

## RESEARCH ARTICLE

# Relationship between heart rate variability and differential patterns of cortisol response to acute stressors in mid-life adults: A data-driven investigation

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## Abstract

Cortisol and heart rate variability (HRV) are well-established biomarkers of the human stress response system. While a relationship between cortisol and HRV is assumed, few studies have found evidence of their correlation within single study designs. One complication for isolating such a relationship may lie in individual variability in the cortisol response to stress such that atypical cortisol responding (i.e., elevated or blunted) occurs. To-date, studies on the cortisol response have employed traditional mean-difference-based approaches to examine average magnitude change in cortisol over time. Alternatively, data-driven trajectory modelling, such as latent growth mixture modelling, may be advantageous for quantifying cortisol based on patterns of response over time. Latent growth mixture modelling was used in  $N = 386$  adults to identify subgroups based on trajectories of cortisol responses to stress. The relationship between cortisol and HRV was tested within subgroups. Results revealed a 'prototypical' subgroup characterised by expected rise and fall in cortisol response to stress ( $n = 309$ ), a 'decline' subgroup ( $n = 28$ ) that declined in cortisol after stress, and a 'rise' subgroup ( $n = 49$ ) that increased in cortisol after stress. Within the 'prototypical' subgroup, greater HRV during stress was associated with decline in cortisol after stress from its maximum ( $r(306) = 0.19, p < 0.001$ ). This relationship failed to emerge in the 'decline' and 'rise' subgroups ( $p > 0.271$ ). Results document different patterns of cortisol response to stress; among those who exhibit a 'prototypical' response, changes in HRV during stress are related to changes in cortisol after stress.

## KEYWORDS

cortisol, heart rate variability, HRV, reactivity, stress, trajectory

## 1 | INTRODUCTION

The hypothalamic pituitary adrenal axis (HPA-Axis) remains the most widely studied biological marker of the stress response as it offers a unique understanding of the body's long-term response to a stressor (Rotenberg & McGrath, 2016). A key output of this system commonly investigated is the secretion of cortisol into the bloodstream (Bozovic

et al., 2013). Cortisol facilitates an increase in blood glucose levels, equipping the body with increased energy to face the demands of stressors (Bozovic et al., 2013). Critically, when cortisol levels become high, this initiates a negative feedback mechanism via the HPA-Axis to inhibit the rate of further cortisol secretion (Bozovic et al., 2013). Thus, cortisol acts dynamically by both preparing the body to face a stressor as well as helping the body return to

homeostasis (Bozovic et al., 2013) and, accordingly, follows a pattern in healthy individuals in response to stressors: rising, peaking, and subsequently falling back towards baseline levels (Kirschbaum & Hellhammer, 2000). Peaks in circulating cortisol, measured from saliva, are detected within 20–30 min after the onset of acute stressors (Kirschbaum & Hellhammer, 2000).

While this pattern of response (rise and fall) of cortisol as indexed in circulation through saliva is associated with a healthy response to stress, there remains sizeable variation among individuals as indexed by magnitude of secretion (i.e., high vs. low) (Bozovic et al., 2013; Kirschbaum & Hellhammer, 1989). Notably, atypical responses as qualified by the magnitude of cortisol release are linked to negative health outcomes (Coyle et al., 2020; Fiksdal et al., 2019; Henckens et al., 2016; Jones & Gwenin, 2021; McEwen, 2008). For example, blunted cortisol responding to acute stressors is associated with unfavourable health outcomes such as obesity, addictions, fibromyalgia, bulimia, and depression as well as increased risk for insomnia (Carroll et al., 2017; Coppens et al., 2018; Reffi et al., 2022) while elevated cortisol responding to acute stressors is related to increase risk for high blood pressure and hypertension (al'Absi et al., 1994; Witbracht et al., 2015). Together, interindividual differences in cortisol production are partially accounted for by known demographic, biological, and psychological factors such as gender, age, corticosteroid binding globulin levels, genetic polymorphisms, personality type, chronic stress, and social support (Foley & Kirschbaum, 2010). Further likely contributing to such variability is the fact that individuals exhibit significantly different basal levels of cortisol (Kirschbaum & Hellhammer, 1989). As one example, average salivary cortisol levels among healthy subjects range from 0.20–1.41  $\mu\text{g}/\text{dl}$  (5.52–28.92  $\text{nmol}/\text{L}$ ) in the morning and between 0.04–0.41  $\mu\text{g}/\text{dl}$  (1.10–11.32  $\text{nmol}/\text{L}$ ) in the afternoon (Bozovic et al., 2013). However, these variables do not fully account for explaining inter-individual differences in cortisol secretion to a stressor (Henckens et al., 2016) and remains an area of ongoing investigation. While the aforementioned associations between blunted or exaggerated response to stress and health are important discoveries, it still remains unclear why some individuals experience such profiles. That is, more research is needed on the precise factors related to individual differences in cortisol response to stress.

