Perseveration and health: An experimental examination of worry and relaxation on autonomic, endocrine, and immunological processes

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ABSTRACT

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The field of psychoneuroimmunology seeks to examine the impact of stress and other psychological processes on physical health. While some theories suggest that processes such as worry may have a significant impact on prolonging the physiological stress response and subsequently increasing risk for long-term health issues, to date, this research has not yet thoroughly examined the impact of worry on physical health processes. The current study sought to combine theories from clinical and health psychology to investigate the impact of experimentally-induced worry and relaxation on cortisol, heart rate variability (HRV), and inflammation. Participants (N = 85) were community members from the New York City area. They completed worry and relaxation inductions within the laboratory while HRV was collected continuously. Three blood samples were taken throughout the study to test for inflammation and cortisol. Results indicated changes in HRV, IL-6, and IFN-γ throughout the study conditions that were not moderated by levels of trait worry. HRV, cortisol, and inflammation did not covary throughout the different experimental conditions and changes in cortisol and/or HRV did not temporally precede changes in inflammation throughout the study. Overall, the findings from the current study offer insight into the contrasting impact that worry and relaxation have on physiological biomarkers and highlights important directions for future research in the field of psychoneuroimmunology.

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Dedication

"You can't stop the waves but you can learn how to surf." – John Kabat Zinn

This work is dedicated to my academic family – Doug Mennin, Jeannie Quintero, and Phillip Spaeth. Thank you for helping me to learn how to surf, helping me to get back up when the waves have knocked me down, and for surfing for me when I have not been capable of doing it myself over the years. It has not always been easy, but your kindness, patience, love, and support has made all of the difference to me, and I could not have gotten through this project (or graduate school) without you. I am so proud of the work that we have done together and I am so excited for everything that there is left to do. I am forever grateful and indebted to the three of you and hope I have given you half as much as you have given me over the years.

1. Introduction

Psychoneuroimmunology and anxiety

Over the past several decades, the field of psychoneuroimmunology (PNI) has emerged in an effort to understand the impact that psychological processes have on immune and nervous system activities. The primary goal of the immune system is to fight against infectious or otherwise harmful agents within the body that threaten overall healthy functioning (Sapolsky, 1994). PNI research and theory have demonstrated a link between negative emotions (i.e., anxiety, fear, sadness, anger) and dysregulated immune system functioning, which may cause infection, inflammation, and an overall increased risk for experiencing a wide variety of chronic health issues (Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002). The interaction between stress and/or negative emotions and autonomic, endocrine, and immune processes is at the nexus of this field of study and has contributed to a growing body of research linking subjective emotional and cognitive experiences and physical health.

Part of the theory guiding PNI research underscores the importance of subjective experiences of stress on the body, as stress leads to inhibition of immunological processes and therefore inhibits the necessary fight against infectious agents within the body. Anxiety, defined as a feeling of nervousness or worry about a real or uncertain potential threat either in the current moment or the future, is a prime emotion to better understand the links between physiological processes in the body and the impact that these subjective emotional experiences have on them. Anxiety disorders represent chronic, excessive, and severe anxiety as part of their diagnostic criteria (American Psychiatric Association, 2013) – i.e. a dysregulated experience of anxiety that is often more severe and debilitating in nature than more normative and briefer states of anxiety.

Although anxiety disorders are categorically distinct, they share a number of common characteristics. Among these, individuals suffering from anxiety experience distorted thinking (Turner, Beidel, & Stanley, 1992; Cox, 1996; Falsetti, Monnier, Davis, & Resnick, 2002), greater emotion dysregulation (Mennin, Heimberg, Turk, & Fresco, 2005; Weiss, Tull, Anestis, & Gratz, 2013) and engage in experiential avoidance of anxiety-inducing stimuli (either real or perceived; Hayes, Wilson, & Gifford, 2015), subsequently putting them at an increased risk for overall psychological and physiological dysregulation compared to their psychologically healthy counterparts. Research has also demonstrated that individuals with anxiety disorders are hypersensitive to contextual cues that may contribute to or maintain anxiety, thus leading to an increased likelihood of experiencing prolonged and/or more frequent physiological activation. PNI research has begun to examine the impact of anxiety on overall health, highlighting that the links between overall psychological and physiological dysregulation (Kemp & Quintana, 2013).

Physiological dysregulation is a criterion for diagnosis in a number of different anxiety disorders (i.e. muscle tension in generalized anxiety disorder, hypervigilance in post-traumatic stress disorder, and numerous physical symptoms such as rapid heartbeat, sweating, and difficulty breathing in panic disorder; American Psychiatric Association, 2013). The physical symptoms associated with an anxiety disorder diagnosis represent the necessity of understanding the long-term physical ramifications of anxiety on an individual's physiological and immunological processes. Taken together, the field of PNI presents an important and necessary perspective in understanding the ways that subjective processes at the person-level may impact overall health and wellbeing. When the body is in a state of prolonged physiological activation, there is a substantially increased risk for an individual to experience systemic, chronic negative health implications. One of the most widely studied systems implicated during times of

prolonged physiological activation is the HPA axis, a neuroendocrine system comprised of the hypothalamus, pituitary gland, and adrenal glands. The HPA axis is involved in adaptations to stress and change. It is also responsible for the regulation of several other processes such as digestion, the immune system, the cardiovascular system, the reproductive system, as well as contributing to fluctuations in mood and emotions. Given the central role that the HPA axis plays in physiological dysregulation, it is important to understand both the physical and psychological mechanisms that may link activation of this system to poor health outcomes in the long term (Tsigos & Chrousos, 1994).

While a number of psychological processes associated with these disorders may impact physical health, much of the current research examining subjective experiences within people with anxiety has been worry. Worry, a characteristic of generalized anxiety disorder (GAD) and common across several other forms of psychopathology, is best defined as thinking repeatedly about things in the future (Borkovec, Robinson, Pruzinsky, & DePree, 1983). The triparate function of worry suggests this cognitive process helps to alarm individuals to potential threats, prompts awareness to unresolved threatening situations, and, lastly, prepares individuals for a necessary 'fight or flight' response (Frijda 1988; Lazarus 1991). Therefore, when worrying, individuals are in an apprehensive state of anticipation of real or perceived subjective threats in the environment, which causes wear and tear on the body over the long term. Despite the objective complications associated with worry both at the emotional and somatic levels, worry may subjectively serve an adaptive and positive function for some individuals, as theoretical understandings of worry have suggested that it is an attempt to prevent negative experiences from occurring or to prepare oneself for the presence of negative experiences (Borkovec 1994; Borkovec 2004). The Contrast Avoidance Model, a theory developed to help explain the utility

of worry and highlights the importance of the maintenance of worry as an adaptive mechanism for reducing the unpleasantness of emotional shifts from positive to negative (Newman & Llera, 2011). According to this theory, worry does not lead to the avoidance of negative emotions. Rather, it sustains negative states once they have already developed.

To date, there have been a number of studies within the worry literature that have begun to test the Contrast Avoidance Model through examining subjective and physiological differences between worry and relaxation conditions among worriers and non-worriers. Indeed, a 2010 study of people with GAD (i.e., high worriers) compared to nonanxious controls demonstrated that those with GAD reported that their worry helped them to cope while viewing emotional film clips, while the reverse was true for the nonanxious group (Llera & Newman, 2010). Most recently, a 2013 study examined the impact of worry and relaxation among low trait worriers, high trait worriers who did not meet diagnostic criteria for GAD, and people diagnosed with GAD. Participants in all groups were randomized to receive either the worry or relaxation condition. Results demonstrated a significant effect of condition, in that individuals in all three groups reported significant increases in subjective ratings of worry from pre-worry to post-worry (Fisher & Newman, 2013). Subjective findings within the relaxation condition demonstrated a significant increase in relaxation from pre- to post-manipulation regardless of group. However, post-hoc analyses found that there were only significant differences between people with GAD and controls in subjective ratings of relaxation, indicating no differences between the GAD and high worry group or the high worry group and controls (Fisher & Newman, 2013). There have been a number of studies that have utilized a similar task design and demonstrated subjective differences in worry throughout the worry manipulation compared to baseline among individuals

with and without diagnoses of GAD compared to healthy controls (Llera & Newman, 2010; Borkovec & Inz, 1990; Oathes, Ray, Yamasaki, Borkovec, Castonguay, & Newman, 2008).

These studies have also examined the role of relaxation between these groups with varying instructions from more imagery-focused relaxation tasks to instructions that encourage participants to engage in several variations of deep-breathing exercises. A number of these studies' findings were replicated by Fisher & Newman's recent work but largely focused on people with GAD compared to healthy controls, and therefore failed to consider the important impact that trait worry, broadly, may have on maintaining negative emotions even when instructed to relax. These findings have generally demonstrated greater subjective levels of relaxation regardless of group (i.e., Borkovec & Inz, 1990; Llera & Newman, 2010); however, in the majority of these studies, participants were randomized to receive either worry or relaxation, rather than both conditions, thus not permitting the ability to fully examine Contrast Avoidance by assessing whether or not participants are able to subjectively and/or physiologically recover from the impact of worry through the utilization of relaxation strategies. Taken together, this theory and associated findings highlights the functionally sustaining nature of worry for some individuals, and this constant state of cognitive activation may have deleterious implications on physical health given what is known about anxiety in dysregulating autonomic and endocrine processes in the body.

The Perseverative Cognition Hypothesis

Given that many anxiety disorders are characterized by high degrees of perseverative processes such as worry and rumination (i.e., thinking back over past losses and failures; Nolen-Hoeksema, Wisco, & Lyubomirsky, 2008), these processes may contribute to physiological dysregulation independent of a formal psychological diagnosis. However, many studies

examining stress and coping have largely failed to examine the impact of worry and rumination in exacerbating or maintaining somatic health complaints and long-term deleterious health outcomes. In an effort to better capture and understand the role that these processes may play in the context of health, the perseverative cognition hypothesis (PCH) was developed as a way to better conceptualize these relationships. The central theory of the PCH links increases in cognitive activation such as worry with negative physical health outcomes. The development of this theory and the body of research associated with it highlights the potential long-term implications of cognitive processes associated with anxiety and depression on physical health and may highlight potential cognitive mechanisms that may link anxiety to increases to overall poor health.

According to the PCH, sustained cognitive activation such as that consistent with chronically worrying or ruminating, may contribute to increased endocrine, immune, and autonomic reactivity, subsequently putting these individuals at an increased risk for experiencing deleterious health effects (Brosschot, Gerin, & Thayer, 2006). Specifically, this theory takes into account the cognitive processes that may precede or come after stressors – highlighting the fact that a stressor in and of itself may not be the most physiologically activating thing in a chain of events for those who are high in perseveration. Essentially, perseveration prolongs a physiological stress response, putting individuals in a chronic state of physiological arousal and subsequently at greater risk for experiencing negative health outcomes (Brosschot et. al, 2006).

Traditional theories of stress and adaptation have conceptualized coping strategies as a means of recovering from stressors and returning to homeostasis. Within this framework, coping serves as a moderator between stress and subsequent health outcomes (Brosschot et al., 2006). In contrast, perseverative cognition is considered a mediator between stress and health, in that these

cognitive processes tend to prolong the stress experience and prohibit the ability for the body to recover physiologically (i.e., putting individuals in constant 'fight or flight' preparation). Therefore, although researchers have established the link between anxiety and chronic health conditions, these perseverative processes may serve as mediators of this relationship. However, it is necessary to first better understand the impact that these processes may have on physical processes that may contribute to poor health, including inflammation, cortisol, and cardiac dysregulation. By clarifying the impact of these processes on these more general markers of physical health, researchers may be better able to determine the potential targets for improving both physical and psychological intervention.

The PCH also builds upon foundational avoidance theories of worry (Borkovec 1994; Borkovec 2004), which emphasize one function of worry as a way to mute somatic responses to threat. A number of studies have shown that worry is associated with greater physiological activation broadly compared to relaxation or neutral conditions (Borkovec & Hu, 1990; Borkovec, Robinson, Pruzinsky, & DePree, 1983). Preliminary research findings in support of the PCH have demonstrated an impact of worry and relaxation on blood pressure, cortisol, heart rate variability (HRV), and immune markers with clear associations between cognitive and physiological variables and some emerging evidence of casual predictions between the two (Ottaviani et al., 2016). These findings are outlined below and highlight a small, but important body of literature that promotes a first step in disentangling this complex relationship.

Inflammation

One of most prominent and widely studied markers of immune functioning and overall physical dysregulation is inflammation, which can be characterized as either acute or chronic. An acute inflammatory response is activated when the body suffers illness or injury, where cytokines

or related proteins are triggered to the affected area in the periphery, promoting the healing process. In an adaptive acute inflammatory response, following repair, these proteins retreat, and the body returns to a state of homeostasis (Ryan & Majno, 1977). This immunological process, however, becomes dysregulated when in a chronic state of autonomic or immunological activation. This constant state of physiological activation subsequently may put an individual at increased risk for experiencing chronic inflammation. Chronic inflammation is a process where the body becomes unable to shut off its inflammatory response leading to a high level of inflammation. In the absence of acute illness or injury, circulating proteins begin to attack healthy tissue. The HPA axis provides bi-directional communication with the immune system, which subsequently leads to the release or inhibition of inflammatory cytokines. The interaction of these systems is important given that dysregulation of either the HPA axis or the immune system can contribute to an increased likelihood of experiencing chronic inflammation.

Although chronic inflammation can be measured via a number of different immunological markers, cytokines tend to be the most frequently used metric in the majority of clinical research, particularly within clinical and health psychology. Cytokines are a specific type of protein, typically characterized as a chemokine, interferon, interleukin, or tumor necrosis factor that circulates spontaneously in the blood. These specific proteins are derived from a number of different cells (i.e., macrophages, lymphocytes, and mast cells). Cytokines may have pro-inflammatory properties (i.e., IL-1β, IL-2, TNF- α, IFN- γ), which promote higher rates of inflammation within the body, or anti-inflammatory (i.e., IL-4, IL-8, IL-12), whose release promotes the reduction of inflammation within the body. Additionally, interleukin-6 (IL-6) has both pro- and anti-inflammatory properties. Within psychological research, IL-6 is largely considered to be a pro-inflammatory cytokine, promoting the presence of inflammation in the

body. Although each of these proteins are derived from different types of cells, they all lead to immunological changes within the body that, over time, can have a significant impact on health and overall physiological functioning.

