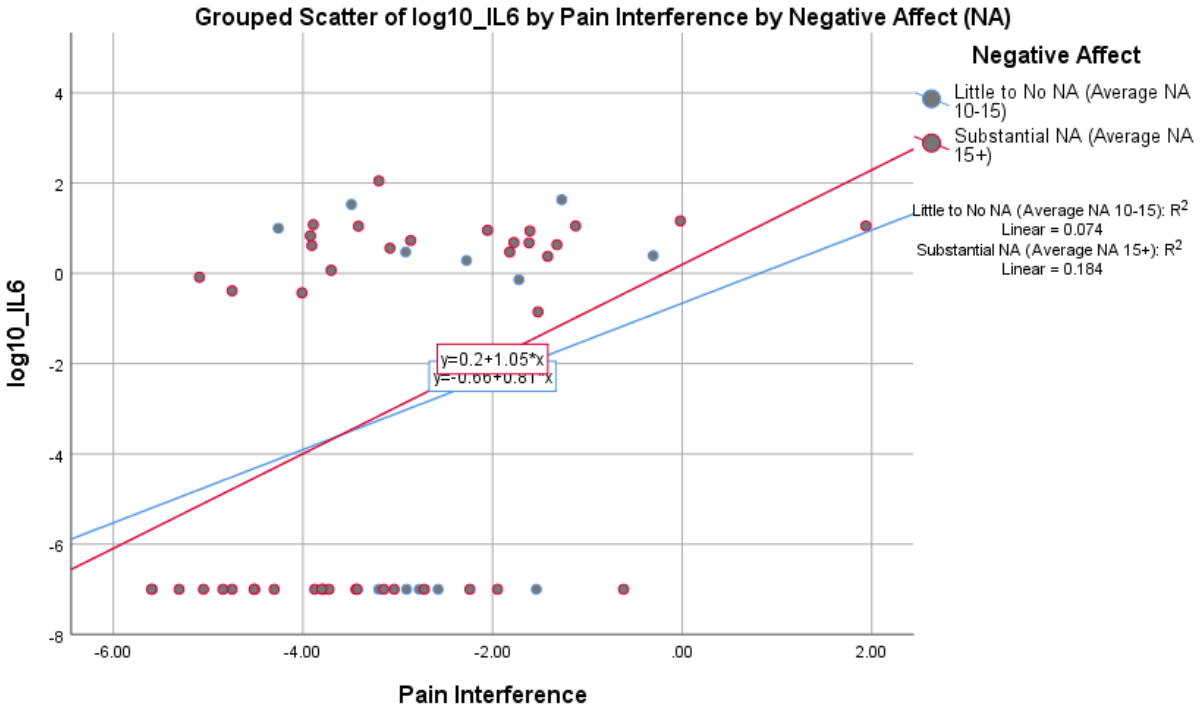


**Figure 1. Moderation of negative affect (NA) on pain interference positively associated with salivary IL-6, breakdown by NA category**

**Table 1. Descriptive characteristics of main study variables and covariates**

| Characteristics | Mean or N (%) | SD |
|---|---|---|
| Pain Interference | 1.00 | 0.840 |
| Acute Pain Intensity | 1.16 | 1.33 |
| Negative Affect | 19.63 | 7.45 |
| IL-6 (mg/L) | -2.39 | 3.79 |
| CRP (mg/L) | 3.77 | 0.573 |
| Age | 20.58 | 1.83 |
| Gender | 41.6% Female | -- |
| BMI | 23.02 | 4.13 |

Note: Log-transformed values are reported for IL-6 and CRP, as were used in all analyses.
Note: BMI represents body mass index.

**Table 2. Correlations between main study variables and covariates**

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|
| 1. Pain Interference | .43** | .31* | -.01 | -.06 | -.003 | .02 | .04 |
| 2. Acute Pain Intensity | - | .23** | -.18 | -.24* | .27** | .08 | .03 |
| 3. Negative Affect | | - | -.04 | .06 | .13 | .06 | .11 |
| 4. IL-6 | | | - | .23* | -.12 | .07 | .05 |
| 5. CRP | | | | - | -.10 | .01 | .02 |
| 6. Gender | | | | | - | .16 | -.23** |
| 7. Age | | | | | | - | .20* |
| 8. BMI | | | | | | | - |

Note: $p < .05$ is represented by *
Note: $p < .01$ is represented by **
Note: gender was coded as 1 = female, 2 = male.
Note: BMI represents body mass index.

