



# An experimental examination of worry and relaxation on cardiovascular, endocrine, and inflammatory processes

Megan E. Renna<sup>a,b,\*</sup>, Michael A. Hoyt<sup>c</sup>, Cristina Ottaviani<sup>d,e</sup>, Douglas S. Mennin<sup>f</sup>

<sup>a</sup> The Comprehensive Cancer Center, Ohio State University College of Medicine, Columbus, OH, USA

<sup>b</sup> The Institute for Behavioral Medicine Research, Ohio State University College of Medicine, Columbus, OH, USA

<sup>c</sup> University of California Irvine, Irvine, CA, USA

<sup>d</sup> Department of Psychology, Sapienza University of Rome, Rome, Italy

<sup>e</sup> Neuroimaging Laboratory, IRCCS Santa Lucia Foundation, Rome, Italy

<sup>f</sup> Teachers College, Columbia University, New York, NY, USA

## ARTICLE INFO

### Keywords:

Worry  
Perseveration  
Inflammation  
Heart rate variability  
Cortisol

## ABSTRACT

**Background:** Worry increases risk for long-term health issues by prolonging the physiological stress response. In contrast, relaxation may ameliorate the psychological and physiological burden resulting from worry. This study examined the impact of experimentally induced worry and relaxation on cortisol, heart rate variability (HRV), and inflammation.

**Method:** Participants (N = 75) completed both a worry and relaxation induction (presented in a fixed order) while HRV was collected continuously. Three blood samples were taken (at baseline, after the worry induction, and after the relaxation induction) to measure IL-6, IFN- $\gamma$ , TNF- $\alpha$  and serum cortisol.

**Results:** There were significant changes in IL-6 ( $p < 0.001$ ), IFN- $\gamma$  ( $p < 0.001$ ), HRV ( $p < .001$ ), and cortisol ( $p < .001$ ) but not in TNF- $\alpha$  ( $p = 0.19$ ) across conditions. HRV increased significantly from baseline to worry and then decreased following relaxation. IL-6 changed significantly between worry and relaxation and continued to increase following relaxation. Cortisol decreased significantly across conditions. Several patterns of covariance between inflammation and HRV and/or cortisol also emerged.

**Conclusions:** These findings offer novel insight into how worry influences the immune system and emphasize the utility of a multi-methods approach to understanding the impact of worry on physical health.

## 1. Introduction

Functionally, worry signals alarm to potential threats, prompts awareness to unresolved threatening situations, and prepares individuals for a ‘fight or flight’ response (Frijda 1988; Lazarus 1991). Theoretical understandings of worry suggest that it reflects attempts to prevent negative experiences from occurring or to prepare oneself for the presence of negative experiences, thus providing an adaptive function for some individuals (Borkovec, 1994; Borkovec et al., 2004). The Contrast Avoidance Model highlights the maintenance of worry as an adaptive mechanism for reducing the unpleasantness of emotional shifts between positive or neutral to negative (Newman & Llera, 2011). In this instance, worry does not necessarily lead to the avoidance of negative emotions but, rather, sustains negative states to make them feel more predictable. Despite the functional dimensions of worry, worrying is

characterized by an apprehensive state of anticipation of real or perceived threats in the environment, which may potentially promote wear and tear on the body over the long term. Thus, worry can be particularly onerous to physiological functioning and overall health.

The perseverative cognition hypothesis (PCH) links increases in worry with negative physical health. Sustained cognitive activation may contribute to increased physiological reactivity, subsequently putting people at an increased risk for experiencing deleterious health effects (Brosschot et al., 2006). The PCH considers that cognitive processes may either precede and/or follow the stressor – highlighting the fact that the actual stressor in and of itself may not be the most salient factor to physiological activation within the chain of events. Essentially, perseverative cognition invokes a prolonged physiological stress response and subsequently increases risk of negative health outcomes (Brosschot et al., 2006). Both trait and state perseverative cognition influence

\* Corresponding author at: Institute of Behavioral Medicine Research, The Ohio State University College of Medicine, Columbus, OH, 43210, United States.

E-mail address: [Megan.Renna@osumc.edu](mailto:Megan.Renna@osumc.edu) (M.E. Renna).

<https://doi.org/10.1016/j.psyneuen.2020.104870>

Received 12 May 2020; Received in revised form 30 July 2020; Accepted 7 September 2020

Available online 16 September 2020

0306-4530/© 2020 Elsevier Ltd. All rights reserved.

physiology.

