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Associations Among Perceived Stress, Pain Sensitivity, And Physiological Reactivity Following An Acute Noxious Stressor

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ASSOCIATIONS AMONG PERCEIVED STRESS, PAIN SENSITIVITY, AND PHYSIOLOGICAL REACTIVITY FOLLOWING AN ACUTE NOXIOUS STRESSOR

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ABSTRACT

There is a growing body of literature that lends support to the relationship of perceived stress and pain perception. However, this relationship is influenced by many factors, including the duration of the stressors and the health of the individual. Additionally, physiological mechanisms underlying this relationship have not been fully characterized. Thus, the current investigation aimed to evaluate relationships between self-reported life stress and subsequent physiological responses (e.g., systolic blood pressure (SBP), plasma cortisol, and circulating resolvins) to an acute noxious stressor. A total of 50 community-dwelling adults without chronic pain participated in the study (50% African-American, 52% female). Prior to pain testing, participants reported perceived stress on the Depression, Anxiety, and Stress Scale – Stress Subscale (DASS21-Stress). They then underwent a cold pressor task (CPT) at 8 degrees Celsius, during which they reported pain intensity and unpleasantness ratings. Blood draws and SBP measurements were collected at baseline, during, and following the CPT. Results indicate that, consistent with hypotheses, higher levels of perceived stress predicted greater pain intensity reports during the CPT. However, despite a significant positive correlation, perceived stress was not found to be a significant predictor of pain unpleasantness ratings in a regression model including covariates. Additionally, higher reported stress predicted significant SBP reactivity during the CPT. In contrast, perceived

stress did not significantly predict baseline SBP, nor SBP recovery in the 5 minutes following the CPT. No significant relationship amongst perceived stress and cortisol levels were found. Finally, a relationship between RvD1 and perceived stress was identified only at a trending level; however, no significant relationships were identified between resolvins D1 or D2 and reports of pain intensity, unpleasantness, or perceived stress. The results of the present study add to the existing literature by incorporating perceived life stress into the study, rather than relying solely on laboratory-induced stressors, and integrating multiple physiological systems into the analyses. Notably, these results may have important clinical implications, suggesting that individuals reporting increased perceived stress may respond more strongly to subsequent stressors. Thus, perceived stress may be important to assess when considering pain sensitivity and physiological reactivity to an acute noxious stressor.

Keywords: Pain sensitivity; physiological reactivity; perceived stress; blood pressure; cortisol; resolvins

DEDICATION

I dedicate this dissertation to my fiercely supportive, intelligent, hilarious, and persevering husband, Thomas Bulls, without whom the completion of this work would have never been possible;

And to my family, none of whom have any idea what I do, but are endearingly proud and enthusiastic about it all the same.

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SPECIFIC AIMS AND HYPOTHESES

There is a growing body of literature that lends support to the relationship of perceived stress and pain perception. However, this relationship is influenced by many factors, including the duration of the stressors and the health of the individual. Additionally, physiological mechanisms underlying this relationship have not been fully characterized. Thus, the current investigation aimed to evaluate relationships between self-reported life stress and subsequent physiological responses to an acute noxious stressor.

Literature considering the relationship of stressors and pain responses is mixed, often depending on the type of stressor evaluated (i.e., work stressor vs. laboratory-induced stressor) and the noxious stimuli used. Research has demonstrated that exposure to acute stressors in healthy participants may result in attenuated pain perception[1,2]. However, this is not consistently reported, and other studies suggest that stressors may exacerbate subsequent pain reports[3–6]. Additionally, in individuals who are exposed to persistent stressors, this relationship may reverse, indicating that perceived stress may serve as a risk factor for increased experimental and clinical reports of pain[7–9]. This suggests that experiencing ongoing life stress may make important changes in one's ability to adaptively modulate pain perception. Indeed, exposure to prolonged stressors results in consistently increased allostatic load, which is believed to diminish endogenous stress regulatory systems[10]. Thus, individuals experiencing ongoing stress may be less

able to effectively modulate acute, incidental pain than their counterparts with less exposure to persistent stressors. Additionally, biomarkers of stressor exposure are not fully understood in this context. However, it is reasonable to suggest that with changes in pain modulation come other physiological changes in systems typically involved in allostasis, including the cardiovascular system, the HPA axis, and anti-inflammatory responses. Though these systems have been studied individually, little is known about systematic physiological reactivity associated with ongoing stress in healthy individuals and how it may be influenced by the experience of pain.

As noted, the aim of this investigation was to characterize relationships among self-reported life stress and subsequent physiological responses to an acute noxious stressor. Within this framework three physiological responses were evaluated: 1) systolic blood pressure, 2) circulating cortisol levels, and 3) a family of pro-resolving factors commonly referred to as resolvins. Specifically, we determined whether individuals who reported higher perceived stress would also report greater pain intensity and unpleasantness in response to an acute noxious stimulus, and whether the higher stress was related to heightened basal cardiovascular and cortisol activity. We also evaluated cardiovascular and cortisol reactivity in response to the acute noxious stimulus. Finally, analyses examining relationships between perceived stress, pain sensitivity, and basal levels of the pro-resolving factor (i.e., resolvins) were conducted. Resolvins are endogenous lipid mediators generated during the resolution phase of acute inflammation, and have been shown to have strong inflammation-resolving actions in animal models[11]. However, this relationship has not been well studied in humans, and the analyses that included resolvins were exploratory in nature. Specifically, one aim of this study was to

test whether circulating resolvin levels in humans are similar to resolvin activity observed in previous studied animal models.

Aim 1: Examine the relationship between perceived life stress and experimental pain sensitivity in a sample of healthy, young adults.

Hypothesis 1: Higher levels of perceived stress will be related to greater pain intensity and unpleasantness ratings during the cold pressor task (CPT).

Aim 2: Determine whether perceived stress is related to resting systolic blood pressure (SBP) as well as pain-induced changes in blood pressure (i.e., reactivity).

Hypothesis 2a: Higher levels of perceived stress will be related to higher resting systolic blood pressure.

Hypothesis 2b: Higher perceived stress levels will predict greater pain-induced systolic blood pressure reactivity even after controlling for resting blood pressure.

Hypothesis 2c: Higher ratings of intensity and unpleasantness during the CPT will partially mediate the relationship between perceived stress and systolic blood pressure reactivity.

Hypothesis 2d: Higher perceived stress will relate to lack of systolic blood pressure recovery, such that systolic blood pressure reactivity stays elevated after the completion of the task.

Aim 3: Examine whether perceived stress is related to basal activity and pain-induced reactivity of the hypothalamic-pituitary-adrenal (HPA) axis as determined by cortisol assessment.

Hypothesis 3a: Higher levels of perceived stress will be related to higher basal cortisol assessed prior to the painful CPT.

Hypothesis 3b: Higher perceived stress levels will predict greater pain-induced cortisol reactivity even after controlling for basal cortisol.

Hypothesis 3c: Higher ratings of intensity and unpleasantness during the CPT will partially mediate the relationship between perceived stress and cortisol reactivity.

Aim 4: Evaluate the relationships among perceived stress levels, pain sensitivity, and basal circulating levels of resolvins. In order to accomplish this aim, we will examine resolvins in blood samples taken before the CPT.

Hypothesis 4a: Higher levels of perceived stress will be related to higher resolvin levels.

Hypothesis 4b: Greater reported pain intensity and unpleasantness during the CPT will be related to higher resolvin levels.

BACKGROUND AND SIGNIFICANCE

Stress and Pain: A Systems Perspective

The concept of stress has been evaluated for decades, from both biological and social science perspectives. Indeed, the term "stress" is used in typical daily conversation as well as scientific literature without a clear operational definition. People often complain of feeling "stressed" but describe very disparate stressors, emotions, and physical sensations related to this perception. Similarly, definitions of stress vary depending on the field examining it. Though originally defined as a "non-specific response of the body to any demand for change"[12], the concept of stress and subsequent physiological and psychological responses has become increasingly complex. Stressors on the human system can include psychosocial events, physiological insults, and changes in environmental conditions, among others, and can range from very simple to increasingly complex situations. Two types of stressors are of interest in this study. The first type of stress is perceived life stress, as measured via the stress subscale of the Depression Anxiety Stress Scale 21 (DASS21[13]) described below. The DASS21-Stress evaluates perceived stress through self-reported ratings of subjective arousal. Questions on this measure assess agitation, irritability and over-reactivity, as well as difficulty relaxing. The second type of stressor to be assessed is exposure to an acute noxious stimulus in the laboratory. The noxious stimulus is the cold pressor task (CPT) in which participants are asked to submerge their hand in cold water for up to one minute at a time.

During the immersion, participants provide subjective ratings of pain intensity and pain unpleasantness. We suggest that these stressors are related, such that increased perceived life stress will correlate positively with more intense and unpleasant subjective ratings during the CPT. Such a relationship would indicate that perceptions of chronic life stressors influence the way that individuals respond to subsequent stressors, even if the nature of those subsequent stressors is different (e.g., acute, fleeting, and/or controllable)[14].

Indeed, perhaps one of the most universally applicable examples of a stressor is pain, which provides the rationale for use of the CPT as a laboratory-induced stressor. The experience of pain is not a passive perceptual phenomenon, but one that can dramatically affect the body's homeostatic regulation systems[15]. Interactions between exposure to stressors and pain have been examined for over half a century[16–18]. Interestingly, different relationships have been found depending on the participant group used and the type of stressor experienced (i.e., whether the stress was induced in the laboratory or elsewhere). In healthy adults, efforts have been devoted to examining the relationship between stressor exposure and subsequent responses to brief, noxious stimuli, such as the CPT. The results of these efforts have been somewhat mixed, suggesting that the relationship between exposure to acute laboratory-induced stressors and pain perception is more complex than originally thought. For instance, findings have emerged showing that healthy humans demonstrate attenuated pain responses after they have been acutely stressed[1,2]. However, at least one study showed that exposure to psychological stressors significantly increases pain intensity during an experimental heat summation stimulus while simultaneously reducing the participants' ability to adaptively

cope[3]. Additionally, psychological and cognitive consequences of stress exposure may actually exacerbate pain perception[4–6]. Consequently, stress loads may carry important implications for subsequent health. It has been suggested that exposure to chronic stressors may relate to the development of many painful conditions, including fibromyalgia[7], chronic low back pain[9], and chronic pelvic pain[8].

Undergoing an acute stressor is typically associated with widespread physiological changes that constitute the 'stress response'[19–23]. The body is in a constant, active process of responding to environmental stressors in order to maintain homeostasis – a construct called "allostasis"[24]. Allostasis is the physiological "protective, coordinated, and adaptive reaction" that ensures interdependent processes responding to stress remain within normal limits[25]. Mounting an allostatic response to a stressor involves mobilization of many varied internal resources to meet the stressful challenge. When a stressor persists for a long period of time, or when repeated stressors occur in rapid succession, allostasis may burn resources faster than the body can replenish them. This cost to the body of allostatic adjustment is called "allostatic load"[25]. Exposure to prolonged stressors results in consistently increased allostatic load, which is believed to diminish endogenous stress regulatory systems and predispose individuals to a number of physical and mental health conditions[10,26–28]. This suggests that exposures to stressors exert a powerful influence on multiple physiological systems, likely those systems typically involved in the adaptive return to homeostasis but which may become impaired after repeated or prolonged stress exposures.

