Heart Rate Variability (HRV), Cortisol, and Trait Anxiety in Mid-Life Adults

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HEART RATE VARIABILITY (HRV), CORTISOL, AND TRAIT ANXIETY IN MID-LIFE ADULTS

by

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A Thesis submitted to the Faculty of the Graduate School, Marquette University, in Partial Fulfillment of the Requirements of the Degree of Master of Science

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ABSTRACT
HEART RATE VARIABILITY (HRV), CORTISOL, AND TRAIT ANXIETY IN MID-LIFE ADULTS

Meghan M. Bennett, M.S.
Marquette University, 2022

Heart rate variability (HRV) and cortisol are well-established biomarkers of the human stress response system. As such, their respective relationships with trait anxiety have been studied. As high HRV indicates healthy emotion regulation while low HRV signifies poor emotion regulation, a negative relationship between HRV and anxiety is found in the literature. Conversely, cortisol both prepares the body for stress and helps it to recover and current studies yield mixed results on its relationship with anxiety. While the link between vagal activity, which mediates HRV, and the HPA-axis, which outputs cortisol, is generally assumed, few studies have examined these biomarkers in the same study design, and specifically how this relationship, if present, manifests over time or in relation to anxiety. Importantly, studying these biomarkers together provides a clearer depiction of the stress response and may better inform treatments aimed at improving biological outcomes in clinical samples, such as highly anxious individuals. Using archival data from 438 mid-life adults, the impetus of the present study was to examine the relationship between cortisol and HRV over time (pre-, during-, post- cognitive and physical stressors) using standard, literature-defined metrics to quantify increased/decreased cortisol and HRV in response to stress. Additionally, we used latent growth mixture modeling (LGMM) to examine trajectories of cortisol and HRV over time in response to stress. Finally, we tested the relationship between all measures of cortisol and HRV to anxiety. Results revealed that in response to a cognitive stressor, higher HRV reactivity was associated with higher cortisol reactivity. LGMM analyses identified differential cortisol and HRV trajectories. Despite this and contrary to hypothesis, we did not find support for a relationship between anxiety and HRV and cortisol metrics, including differential trajectories of change, in the full sample. In exploratory analyses, we did find that individuals with greater anxiety exhibited less cortisol reactivity to the cognitive stressor when we restricted our analysis to a “clinically-detectable” anxious sub-group. Results hold clinical relevance in that targeting moment-to-moment adaption to stress via HRV may help promote more adaptive cortisol responding via changing cortisol output. However, more research is needed to better understand this relationship.
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In no particular order, I would like to thank my committee members, my mentor, and my partner. I would like to thank the Graduate School and all of the Marquette University administration.
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Introduction

According to Darwin’s Origin of Species, it is not the most intellectual of the species that survives; it is not the strongest that survives; but the species that survives is the one that is best able to adapt and adjust to the changing environment in which it finds itself. (Megginson, 1963, p. 4)

Fear is the response to an imminent or real threat (American Psychiatric Association, 2013). For example, fear of a snake that has just moved on the path ahead of you as you round a corner in your walk activates the hypothalamic-pituitary-adrenal (HPA)-Axis and initiates a cascade of stress hormone release, a biological responses that in part activates heart rate and muscle activity. Activation of this system initiates action to help you avoid harm and is therefore considered adaptive (Rotenberg & McGrath, 2016). In contrast, anxiety is defined as the anticipation of future threat; that is, a response to a real or imagined stimuli that one perceives as threatening, but which may or may not put the organism in danger and for which the outcome is unknown. Clinically, anxiety is the most common psychiatric disorder, surpassing depression, with an estimated 12-month prevalence for anxiety disorder diagnoses in the United States at 18.1% (Chalmers et al., 2014; Kessler et al., 2005). Anxiety is also costly to treat, in part because it is associated with a host of long-term deleterious psychological (e.g., the development of diagnosable anxiety disorders) and physical (e.g., cardiovascular disease) outcomes (Sylvers et al., 2011). An approximate $42.3 billion is spent annually on treating this disorder across associated health care costs or estimated loss of work revenue (Greenberg et al., 1999). Despite the prevalence, cost-burden, and clinical certification of anxiety, the associated biological mechanisms of this disorder are still not
fully understood. Critically, a clearer understanding of anxiety’s biological underpinnings may be important to inform treatment approaches that target the underlying causes of anxiety and mitigate its deleterious effects.

Because of the link between anxiety and biological response to threat, anxiety can manifest in a variety of psychological, biological, and behavioral presentations, and be measured in such responses. First, psychological cardinal symptoms of anxiety include worry, hypervigilance, avoidance, and apprehension (Sylvers et al., 2011), while self-report measures are a primary tool in assessing these symptoms (Sylvers et al., 2011). Spielberger et al. (1983) defines trait anxiety as a relatively stable tendency of the individual to manifest anxiety states, differing from state anxiety, which is described as an unpleasant emotional state rooted in fear. Differences in state vs. trait anxiety are measured using the State-Trait Anxiety Inventory (STAI), considered the gold-standard self-report scale (Kayikcioglu et al., 2017). Using such metrics, trait anxiety is defined as individual differences in how people tend to perceive stressful situations as threatening and to respond to such situations with heightened and often prolonged intensity (Spielberger et al., 1983). Importantly, ‘intensity’ is defined here as hormonal and physiological response to perceived threat (Spielberger & Reheiser, 2009). Thus, variation in two neurobiological systems associated with stress hormones and physiology, the HPA-Axis and the autonomic nervous system (ANS), respectively, may be particularly indicative of trait anxiety.

**Biological Mechanisms of the Stress Response System**

The HPA-Axis is the body’s hormonal response system and regulator of the stress response (Rotenberg & McGrath, 2016). When a person encounters perceived threat, the
hypothalamus releases a corticotrophin-releasing hormone, which in turn triggers the pituitary gland to release adrenocorticotropic hormones (ACTH) into the blood stream. The ACTH travels down to the adrenal glands prompting the release of cortisol, a steroid hormone considered the main output of HPA-axis activation (Bozovic et al., 2013). Thus, cortisol output serves as a reliable metric of biological response to stress (Bozovic et al., 2013). Importantly, when cortisol levels in the blood become high, it induces a negative feedback mechanism for termination of the stress response (Bozovic et al., 2013). Thus, while a metric of activation of stress response, high cortisol after stress is considered adaptive in that it serves an important role in shutting down further secretion of stress hormones.

Cortisol also follows a known time course in healthy individuals. Upon exposure to a stressor, the ACTH responds within in the first 5 minutes, while cortisol secretion lags by 5-20 minutes (Bozovic et al., 2013). Thus, peak blood levels for measuring cortisol are established within approximately 10-30 minutes following stress exposure. Additionally, healthy individuals display a robust diurnal rhythm of cortisol response irrespective of experienced stress, with cortisol levels peaking approximately 30 minutes after waking, then declining throughout the day to reach their lowest in the evening (Miller et al., 2007). As the transfer of cortisol from blood to saliva takes place between 2-3 minutes, cortisol can be measured in saliva, and due to its noninvasive nature and ease at collecting samples across multiple time points, salivary measures of cortisol have become a commonly used marker of cortisol output. After stress, rises in cortisol measured in saliva are well-detected and occur approximately 20 minutes after exposure to the stressor, before returning to baseline (e.g., pre-stress) levels (Kirschbaum &
Nevertheless, there is great variability in cortisol output. As one example, average salivary cortisol levels among healthy subjects range from 0.20 – 1.41 μg/dl (5.52 – 28.92 nmol/l) in the morning and between 0.04 – 0.41 μg/dl (1.10 – 11.32 nmol/l) in the afternoon (Bozovic et al., 2013).

One of the main targets of cortisol activation is the autonomic nervous system (ANS), which unconsciously regulates bodily functions. Divided into sympathetic and parasympathetic branches, these systems work in a largely opposite fashion, activating or inhibiting internal organs in response to perceived stress. One main target of the ANS is the heart, as the ANS regulates heart rate through both sympathetic fibers and parasympathetic innervations mediated by the vagus nerve (Sloan et al., 2017). Sympathetic activation of the heart speeds up heart rate, while parasympathetic activation decreases is (Thayer & Sternberg, 2006). One commonly-used marker of non-linear adaptation of the heart to threat is called heart rate variability (HRV) and HRV has been utilized as an individual-differences marker in the body’s adaptability to stress. More concretely, HRV measures the heart’s flexible responding, which changes in response to stress as well as over the day. That is, both cortisol and HRV follow time course patterns in response to stress, while changes in HRV are also known to vary among individuals (Friedman, 2007). For instance, high HRV, qualified by a relatively large inter-beat variability (Berntson et al., 1997; Friedman, 2007; Rajcani et al., 2018) is associated with intact emotion regulation, a main mechanism for the control of perceived stress or threat. Conversely, low HRV is thought to reflect emotion dysregulation (Pulopulos et al., 2018). Critically, although cortisol influences heart rate, individual differences in underlying HRV prior to, during, and after stress has been heralded as an important driver
at anxiety reduction (Pulopulos et al., 2018). That is, while cortisol influences HRV, HRV also appears to serve as its own negative feedback targeting the stress response itself. As a metric of HRV and specifically tied to activation of the parasympathetic modulation of the heart, the root mean square successive difference (RMSSD) is a widely used measure as it is considered to be mostly free of respiratory influences that are not of cardinal interest when studying activation of the heart as it relates to stress (Hill & Siebenbrock, 2009). To our knowledge, there are not well-established normative values of what HRV, or RMSSD, variability should be in healthy individuals, which may be, in part, due to extreme individual differences of HRV. Considering fear’s association with activation of the HPA-axis, and in turn the ANS and associated HRV, trait anxiety may be an important individual difference factor that contributes to this inconsistency (Thayer et al., 1996). Indeed, current theory dictates that the etiology of anxiety may be rooted in abnormal biological response to stress (Sapolsky, 1994).

**Cortisol and Anxiety**

Despite a strong theoretical assertion that stress responding is atypical in individuals with anxiety, prior literature has found disparate evidence for whether cortisol output after stress is indeed atypical, and whether it is increased or decreased in this population. First, a growing number of studies demonstrate blunted cortisol response (e.g., less rise after stress compared to healthy individuals) immediately following and recovering from stress among anxious individuals (de Rooij et al., 2010; Fiksdal et al., 2019; Jezova et al., 2004). More specifically, compared to peers with low trait anxiety, adults with comparatively higher trait anxiety exhibit lower cortisol output 15, 30, and 90
minutes after stress (Jezova et al., 2004). Similarly, in a large sample of middle-aged adults salivary cortisol was measured at baseline, peak response to stressor, and recovery within 30 minutes of stress exposure. Findings revealed that adults with higher trait anxiety experienced blunted cortisol in peak response to the stressor compared to their low-anxious counterparts, but cortisol at baseline and recovery did not significantly differ between anxious and non-anxious individuals (de Rooij et al., 2010). Other work has found that the recovery of cortisol to pre-stress levels is altered in those with high anxiety and adds further evidence to a “blunted cortisol” theory of anxiety. For instance, Fiksdal and colleagues (2019) found that greater anxiety was related to flattened cortisol slope back to baseline after stress, meaning it took these individuals longer to return to a baseline cortisol level. Takahashi et al. (2005) examined participants’ baseline, stress reactivity, and post-stress cortisol levels and found respondents’ trait anxiety was significantly positively correlated with both pre-stress cortisol levels, and that pre-stress cortisol levels were inversely associated with stress-induced cortisol responding. That is, lower pre-stress cortisol was related to greater stress-induced cortisol. In particular, these findings suggest a functional relationship between cortisol measured at different timepoints. One possibility is that trait anxiety is associated with time course of cortisol response, not just overall cortisol output. Notably, prior studies have used varied stressors ranging from a public speech task, Stroop test, and cognitive tasks and either studied these effects in healthy individuals without a diagnosis of anxiety (but with trait anxiety symptoms; e.g., Fiksdal et al., 2019) or in clinically-diagnosed patients with anxiety (e.g., de Rooij et al., 2010; however, only 2% clinically diagnosed). Thus, it appears that
neither the type of stressor probe used nor the diagnostic status of participants seems to alter the finding that greater anxiety symptoms is tied to lower cortisol output.

In contrast to the above, other studies suggest increased cortisol in this population immediately through 60 minutes post-stress (Furlan et al., 2001; Yoon & Joormann, 2012). This is particularly true among individuals with socially related anxieties. For example, Yoon & Joormann (2012) found that those with social anxiety experienced an increased, and significantly different, cortisol response to the stressor compared to healthy controls. Likewise, Furlan and colleagues (2001) found that individuals with social phobias responded with higher cortisol levels compared to healthy controls in response to a social stress task, but not to physical exercise. Thus, at least for some individuals for which their anxiety manifests in social contexts, heightened cortisol may depend on the type of stressor.