Meta-analytic findings demonstrate a significant decrease (Hedge's  $g = .15$ ) in heart rate variability (HRV) throughout experimental manipulations of perseverative cognition (Ottaviani, Verkuil, Medea, Couyoumdjian, Thayer, Lonigro, & Brosschot, 2016). Among correlational studies, there is a significant (Hedge's  $g = .27$ ) association between higher levels of perseverative cognition and decreased HRV (Ottaviani et al., 2016). In testing factors that may ameliorate the negative impact of worry on HRV among psychologically healthy undergraduate students, those instructed to relax or those given a neutral mentation condition, compared to those instructed to worry, had significantly higher HRV (Llera & Newman, 2010). Differences in HRV between worry and relaxation conditions have been replicated in people with GAD and healthy controls (Fischer & Newman, 2013).

Worry is also linked to cortisol reactivity (Brosschot et al., 2006; Thomsen et al. 2004). Worry was associated with greater cortisol reactivity across healthy individuals as well as those diagnosed with social anxiety disorder during a social evaluative threat task (Lewis, Yoon, & Joorman, 2017). In addition, trait worry was associated with increased cortisol during the recovery period post-task (Lewis et al., 2017). Greater worry and rumination contributes to increased (Hedge's  $g = .36$ ) cortisol levels from baseline to post-manipulation (Ottaviani et al., 2016). Correlational studies demonstrated a significant relationship (Hedge's  $g = .32$ ) between higher perseveration and higher cortisol levels (Ottaviani et al., 2016).

Worry may also link anxiety to inflammation. Cytokines and the C-reactive protein (CRP) are associated with GAD among both adults and children (Bankier et al., 2008; Copeland et al., 2012). However, less is known regarding how worry, independent of GAD, provokes inflammation. In one study of earthquake survivors, participants who experienced higher trait worry had fewer natural killer cells than those with low worry (Segerstrom et al., 1998). Further, pregnant women of high socioeconomic status who experienced greater degrees of perseverative thinking (including both worry and rumination) had higher levels of interleukin-6 (IL-6; Mitchell & Christian, 2019). Investigating the experimental influence of worry on inflammation can elucidate pathways to how worry disrupts the immune system.

Increased inflammation is a significant downstream consequence of reduced cortisol and lowered HRV; however, experimental and correlational findings of how these markers interact remain somewhat limited. Decreased HRV is associated with higher levels of CRP and IL-6 among healthy men (Lampert et al., 2008). Prospectively, chronic low levels of HRV are associated with increases in inflammation (Katon, Maj, & Sartorius, 2011; Kissane, Maj, & Sartorius, 2011; Thayer, Yamamoto, & Brosschot, 2010; see Williams et al., 2019 for a recent meta-analysis). Cortisol is typically inversely correlated with inflammation (Petrovsky et al., 1998; Shelton et al., 2015). No research has tested how HRV, cortisol, and inflammation relate across time during experimentally induced worry.

### 1.1. The Current Study

The current study builds off of both the contrast avoidance model of worry (Newman & Llera, 2011) and the perseverative cognition hypothesis (Brosschot et al., 2006) to better understand how worry prolongs the psychological stress response, subsequently creating sustained physiological activation. Understanding how worry disrupts biological functioning across multiple systems can provide insight into potential mechanisms linking anxiety to long-term physical health problems. This study examined dynamic changes in HRV, inflammation, and cortisol throughout an experimental manipulation of worry and relaxation in a community sample of adults. Inflammation, cortisol, and HRV were assessed at baseline and throughout the worry and relaxation conditions. Experimentally-induced worry was examined in this study, as opposed to rumination, to better understand how worry primes the body for threat, thus influencing biology and creating a pathway for long-term

health problems. The worry condition preceded relaxation to test whether worry contributed to heightened physiological dysregulation compared to baseline and if relaxation subsequently contributed to a return to baseline physical functioning. Such findings could further provide valuable insight into how the deleterious physiological effects of worry can be intervened on to improve physical health. It was hypothesized that inflammation and cortisol would increase from baseline to the worry condition and decrease from the worry to relaxation condition while HRV would decrease during worry and increase during relaxation. Finally, we tested how inflammation, cortisol, and HRV are related during baseline and the experimental conditions. Consistent with previous research, we posited that HRV, cortisol, and inflammation would covary across conditions.