Increased rates of pro-inflammatory cytokines are associated with a number of deleterious health outcomes. Higher and prolonged levels of such markers contributes to a higher likelihood of experiencing congestive heart disease (Kaptoge et al 2013), inflammatory bowel disease (Halpin & Ford, 2012), Alzheimer's disease (Swardfager, Lanctôt, Rothenburg, Wong, Cappell, & Herrmann, 2010), chronic obstructive pulmonary disease (Gan., Man, Senthilselvan, & Sin, 2004), cancer (Heikkilä et. al, 2009), and stroke (Kuo, Yen, Chang, Kuo, Chen, & Sorond, 2005). Although these findings are largely correlational, the vastness of the literature examining the relationship between chronic inflammation and physical diseases highlights the need to better understand physical and psychological risk factors for high rates of inflammation in an effort to better prevent these long-term chronic health issues. Within coronary heart disease, a meta-analysis of prospective studies demonstrated higher levels of c-reactive protein (CRP), a protein circulating in the body that is produced as a direct result of higher levels of inflammation, being prospectively associated with development of the disease (Danesh et. al, 2000). Generally, high levels of inflammation are often a risk factor for metabolic syndrome, a non-specific condition diagnosed by assessing the presence of higher than average levels of triglycerides and/or fasting glucose, lower than average levels of HDL cholesterol, high blood pressure, and obesity. Metabolic syndrome is associated with heart disease, stroke, diabetes, and mortality broadly (Kaur 2014). As stated above, the HPA axis contributes to the regulation of the immune system, and the increase of inflammatory cytokines may therefore suggest a potential mediator in HPA axis dysregulation and these negative health outcomes.

Given the numerous negative health implications of high levels of pro-inflammatory cytokines, scientists and researchers have become increasing interested in understanding the link between inflammation and physical health. In addition, there has been an increasing amount of research and media coverage that have documented different methods for reducing inflammation, including specific diets, over the counter and prescription medications and lifestyle changes (including alterations in smoking, alcohol consumption, exercise, etc.). Although inflammation has been examined more widely within health psychology in relation to acute and chronic stress, clinical psychology has begun examining these variables and their relation to psychological traits and symptoms more and more over the past two decades.

Anxiety disorders have been shown to lead to increases in a number of inflammatory conditions such as inflammatory bowel disease, rheumatoid arthritis, and asthma (O'Donovan et. al, 2010). Patients with chronic obstructive pulmonary disease (COPD) have 5.3 times higher rates of anxiety disorders than a physically healthy comparison group (Karajgi, Rifkin, Doddi, & Kolli, 1990). Further, research examining anxiety disorders within patients with inflammatory bowel disease (IBD) found that rates of GAD, panic disorder (PD), and obsessive-compulsive disorder (OCD) were higher in patients with IBD than in general community samples of physically healthy individuals (Walker et. al, 2008). Within people with coronary heart disease (CHD) prevalence rates of SAD and GAD have shown to be 21.3% and 18.7%, respectively, with both of these rates substantially higher than in psychologically healthy individuals (Todaro, Shen, Raffa, Tilkemeier, & Niaura, 2007). This highlights the integral role that anxiety plays in physical health. However, to date, similar to the issues in examining inflammation and physical health, it is difficult to disentangle this relationship to determine whether anxiety causes an increased likelihood for experiencing these health conditions, or, rather, if these illnesses

increase the likelihood of feeling anxious. This remains a theoretical and empirical question within the field of psychoneuroimmunology. Given these demonstrated links of anxiety and inflammation with numerous physical health conditions, it is important to elucidate the ways in which these immunological and psychological processes may interact with one another.

There has been a large amount of research that has examined the relationship between depression and inflammation over the past several decades. A 2010 meta-analysis synthesizing these results highlighted a significant difference between individuals with a depression diagnosis and healthy controls, but only within a limited number of inflammatory markers (i.e., IL-6 and TNF-α; Dowlati et. al, 2010). In terms of anxiety, a number of studies have examined the relationship between anxiety symptoms broadly and inflammation (Duivis, Vogelzangs, Kupper, de Jonge, & Penninx, 2013; Pitsavos, Panagiotakos, Papageorgiou, Tsetsekou, Soldatos, & Stefanadis, 2006) as well as the physiological impact of chronic stress (McEwen, 2004; Miller, Chen, & Zhou, 2007). An anxiety diagnosis, however, is highly relevant to understanding inflammatory dysregulation, as it is characterized by a chronic state of preparation and defense to possible threats and may, therefore, put the body at potential risk for negative health effects from this more or less constant state of heightened physiological arousal. These disorders in particular represent an essential avenue for future research, as immune functioning may be more dysregulated in people with anxiety disorders than those simply high general state anxiety symptoms, which tend to be relatively non-specific. Therefore, measures of trait anxiety may not be specific enough to detect psychological mechanisms that underlie the link to exacerbated inflammatory cytokines.

Emerging research over the past several decades has provided some preliminary evidence for the impact of a diagnosis of an anxiety disorder on inflammatory biomarkers, but the majority

of this research has been conducted within PTSD and OCD with little consensus on the impact of diagnosis on inflammatory markers. A 2009 systematic review of PTSD and chronic inflammation indicated that individuals with PTSD have significantly higher levels of inflammatory dysregulation in comparison to healthy controls (Gill, Saligan, Woods, & Page, 2009). Researchers recently published an updated meta-analysis examining levels of different cytokines among individuals with PTSD and healthy controls. These findings demonstrated significantly higher levels of IL-6, IFN- γ, and IL-1β in people with PTSD compared to healthy controls (Passos et al., 2015). Within OCD research examining inflammation, a 2012 systematic review and meta-analysis showed that there were no significant differences in IL-6 or TNF-α between people with OCD and healthy controls (Gray & Bloch, 2012). However, there was a significant difference in IL-1β between the two groups. These results differed between individuals who had a comorbid depression diagnosis versus no depression comorbidity and among adults and children, as this meta-analysis included both age groups (Gray et. al, 2012), highlighting an important role that depression may play in understanding the link between anxiety disorders and inflammation. Further, across other anxiety disorders (i.e., generalized anxiety disorder, social anxiety disorder, and panic disorder), the evidence has generally been sparse and somewhat mixed (Furtado & Katzman, 2015). A recent meta-analysis found an overall significant difference between physically healthy adults with anxiety, obsessive-compulsive, and traumatic stress disorders and healthy controls in terms of pro-, but not anti-inflammatory markers (Renna, O'Toole, Spaeth, Lekander, & Mennin, 2018). In terms of breakdown between specific diagnostic groups, we found that those with PTSD had significantly different levels of pro-inflammatory cytokines compared to controls but that those with other anxiety disorders, including PD, GAD, SAD, and OCD did not. However, the majority of the

research linking anxiety disorders and inflammatory cytokines were within PTSD and the study was therefore underpowered to detect these between-group differences. Additionally, contrary to findings of other meta-analytic investigations, co-morbid depression did not moderate these results (Renna et al., 2018). Taken together, these findings indicate an overall significant impact that anxiety may have on inflammatory functioning. The findings from both depression and anxiety disorders research have demonstrated that some, but not all, of the inflammatory markers tested differ between people with psychopathology and healthy controls. Currently, the field of psychology has not yet disentangled why this may the case – however, it may be that these psychological processes impact the different cells that produce these cytokines differently, which in turn promotes greater dysregulation in some systems (i.e., macrophages that produce TNF- α, IL-6, and a number of other cytokines in the interleukin family). However, to date, no studies have examined mechanisms that may have impacted this relationship and future research is well geared to begin to disentangle how anxiety may influence inflammation and vice versa. According to the PCH, it may be hypothesized that cognitive processes such as worry and rumination may underlie the link between anxiety and high levels of pro-inflammatory cytokines.

Heart Rate Variability

Although research examining the long-term negative health impact of perseverative cognition is sparse in terms of its relationship to inflammatory processes, empirical examinations of the PCH as well as worry and rumination more broadly have demonstrated considerable evidence to support this theory. Perseverative cognition impacts the autonomic nervous system (ANS), comprised of the excitatory sympathetic nervous system (SNS) and the inhibitory parasympathetic nervous system (PNS). The body's ability to rapidly shift between excitatory

and inhibitory states demonstrates flexibility in heart rate. To date, research examining the physiological implications of perseveration has largely focused on autonomic measures such as heart rate variability (HRV). HRV is considered to be a marker of shifts between the SNS and PNS, with several metrics of HRV focusing on this autonomic balance while others are considered to be a putative measure of PNS responding (Allen, Chambers, & Towers, 2007). These putative measures of PNS responding are those most closely linked to psychopathology and particularly anxiety disorders (Applehans & Leuken, 2006). HRV is traditionally measured by the variation in beat-to-beat intervals of the heart that is thought of as a proxy for emotional responding (Applehans & Leuken, 2006). Higher HRV is largely considered to be more adaptive, as it demonstrates greater autonomic flexibility. Indeed, persistently low HRV is associated with higher risk for cardiovascular disease (i.e., congestive heart failure, stroke, hypertension, heart failure) as well as poor outcomes as a result of these conditions (Stauss 2003).

Autonomic processes such as HRV have been widely studied in relation to perseverative cognition. Previous research has demonstrated that individuals higher in trait worry are more likely to experience lower baseline HRV (i.e., greater cardiac rigidity) compared to those less prone to worrying (Lyonfields, Borkovec, & Thayer, 1995; Thayer, Friedman, & Borkovec, 1996; Brosschot, Van Dijik, & Thayer, 2007). In response to an experimental stressor task, high trait worriers also demonstrated slower HRV recovery (i.e., shifting from lower HRV during the task and returning to baseline HRV after the task) compared to low trait worriers (Verkuil, Brosschot, Beurs, & Thayer, 2009). Further, in comparing rates of HRV during state-level subjective rates of worry and rumination in response to emotion-eliciting film clips among physically and psychologically healthy participants, research has shown that worry is more closely associated with HRV than rumination (Aldao, Mennin, & McLaughlin, 2013). Taken

together, these findings highlight the integral role that worry plays in promoting autonomic dysregulation, subsequently putting individuals at risk for negative cardiovascular outcomes in the long-term.

Examinations of perseverative cognition in healthy participants have demonstrated similar findings to those utilizing high and low trait worry samples. Among psychologically healthy undergraduate students, those instructed to worry, compared to those instructed to relax or those given a neutral mentation condition had significantly lower vagal activity throughout the experiment (Llera & Newman, 2010). Independent of experimental manipulation, research findings indicate that when HRV is measured among physically healthy men and women, those who reported greater emotional stress during the week prior to study participation had lower levels of resting HRV compared to those who reported low levels of emotional stress (Dishman, Nakamura, Garcia, Thompson, Dunn, & Blair, 2000). A recent meta-analysis examining the impact of perseverative cognition (defined as either worry or rumination, either at the state or trait level) on HRV and other health-related processes found a significant decrease in HRV throughout experimental manipulations of perseverative in individuals free of psychological or physical diagnoses. Among correlational studies, there was also a significant association between higher levels of perseverative cognition and decreased HRV (Ottaviani, Verkuil, Medea, Couyoumdjian, Thayer, Lonigro, & Brosschot, 2016). Taken together, these findings highlight that worry, independent of psychopathology, contributes to significantly lower HRV compared to low levels of worry and compared to experimental conditions aimed at promoting relaxation or rumination. These findings are significant as they suggest not only simple baseline relationships between these variables but a possible more mechanistic role that perseverative cognition may play in exacerbating HRV dysregulation independent of chronic and excessive

rates of these cognitive processes common to GAD and MDD. It is therefore imperative to better understand the autonomic and immunological impact of worry on worsened physical health.

HRV also tends to be lower among people with formal diagnoses of anxiety disorders compared to healthy controls, and this comparison is especially salient for disorders characterized mainly or in part by worry. Compared to healthy controls, a 2014 meta-analysis found that people diagnosed with GAD, PTSD, SAD, and PD, but not OCD, had significantly lower HRV (Chalmers, Quintana, Abbott, & Kemp, 2014). These findings highlight the integral role that anxiety processes may have on autonomic functioning but fail to demonstrate whether there are mechanisms underlying the link between an anxiety disorder diagnosis and lower HRV. Further, it remains unclear whether decreased HRV may contribute to further negative health implications, such as higher rates of pro-inflammatory cytokines.

Cortisol

In addition to autonomic outcomes used to assess the PCH, cortisol has been used frequently in relation to both experimental inductions of perseverative cognition and trait level perseverative cognition. Cortisol is a hormone that is produced via the adrenal gland, often in response to stress, and therefore serves as a potent marker for HPA activation and has subsequently been widely used to understand the impact of stress on the body. Specifically, cortisol is released by the adrenal gland in times of increased stress, which subsequently contributes to a cascade of immunological and autonomic changes in the body. Cortisol promotes production of glucose in the body, which aids individuals in better preparing to fight or flee real or perceived threats in the environment. Cortisol regulates a variety of processes in the body including the immune system and metabolism. This hormone has been referred to as “the stress hormone” due to its modulation of changes in the body that typically occur as a result of stress

(i.e., blood glucose levels; Dickerson & Kemeny, 2004). Cortisol levels naturally vary throughout the day among individuals, with levels typically being highest at within 20-30 minutes after waking (Miller, Chen, & Zhou, 2007) and gradually decreases throughout the day. The overall impact of too much cortisol has been shown to have detrimental physical and psychological impacts, including weight gain, high blood pressure, and mood swings. In addition, low levels of cortisol may result in weight loss, fatigue, and dizziness (Lupien, Maheu, Ficco, & Schramek, 2007). Given the substantial impact that cortisol may have, both physically and psychologically, this hormone has proven to be a substantial marker of immune functioning in a variety of different populations. Cortisol levels are typically collected as baseline measurements to assess differences between groups, following experimental inductions, and as a marker of diurnal physiological variability (Segerstrom & Miller, 2004).

There has been a substantial amount of research examining perseverative processes in relation to cortisol. A 2006 review of studies supporting the PCH indicated that worry and rumination are linked to cortisol reactivity (i.e., increased or decreased production of cortisol as a result of a stressor; Brosschot et. al, 2006; Thomsen et. al, 2004). Cortisol has also been examined in relation to rumination, particularly while participants undergo social evaluative stressor tasks, as these tasks have been shown to be particularly able to elicit cortical responses among participants both with and without psychopathology (Dickerson & Kemeny, 2004). Research by Zoccola and colleagues (2008) demonstrated that following a social evaluation task (SET) participants experienced higher rumination compared to a control condition. This heightened rumination was subsequently related to a prolonged and amplified cortisol response, a physiological marker commonly used to assess HPA axis activity and dysregulation. Similar research by this group has found that compared to a distraction condition, healthy individuals

who were explicitly instructed to ruminate in response to the Trier Social Stress Test (TSST) had significantly higher levels of saliva-derived cortisol post-task (Zoccola, Figueroa, Rabideau, Woody, & Benencia, 2014). Higher daily levels of self-focused rumination (i.e., perseverating on past failures or flaws within oneself) has also been shown to be associated with higher mean daily cortisol levels compared to individuals low in rumination (Huffziger, Ebner-Priemer, Zamoscik, Reinhard, Kirsch, & Kuehner, 2013). In terms of worry, experimental manipulations of worry during a stressful context were associated with greater cortisol reactivity across healthy individuals as well as those diagnosed with SAD. In addition, worry was associated with increased cortisol during the recovery period post-task (Lewis, Yoon, & Joorman, 2017). The 2016 meta-analysis on the impact of perseverative cognition on physical health demonstrated a significant impact of experimental manipulations of perseveration on cortisol, such that greater perseveration led to increases in cortisol levels from baseline to post-manipulation. Further, correlational studies demonstrated a significant relationship between higher perseveration and higher cortisol levels (Ottaviani et al., 2016). Taken together, these findings indicate that cortisol can be significantly impacted both at baseline and throughout experimental manipulations of stress and related processes in psychologically healthy individuals. Although it is difficult to determine causality in these studies, it appears that worry and rumination may play an important role in influencing cortisol levels. Importantly, many of these studies highlight cortisol reactivity, where cortisol may be either increasing or decreasing during an experimental manipulation, highlighting a need for increased research within the field to better understand which processes may exacerbate or inhibit cortisol production.