Still yet, other work has failed to find atypical cortisol responses tied to anxiety (Henckens et al., 2016; Souza et al., 2015; Young et al., 2004). Elnazer & Baldwin (2014) conducted a structured review of the literature and found no unifying HPA-Axis response across anxiety disorders (e.g., generalized anxiety disorder, panic disorder, specific phobias, and social anxiety disorder). Furthermore, their findings revealed inconsistent HPA-Axis functioning within each disorder and even among studies with similar methodology. They highlight small sample sizes and variations in clinical samples as potential contributors to these inconsistencies and point to the need for a better consensus in order to inform future treatment approaches. One possibility for inconsistent results may be with respect to the time course of cortisol not being adequately studied in this literature. While many of the above studies have investigated slope differences (e.g.,
rises and falls) of cortisol over time in anxious individuals (Fiksdal et al., 2019; Michels, Sioen, Braet, et al., 2013; Murdock et al., 2017; Rotenberg & McGrath, 2016), no study to our knowledge has investigated incidences of delayed or rapid onset of cortisol after stress as it relates to anxiety. Although under-studied, trajectory of cortisol may be an illuminating predictor of anxiety. Another possibility is that there may be other biological factors related to the HPA-Axis and cortisol that are tied to anxiety and thus driving discrepant findings.

**Heart Rate Variability (HRV) and Anxiety**

One such variable is HRV, given that heart rate is a target of cortisol, while HRV in turn modulates the stress response. As opposed to cortisol, which produces inconsistent findings, the relationship between HRV and anxiety appears well-established, further suggesting that HRV may be an important mediating variable for understanding anxiety-related changes in cortisol. Indeed, many studies demonstrate decreased HRV associated with anxiety (Chalmers et al., 2014, 2014; Chang et al., 2013; Kemp et al., 2012; Pittig et al., 2013; Thayer et al., 1996; Watkins et al., 1998) and results of a large meta-analysis, by Chalmers and colleagues (2014), provide further support for reduced resting HRV among anxious individuals compared to healthy controls. In a review, Friedman (2007) also found convergent evidence in the literature for a negative association between HRV and anxiety. In terms of specific evidence, Watkins and colleagues (1998) studied the relationship between resting baseline HRV and anxiety among healthy adults and found that, compared to those with low trait anxiety (STAI scores below 31), the high trait anxiety group (STAI scores above 41) demonstrated significantly decreased vagal control
of the heart. Extending this investigation to a clinical group, Klein et al. (1995) compared individuals with panic disorder (PD) to non-anxious controls and findings suggested that reduced HRV may be a characteristic of PD. HRV appears to also be a good metric of anxiety amidst comorbid populations. For example, Chang and colleagues (2013) studied baseline HRV among anxious individuals with and without comorbid depression compared to healthy controls and found reduced HRV among the purely anxious group with an even greater reduction in HRV among the comorbid group. Notably, the authors excluded patients on antidepressants, as previous studies (Hu et al., 2019; Licht et al., 2009) suggested antidepressant use may moderate HRV. Similarly, Kemp et al. (2012) examined resting HRV in an unmedicated sample of adults with comorbid anxiety and depression, depression alone, and healthy controls. Findings yielded reduced HRV among the comorbid and purely depressed groups compared to healthy controls, but with the greatest reduction in the comorbid anxious group.

Expanding on the literature demonstrating reduced HRV at resting baseline, Friedman and Thayer (1998) found that anxious individuals also presented reduced HRV compared to healthy controls in response to a laboratory stressor. Likewise, Thayer et al. (1996) compared autonomic functioning between anxious and non-anxious controls at resting baseline, relaxation, and worry periods and found lower cardiac vagal control in the anxious group across all conditions. Furthermore, Pittig et al. (2013) investigated HRV among a sample of individuals with heterogeneous anxiety disorder diagnoses compared to healthy controls at baseline and in response to a stressor. Anxious individuals (regardless of type of formal diagnosis) exhibited significantly lower HRV at baseline and during the first 30 seconds of the stressor task compared to healthy controls,
and these effects were independent of gender and age. This suggests that low HRV may be a unifying feature of trait anxiety.

Notably, the above studies consistently found reduced HRV at resting and in response to a stressor among individuals with both clinical (e.g., Chalmers et al., 2014; Klein et al., 1995) and subclinical levels of anxiety (e.g., Watkins et al., 1998) suggesting that neither the diagnostic status of individuals or state of arousal (relaxed vs. stressed) changes the pattern of findings (however, see Rajcani et al., 2018) which showed opposite effects, though in a small sample size [$N = 19$]).

**Concurrent Measures of HRV and Cortisol and Anxiety**

As cortisol and HRV are two commonly studied biological underpinnings of trait anxiety, some researchers have studied these variables together within a single study design (Michels, Sioen, Braet, et al., 2013; Murdock et al., 2017; Pulopulos et al., 2018). The impetus for this approach is not only because they are both instrumental in the stress response, but also because prior literature suggests they may have an influential relationship on one another (Aimie-Salleh et al., 2019; Rotenberg & McGrath, 2016). Notably, however, no study investigating the relationship between stress-induced cortisol and HRV has been done with respect to anxiety, and we report findings below only as they appear in healthy populations, particularly in adolescents, or in relationship to other psychological states other than anxiety. First, Pulopulos et al. (2018) explored whether cortisol response to a laboratory-based stressor was associated with anticipation-induced or stress task-induced HRV changes among a sample of 171 healthy unmedicated adults. Salivary samples were taken immediately before introduction to the task, immediately after the Trier Social Stress Test (TSST), a commonly-employed lab-based social stress
test, and at 5 points in time in the hour following the TSST to measure cortisol. HRV was measured continuously from the beginning of the session until 15 minutes following the TSST. Results revealed that HRV changes during the stress-task were uncorrelated with cortisol increase or recovery. In contrast, however, a greater decrease in HRV during the *anticipation* of stress was correlated with higher stress task-induced cortisol increase, but not cortisol recovery. These findings indicate that anticipatory HRV, reflective of individual differences in stress regulation before interaction with a stressor, may be uniquely important for studying inter-individual differences in cortisol response to stress.

Another study examining the relationship between HRV and cortisol looked at these variables in relation to hostility in a sample of 213 adults (Murdock et al., 2017). The researchers investigated if high self-regulatory strength, as reflected by high stress-induced high-frequency HRV (HF-HRV), moderated the effects of hostility on cortisol secretion. HRV was measured at rest and during the TSST. Saliva samples were collected immediately before, and six times over a 50 minute time period following, the TSST, to measure cortisol secretion over time. Results indicated that individuals scoring high on a self-report measure of hostility were less likely to demonstrate high changes in cortisol over time when they had high stress-induced HF-HRV. That is, hostility-related changes in cortisol were moderated by high HRV. These findings suggest that targeting stress-induced HF-HRV may be an appropriate intervention for reducing the impact of hostility on health outcomes. Notably, Murdock and colleagues (2017) point out that this may, in part, account for why higher cortisol sensitivity tends to appear more among mood disordered populations compared to healthy controls (Quax et al., 2013; Young et al., 2001).
Finally, in the only study to-date that has investigated the relationship between cortisol, HRV, and anxiety Michels et al. (2013) found that among children anxiety, peer problems, anger, and sadness were associated with reduced HRV. Furthermore, they found that reduced vagal parasympathetic activity was moderately associated with higher salivary cortisol output and a steeper cortisol diurnal decline. Saliva was collected at home during two consecutive weekdays at four time points and HRV was measured in a quiet room for a 10 minute interval and qualified by RMSSD.

In summary, the literature reviewed above indicates that a negative relationship between HRV and anxiety is well-established, while the relationship between salivary cortisol and anxiety is less determined. Nevertheless, there is strong evidence to suggest an interaction between HRV and salivary cortisol. Discrepant findings of cortisol among anxious individuals suggests that there is a yet-unidentified mechanism influencing the cortisol stress response in this population. Based on the literature surveyed, we hypothesize that HRV may be a meaningful moderating variable of the stress response system in those with high anxiety.

Present Study

While numerous studies have examined salivary cortisol and HRV separately, far fewer have investigated the relationship between them, and specifically how this relationship, if present, manifests over time. Importantly, studying these biomarkers together provides a better depiction of the stress response than either alone (Aimie-Salleh et al., 2019; Rotenberg & McGrath, 2016), which could hold implications for making accurate treatment recommendations aimed at improving biological outcomes in clinical samples. Furthermore, well understood patterns of cortisol and HRV demonstrate that
these measures fluctuate over time, and in response to stressors, among healthy populations. Additionally, there is variation in these patterns accounted for by individual differences. Therefore, the impetus of the present study is to examine the relationship between cortisol and HRV over time (pre-, during-, post- stress) as well as their relationship to trait anxiety.

Ultimately, this investigation may lead to a better understanding of these biological variables, but could yield clinical significance for informing targets of anxiety intervention. As cortisol is the main output of the stress response it is a logical target of intervention. However, there are real barriers to using cortisol as an intervention target, including collecting cortisol for assay or manipulating cortisol in the moment. Additionally, as outlined above, cortisol follows a delayed response after stress, peaking typically only 20 minutes later, making it difficult to assess real-time moment-to-moment changes as they occur in the face of a stressor despite the fact that clinically, targeting momentary response to stress is a main goal of therapeutic interventions. For these reasons while readily manipulating cortisol levels may be difficult to achieve, controlling HRV is more easily accessible and measured in real time. Indeed, prior research has shown promise for HRV biofeedback as an adjunct treatment modality for reducing stress among anxious individuals (Henriques et al., 2011; Lehrer & Eddie, 2013). Among those with major depressive disorder, highly comorbid with anxiety, HRV biofeedback has demonstrated reduction in depressive symptoms in as little as 4 training sessions and twice daily 20-minute practice periods at home between sessions (Karavidas et al., 2007). However, more research is needed to determine whether HRV is related to cortisol in this population.
The present study will utilize a large, nationally-representative archival dataset from the Midlife in the United States Study (MIDUS), which has data on salivary cortisol and HRV over the course of a psychological and physical stressor. Adults in this dataset were also characterized on trait anxiety. Thus, this data provides a valuable and relevant tool for testing of the following aims:

**Specific Aims**

**Aim 1a**

*Examine variability in the change in salivary cortisol over time (baseline, reactivity to stressor, recovery).* Prior literature demonstrates that individuals differ in their cortisol response to stressors, at baseline, and recovery; thus, it will be important to understand differences of this response for respondents in our sample. The intent of this aim is descriptive in order to quantify change in cortisol over time using pre-established metrics of response, including an area under the curve with respect to cortisol increase (AUC), slope to capture the direction of change over time, change in cortisol from baseline to maximum level after stress, and change in cortisol from maximum level to recovery.

**Aim 1b**

*Re-evaluate change in cortisol over time with a focus on differential trajectories of cortisol (baseline, reactivity to stressor, recovery).* Discrepancies in the literature on cortisol secretion in anxious populations may, in part, result from lack of a clear understanding as to how cortisol varies at different points in time. The aforementioned cortisol metrics in Aim 1a are based on pre-existing traditional methods of analysis and do not calculate differential timing in rise and decline of cortisol. For example, anxiety...
may not be correlated with magnitude of cortisol rise during stress, but may be related to
an earlier or delayed onset of cortisol rise. Thus, it will also be important to quantify the
trajectory of cortisol over time in this sample.

Aim 1c

*Test if trait anxiety is associated with change in cortisol as measured by the above
metrics.* We will examine associations between levels of trait anxiety and cortisol
secretion in our sample to better understand if trait levels of anxiety are correlated with
cortisol change as quantified across Aims 1a and 1b.

Aim 2a

*To examine variability in the change in HRV over time (baseline, reactivity to stressor,
recovery).* Prior literature suggests that high HRV over time and in response to stressors
is an indication of emotion regulation; whereas low HRV signals emotion dysregulation.
Similar to Aim 1a, it will be important to descriptively evaluate how HRV changes over
time in our sample, again using pre-established metrics, including change in HRV from
baseline to stress, and change from stress to recovery.

Aim 2b

*Re-evaluate change in HRV over time with a focus on differential trajectories of HRV
(baseline, reactivity to stressor, recovery).* Identical to Aim 1b above, we will examine
differential timing in rise and decline of HRV over time through quantification of the
rapid vs. delayed trajectory of HRV over time.

Aim 2c
Test if trait anxiety is associated with change in HRV as measured by the above metrics.

Again, we will examine associations between levels of trait anxiety and HRV in our sample to better understand if trait levels of anxiety are correlated with change in HRV as quantified across Aims 2a and 2b.

**Aim 3a**

Examine the relationship between change in HRV (baseline, reactivity to stressor, recovery) and change in cortisol (baseline, reactivity to stressor, recovery). Some prior findings have shown a relationship between changes in HRV during stress and cortisol response and/or recovery in healthy samples (Johnsen et al., 2012; Weber et al., 2010). Other research shows that HRV during the anticipation of stress (but not during stress) may be associated with cortisol stress response (Pulopulos et al., 2018). Therefore, studying the relationship between HRV and cortisol in the context of time with regard to pre-, peri-, and post- stress may be critical to furthering our understanding of these mechanisms. Furthermore, there is unexplained variance in the cortisol response; illuminating a possible association between HRV and cortisol that could, in part, help explain such variance. Thus, we aim to investigate the relationship between HRV and the cortisol stress response.