## 2. Methods

### 2.1. Participants

Participant demographics are presented in Table 1. Participants were community members between the ages of 18 and 65 years. All participants were required to be over 18 years of age and be able to read and understand English. Exclusion criteria included complicating autoimmune or inflammatory diseases (e.g., rheumatoid arthritis, hepatitis, inflammatory bowel disease), medications that alter immunological or cardiovascular functioning (e.g., statins, beta-blockers, blood pressure medicine), diagnosed heart conditions, or diagnoses of bipolar I disorder, alcohol or substance dependence, active psychosis, or blood injury/injection phobia.

### 2.2. Measures

**Sociodemographic information.** A questionnaire assessing sociodemographic variables was administered, including race, ethnicity, gender, and age. Biobehavioral information regarding exercise, alcohol use, caffeine intake, current medications, or smoking behavior, and body mass index (BMI) was also collected.

### 2.3. Worry/Relaxation Manipulation

The worry and relaxation conditions were modeled off of previous research utilizing worry and relaxation manipulation tasks (Llera & Newman, 2010; Borkovec & Inz, 1990; Fisher & Newman, 2013). Prior to completing the experimental portion of the study, participants listed a number of items that they "worry about the most", ordering them as the things that they worry about *most* frequently to *least* frequently. Participants were then instructed (via computer prompt) to: "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

**Table 1**  
Participant Characteristics

	M (SD)	n	%
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, BMI = body mass index.

refocus your thoughts back onto your worry topic" (Fisher & Newman, 2013). Participants completed this induction for 10 minutes while receiving the prompt on the computer throughout the induction period.

For the relaxation condition, participants were instructed (via computer prompt) to: "Shift your breathing to 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. The 10-minute duration is a departure from the original three-minute design (Fischer & Newman, 2013). Longer durations of preservative cognition optimize outcome effects by increasing intensity of worry compared to briefer, more discrete inductions (Ottaviani et al., 2016).

## 2.4. Physiological Assessment

Participants' HRV was monitored throughout the experimental portion of the study using the Polar<sup>TM</sup> RS800CX Watch system. This ambulatory psychophysiological measurement device collects HRV data via a band with two electrodes placed across the participants' upper abdomen using a sampling frequency of 1,000 Hz for the electrocardiogram (ECG) signal. The Polar Watch system demonstrates reliability and validity in HRV measurement compared to an electrocardiogram (Hernando et al., 2018; Porto and Junqueira 2009). HRV was collected continuously during each discrete segment of the experiment. Data was then uploaded from the device onto a computer for processing and analysis. Root mean squares of successive RR intervals (RMSSD) was obtained and analyzed using CMetX software (Allen et al., 2007).

## 2.5. Inflammation and Cortisol Measurement

Inflammatory cytokines were assessed via serum-derived IL-6, TNF- $\alpha$ , and IFN- $\gamma$ . 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 5 mL vacutainer per time point by a trained nurse or phlebotomist. Nurses/phlebotomists inverted the tube 8–10 times post-collection prior to immediate processing and storage. All 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). A multiplex assay kit (Meso Scale Diagnostics, Rockville, MD, U.S.A.) was used to measure IL-6, TNF- $\alpha$ , and IFN- $\gamma$ . Cortisol samples were also analyzed in batches by ELISA. All data was reported in picograms (pg) per milliliter (ml) of serum and nondetectable amounts of cytokines and cortisol were defined as levels < 0.1 pg/ml.

## 2.6. Manipulation Check

The Worry Visual Analog Scale (WVAS; Wichelns et al., 2016) was used to assess subjective changes in participant worry throughout the experiment. This measure contains both an anchor sheet and a score sheet. The anchor sheet asks a participant to describe five personal situations with 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, corresponding to how much worry they are experiencing "at the current moment." Participants also completed an anchor sheet asking for situations that lead them to feel relaxed (RelaxVAS). Similar to the WVAS anchor scale, participants indicated situations representing relaxation levels at 0, 25, 50, 75, and 100 and were then asked to rate how relaxed they were feeling "at the current moment". Using their anchor sheet as guides, the participant rapidly provided a rating of worry and relaxation at baseline, immediately following both mentation conditions, and twice throughout the wait period prior to the blood

draw.