Despite the strongly suggested link between stressor responses and pain, the physiological mechanisms underlying this relationship have not been fully characterized.

Simple causal relationships are ineffective in explaining the complex physiological responses to perceived stress, suggesting that multiple systems should be considered in concert. The fact that the human body is a complicated, multifaceted system involving many co-occurring responses doubtlessly influences this gap in our collective knowledge. However, there have been many efforts to identify key physiological systems that may mediate the effect of perceived stress on pain perception. Systems involved in this relationship include the HPA axis (particularly changes in circulating levels of cortisol)[29], cardiovascular responses such as change in systolic blood pressure[30,31], and release of inflammatory cytokines (e.g., IL-6)[32,33]. Different basal levels of stress may affect both the baseline presentation of these physiological systems (i.e., resting blood pressure) in the laboratory as well as their potential for reactivity in response to noxious stressors.

When examining physiological responses to noxious stimuli, multiple phases of the stress response can be evaluated for adaptive reactions. Baseline physiology may be important because perceived life stress is ongoing, not instigated in the laboratory. Thus, differences may exist between high- and low-stress individuals at the time of study entry that influence subsequent physiological responses to stressful situations. Secondly, reactivity may be important as it characterizes the magnitude of the stress response during the noxious stimulation. It is suggested that increased physiological responses would indicate a system preemptively prepared to react strongly to noxious experiences. Finally, recovery of the physiological system to baseline following the noxious event allows evaluation of the individual's return to homeostasis. Failure to return to baseline is termed "dysfunctional recovery"[25] and has been related to increased health complications in both the HPA axis[34] and the cardiovascular system[35–38].

The HPA Axis: Cortisol

Many researchers have devoted considerable attention to the HPA axis and its role in allostasis. This hormonal response system can be activated by a broad range of physiological and psychological stressors and has a direct influence on the secretion and release of cortisol. Cortisol plays a powerful role in the stress response because of its widespread influence on a number of systems including the central nervous system, where it influences learning, memory, and emotion through limbic structures; the metabolic system, where it regulates glucose storage, regulation and utilization; and the immune system, where it initiates and regulates a number of inflammatory responses[29]. A sustained level of elevated cortisol elicited by chronic stressors is thought to break down muscle, bone, and neural tissue that predispose the dysregulation of biological systems[15,29].

The relationship of cortisol and stress has been evaluated in a number of contexts, including animals and human participants. Hundreds of studies have specifically investigated the effects of psychological stressors on cortisol activation, but only two main conclusions can be definitively culled from this literature: 1) psychological stressors as well as physiological ones have the ability to activate the HPA axis, and therefore, influence circulating cortisol levels, and 2) the effects of psychological stressors on physiological systems are variable[39]. Certainly, these conclusions seem to negate early theories that stress responses are non-specific and respond equally to all psychological and physiological insults[40]. However, the question then remains: what is it that causes different responses to various psychological and physiological stressors? Some have

suggested focusing on the specific characteristics of the stressor, such as whether the situation causing stress is novel[41], unpredictable[42], uncontrollable[43,44], or threatening, with the potential for harm or loss[45,46]. While no definitive answer emerges, reviews have concluded that tasks containing both uncontrollable and socially evaluative elements often result in the largest cortisol hormone changes as well as the longest recovery times necessary to return to baseline[39]. Interestingly, the exposure to a laboratory stressor that produces pain might often involve all of these exacerbating characteristics.

Despite years of attention to cortisol and stress, the influence of cortisol on pain perception, particularly in healthy adults reporting high subjective levels of stress, has not been widely studied. Of the limited research that exists, it appears that increases in cortisol after noxious stimulation may be associated with pain reduction in healthy adults[47]. Additionally, a preliminary report in 2002 suggested that greater cortisol concentrations assessed during rest prior to a cold pressor test (CPT) predicted lower pain reports during and after the task in healthy men[48]. This seems to indicate that stress-related increases in cortisol concentrations may contribute to attenuated pain perception when experiencing acute stress. However, when investigating this relationship while adding additional stressors, results become complicated. The same group examined cortisol in the context of a CPT that followed a laboratory-induced stress task. Blood samples were obtained for cortisol measurement at 5 timepoints: after an initial rest period, after the stressful task (public speaking), after a subsequent rest period, immediately following a CPT, and approximately 20 minutes after the CPT. They found that cortisol responses did not mediate the relationship between stress effects and pain ratings[49]. It is clear that some sort of link exists between cortisol, stress, and pain.

However, it is not as certain whether baseline and/or reactive levels of cortisol play a role in subsequent reports of pain perception, or that those relationships differ based on perceived stress experienced outside of the laboratory. To date, it does not appear that anyone has specifically assessed the relationship between perceived life stress levels with reports of pain during a noxious stimulus in healthy young adults without induction of acute stress (i.e., the Trier Social Stress Test) in the laboratory procedures, nor have cortisol levels been examined for a potential role in this relationship.

The Cardiovascular System

Another physiological system that has been linked to stress and the experience of pain is the cardiovascular system. In particular, blood pressure has been evaluated in its relationship to stress, whether the stressor was induced in the laboratory[30] or by naturally occurring psychosocial stressors[31]. It has been suggested that cardiovascular reactivity to stressful environmental stimuli may share mechanisms with systems of pain modulation, possibly including baroreceptor neural pathways and endogenous opioid systems[50,51]. The coordination of these systems may act to reduce the negative impact of the stressor. Specifically, systolic blood pressure has been found to mediate the relationship between laboratory-induced stress and pain perception following a cold pressor task in healthy adults[49]. Thus, for this study, systolic blood pressure will be used as an indicator of cardiovascular reactivity to the noxious stimuli.

Much like cortisol, the relationship of blood pressure to pain often depends on the context in which it is measured. For example, cardiovascular reactivity to a stressful task has been demonstrated to relate to subsequent pain sensitivity in healthy adults[5]. In

contrast, several other groups have demonstrated that both resting blood pressure and blood pressure reactivity to stress are inversely related with pain sensitivity, also in healthy adults[52–55]. Though many studies use the CPT as a task of noxious stress, one group suggests that beta-adrenergic influences were either minimal or quickly resolved under conditions where control was perceived to be out of the participant's hands, including a CPT exposure[56]. Thus, the literature is mixed in regard to when and how cardiovascular function might affect subjective pain experiences. Often it appears that the stressors are induced in the laboratory, limiting our ability to extend these findings to healthy participants undergoing perceived life stress. In particular, it is unknown how higher blood pressure at baseline or during a noxious stressor may influence pain perception in individuals experiencing higher levels of life stress outside of the laboratory setting. It's also possible that those with greater levels of stress may experience more dysfunctional recovery, such that their blood pressure would remain elevated long after the conclusion of the aversive stimulus.

Interestingly, exaggerated blood pressure reactions to stressors may predispose one to develop standing hypertension over time in an effort to diminish pain or aversion. It is clear that prolonged exposure to stressors changes the cardiovascular response to aversive stimuli, though not quite as clear how these changes occur. Some studies show that chronic hypertension is associated with decreased sensitivity to pain[57], though there are other studies to suggest that at some point hypertension becomes a risk rather than protective factor for pain perception[58]. Additionally, it has been suggested that dysfunctional recovery, or continued elevation of blood pressure following an aversive event, is a strong indicator of subsequent cardiovascular disease in the future[35–38].

Inflammation, Pain, and Resolvins

Systemic inflammatory processes directly influence pain processing. Work performed in rodents and humans has demonstrated that psychological stress can stimulate production of pro-inflammatory agents[32,33,59]. A review of the literature suggests that a variety of inflammatory markers are likely to play a role in the body's pro-inflammatory response to pain[60]. However, much less is known about how inflammatory pain is resolved. It is clear now that resolution of acute inflammation is not a passive process, but one that requires recruitment of active biochemical processes[11]. One such biochemical implicated in the control of inflammation is resolvins.

Resolvins are endogenous lipid mediators generated during the resolution phase of acute inflammation, and have been shown to have strong inflammation-resolving actions in animal models[11]. Resolvins consist of subgroups including the D-series and E-series that are biosynthesized from the omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), respectively. Biosynthetic enzymes necessary for metabolism of the omega-3 acids to RvD and RvE species have low expression levels in most tissues in non-injured states[11]. This suggests that metabolism of resolvins is not an automatic process, but rather one that occurs in response to an inflammatory event. The analgesic effects of resolvins in animal models have been shown to be mediated by specific receptors widely available in the body, acting by reducing inflammation, glial activity, and spinal cord synaptic plasticity specific to inflammatory pain[11]. There is little existing literature on how resolvins might function in humans, both in relation to perceived stress and in any pain-alleviating capacity they may exhibit. However, it has

been suggested that resolvins have therapeutic potential in the prevention and treatment of inflammatory disorders. Without pro-resolvin factors, the return to homeostasis following an injury or infection is hampered and chronic inflammation can occur. Chronic inflammation has been linked to a variety of serious health concerns, some of which overlap with risks of other systems in this study, such as cardiovascular disease and cancer[24,61]. Despite the lack of human models at this time, it is suggested that resolvins may function as part of a pain response system in concert with the cardiovascular system and/or HPA axis. The study of resolvins in relation to pain and perceived life stress in humans is a novel addition to the field. Importantly, evaluation of new markers of inflammatory resolution (i.e., resolvins) in conjunction with more well-known biomarkers, such as cortisol, allows a new and integrative investigation into systemic responses to stressors.

Clinical Significance

Theoretical models have proposed that repeated stressful experiences might elicit lasting negative physiological changes, which result in chronic health concerns. Indeed, higher allostatic load is associated with worse health outcomes[62]. Specifically, extended HPA activation could lead to a wide array of negative physiological changes that can have long-term health effects[24,46], including diabetes, hypertension, cancer, and cardiovascular disease. One suggested mechanism for this phenomenon is repeated activation of the HPA system as a result of frequent stressor exposure. A failure to shut down the response after stressor termination may be another potential mechanism[39]. Additionally, clinical evidence is mounting for specific effects of stress on cardiovascular

systems[24]. For example, it is typical for blood pressure to rise and fall throughout a normal day. However, repeatedly elevated blood pressure – as in the event of a stressor – may increase atherosclerotic plaques and stiffness of large arteries leading to greater risk of cardiovascular disease[63,64]. Finally, inflammation is widely associated with various clinical pain conditions, including post-operative pain, low back pain, cancer pain, temporomandibular joint disease, and arthritis, among others[65]. There is also a possibility that fibromyalgia and irritable bowel syndrome are related to chronic ongoing and subthreshold inflammation[11]. Finally, chronic inflammation due to unresolved infection or injury can result in many health conditions such as atherosclerosis, asthma, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, and obesity, among others[61], suggesting an important role for pro-resolving factors in maintaining health.