**Aim 3b**

Test the moderating effects of trait anxiety on the relationships between change in HRV and change in cortisol. This study will ultimately test whether the relationship between HRV and cortisol is moderated by trait anxiety. Testing this association will help
illuminate whether HRV as a treatment target for remediation of atypical stress responding will be relevant for those with high trait anxiety.

Methods

Study Protocol

In 1995, to examine the role of behavioral, psychological, and social factors in age-related differences in well-being and health, an interdisciplinary team collaborated to conduct a national survey (N = 3,487) of Midlife Development in the U.S. (MIDUS). This study was innovative as it allowed researchers to study a large sample of participants through a wide scientific scope by conducting phone interviews and self-administered surveys. Based on its success, the National Institute of Aging awarded a grant to the Institute of Aging at University of Wisconsin-Madison to conduct a follow-up longitudinal study of the MIDUS respondents, thus leading to the collection of MIDUS 2 data in 2002-2008. The MIDUS 2 study expanded on MIDUS 1 through inclusion of biological and neurological assessment and therefore MIDUS 2 consisted of five separate research projects, each collecting different information from sample respondents. Furthermore, MIDUS 2 added a sample of 600 African Americans from Milwaukee, WI to improve the focus on health and well-being of African Americans in the U.S..

The study was extended for a third round of funding in 2011-2016 as the Midlife Development in the U.S. (MIDUS) Refresher study Survey, which recruited a large sample of midlife adults (N = 3,577) to replenish the original MIDUS 1 baseline cohort. A second aim was to provide increased sample sizes for testing hypotheses exploring key variables (e.g., psychological, biological, socioeconomic status) in mid- and later-life health. Therefore, after completing project 1 respondents were eligible to participate in 4
additional projects. Data for these projects were obtained during respondents 24 hour stay at one of 3 Clinical Research Units (CRU), UCLA, Georgetown, and University of Wisconsin-Madison, and processed at the Columbia University Medical Center in Dr. Richard Sloan’s laboratory (Appendix A).

Participants

The present study will use data obtained from the publicly available dataset MIDUS Refresher Biomarker Project, which is one of five projects developed from the MIDUS Refresher study Survey. From 2012-2016, the MIDUS Refresher Biomarker study collected data from 863 (n = 746 main sample, n = 117 African Americans from Milwaukee, WI) of the respondents who had completed the initial MIDUS Refresher Survey. Respondents’ ages at the time of biomarker data collection ranged from 25 to 78 with females representing 52.1% of the sample and males comprising 47.9% of the sample.

Procedures

Data for the MIDUS Refresher Biomarker Project were collected after an overnight stay at CRU. Details on the project’s protocols in full can be found on Midlife in the United States (MIDUS Refresher): Biomarker Project, 2012-2016 (ICPSR 36901) website (https://www.icpsr.umich.edu/web/ICPSR/studies/36901). In the morning on the second day of the CRU stay, data was collected from respondents according to the Psychophysiology Challenge Protocol, a standard laboratory-based stress reactivity protocol. Information on respondents’ heart rate, blood pressure (BP), and breathing while at rest and during challenging tasks was recorded.
Respondents started seated, at rest, to gather baseline measures over 11 minutes and then were asked to perform a Stroop color-word matching task or simple arithmetic task for 6 minutes (counterbalanced), followed by a 6 minute recovery period before completing the second challenging cognitive task. After completion of the two challenging tasks and resting recovery periods, respondents were asked to get out of the chair and stand still for a 6-minute orthostatic stressor. Finally, the respondents returned to resting in their chair for 30 minutes (Appendix B).

**Cortisol**

At five predesignated time points during the laboratory-based stress reactivity protocol, respondents removed a cotton swab from the Salivette®, put it in their mouth, rolled it around until saturated, and then placed it back in the tube and replaced the cap for saliva collection. At the end of each session saliva salivettes were stored in a freezer at -80 degrees Fahrenheit. At the time of assay cortisol samples were thawed and centrifuged at 3000 rpm for 5 minutes for cortisol assay (Appendix C). The first salivary cortisol sample was collected pre-protocol while respondents were standing before attaching electrocardiogram (ECG) leads and other monitors for cardiovascular monitoring. Notably, prior literature suggests the importance of a habituation period in order to avoid elevated cortisol baseline levels (Goodman et al., 2017). Next, a sample was collected while respondents were seated at baseline, immediately before beginning the seated baseline physiological recording. The third sample, post-cognitive stress, was obtained while seated after completion of both cognitive stress tasks and their corresponding recovery periods. Fourth, a sample was taken standing, directly following
the orthostatic challenge. Finally, the last sample, recovery, was taken while seated 30 minutes after the orthostatic challenge.

When preparing the saliva samples for assay, the testing lab detected some cases in which there was insufficient saliva present in the tube to run cortisol assay and these samples were included in the dataset but flagged. For the purposes of the current study, participants with insufficient cortisol assays were removed from analyses. Additionally, a small number of cases were identified during data cleaning for whom the session start time or the time participant got out of bed that morning was missing or later than expected due to recording error. Information on wake time was used as a covariate in analysis given the expected diurnal rise and fall of cortisol based on wake time, but which is not a focus of this investigation. Missing session start time cases were handled with an imputed value which was computed using the site-specific mode (50th percentile) of the lag time in minutes from the session start time to the 1st saliva collection time. Imputed values for missing wake time cases were computed using the average lag time in minutes from the blood collection time to the wake time. All cases with imputed values were flagged and included in the dataset. Of the entire sample \((N = 863)\), data across all 5 cortisol samples were available for \(N = 757\) respondents.

**Heart Rate Variability**

Throughout the Psychophysiology Challenge Protocol described above, participants’ cardiovascular reactivity was evaluated by continuous measurement of ECG and BP was measured at the finger using brachial pressure. The beat-to-beat ECG and BP waveforms were then analyzed to calculate numerous indices of HRV defined as variability in the interval between consecutive R waves. While heart rate supplies a
measure of the number of heart beats per minute, HRV reflects the variation in time intervals between consecutive heart beats (Shaffer & Ginsberg, 2017). Data collected from ECG were analyzed with a specified 300 second epoch duration and compartmentalized into 7 epochs (Figure 1). Due to the ease of recording short-term measures of HRV (~5 minutes), this convention appears to be the most widely reported source of published HRV data (Shaffer & Ginsberg, 2017).

The MIDUS Refresher Biomarker Project collected several measurements of HRV and the RMSSD was selected for analysis in the present study due to its reliable and common use in the literature (Hill & Siebenbrock, 2009; Malik et al., 1996). RMSSD indicates “the beat-to-beat variance in heart rate and is the primary time-domain measure used to estimate the vagally mediated changes reflected in HRV” (Shaffer & Ginsberg, 2017). Of the entire sample ($N = 863$), data across all 7 HRV epoch measurements were available for $N = 503$ respondents.
Figure 1. Sequence of 90-minute stress protocol.

Trait Anxiety

Trait anxiety for the present study was measured by participants’ overall scores on the STAI (Appendix D). The STAI is a self-evaluation questionnaire that consists of 20 items with rankings 1-4 (1 = Almost never; 2 = Sometimes; 3 = Often; 4 = Almost always) and asks respondents to circle the number that “best describes how you generally feel” (e.g., “I worry too much over something that really doesn’t matter”). Of the entire sample ($N = 863$) survey data collection was utilized from project 1, which included a 30-minute phone interview followed by two 50-page mailed self-administered
questionnaires, one of which was the STAI. Scores in the dataset ranged from 20 to 67 ($M = 35.16$), with higher scores indicating more trait anxiety. The STAI is the gold-standard self-report scale used to measure both trait and state anxiety and is widely used (Balsamo et al., 2018; Spielberger et al., 1983). Every participant that provided biomarker data also provided STAI responses ($N = 863$), therefore, no instances of missing data for the STAI were identified.

**Additional Variables**

Based on recommendations in the literature (O’Neal et al., 2016; Silvia et al., 2014; Sin et al., 2016) the following variables will be used in analyses as covariates in an effort to increase clarity and understanding of discrepancies in the literature: age, gender, race, antidepressant use, time of cortisol collection lagged from wake time, and smoking status.

**Age.** Age has been identified as a highly important contextual factor in analyzing variables related to individuals’ stress response. For example, HRV tends to decrease with age (Umetani et al., 1998). The present study considers participants spanning several decades (ages 25 to 75), and as such age will be used as a covariate to better understand variability in the sample across measures.

**Gender.** Gender may hold important implications for HRV and salivary cortisol response and therefore will be included for analysis in the present study to better illuminate and clarify differences. According to previous studies, the upper and lower limits of HRV appear to be dependent on gender, and gender appears to moderate the rate at which HRV declines with age (Umetani et al., 1998). Specifically, under age 30 women demonstrated lower HRV compared to men, but these differences diminished
after age 30 and disappeared by age 50 (Umetani et al., 1998). A meta-analysis conducted by Zorn and colleagues (2017) also suggests differing cortisol responses to stressors among men and women.

**Antidepressants.** Previous studies demonstrate that antidepressants may yield significant effects on individual’s stress response system (Hu et al., 2019; Kemp et al., 2012; Licht et al., 2009). As a part of the Refresher Biomarker Project protocol, comprehensive data about medications participants were taking at time of data collection were recorded. Participants were directed to bring all their medications, in original bottles, to their visit to CRU to ensure proper recording of all medication names and dosages. Current antidepressant use was included as a dichotomous variable (i.e., yes or no) for each participant in the data set.

**Race.** Prior literature has suggested race as an important factor in determining normative and stress response data of HRV. For example, Sin and colleagues (2016) found higher HRV among African Americans compared to their White counterparts in a large study. As such, race of participants will be examined in our sample as it relates to these factors.

**Smoking Status.** Prior literature has suggested smoking is an important factor in individual stress response (Adam et al., 2017). As such, smoking status (dichotomous variable indicating current smoker: yes/no) of participants will be examined in our sample.

**Time of Cortisol Collection.** Cortisol follows a known diurnal rhythm, with levels peaking in the morning, dropping throughout the day, and at its lowest in the evening (Miller et al., 2007). The cortisol awakening response (CAR), defined by a
change in cortisol secretion within the first hour of awakening, peaks within about 30-40 minutes of waking and thus is an important factor to consider in salivary cortisol collection (Steptoe & Serwinski, 2016). Presently, differences in CAR are not the subject of the present inquiry. As such, only participants who have data available for the duration between awakening and time of first cortisol collection will be included in the present study so that lag time between cortisol collection and waking can be controlled for.

**Data Analysis**

Prior to data analysis, outliers will be reviewed following guidelines from previous HRV and cortisol research (Pulopulos et al., 2018) and values defined as values ±3 SD will be removed from analysis. If cortisol and HRV measurements are not approximately normally distributed, we will apply the correct transformation prior to data analysis. For regression analyses (Aims 3a and 3b), distribution of independent variables will be assessed for normality and the appropriate transformations will be used to achieve normal distributions. Results of hierarchical regressions will be evaluated for meeting the assumptions of the linear model including confirming that errors are independent, residuals are homoscedastic and normally distributed, and there are no problems with multicollinearity among predictors.

Owing to a substantial number of proposed tests, we will use a Bonferroni correction for multiple comparisons for the testing of Aims 1c, 2c, 3a, and 3b, our main study aims examining the relationship between anxiety and cortisol indices (Aim 1c), anxiety and HRV indices (Aim 2c), HRV and cortisol (Aim 3a), and the moderating role of anxiety on HRV and cortisol (Aim 3b). Combined, a total of 10 tests will be done, thus detection of significant effects will be assessed at $\alpha = .005$ ($.05/10 = .005$).
Aim 1a

Examine variability in the change in salivary cortisol over time (baseline, reactivity to stressor, recovery). Four metrics will be calculated for each participant: (1) an area under the curve (AUC) based on magnitude response of cortisol across the five timepoints using the formula from Pruessner et al. (2003), (2) a slope calculated using the collection times across the five timepoints to capture the direction of change over time, (3) change in cortisol from baseline to maximum cortisol level using a difference score (maximum – baseline), referred hereafter as “cortisol reactivity”, and (4) change in cortisol from maximum to recovery using a difference score (maximum – recovery), referred hereafter as “cortisol recovery”.

Across the entire sample, means and standard deviations as well as ranges of these metrics will be calculated and reported for descriptive purposes. Associations with cortisol metrics and the following variables will also be tested using appropriate Pearson’s correlations and independent samples t-tests for the identification of relevant covariates in further analyses: (a) age, (b) gender, (c) race, (d) antidepressant use (dichotomous variable: 0=no; 1=yes); (e) smoking status (dichotomous variable; 0=no; 1=yes), and (f) lagged time of day first cortisol sample was collected with respect to waking.