One such factor is vagal activity based on the fact that it is governed by the autonomic nervous system and is assumed to exert an inhibitory influence on the HPA-Axis during stress. In this way, vagal activity contributes to ushering the return of the body to homeostasis (Thayer & Sternberg, 2006). Indeed, while cortisol facilitates an increase in heart rate and blood pressure when faced with a stressor, sustained vagal response to stressors also facilitates top-down processes influencing HPA-Axis responding (Bozovic et al., 2013; Sloan et al., 2017). Given their shared function, cortisol and vagal activity are widely utilised independent markers of the biological stress response (Rotenberg & McGrath, 2016; Thayer & Sternberg, 2006). One common index of vagal activity, heart rate variability (HRV), captures the variation in intervals between heart beats (Sloan et al., 2017) and is specifically cited in its relationship to

adaptive stress responding. Low HRV is linked with emotion dysregulation, psychopathology, increased risk for cardiovascular disease, and mortality (Sin et al., 2016; Thayer et al., 2010, 2012), and has been theorized to represent non-adaptive stress responding (Thayer et al., 2012). Conversely, high HRV qualified by a relatively large inter-beat variability, is linked with a greater ability to exhibit context appropriate responses such as greater biological recovery and self-reported control of emotions after cessation of a stressor (Thayer et al., 2012; Weber et al., 2010).

To-date, some limited research has examined the relationship between HRV and cortisol during stress (Glier et al., 2022; Murdock et al., 2017; Pulopulos et al., 2018). In one such study, Pulopulos et al. (2018) found that lower HRV during anticipation of stress was correlated with higher stress task-induced cortisol (Pulopulos et al., 2018). Other work measured HRV during the Trier Social Stress Test in adolescents, finding that decreases in HRV in response to stress were associated with steeper increases in cortisol reactivity (Glier et al., 2022).

Despite such findings, a number of studies have failed to find a significant association between cortisol and HRV (Altemus et al., 2001; Bosch et al., 2009; Looser et al., 2010; Marca et al., 2011). Discrepant results demonstrate possible gaps in our understanding of the interplay between cortisol and HRV and, precisely, the conditions whereby a relationship between the two may exist. Notably, these studies have relied on traditional methods for quantifying cortisol reactivity to stress by using mean-difference-based approaches that allow for an assessment of average magnitude change over time. By contrast, to better understand variability in individual differences in cortisol responses to stress, data-driven trajectory modelling, such as latent growth mixture modelling (LGMM), may be advantageous (Felt et al., 2017). This approach enables the identification of interindividual (e.g., between-individual) differences in cortisol change based on intraindividual (e.g., within-individual) change (Ram & Grimm, 2009). Critically, trajectory modelling can offer insight to an individual's entire stress response profile (e.g., rise, peak, and fall over time) and therefore may help to incrementally document interindividual variability in cortisol responding beyond traditional statistical techniques (Glier et al., 2022; Van Ryzin et al., 2009). Despite the demonstrated utility of such analyses, they have been relatively underutilised, in part likely due to their requirement of large sample sizes ( $>200$ ) and multiple sampling time points ( $\geq 3$ ) (Felt et al., 2017).