## 2.7. Procedures

All participants completed written informed consent and all procedures were approved by the Institutional Review Board (IRB) at Teachers College, Columbia University. Study procedures took place between 9:00AM – 12:00PM to control for diurnal variation in inflammation and cortisol. Participants fasted on the morning of their visit. Following informed consent, participants completed the WVAS and RelaxVAS anchor scales and baseline ratings of worry and relaxation. The Polar Watch was then attached and nurses inserted the angiocath to the participant's non-dominant arm. Participant's baseline blood sample was drawn via two 5-ml tubes approximately 35–40 minutes after arriving for their study visit. 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 underwent a thirty-minute rest period where they were asked to sit quietly without speaking to the experimenter, using their phones, or reading. Previous research has demonstrated that longer wait times post-experimental inductions or stressors are associated with stronger effects in examining both inflammation and cortisol changes (Steptoe et al., 2007; Dickerson & Kemeny, 2004). At the end of the rest period, nurses/phlebotomists drew two 5 mL tubes of blood through the angiocath. Participants then underwent the same sequence of procedures for the relaxation condition, including the thirty-minute wait period and subsequent blood draw. 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 to assess their demographics and physical health.

## 2.8. Data Analysis Plan

All analyses were completed within SPSS software version 26. All biological variables were log transformed to better approximate normality of residuals. Age, biological sex, race, BMI, medication use, smoking status, and alcohol and caffeine use were controlled for in all analyses.

In order to verify that the worry and relaxation manipulations induced worry or relaxation respectively, paired sample t-tests were employed for WVAS and RelaxVAS ratings. 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 to examine changes in inflammation, cortisol, and HRV across baseline, worry, and relaxation conditions. Pairwise comparisons were employed to identify differences between conditions. Mixed linear models (MLM) were used to test covariance of HRV, inflammation, and cortisol throughout the three conditions. All mixed models used restricted maximum likelihood estimation and accounted for the repeated assessments of each participant.

## 3. Results

### 3.1. Manipulation Check

See Table 2 for how mean worry and relaxation ratings differed between conditions. Overall, results indicated a significant increase in subjective ratings of worry following the worry task for all participants ( $t = 9.83$ ,  $df = 79$ ,  $p < 0.001$ , Cohen's  $d = 1.54$ ). Further, there was a significant increase in subjective ratings of relaxation following the relaxation task compared to baseline ( $t = 7.34$ ,  $df = 79$ ,  $p < 0.001$ , Cohen's  $d = 1.15$ ). Further, the worry condition led to a significant reduction in relaxation across all participants ( $t = 6.06$ ,  $df = 79$ ,  $p < 0.001$ , Cohen's  $d = .95$ ), while the relaxation condition led to a significant reduction in worry across all participants ( $t = 14.42$ ,  $df = 79$ ,  $p <$

**Table 2**

Paired sample t-test results comparing experimental inductions

Experimental Inductions	Paired Differences		95% CI		<i>t</i>	<i>df</i>	Sig. (2-tailed)
	<i>M</i>	<i>SD</i>	<i>Lower</i>	<i>Upper</i>			
Baseline WVAS Rating	-27.78	25.28	-33.40	-22.15	-9.83	79	<0.0001
Post Worry Task WVAS	-19.58	23.70	-24.89	-14.27	-7.34	79	<0.0001
Baseline RelaxVAS Rating	15.55	23.42	10.44	20.69	6.05	79	<0.0001
Post Relaxation Task RelaxVAS	15.76	24.46	10.45	21.07	5.90	79	<0.0001
Baseline WVAS Rating							
Post Relaxation Task WVAS							
Baseline RelaxVAS Rating							
Post Worry Task RelaxVAS							

Note. The paired sample t-tests were conducted to show differences in mean worry and relaxation ratings between the noted conditions. 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.

.0001, Cohen's *d* = 2.25).

### 3.2. HRV

Means and standard deviations of HRV across each condition are presented in Table 3. A repeated measures ANOVA revealed a significant difference in RMSSD between the three conditions ( $F [2,70] = 16.15, p < .001, \eta_p^2 = .19$ ). Pairwise comparisons revealed a significant difference such that RMSSD decreased significantly from the baseline to worry conditions ( $p = .02$ ), and that RMSSD was significantly higher in the relaxation condition compared to both the baseline ( $p < .01$ ) and worry ( $p < .001$ ) conditions.

### 3.3. Cortisol

Means and standard deviations of the cortisol data across each condition are presented in Table 3. A repeated measures ANOVA revealed a significant difference in cortisol between the three conditions ( $F [1,76] = 18.22, p < .001, \eta_p^2 = .19$ ). Pairwise comparisons revealed a significant difference across all conditions. This difference occurred in the opposite direction of what was anticipated, with cortisol being the highest during baseline. Cortisol decreased significantly from the baseline to worry condition ( $p < .01$ ) and continued to decrease from the worry to relaxation condition ( $p = .02$ ). Cortisol was also significantly lower during the relaxation condition compared to baseline ( $p < .01$ ).