Aim 1a Hypothesis: Given the exploratory and descriptive intent of this aim, we do not have specific hypotheses with regard to the variability of cortisol metrics in this sample.

Aim 1b

Re-evaluate change in cortisol over time with a focus on differential trajectories of cortisol (baseline, reactivity to stressor, recovery). To examine if individual trajectories
of cortisol exist across participants, we will employ latent growth mixture modeling (LGMM) to study the existence of yet identified differential trajectories of cortisol across the three time points. LGMM is a data-driven technique to identify interindividual (e.g., between-individual) differences in cortisol change based on intraindividual (e.g., within-individual) change (Ram & Grimm, 2009). LGMM is ideally suited for large samples sizes (like in the present study) so long as at least three data points for the same measure can be supplied over time, which we have confirmed for this sample. Sub-groups identified via LGMM using data-driven methods will be subsequently qualified according to their cortisol profile (e.g., “blunted early responders” vs. “normal”). To complete LGMM we will use the R lcmm package; relevant covariates identified in Aim 1 and time of first cortisol collection will be added and controlled for in the model and BIC will be used for model selection.

Aim 1b Hypothesis: We anticipate that three sub-groups will emerge: (1) a “normal” group based on prototypical rise and fall of cortisol over the testing session, (2) a “late-onset”, and (3) an “early-onset” group, with the respective latter groups qualified with respect to the normal trajectory.

Aim 1c

*Test if trait anxiety is associated with change in cortisol as measured by the above metrics.* Continuous STAI scores will be used in Pearson’s correlations to test its association with cortisol metrics of change identified in Aim 1a. Differences in STAI scores will be assessed using either independent samples t-tests or ANOVA (depending on the number of identified sub-groups) for those sub-groups identified via LGMM in Aim 1b.
Aim 1c Hypothesis: We hypothesize that trait anxiety will correlate with cortisol metrics and in accordance with most previous literature, we hypothesize that this relationship will be negative, such that greater trait anxiety will be related to blunted cortisol responding. Importantly, we hypothesize that this difference may not present across all metrics, but rather may be specific to changes in magnitude of response (Aim 1a) or latent trajectory (Aim 1b).

Aim 2a

To examine variability in the change in HRV over time (baseline, anticipatory, reactivity to stressor). Three metrics will be calculated for each participant: (1) overall HRV collapsed across timepoints, (2) change in HRV from baseline to anticipation of the stressor using a difference score (anticipation – baseline), referred hereafter as “HRV anticipation”, and (3) change in HRV from anticipation to reactivity to stressor using a difference score (reactivity – anticipation), referred hereafter as “HRV reactivity”. Across the entire sample, means and standard deviations as well as ranges of these metrics will be calculated and reported for descriptive purposes. Associations with HRV metrics and the following variables will also be done using appropriate Pearson’s correlations and independent samples t-tests for the identification of possible covariates in further analyses: (a) age, (b) gender, (c) race, (d) antidepressant use (dichotomous variable: 0 = no; 1 = yes); and (e) smoking status (dichotomous variable; 0 = no; 1 = yes).

Aim 2a Hypothesis: Given the exploratory and descriptive intent of this aim, we do not have specific hypotheses with regard to the variability of HRV metrics in this sample.

Aim 2b
Re-evaluate change in HRV over time with a focus on differential trajectories of HRV (baseline, reactivity to stressor, recovery). Identical to 1b, we will employ LGMM to examine sub-groups of individuals based on trajectories of HRV over time. Sub-groups identified via LGMM will be qualified according to their HRV profile and we will again complete LGMM using the R lcmdm package; relevant covariates identified in Aim 2a will be added and controlled for in the model and BIC will again be used for model selection.

**Aim 2b Hypothesis:** We anticipate that three sub-groups will emerge: (1) a “normal” group based on prototypical rise and fall of HRV over the testing session, (2) a “late-onset”, and (3) an “early-onset” group, with the respective latter groups qualified with respect to the normal trajectory.

**Aim 2c**

*Test if trait anxiety is associated with change in HRV as measured by the above metrics.* Continuous STAI scores will be used in Pearson’s correlations to test its association with HRV metrics of change identified in Aim 2a and differences in STAI scores will be assessed using either independent samples t-tests or ANOVA (depending on the number of identified sub-groups) for those sub-groups identified via LGMM for HRV trajectories in Aim 2b.

**Aim 2c Hypothesis:** We hypothesize that trait anxiety will be negatively correlated with HRV as measured by the above metrics. That is, we predict that individuals scoring higher on the trait anxiety will (a) demonstrate lower HRV, (b) less change in HRV over time, and (c) delayed rise in HRV during stress compared to those scoring low on trait anxiety.

**Aim 3a**
Examine the relationship between change in HRV (baseline, reactivity to stressor, recovery) and change in cortisol (baseline, reactivity to stressor, recovery). First, we will calculate the correlation matrix for all cortisol metrics (cortisol AUC, cortisol reactivity, cortisol recovery, slope, or cortisol trajectory) and HRV metrics (overall HRV, HRV anticipation, HRV reactivity, HRV trajectory group membership) to determine significant correlations between HRV and cortisol for use in follow-up hierarchical regression analyses.

Aim 3a Hypothesis: We hypothesize that, across the sample, greater HRV will related to less cortisol. Second, we will use a hierarchical regression analysis to test the relationship between HRV and cortisol controlling for covariates. Step 1 of the model will include the HRV index correlated with cortisol as identified above as the main predictor variable; the outcome variable will be one of the pre-identified cortisol indices. In step 2, we will include covariates of interest as identified in Aims 1a and 2a.

Aim 3b

Test the moderating effects of trait anxiety on the relationships between change in HRV and change in cortisol. We will repeat the hierarchical linear regression from Aim 3a but will include STAI continuous scores in Step 2. In the critical step 3, we will create an interaction term between HRV index and STAI to test its moderating effect on cortisol.

Aim 3b Hypothesis: We hypothesize that the relationship between greater HRV and less cortisol will be driven by individuals with low trait anxiety and that this relationship will be comparatively weaker – or absent – in individuals with high trait anxiety.

Results
Participants

In surveying all aforementioned variables, complete relevant data were available from $N = 480$ respondents. In order to test study aims, outliers were reviewed following guidelines from previous HRV and cortisol research (Pulopulos et al., 2018) and values $\pm 3$ SD were removed from analysis. This retained a final sample size of $N = 438$ for data analyses.

The sample of $N = 438$ comprised of 52.7% females ($N = 231$) and 47.3% males ($N = 207$) with an age range of 25 to 75 ($M = 48.18, SD = 12.21$). Individuals were asked about their race via the question “What are your main racial origins—that is, what race or races are your parents, grandparents, and other ancestors”; data regarding racial origins were available for 376 participants (0.5% Native Hawaiian or Pacific Islander; 1.6% Native American or Alaska Native Aleutian Islander/Eskimo; 1.8% Asian; 5.9% Other; 5.5% Black and/or African American; 70.5% White). Smoking status (i.e., “do you currently smoke cigarettes regularly?”) was available for 149 participants in our sample (Yes, $N = 39$; No, $N = 110$). Information about antidepressant use was available for 388 participants (Yes, $N = 65$; No, $N = 323$). Based on the availability of all relevant covariates, a final sample of $N = 438$ was used for analyses involving cortisol and HRV, with the exception of LGMM for HRV ($N = 388$). Regarding the LGMM calculation for HRV, some covariates were necessary to include in the model (see below); thus, trimming the sample size to those with available values for covariates (Table 1). To note, these numbers are higher than the required sample size of $N = 114$ (estimating $\alpha = .05$).
and the sample size of $N = 171$ (estimating corrected $\alpha = .005$) identified in our power analyses to detect medium-sized effects ($f^2 = .15$) with a conserved power of 80% (completed using G*Power; Faul et al., 2007).

<table>
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<tr>
<th>Table 1</th>
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<td>Sample demographics.</td>
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<tr>
<td>Smoking status (yes)</td>
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<td>Antidepressant use (yes)</td>
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Note: Data on race were available for a total of $n = 376$ individuals. Smoking status data were available for $n = 149$, and antidepressant use data for $n = 388$.

**Testing Aim 1a**

Four cortisol metrics were calculated for each participant ($N = 438$): AUC ($M = 4.99$, $SD = 0.89$), slope ($M = -0.03$, $SD = 0.25$), cortisol reactivity ($M = 0.15$, $SD = 0.36$), and cortisol recovery ($M = 0.22$, $SD = 0.33$). With respect to the relationship between these cortisol metrics and demographics, we found evidence of gender differences. First, cortisol reactivity was higher in men ($M = .21$, $SD = .37$) compared to women ($M = .11$, $SD = .34$), $t(436) = -2.95$, $p = .003)$. Second, men had greater AUC ($M = 5.15$, $SD = 0.89$) than women ($M = 4.85$, $SD = .87$; $t(436) = -3.57$, $p < .001$). Finally, men had a positive slope ($M = 0.0002$, $SD = 0.26$) while women had a negative slope ($M = -0.05$, $SD = 0.23$), $t(436) = -2.20$, $p = .028$. In addition, age was significantly
correlated with AUC \((r (436) = .16, p < .001)\), such that older individuals had greater AUC.

No significant relationships were detected between any of the cortisol metrics and smoking status \((p > .175)\), antidepressant use \((p > .533)\), race \((p > .144)\), or wake lag time \((p > .142)\). Based on the above significant effects, gender and age were included as covariates in relevant follow-up analyses involving cortisol.

**Testing Aim 1b**

To examine if individual trajectories of cortisol existed across participants, we employed LGMM using the R `lcmm` package; relevant covariates identified in Aim 1 - gender and age, and time of first cortisol collection - were added and controlled for in the model. We compared five models ranging from one to six classes to ensure we selected the optimal model based on fit. According to standard conventions, model selection was based on specific selection criteria including the lowest BIC, assuring that greater than 5% of participants were retained in the smallest class subgroup, and theoretical guidance (Nguena Nguefack et al., 2020). A 3-group solution with the lowest BIC (12515.36) retained acceptable latent group sizes and also was clinically interpretable, thus this was selected as the best model fit. The 3-group solution included a “normal” group based on prototypical rise and fall of cortisol over the testing session (79% of the sample), a “decline” group that started high and declined throughout the testing session (10% of the sample), and a “rise” group that started low and increased throughout the testing session (11% of the sample) as shown in Figure 2.
In post-hoc analyses, we examined significant differences in the cortisol values across time within each of the sub-groups. We found that, in the “normal” sub-group, cortisol was significantly higher at the post-cognitive stressor timepoint with respect to the baseline ($t(343) = 3.27, p = .001$), and the 30-minutes post-protocol timepoints ($t(343) = 4.02, p < .001$). Additionally, the 30-minutes post-protocol timepoint and baseline did not differ ($t(343) = -1.00, p = .318$). In the “decline” sub-group, we found that cortisol was significantly lower at each subsequent timepoint following arrival ($p’s < .010$).

Finally, within the “rise” sub-group, we found that cortisol at the post-cognitive stressor was significantly higher compared to baseline ($t(50) = 4.29, p < .001$), and significantly lower compared to the 30-minutes post-protocol timepoint ($t(50) = -2.54, p = .014$). Results indicated no significant differences in cortisol between the post-cognitive stressor and post-physical stressor ($t(50) = -1.66, p = .104$); further in comparing cortisol 30 minutes post-protocol to that of arrival, individuals remained significantly higher than arrival ($t(50) = 5.83, p < .001$). Although the three sub-groups followed significantly different trajectories throughout the protocol ($p’s < .001$), results from a one-way ANOVA revealed that they did not differ significantly from one another at the final timepoint, 30-minutes post-protocol ($F(2, 435) = 1.01, p = .366$).
Testing Aim 1c

Continuous STAI scores were used in Pearson’s correlations to test its association with cortisol metrics of change identified in Aim 1a. Trait anxiety was negatively correlated with AUC while controlling for gender and age, although this effect did not surpass our Bonferroni correction ($r(436) = -0.10, p = .039$). This suggests that greater anxiety was associated with less cortisol output over the duration of the protocol. STAI scores were not found to significantly correlate with any other cortisol metrics ($p$’s > .197). Differences in STAI scores were assessed using a one-way ANOVA for the three sub-groups identified via LGMM in Aim 1b. There was not a significant effect of sub-groups on STAI scores across the three conditions ($F(2, 435) = 0.073, p = .930$).