In each case, sustained physiological responses tested in this study indicate greater likelihood for the development of chronic, disabling conditions. As noted above, dysregulation of the individual systems often share increased risk for certain conditions, such as cancer or cardiovascular disease. It should not be lost that many of these disorders come with high risk of ongoing, unremitting pain and potentially serious implications for one's mortality. Were these phenomena to be linked to ongoing stress early on in otherwise healthy individuals, it is possible that a group of individuals at heightened risk for development of stress-related health conditions could be identified. Additionally, if this hypothesis were to be found true, early interventions in stress management for these individuals might curb subsequent risk of such health conditions.

Summary

It has been repeatedly demonstrated that stress alters the pain experience in a variety of ways. Additionally, stress may influence multiple physiological systems involved in allostasis and allostatic load, including the HPA axis, the cardiovascular system, and inflammation resolution processes. Although the interdependent relationships between these systems are complicated, perceived ongoing stress may be an especially important psychosocial variable to study because it occurs frequently in everyday life and has implications for long-term health outcomes. Indeed, investigators have posited that perceived stress leads to changes in one's ability to modulate pain effectively, and physiological reactivity to noxious stressors may be an illustration of that phenomenon. Identification of relationships between perceived stress, physiological reactivity, and increased pain sensitivity may allow further investigation into a group at risk for development of chronic health conditions, including chronic pain.

To our knowledge, the relationship between perceived stress, multiple biomarkers of physiological reactivity, and pain sensitivity has never been specifically examined. Thus, the proposed investigation determines if a link between perceived stress and physiological activity following a noxious stimulus exists. To do so, we evaluated both basal physiology and the magnitude of physiological reactivity. We suggest that these physiological responses typically act in concert to alleviate stress and return to homeostasis, and that higher perceived stress may impair one or all of these systems from reacting adaptively. Then, we examined whether that relationship can be partially explained by pain intensity and unpleasantness reported during the noxious task. Finally,

an exploratory aim was conducted to identify any relationship between a pro-resolution biomarker, resolvin, and stress levels and/or pain sensitivity

RESEARCH DESIGN AND METHODS

The current study is part of a larger investigation completed in 2014 that aimed to enhance the understanding of the role of minority aging in endogenous pain modulation[66]. The initial study compared the pain report of a young, healthy cohort with older counterparts from the Understanding Pain and Limitations in Osteoarthritic Disease study (UPLOAD) conducted at the University of Alabama at Birmingham (UAB). For the purposes of these analyses, only the younger group of participants was examined. The cohort consists of 50 healthy adults with no chronic health conditions, aged 19-34. Participants were selected to be approximately half female (52%) and half African-American, half non-Hispanic white.

Participants

Participants were recruited via posted fliers around the UAB campus and word-of-mouth referral. All procedures were reviewed and approved by the UAB Institutional Review Board. Potential participants provided informed consent and were compensated for their participation. Interested parties were screened with a Health History questionnaire via phone to ensure that inclusion criteria were met. These criteria included age between 19 and 35 years, self-reported ethnicity of African-American or non-Hispanic white, and absence of comorbid conditions, with the most important considerations involving no evidence of: 1) uncontrolled high blood pressure or heart

disease; 2) a chronic or acute pain condition; 3) decreased peripheral sensitization; and 4) a diagnosed and/or medicated psychiatric disorder. Other rule-outs included history of seizures, severe eczema, rheumatic disease, or any other chronic medical condition that may interfere with typical pain processing. If all criteria were satisfied, the participant was scheduled for the testing session at the Clinical Research Unit at UAB.

Procedures

A flow diagram depicting the progression of the study session is presented in Figure 1.

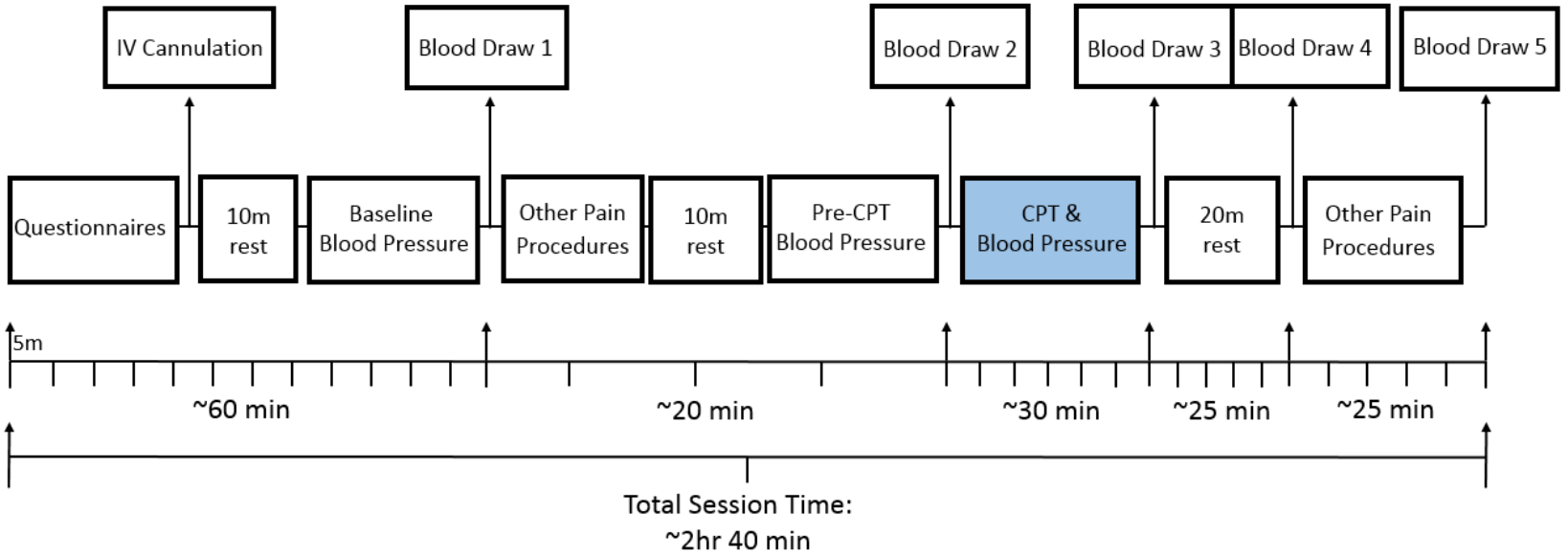

Figure 1: Overview of Study Procedures

Questionnaires

Prior to completing any pain testing, the participants completed pencil-and-paper questionnaires in the Clinical Research Unit at UAB. The following demographic and health data were obtained: self-reported sex, age, ethnicity, years of school completed, smoking status (e.g., non-smoker, occasional, or daily smoker), corticosteroid use, and height and weight for BMI calculation. For this investigation, responses on the Depression, Anxiety and Stress Scale – Stress Subscale (DASS21-Stress) and the Pain Anxiety Symptoms Scale (PASS-20) were assessed in relation to pain sensitivity. The DASS21-Stress subscale served as the measure of perceived stress, describing the participant's responses to their life stress (agitation, irritability, etc.). In contrast, the PASS-20 was used to account for pain-related anxiety, which encompasses specifically pain-related (or, in this case, laboratory-related) fear, unease, and/or worry about the session itself.

Depression, Anxiety, and Stress Scale

The short form of the Depression Anxiety Stress Scales (DASS21) is a 21-item self-report instrument designed to measure the three related negative emotional states of anxiety, depression, and stress[13]. A separate scale is derived for anxiety, depression, and stress. Scales are divided into 7 items each, in which the participant is asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week. For the purposes of this study, the DASS21-Stress subscale was used. Questions on the DASS21-Stress subscale include "I felt that I was rather touchy", "I tended to over-react to situations", or "I found myself getting agitated", among others.

The DASS21 scales have been shown to have high internal consistency as well as meaningful discriminatory power in a variety of settings.

Pain Anxiety Symptoms Scale (PASS-20)

The short form of the Pain Anxiety Symptoms Scale (PASS-20) is a 20-item questionnaire that is administered to assess pain-related anxiety responses[67]. The PASS-20 evaluates pain-specific anxiety symptoms with four 5-item subscales, including cognitive anxiety responses, escape and avoidance, fearful thinking, and physiological anxiety responses. All items are rated on a frequency scale from 0 (never) to 5 (always). The short form of the PASS maintained good internal consistency, criterion validity, and construct validity when compared to the long form PASS. The PASS-20 was used as a covariate in analyses to account for pain-related fear and stressors that may be unique to the laboratory setting, but that do not reflect perceptions of life stress (as measured by the DASS21-Stress).

Quantitative Sensory Testing (QST)

During this session, a number of standard pain testing procedures were performed prior to the CPT (described below), including: heat pain thresholds and tolerances assessed at the knee and ipsilateral forearm; pain intensity ratings in response to repetitive 44˚, 46˚, and 48˚ C thermal heat pulses at the knee and ipsilateral forearm; pressure pain thresholds at the knee, forearm, trapezius, and quadriceps; and pain intensity ratings in response to single and repetitive punctate mechanical stimuli applied at the knee and hand. For the purposes of this study, only the CPT was assessed in

relation to cortisol because the CPT has been shown to be the best quantitative sensory testing (QST) modality for stimulating physiological reactivity in both the cardiovascular and HPA systems. There is evidence, for example, that the CPT elicits significant cortisol reactivity[47,48] as well as elevated systolic blood pressure[49]. Though it is unclear whether a CPT will affect resolvin reactivity, it is reasonable to use this task to establish initial relationships with resolvins and noxious stimuli given its effect on other physiological systems. Additionally, though other pain tasks were performed during the study session, the CPT is the only task in which blood draws occurred directly before, immediately after, and at a short time later (20 minutes), allowing evaluation of baseline, pre-stimulus, immediate post-stimulus, and delayed change in physiological systems. This is particularly important for cortisol measurement, as maximum circulating cortisol change can take up to 20 minutes after stressor exposure. Additionally, the CPT is the only task that involves pre-, during-, and post-stimulus blood pressure readings. Thus, the CPT allows comparison of multiple physiological systems due to the timing of blood pressure readings and blood draws that would not be possible with any other task in the session.

Cold Pressor Task

Trials were assessed at 16, 12, and 8 degrees Celsius. For each immersion trial, the participant was instructed to place their right hand up to the wrist in the water bath while avoiding touching the bottom and sides of the metal basin. The participants were informed that they were able to remove their hand from the cold water if the stimulus was too painful for them to endure. At the completion of the CPT (60 seconds, or at time of

withdrawal) the participant was prompted for ratings of pain intensity and pain unpleasantness on the 0-100 scale.