Cortisol levels have also shown to be dysregulated among individuals with formal anxiety disorder diagnoses as well. Among individuals diagnosed with GAD, given its

characterization by high degrees of perseverative processes such as worry, findings have been less consistent. Particularly, a 2010 cross-sectional study demonstrated that individuals with GAD, compared to healthy controls, had lower levels of cortisol concentrations in the hair, one commonly used marker of short-term cortisol secretion (Steudte, Stalder, Dettenborn, Klumbies, Foley, Beesdo-Baum, & Kirschbaum, 2011). Salivary cortisol is also associated with greater symptom severity among people with GAD as well as trait worry (Mantella et. al, 2008). This is generally consistent among basal serum-derived cortisol levels in individuals with panic disorder (Staufenenbiel, Pennix, Spijker, Elzinga, & Rossum, 2013), although other findings have demonstrated higher cortisol levels among participants with panic disorder (Wedekind, Bandelow, Broocks, Hajak, & Ruther, 2000). These findings are not always clear, however, as greater PTSD symptom severity is associated with lower levels of morning and evening cortisol levels (Doruk, Gulsun, & Balikci, 2015) and has been shown to be significantly lower than in healthy controls (Yehuda, Teicher, Trestman, Levengood, & Siever, 1996). A 2007 meta-analysis comparing basal cortisol levels between people with PTSD and healthy controls found no significant differences between the two groups, with significantly lower levels among participants with PTSD only being present when cortisol was assessed through plasma or serum, but not salivary cortisol (Meewisse, Reitsma, Vries, Gersons, & Olff, 2007). Across all anxiety disorders, correlational research has shown significantly higher cortisol awakening levels in people with anxiety disorders compared to controls as well as higher cortisol levels one-hour post-awakening (Vreeburg et. al, 2010). Although findings have been mixed, the extant literature indicates that regardless of formal anxiety disorder diagnosis, processes that may underlie these disorders, such as worry and rumination, may associate with dysregulated patterns of cortisol responding and higher levels of basal cortisol levels compared to controls. Cortisol therefore

represents a worthwhile area of investigation in understanding the physiological impact of perseveration.

Integrative Research of Physical Health Outcomes and Perseverative Cognition

Taken together, previous research examining the PCH in relation to HPA axis and HRV dysregulation have demonstrated that people who are high in perseverative processes such as worry and rumination, both at the state and trait level, are more likely to experience lower HRV and higher cortisol responses compared to those who are low in these perseverative processes. To date, however, there has been no research examining whether immunological outcomes such as cytokine levels (a marker typically used to assess chronic inflammation) are associated with increased rates of worry and/or rumination. Research has begun to explore relationships between HRV and inflammation along with cortisol and inflammation given the influence that the activation of the HPA axis has on the cascade of increases in these processes. A 2008 study of physically and psychologically healthy men found that decreased HRV, interpreted as overall autonomic rigidity, was associated with higher levels of CRP and IL-6 (Lampert et. al, 2008). Prospectively, chronic low levels of HRV are associated with increases in inflammation, which, as mentioned above, may subsequently put individuals at an increased risk of experiencing negative health outcomes (Katon, Maj, & Sartorius, 2011; Kissane, Maj, & Sartorius, 2011; Thayer, Yamamoto, & Brosschot, 2010). The study by Zoccola and colleagues (2014) that found greater post-task cortisol in individuals who were instructed to ruminate rather than distract found that CRP levels were significantly higher in the rumination condition and did not return to baseline by the end of the experiment. Individuals in the distraction condition, although demonstrating increases in CRP during the TSST, experienced these levels returning to baseline by the end of the experiment. A recent meta-analysis found that higher perseverative cognition

was associated with greater blood pressure, higher heart rate, higher levels of cortisol, and lower HRV in both experimental and correlational studies (Ottaviani, Thayer, Verkuil, Lonigro, Medea, Couyoumdjian, & Brosschot, 2016). Given that the majority of this research has been correlational in nature, it is difficult to disentangle whether changes in cortisol and HRV contribute to higher levels of pro-inflammatory cytokines. Additionally, to date, no research has examined the combination of these autonomic and immunological processes within the context of perseveration.

Although there have been a number of studies that have looked at the experimental impact of perseveration on HRV and cortisol, to date, there has been no research that has examined inflammation within this context. A number of studies have examined increases in inflammation as a result of social stressors to understand the impact of stress on inflammation (Steptoe, Hamer, & Chida, 2007). Results of this 2007 meta-analysis indicated a small but significant impact of experimental stressors on inflammation from pre to post intervention, signifying significant increases in pro-inflammatory cytokines from baseline to post-task. However, less is known in regard to how the perseverative processes of worry and/or rumination may impact immunological functioning. The results of this meta-analysis examining have demonstrated that a significant increase in IL-6 and IL-1β across 13 studies from baseline to post-task and marginally significant increases in the c-reactive protein (CRP; Steptoe et. al, 2007). The majority of tasks utilized a traditional or modified version of the TSST (9 studies) highlighting an important role of social stress on inflammation. However, all of these studies utilized either physically and psychologically healthy individuals or individuals with chronic health conditions with the exception of one study that utilized participants diagnosed with major depressive disorder (MDD). Since the publication of this meta-analysis in 2007, there continues

to be no experimental manipulations of inflammation in relation to worry. Although the research examining inflammation and cortisol by Zoccola and colleagues (2014) has highlighted the importance of the perseverative processes and immunology link, there continues to be a discrepancy in our understanding of the impact of worry and how these physiological processes may be altered as a result.

The PCH and subsequent research supporting this theory highlights the role that worry may have on activating dysregulated physiological responses in the body such as through inflammation, cortisol, and HRV via increased activation of the HPA axis. This small but important body of research examining experimental manipulations of perseverative cognition highlight autonomic and cortisol outcomes and point towards a potential causal role that perseveration may have on these processes. Given that dysregulation of HRV, inflammation, and cortisol have been shown to promote deleterious health outcomes in the long term, it may be important to understand the mechanistic role that perseveration may play in exacerbating these outcomes. The examination of such data could have important implications in highlighting the necessity of reducing perseveration in an effort to promote better physical health via increased regulation of these physiological processes.

The Current Study

This study sought to examine dynamic changes in chronic inflammatory proteins, HRV, and cortisol throughout a worry and relaxation manipulation in individuals with varying degrees of trait worry. Participants underwent an experimental manipulation of worry and relaxation. Inflammatory cytokines, cortisol, and HRV recordings were collected throughout the experiment. The aims and specific hypotheses for this project were as follows:

1.To examine the interrelationships of inflammatory cytokines, heart rate variability, and cortisol at a baseline measurement and throughout an experimental manipulation of worry and relaxation. I hypothesized that inflammatory cytokines (pro-inflammatory markers IL-6, TNF- α, IL-1β, and IFN- γ), HRV, and cortisol would covary at baseline and throughout the worry and relaxation manipulations.

1. **To determine if trait worry moderates the relationship between inflammatory cytokines, heart rate variability, and cortisol at baseline and throughout the worry and relaxation inductions.** It was hypothesized that individuals high in trait worry would not demonstrate significant differences in inflammatory cytokines, cortisol, and HRV at baseline compared to people low in trait worry. Further, I hypothesized that there would be a significant change in inflammatory cytokines, HRV, and cortisol from baseline to post worry induction both in people who experience high and low degrees of trait worry. Among people higher in trait worry, I hypothesized that there would be significantly greater levels of inflammatory cytokines and cortisol and significantly lower HRV post worry induction than low trait worriers. Finally, it was hypothesized that individuals higher in trait worry would maintain increased levels of pro-inflammatory cytokines and cortisol as well as low HRV from the worry to relaxation inductions. Among people low in trait worry, I hypothesized that there would be a significant change in these markers post-worry induction compared to post-relaxation induction, such that inflammatory cytokines would be significantly lower post relaxation compared to post worry manipulations. Among low trait worriers, I also hypothesized that they would experience significantly greater HRV and lower cortisol levels following the relaxation

manipulation compared to worry manipulation and subsequently return to their baseline levels of physiological functioning.

2. **To examine temporal relationships among inflammatory cytokines, heart rate variability, and cortisol through baseline and the worry and relaxation manipulations.** As an additional exploratory hypothesis, I hypothesized that changes in HRV and cortisol would precede the changes in inflammatory cytokines throughout the experiment. Specifically, I hypothesized that changes in HRV and/or cortisol from baseline to the worry condition would precede changes in inflammation from the worry to relaxation condition. As an extension of this, I further hypothesized that trait worry would moderate this relationship, in that trait worry would interact with changes in HRV and cortisol to differentially predict changes in inflammation among high and low trait worriers. I hypothesized that the impact of changes in HRV and/or cortisol on inflammation would be greater among individuals high in trait worry compared to low trait worriers.

2. Methods

Participants

Participants were community members between the ages of 18 – 65 years old. All participants were required to be over 18 years old, be able to read and understand English, and be free of any autoimmune or inflammatory diseases. Participants were also excluded if they were on a consistent dose of medications that specifically has been shown to alter immunological functioning (i.e. daily prescribed use of NSAIDS, steroids, or SSRIs) or suffered from any diagnosed heart conditions. Exclusion criteria also included the presence of bipolar I disorder, alcohol or substance dependence, or active psychosis. Finally, due to the serum extractions,

participants were excluded if they endorsed a blood injury/injection phobia during a prelimnary phone screen (see below for phone screen details).

Screening Tools

All people interested in participating in the current study completed a brief phone screen to determine basic eligibility criteria. Phone screens were conducted by research assistants in the Regulation of Emotion in Anxiety and Depression (READ) Lab at Teachers College, Columbia University. First, information was obtained regarding the individual's age, occupation/student status, medication usage, and physical health (e.g. heart conditions, autoimmune and/or inflammatory conditions). All interested individuals were then screened using the Penn State Worry Questionnaire (PSWQ; see below). Although a formal cutoff score was not used, ensuring proper representation of four different quadrants of PSWQ scores aided in the stratification of recruitment. These quadrants represented low trait worry (PSWQ scores below 30), low-moderate trait worry (PSWQ scores between 31 and 45), moderate-high trait worry (PSWQ scores between 46 and 64), and high trait worry (PSWQ scores between 65 and 80), with approximately 25% of all participants being represented in each group. This type of stratification ensured a sufficient breath of worry symptoms are represented within the total sample. This stratification was important in light of previous research that suggested low trait rates of perseveration for individuals may not be successful in altering physiological outcomes as the result of the experiment (Ottaviani et al., 2016). However, at the same time, this grouping was consistent with ideas posited by the PCH in that chronic and excessive levels of worry characterized by a diagnosis of GAD may not be necessary in order to demonstrate perseverative cognition altering physiological activity. Based on previous research examining cutoffs for the PSWQ in identifying worry that may be consistent with a diagnosis of GAD (Mennin, Fresco,

Heimberg, & Turk, 1999; Ruscio 2002; DiNardo & Barlow, 1988), a cutoff score of 56 or above was utilized to categorize high trait worriers in all analyses.

The phone screen also contained questions related to diagnostic exclusionary criteria. As stated above, these diagnostic exclusions included bipolar I disorder, alcohol or substance dependence, active psychosis, and/or a blood injury/injection phobia. Potential participants were asked several questions to assess the potential presence of the disorders. The screening question for bipolar disorder was the following: "Have you ever been diagnosed with bipolar disorder?" The screening question for alcohol and/or substance dependence was the following: "Have you ever had a problem with alcohol and/or drugs? If so, is this something that you are currently struggling with?" In order to assess for active psychosis, participants were asked, "Have you ever heard things that other people could not hear or saw things that other people could not see?" Responses for each of these questions were recorded as a yes or no. Any individual who endorsed a yes for any of these questions was informed that they are unable to participate.

Potential participants were also asked a series of questions relating to a diagnosis of blood injury/injection phobia (i.e., are you especially afraid of needles, seeing blood, or getting a shot?). Potential participants were also asked if they have ever fainted in the context of being in a doctor's office or getting a shot/blood drawn to assess whether the potential participant would be likely to experience a vasovagal response during the experiment. If individuals responded yes to any of these screening items, they were informed that they were unable to participate in the study.

If participants were deemed able to withstand the blood draw portion of the study based off of the screening questions, were within the age specifications, and did not endorse any chronic illnesses that are known to impact cardiovascular or immunological functioning, a study

visit was scheduled by the research assistant conducting the phone screen. All information obtained during the phone screen was recorded in a password-protected Microsoft Excel sheet within the READ Lab at Teachers College, Columbia University. Following scheduling of the study visit, all responses were de-identified with a unique subject ID number.

Self-Report Measures

Penn State Worry Questionnaire (PSWQ): The PSWQ (Meyer, Miller, & Metzger, 1990) is considered to be the gold standard measure of pathological worry. This 16-item self-report measure requires all participants to respond these questions on a 5-point Likert-type scale ranging from 1 (*not at all typical of me*) to 5 (*very typical of me*), which are summed to create a total score representing how worried an individual typically (otherwise known as trait worry). These total sum scores range from 16-80, with scores of 65 and above indicating a high degree of worry congruent with a diagnosis of GAD (Fresco, Mennin, Heimberg, & Turk, 2003). Psychometric validation of the PSWQ has yielded strong reliability and validity among healthy individuals as well as people diagnosed with GAD (of which worry is a key diagnostic characteristic; Meyer et. al, 1990).

Sociodemographic questions. A questionnaire assessing a number of sociodemographic variables that have been shown to be associated with inflammation will also be administered as part of the questionnaire battery. This measure will collect data including race, ethnicity, height, weight, gender, and age. In addition, information will be collected regarding whether participants exercise, drink alcohol, drink caffeine, or smoke (and, if so, how much and how often they engage in these behaviors). Finally, information will be collected as to whether the participant suffers from a chronic illness and, if so, what type of illness. This will allow me to control for all necessary variables that may contribute to artificial increases in chronic inflammation.