Testing Aim 2a
Three HRV metrics were calculated for each participant ($N = 438$) in the sample: HRV reactivity ($M = -0.09$, $SD = 0.15$), HRV anticipation ($M = 0.02$, $SD = 0.07$), and overall HRV ($M = 1.25$, $SD = 0.22$). With respect to HRV metrics, we found significant gender differences on overall HRV ($t(436) = 2.63$, $p = .009$), such that women ($M = 1.28$, $SD = 0.23$) had greater HRV than men ($M = 1.22$, $SD = 0.22$). Overall HRV also varied by antidepressant use ($t(386) = 2.68$, $p = .008$), such that participants on antidepressants had lower HRV ($M = 1.17$, $SD = 0.25$) compared to participants not on antidepressants ($M = 1.25$, $SD = 0.21$). Age was also significantly correlated with HRV reactivity ($r(436) = .10$, $p = .038$) and overall HRV ($r(436) = -.31$, $p < .001$): older individuals had higher HRV reactivity and lower overall HRV. No significant relationship was detected between any other HRV metric and smoking status ($p > .142$), race ($p > .113$), or wake lag time ($p > .063$). Based on the above significant effects, Emp

**Testing Aim 2b**

Identical to aim 1b, we employed LGMM to examine sub-groups of individuals based on trajectories of HRV over time. Sub-groups identified via LGMM were qualified according to their HRV profile and LGMM again was completed using the R `lcmm` package; relevant covariates identified in Aim 2a - gender, antidepressant use, and age - were added and controlled for in the model and BIC was again used for model selection.

Prior to LGMM analysis, participants with missing data across relevant covariates were removed from analysis ($n = 50$ missing antidepressant information) because LGMM cannot fit individuals with missing data into classes. This left $N = 388$ for LGMM analysis, for which five models were tested ranging from one to six classes to again ensure we selected the optimal model based on fit. According to standard conventions,
model selection was based on specific selection criteria including the lowest BIC, assuring that greater than 5% of participants in the smallest class subgroup were retained, and theoretical guidance (Nguena Nguefack et al., 2020). A 2-group solution with the lowest BIC (17149.61) retained acceptable latent group sizes and was also clinically interpretable; thus the 2-group solution was selected to model trajectories of HRV. The 2-group solution included a “high and variable” HRV group (58% of the sample), a “low and variable” HRV group (42% of the sample).

In post-hoc analyses, we examined differences in the HRV values across time within each of the sub-groups. We found that, in the “high and variable” sub-group, HRV was significantly lower during the first cognitive stressor in comparison to the second baseline timepoint ($t(218) = -10.64, p < .001$) and the first recovery timepoint ($t(207) = -7.83, p < .001$). Additionally, HRV was significantly lower during the second cognitive stressor in comparison to the first ($t(212) = -7.87, p < .001$) and second ($t(224) = -10.04, p < .001$) recovery periods. Finally, HRV at the second recovery period was significantly lower than HRV at the second baseline ($t(224) = -3.03, p = .003$). This “high and variable” group did not differ in HRV when comparing cognitive stressor 1 and cognitive stressor 2 timepoints ($t(218) = 0.02, p = .985$), meaning that individuals within this group changed in their HRV between rest and stress timepoints, but maintained the same HRV values during each stressor. By contrast, the “low and variable” group was also significantly lower during the first cognitive stressor compared to the second baseline timepoint ($t(156) = -5.38, p < .001$) and first recovery period ($t(153) = -7.30, p < .001$). Additionally, HRV was significantly lower during the second cognitive stressor in comparison to the first ($t(159) = -3.68, p < .001$) and second ($t(162) = -6.02, p < .001$)
recovery periods. However, in contrast to the “high and variable” group, the “low and variable” group demonstrated significantly increased HRV at the second recovery timepoint in comparison to the second baseline timepoint ($t(162) = 3.35, p = .001$). Furthermore, unlike the “high and variable”, the “low and variable” group demonstrated increased HRV during the second cognitive stressor in comparison to the first cognitive stressor ($t(156) = -2.44, p = .016$). Finally, independent samples t-tests revealed that the two groups differed significantly on measures of HRV reactivity ($t(386) = -4.47, p < .001$), and overall HRV ($t(386) = 22.16, p < .001$) such that the “high and variable” group exhibited lower HRV reactivity and higher overall HRV than the “low and variable” group.

**Figure 3.** HRV trajectories identified in LGMM analysis.
Testing Aim 2c

Continuous STAI scores were used in Pearson’s correlations to test its association with HRV metrics of change identified in Aim 2a, controlling for relevant covariates, and no significant correlations emerged ($p > .402$). Likewise, differences in STAI scores were assessed using independent samples t-tests for the two sub-groups identified via LGMM for HRV trajectories in Aim 2b, and no significant differences emerged ($t(386) = 0.45, p = .654$).

Testing Aim 3a and b

First, we calculated a correlation matrix for all cortisol metrics (cortisol AUC, cortisol slope, cortisol reactivity, and cortisol recovery) and HRV metrics (overall HRV, HRV anticipation, and HRV reactivity) (Table 2). Results indicated that HRV reactivity and cortisol reactivity were significantly correlated ($r(436) = .11, p = .024$), such that greater cortisol reactivity was associated with greater HRV reactivity as shown in Figure 4. That is, greater rise in cortisol during the stressors was associated with greater rise in HRV during the same tasks. No other metrics were significantly correlated ($p > .128$). Additionally, chi-square results revealed that the cortisol subgroups and HRV subgroups identified in trajectory analyses were unrelated to one another ($\chi^2 (2) = .11, p = .945$).
Based on the correlation between cortisol reactivity and HRV reactivity, we carried these two variables forward into a hierarchical regression analysis to test the relationship between HRV reactivity and cortisol reactivity accounting for covariates. For all regression analyses, all assumptions of the linear model were met: errors were independent, residuals were homoscedastic and normally distributed, and there were no
problems with multicollinearity among predictors. Step 1 of the model that examined HRV reactivity as a predictor of cortisol reactivity was significant ($F(1, 436) = 5.10, p = .024; R^2 = .012$). A total of 1.2% of the variance in cortisol reactivity was explained by HRV reactivity.

With the inclusion of covariates of interest (age and gender) in step 2, the model continued to be significant $F(3, 434) = 3.96, p = .004; R^2 = .035; R^2$ change = .023) such that HRV reactivity continued to be a significant independent predictor of cortisol reactivity ($B = .23, SE = .11, p = .045$) (Table 3).

Finally, to test the moderating effects of trait anxiety on the relationships between change in HRV and change in cortisol we repeated the hierarchical linear regression but included STAI continuous scores in step 2. Results indicated STAI was not independently related to cortisol reactivity ($B = -.002, SE = .002, p = .232$). In step 3, we created an interaction term, HRV reactivity x STAI, to test its moderating effect on cortisol. When including this interaction term, the model was not statistically significant ($p = .569$). Further, STAI interaction did not moderate the effect of HRV reactivity on cortisol reactivity ($B = .007, SE = .01, p = .569$). HRV reactivity also ceased to be an independent predictor of cortisol reactivity after inclusion of the interaction term ($p > .964$) (Table 3).
Table 3
Hierarchial linear regression testing the moderating effects of trait anxiety on the relationship between HRV and cortisol reactivity.

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*Note: Dependent variable: Cortisol reactivity.*

**Exploratory Analyses**

**Restriction to an Anxious Sub-group**

The sample reported herein – as a whole – had comparatively low anxiety as indexed by the STAI using published guidelines suggesting that scores < 38. A score of 38 appears as a reliable cut-off given published evidence that this value is associated with clinically-detectable anxiety (Kayikcioglu et al., 2017). Thus, we repeated all analyses above within a sub-sample of individuals with STAI scores > 38. This allowed us to re-test the relationship between anxiety, cortisol, and HRV in individuals who may have clinically-detectable symptoms. Our “highly anxious” sub-sample amounted to N = 152 individuals. To note, this sample is still larger than the N = 114 required (estimating α = .05) to detect medium-sized effects based on our power analysis.
In this sample, continuous STAI scores were again used in Pearson’s correlations to test its association with cortisol metrics of change identified in Aim 1a. Trait anxiety was negatively correlated with cortisol reactivity while controlling for gender and age ($r(150) = -.17, p = .040$). STAI scores were not found to significantly correlate with any other cortisol metrics ($p > .099$). Differences in STAI scores were assessed using a one-way ANOVA for the three sub-groups identified via LGMM in Aim 1b and no significant differences emerged ($F(2, 149) = 0.18, p = .837$).

Continuous STAI scores were used in Pearson’s correlations to test its association with HRV metrics of change identified in Aim 2a, controlling for relevant covariates, and no significant correlations emerged ($p > .104$). Likewise, differences in STAI scores were assessed using independent samples t-tests for the two sub-groups identified via LGMM for HRV trajectories in Aim 2b, and no significant differences emerged ($t(137) = 0.69, p = .491$).

Finally, we calculated a correlation matrix for all cortisol metrics (cortisol AUC, cortisol reactivity, and cortisol recovery) and HRV metrics (overall HRV, HRV anticipation, and HRV reactivity). None of the metrics were significantly correlated ($p > .120$). Additionally, chi-square results revealed that the cortisol subgroups and HRV subgroups identified in trajectory analyses were unrelated to one another ($\chi^2 (2) = .89, p = .641$). Thus, the hierarchical linear regression testing the association between cortisol and HRV metrics was not completed in this sub-sample.

**Group Differences among LGMM-identified Trajectories**
In addition, as we found no differences in STAI among the LGMM-identified trajectories, we were interested in other, related, measures that may differ among these individuals. Namely, we found the differential trajectories interesting and wished to explore if these individuals differed in other, meaningful ways besides anxiety severity. Here, our focus was on measures of stress, trauma, depression, and well-being given that these constructs are highly related to anxiety.

We found that subgroup differences in HRV ($p = .387$) and cortisol ($p = .354$) did not emerge on a measure of trauma, the Childhood Trauma Questionnaire (CTQ) nor did group differences among HRV ($p = .459$) or cortisol ($p = .900$) emerge on a measure of depression, the Center for Epidemiological Studies Depression (CESD) scale. On a measure of subjective well-being, the Satisfaction with Life Scale, there were no differences between HRV groups ($p = .906$). However, cortisol groups differed on this measure ($F(2, 435) = 3.51, p = .027$), such that those in the “decline group” ($n = 43$) had significantly higher ratings of subjective well-being ($M = 5.22, SD = 1.23$) compared to those in the “normal” ($n = 344$) group ($M = 4.65, SD = 1.35$). Those in the “rise” ($n = 51$) group ($M = 4.73, SD = 1.33$) did not significantly differ from either of the other two groups. Finally, in examining anxious arousal via the Mood and Symptom Questionnaire (MASQ), we found that differences between the two HRV groups were trending toward significance ($p = .080$), such that the “low and variable” group reported greater anxious arousal compared to the “high and variable” group.

**Discussion**
This investigation examined the relationship between cortisol and HRV during cognitive and physical stress with a particular focus on metrics of change and the moderating effect of anxiety. Several important results emerged: first, we found that HRV was a significant predictor of cortisol, such that higher HRV reactivity to cognitive stress was associated with higher cortisol reactivity to the same stressor. Notably, this effect persisted after controlling for age and gender differences in HRV and cortisol. We also found evidence of differential cortisol and HRV trajectories over the course of the 90-minute stress protocol: three sub-groups emerged with respect to their cortisol release qualified as a “normal” group based on prototypical rise and fall of cortisol over the testing session, a “decline” group that started high and steadily declined, and a “rise” group that started low, increased, plateaued, and then continued to rise. By contrast, two groups emerged with respect to their HRV: a “high and variable” group, and a “low and variable” group. Despite this and contrary to hypothesis, we did not find support for a relationship between anxiety and HRV and cortisol metrics in the full sample. Namely, STAI scores were not related to cortisol or HRV metrics and did not differ among cortisol and HRV sub-groups identified in the LGMMs. Finally, we found no moderating effect of trait anxiety on the relationship between changes in HRV and cortisol. In contrast and in exploratory analyses, we did find that individuals with greater anxiety exhibited less cortisol reactivity to the cognitive stressor when we restricted our analysis to a “clinically-detectable” anxious sub-group. Finally, although cortisol sub-groups identified by the LGMM did not differ in anxiety, we found that individuals in the “decline” sub-group experienced more subjective well-being in exploratory analyses.
The lack of an association between cortisol and/or HRV and anxiety was unexpected. Nonetheless, as we note in the introduction, there are mixed findings regarding the relationship between cortisol and anxiety, with prior studies finding a negative relationship (i.e., greater anxiety relates to less cortisol) (de Rooij et al., 2010; Fiksdal et al., 2019; Jezova et al., 2004), a positive relationship (i.e., greater anxiety relates to greater cortisol) (Furlan et al., 2001; Yoon & Joormann, 2012), or no relationship (Henckens et al., 2016; Souza et al., 2015; Young et al., 2004). Thus, our findings of no relationship between cortisol and anxiety fits with some – but not all – of this literature. By contrast, there is prior strong evidence for a negative relationship between resting HRV and trait anxiety, and a negative relationship between HRV during stress and trait anxiety. Thus, our null effects run counter to this existing framework.