### 3.4. Inflammation

IL-6. Means and standard deviations of the IL-6 data across each condition are presented in Table 3. A repeated measures ANOVA revealed a significant difference in IL-6 between the three conditions ( $F [1,77] = 24.39, p < .001, \eta_p^2 = .24$ ). Pairwise comparisons revealed a significant difference was found between baseline and relaxation ( $p < .001$ ), with IL-6 levels being higher during relaxation compared to baseline. There was also a significant difference in IL-6 between the worry and relaxation conditions ( $p < .01$ ), indicating that IL-6 was higher during relaxation compared to worry. In contrast, there was no

**Table 3**

Means and Standard Deviations of Outcome Variables

	Baseline	Post Worry	Post Relaxation
HRV	43.77 (24.4)	39.89 (20.1)	53.18 (26.0)
IL-6	.45 (.3)	.49 (.3)	0.57 (.3)
TNF- $\alpha$	1.82 (.5)	1.98 (1.2)	1.90 (.9)
IFN- $\gamma$	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. These values represent each variable prior to log transformation. IL-6 = interleukin 6; TNF- $\alpha$  = tumor necrosis factor alpha; IFN- $\gamma$  = interferon gamma; HRV = heart rate variability defined by rMSSD; *M* = Mean; *SD* = Standard Deviation.

difference between baseline and worry ( $p = .11$ ).

TNF- $\alpha$ . Results of the RM ANOVA demonstrated no significant changes in TNF- $\alpha$  between conditions ( $F [1,77] = .20, p = .65, \eta_p^2 = .003$ ). Pairwise comparisons corroborated these findings, indicating no changes between the specific conditions (all  $ps > .25$ ). Means and standard deviations for each condition are presented in Table 3.

IFN- $\gamma$ . Means and standard deviations of the IFN- $\gamma$  data across each condition are presented in Table 3. A repeated measures ANOVA revealed a significant difference in IFN- $\gamma$  between the three conditions ( $F [1,77] = 7.37, p < .01, \eta_p^2 = .09$ ). Pairwise comparisons demonstrated a significant difference between the baseline and relaxation conditions ( $p < .01$ ), indicating that IFN- $\gamma$  was higher during the relaxation condition compared to baseline. Although there were no other significant differences between baseline and the worry condition ( $p = .30$ ) or worry and relaxation ( $p = .31$ ), the trend of means indicated that IFN- $\gamma$  increased from the baseline to worry conditions and then continued to increase from worry to relaxation.

### 3.5. Covariance

Table 4 provides information on baseline correlations between all variables. Separate covariance models were run to determine the relationship of change with each inflammatory marker and HRV and cortisol separately. The inclusion of both random slopes and intercepts did not significantly improve model fit.

Covariance results are presented in Table 5. There was also significant covariance among IL-6 and cortisol ( $p = .01$ ) and IFN- $\gamma$  and cortisol ( $p < .001$ ). TNF- $\alpha$  and RMSSD covaried across conditions ( $p = .03$ ). No other relationships were significant across time.

## 4. Discussion

This study examined the differential impact of worry and relaxation on HRV, cortisol, and inflammation and calls upon the contrast avoidance model of worry and the PCH to better understand how worry may contribute to sustained physiological dysregulation (Brosschot et al., 2006; Newman & Llera, 2011). To date, this is the first known study to

**Table 4**

Baseline correlations between study variables

	1	2	3	4	5	6	7
1. HRV	-						
2. IL-6	-.06	-					
3. TNF- $\alpha$	-.14	.15	-				
4. IFN- $\gamma$	-.05	.71**	.14	-			
5. Cortisol	.06	-.01	.01	.03	-		
6. WVAS	.05	-.12	-.16	-.17	-.06	-	
7. RelaxVAS	-.18	.02	.14	.14	-.02	-.27*	-

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



**Table 5**  
Covariance of outcome variables across conditions

Measure	b	SE	95% CI	p-value
<i>HRV &amp; Cortisol</i>				
HRV & Cortisol	.11	.08	-.05 - .26	.17
<i>Inflammation &amp; Cortisol</i>				
IL-6 & Cortisol	-.21	.08	-.37 - -.05	.01
TNF- $\alpha$ & Cortisol	.03	.04	-.04 - .10	.39
IFN- $\gamma$ & Cortisol	.18	.05	.09 - .27	< .001
<i>Inflammation &amp; HRV</i>				
IL-6 & HRV	.04	.07	-.09 - .17	.56
TNF- $\alpha$ & HRV	-.07	.03	-.14 - -.01	.03
IFN- $\gamma$ & HRV	-.07	.04	-.15 - .02	.14

Note. IL-6 = interleukin 6; TNF- $\alpha$  = tumor necrosis factor alpha; IFN- $\gamma$  = interferon gamma; HRV = heart rate variability defined by RMSSD; df = degrees of freedom; CI = confidence interval.

examine how contrasting worry and relaxation conditions impact inflammation and covariance among multiple physiological indicators.