Worry/Relaxation Manipulation

The worry and relaxation conditions were modeled off of previous research utilizing worry manipulation tasks to assess contrast avoidance (Llera & Newman, 2010; Borkovec & Inz, 1990; Fisher & Newman, 2013). Prior to completing the experimental portion of the study, participants were asked to list a number of items that they worry about the most, rating them as things that they worry about most frequently to least frequently. Upon beginning the worry manipulation, participants received instructions on the computer of the following: "Pick your most worrisome topic and worry about it as intensely as you can in your usual way for the next few minutes. If at any point your mind wanders off track, simply refocus your thoughts back onto your worry topic." (Fisher & Newman, 2013). Participants completed this induction for 10 minutes while receiving a prompt on the computer screen for 10 seconds every minute. For the relaxation condition, participants received the following instructions presented to them on the computer screen: "Shift your breathing from your stomach rather than from your chest. Also, slow your breathing rate down to a rate slower than usual but not so slow that it is unpleasant or uncomfortable. You might do this by counting from one to three as you breathe in evenly and then again as you evenly exhale." Participants completed this induction for 10 minutes while receiving a reminder of the instructions on the computer screen for 10 seconds every minute.

Previous research has utilized these tasks among low trait worriers, high trait worriers, and individuals diagnosed with GAD (Fisher & Newman, 2013). Despite a small sample size (N = 23), previous research from our lab has shown a similar worry manipulation has led to significantly greater subjective ratings of worry from baseline to post-task in a sample of undergraduate students with varying degrees of trait worry as measured by the PSWQ (for information on the subjective measure used, see manipulation check section below). These

findings demonstrate the utility of these manipulations within experimental research on perseveration. Although the task in the original study (Fisher & Newman, 2013) was only three minutes per condition and the proposed study utilizes 10-minute long inductions, researchers have posited that longer durations of perseveration may promote stronger outcome effects by increasing the intensity of experimentally-induced worry compared to these briefer, more discrete episodes (Ottaviani et al., 2016).

Physiological Assessment

Participants' heart rate variability (HRV) was monitored throughout the experimental portion of the study using the Polar Watch system. This ambulatory psychophysiological measurement device collects HRV data via a band with two electrodes placed across the participants' upper abdomen. The sampling rate for this device provided adequate sampling to obtain HRV statistics. Specifically, a sampling rate of 1024hz is at the level required to ascertain levels of HRV. HRV requires sampling rates that are higher in order to promote greater sensitivity in detecting interbeat interval differences (Allen et al., 2007). HRV data was collected continuously throughout the experimental portion of the data during each discrete segment of the experiment (i.e., baseline recording, worry manipulation, worry recovery/wait period, relaxation manipulation, relaxation recovery/wait period). Data was then uploaded from the device onto a computer in the READ Lab for processing and analysis.

Mean absolute successive interbeat interval differences (MSD) values, a commonly used metric of HRV that assesses the average differences in beat-to-beat intervals of the heart, was analyzed and obtained using CMetX (Allen et al., 2007). MSD is a putative measure of HRV in the clinical psychology literature (Allen et al., 2007).

Inflammatory and Cortisol Measurement

Inflammatory cytokines were assessed via serum-derived IL-6, TNF- α, IL-1β, and IFN- γ. An angiocath was inserted into the participant's non-dominant arm in order to obtain serum samples to test for inflammatory cytokines and cortisol. Blood was drawn at three time points (baseline, post worry, post relaxation) and collected via one gold-top 5mL tube per time point by trained nurses or phlebotomists at the CTSC. Nurses/phlebotomists inverted the tube 8 -10 times post-collection and immediately transferred the blood samples to the Core Lab for preparation, storage, and processing. All serum samples were centrifuged at 1200 x g for 15 min at four degrees Celsius and stored at -80 degrees Celsius until assay.

Inflammatory markers were analyzed in duplicate in batches utilizing enzyme-linked immunosorbent assay (ELISA) by the Core Lab. A multiplex assay kit was used to measure IL-6, TNF- α, IL-1β, and IFN-γ. Cortisol samples were also be analyzed in batches using ELISA methods. The results of both of these analyses were disseminated via an online results system created and monitored by the Clinical and Translational Science Center's (CTSC) Core Lab of Weil Cornell Medical College. All data was reported in picograms (pg) per milliliter (ml) of serum and nondetectable amounts of cytokines and cortisol was defined as levels < 0.1 pg/ml.

Manipulation Check

The Worry Visual Analog Scale (WVAS; Wichelns, Renna, & Mennin, 2016) was used to assess subjective changes in participant worry throughout the experiment. Worry is defined on the WVAS as, "talking a lot to ourselves about things that we are concerned about happening in the future". This measure contains two sheets: an anchor sheet and a score sheet. On the top of each sheet is a line representing 0 to 100. The anchor sheet asks a participant to describe five situations, personal to them, that represent differing degrees of worry. The score sheet asks a participant to refer to their anchor sheet and give themselves a score between 0 and 100,

according to how much worry they are experiencing "at the current moment." Using their anchor sheet as a guide, the participant rapidly provided a score at baseline, immediately following both mentation conditions, and twice throughout the wait period prior to the blood draw. Participants then immediately completed the WVAS after each blood draw. Previous research has demonstrated that the WVAS is a reliable measure of state-level worry and it has shown acceptable levels of convergent and discriminant validity among unselected undergraduate and clinical samples (Wichelns et. al, 2016). Participants also completed an anchor sheet asking for situations that lead them to feel relaxed. Similar to the WVAS anchor scale, participants indicated situations representing relaxation levels at 0, 25, 50, 75, and 100. Subjective 0 – 100 rating of their level of relaxation were collected at baseline, immediately following the relaxation condition as well as twice throughout the wait period prior to the third and final blood draw. The utilization of these measures allowed the examination of subjective levels of worry and relaxation throughout the experiment in order to determine whether or not the experimental manipulations altered these experiences for participants.

Procedures

All data collection took place at the Clinical and Translational Science Center (CTSC) of Weil Cornell Medical College. All study procedures took place between 9:00AM – 12:00PM to control for diurnal variation of inflammatory and cortisol changes and variability. Participants completed informed consent with a research assistant, followed by completing paperwork required by the CTSC for the blood draw portion of the study. Following informed consent, participants completed a baseline packet of subjective measures including the WVAS and RelaxVas anchor sheets, a baseline rating of worry and relaxation, and the PANAS. Participants then were instructed on how to properly put on the Polar Watch and band and were given privacy

to do so. They were then brought down the hall to a phlebotomy room where a CTSC nurse inserted the angiocath to the participant's non-dominant arm and drew the first blood sample via two 5-ml tubes (T0). Participants then completed a resting baseline for the psychophysiological assessment for five minutes followed by the worry mentation for 10 minutes. Immediately following the worry mentation, participants were instructed to undergo a thirty-minute rest period in order to allow time for immunological and cortisol changes to take place. Previous research has demonstrated that longer wait times post-experimental inductions or stressors are associated with stronger effects in examining both inflammation and cortisol (Steptoe et. al, 2007; Dickerson & Kemeny, 2004). During this thirty-minute rest, participants completed the WVAS every 10 minutes. At the end of the rest period, nurses/phlebotomists drew two 5mL tubes of blood through the angiocath (T1). Participants then underwent the same sequence of procedures for the relaxation condition, including the thirty-minute wait period and subsequent blood draw (T2). Following completion of the worry and relaxation conditions, wait periods, and blood draws, participants were detached from the psychophysiological equipment and the nurse removed the angiocath. Lastly, participants completed self-report questionnaires on Qualtrics to assess trait-level worry, rumination, anxiety, depression, and emotion dysregulation, as well as questions related to their demographics and physical health. Upon completion of the questionnaires, participants were compensated $50, debriefed, and dismissed.

Data Analysis Plan

Power analyses were conducted using G Power software. A power analysis conducted in an effort to detect a medium effect size (Cohen's $d = 0.50$) with 80% power yielded the necessity of a total sample size of at least 75 participants total (i.e., approximately 37 participants per group). All analyses were completed within SPSS software. All inflammatory, cortisol, and HRV data

will first be examined for normality, as these metrics are oftentimes skewed. If data is deemed to be non-normal, a log-10 or square root transformation will be used in an effort to normalize the skew of the data. Effect sizes are also reported for all analyses to determine the magnitude of each effect for each result.

Given that cortisol, HRV, and inflammation have been shown to be associated with several sociodemographic factors, bivariate correlations were run between all physiological outcome variables at baseline and BMI, gender, race, age, and smoking status to determine whether these variables were significantly related to the outcome variables. Analyses were run with all covariates in the model based off of previous findings suggesting doing so when working with markers of immune function (O'Connor et al., 2009).

In order to first determine whether or not the worry and relaxation manipulations promoted increased worry or relaxation across participants, paired sample t-tests were employed for WVAS and RelaxVAS ratings. Comparisons were made between baseline WVAS and RelaxVAS and post worry induction WVAS and RelaxVAS ratings to determine whether the worry induction significantly increased participants' subjective state-level ratings of worry while reducing relaxation. An additional paired sample t-test was run to assess changes in WVAS and RelaxVAS ratings both from baseline and post worry to the post relaxation induction to determine whether the relaxation condition significantly increased subjective state-level ratings of relaxation while concurrently reducing state-level subjective ratings of worry.

In order to examine changes in chronic inflammation, cortisol, and HRV across baseline, worry, and relaxation, separate repeated measures ANOVAS (i.e., one test for cortisol, one test for HRV, and one test for each type of inflammatory marker) were conducted collapsing all participants together. In order to examine Aim 1 and assess how inflammatory cytokines, HRV,

and cortisol relate to one another at baseline and throughout the experimental conditions, mixed linear models (MLMs) were used to assess how these variables co-vary throughout the three study conditions (i.e., baseline, worry, relaxation). Separate models were used for each type of inflammatory marker and its relationship to HRV and cortisol. Models were based on random intercept or random slope and intercept if it was found that the addition of both random effects significantly improves the model, as indicated by comparing -2LL values for both analyses.

Independent sample *t*-tests were conducted for HRV, cortisol, and each inflammatory marker in order to assess the first hypothesis of Aim 2 and determine how these variables compare at baseline between people high and low in trait worry. Two separate 2 (high and low trait worry) x3 (baseline, post worry, post relaxation) ANOVAs were conducted in order to test the second hypothesis of Aim 2 of the current study with a specific interest in examining the within-between interaction. Separate ANOVAs were run for each inflammatory marker as well as for HRV and cortisol. As applicable, the non-parametric equivalents of these tests were run for any data that were deemed as non-normal if it was not possible to correct the data via a log10 or square root transformation. Specifically, Mann-Whitney U tests were used to examine differences in physical functioning between the participants high and low in trait worry. Further, Friedman tests for repeated measures of related samples was utilized as an equivalent of the RM ANOVA to test for changes in physical functioning throughout the three conditions (baseline, worry, relaxation). Given that post hoc tests are not possible when using nonparametric tests, the Friedman tests were followed by separate Wilcoxon Signed Rank tests to determine the specific breakdown of differences across conditions (e.g. differences between baseline and worry, worry and relaxation, etc.)

In order to examine Aim 3, time-lagged mixed linear models (MLMs) were utilized. Data was restructured in such a way that time-lagged values for HRV and cortisol were computed by including baseline and post worry values while inflammatory data was restructured to include post worry and post relaxation values. The resulting restructured data set included two different timepoints (baseline-post worry and post worry-post relaxation). Time was be entered as a repeated measure at Level 1 for all models. HRV or cortisol was included as a continuous variable at another level with each inflammatory marker included as the dependent variable in separate models. In order to examine the second hypothesis of Aim 3 and examine moderation of this effect, each model was re-run with group (high versus low trait worry) entered as an additional covariate with the interaction between either HRV or cortisol and group entered as a separate fixed factor. Models were based on Maximum Likelihood Estimation. Models were also based on random intercept or random slope and intercept if it is found that the addition of both random effects significantly improves the model, as indicated by comparing -2LL values for both analyses. If not, only a random intercept was included. Effect sizes are also reported for all analyses to determine the magnitude of each effect for each result.

3. Results

Missing Data and Outliers

Ninety participants were enrolled in the current study. Outliers were examined for all outcome variables and removed. Overall, five participants were excluded from analyses for having values more than 1 standard deviation above the mean on at least one outcome variable, resulting in a total final sample of 85. The vast majority of IL-1β levels for participants were below a detectable limit – only 18 of a possible 255 samples had levels > 0.1. As a result, IL-1β was not utilized in any of the subsequent analyses. In addition to not having samples for this

marker, seven participants did not have their blood drawn and were subsequently excluded from analysis. Three of these participants fainted. One participant had blood pressure that was deemed too high to participate based on the nurses' recommendation at the CTSC. An additional participant was suspected of being under the influence of drugs and/or alcohol upon presentation to the study and was subsequently dismissed. Finally, two participants completed their baseline blood draw but terminated their participation in the study prior to completing all study procedures.

An additional 10 participants were missing HRV data for some portion of the collection period. Of these missing data, four were due to errors by the research assistant running the study (e.g., not starting or stopping the HRV recording at the appropriate time, forgetting to start HRV recordings during the experiment). Four participants had HRV data collected throughout the study but due to errors on the Polar Watch device the data was unable to be downloaded from the device and processed. Finally, two participants had partially collected HRV data but did not have data for all three timepoints due to space issues on the Polar Watch.

Demographic Characteristics

Participants' mean age was 30.88 years old (*SD* = 11.42). The sample was predominantly female (*n* = 53, 62.4%). The sample also demonstrated racial diversity overall. Self-reported race for this sample was: 39.2% White (*n* = 31), 15.2% African American (*n* = 12), 24.1% Asian American or Pacific Islanders (*n* = 19), 5.1% as mixed race (*n* = 4), 1.3% as 'other' (*n* = 1), and 15.2% of the sample self-identified their race as Hispanic/Latino (*n* = 12). Overall, 57.5% of participants (*n* = 46) were undergraduate or graduate students, 31.3% were employed either full-time or part-time (*n* = 25), and 11.3% unemployed (*n* = 9) and one person did not report these data. Participants had a mean screening PSWQ score of 49.20 (*SD* = 14.73) with 33 participants

being categorized as high worriers, indicating overall success in recruiting participants into the different worry quadrants.

Examination of Covariates

Correlations between potential covariates and all physiological outcome variables at baseline are presented in Table 1. Ten participants enrolled in the study were prescribed a psychiatric medication while 14 were prescribed medication for a medical or physical issue or as a method of birth control. Bivariate correlations demonstrated no significant relationships between medication usage and any of the outcome variables (all *p*s > .34). In terms of age, there was a significant relationship between HRV (r = -.40, p < .001). Age was also significantly related to baseline IL-6 (r = .29, p < .01) and TNF- α (r = .30, p < .01). There were no significant relationships between age and IFN-γ (r = .04, p = .69) or cortisol (r = -.11, p = .52). There were no significant relationships between race and any of the outcome variables (all *p*s > .17). In terms of gender, a point biserial correlation indicated that there was a significant association between gender and TNF- α (r = -.34, p = .001). No other significant point biserial correlations between gender and outcome variables emerged (all *p*s > .06). Given point biserial correlations assume one continuous and one categorical variable that is dummy-coded, the direction of the associations between outcome variables and gender can be ignored.