One explanation for a lack of such findings could be that the relationship between HRV and cortisol with anxiety exists only in individuals high in trait anxiety and is not detectable at moderate levels. While the STAI ranges from 20-80, the highest score in our sample was 67, and only 14.4% had scores considered in the highly anxious range (45 or higher) (Kayikcioglu et al., 2017). Thus, we re-completed all analyses in an exploratory fashion to test whether these relationships existed in a more anxious “sub-group” reflecting 34.70% of the full sample. In doing so, we still failed to detect a significant relationship between HRV and anxiety. To note, sample sizes in both analyses (e.g., full and reduced samples) were powered to detect moderate effects. However, we did find a significant relationship between anxiety and cortisol reactivity, such that individuals higher in trait anxiety exhibited less cortisol reactivity to the cognitive stressor. This finding aligns with a previous study that also utilized a large sample size ($N = 725$)
showing that adults with higher trait anxiety outputted less cortisol in peak response to a stressor, but that cortisol recovery did not differ in respect to anxiety (de Rooij et al., 2010). Notably, the previous study demonstrating these results used not only a cognitive stress task (Stroop), but also a social stress task (a speech test) and calculated peak cortisol response in the same manner as the present study. Taken together, these findings suggest that highly anxious individuals, may demonstrate a blunted cortisol response when confronted with a stressor compared to their low-anxious counterparts.

Our study consistently failed to detect a relationship between HRV and anxiety, even in our anxious sub-group. One consideration is that much of the previous work has identified this negative relationship in regards to resting HRV which was not tested directly in our study. Rather we examined anticipation of HRV as individuals prepared for a stressor and reactivity to a stressor. Therefore, it could be that the negative relationship between HRV and anxiety is more robust in resting states outside the prospect of stress. Indeed, as HRV is expected to decrease in response to stress in healthy populations, it would need to decrease significantly more to be detected as a difference in anxious populations. Still, there are some studies that do demonstrate the relationship during reactivity to stress (Friedman & Thayer, 1998; Pittig et al., 2013; Thayer et al., 1996). Thus, further investigation of the relationship between HRV and stress reactivity among anxious adult populations is needed to clarify the nuances of this relationship.

We did find that higher HRV reactivity was associated with higher cortisol reactivity, and this relationship was not in the expected direction. That is, we found that individuals with high cortisol reactivity (an increase in cortisol secretion during stress) have higher HRV reactivity (increased HRV during stress) and conversely, individuals
with low HRV reactivity scores (decreased HRV during stress) have lower cortisol reactivity (a decrease in cortisol secretion during stress). This suggests the potential of a compensatory effect, such that when cortisol is heightened in response to stress, HRV also increases to promote regulation and a return to homeostasis. To our knowledge only three other investigations have examined changes over time in cortisol and HRV metrics in the same study design. One of these studies found that HRV measures during a stress-task were uncorrelated with cortisol; but that lower HRV during *anticipation* of stress was correlated with higher stress task-induced cortisol, but not cortisol recovery (Pulopulos et al., 2018). Another study found that high stress-induced HRV during a social stress task buffered the moderating effect of hostility on increased cortisol secretion (Murdock et al., 2017). The third study measured HRV and cortisol at rest from children at home over two consecutive weekdays and found that lower HRV was associated with greater cortisol output (Michels, Sioen, Clays, et al., 2013). Two of these studies examined these variables during a social stress task (Murdock et al., 2017; Pulopulos et al., 2018), and the third study examined them in children not during a stress task (Michels, Sioen, Clays, et al., 2013). Therefore, the present study is the first study to our knowledge that extends the literature to include the examination of this relationship over time during a cognitive and physical stress task in healthy adults. Notably, the existence of this relationship suggests HRV as a potential treatment target for improving effective regulation of stress.

Indeed, HRV can be manipulated in real-time as it can be regulated by breathing techniques (Henriques et al., 2011). Prior research has already established promise for HRV biofeedback as an adjunct treatment modality for reducing stress among anxious
individuals (Lehrer & Eddie, 2013). However, this work points to a need for further investigations to clarify the mechanisms of change of HRV biofeedback. Our results suggest one mechanism could be the vagal-HPA axis link in that targeting HRV may work not only by providing immediate benefit in the moment-to-moment responses, but also via promoting longer term benefits by altering cortisol over time. While these biomarkers of stress are typically studied independently, these results suggest merit in continued research of how they function together. Considering these two biomarkers of the stress response that are linked with health outcomes, Aimie-Salleh and colleagues (2019) conducted a study examining HRV and salivary cortisol separately and together. Results indicated that a fused biomarker (HRV-SCort) representing both the HPA axis (salivary cortisol) and the ANS (HRV) performed better in discriminating the stress response than either biomarker on its own. Taken together with the prior literature, results from this study suggest that while a relationship between vagal activity and the HPA axis is generally assumed (Thayer & Sternberg, 2006) a closer investigation of the nuances in this relationship is valuable to informing clinical prognosis and interventions.

In addition to a relationship between HRV and cortisol reactivity in our sample, we also found evidence of differential trajectories on the five cortisol and seven HRV measures over time. For cortisol, three groups emerged. Most of the sample (79%) followed a “normal” trajectory that peaked at timepoint three, post-cognitive stressor, and then returned to baseline levels by recovery. Notably, cortisol samples collected at timepoint three occurred approximately 24 minutes after engagement with the first cognitive stressor. This is aligned with the expected delayed response observed in cortisol which typically peaks 20 minutes after a stressor. Individuals in the “normal” group then
returned to baseline levels by cortisol sample five, which occurred 30 minutes after the
physical stressor (a task that required participants to stand still for 6 minutes).
Interestingly, two additional sub-groups emerged that followed opposite patterns (starting
high and dropping versus starting low and rising) throughout the protocol, but ending at
the same levels as the “normal” group 30 minutes post-protocol. A “rise” group (11%)
emerged that started with lower levels of cortisol compared to the other two groups,
which also increased at timepoint three, but then continued to increase until timepoint
five, ending higher than within-group baseline levels. A “decline” group (10%) also
emerged that started higher at baseline compared to the other two groups and declined
throughout the testing protocol, ending at approximately the same levels as the “normal”
group. To note, groups were only differentiated by cortisol before and during the stress
protocol as they exhibited equivalent cortisol values at the end of the protocol.

With respect to the differential trajectories of cortisol release, exploratory
analyses revealed that those in the “decline” group scored higher on subjective ratings of
well-being, compared to those in the “normal” group. This finding is curious given that
these individuals demonstrated higher baseline levels of cortisol, and decreased cortisol
in response to stressors, a pattern that might suggest maladaptive or atypical responding,
but had recovery levels almost identical to those in the “normal” group. One explanation
is that subjective well-being might serve as a stress buffer for individuals with high
baseline cortisol levels. However, more work is needed to explore this possibility.

In regard to HRV, a roughly even split occurred among the sample with over half
following a “high and variable” trajectory and just below half following a “low and
variable” trajectory. The two groups significantly differed on two measures of HRV: the
"high and variable" group demonstrating higher overall HRV and lower HRV reactivity (decreased in HRV from baseline to second cognitive stressor – time point five) than the "low and variable" group. Both sub-groups decreased from baseline to the first cognitive stressor, increased during the first recovery period, decreased during the second cognitive stressor, after which they increased during the second recovery period and decreased again during the physical stressor. However, examination of within-group differences revealed unique patterns in each group such that the “high and variable” group demonstrated decreased HRV during the final recovery period in comparison to baseline, while the “low and variable” group demonstrated increased HRV at the second recovery timepoint in comparison to baseline. Furthermore, while the “high and variable” group demonstrated equivalent HRV values during both cognitive stressors, the “low and variable” group demonstrated increased HRV during the second cognitive stressor in comparison to the first cognitive stressor.

An advantage of the present study design is that it explores the trajectories over the course of two recovery periods and two cognitive stressors. It is expected, based on the literature, that decreases in HRV occur under stress (Endukuru & Tripathi, 2016; Pulopulos et al., 2018). Therefore, both trajectories exhibiting decreased HRV during the first cognitive stressor compared to resting or recovery timepoints is aligned with expected responding. However, it is interesting that the “low and variable” group, but not the “high and variable” group, demonstrated increased HRV during the second cognitive stressor compared to the first stressor and exhibited increased HRV during the second recovery period compared to baseline. One explanation is that an increase in HRV during stress is a compensatory effect driven by a dysregulated stress response system and that
while individuals low in overall HRV might respond in the same pattern (decrease HRV) as individuals with high overall HRV to a single stressor, they might be uniquely comprised when faced with consecutive stressors. Indeed, when examining anxious arousal as measured by the MASQ, we found that differences between the two HRV groups were trending toward significance ($p = .080$) such that the “low and variable” group reported greater anxious arousal compared to the “high and variable” group, but this did not reach significance. Thus, we did not find that anxiety or other exploratory measures differed between HRV trajectories. Future work is necessary to understand how HRV trajectories in response to stress differ as a function of physical or mental health status or other identifiable markers.

Further interrogation of group differences in the trajectories that emerged in the current study could also help to clarify the positive relationship that was found between HRV reactivity and cortisol reactivity. The “high and variable” group demonstrated significantly higher overall HRV, and this group decreased in HRV after the cognitive stress tasks compared to their HRV levels just prior to the first cognitive stressor. This could reflect that the higher HRV prior to the tasks served an adaptive function in preparing the individual to face the stress tasks ahead, and then a decrease in HRV followed as their sympathetic nervous system activated while engaged in the stressful tasks. In contrast, the “low and variable” group that demonstrated significantly lower overall HRV, exhibited an increase in HRV after the cognitive stressors compared to baseline. Thus, these individuals in preparation for the stressor at baseline were presumably more dysregulated (lower HRV) but then increased HRV during the stressors and this remained high at the second recovery period. These trajectories suggest that an
increase in HRV during stress might serve a compensatory effect, due to a comprised ability to prepare to face an anticipated stressor. Alternatively, the positive relationship between HRV reactivity and cognitive reactivity in the full sample indicate that this compensatory effect could be driven by a comprised ability to respond during stress (increased cortisol), such that increased cortisol secretion during stress prompts increased HRV to help regulate. In this case, an increase in HRV and an increase in cortisol during stress might represent an over-exertion of effort to regulate related to a comprised ability to respond to stress. However, this could also reflect normal, healthy responding that when cortisol is low, HRV can adaptively remain low, but that when cortisol is heightened, an increase in HRV is adaptive. As few studies to date have examined HRV across repeated cognitive stressors in the same study design future work is needed to understand what can be expected and considered an adaptive pattern in healthy populations. The present study offers a starting point of how HRV patterns emerged in one sample of mid-life adults, but replications are needed to understand if these results generalize to other populations and to interpret what these patterns signify. Our findings suggest future work may benefit from examining variability across stress conditions and resting conditions and considering these in the context of performance metrics on stress tasks. Additionally, while our reactivity measures captured how individuals changed in cortisol and HRV in response to stress they do not reflect how much individuals changed. It is possible that the magnitude of change would provide valuable insight that is left out of the picture when examining only direction (increase or decrease) of change, and future studies should examine both.
A limitation noted in previous work examining HRV and cortisol over time in the same study design has been that the samples have been either primarily male or female not allowing for the comparison across genders (Held et al., 2021). A major advantage of the present study is the large sample size which afforded sufficient variability across genders, ages, and impact of smoking and antidepressants. Aligned with previous work (Foley & Kirschbaum, 2010; Zorn et al., 2017) we found evidence for gender differences in cortisol-mediated response to stress, such that men had greater cortisol reactivity, AUC, and a positive slope compared to women who had lower cortisol reactivity, less AUC, and a negative slope. Notably, gender differences to stress have primarily been explored in response to psychosocial stress, and the present study extends these findings to cognitive and physical stress. Gender differences also emerged on HRV metrics such that women had greater overall HRV than men, demonstrating superior ANS regulation. This finding helps add clarity to the current literature on gender differences in HRV. An inverse relationship appears at rest (Zachariah & Joseph, 2018) with men demonstrating higher HRV and while this relationship exists it seems to diminish with age (Umetani et al., 1998). Notably, the present study utilized RMSSD as a measure of HRV which is a measure of vagal activity sensitive to short-term changes. Zachariah and Joseph (2018) also examined gender differences during a cognitive stress task and found women had greater HRV than men during the stress task but not at rest, and only on measures of RMSSD but not on other measures of HRV. Thus, gender differences in HRV may be context-dependent. More work is needed to explore these differences as HRV is often utilized as an important biomarker for predicting health outcomes (Zachariah & Joseph, 2018). Additionally, and as expected based on previous findings, age differences emerged
such that older individuals had more AUC and lower overall HRV (Umetani et al., 1998), but also increased HRV reactivity (increase in HRV during stress). Finally, adding support to results from previous studies we found that participants on antidepressants had lower overall HRV (Hu et al., 2019; Kemp et al., 2012; Licht et al., 2009). Importantly, in trajectory analyses we added these covariates, controlling for them in the model; yet, different trajectories of cortisol and HRV over time were still evident in our sample. This suggests that there are unaccounted factors other than age, gender, and antidepressant use that drive individuals into different stress response patterns.