As hypothesized, participants' HRV was significantly lower during the worry condition compared to both baseline and relaxation. Further, RMSSD during the relaxation condition was significantly higher than baseline, demonstrating a significantly relaxing physiological effect of the condition. The worry condition, on the other hand significantly lowered RMSSD. 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 (Chalmers et al., 2016; Fisher & Newman, 2013). Our findings, coupled with this previous research, highlight how worry perturbs cardiovascular function, thereby putting worriers at risk for long-term cardiovascular problems. In contrast, our findings highlight the utility of relaxation in ameliorating this cardiovascular dysregulation, thus offering the potential to decrease the deleterious effects of low HRV among people who worry.

This is the first known study to date that assesses experimental changes in inflammation throughout worry and relaxation. Although the IL-6 and IFN- $\gamma$  findings were in contrast to the study hypotheses, given the contrasting conditions within a relatively short time window, it is possible that experimental change for some inflammatory markers may be relatively slow and therefore less amenable to this type of study design. Subsequently, it will be important for future research to separate out these conditions (e.g., worry and relaxation) to determine whether IL-6 may be amenable to experimental change within one of these conditions rather than both. Consistent with study hypotheses, there was a significant increase in IFN- $\gamma$  from baseline and worry and a significant decrease from worry to relaxation. While IL-6 and IFN- $\gamma$  demonstrated some experimental changes throughout the different conditions, findings related to TNF- $\alpha$  showed that it did not change significantly throughout the conditions. Consistent with the PCH, this study provides preliminary insight into how worry influences inflammation. In doing so, these findings highlight a potential cognitive mechanism through which psychological symptoms, such as anxiety or depression, may be associated with long-term health problems.

The cortisol findings highlighted that cortisol decreased following relaxation but was not changed in the expected direction following the worry condition. Cortisol can demonstrate both higher and lower levels following experimental induction among participants with distress symptoms (e.g., anxiety, depression, rumination). Among 16 studies utilizing an experimental emotion induction, 50% demonstrated a negative effect size, indicating that cortisol levels went down throughout the stressor period. An additional 25% of studies had positive effect sizes while remaining study results were null (Dickerson & Kemeny 2004). The current study's decrease in cortisol from baseline through the worry and relaxation conditions may therefore be relatively consistent with similar types of experimental inductions where participants demonstrated less cortisol reactivity in response to an experimental laboratory task than 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 et al., 2017). Previous research 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 et al., 2018).

This study also extends previous research looking at physiological implications of perseverative cognition by testing how cortisol, inflammation, and RMSSD correlate in the context of experimentally induced worry in addition to how they covary across conditions (Ottaviani et al., 2016). Covariance between IL-6 and IFN- $\gamma$  with cortisol are in line with research that highlights experimentally induced cortisol responses relating to changes in CRP (Laurent et al., 2016). Interestingly, in the study by Laurent 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. The covariance between RMSSD and TNF- $\alpha$  are consistent with recent research examining baseline correlations between HRV and inflammation when examining relationships during single timepoints (Williams, Koenig, Carnevali, Jarczok, Sternberg, & Thayer, 2019). Our findings suggest that worry provokes the dysregulation of multiple biological systems at once, expanding previous research looking at correlations among biomarkers within a single timepoint or during baseline resting conditions alone.

Taken together, the findings from this study lead to larger questions within psychoneuroimmunology as to why some biomarkers, but not others, change during experimental manipulations. 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 worrying.

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. Collection methods for all biomarkers in this study were consistent with previous research. A previous meta-analysis highlighted heterogeneity of data collection methods and analytical techniques for cortisol as one potential explanation for discrepancies between findings (Liu et al., 2017). Evidence for some but not all of the markers assessed in this study changing throughout the experimental conditions warrants further investigation in future research. Taken together, these findings provide provisional evidence for differential biological responding to worry. HRV, cortisol, and inflammation are mediated by different cellular mechanisms that may be more or less sensitive to worry or experimental manipulation. Because high inflammation and constricted HRV are risk factors for numerous health conditions, it is important to better disentangle what patterns of physiological activation are related to worry and other forms of perseveration.