Biomarkers & Technical Issues

Blood was drawn at 5 different times throughout the session: 1) at the beginning of the session, prior to any pain testing; 2) immediately before the CPT; 3) immediately following the CPT; 4) 20 minutes after the end of the CPT; and 5) at the very end of the session, following subsequent pain testing. At each timepoint, plasma was immediately isolated and stored at -80C. Additionally, plasma aliquots for resolvin measurements were treated with BHT and overlaid with Ar gas to prevent fatty acid oxidation. A depiction of the entire study can be seen in Figure 1, while the timeline for cortisol blood draws can been seen in Figure 2. Resolvin extractions from plasma were performed by Avanti Polar Lipids (Alabaster, AL). Plasma cortisol and resolvin type D1 and D2 assays were performed by Dr. Barbara Gower's laboratory (UAB Department of Nutrition Sciences, Division of Physiology & Metabolism) using commercially available ELISA kits, which allow quantitative analyses of circulating biomarker levels in the blood. Plasma cortisol was assayed on a TOSOH 600 II analyzer using the immunofluorescence method (TOSOH Bioscience – South San Francisco, CA). For resolvin assays, ELISA kits were obtained from Cayman Chemical (Ann Arbor, MI). The RvD1 kit has a range from 3.3-2,000 pg/mL and a sensitivity (80% B/B_0) of approximately 15 pg/mL. The RvD2 kit has a range from 1.6-1,000 pg/mL and a sensitivity (80% B/B_0) of approximately 10 pg/mL.0

Previous studies have shown that the response magnitude and time course of salivary cortisol levels after awakening are significantly related to various psychological and physical conditions (e.g., pain and stress[68–70]). Additionally, cortisol follows a diurnal rhythm throughout the day, suggesting that taking samples at different times throughout the day might complicate comparisons amongst participants. Thus, the start time for each session was standardized within 1-2 hours across participants to minimize cortisol variability due to time of day (starting 11am -12:30pm).

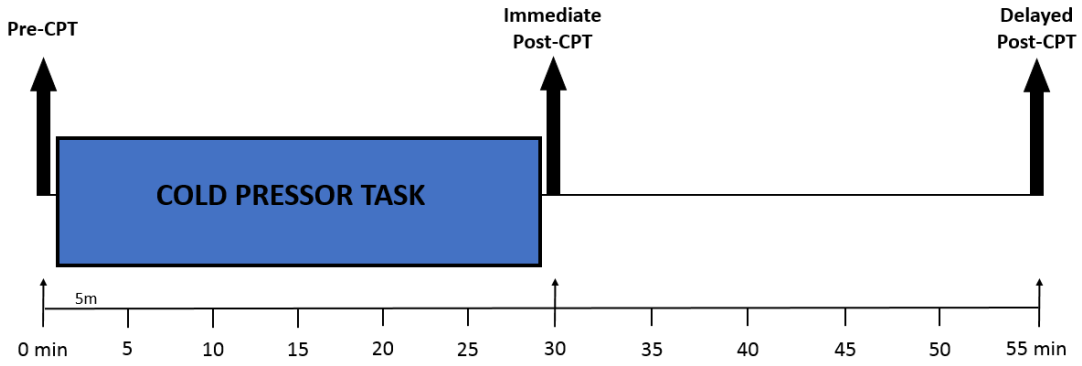

Figure 2: Cortisol Timing

Cardiovascular Measurement

The timeline of blood pressure readings relevant to the study can be found in Figure 3. At the beginning of the session, the participant's baseline blood pressure and heart rate was taken using a digital sphygmomanometer. The same device was used to take blood pressure during the CPT, as well as recovery blood pressures at 1, 3, and 5 minutes after the completion of the CPT. For the purposes of this study, blood pressure was analyzed as either "baseline" (before any CPT pain is applied) or "reactivity"

(change between baseline and during-CPT blood pressure). To determine systolic blood pressure reactivity, area under the curve (AUC) calculations was performed comparing pre-CPT SBP to during-CPT SBP, while controlling for baseline SBP. This calculation yielded two variables, AUC with respect to ground (AUCg) and AUC with respect to increase (AUCi). AUCg represents total cortisol magnitude, or the overall response, while AUCi characterizes the increase or pattern of response over time[71].

Additionally, a measure of blood pressure recovery was assessed using similar AUC calculations. The recovery period included SBP readings during the CPT as well as 1, 3 and 5 minutes following the conclusion of the CPT. AUCg and AUCi calculations were both performed for this recovery period.

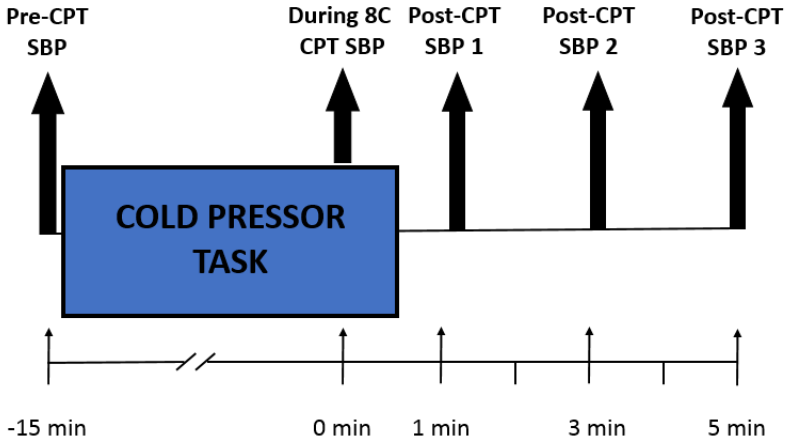

Figure 3: Systolic Blood Pressure Timing

Cortisol Measurement

At the beginning of the study session, a clinical research unit (CRU) nurse placed an intravenous catheter in the arm opposite the arm used for sensory testing. As

previously mentioned, blood was drawn at five separate time points throughout the study session (see Figure 1). For the purposes of this study, plasma cortisol was analyzed at four different time points: baseline, immediately prior to the CPT (hereafter referred to as "pre-CPT"), immediately following the CPT (referred to as "immediate post-CPT"), and approximately 20 minutes after the CPT (hereafter referred to as "delayed post-CPT"). To determine cortisol reactivity, AUC calculations were performed comparing pre-CPT cortisol levels to immediate post-CPT and delayed post-CPT, while controlling for baseline cortisol. AS with the SBP calculations, this procedure yielded both AUCg and AUCi levels. Baseline cortisol levels may be confounded by anxiety and IV stick, so for AUC calculations, pre-CPT was used as the initial cortisol level for comparison to the subsequent responses.

Resolvin Measurement

Resolvin samples were collected at the baseline blood draw of the study session. Resolvins D1 (RvD1) and D2 (RvD2) were both assayed via ELISAs. RvD1 and RvD2 are derived from docosahexaenoic acid (DHA) and each have unique stereochemical assignments differentiating them[72]. Prior research in animal models indicates that RvD1 and RvD2 have similarities in function as anti-nociceptive, anti-inflammatory, and pro-resolving agents; however, their exact mechanisms via G-coupled protein receptors may be unique[73]. Both were included in this study in an effort to characterize resolvin D pathways.

As of now, there are minimal existing data regarding resolvin responses to noxious stimuli among humans. No human literature exists suggesting a timecourse for

resolvin responses or resolution following an experimental noxious task. Because there is not yet a theoretical basis suggesting any directional relationships between stress, experimental pain sensitivity, and resolvin reactivity, analysis of plasma resolvin levels was limited to the baseline timepoint.

Data Analysis

There were two outcome variables (ratings of pain intensity, ratings of pain unpleasantness) obtained during each cold water exposure for a total of 6 potential outcome variables between all three temperatures assessed. However, for the proposed project, we focused on pain ratings obtained during the 8˚ C cold water immersion because 1) this temperature likely leads to greater physiological reactivity than the other two temperatures, and 2) investigators using a single cold water exposure demonstrate significant cortisol reactivity approximately 15 minutes after the CPT[47,48]. Therefore, post-CPT cortisol levels in the proposed investigation are most likely reflective of cortisol responses to the 8˚ C cold water immersion. Additionally, pain unpleasantness ratings served as a primary dependent variable associated with the affective-motivational aspect of pain, whereas pain intensity ratings are associated with sensory-discriminative aspect of pain[74]. This was of interest when evaluating which, if any, differences exist in physiological reactivity and pain experiences. Thus, there were a total of two primary dependent variables, pain intensity and pain unpleasantness ratings, during the 8˚ C cold water immersion for the proposed investigation.

The following demographic variables were statistically controlled for: age, gender, education, and pain-related anxiety (as measured by the PASS-20). Education

was categorically coded by highest level completed (1 = High School, 2 = 2-year degree, 3 = 4-year degree, 4 = MA). The PASS-20 was included to account for any pain-related anxiety specifically related to the study session that may influence self-reported pain and/or physiological reactivity during the session, rather than a broad measure of perceived stress as demonstrated by the DASS21-Stress. Multicollinearity was assessed for all study variables. In addition, we controlled for smoking status (0 = not a current smoker; 1 = current smoker), use of corticosteroid medications (0 = no corticosteroid medication use; 1 = current use of corticosteroid medications) and BMI, as these variables are known to interact with physiological reactivity, particularly cortisol[39,75].

Cohen's f^2 effect sizes are presented where appropriate following the conventions of Cohen for tests of linear relationships[101]. Per Cohen's guidelines, $f^2 = 0.02$ (or 2% predicted variance) is considered a small effect, $f^2 = 0.15$ (15% predicted variance) a medium-sized effect and $f^2 = 0.35$ (35% predicted variance) a large effect. All data was analyzed using SPSS, version 22 (IBM; Chicago, IL).

All analyses were first inspected without adjusting for covariates followed by inspection of fully adjusted models. However, only adjusted analyses were used to test hypotheses.

Aim 1

Hypothesis 1. Linear regression models were used to determine the predictive utility of perceived stress for ratings of pain intensity and unpleasantness during the 8˚ C cold water immersion.

Aim 2

Hypothesis 2a. Linear regression models were used to determine the predictive utility of perceived stress for higher baseline systolic blood pressure.

Hypothesis 2b. Linear regression models were used to determine the predictive utility of perceived stress for higher systolic blood pressure reactivity as assessed during the 8˚ C cold water immersion, while controlling for baseline blood pressure readings.

Hypothesis 2c. The bootstrapping technique and macro created and described by Preacher and Hayes[77] was used to test whether the relationship between stress and systolic blood pressure reactivity during the CPT is partially mediated by subjective pain ratings of intensity and/or unpleasantness. Bootstrapping is a nonparametric resampling procedure that has been shown to be a viable alternative to other normal-theory tests of the intervening mediator between the independent and dependent variable[78]. This analysis provides advantages to other mediational approaches, particularly as it does not violate standards of normality. Additionally, this particular analysis is recommended for studies with smaller sample sizes as it is more powerful than some of the alternative analyses. A 95% confidence interval was obtained to help minimize potential Type 1 error related to the test of mediation[79]. As described above, pain intensity and unpleasantness during the 8˚C CPT were used, resulting in 2 separate mediational analyses. Additionally, because pre-CPT blood pressure may be influenced by previous pain testing, basal systolic blood pressure levels were added as an additional covariate when assessing systolic blood pressure reactivity in this model.