The present study is not without limitations. Of note, although we explored gender effects in our analyses, we did not include variables related to hormonal changes associated with menstruation or pregnancy but which are important considerations when understanding differences in the stress response (Umetani et al., 1998). Furthermore, the archival data set we used is from 2012-2016 and gender was coded with outdated methods as a dichotomous variable (female vs. male). Therefore, we were unable to capture potential gender differences across the full spectrum of gender. Additionally, while we did not find differences between racial groups, this could be due to the fact that the sample was lacking in racial diversity (over 70% White). Prior literature has suggested race as an important factor in determining normative and stress response data of HRV (Sin et al., 2016). Thus, future work is needed to explore whether racial differences emerge. Finally, we only examined one measure of anxiety and only two types of stressors. Thus, the generalizability of our results is limited in that they do not extend to anxiety disordered populations per se, or to psychosocially stressful situations,
which are a major part of daily life that may be particularly relevant for anxious individuals.

It is well-established that individuals differ in their stress response both via the HPA-axis (i.e., cortisol) and vagal activity (e.g., HRV). Further, it is known that these stress responses change over time and in response to stressors and that this change is dependent on age, gender, and antidepressant use (Hu et al., 2019; Kemp et al., 2012; Licht et al., 2009; Umetani et al., 1998; Zachariah & Joseph, 2018). The present study demonstrated that even after controlling for known variables, different trajectories emerged for how cortisol and HRV changed over time in response to three different stressors in a large sample of adults. This indicates that there are indeed unexplained individual differences over time in cortisol and HRV responses to stressors. Surprisingly, we found that anxiety levels did not help account for any of these differences. Interestingly, our results revealed that the only cortisol and HRV measures that tracked with one another were directions of change on HRV and cortisol reactivity such that a decrease in HRV during stress (low HRV reactivity) was associated with a decrease in cortisol during stress (cortisol reactivity). Results from the present study highlight the importance of the vagal-HPA-axis link as evidenced by an interplay between the changes in HRV and cortisol stress responding. Results hold clinical relevance in that it may be possible to promote adaptive long-term responding to stress (desired change in cortisol in response to stress) via targeting short-term adaptation to stress, HRV, through biofeedback. However, more research is needed to understand this relationship. Further examination of these two dynamic biological markers will provide important clarification.
for understanding the human stress response system, and ultimately help to improve interventions for when this system is disrupted.
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APPENDICES

Appendix A
GENERAL STUDY OVERVIEW
Taken from “36901-Documentation-Overview.PDF” found at:
https://www.icpsr.umich.edu/web/ICPSR/studies/36901/datadocumentation

MIDUS REFRESHER BIOMARKER PROJECT (P4) OVERVIEW

The Biomarker Project is one of 5 projects comprising the MIDUS Refresher “Integrative Pathways to Health and Illness”. The overarching objective of MIDUS is to investigate linkages between sociodemographic, psychosocial, and biological variables to account for later life profiles of morbidity and mortality. The MIDUS Refresher allows for expansion of the Biomarker sample to facilitate analyses that pay attention simultaneously to age, gender, and socioeconomic variation in how psychosocial and biological variables are linked. In addition, the Refresher sample will permit assessment of the impact of the ongoing economic recession on the health of diverse-aged adults via comparison with the extant Biomarker data from the MIDUS 2 sample. It will also lay the foundation for parallel longitudinal studies of same-aged adults from different birth cohorts on whom unfolding health trajectories are studied as the product of interacting domains of influence (biological, psychological, social) in a changing historical context (economic recession).

The Refresher Biomarker Project (P4) supported this goal primarily through assessment of a variety of biological indicators of physiology and health according to the basic protocol implemented at MIDUS 2. The protocol also included assessments of additional aspects of psychosocial experience. Modifications to that basic protocol and psychosocial assessments are noted below as appropriate. Data was collected during a 24 hour stay at one of 3 Clinical Research Units (CRU). The following provides basic information about the sample, recruitment, and data collection procedures. Additional information about the basic assessments and the MIDUS longitudinal sample (i.e. MIDUS 2) can be found in:


The Sample

MIDUS participants were eligible for Biomarker data collection if they:
- Completed the MIDUS Refresher Survey project Phone Interview and Self-Administered Questionnaire
- Lived in the continental U.S.

Eligible participants were assigned to one of the three data collection sites (UCLA/Site 1, University of Wisconsin (UW)/Site 2, or Georgetown University/Site 3) based on the region (West Coast, Midwest, East Coast, respectively) in which they lived.

Recruitment

Recruitment was a two-step process. Staff at a given data collection site sent a recruitment packet (letter and brochure describing the study) to individuals assigned to their site. Within a few weeks designated staff at that site made a follow-up call to answer any questions the
individual may have had, then attempted to schedule a CRU visit and facilitated travel arrangements.

**Informed Consent**
Verbal consent is obtained by phone when individuals agree to participate and schedule a CRU visit. Written consent is obtained at the clinic prior to beginning study procedures.

**Data Collection Procedures**
All biomarker assessments, with the exception of sleep assessments, were completed during the overnight stay at the regional CRU. The protocol for the visit was standardized across the three sites so that assessments were completed as follows:

*Day 1* (late afternoon or evening of the day the person arrived at the CRU)
- Medication Chart
- Medical History
- Self-Administered Questionnaire (SAQ)
- Pittsburgh Sleep Questionnaire (PSQ)
- Physical Exam (Vitals)
- 12 hour Urine Collection began

*Day 2* (the morning of the day the person left the CRU)
- 12 hour Urine Collection ended
- Fasting Blood Draw
- Psychophysiology Experimental Protocol
- Physical Exam (Functional Assessments)

The following assessments could be completed on Day 1 or Day 2 to provide flexibility in accommodating availability of clinical staff:
- Bone and muscle function (Densitometry (DXA) Scan, Jump/Balance) as well as Gait assessments. These assessments are done at UW only.
- The Ankle Brachial Index Assessments (ABI). These assessments were done at UCLA only.
Appendix B

OVERVIEW OF PSYCHOPHYSIOLOGY PROTOCOL AND HRV MEASURES

Taken from “36901-Documentation-Psychophysiology_Protocol.pdf” found at: https://www.icpsr.umich.edu/web/ICPSR/studies/36901/datadocumentation

**Overview of Protocol**

**Purpose.** The psychophysiology protocol in the MIDUS Biomarker project is a widely used laboratory based, experimental procedure designed to measure cardiovascular reactivity to and recovery from stress.

**Procedure.** During the protocol, participants' physiological outcomes are measured during a seated, resting baseline period followed by two cognitive/psychological stressor tasks, also in a seated position. The cognitive tasks are a mental arithmetic task called MATH (Morgan And Turner Hewitt arithmetic; Turner et al, 1986) and a Stroop color-word matching task. After each cognitive stress task, participants undergo another seated, resting period to assess physiological recovery to stress. The last period in the procedure is an orthostatic stressor. Participants move from a seated to standing position and remain standing for several minutes.

**Physiological signal collection.** Cardiovascular reactivity is assessed via continuous measurement of the electrocardiogram (ECG) and blood pressure measured at the finger and corrected to brachial artery standards. The beat-to-beat ECG and BP waveforms are then analyzed to calculate heart rate, several indices of heart rate variability (HRV), systolic and diastolic BP, and indices of BP variability (BPV). Heart rate variability is operationalized as variability in the series of intervals between consecutive R waves (the first upward deflection of the electrocardiogram following the Q-wave, arising from ventricular depolarization) (Figure 1). In addition, reactivity of the Hypothalamic Adrenal Pituitary (HPA) axis is measured via collection of saliva samples for cortisol assay.

**Theory and method.** Throughout this guide, relevant references are cited to provide investigators information about the methodology used in this protocol. We offer the following references, for an introduction and review of cardiac psychophysiology, and the types of questions that can be investigated with this type of protocol (Carney, Freedland, & Veith, 2005; Gorman & Sloan, 2000; Shcheslavsky, Burg et al., (2010); Sloan, McCredie et al., 2007). A recent special issue of *Biological Psychology* (Allen & Chambers, 2007) on cardiac vagal control is a good resource. Investigators are encouraged to review the literature in more depth. Relevant key words for literature searches include: heart rate reactivity, heart rate variability (also referred to as “RR interval variability” or as a related measure, “respiratory sinus arrhythmia”), stress reactivity, and stress recovery.

The cognitive/psychosocial measures are described more fully in the next section. Details about the physiological measures and how they are processed appear later in this section after the comprehensive description of the protocol.

**Psychological Stressor Measures**

**Stroop Color-Word Task.** In this modified version of the Stroop task, one of four color name words (blue, green, yellow or red) is presented on a computer screen in a font color that is either congruent or incongruent with the name. The color name stimulus appears on screen, and participants press one of four keys on a keypad corresponding to the color of the letters in the word, not the color name. To roughly standardize the stressfulness of the task, the rate of presentation of the stimuli varies as a function of task performance. Greater accuracy leads to a more rapid presentation rate. Poorer accuracy leads to a slower rate. Overall, participants achieve an accuracy of about 67%. Response data for each trial, including the stimulus features, response latency, and response accuracy are stored in a file for later analysis.

**Mental Arithmetic.**

The Morgan And Turner Hewitt (MATH) task is a computer-administered mental arithmetic task designed for
use as a psychological stressor in laboratory studies of cardiovascular reactivity (Turner, Hewitt et al., 1986; Turner, Sims, Carroll, Morgan, & Hewitt, 1987). Task problems involve the addition or subtraction of two numbers. Problem difficulty can vary across five levels, ranging from problems of 1-digit ± 1-digit numbers (level 1) to 3-digit ± 3-digit numbers (level 5). The task always begins at level 3; difficulty level thereafter is determined at each trial by response accuracy on the previous trial. Correct responses were followed by one step up in difficulty, or if already at level 5, difficulty remains at level 5 until an incorrect response. Incorrect responses were followed by one step down in difficulty, or if already at level 1, difficulty remains at level 1 until a correct response.

Each trial consists of three elements presented on screen in succession. First, one math problem is presented for 2.0 sec. Then, the word 'Equals' appears alone on screen for 1.5 sec, giving the participant more processing time. A solution to the problem then appears for up to 1.0 sec, during which the participant presses one of two keys on a keypad to indicate whether the presented solution to the problem is correct or not. The next trial is presented as soon as a response key was pressed. Failure to respond within the one-second solution screen is recorded as an incorrect response, with a response time of 1.0 sec, and the next trial is presented.

Trials continued for the full duration of the mental arithmetic protocol period; total number of trials varies based on the participant’s response times. The ratio of addition to subtraction problems is 3:7. The ratio of correct to incorrect problem solutions presented on screen is 1:1. Response data, including problem content, level, and response time and accuracy, are collected for each trial and stored in a file for later analysis. For MIDUS, the original task specifications by Turner et al. (Turner et al., 1986) were modified to extend the task length from 4 to 6 minutes.

**Psychophysiology Protocol Description**

The following is a detailed description of the data collection protocol, including equipment setup, protocol order, and data processing.

**Protocol Flowsheet:**

MIDUS staff who conducted this protocol used a data collection form called the psychophysiology flowsheet. A copy of this form with variable names inserted is in Section B (above).

The first two pages included questions about handedness, physical characteristics of the participant, and other factors that may influence experimental outcomes (e.g., consumption of caffeine, nicotine etc.), as well as a template of the protocol order. A more detailed version of this template appears in Table 1 below. The remainder of the flowsheet contains a more complete description of the protocol, instructions to research staff, instructions to participants, descriptions of the stress tasks, etc. Throughout the protocol, staff were instructed to record information at designated locations on flowsheet. This information as well as responses to the items at the beginning of the flowsheet were data-entered and included in the MIDUS Refresher Biomarker data file just prior to the physiology data described below and the variables begin with RA4V. Details about saliva sample collection are included in Section A above.

**Monitoring Device Setup.**

Electrocardiograph (ECG) electrodes were placed on the left and right shoulders, and in the left lower quadrant. Stretch bands were placed around the participant's chest and abdomen to measure respiration. A Finometer blood pressure cuff was placed on the middle finger of the non-dominant hand, and a Finometer blood pressure arm cuff was placed on the upper arm on the same side as the finger cuff. The participant was then seated and a numeric keypad, for responding to the stress tasks, was secured in a comfortable position relative to the dominant hand. The monitoring devices were then calibrated in the seated position.
Notes:

The order of the Math and Stroop tasks was automatically and randomly selected at the time of data collection. Thus, each task was either in position 5 or 7 of the protocol for each session. A Task presentation order variable is included in the psychophysiology flowsheet data set.

Protocol Order.

The general protocol order was as follows (details are in Table 1): seated baseline (11 minutes); psychological stress task 1 (mental arithmetic or Stroop task - 6 minutes); recovery 1 (6 minutes); psychological stress task 2 (mental arithmetic or Stroop task - 6 minutes); recovery 2 (6 minutes); orthostatic stressor (standing/upright) (6 minutes). No recovery data were collected after exposure to the orthostatic stressor. Participants were instructed to remain silent throughout the procedures. After the second recovery period, participants were assisted in moving to a standing position. The monitoring devices were recalibrated, then the orthostatic stress period began.