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, physiology being altered via worry inductions and improved through relaxation training, even via short experimental manipulations is an important step in translating these basic findings to more applied research within clinical health psychology. The inclusion of both conditions in a fixed order 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. Further, it would be beneficial for future research to follow participants longitudinally to examine whether the effects of brief relaxation trainings have physical health benefits over the long-term.

Future research should seek to expand these findings by examining 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. 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 et al., 2012). High worriers may also be less likely to engage in a healthy lifestyle. These individuals may suffer from sleep disturbances, exercise less frequently, and utilize more alcohol, smoking, food, or drugs in an effort to regulate their negative emotions, subsequently putting them at risk for experiencing a number of poor health outcomes over the long term (Michopoulos et al., 2017; Pederson 2017).

This study had several limitations. 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. Further, although a 30-minute wait period was considered acceptable based off of previous studies (Steptoe et al., 2007), results 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. All participants completed their visit in the morning to control for time of day; however, the cortisol awakening response may have partially accounted for cortisol decreasing throughout the study. Further, although participants had their first blood draw approximately 35–40 minutes after arriving for their visit, the environment where the experiment took place may have impacted worry, thus influencing physiological stress at baseline. This study did not 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.

Findings from this study provide novel insights for understanding how worry perturbs biological systems. Although future research is needed, our findings highlight a direct link between worry and the immune, cardiovascular, and endocrine systems while controlling for several important covariates. To date, there has been no published studies of contrasting experimental conditions of worry and relaxation in exacerbating immune dysregulation. Such work is important in more fully examining how perseverative processes such as worry may interact with biomarkers to influence physical health. An important next step is to further examine the physiological processes linking worry and subsequent anxiety to chronic illnesses in an effort to better understand ways to intervene on this relationship. In doing so, findings 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.

## Funding

This work was supported in part by National Institute of Health grants 1SC1CA187494 and T32 CA229114.

## Declaration of Competing Interest

The authors have no conflicts of interest to report.