On average, participants had a mean body mass index (BMI) of 24.76 (SD = 5.90). Three participants indicated that they were diagnosed with a medical condition (one polycystic ovarian syndrome, one irritable bowel syndrome, one obstructive sleep apnea). BMI was significantly and positively correlated with baseline TNF- α (r = .33, p < .01) and IL-6 (r = .30, p < .01). There were no other significant relationships between BMI and the other outcome variables (all *p*s > .10). Finally, smoking status was significantly associated to both IL-6 (r = -.77, p < .001)

and IFN-γ (r = -.82, p < .001) while no other significant correlations emerged with the other outcome variables (all ps > .38). Similar to the dummy-coded gender variable, the point biserial correlation of the relationship between smoking status and all outcome variables can ignore the direction of the associations.

Although a number of proposed covariates were not associated with the outcome variables at baseline, previous research has suggested the necessity of controlling for these and a number of other variables despite their relationship to outcome given the strong influence that they have demonstrated on inflammation and other biomarkers in previous studies (O'Connor et al., 2009). Given this, all proposed covariates were controlled for in the subsequent analyses when possible as a conservative assessment of the influence of such variables on immune, endocrine, and cardiovascular function.

Normality Tests

Skew and kurtosis were examined to determine whether or not the inflammatory, cortisol, and HRV data were normally distributed. HRV data was derived from the MSD variable. The MSD was non-normal at baseline (Shapiro Wilk = .93, p = .001), worry (Shapiro Wilk = .92, p = .001), and relaxation (Shapiro Wilk = .96, p = .02). Performing a square root transformation on the MSD variable corrected the skew and kurtosis at baseline (Shapiro Wilk = .99, p = .70), worry (Shapiro Wilk = .98, p = .20), and relaxation (Shapiro Wilk = .99, p = .73), respectively. Results indicated that IL-6, TNF-α, IL-1b, and IFN-γ were not normally distributed. Square root and log 10 transformations were utilized, but these transformations did not normalize the data. Further, the cortisol data were not normally distributed. Square root and log 10 transformations did not normalize the data. Tables 2, 3, 4, 5, and 6 provide information regarding normalizing the

data for HRV, cortisol, IL-6, TNF-α, and IFN-γ, respectively. The inclusion versus exclusion of outliers did not impact normality of the data.

Manipulation Check

Means and standard deviations for the WVAS and RelaxVAS are presented in Figure 1. In order to examine whether or not the worry and relaxation tasks promoted an increase in subjective worry and relaxation, independent sample *t*-tests were conducted to examine changes from baseline to post-worry or post-relaxation among all participants. Overall, results indicated a significant increase in subjective ratings of worry following the worry task for all participants (t = -9.83, df = 79, p = 0.00, cohen's d = -1.54). Further, there was a significant increase in subjective ratings of relaxation following the relaxation task compared to baseline (t = -7.34, df = 79, p = 0.00, cohen's d = -1.15). Further, the worry condition led to a significant reduction in relaxation across all participants (t = 6.06, df = 79, p = 0.00, cohen's d = 0.95), while the relaxation condition led to a significant reduction in worry across all participants (t = 14.42, df = 79, p = 0.00, cohen's d = 2.25). These results can be found in Table 3.

Aim 1: Covariation of immune markers, cortisol, and HRV

HRV data was able to be corrected via transformation while the inflammatory and cortisol data remained non-normal despite transformation. Despite the violation of assumptions of normality, the covariation analysis was run to examine Aim 1. Separate covariance analyses were run to determine the relationship of change with each inflammatory marker and HRV and cortisol separately. Results are presented in Table 5. In terms of model fit, random slopes were included in all models. The inclusion of both random slope and intercept did not improve the models based on -2LL values. Therefore, the final results of the model including random slope only are reported. Each model controlled for all related sociodemographic variables.

Overall, the three variables did not significantly covary throughout the different conditions when examining IFN-γ, IL-6, and TNF-α in separate models. In examining the manner in which HRV and cortisol alone covaried throughout the study conditions, results demonstrated that there was not a significant relationship between the two across time (p = .18), indicated that HRV and cortisol did not have a similar rate of change to one another throughout the three study conditions.

Aim 2: HRV Data

All HRV analyses utilized the square root transformed MSD variables. Means and standard deviations of the non-transformed variables for each condition are presented in Table 4. Age was controlled for in all of the following analyses given the significant correlation between age and baseline MSD.

Across all participants, paired sample *t*-tests revealed a significant different in MSD between baseline and the worry condition (t = 2.92, df = 74, p = 0.01, cohen's d = .68) and between baseline and the relaxation condition (t = -3.35, df = 71, p = 0.001, cohen's d = -.80) as well as a significant difference in MSD between the worry and relaxation conditions (t = -5.25, df = 70, p = 0.00, cohen's d = -1.26). A repeated measures ANOVA revealed a significant difference in MSD across all participants between the three conditions (F [2,69] = 14.17, p = .00, partial eta squared = .29). Pairwise comparisons corroborated findings from the paired sample *t*-tests, revealing a significant difference between the different conditions such that MSD decreased significantly across all participants from the baseline to worry conditions, and that MSD was significantly higher in the relaxation condition compared to both the baseline and worry conditions.

There was not a significant difference in baseline MSD between participants high and low in trait worry (t = .79, df = 70, p = 0.44, cohen's d = .13). Overall, participants high in trait worry demonstrated higher HRV compared to low trait worriers. The group (high versus low PSWQ) x condition interaction yielded a trending difference in MSD across conditions (F [2,69] = 3.20, p = .07, partial eta squared = .05). Overall, all participants experienced decreased MSD during the worry condition compared to baseline, but there was a significantly greater decrease in MSD among high trait worriers compared to low trait worriers. Both groups experienced a significant increase in MSD between the worry and relaxation conditions. For both groups, MSD during the relaxation condition was greater than baseline.

Aim 2: Cortisol Data

Means and standard deviations of the cortisol data across each condition are presented in Table 4. Given non-normality of the data that could not be corrected via transformations, nonparametric tests were used to assess changes over time throughout the baseline, worry, and relaxation conditions and to determine baseline differences between individuals high and low in trait worry. Results of the Mann-Whitney U test indicated that there was not a significant difference in baseline cortisol between groups (U = 706.00, p = 0.71).

Results of the Friedman test indicated an overall significant difference among all participants in cortisol between the baseline, worry, and relaxation conditions (X^2 = 25.22, p < .001, cohen's d = 1.32). Wilcoxon Signed Ranks tests to follow up on this change indicated significant differences among each condition (all ps < .01). Specifically, contrary to study hypotheses, results indicated that cortisol at baseline was significantly higher than during the worry condition (z = -3.71, p < .001, cohen's d = -.89). Further, consistent with study hypotheses, cortisol differed significantly between the worry and relaxation conditions, with

cortisol being significantly lower during relaxation compared to worry (z = -2.90, p < .001, cohen's d = -.67). Cortisol was also significantly lower following relaxation compared to baseline (z = -4.02, p < .001, cohen's d = -.98).

While the data violated the assumptions of normality, the group*condition interaction was run to determine the impact of group on cortisol change throughout the experiment. The group (high versus low PSWQ) x condition interaction yielded no significant impact of worry across conditions (F [2,76] = .403, p = .53, partial η^2 = .005). Descriptive and available test statistics corroborated this finding, demonstrating a similar trend across time for both high and low trait worriers. Cortisol levels, overall, were lower among high trait worriers compared to low throughout the experiment, with slight decreases in cortisol among both groups following the worry condition which continued to decline slightly following relaxation. The parametric RM ANOVA corroborated the findings of the Friedman Test and identified a significant difference in cortisol between the three conditions (F = 14.11, p < .001, partial η^2 = .16).

Aim 2: Inflammation Data

Means and standard deviations of the inflammatory variables for each condition are presented in Table 4. Due to the fact that all inflammatory data were not normally distributed and could not be corrected via a lg10 or sqrt transformation, nonparametric tests were used. However, results of the group*condition interaction are presented below in order to understand the way that group influenced the current findings.

IL-6. Results of the Mann-Whitney U test indicated that there were no significant differences in baseline IL-6 between participants high and low in trait worry (U = 616.50, p = 0.16). Results from the Friedman test indicated a significant difference in IL-6 across the three conditions (χ^2 = 32.03, p < .001, cohen's d = 1.59) for all participants. While there were no

significant differences in IL-6 between baseline and the worry condition (z = -1.27, p = 0.20, cohen's d = -.28), a significant difference did emerge between the baseline and relaxation condition (z = -4.45, $p < 0.01$, cohen's d = -1.12). Further, there was a significant difference in IL-6 between the worry and relaxation conditions (z = -4.50, $p < 0.001$, cohen's d = -1.14). Results indicated that although there was a slight, although non-significant increase in IL-6 levels between baseline and the worry condition, IL-6 levels continued to significantly increase throughout the relaxation condition, with the highest levels of IL-6 being present following relaxation compared to the other two conditions.

While the data violated the assumptions of normality, the group*condition interaction was run to determine the impact of group on IL-6 change throughout the experiment. The group*condition interaction yielded no significant impact of worry on changes in IL-6 throughout the study conditions (F [2,76] = .002, p = .96, partial η^2 = .000). The available test statistics and mean changes across conditions for each group demonstrated that participants in the high worry group experienced a significantly greater increase in IL-6 following the worry condition compared to low trait worriers. IL-6 continued to increase among both groups following the relaxation condition, indicating no significant differences between the two groups following relaxation. The parametric RM ANOVA corroborated the findings of the Friedman Test and identified a significant difference in IL-6 between the three conditions (F = 16.82, p < .001, partial η^2 = .18).

TNF-α. There were no significant differences in TNF-α between groups (U = 710.50, p = 0.63). Results of the Friedman test demonstrated no significant changes in TNF-α across participants between conditions (χ^2 = 3.30, p = .19, cohen's d = .41). Follow-up Wilcoxon Sign Rank Tests corroborated this finding, demonstrating no differences in TNF-α between baseline

and worry (z = -.70, p = .48, cohen's d = -.15), baseline and relaxation (z = -1.42, p = .16, cohen's d = -.32), or worry and relaxation (z = -.63, p = .53, cohen's d = -.14).

While the data violated the assumptions of normality, the group*condition interaction was run to determine the impact of group on TNF-α change throughout the experiment. The group*condition interaction yielded no significant impact of worry on changes in TNF-α throughout the study conditions (F [2,76] = 2.34, p = .13, partial η^2 = .03). Available test statistics and variable means demonstrated little to no change in TNF-α among low trait worriers throughout the different study conditions. High trait worriers demonstrated an increase in TNF-α following the worry condition compared to baseline which decreased marginally following relaxation. The parametric RM ANOVA corroborated the findings of the Friedman Test and identified no significant difference in TNF-α between the three conditions (F = .615, p = .44, partial η^2 = .01).

IFN-γ. Results indicated no significant differences in IFN-γ between participants high and low in trait worry (U = 649.00, p = 0.27). Across all participants, results of the Friedman test indicated a significant difference in IFN-γ across the three conditions (χ^2 = 49.24, p < .001, cohen's d = 2.42). Follow-up Wilcoxon Sign Rank Tests corroborated this finding, indicating a significant difference in IFN-γ between baseline and the worry condition (z = -5.36, p < .001, cohen's d = -1.46). A significant difference also emerged between the baseline and relaxation conditions (z = -6.07, p < .001, cohen's d = -1.79) in addition to the worry and relaxation conditions (z = -2.86, p < .01, cohen's d = -.66).

Similar to the IL-6 and TNF-α data, the group*condition interaction was run to determine the impact of group on IFN-γ change throughout the experiment. The group*condition interaction yielded no significant impact of worry on changes in IFN-γ throughout the study

conditions (F [2,76] = .095, p = .76, partial η^2 = .001). Examining the available test statistics and mean changes across conditions for each group, it appeared that participants in the high worry group experienced a significantly greater increase in IFN-γ following the worry condition while low trait worriers demonstrated a significant decline in IFN-γ between these two conditions. IFN-γ continued to increase among high trait worriers following the relaxation condition while it continued to decrease among low trait worriers from the worry to relaxation condition. The parametric RM ANOVA corroborated the findings of the Friedman Test and identified no significant difference in IFN-γ between the three conditions (F = .615, p = .44, partial η^2 = .01).

Aim 3: Changes in cortisol and HRV preceding inflammatory change

Transformed data was utilized for the HRV data while inflammatory and cortisol data were non-transformed in this analysis. Although assumptions of normality were violated, mixed linear models were utilized to examine if changes in HRV and cortisol temporally preceded changes in each inflammatory marker across the study conditions, and whether this pattern was dependent upon group status (e.g., high or low trait worry). Each model was run with a random intercept as well as with a random slope and intercept. Overall, the inclusion of a random slope did not improve the model fit based on the -2LL. Subsequently, results are reported below based on random intercept models only.

IL-6. Results of the mixed linear model revealed that changes in HRV from baseline to post worry did not significantly predict changes in IL-6 from post worry to post relaxation (F[1, 132.13) = .007, p = .93, Cohen's d = .02). When examining the interaction between HRV and trait worry, there was a trend in the relationship between changes in HRV from baseline to worry and changes in inflammation from worry to relaxation (F[1, 132.11) = 3.26, p = .07, Cohen's d = .40). This model did produce a moderate effect size, highlighting a probable influence of HRV

on IL-6 change. Changes in cortisol from baseline to post worry also did not significantly predict changes in IL-6 from worry to relaxation (F[1, 151.78) = 1.48, p = .23, Cohen's d = .27). Trait worry also did not interact with cortisol to significantly influence this relationship (F[1, 150.26) = .02, p = .90, Cohen's d = .03).

IFN-γ. Similar to IL-6, changes in HRV from baseline to post worry did not significantly predict changes in IFN-γ from post worry to post relaxation (F[1, 131.62) = 2.11, p = .15, Cohen's d = .32) although the effect size was of a moderate magnitude. The inclusion of trait worry within the model demonstrated that worry did not interact with HRV to predict changes in inflammation (F[1, 86.05) = .04, p = .85, Cohen's d = .04). Changes in cortisol from baseline to post worry also did not predict changes in IFN-γ from worry to relaxation (F[1, 114.99) = .03, p = .86, Cohen's d = .04). Trait worry did not significantly interact with cortisol to influence changes in inflammation (F[1, 120.81) = 1.06, p = .31, Cohen's d = .23).

TNF-α. Changes in HRV from baseline to post worry did not significantly predict changes in TNF-α from post worry to post relaxation (F[1, 152.38) = .65, p = .42, Cohen's d = .18). The interaction between changes in HRV and trait worry in influencing changes in TNF-α was not significant (F[1, 97.64) = .29, p = .59, Cohen's d = .12). Changes in cortisol from baseline to post worry also did not predict changes in TNF-α from worry to relaxation (F[1, 119.96) = .16, p = .69, Cohen's d = .09). There was no significant influence of trait worry on this relationship (F[1, 117.59) = 1.91, p = .17, Cohen's d = .31).