Hypothesis 2d. Linear regression models were used to determine the predictive utility of perceived stress for SBP recovery, defined as the change in blood pressure from during the CPT task to five minutes post-CPT (using AUC measures).

Aim 3

Hypothesis 3a. Linear regression models were used to determine the predictive utility of perceived stress for higher basal cortisol levels.

Hypothesis 3b. Linear regression models were used to determine the predictive utility of perceived stress for higher cortisol reactivity as assessed during the 8˚ C cold water immersion, while controlling for baseline cortisol level

Hypothesis 3c. The same bootstrapping technique described above was used to determine if subjective pain ratings of intensity or unpleasantness mediate the relationship between perceived stress and cortisol reactivity during the 8˚ C cold water immersion. Similarly, basal levels of cortisol were added as a covariate in analyses assessing cortisol reactivity in this model.

Aim 4

Hypothesis 4a. Due to the exploratory nature of this aim, Pearson correlations were performed to evaluate the relationship of perceived stress and basal resolvin levels.

Correlations were chosen to establish initial relationships of stress and resolvins, as there is not yet a theoretical basis from the literature that guides further analysis.

Hypothesis 4b. Similarly, Pearson correlations were performed to evaluate the relationship of pain intensity and unpleasantness with basal resolvin levels.

RESULTS

Characteristics of the 50 healthy adults who participated in the study are presented in Table 1. The mean age of the group was 23.68 (±4.15), with an approximately equal number of men and women (52% female).The ethnic representation in the group was equally divided between NHW and AA participants (50% each). The mean body mass index (BMI) was 27.20 (±7.59). Neither acute pain in the two weeks before the study session or a history of chronic pain was endorsed by any of the participants. None reported taking any prescribed or over-the-counter analgesics or corticosteroid medications prior to the study session. All participants reported obtaining a high school diploma, with 56% reporting higher educational attainment. A small percentage of the study sample reported smoking cigarettes "some days" (10%), while none reported smoking cigarettes daily.

Responses to psychological questionnaires are also presented in Table 1. Scores on the DASS21-Stress subscale, an index of perceived stress, ranged from 0-14 with a mean of 4.18 (±3.62). Scores on the PASS-20, which measures pain-related anxiety responses, ranged from 1-66, with a mean score of 26.10 (±17.94). The bivariate correlations of the DASS21-Stress and covariates with outcome variables are presented in Table 2.

Table 1. Descriptive sample characteristics

N	50
Gender (%F)	26 (52%)
Ethnicity	
Non-Hispanic White	25 (50%)
African-American	25 (50%)
Age (Yrs)	23.68 (4.15)
Education	
High School	22 (44%)
2-yr Degree	4 (8%)
4-yr Degree	18 (36%)
MA	6 (12%)
Body Mass Index	27.20 (7.59)
Smoking	
"Every Day"	0 (0%)
"Some Days"	5 (10%)
"Not at All"	45 (90%)
DASS21-Stress	
Mean (SD)	4.18 (3.62)
Range	0 – 14
PASS-20	
Mean (SD)	26.10 (17.94)
Range	1 – 66

Note:
DASS21-Stress: Depression, Anxiety, and Stress Scale (21-item)
PASS-20: Pain Anxiety Symptoms Scale (20-item)

Table 2: Correlations between perceived stress, physiological measures, and covariates

	Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	DASS21-Stress N=50																	
2	Gender N=50	-.108																
3	Ethnicity N=50	.050	.000															
4	Age N=50	.293*	.110	-.224														
5	Education N=50	.272	.030	-.536±	.689±													
6	PASS-20 N=50	.417±	.217	.066	.270	.216												
7	Smoking N=50	-.039	.080	-.067	-.058	.167	.133											
8	BMI N=50	.127	.055	.340*	.205	-.212	.169	-.125										
9	Pain Int N=49	.371±	.059	.105	-.037	-.099	.310*	.019	.106									
10	Pain Unpl N=49	.340*	.099	.048	.066	.050	.263	-.006	.106	.922±								
11	Baseline SBP N=50	.232	-.216	.223	.114	-.080	.214	-.025	.606±	.143	.065							
12	AUCg – SBP N=50	.196	-.432±	.202	-.128	-.254	.044	.002	-.395*	.145	.078	.750±						
13	AUCi – SBP N=50	.061	-.114	-.211	-.300*	-.031	-.171	.291*	-.333*	.035	.093	-.339*	.142					
14	AUCg – Rec N=50	.232	-.317*	.160	-.115	-.228	.022	.096	.423±	.255	.185	.729±	.855±	.195				
15	AUCi – Rec N=50	-.021	.269	.093	.260	.079	.101	-.109	.256	.099	.058	.167	-.381±	-.722±	-.051			
16	Baseline Cort N=49	-.029	-.079	-.407±	.025	.345*	-.135	.235	-.333*	-.236	-.174	-.205	-.104	.224	-.132	-.196		
17	AUCg – Cort N=47	-.002	-.142	-.282	.112	.226	-.068	.141	-.412±	-.165	-.097	-.201	-.007	.269	-.146	-.359*	.791±	
18	AUCi – Cort N=47	.147	-.162	.297*	.092	-.118	.220	-.025	.091	.069	.000	.211	.223	.003	.134	-.127	-.445±	-.091

± = $p<0.01$, * = $p<0.05$

DASS21-Stress = Stress Subscale of the DASS21; BMI = Body Mass Index; Pain Int = Pain Intensity; Pain Unpl = Pain Unpleasantness; SBP = Systolic Blood Pressure, AUCg = Area under the curve with respect to ground; AUCi = Area under the curve with respect to increase; Cort = Cortisol; Rec = Recovery

Each of the 50 participants completed the CPT in its entirety. However, upon inspecting the data during assumptions testing, one significant outlier became clear with a rating of 8/100 for intensity. This rating was > 3 standard deviations below the mean CPT intensity rating. After further inspection, this participant was found to possess multiple data inconsistencies pertaining to the CPT pain ratings. Due to concerns about data validity, this participant was removed from all analyses including subjective rating data using the 0-100 scale (e.g., ratings of pain intensity and unpleasantness). Additionally, the height machine at the CRU was malfunctioning for one participant and their height could not be recorded. Thus, the single missing BMI data point for this participant was estimated using mean imputation. Finally, due to difficulties with blood collection in some participants, three participant did not have complete cortisol data. One individual was not able to give blood at any timepoint, with two more only giving baseline blood samples. Only 40 of the 50 participants were able to donate blood for resolvin analysis due to delays in storage appropriate for these assays. Of these 40, ultimately 25 samples were included in resolvin assays due to technical errors resulting in dropouts. Technical errors in this case involved erroneously combining multiple timepoints of plasma into one sample per participant, negating our ability to make inferences about the initial timepoint alone. Valid sample sizes for each analysis have been included in the relevant tables.

Additional assumptions testing revealed that ratings of pain intensity, pain unpleasantness, and baseline cortisol violated the assumption of normality (Shapiro Wilks < .001). These violations were not resolved by logarithmic transformation; thus, non-transformed data were included in analyses for these measures. Additionally, while logarithmic transformation did resolve violations for the baseline SBP and SBP area under

the curve with respect to ground measures (Shapiro Wilks > 0.05), use of transformed data did not result in statistically different results; thus, for ease of interpretation, non-transformed data were used in these analyses.

Aim 1

Perceived stress was significantly correlated with both pain intensity ($r(47) = .371$, $p = .009$) and pain unpleasantness ($r(47) = 340$, $p = .017$). Bivariate correlations can been seen in Table 2. As shown in Table 3, regression analyses including covariates showed perceived stress was a significant predictor of pain intensity ratings at 8° C ($b = 3.21$, $t(40) = 2.36$, $p = 0.023$). However, in adjusted analyses, perceived stress no longer significantly predicted unpleasantness (($b = 2.81$, $t(40) = 1.89$, $p = 0.066$). Due to these results, only pain intensity was examined during subsequent mediational analyses.

Table 3. Regression analysis assessing reported pain intensity and unpleasantness during the 8˚C CPT as a function of perceived stress (N=49).

Dependent Variable	b (SE)	β	t score	p value	f^2
Pain Intensity	3.21 (1.36)	.40	2.36	.023	0.14
Pain Unpleasantness	2.81 (1.49)	.34	1.89	.066	0.09

Note: adjusted for gender, ethnicity, age, education level, BMI, smoking frequency, and PASS20 total score.

Aim 2

Systolic blood pressure measurements over the course of the session can be seen in Figure 4. Perceived stress was not significantly correlated with baseline SBP, SBP reactivity indicated by area under the curve with respect to ground (SBP-AUCg), or SBP reactivity indicated by area under the curve with respect to increase (SBP-AUCi); all p's > 0.05. After adjusting for covariates, regression analyses demonstrated that heightened perceived stress was significantly associated with an increase in SBP-AUCi (b = 13.47, $t(40) = 2.12$, p = 0.04, $f^2 = 0.11$) but not baseline SBP or SBP-AUCg (p's > 0.05; see Table 4). Ratings of pain intensity did not mediate the relationship between perceived stress and SBP-AUCi (a x b = .52, 95%CI -4.92 to 7.33; Figure 5).

Analyses were also performed examining the predictive utility of perceived stress for systolic blood pressure recovery with respect to ground (SBP-AUGg-Recovery) and increase (SBP-AUCi-Recovery). Results trended towards significance when considering SBP-AUCg-Recovery (b = 3.36, $t(40) = 1.96$, p = 0.057; $f^2 = 0.10$); results examining SBP-AUCi-Recovery were non-significant (b = -1.55, $t(40) = -.73$, p = .47; Table 4).