Data Processing Criteria

The physiological monitoring equipment (ECG, Finometer, Inductotrace respirometer) ran continuously throughout the protocol and produced raw waveform data. These raw data were processed according to standardized algorithms (Task Force, 1996) to create variables (see Key Variables) that can be used in analyses. Analytic data are provided in MIDUS by period and by epoch within each period. The MIDUS Refresher Biomarker (P4) data includes one set of data from the psychophysiology session, which uses 300 second epochs of data. This section defines these terms and describes the criteria used to select raw physiological waveform data for processing to generate key variables.

Periods.

The protocol was divided into periods based on experimental conditions and participants’ activity. Physiological outcome variables are computed separately for each protocol period and are identified by Period, as specified in Table 1, in the data sets.

Periods in **BLUE** font in Table 1 represent data included in the MIDUS data set for analyses.

Other periods represent interim periods used for calibrating equipment and other purposes not relevant to hypothesis testing. Raw physiological waveform data from the interim periods are preserved at the CUMC site but are not analyzed.

Each period name as shown in Table 1 is part of the variable names for all data from that period.

Data Epochs for Analysis

Within each protocol period, data were analyzed in specified epochs of time, based on different criteria and different types of research questions.

Data were analyzed with a specified 300 sec epoch duration.

For the 11 min baseline period, we attempt to provide 2 epochs of 300 sec each. Cases with unscorable intervals of data may have only 1 Baseline epoch (due to noisy signal). The stress tasks, recovery periods and upright stressor were all 6 min periods. For these, one epoch of data is included in the data set.

Salivary Cortisol Samples: Table 1 indicates the order and timing of the saliva samples. In the data set, saliva sample numbers (corresponding to numbers in Table 1) indicate the specific protocol context of the sample. For example, sample #4 was collected after the orthostatic stressor period. If sample #4 has a missing value in the data set, it means that participant does not have cortisol data related to orthostatic stress reactivity (period U1). Likely, it also indicates that the participant did not complete the U1 period in the protocol.

Physiological Measures

The following sections describe the 3 sets of physiological measures (HRV, BP, Respiration) and conventions
used in naming variables. Across all three sets of measures, the following characters are used to represent the indicated period when they were obtained during the protocol:

**Key to Period and Epoch indicators in variable names:**

<table>
<thead>
<tr>
<th>B1</th>
<th>BASELINE period, epoch1 (of usually 2 epochs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B2</td>
<td>BASELINE period, epoch2 (of usually 2 epochs)</td>
</tr>
<tr>
<td>M1</td>
<td>MATH period, epoch1 (of 1 epochs)</td>
</tr>
<tr>
<td>S1</td>
<td>STROOP period, epoch1 (of 1 epoch)</td>
</tr>
<tr>
<td>R1</td>
<td>first RECOVERY period (1 epoch)</td>
</tr>
<tr>
<td>R2</td>
<td>second RECOVERY period (1 epoch)</td>
</tr>
<tr>
<td>U1</td>
<td>UPRIGHT period (1 epoch)</td>
</tr>
</tbody>
</table>

**A. Electrocardiogram Measures: Heart Rate, Heart Rate Variability**

Acquisition and Processing of ECG Signals. Beat-to-beat analog ECG signals were collected then digitized at a sampling rate of 500 Hz by a 16-bit National Instruments analog-to-digital (A/D) board installed in a microcomputer. ECG waveforms were submitted to proprietary event detection software to identify R waves. Following established procedures, (Berntson, Quigley, Lang, & Boysen, 1990; Dykes, Ahmann et al., 1986), research staff visually reviewed all ECG waveforms to correct interactively any software errors in identifying normal R waves. The resulting series of normal RR intervals was used to calculate the cardiac variables heart rate (HR) and several standard indices of HRV.

**Heart rate.** Heart rate is calculated as an average of all valid RR intervals for a specified length of time. HR data in the MIDUS data set have been converted from RR interval units (milliseconds) to beats per minute units.

**Heart rate variability.** Time domain indices of RR interval variability include the standard deviation of RR intervals (SDRR) and the root mean squared successive differences (rMSSD). Frequency domain measures include spectral power in the low (0.04-0.15 Hz (LF-HRV)) and high (0.15-0.50 Hz (HF-HRV)) frequency bands. The spectra of RR interval series were calculated using an interval method for computing Fourier transforms similar to that described by DeBoer, et al. (DeBoer, Karemaker, & Strackee, 1984). Prior to computing Fourier transforms, the mean of the RR interval series is subtracted from each value in the series and the series then is filtered using a Hanning window (Harris, 1978) and the power, i.e., variance (in msec2), over the LF and HF bands is summed. Estimates of spectral power are adjusted to account for attenuation produced by this filter (Harris, 1978).

**A. Key ECG Variables and Naming Conventions**

a. **Key Variables.**

The key cardiac variables from the psychophysiology session used by CUMC investigators are listed below. These output variables are standardized based on conventions for measuring heart rate and heart rate variability parameters (Task Force, 1996):

- **HR:** Average heart rate, beats per minute units
- **SDRR:** Standard deviation of RR intervals, msec units
- **rMSSD:** Root mean squared successive differences, msec units
- **LF_HRV:** Low frequency RR interval variability, bandwidth 0.04-0.15 Hz, msec² units
- **HF_HRV:** High frequency RR interval variability, bandwidth 0.15-0.40 Hz, msec² units

The data file includes both original and log transformed versions of all HRV variables (the last 4 variables
Appendix C

CORTISOL ASSAY

Taken from “36901-Documentation-Blood_Urine_Saliva.pdf” found at: https://www.icpsr.umich.edu/web/ICPSR/studies/36901/datadocumentation

II. Saliva Assays

A. Sample Collection:
Saliva samples are collected during the Psychophysiology Challenge Protocol to complement assessments of stress reactivity based on Heart Rate Variability. Five samples are collected (pre-protocol, baseline, post-cognitive challenge, immediately post-orthostatic challenge, and 30 minutes post-protocol). Samples are then frozen and shipped to the MIDUS Biocore lab for storage and subsequent Cortisol assay. See the Documentation for Psychophysiology Data for details about saliva sample collection.

B1. Assay Details – Saliva Cortisol

Salivary cortisol is assayed at the iCTR for the MIDUS Refresher phase. At MIDUS 2 the assays were done at Dresden Lab Service. See the Data Adjustment section for more information.

The samples for this assay are collected on cotton swabs in salivettes (Sarstedt Cat. #51.1534) and frozen. At the time of assay, they are thawed and centrifuged at 3000 rpm for 5 min, resulting in a particulate-free, clear fluid of low viscosity.

Salivary Cortisol:
Concentrations of free cortisol (the only type found in saliva) were determined using radioimmunoassay (catalog #07-221106 from MP Biomedicals, Solon, OH).21

May 18, 2017

B. P4 Bioassays from urine and saliva samples (specimen type).

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>Method</th>
<th>Performed at</th>
<th>Assay Range[22]</th>
<th>Inter-assay Variability</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (urine)</td>
<td>Colorimetric</td>
<td>ICTR</td>
<td>0-400 mg/dL[23]</td>
<td>inter-assay CV: 2.39-4.17%[24]</td>
<td>Male: 1.7-4.0g/day</td>
</tr>
<tr>
<td>Cortisol (urine)</td>
<td>HPLC/mass spectrometry</td>
<td>ICTR</td>
<td>min 0.00125 mg/dL</td>
<td>inter-assay CV: 3.5±1.7%[23]</td>
<td>3.5-45 μg/dl[20]</td>
</tr>
<tr>
<td>Cortisol/creatinine ratio (urine)</td>
<td>Calculated</td>
<td>Univ. of Wisconsin (CoE Lab)</td>
<td>N/A</td>
<td>N/A</td>
<td>Male: 1.19 μg/g</td>
</tr>
<tr>
<td>Cortisone (urine)</td>
<td>HPLC/mass spectrometry</td>
<td>ICTR</td>
<td>min 0.00125 mg/dL</td>
<td>inter-assay CV: 7.82-14.29%[24]</td>
<td>17-129 μg/dl[20]</td>
</tr>
<tr>
<td>Cortisone/creatinine ratio (urine)</td>
<td>Calculated</td>
<td>Univ. of Wisconsin (CoE Lab)</td>
<td>N/A</td>
<td>N/A</td>
<td>Age (g/dl)</td>
</tr>
<tr>
<td>Norepinephrine (acidified urine)</td>
<td>HPLC, electrochemical</td>
<td>ICTR</td>
<td>min 0.488 ng/mL</td>
<td>inter-assay CV: 10.45-14.77%[25]</td>
<td>15-80 ng/dl[27]</td>
</tr>
<tr>
<td>Epinephrine (acidified urine)</td>
<td>HPLC, electrochemical</td>
<td>ICTR</td>
<td>min 0.488 ng/mL</td>
<td>inter-assay CV: 12.59-14.61%[25]</td>
<td>0-20 ng/dl[25]</td>
</tr>
<tr>
<td>Dopamine (acidified urine)</td>
<td>HPLC, electrochemical</td>
<td>ICTR</td>
<td>min 0.488 ng/mL</td>
<td>inter-assay CV: 10.15-18.48%[25]</td>
<td>65-400 ng/dl[25]</td>
</tr>
<tr>
<td>Cortisol (saliva)</td>
<td>RIA</td>
<td>ICTR</td>
<td>0.69-68.98 nM</td>
<td>inter-assay CV: 29.44%[26]</td>
<td>3-25 nM[26]</td>
</tr>
</tbody>
</table>
XII. Salivary cortisol

During MIDUS 2, cortisol was measured at Dresden Lab Service by ELISA using kits manufactured by IBL International. For MIDUS R, saliva samples were sent to ICTR, where they were initially assayed using ELISA kits from Salimetrics LLC (Carlsbad, CA). The data obtained with these assays was deemed unreliable, so the lab re-assayed all the samples by RIA using kits from MP Biomedicals. From their comparison data the following equation was generated to adjust their RIA data to get it in line with the earlier IBL-ELISA data:

\[ [\text{cortisol}] = 0.7629 \text{ (reported value)} - 0.0544^{14} \]

By the time ICTR decided to try RIA, some of these early saliva samples were depleted. For these samples the Salimetrics data were adjusted to align them with IBL data using the following equation:

\[ [\text{cortisol}] + 1.7973 \times \text{(Salimetrics value)} + 1.0035^{14} \]
Appendix D
STAI
Taken from “36901-Documentation-Psychosocial_Constructs.pdf” found at: https://www.icpsr.umich.edu/web/ICPSR/studies/36901/datadocumentation

SPIELBERGER TRAIT ANXIETY INVENTORY

Note: throughout the following "R" indicates item is reverse coded before constructing the scale score.

**Scales/Items:**
**TRAIT ANXIETY [RA4QTA_AX]:**

*Items:* 20 items - Question 7(a-t).

Circle the number that best describes how you *generally* feel.

a. "I feel pleasant. "(R)
b. "I tire quickly. "
c. "I feel like crying. "
d. "I wish I could be as happy as others seem to be. "
e. "I am losing out on things because I can’t make up my mind soon enough. "
f. "I feel rested. "(R) 
g. "I am “calm, cool, and collected”. (R)
h. "I feel that difficulties are piling up so that I cannot overcome them. "
i. "I worry too much over something that really doesn’t matter. "
j. "I am happy. "(R)
k. "I am inclined to take things hard. "
l. "I lack self-confidence. "
m. "I feel secure. "(R)
n. "I try to avoid facing a crisis or difficulty. "
o. "I feel blue. "
p. "I am content. "(R)
q. "Some unimportant thought runs through my mind and bothers me. "
r. "I take disappointments so keenly that I can’t put them out of my mind. "
s. "I am a steady person. "(R)
t. "I get in a state of tension or turmoil as I think over my recent concerns and interests. "

**Coding:** 1 Almost never; 2 Sometimes; 3 Often; 4 Almost always.

**Scaling:** Items marked with (R) were reverse-coded so that high scores reflect higher standing in the scale. Unless otherwise indicated above, scale scores were computed by summing across all items for which there was no missing data. Mean substitution was used in cases with only one missing value.
Psychometrics:

<table>
<thead>
<tr>
<th>Sample (N)</th>
<th>Alpha</th>
<th>Mean</th>
<th>Std. dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>MR Total Sample (863)</td>
<td>.906</td>
<td>35.215</td>
<td>9.208</td>
</tr>
<tr>
<td>MR Main RDD Sample (746)</td>
<td>.907</td>
<td>34.796</td>
<td>9.105</td>
</tr>
<tr>
<td>MR African American Sample</td>
<td>.897</td>
<td>37.928</td>
<td>9.454</td>
</tr>
</tbody>
</table>

Source(s):