4. Discussion

This study sought to examine the differential impact of worry and relaxation on several physiological markers of health, including HRV, cortisol, and four different inflammatory markers (IL-6, IL-1b, TNF-α, and IFN-γ). Specifically, the current study builds off of empirical

work of both the contrast avoidance model of worry (Newman & Llera, 2011) and the perseverative cognition hypothesis (Brosschot et al., 2006) to better understand the ways in which the cognitive process of worry may prolong a psychological stress response, subsequently creating sustained physiological activation. The impact of worry and relaxation was examined among both high and low trait worriers, and it was first hypothesized that there would be no differences between all of these variables between the two groups at baseline. I also hypothesized that there would be a significant change in HRV, cortisol, and inflammatory markers from baseline to following the worry condition across all participants. Following the relaxation condition, it was hypothesized that low trait worriers would return to their baseline physiological functioning while high trait worriers would not. Further, this study sought to examine whether HRV, cortisol, and inflammation covaried throughout the different conditions. Lastly, an exploratory aim of the current study sought to examine whether changes in cortisol and/or HRV temporally preceded changes in inflammatory markers throughout the study conditions and whether this relationship was influenced by trait worry. To date, this is the first known study to examine contrasting worry and relaxation conditions and how such conditions impact inflammatory functioning.

Summary and Interpretation of Study Findings

The hypothesis that high and low trait worriers would not differ in terms of physiological functioning at baseline was supported. Findings indicated that participants high and low in trait worry did not have significantly different HRV at baseline. Interestingly, the pattern of HRV responding was in the opposite direction of what would be typically expected, in that low trait worriers had lower HRV than high trait worriers at baseline. This finding is contrary to previous findings demonstrating lower HRV in high trait worriers or individuals diagnosed with GAD

compared to low trait worriers or controls (Thayer, Friedman, & Borkovec, 1996). Consistent with study hypotheses, there were no differences in inflammatory functioning across any of the inflammatory markers when comparing individuals with high and low trait worry. There were also no significant differences between the two groups in terms of baseline cortisol levels, consistent with some previous research that has examined hair cortisol concentrations in participants diagnosed with GAD and healthy controls (Steudte et al., 2011). To date, this is the first study that has examined differences in systemic inflammation and cortisol among individuals with high and low trait levels of worry independent of psychological diagnoses. However, there have been several previous studies that have examined inflammation in the context of GAD compared to healthy controls. These studies demonstrated non-significant or small differences between these groups (Koh & Lee, 1998; Vieira et al., 2010), demonstrating that individuals experiencing chronic, pervasive anxiety and worry did not differ in terms of inflammation from their healthy counterparts. The current findings are well in line with this previous research, although additional work is needed to determine whether there are other processes associated with anxiety that may be contributing to inflammatory and endocrine dysregulation among individuals.

Overall, participants across both groups showed a significant difference in HRV across the baseline, worry, and relaxation conditions. Notably, as hypothesized, participants' HRV was significantly lower during the worry condition compared to both baseline and relaxation regardless of group. Further, HRV during the relaxation condition across all participants was significantly higher than even the baseline condition, demonstrating a significantly relaxing physiological effect of the condition. The worry condition, on the other hand, increased physiological activation as demonstrated by lower HRV across individuals both high and low in

trait worry. When examining differential physiological functioning between the high and low trait worry groups, we found partial support that changes in physiology differed across the conditions. Results for HRV demonstrated a trending interaction between group (high versus low worry) and condition (baseline, worry, relaxation) with a moderate effect size, demonstrating a trend towards significant differences in HRV across the conditions based on whether someone was prone to high levels of trait worry or not. This finding contributes to a larger body of research examining changes in HRV and its association with worry in both laboratory-based and self-report studies. Indeed, a recent study looked at worry as a transdiagnostic symptom of anxiety disorders and found that trait worry, more so than an anxiety diagnosis, was associated with lower levels of HRV during a resting state/baseline collection (Chalmers, Heathers, Abbott, Kemp, & Quintana, 2016). While the current findings highlight a pattern of results consistent with this previous research, the non-significant group by condition interaction is also consistent with some research looking at worry manipulations such as the one used in this study in high trait worriers which found a trending effect (Fischer & Newman, 2013). The interaction in this study was trending with a moderate effect size and these findings may therefore be consistent but slightly less strong with that of Fischer and Newman (2013).

In terms of inflammatory findings, this study found a significant difference in both IL-6 and IFN-γ between the baseline and the worry conditions. However, there was no significant change in TNF-α between the baseline and the worry conditions. This finding is inconsistent with the hypothesis that there would be significant changes in inflammation across both groups between baseline and worry, as it was expected that the worry condition would contribute to an increased physiological stress response across all inflammatory markers. When comparing inflammatory levels from baseline to relaxation and worry to relaxation results appeared to be

more mixed. Notably, for IL-6, while there were no significant differences from baseline to worry, follow-up analyses demonstrated a significant difference between the baseline and relaxation conditions as well as from the worry to relaxation conditions. Further, rates of change in IL-6 was similar between the two groups, with slight increases in IL-6 levels observed throughout the experiment. This finding highlights the likelihood of inflammatory change potentially occurring at a slower rate with levels continuing to change throughout the experimental portion of the study. Although this is in contrast to the study hypotheses, given the contrasting conditions within a relatively short time window, it is likely that experimentally-induced inflammatory change for some inflammatory markers may be relatively slow and therefore less amenable to this type of study design. Subsequently, it may be important for future research to separate out these conditions (e.g., worry and relaxation) to determine whether a slow-changing marker such as IL-6 may be amenable to experimental change within one of these conditions rather than both. Lastly, consistent with study hypotheses, there was a significant difference in IFN-γ across all conditions in a manner that was expected between baseline and worry and then decreased from worry to relaxation. Although the group by condition interaction was not significant, examining means across conditions for participants high and low in trait worry demonstrated a somewhat different pattern of IFN-γ activity between the two groups. Notably, IFN-γ levels increased for high trait worriers between baseline and worry and then continued to increase during the relaxation condition. Among low trait worriers, the opposite pattern was observed – IFN-γ levels decreased from baseline to worry and then continued to reduce throughout relaxation. While IL-6 and IFN-γ demonstrated some experimental changes throughout the different conditions, findings related to TNF-α showed that it did not change

significantly throughout the conditions among either high or low trait worriers. TNF-α may therefore be less susceptible to experimental change compared to other inflammatory markers.

Taken together, the inflammatory findings from the current study lead to larger questions within the field of psychoneuroimmunology as to why some seemingly related biomarkers, but not others, may change throughout the course of experimental manipulations. The inflammatory markers chosen for the current study were based off of a previous meta-analysis examining differences in inflammation among individuals with anxiety disorders versus healthy controls which highlighted these specific markers to be different among the two groups and therefore seemed to be particularly salient among people high in trait anxiety (Renna et al., 2018). A more in-depth understanding of these markers and their susceptibility to change in psychological experiments likely needs to be derived from a greater understanding of the composition of each of these different proteins. This may also be an important step in disentangling the specific biological implications of processes such as worrying on the body, as greater specificity in biomarkers may help to gain insight into specific physical targets that are impacted while perseverating.

Overall, findings demonstrated a significant change in cortisol throughout the different study conditions, in that cortisol actually decreased from baseline to worry across participants and continued to decrease following relaxation. This finding is partially in support of the study hypotheses, as it highlights that cortisol functioning does decrease following experimentally-induced relaxation, but it is not necessarily impacted in the expected direction following experimentally-induced worry. Further, the inclusion of both worry and relaxation conditions in a fixed order makes it difficult to ascertain the differential impact of the two conditions. Although the interaction between group (high versus low trait worry) and condition was not

significant, the general pattern of mean change of cortisol throughout the condition demonstrated similar changes between both high and low trait worriers. In terms of methodological considerations, a possible interpretation of these lack of results is that cortisol is not necessarily sensitive to these types of experimental inductions, as this was the first study to look cortisol responses as a result of experimental inductions of worry and relaxation. The current study utilized serum-derived cortisol levels in order to reduce participant burden, as cortisol levels could be obtained through the same sample as inflammatory markers. However, serum-derived cortisol is less common within the field compared to saliva-derived samples via the passage drool method. As a result, it may also be difficult to interpret the current findings in the context of other cortisol research within the field using different sampling methods.

Previous research looking at cortisol has found both higher and lower levels of cortisol following experimental induction among participants with distress symptoms (e.g., anxiety, depression, rumination) making it difficult to contextualize the current findings within the larger body of literature in the field. The 2004 meta-analysis by Dickerson and Kemeny highlighted 16 studies utilizing an experimental emotion induction, which were comprised of both idiographic and nomothetic inductions. Overall, 8 of the 16 studies demonstrated a negative effect size, indicating that cortisol levels went down throughout the stressor period. Four of these 16 studies had positive effect sizes while four results were null. Taken together with these findings, the current study's decrease in cortisol from baseline through the worry and relaxation conditions may be relatively consistent with other similar types of experimental inductions. One potential theory from the examination of previous research is that the findings from the current study might highlight a blunted cortisol response, in that participants actually demonstrated less cortisol reactivity in response to a stressor than what was anticipated. If so, this finding is

consistent with a previous meta-analysis demonstrating a blunted cortisol response among women with both anxiety and depressive disorders compared to healthy controls (Zorn, Schur, Boks, Kahn, Joels, & Vinkers, 2017). More recent research has also shown an association between anxiety symptoms and less cortisol reactivity to a TSST compared to depressive symptoms (Fiksdal, Hanlin, Kuras, Gianferante, Chen, Thoma, & Rohleder, 2019). While this study did not examine worry specifically, it highlights important potential pathways through which symptoms associated with anxiety or anxiety disorders may impact cortisol reactivity. However, overall, findings remain mixed within the field, as the findings from the current study are inconsistent with other recent research looking at baseline correlations between worry/rumination and cortisol as well as cortisol reactivity during a TSST. This study found that worry was associated with higher cortisol reactivity following the TSST compared to rumination in both healthy controls and individuals diagnosed with social anxiety disorder (Lewis, Yoon, & Joorman, 2018). Independent of explanations for the lack of cortisol findings in the current study, research within the field of psychoneuroimmunology has highlighted significant variability in cortisol reactivity to stressors both across and within individuals depending upon stress paradigms, time or day, and other behavioral and health factors (Zänkert, Bellingrath, Wüst, & Kudielka, 2018). Further, some research has demonstrated that in contexts of stressors, cortisol levels may initially increase but decrease again to baseline once a stressor has been mastered, resulting in blunted reactivity patterns (Daruna 2012). This research is in line with more recent findings demonstrating that higher levels of reappraisal, or thinking differently about things when upset in order to change the emotional impact of certain thoughts, predicted stronger HPA habituation (e.g., more blunted cortisol responses) in response to stress compared to participants low in reappraisal abilities (Roos, Janson, Sturmbauer, Bennett, & Rohleder, 2019).

Taken together, these findings highlight importance in being able to change cortisol through experimental stressors as both increases and decreases in cortisol appear to be found within the literature. Future research should continue to examine what psychological processes may promote cortisol change and the specific processes implicated in the directionality of such changes given mixed findings overall.

In terms of concordance, the hypothesis that cortisol, HRV, and inflammatory markers would covary across the different study conditions was not supported by these data. These findings are in contrast to some recent research that highlights experimentally induced cortisol responses being aligned with changes in c-reactive protein (CRP), a circulating protein in the body that is produced as a direct result of inflammatory release within the bloodstream (Laurent, Lucas, Pierce, Goetz, & Granger, 2016) and is often used as a marker of chronic inflammation. Interestingly, in the study by Lauren and colleagues (2016), the similarities in change between CRP and cortisol was strongest among participants with higher negative affect following a TSST, highlighting the unique role that negative emotional states may have in altering physiology. Recent research synthesizing findings that examine baseline correlations between different metrics of HRV and inflammation have shown some evidence for associations between these two biomarkers within single timepoints. Overall, researchers found that SDNN, one metric of HRV that represents both sympathetic nervous system and parasympathetic nervous system activity was most closely and consistently associated with inflammation (Williams, Koenig, Carnevali, Jarczok, Sternberg, & Thayer, 2019). Interestingly, given heterogeneity in types of experimental manipulations, the authors did not include any data associated with experimental change or change over time in this meta-analysis and it is therefore difficult to draw conclusions regarding whether these associations are maintained in the context of a

laboratory-based stressor such as the one utilized in the current study. Lastly, the exploratory hypothesis to examine if cortisol and/or HRV temporally preceded changes in inflammation throughout the study was not supported. Additionally, trait worry did not moderate this temporal relationship. These findings highlight that the faster-changing markers of HRV and cortisol did not necessarily change before inflammation across conditions. Given that not all biomarkers in the current study demonstrated significant changes across time, this finding was anticipated. However, similar to examining covariance among biomarkers, it does highlight interesting considerations for future research and our understanding of biomarkers and their interactions more broadly.

Concordance among potential biomarkers is an important issue within the fields of clinical and health psychology, as it helps direct us towards the ways that physiological implications may be similar or different depending on the type of experimental stressor. Further, it is important to consider time course when examining concordance in experimental studies. Namely, given the relatively slow-changing nature of inflammation compared to HRV and cortisol, it may be that although there is not concordance between inflammation and these faster changing markers, it may not be anticipated when considering the biological composition of these different markers. While this finding was in contrast to the study hypotheses, it does highlight potential important implications for assessing biomarkers within both experimental and longitudinal designs and highlights the importance of using a multi-method approach to understand the impact of perseveration on physiology. Studies that only utilize a single marker (e.g., HRV) and determine that it is possible to change physiology experimentally may miss important information about other, slower-moving, physiological biomarkers have the potential to continue to worsen physical health over the long term.

Considerations and Future Directions

An important strength of the current study is the utilization of multiple biomarkers within an idiographic experimental manipulation. Much of the previous research examining contrasting conditions such as these have focused on measuring a single biomarker or self-reported changes in purported cognitive and/or emotional mechanisms throughout the inductions. While this previous research offers important considerations, the utilization of a single biomarker fails to capture the intricacies of biology via targeting multiple systems within the body. Further, the utilization of contrasting conditions of worry and relaxation provide some insight into the ways in which physiology can be altered and subsequently improved through tasks aimed at inducing either worry or relaxation, respectively. Although further research is needed to help understand the nature and stability of these findings, the idea that physiology may be negatively altered via worry inductions and improved through relaxation training, even via short experimental manipulations is an important one in translating these basic findings to more applied research within clinical health psychology. Future research should attempt to build upon these findings to examine whether relaxation training delivered in brief formats may help to ameliorate short-term negative health implications for individuals with high degrees of trait worry. Further, it would be most beneficial for future research within this domain to follow participants longitudinally to examine whether the effects of brief relaxation trainings have physical health benefits over the long-term, as this is something that the current study was unable to assess.