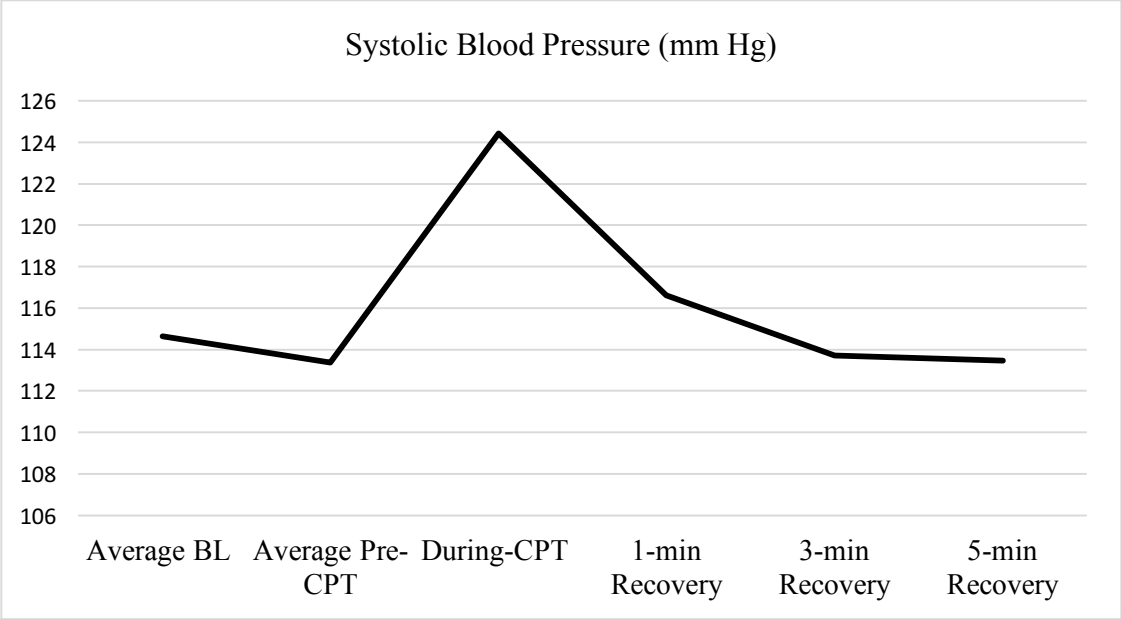

Figure 4: Systolic Blood Pressure Measurements over Time

Table 4. Regression analysis assessing cardiovascular measures during the 8˚C CPT as a function of perceived stress (N=50).

Dependent Variable	b (SE)	β	t score	p value	f^2
Baseline SBP	.27 (.51)	.07	0.54	.60	0.01
SBP-AUCg	8.15 (7.66)	.11	1.06	.29	0.03
SBP-AUCi	13.47 (6.35)	.31	2.12	.040	0.11
SBP-AUCg-Recovery	3.36 (1.71)	.215	1.96	.057	0.10
SBP-AUCi-Recovery	-1.55 (2.13)	-.12	-0.73	.47	0.01

Note: adjusted for gender, ethnicity, age, education level, BMI, smoking frequency, and PASS20 total score.

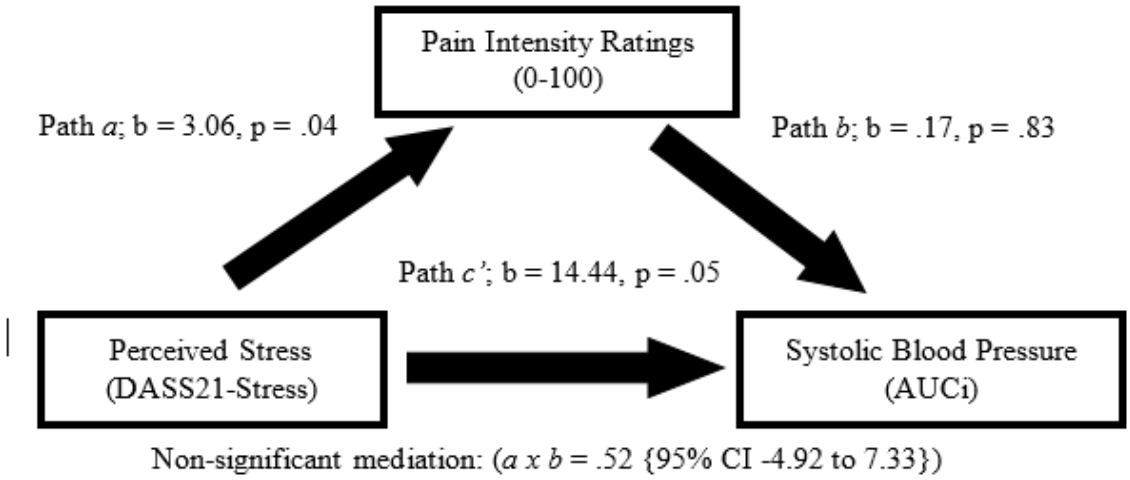

Figure 5: Mediation analysis showing the indirect effect of pain intensity ratings on the relationship between perceived stress and SBP-AUCi. Note: adjusted for gender, ethnicity, age, education level, BMI, smoking frequency, and PASS20 total score.

Aim 3

Plasma cortisol measurements over the course of the session can be seen in Figure 6. Perceived stress was not significantly correlated with baseline cortisol, cortisol reactivity indicated by area under the curve with respect to ground (Cort-AUGc), or cortisol reactivity indicated by the area under the curve with respect to increase (Cort-AUCi). Regression analysis determined that perceived stress was not significantly associated with baseline cortisol nor any measure of cortisol reactivity in either adjusted or unadjusted models (all p's > 0.05; Table 5). Given the lack of associations, proposed mediational analyses were not performed.

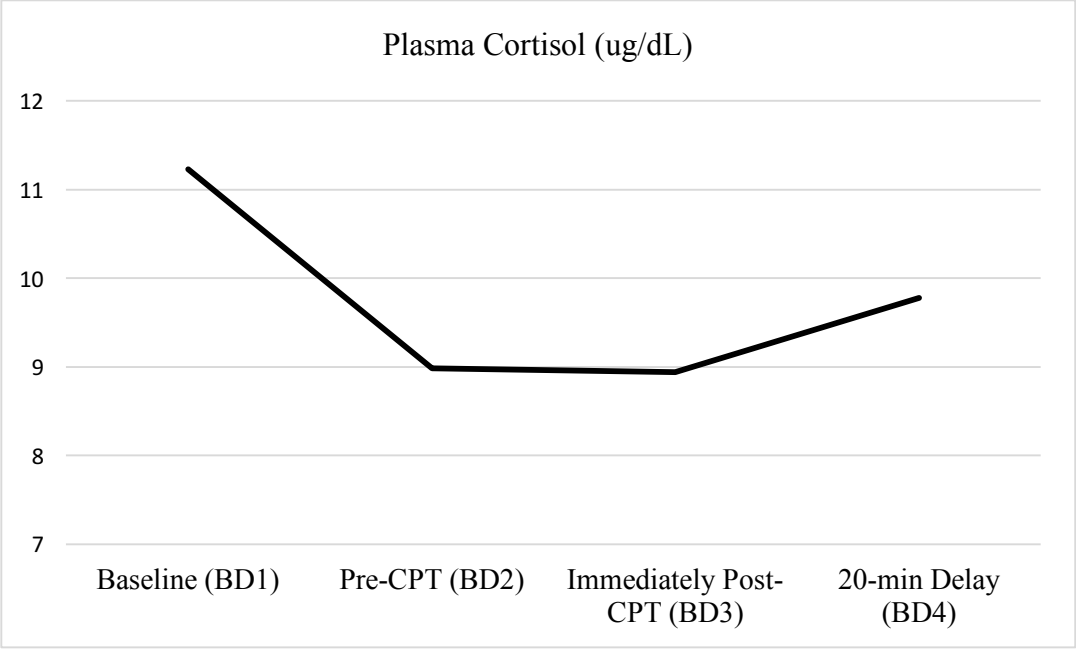

Figure 6: Plasma Cortisol Measurements over Time

Table 5. Regression analysis assessing cortisol measures as a function of perceived stress (Baseline N=49, Reactivity N = 47).

Dependent Variable	b (SE)	β	t score	p value	f^2
Baseline Cortisol	.04 (.17)	.03	.21	.84	0.00
Cortisol-AUCg (Timepoints 2 to 4)	-.10 (5.47)	-.00	-.02	.99	0.00
Cortisol-AUCi (Timepoints 2 to 4)	-.08 (3.66)	-.00	-.02	.98	0.00

Note: adjusted for gender, ethnicity, age, education level, BMI, smoking frequency, and PASS20 total score.

Aim 4 – Resolvin Correlations

Resolvins D1 and D2 were each examined for relationships with pain intensity, pain unpleasantness, and perceived stress levels. Both RvD1 and RvD2 were significantly correlated with each other (Figure 7), indicating possible cross-reactivity ($r(23) = .944$, $p < 0.001$). Neither was significantly correlated with ratings of pain during the CPT (all p's > 0.05). RvD1 exhibited a trend towards significance when examined with the DASS21-Stress responses ($r(22) = .381$, $p = .060$); this was not true of RvD2 ($r(22) = .328$, $p = 0.11$).

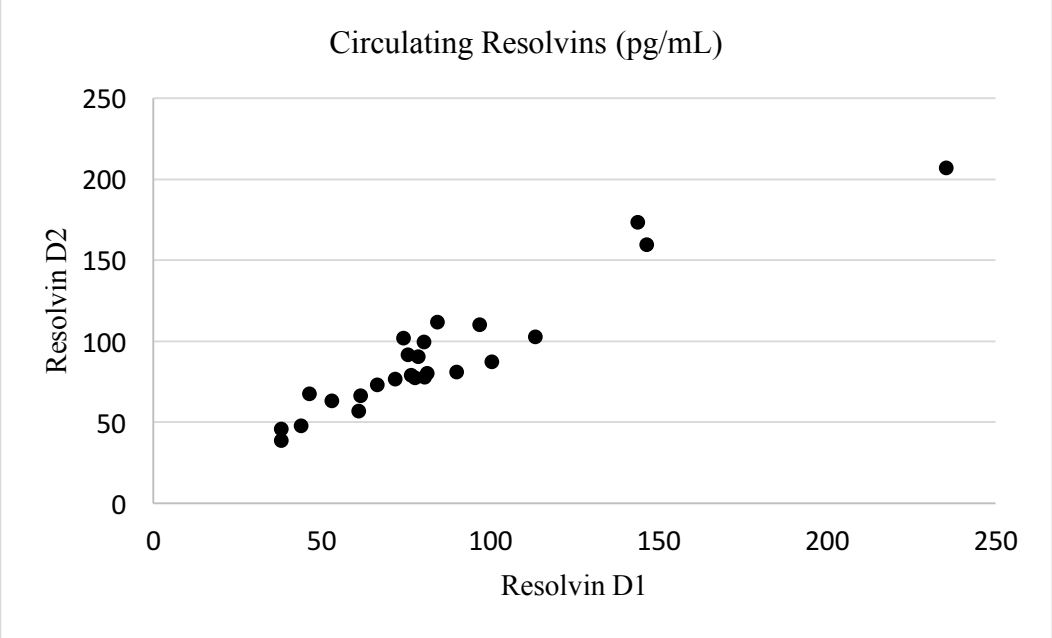

Figure 7: Circulating Resolvins RvD1 and RvD2

Table 6: Correlations between RvD1, RvD2, perceived stress, and subjective pain **r**eports

	Variable	1	2	3	4
1	Resolvin D1 N=25				
2	Resolvin D2 N=25	.944** p = .000			
3	Pain Int N=24	-.288 p = .172	-.150 p = .485		
4	Pain Unpl N=24	-.267 p = .208	-.128 p = .552	.771** p = 0.000	
5	DASS20-Stress N=25	.381 p = .060	.328 p = .109	.208 p = .329	.217 p = .309