**Table 3. Linear regression data of moderation effects and main effects**

| **Moderation Effects** | **IL-6** | **CRP** |
|---|---|---|
| | ***p-value, β (95% CI)*** | ***p-value, β (95% CI)*** |
| Pain Interference X NA | 0.03, 0.20 (0.02, 0.43)* | 0.64, -0.01 (-0.04, 0.02) |
| Acute Pain Intensity X NA | 0.38, 0.03 (-0.04, 0.11) | 0.99, -5.06E-6 (-0.01, 0.01) |
| **Main Effects** | **IL-6** | **CRP** |
| | ***p-value, β (95% CI)*** | ***p-value, β (95% CI)*** |
| Pain Interference | 0.90, -0.08 (-1.41, 1.25) | 0.73, -0.03 (-0.21, 0.15) |
| Acute Pain Intensity | 0.12, -0.44 (-0.99, 0.11) | 0.02, -0.10 (-0.19, -0.02)* |
| Negative Affect | 0.83, -0.01 (-0.12, 0.09) | 0.46, 0.01 (-0.01, 0.02) |

Note: $p < .05$ is represented by *

Note: NA represents negative affect.

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**ACADEMIC VITA**

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## EDUCATION

**Bachelor of Science: Biobehavioral Health** *May 2020*
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## CLINICAL EXPERIENCE

*Medical Scribe* *May 2018 – Jan 2019*
**UPMC Pinnacle Legacy Hospitals and Affiliated Practices** Harrisburg, PA

- Assisted physicians in the Emergency Department for over 300 hours by writing history of present illness, review of systems, and physical exams for each patient seen

## RESEARCH EXPERIENCE

*Research Assistant* *Aug 2018 – Present*
**Stress and Health Laboratory, Department of Biobehavioral Health (BBH), Penn State** University Park, PA

- Conduct literature reviews and analyze psychological and physiological data towards an honors thesis
  - Investigating how reported levels of pain predict levels of CRP and IL6 salivary inflammatory markers, and how negative affect may moderate this relationship
- Assist an active dissertation study by helping with survey formatting and running participants at baseline visit

## TEACHING EXPERIENCE

*Teaching Assistant* *Jan 2018 – May 2018*
**Department of Biology, Penn State** University Park, PA

- Aid Dr. Denise Woodward in an introductory Biology Lab course through preparing lab lectures, grading assignments, developing quizzes, and answering student questions in class, during office hours, and via email

*Teaching Assistant* *Aug 2018 – present*
**Department of Organic Chemistry, Penn State** University Park, PA

- Aid Dr. Sheryl Dykstra through working in the organic chemistry laboratory instrument room to help students run samples using 60 MHz $^{1}$H NMR, IR, GC-MS, and UV-VIS photometers, and assisting them to interpret results

## LEADERSHIP EXPERIENCE

**College of Health and Human Development Ambassadors Treasurer** *Aug 2018-May 2019*

- Coordinate finances for HHD Ambassadors to increase involvement of events run through the college

**Schreyer Honors College Scholar Ambassador Team** May *2017-present*

- Assist the Schreyer Honors College planning and execution of events, including recruitment of new scholars and philanthropy initiatives for the college

**Schreyer Student Council Co-Involvement Chair** *Sept 2017-May 2018*

- Help organize bonding events for students involved with Schreyer Student Council, including involvement in the annual Homecoming parade

## VOLUNTEER EXPERIENCE/SERVICE

*Volunteer,* LifeLink PSU-assisted young adults with special needs with their course work *8/2018 – 12/2018*
*Member,* Springfield special THON organization-raising money and awareness for childhood cancer *8/2016 – 5/2018*
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*Volunteer,* Central PA Annual Greek Cultural Festival at Holy Trinity Greek Orthodox Cathedral *Annually in May*

## AWARDS

Bausch & Lomb 2016 Science Award recipient
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## TECHNICAL SKILLS AND RELATED EXPERIENCE

Foreign Languages: Greek (conversational); Spanish (conversational)
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