Although the use of multiple biomarkers is a strength of the current study and provides novel data not yet available in the field, there is a substantial degree of heterogeneity regarding how these data are collected within the field. The current study examined MSD as a metric of HRV which has been consistently utilized in research on emotion regulation and anxiety

(Applehans & Leukin, 2006) and has subsequently been shown to be associated with psychological symptoms and disorders, a number of other metrics, such as SDNN, may be more closely associated with inflammation and cortisol (Williams et al., 2019). In terms of cortisol collection, although this study utilized serum-derived cortisol, cortisol can also be assessed via saliva or hair samples. A previous meta-analysis has highlighted heterogeneity of data collection methods and analytical techniques for cortisol as one potential explanation for discrepancies between findings (Liu, Ein, Peck, Huang, Pruessner, & Vickers, 2017). Other findings have shown that cortisol in saliva is typically 10-35% lower than in the blood due to the transformation of cortisol as it enters into saliva (Vining, McGinley, Maksvytis, & Ho, 1983) although the two methods of collection correlate highly with one another. Overall, collection methods in the current study for all physiological outcome variables were consistent with previous research. However, an important step in better contextualizing these findings and others in the field is to establish best practices for collecting, analyzing, and interpreting these types of data.

The experimental manipulations of worry and relaxation were based off of previous studies utilizing a similar design (Llera & Newman, 2010; Borkovec & Inz, 1990; Fisher & Newman, 2013). However, the conditions in the current study were significantly longer (10 minutes) than those used in previous research. Findings indicated that this longer induction time appropriately and significantly increased both worry and relaxation during the respective conditions, highlighting that a longer duration of induction may be successful in experimental studies in addition to the previously utilized briefer designs. A 10-minute study design may be particularly beneficial for designs testing biomarkers such as inflammation that are relatively slow and less sensitive to experimental change, as a more salient and longer-lasting stressor may

be necessary to elicit immunological change in an experimental setting. However, previous studies utilizing this shorter duration of worry and relaxation inductions did not assess inflammation and it is therefore not possible to compare the findings from this study with findings from briefer designs. Further, previous studies utilizing these types of experimental inductions often randomized participants to undergo either a worry or relaxation induction rather than both, highlighting both a strength of the current study and potential limitation. The inclusion of both conditions in a fixed order in the current study allowed for examining the differential impact of worry and relaxation and the ways in which the body may "bounce back" physiologically after brief, relatively intense periods of worry. However, the inclusion of both conditions may have created a within-subject contamination effect, subsequently making it difficult to discern the impact of either condition in isolation. Future research may seek to expand upon the current study by utilizing a larger sample and randomizing participants to receive either worry or relaxation while simultaneously assessing changes in immune functioning before and after the inductions.

Although the worry and relaxation manipulations were based off of previous research looking at stress inductions among individuals high in trait worry and diagnosed with GAD within the clinical psychology literature, other types of experimental stress inductions may be beneficial in altering inflammatory, endocrine, and cardiovascular reactivity. Clinical health psychology has most frequently utilized stressors comprised of social evaluative threat, and in particular the Trier Social Stress Test, to elicit experimentally induced endocrine change both in physically and psychologically healthy individuals (Dickerson & Kemeny, 2004) and those diagnosed with psychological disorders (Burke, Davis, Otte, & Mohr, 2005). Research on cortisol responsivity particularly has highlighted that biomarkers are sensitive to the types of

stressors that are induced experimentally, and a stressor that activates on system within the body may not activate another (Baum & Grunberg, 1995). While social evaluative threat has been shown to be a salient stressor in evoking physiological change, these types of tasks may not be as salient for individuals who engage in high degrees of negative self-referential processing such as worry, rumination, and self-criticism (Mennin & Fresco, 2013) as their stressors become prolonged as a result of persistent thought patterns related to negative information about one's self in their past, present, and anticipated future. Several other theories regarding physiological change have been proposed in terms of alterations as a result of experimental change. Along with social-evaluative threat and emotion inductions, cognitive tasks and tasks involving uncertainty may also be salient. Future research should attempt to further study methods that most strongly promote physiological change in different populations to better understand salient stressors. A paradigm such as the one used in the current study subsequently has the potential to help better understand the impact of such processes on health and highlight ways that stressors above and beyond social evaluative threat may alter physiology.

An important consideration in this study is that it did not necessarily utilize individuals diagnosed with an anxiety disorder or other form of psychopathology, and rather relied on examining differences in high and low trait worry based on self-reported scores on the PSWQ. While this approach allowed me to examine a broader sample of individuals and there was a sufficient amount of variability in terms of PSWQ scores, stratifying based on high and low scores may have resulted in some participants in different groups looking more similar than different. Although study hypotheses adjusted for this in proposing that there would be no baseline differences between the two groups, it may be important for future research to more systematically examine differences among individuals high in trait worry in a way that may be

consistent with a diagnosis of GAD (e.g. PSWQ scores above 65; Meyer et al., 2000) and people with very low degrees of trait worry to better understand the differential impact of trait worry. The use of a PSWQ score of 56 was utilized to capture high worry that was not necessarily consistent with GAD, as splitting the current sample on the cut score of 65 resulted in substantially unequal groups (e.g., only 12 participants out of 85 with PSWQ > 65). Additionally, given that all participants commuted to the hospital where the clinic where the study took place was on the morning of the experiment, an additional consideration is that all participants, independent of group, may have experienced higher than average anticipatory anxiety at baseline that may have created a contamination effect in terms of the current findings. They may also have experienced higher baseline anxiety as a result of anticipation of the blood draw and interacting with the experimenter and nursing staff at the CTSC. The mean baseline WVAS rating across all participants was approximately 38 out of a possible 100, highlighting worry was present at a moderate level upon entering the clinic prior to undergoing any other experimental procedures.

Although baseline state anxiety was not controlled for in these analyses, these findings do offer some insight into the ways that trait anxiety may dysregulate physiological functioning. While the findings examining changes across conditions for each biomarker was mixed, looking at high trait worry specifically highlights a dysregulated pattern of physiological responding, and offers several important considerations for future research. It may be worthwhile for future research to more specifically examine physiological changes for high trait worriers and look at within-subject changes rather than between-subject differences. Further, it is also important to also examine other potential trait-level variables that may impact physiological functioning, such

as trait anxiety or depression. This is an important next step in identifying how symptoms associated with psychopathology may impact endocrine, cardiovascular, and immune function.

In addition to studying the ways that trait anxiety, depression, and other symptoms associated with psychopathology in differentially predicting trajectories of change in immune, endocrine, and cardiovascular function throughout the study, future research may benefit from examining the ways in which these physiological variables covary with psychological symptoms. Particularly, future research may benefit from examining whether subjective changes in worry and relaxation throughout experimental inductions covary with physical symptom changes. Further, it would be beneficial for these results to be better understood in the context of chronic stress literature within clinical health psychology. Chronic stress represents a consistent state of physiological activation and psychological stress due to uncontrollable or unpredictable stressors with no definitive endpoint, such as physical disabilities, caregiving, or having a low socioeconomic status (Baum, 1990). Chronic stress is associated with overall greater immunosuppression as well as increased levels of IL-6 and CRP (Segerstrom & Miller, 2004), highlighting a substantial negative impact of chronic stress on physical functioning. Indeed, both chronic stress and depression or anxiety may be somewhat related in their impact on inflammatory markers (Slavich & Irwin, 2014). However, experimental examinations of chronic stress and worry have not yet disentangled the unique contributions that perseverative processes such as worry may have on cardiovascular, endocrine, and immune function over and above chronic stress. Future research should therefore attempt to examine unique contributions of chronic stress and perseveration in exacerbating of maintaining physiological dysregulation. Further, it may be imperative to understand whether or not chronic stress moderates the impact of worry and other perseverative processes on physical functioning. If so, future research may

attempt to establish a better understanding of ways that this impact can be buffered through psychological interventions to target both stress and worry with the goal of reducing the likelihood of long-term negative health effects.

An important next step in terms of research is to examine the physiological, cognitive, and behavioral mechanisms that may underlie the impact of worry on physical functioning. In response to anxiety and fear, the stress response is exacerbated among individuals with heightened emotions associated with anxiety disorders. The stress response may, in turn, promote a cascade of psychological and physiological processes, which is mediated by hypothalamic-pituitary-adrenal axis (HPA) dysregulation (Michopoulos et al., 2017). Through glucocorticoid insensitivity, HPA dysregulation is theorized to contribute to a state of low-grade inflammation, which might put an individual at risk for negative health consequences (Cohen, Janicki-Deverts, Doyle, Miller, Frank, Rabin, & Turner, 2012). Cognitive processes such as worry (McEvoy, Watson, Watkins, & Nathan, 2013), self-criticism (Sowislo & Orth, 2013), and intolerance of uncertainty (Carleton, 2012) may impact physiological responding, as these processes tend to prolong physiological activation through cognitive activation that is difficult to control. Finally, in terms of behavioral mediators, people who experience high degrees of worry and rumination may be less likely to engage in a healthy lifestyle. Notably, these individuals may be more likely to suffer from sleep disturbances, be less likely to engage in exercise, and may utilize alcohol, smoking, food, or drugs in an effort to regulate the negative emotions associated with these cognitive processes, subsequently putting them at risk for experiencing a number of poor health outcomes over the long term (Michopoulos et al., 2017; Pederson 2017). While the current study assessed whether or not people smoked, it did not assess a number of these other important health behaviors and therefore was not able to examine the impact of behavioral habits

on the physiological outcome variables of interest. Taken together, an essential avenue for future research is to take a more mechanistic approach to assessing the link between perseverative processes, such as worry, and physical health. Although worry may serve as one potential mechanism linking negative emotions to poor physical health, there may be other processes at play that may have the potential to exacerbate physiological dysregulation.

Given the association between emotionality and increased physiological activation (Brosschot, Gerin, & Thayer, 2006), most recently I have begun to craft a theoretical model guided by the emotion dysregulation model of generalized anxiety (Mennin, Heimberg, Turk, & Fresco, 2005) and the PCH (Brosschot et al., 2006). The emotion dysregulation model, which highlights how primary and secondary emotions promote increases in perseverative processes such as worry, rumination, and self-criticism, has been used to guide an understanding of the ways that thoughts and emotions are connected in individuals experiencing chronic generalized anxiety. In comparison, the PCH highlights the ways that perseveration contributes to sustained physiological activation, putting an individual who experiences chronic worry and rumination at risk for long-term health issues. Overall, this new model posits the necessity of understanding the role that primary and secondary emotional responses have in prolonging cognitive and physiological activation and the central role that subsequent maladaptive emotion regulation strategies (both cognitive and behavioral) play in promoting poor physical health over the long term. An important assumption of this model is that secondary emotional responding (i.e., anger, sadness, and/or anxiety) promotes perseverative processes such as worry and rumination subsequently prolonging physiological activation via emotional and behavioral dysregulation. It therefore integrates past theories to link emotions, perseveration, and physiology. Future research should therefore attempt to better understand the impact of emotional functioning in the context

of perseverative thinking and physiological dysregulation. Negative emotions have been shown to promote the release of proinflammatory cytokines associated with numerous chronic illnesses (for a review, see Kiecolt-Glaser, McGuire, Robles, & Glaser, 2002) but there has yet to be a comprehensive understanding of the ways that emotions interact with immune function to exacerbate or prolong physical health issues. Although this study provides one step in better testing this model, emotionality was not accounted for in analyses and future research may benefit from examining how emotion dysregulation may interact with perseveration to examine whether or not it contributes to increased physiological dysregulation.

Given that the current study explicitly excluded participants who had medical conditions that are characterized by cardiovascular and immune dysregulation, it will also likely be important to expand upon this work to better understand these results in the context of individuals with chronic illnesses. Worry and rumination have been shown to be salient cognitive processes among several medical populations including cancer patients (Lampic, Wennberg, Schill, Brodin, Glimelius, & Sjödén, 1994; Berman et al., 2014) and survivors (Morris & Shakespeare-Finch, 2011). Given that findings from this study and others have highlighted some impact of worry on immune, cardiovascular, and endocrine function, it may be important for future research to better understand the ways in which perseverative processes such as worry and rumination may exacerbate or maintain some physiological dysregulation or pain experienced by these patients. Such an understanding would provide valuable insight into the ways in which we may be better able as a field to intervene on cognitive aspects of a person's experience that may provide less suffering and impairment physically.

While the inclusion of adult participants age 18-65 allowed this study to generalize more broadly to physically healthy adult populations, it is important to note that the physical health

profiles among participants of different age groups (e.g. emerging adults versus older adults) may influence inflammatory, endocrine, and cardiovascular function. Although age was appropriately examined as a potential covariate and older participants were no more likely to be on medication or be diagnosed with a medical condition than younger participants in the current study, there has been some research suggesting that health risk behaviors among adolescents and young adults may promote changes in physiological functioning later in adulthood (Cromwell, Puzia, & Yaptangco, 2015). It may therefore be important to disentangle the unique physiological profiles of different age groups in future research utilizing larger sample sizes or limiting the studies to more specific age ranges to better understand these nuances. Further, a number of studies specifically looking at cortisol responding and endocrine function limit samples to only male or female participants in order to control for hormonal differences. In order to increase both generalizability and feasibility, this was not done in the current study but may be an important direction for future research, particularly given that no research to date has examined sex differences in covariance of biomarkers or the impact of sex on differential inflammatory responding to experimental manipulations of stress and worry.

Limitations

This study had several limitations which should be noted. First, given the contrasting conditions and length of time that previous studies have demonstrated is essential to demonstrate inflammatory change (Steptoe et al., 2007), it is difficult to disentangle whether inflammatory change following the relaxation induction was due exclusively to relaxation or whether a longer wait period was necessary following the worry condition. Along these lines, it is possible that the wait period for the blood draws following the worry and relaxation conditions were not long enough to detect a substantial change in inflammatory markers. Although a 30-minute wait

period was considered acceptable in a 2007 meta-analysis (Steptoe et al., 2007), resulted varied and overall found that longer wait periods following experimental manipulation (e.g., upwards of 120 minutes) had higher effect sizes when examining inflammatory change compared to those studies that had briefer wait periods. Due to limitations in terms of space and compensation, we were unable to accommodate a longer wait period which may have subsequently impacted the current findings in relation to the inflammation data specifically. Additionally, participants were grouped based on their PSWQ scores as either 'high' or 'low' trait worry. However, as stated previously, these groups did not necessarily distinguish between individuals who had pathological levels of worry characteristic of GAD. Therefore, it is possible that breaking up the groups in this way may have resulted in non-severe worriers categorized as 'high' worry, and subsequently may not have high enough levels of worry to create a physiological distinction from the low trait worriers. Additionally, as discussed above, the environment where the experiment took place may have impacted worry and anxiety at baseline. In terms of experimental design, an additional limitation is that the current study was unable to follow participants across time, which may limit the generalizability of the current findings in identifying the impact that acute states of worry may have on physical health across time. Although previous research within this regard has yet to be conducted, it is reasonable to posit that should brief acute inductions of worry alter physiology, more chronic and pervasive episodes of worry in one's everyday life outside of the laboratory are likely to create a larger 'sum' of physiological dysregulation and subsequently increase the likelihood of an individual experiencing poor long-term health outcomes.