± = $p<0.01$, * = $p<0.05$

DASS21-Stress = Stress Subscale of the DASS21; Pain Int = Pain Intensity; Pain Unpl = Pain Unpleasantness; SBP = Systolic Blood Pressure

DISCUSSION

The first aim of the study was to assess the relationships between perceived life stress and experimental pain sensitivity in a sample of healthy, young adults. Consistent with predictions, higher levels of perceived stress predicted reports of greater pain intensity during the CPT. However, despite a significant positive correlation, perceived stress was not found to be a significant predictor of pain unpleasantness ratings in a regression model including covariates. The second aim was to determine whether perceived stress related to SBP before, during, and after a CPT. Consistent with hypotheses, higher reported stress predicted significant SBP reactivity during the CPT. It should be noted that the unadjusted analysis (i.e., Pearson correlation) did not reveal a significant relationship between perceived stress and AUC-SBPi. It was not until this relationship was adjusted for participant's gender, ethnicity, age, education level, and pain-related anxiety (i.e., regression) that it became significant. It appears that statistical adjustment for the confounding effects of these other study variables helped to provide an undistorted estimate of the relationship between perceived stress and AUC-SBPi during the acute noxious stressor. Perceived stress did not significantly predict baseline SBP, nor SBP recovery in the 5 minutes following the CPT. Additionally, we assessed whether higher ratings of pain intensity during the CPT mediated any relationships found between perceived stress and SBP. Contrary to hypotheses, subjective pain intensity did not mediate the relationship of perceived stress and SBP reactivity. Thirdly, we examined

whether perceived stress might be related to basal and/or pain-induced cortisol reactivity during the CPT, similar to investigations of SBP. Contrary to predictions, no significant relationship amongst perceived stress and cortisol levels were found. Finally, for the fourth and final aim, we assessed an exploratory hypothesis investigating any relationships between circulating resolvin levels at baseline with reported stress levels, pain intensity, and pain unpleasantness during the CPT. No significant relationships were identified between resolvins D1 or D2 and reports of pain intensity, unpleasantness, or perceived stress. A relationship between RvD1 and perceived stress was identified only at a trending level ($p = 0.06$).

Aim 1

Prior research suggests that the relationship between perceived stress and pain sensitivity is complicated by many factors, including setting (e.g., laboratory pain) and chronicity of the stressor. In the present study, increased reported stress over the past week significantly predicted greater pain intensity ratings at 8° C, but did not predict pain unpleasantness ratings after adjusting for covariates (trending $p = 0.066$). This finding is most likely related to differences in the pain experience that intensity and unpleasantness ratings capture. Pain intensity ratings tend to be associated with the sensory-discriminative aspect of pain, while pain unpleasantness tends to relate to the affective-motivational aspect of pain[73]. Typically, the sensory-discriminative dimension of pain has been associated with processing in the primary and secondary somatosensory cortex and includes the perceived intensity, location, and quality of the painful sensation. In contrast, the affective-motivational dimension of pain reflects perceived distress or suffering as a

result of the noxious stimuli[80,81]. Thus, reports of perceived basal stress levels more closely indicate heightened awareness of the quality of the painful experience, rather than the distress associated with it. The results of the present study corroborate existing literature indicating that exposure to psychological stressors significantly increases pain intensity during experimental noxious stimuli[3].

It has been suggested that exposure to chronic stressors may relate to the development of many chronic health conditions. Notably, many of these conditions involve chronic pain, such as fibromyalgia[7], chronic low back pain[9], and chronic pelvic pain[8]. Additionally, responses to noxious laboratory stimuli have been linked to clinical pain outcomes, including reports of daily pain experienced by healthy adults with no chronic pain conditions[82]. It is not possible to determine causality due to the cross-sectional nature of this study; however, it is possible that life stressors may significantly relate to pain processing, as evidenced in the QST results here. If so, and the stressors were prolonged, long-term changes in pain processing may confer increased risk for the subsequent development of chronic pain conditions.

Aim 2

In the present study, heightened perceived stress reports significantly predicted increased SBP reactivity during the 8° C CPT. To our knowledge, this is the first study to demonstrate an increase in SBP reactivity to a noxious stimulus in the context of general life stress rather than stress induced in the lab (e.g., by a Trier Social Stress Test or similar). This study first assessed basal perceived stress levels and then examined SBP prior to, during, and immediately after a noxious stimulus in normotensive individuals. In

contrast, much of the research on this topic has been focused on examining elevated resting blood pressure and subsequent effects on acute pain sensitivity[83]. These results show that even in healthy, normotensive individuals with non-elevated resting blood pressure, changes in cardiovascular reactivity to a noxious stimulus can be observed. One explanation for this phenomenon is that cardiovascular reactivity to stressful environmental stimuli may share mechanisms with systems of pain modulation, including baroreceptor neural pathways, noradrenergic mechanisms, and endogenous opioid systems[50,51]. For example, a central autonomic network has been proposed to reflect integrated brain regions that coordinate responses to environmental stimuli[84], and those regions (e.g., the nucleus tractus solitarius, or NTS) implicated in coordination of the cardiovascular system have also been associated with antinociceptive pathways[51]. Through a baroreceptor feedback loop, descending pain inhibitory pathways may be able to self-regulate their activity through actions in autonomic centers of the spinal cord modulating cardiovascular function[85]. Given this research, it is reasonable to consider that ongoing stressors might impact the feedback loop between cardiovascular activity and pain inhibition. Specifically, increased perceived life stress may lead to increased pain sensitivity and primed cardiovascular reactivity in an adaptive effort to engage this feedback loop, minimizing the impact of subsequent pain or stress.

Contrary to predictions, baseline SBP was not significantly related to perceived life stress. A potential explanation for this null finding may relate to the attenuated range of baseline SBP allowed, which included limits necessary to characterize this group as healthy and non-hypertensive. A maximum resting blood pressure limit was set at 150/95 in participants reporting no current or past diagnosis of high blood pressure, thus

restricting the ranges allowed in the study. However, given the strength of the relationship between SBP reactivity and perceived stress versus the dearth of a relationship with basal SBP, an alternate conclusion may be considered. It is reasonable to posit that acute instances of perceived life stress may first impact one's ability to effectively react to stressors. Speculatively, if indeed rises in blood pressure result in lessened pain or suffering, hypertension may begin as small, individual increases to mitigate specific stressors in a premorbid state before ultimately becoming chronic as an adjustment to ongoing, unrelenting stressors. Also in this study, SBP recovery was marginally related to perceived life stress ($p = 0.057$). This may indicate a tendency towards more dysfunctional recovery following an acute stressor, but not so strongly as effective reactivity during the stressor itself. However, this conclusion remains speculative based on the trending significance, and should be evaluated in a larger sample over time before drawing strong conclusions.

As noted previously, clinical evidence suggests that repeatedly elevated blood pressure (e.g., in response to ongoing stressors) may increase risk of negative cardiovascular complications such as atherosclerotic plaques and large artery stiffening[63,64]. These developments likely lead to greater risk of subsequent cardiovascular disease. In conjunction with the marginally significant dysfunctional recovery results, we posit that experiencing repeated stressors resulting in acute hypertensive events might predispose one to develop subsequent cardiovascular disease. This is thought to occur because increased blood pressure reactivity may diminish subsequent pain or aversion, leading a chronically stressed system to embrace hypertension in order to mitigate further suffering[83]. Perhaps Selye's 1948 description of

hypertension as a "disease of adaptation" continues to have merit today[86]. Given the high rate of cardiovascular disease in the general population[87], attention to risk factors such as repeated stress is warranted. The results of this study lend credence to the need to study cardiovascular risk factors in response to perceived life stress, even in young, healthy people with no existing cardiovascular or other longstanding health problems. Interestingly, as noted before, the predictive utility of perceived stress was significant only for SBP reactivity during the CPT, not for basal SBP. This is an important clinical implication, as it suggests that 1) individuals may not demonstrate increased resting SBP related to their stress, but may be less equipped to handle subsequent stressful events like a noxious task and 2) this effect may be missed in routine clinical examinations by medical professionals, who typically evaluate resting blood pressure rather than reactivity to a stressor. Thus, continued research should be conducted examining chronicity of stressors and their impact on blood pressure reactivity. Were these results to be replicated in studies in other studies of healthy individuals, resting basal BP measurements in the clinic would not suffice to indicate increased risk of subsequent cardiovascular disease.

Aim 3

Despite hypotheses to the contrary, there were no significant predictive relationships between perceived stress and either basal or reactive cortisol levels. In fact, no significant cortisol change was observed as a result of the CPT itself, irrespective of stress levels ($p > 0.05$). This result was unexpected, given that multiple studies have demonstrated adrenocortical activity in response to the CPT[48,49,88]. Several factors may have influenced the lack of results in this study. For example, some previous studies

indicate that it can take 30-45 minutes for cortisol to reach peak levels following a noxious task[89,90]. The blood draw in this study was taken 20 minutes after the CPT was completed. Thus, the timing of this study may have been too abbreviated to accurately capture the maximum peak of cortisol change. Another area of difference between our study and others is the water temperature used. It is possible that the CPT was too mild to elicit a strong cortisol response at 8° C, in contrast to one group's successful cortisol responsivity to average temperatures of 0-4° C[49]. Similarly, it may also be possible that the stressors the participants were reporting over the past week were too mild to elicit a strong cortisol response, or that the chronicity of said stress was too short to result in lasting basal or reactive cortisol changes. Much of the research evaluating cortisol reactivity and pain perception has been conducted with lab-induced, acute, strong stressors where the reactivity can be evaluated quickly thereafter. The nature of the perceived life stress used in this study may have made it unlikely to result in any resulting cortisol effects in the laboratory. These results underline the supposition that cortisol's role in both stress and pain perception is complicated and difficult to ascertain.

Another consideration for evoking cortisol change is that stress responses are not non-specific, despite early theories. It does not appear that responses occur equally to all stressors, and some added psychological or physical stressors can better induce HPA reactivity than others. For example, al'Absi and team specifically informed participants that the CPT was used to induce pain, which may have enhanced its stressful qualities[49]. Giving such instructions with the CPT differentiated it from its typical use as a "passive" stressor and resulted in not only significant cortisol change in response to the CPT, but also positive correlations between ratings of pain and changes in cortisol. Participants in

the current study were not informed that the CPT was specifically used to induce pain, and as such may have escaped increased stress as a result.