## References

- Allen, J.J., Chambers, A.S., Towers, D.N., 2007. The many metrics of cardiac chronotropy: A pragmatic primer and a brief comparison of metrics. *Biological Psychology* 74 (2), 243–262.
- Borkovec, T.D., 1994. The nature, functions, and origins of worry. In: Davey, G.C.L., Tallis, F. (Eds.), *Wiley Series in Clinical Psychology. Worrying: Perspectives on Theory, Assessment and Treatment*. John Wiley & Sons, pp. 5–33.
- Bankier, B., Barajas, J., Martinez-Rumayor, A., Januzzi, J.L., 2008. Association between C-reactive protein and generalized anxiety disorder in stable coronary heart disease patients. *European heart journal* 29 (18), 2212–2217.
- Borkovec, T.D., Alcaine, O., Behar, E., 2004. Avoidance theory of worry and generalized anxiety disorder. In: Heimberg, R.G., Turk, C.L., Mennin, D.S. (Eds.), *Generalized Anxiety Disorder: Advances in Research and Practice*. Guilford Press, pp. 77–108.
- Borkovec, T.D., Inz, J., 1990. The nature of worry in generalized anxiety disorder: A predominance of thought activity. *Behaviour Research and Therapy* 28 (2), 153–158.
- Brosschot, J.F., Gerin, W., Thayer, J.F., 2006. The perseverative cognition hypothesis: A review of worry, prolonged stress-related physiological activation, and health. *Journal of Psychosomatic Research* 60 (2), 113–124.
- Chalmers, J.A., Heathers, J.A., Abbott, M.J., Kemp, A.H., Quintana, D.S., 2016. Worry is associated with robust reductions in heart rate variability: a transdiagnostic study of anxiety psychopathology. *BMC Psychology* 4 (1), 32.
- Copeland, W.E., Shanahan, L., Worthman, C., Angold, A., Costello, E.J., 2012. Generalized anxiety and C-reactive protein levels: a prospective, longitudinal analysis. *Psychological medicine* 42 (12), 2641–2650.
- Dickerson, S.S., Kemeny, M.E., 2004. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychological Bulletin* 130 (3), 355.
- Fisher, A.J., Newman, M.G., 2013. Heart rate and autonomic response to stress after experimental induction of worry versus relaxation in healthy, high-worry, and generalized anxiety disorder individuals. *Biological Psychology* 93 (1), 65–74.
- Frijda, N.H., 1988. The laws of emotion. *American Psychologist* 43 (5), 349.
- Hernando, D., Garatachea, N., Almeida, R., Casajús, J.A., Bailón, R., 2018. Validation of heart rate monitor Polar RS800 for heart rate variability analysis during exercise. *The Journal of Strength & Conditioning Research* 32 (3), 716–725.
- Laurent, H.K., Lucas, T., Pierce, J., Goetz, S., Granger, D.A., 2016. Coordination of cortisol response to social evaluative threat with autonomic and inflammatory responses is moderated by stress appraisals and affect. *Biological Psychology* 118, 17–24.
- Lazarus, R.S., 1991. Progress on a cognitive-motivational-relational theory of emotion. *American Psychologist* 46 (8), 819.
- Lewis, E.J., Yoon, K.L., Joormann, J., 2017. Emotion regulation and biological stress responding: associations with worry, rumination, and reappraisal. *Cognition and Emotion* 1–12.
- Liu, J.J., Ein, N., Peck, K., Huang, V., Pruessner, J.C., Vickers, K., 2017. Sex differences in salivary cortisol reactivity to the Trier Social Stress Test (TSST): A meta-analysis. *Psychoneuroendocrinology* 82, 26–37.
- Llera, S.J., Newman, M.G., 2010. Effects of worry on physiological and subjective reactivity to emotional stimuli in generalized anxiety disorder and nonanxious control participants. *Emotion* 10 (5), 640.
- Mitchell, A.M., Christian, L.M., 2019. Repetitive negative thinking, meaning in life, and serum cytokine levels in pregnant women: Varying associations by socioeconomic status. *Journal of Behavioral Medicine* 42 (5), 960–972.
- Newman, M.G., Llera, S.J., 2011. A novel theory of experiential avoidance in generalized anxiety disorder: A review and synthesis of research supporting a contrast avoidance model of worry. *Clinical Psychology Review* 31 (3), 371–382.
- Ottaviani, C., Thayer, J.F., Verkuil, B., Lonigro, A., Medea, B., Couyoumdjian, A., Brosschot, J.F., 2016. Physiological concomitants of perseverative cognition: A systematic review and meta-analysis. *Psychological Bulletin* 142 (3), 231–259.
- Petrovsky, N., McNair, P., Harrison, L.C., 1998. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. *Cytokine* 10 (4), 307–312.
- Porto, L.G.G., Junqueira Jr, L.F., 2009. Comparison of time-domain short-term heart interval variability analysis using a wrist-worn heart rate monitor and the conventional electrocardiogram. *Pacing and Clinical Electrophysiology* 32 (1), 43–51.
- Segerstrom, S.C., Solomon, G.F., Kemeny, M.E., Fahey, J.L., 1998. Relationship of worry to immune sequelae of the Northridge earthquake. *Journal of Behavioral Medicine* 21 (5), 433–450.
- Shelton, M.M., Schminkey, D.L., Groer, M.W., 2015. Relationships among prenatal depression, plasma cortisol, and inflammatory cytokines. *Biological Research for Nursing* 17 (3), 295–302.

- Steptoe, A., Hamer, M., Chida, Y., 2007. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain, Behavior, and Immunity* 21 (7), 901–912.
- Thomsen, D.K., Mehlsen, M.Y., Hokland, M., Viidik, A., Olesen, F., Avlund, K., Zachariae, R., 2004. Negative thoughts and health: Associations among rumination, immunity, and health care utilization in a young and elderly sample. *Psychosomatic Medicine* 66 (3), 363–371.
- Wichelns, G.A., Renna, M.E., Mennin, D.S., 2016. Preliminary validation of subjective anchor scales for worry and rumination. *Cognitive Therapy and Research* 40 (5), 1–16.
- Williams, D.P., Koenig, J., Carnevali, L., Sgoifo, A., Jarczok, M.N., Sternberg, E.M., Thayer, J.F., 2019. Heart Rate Variability and Inflammation: A Meta-Analysis of Human Studies. *Brain, Behavior, and Immunity*.
- Zänker, S., Bellingrath, S., Wüst, S., Kudielka, B.M., 2018. HPA axis responses to psychological challenge linking stress and disease: What do we know on sources of intra- and interindividual variability? *Psychoneuroendocrinology*.
- Zorn, J.V., Schür, R.R., Boks, M.P., Kahn, R.S., Joëls, M., Vinkers, C.H., 2017. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. *Psychoneuroendocrinology* 77, 25–36.