Further, a number of potential covariates were assessed during the study that may have impacted physiological outcomes, including age, medication usage, gender, race, smoking status,

and BMI. However, there are several other variables that may impact these data, including sleep and exercise, which was not assessed in the current study. Previous research has demonstrated that sleep may have a significant impact on endocrine and immune functioning in healthy adults (Spiegel, Leproult, & Van Cauter, 1999). There has also been some evidence that vigorous levels of physical activity may decrease inflammation (Ford 2002). In contrast, moderate to high levels of physical activity has been shown to increase circulating cortisol levels in the blood (Hill, Zack, Battaglini, Viru, Viru, & Hackney, 2008). Given that amount, type, and frequency of physical activity in the morning before the experiment took place was not assessed it is not possible to determine its overall impact on the current findings. Among women, menstrual cycle is another potentially important covariate to consider (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999) which was also not assessed in this study but may have important implications for cortisol output. Although this study did control for medication usage which included birth control, future research should take a more nuanced approach to these potential covariates and consider a broader array of factors that may impact physiological functioning. Given previous recommendations to further exclude participants based on a number of covariates shown to significantly influence immune functioning (O'Connor et al., 2009), future research may also attempt to further refine the inclusion criteria to account for these recommendations beyond what was done in the current study. Lastly, all of the outcome variables were non-normal prior to transformation based on the Shapiro-Wilk tests across all study conditions. While HRV was able to be normalized via a square root transformation, the cortisol and inflammatory data was not able to be normalized. Although parametric tests were utilized in order to examine hypotheses related to concordance, temporal spacing of biomarkers, and the group by condition interaction, these results should be interpreted with caution as the data violated assumptions

necessary to complete these analyses and interpret the findings with confidence. Data normality is a larger issue in the context of biomarkers, as data is most typically non-normal and there are no standards in terms of transforming variables to increase normality or conducting non-parametric tests. It is therefore difficult to fully interpret these findings within the larger body of available research given that many studies do not report normality and whether or not data has been transformed prior to analysis.

Conclusions

Despite these limitations, this study offers an ability to advance the field's understanding of the differential impact of worry and relaxation on physiology independent of psychological disorders. To date, there has been no published findings of contrasting experimental conditions of worry and relaxation in exacerbating immune dysregulation. Such work is important in more fully examining how the processes associated with psychopathology may interact with biomarkers to influence physical health outcomes. Further, it highlights ways in which different physiological biomarkers may relate to one another which may provide important information for future research taking a multi-method approach to study the physical impact of worry and other perseverative processes. Overall, although additional research is needed, the current study provides some preliminary evidence that trait worry contributes to dysregulation of cardiovascular, endocrine, and immunological processes. An important next step from this study is to better examine the physiological processes linking worry and subsequent anxiety to chronic illnesses in an effort to better understand ways to intervene on this relationship. Further, a better understanding within these domains may have the potential to impact translational research and highlight avenues for future intervention work in ameliorating symptoms of worry and other

related processes and subsequently alter physiology and reduce the likelihood of developing long-term physical health issues.

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Appendix

Table 1
Participant Characteristics

	M (SD)	n	%
Baseline PSWQ	49.20 (14.7)		
Age	30.88 (11.4)		
% Female		53	62.4%
Race			
White		31	39.2%
African American		12	15.2%
Asian American		19	24.1%
Hispanic/Latino		12	15.2%
Mixed Race		4	5/1%
Other		1	1.3%
% Students		46	57.5%
% Employed		25	31.3%
% Unemployed		9	11.3%
BMI	24.76 (5.9)		

Note. M = mean, SD = standard deviation, % = percentage of participants, PSWQ = Penn State Worry Questionnaire, BMI = body mass index.

Table 2

Physiological Outcome Measure Correlations and Associated Covariates at Baseline

Measure	HRV	IL-6	TNF-α	IFN-γ	Cortisol	BMI	Gender	Age	Medication	Race	Smoking
HRV	-										
IL-6	-.01	-									
TNF-α	-.13	.36**	-								
IFN-γ	.03	.18	.40**	-							
Cortisol	.09	.12	-.04	.07	-						
BMI	-.13	.34*	.28**	-.03	-.16	-					
Gender	-.02	-.19	-.35**	-.14	.08	-.50**	-				
Age	-.37**	.33**	.20	-.07	-1.0	.44**	-.25*	-			
Medication	.13	.02	-.07	-.10	.22	-.01	.19	.00	-		
Race	.06	-.05	.13	-.17	.04	.07	-.02	.04	.05	-	
Smoking	.10	-.77**	.07	-.82**	-.01	-.03	.14	-.09	.07	.07	-

Note. **$p < .01$, *$p < .05$. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability.

Table 3
Normality Tests for HRV

	Statistic	df	Sig.
Baseline			
Non-transformed	.937	71	.001
Lg-10 transformation	.953	71	.010
Sqrt transformation	.987	71	.695
Worry			
Non-transformed	.928	71	.001
Lg-10 transformation	.946	71	.004
Sqrt transformation	.976	71	.195
Relaxation			
Non-transformed	.963	71	.037
Lg-10 transformation	.936	71	.001
Sqrt transformation	.988	71	.727

Note. HRV = heart rate variability; Lg-10 = log10; sqrt = square root; df = degrees of freedom; sig = significance level.

Table 4
Normality Tests for Cortisol

	Statistic	df	Sig.
Baseline			
Non-transformed	.890	77	.000
Lg-10 transformation	.982	77	.333
Sqrt transformation	.948	77	.003
Worry			
Non-transformed	.882	77	.000
Lg-10 transformation	.981	77	.318
Sqrt transformation	.943	77	.002
Relaxation			
Non-transformed	.830	77	.000
Lg-10 transformation	.964	77	.026
Sqrt transformation	.909	77	.000

Note. Lg-10 = log10; sqrt = square root; df = degrees of freedom; sig = significance level.

Table 5
Normality Tests for IL-6

	Statistic	df	Sig.
Baseline			
Non-transformed	.875	78	.000
Lg-10 transformation	.967	78	.032
Sqrt transformation	.944	78	.002
Worry			
Non-transformed	.771	78	.000
Lg-10 transformation	.978	78	.209
Sqrt transformation	.914	78	.000
Relaxation			
Non-transformed	.903	78	.000
Lg-10 transformation	.992	78	.928
Sqrt transformation	.971	78	.076

Note. IL-6 = interleukin 6; Lg-10 = log10; sqrt = square root; df = degrees of freedom; sig = significance level.

Table 6
Normality Tests for TNF-α

	Statistic	df	Sig.
Baseline			
Non-transformed	.974	78	.115
Lg-10 transformation	.993	78	.950
Sqrt transformation	.992	78	.897
Worry			
Non-transformed	.494	78	.000
Lg-10 transformation	.861	78	.000
Sqrt transformation	.690	78	.000
Relaxation			
Non-transformed	.561	78	.000
Lg-10 transformation	.915	78	.000
Sqrt transformation	.769	78	.000

Note. TNF-α = tumor necrosis factor-alpha; Lg-10 = log10; sqrt = square root; df = degrees of freedom; sig = significance level.

Table 7
Normality Tests for IFN-γ

	Statistic	df	Sig.
Baseline			
Non-transformed	.879	78	.000
Lg-10 transformation	.987	78	.627
Sqrt transformation	.953	78	.006
Worry			
Non-transformed	.803	78	.000
Lg-10 transformation	.979	78	.221
Sqrt transformation	.913	78	.000
Relaxation			
Non-transformed	.494	78	.000
Lg-10 transformation	.953	78	.007
Sqrt transformation	.772	78	.000

Note. IFN-γ = interferon gamma; Lg-10 = log10; sqrt = square root; df = degrees of freedom; sig = significance level.

Table 8

Paired Sample t-Test Results Comparing Experimental Inductions

Experimental Inductions	Paired Differences		95% CI				
	M	*SD*	*Lower*	*Upper*	*t*	*df*	*Sig. (2-tailed)*
Baseline WVAS Rating Post Worry Task WVAS	-27.78	25.28	-33.40	-22.15	-9.83	79	0.00
Baseline RelaxVAS Rating Post Relaxation Task RelaxVAS	-19.58	23.70	-24.89	-14.27	-7.34	79	0.00
Baseline WVAS Rating Post Relaxation Task WVAS	15.55	23.42	10.44	20.69	6.05	79	0.00
Baseline RelaxVAS Rating Post Worry Task RelaxVAS	15.76	24.46	10.45	21.07	5.90	79	0.00

Note. WVAS = Worry Visual Analogue Scale; RelaxVAS = Relaxation Visual Analogue Scale, M = mean; SD = standard deviation; CI = confidence interval; df = degrees of freedom; sig = significance value.

Table 9

Means and Standard Deviations of Outcome Variables

	Baseline	Post Worry	Post Relaxation
HRV	32.24 (16.8)	29.87 (13.8)	36.62 (16.3)
IL-6	0.45 (0.3)	0.49 (0.3)	0.57 (0.3)
TNF-α	1.82 (0.5)	1.98 (1.2)	1.90 (0.9)
IFN-γ	4.17 (2.4)	4.19 (2.8)	4.20 (4.2)
Cortisol	14.73 (6.0)	13.19 (5.4)	12.33 (5.5)

Note. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability; M = Mean; SD = Standard Deviation.

Table 10

Means and Standard Deviations of Outcome Variables by Group

		Baseline	Worry	Relaxation
HRV				
	Low PSWQ	31.62 (18.6)	30.03 (16.2)	35.28 (16.5)
	High PSWQ	33.69 (13.9)	30.25 (9.1)	38.52 (16.1)
Cortisol				
	Low PSWQ	14.90 (6.5)	13.42 (5.9)	12.59 (6.1)
	High PSWQ	14.57 (6.2)	13.10 (5.6)	12.33 (5.2)
IL-6				
	Low PSWQ	.46 (0.2)	.48 (0.2)	.56 (0.3)
	High PSWQ	.42 (0.3)	.50 (0.4)	.58 (0.4)
TNF-α				
	Low PSWQ	1.81 (0.6)	1.80 (0.5)	1.80 (0.6)
	High PSWQ	1.84 (0.4)	2.24 (1.7)	2.04 (1.3)
IFN-γ				
	Low PSWQ	4.46 (2.6)	4.20 (2.4)	4.10 (2.4)
	High PSWQ	3.77 (2.0)	4.16 (3.4)	4.32 (5.9)

Note. HRV = heart rate variability; IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; PSWQ = Penn State Worry Questionnaire.

Table 11
Correlations Between Outcome Variables at Baseline

Measure	HRV	IL-6	TNF-α	IFN-γ	Cortisol
HRV	-				
IL-6	-.01	-			
TNF-α	-.13	.36**	-		
IFN-γ	.03	.18	.40**	-	
Cortisol	.09	.12	-.04	.07	-

Note. **$p < .01$, *$p < .05$. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability.

Table 12

Correlations Between Outcome Variables After Worry Condition

Measure	HRV	IL-6	TNF-α	IFN-γ	Cortisol
HRV	-				
IL-6	-.04	-			
TNF-α	-.09	.68**	-		
IFN-γ	.03	.41**	.65**	-	
Cortisol	.04	-.16	-.17	-.10	-

Note. **$p < .01$, *$p < .05$. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability.

Table 13

Correlations Between Outcome Variables Following Relaxation Condition

Measure	HRV	IL-6	TNF-α	IFN-γ	Cortisol
HRV	-				
IL-6	-.22	-			
TNF-α	-.29*	.47**	-		
IFN-γ	-.30*	.37**	.84**	-	
Cortisol	.02	-.13	-.09	-.08	-

Note. **$p < .01$, *$p < .05$. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability.

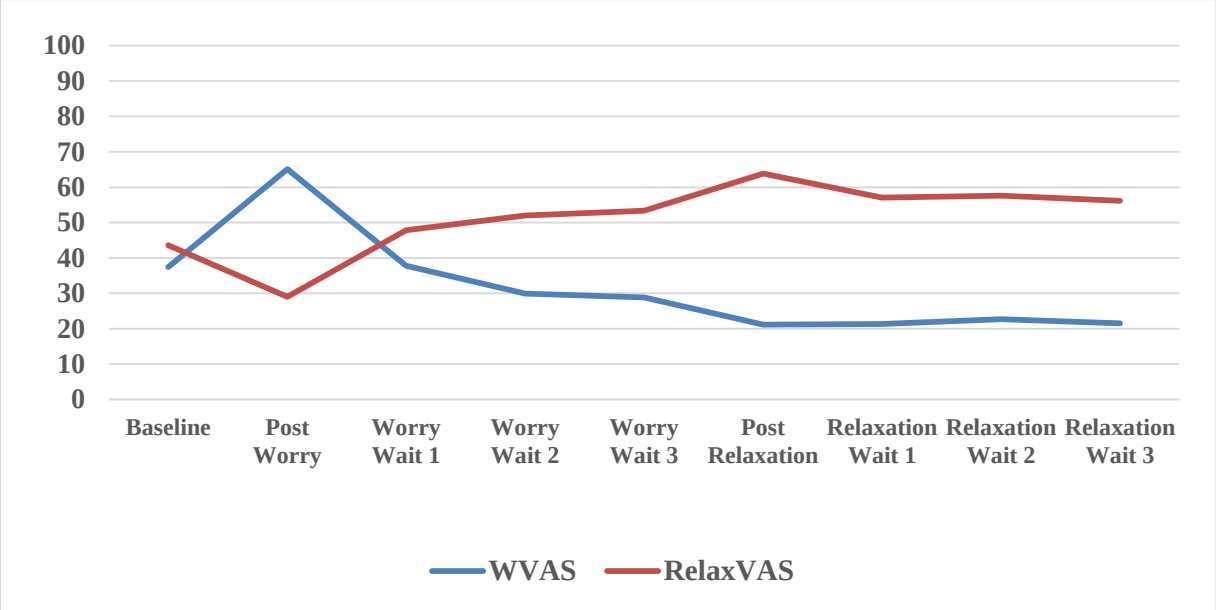
Table 14

Covariance of outcome variables across conditions

Measure	F	df	95% CI	*p*-value	Cohen's d
IL-6, HRV, & Cortisol	1.51	170.75	-.01 - .003	.22	.27
TNF-α, HRV, & Cortisol	.008	109.52	-.02 - .02	.93	.02
IFN-γ, HRV, & Cortisol	1.80	115.95	-.02 - .10	.18	.30
HRV & Cortisol	1.17	148.72	-.01 - .04	.28	.24

Note. IL-6 = interleukin 6; TNF-α = tumor necrosis factor alpha; IFN-γ = interferon gamma; HRV = heart rate variability; df = degrees of freedom; CI = confidence interval.

Figure 1.
Means of WVAS and RelaxVAS throughout experiment



Note. WVAS = Worry Visual Analogue Scale; RelaxVAS = Relaxation Visual Analogue Scale.