Finally, these results may indicate differences in the timeline of cardiovascular and HPA responses to stressors. SBP may be more variable and quicker to respond to acute or marginally increased perceived stress over a recent period of time. Cortisol responses to ongoing life stressors may change at a slower rate, indicating that cardiovascular measures may be more appropriate to assess as a first-line look at physiological reactivity to perceived stress. Despite our hypotheses, it does not appear that at this early stage, cardiovascular and HPA function act in concert to respond to stressful stimulation in the presence of increased life stress. However, this conclusion does not unequivocally state that cortisol changes are non-existent in stressed but healthy individuals. It will be important in future research to establish what types of stress (e.g., psychological vs. physical, chronicity, settings) specifically stress the adrenocortical system and increase risk for extended HPA activation and subsequent health concerns. Given the severity of these implications, future research is needed to better characterize the relationship between ongoing life stress, acute stressors, and cortisol reactivity.

Aim 4

The final aim of this study was a preliminary analysis attempting to identify relationships between circulating RvD1 and RvD2 levels with subjective pain intensity and unpleasantness reports, in addition to reports of perceived stress. No significant relationships were identified between either resolvin type and any of the subjective ratings. A trending relationship was identified between RvD1 and the DASS21-Stress

responses; however, this relationship was heavily dependent on the highest rated DASS21-Stress level (14 of 21), and the significance of the correlation fell dramatically if that participant was removed. Given the attenuated range of DASS21-Stress scores and the small sample size with resolvin data, it is not possible to identify whether this relationship is valid at this time. The results should be replicated with a larger group selected for a wider range of perceived stress responses. However, given the trend towards significance between RvD1 and perceived stress that was absent in the subjective pain reports, it may be that resolvins are better suited to characterize ongoing and/or uncontrollable stressors (e.g., those outside the lab) rather than acute, controllable noxious stimulation such as the CPT.

Analyses revealed that RvD1 and RvD2 were significantly positively correlated. In part, this may be due to the assays chosen. ELISAs function well as a preliminary screening assay and were chosen for the relative ease, cost, and ability to identify initial relationships amongst resolvins, perceived stress, and pain reports. However, there may be overlap in the resolvins detected as RvD1 and RvD2 given their close structural similarity. Ideally, mass spectrometry would be used to evaluate circulating plasma resolvin levels in future studies. Mass spectrometry has the advantage of heightened specificity, though it is difficult and expensive to pursue with large samples. Given previous research demonstrating both similarities and differences in the function of the D family of resolvins, greater specificity in follow-up analyses is warranted. For example, studies in animal models indicate that RvD1 and RvD2 have similarities in function as anti-nociceptive and pro-resolving agents[11,91]. However, they may utilize different G-protein coupled receptors to exact their action[72]. Additionally, functional differences have

been investigated in RvD1 and RvD2. RvD1 has been shown to reduce inflammatory and postoperative pain in rodent models[92,93] and is an effective antihyperalgesic agent in a rat model of adjuvant-induced arthritis[94,95]. By contrast, RvD2 may block synaptic plasticity that contributes to the development and maintenance of inflammation-induced pain[96]. Additionally, rodent models suggest that administration of D-series resolvins may be differentially effective in reducing painful and depressive symptoms in animals following myocardial infarction (specifically, RvD1[97]) or with fibromyalgia (primarily RvD2[98]). Given these results, using assays that allow greater specificity may aid in establishing relationships amongst resolvins, pain reports, and psychological measures in humans.

Finally, as is expected when translating from animal to human research, the methodology between previous studies and the current study are significantly different. For example, in many animal studies, resolvins are administered via peripheral or spinal injection, with pain behaviors subsequently observed in response to heat or mechanical stimulation[92]. Alternatively, some research has examined the role of resolvins in human cellular models[99]. However, little is known about any relationships between circulating resolvins in the human body and an acute, focal noxious stimulus, such as was described here. The methods used provided a general overview of systemic resolvin D pathway function in the context of nonspecific, general life stressors. Despite the small sample, the results have helped to hone in on areas to explore with altered methods. The first would be to further evaluate any relationships between stress and circulating levels of resolvins. For example, though pro-inflammatory factors were not assessed in this study, psychosocial stress has been associated with increased pro-inflammatory activity (e.g., C-reactive protein, or CRP levels[100]). Speculatively, higher levels of circulating resolvins

may be part of a physiological response to offset the impact of increased inflammation. Thus, a study involving individuals with a larger range of perceived stress scores may result in stronger correlations with resolvins. Similarly, the type and chronicity of the stressors reported may also play a role in ongoing resolvin circulation levels. It is possible that a chronic stressor (e.g., a mentally taxing job, ongoing psychological abuse, etc.) being reported may elicit resolvin responses differently than an acute stressor (e.g., an upcoming exam or presentation). It is unknown what the perceived stress levels reported in this study were regarding; however, the average stress level reported is low (m = 4.18 of 21 possible points, characterized as normal), so it is unlikely that the majority of the participant were experiencing significant ongoing stressors. Secondly, if possible, using noxious stimulation in a QST session may be better suited to evaluating resolvin reactivity throughout the session rather than correlating to baseline levels. At this time, there is not a significant basis on which to determine an appropriate timecourse for resolvin reactivity. However, if resolvins are accurately conceptualized as "pro-resolving" factors, they may be more active following an acute noxious or psychological stressor rather than at a baseline measurement. Third, a novel area in which to explore the impact of resolvins would be in individuals with a chronic inflammatory and/or painful condition. Preliminary data from our lab examined resolvin receptor expression in a small number of knee osteoarthritis patients and identified a significant positive relationship between resolvin receptors and reports of pain intensity and unpleasantness during the cold pressor task. Speculatively, cross-sectional resolvin research in humans using QST measures may be better suited to evaluate relationships in individuals with chronic pain conditions, as their circulating resolvin levels may be higher to account for their

inflammatory condition. That speculation would need to be verified with a significant amount of research evaluating circulating resolvins and receptor expression in individuals with inflammatory conditions.

Limitations

The findings of the present study should be interpreted in light of its limitations. First, a potential limitation of the study is that the chosen sample (e.g., young, healthy college students) may not be representative of the population as a whole. Though they certainly perceive stressors in their day-to-day lives, college students may perceive themselves as being more adept at coping with stress and/or enjoy enhanced resources to do so. Results may have been different were this group comprised of community-dwelling adults of various age and socioeconomic status ranges. Additionally, we were unable to pinpoint the exact type of stressor(s) that the participants were reporting, making it impossible to differentiate between longstanding chronic stressors and acute or fleeting stressful events that the participant is experiencing specific to the week before presenting for the study. To attenuate the risk that the stress was directly related to the session, a measures of pain-specific anxiety was given and the results accounted for; however, outside of this one possible contributor, it is possible that participants were reporting their reactions to a variety of stressors. Third, these data were gathered from one sample in young, healthy participants in the southeastern United States, thus necessitating replication of our findings in other settings. In particular, groups with chronic pain and/or cardiovascular disease would be of particular interest, as well as individuals selected to reflect a significant range of perceived stress. Fourth, the small

sample size, particularly with respect to the exploratory resolvin analyses, limited the scope of the conclusions drawn in this study. Finally, due to the cross-sectional nature of the study, we were unable to determine causality of the relationships determined. However, as perceived life stress is reported prior to any QST testing or physiological measures and specifically asks the participants to rate the week before the session, we suggest that the ongoing perceived stress predates the subsequent testing for cautious interpretation.

Summary

The purpose of the present study was to characterize relationships between self-reported life stress and subsequent responses to an acute noxious stressor. Three physiological responses were evaluated: 1) systolic blood pressure, 2) circulating cortisol levels, and 3) circulating basal resolvin levels. We were able to demonstrate that perceived stress levels significantly predicted reports of pain intensity during the CPT. Additionally, we found that heightened perceived stress also related to increased SBP reactivity during the CPT, though pain intensity did not help to explain that relationship. No significant relationships were found with perceived stress and cortisol, whether basal or reactive. Additionally, no significant relationships were identified between either resolvin D1 or D2 and pain intensity, pain unpleasantness, or perceived stress reports. Overall, the results of the present study add to the existing literature by incorporating perceived life stress into the study, rather than relying solely on laboratory-induced stressors, and integrating multiple physiological systems into the analyses. To our knowledge, there has not been a study investigating both laboratory pain and ongoing life

stressors in this context. This is important because investigating ongoing perceived life stressors may more closely replicate stress individuals experience in regular life that impact ability to modulate subsequent stressors in research studies. Additionally, this study indicates that even in young, healthy individuals, changes can be observed in cardiovascular reactivity to an acute noxious stimulus. These results may have important clinical implications, suggesting that at early stages individuals reporting increased stress respond more strongly to subsequent stressors. The experience of chronic, ongoing stress is a phenomenon that has important implications for physiological and psychological health problems, including a variety of disorders that involve chronic pain. Thus, if the perceived stress reported was consistently ongoing with multiple subsequent acute stressors experienced, the individual may be at greater risk for developing standing hypertension and other cardiovascular conditions. Importantly, basal SBP was not affected, indicating that this risk may be overlooked in a standard checkup. Taken together, the results of this investigation suggest that perceived life stress is important to assess when considering pain sensitivity and physiological reactions to an acute noxious stressor. Additionally, further research is warranted to evaluate these results across a variety of stressors and possibly evaluate short interventions (e.g., distress intolerance, stress management techniques) to mitigate risk of cardiovascular disease.

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APPENDIX A

INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

MEMORANDUM

TO: Hailey W Bulls, MA
Principal Investigator

FROM: Sally Blake Headley, CIP
On behalf of IRB 01 Sally Blake Headley, CIP

DATE: August 13, 2015

RE: F130729001
Ethnic Differences in Endogenous Pain Modulation Across the Adult Lifespan

The IRB 01 met on **August 12, 2015** and **approved** the protocol referenced above. The approval form is attached. **This approval will expire and no longer be valid on August 12, 2016.**

Please note the following as related to this review:

- The IRB noted approval of this protocol expired on August 6, 2015 and acknowledged the memorandum indicating that no activity has taken place since the lapse in approval. The IRB recommends submitting renewal materials 4-6 weeks before the protocol expiration date to avoid a lapse in approval. Allowing IRB approval to lapse constitutes non-compliance. Any further instance of non-compliance may be considered serious and/or continuing non-compliance, which must be reported in accordance with POL024, UAB Policy on Reporting to Institutional Officials and Regulatory Agencies.
- The IRB reviewed the Problem Summary Sheet submitted with this renewal. The dates of the Problem Summary Sheet are 10/25/13 to 08/06/14. It lists 1 event in Table A and 0 events in Table B.
- The IRB noted that this protocol was permanently closed to enrollment and may qualify, in the future, for expedited review under Category 8. Expedited review would be appropriate where:
  a. (i) the research is permanently closed to the enrollment of new subjects; and
     (ii) all subjects have completed all research-related interventions; and
     (iii) the research remains active only for long-term follow-up of subjects; or
  b. no subjects have been enrolled and no additional risks have been identified; or
  c. the remaining research activities are limited to data analysis.

  Subsequent changes in the protocol may result in convened IRB review being required. ***If this protocol meets the requirements above and you would like move it to expedited review, please mark the "Expedited Review" box at the top of the IPR and submit a copy of this memorandum with next year's renewal materials.***