The present study used LGMM to isolate discrete patterns of salivary cortisol responding to acute stress within a large sample of individuals ( $N = 386$ ). Within subgroups identified via LGMM, we subsequently tested the relationship between salivary cortisol and HRV. With respect to LGMM results, given the exploratory nature of this analytic approach, we did not have strong a priori hypotheses regarding what these trajectories would resemble, particularly with respect to number of trajectories, proportion of the sample, and patterns of change. However, we hypothesised that a single cortisol trajectory would be improbable, given the variability in cortisol response to stress, and that a multiple trajectory model would

emerge as a better fit for the data. In addition, we anticipated the largest number of participants would have a 'prototypical' trajectory defined by cortisol rising, peaking, and then falling back towards pre-stress levels as this pattern is frequently observed (Kirschbaum & Hellhammer, 2000). With respect to an association between cortisol and HRV, we expected that an association between cortisol and HRV may exist in some—but not all—subgroups defined by differential cortisol response to stress, which may help shed light on why discrepant findings in the literature exist with respect to the presence and/or absence of a cortisol and HRV relationship.

## 2 | METHODS

### 2.1 | Participants

The Midlife in the United States (MIDUS) Refresher study is a longitudinal investigation of health, well-being, and ageing, which collected self-report written and phone interview data from 3577 adults in the US. The present study used data obtained from the publicly available Midlife in the United States (MIDUS) Refresher Biomarker Project (data available via public repository hosted by the Inter-university Consortium for Political and Social Research: <https://www.icpsr.umich.edu>), a sub study of the MIDUS Refresher which involved 863 adults who partook in an acute stress protocol. Data reported herein are from participants who completed survey data and a laboratory-based stress test between the years of 2012 and 2016. Further demographics are described below in Results.

### 2.2 | Procedure

Participants stayed overnight at one of three research facilities (i.e., UCLA, University of Wisconsin, Georgetown University) for a 2 day visit. Participants were asked to avoid caffeine and nicotine after midnight. On the morning of the second day, data was collected from participants in response to a standardized laboratory-based experimental stress-induction protocol lasting approximately 90 min (Love et al., 2010). A detailed protocol is available on the Midlife in the United States (MIDUS Refresher): Biomarker Project, 2012–2016 (ICPSR 36901) website (<https://www.icpsr.umich.edu/web/ICPSR/studies/36901>).

Briefly, upon arrival at the laboratory participants provided a saliva sample and cardiovascular equipment was then calibrated followed by participants completion of practice trials of two tasks demonstrated to elicit a psychological and HPA-axis stress response (Skoluda et al., 2015): a Stroop colour-word matching task and a mental arithmetic task (Stroop, 1935; Turner et al., 1986). Prior to completion, participants sat quietly at rest, to gather baseline measures over 11 min and then were asked to perform the first cognitive stress task. Each stress task lasted 6 min and was followed by a 6 min recovery period; the order of the stress tasks was counter-balanced and randomized across participants.

### 2.3 | Cognitive stress tasks

The Stroop task involved a colour name presented on a computer screen in a font that was either congruent or incongruent with the name (e.g., "blue" presented in a blue font colour or "blue" presented in a red font colour). Participants were instructed to use a keypad to select the answer that matched the font colour as opposed to the colour name. Participants were instructed to do this as quickly and as accurately as possible (Love et al., 2010).

The Morgan and Turner Hewitt (MATH; Turner et al., 1986) mental arithmetic task presented a series of addition and subtraction problems on a computer screen, each followed by an answer that the participant was asked to indicate using a 'yes/no' selection on the keypad as either correct or incorrect. Participants were asked to complete their responses as quickly and accurately as possible, and problems varied in difficulty ranging from problems of 1-digit to 3-digit numbers. Incorrect responses were followed by problems of lower difficulty, while correct responses were followed by problems of higher difficulty.

### 2.4 | Cortisol

Throughout the administration of the cognitive stress tasks, participants provided a saliva sample for assay of cortisol (Bozovic et al., 2013). Salivary cortisol is more easily-obtained in a non-invasive way has been shown to not elicit additional stress from the participant during collection (Bozovic et al., 2013). At five pre-designated time points during the laboratory-based stress protocol (see below for further details), participants provided a salivary sample collected via a Salivette® cotton swab. Swabs were placed directly in the mouth by the participant and saturated, and subsequently placed it back in the storage contained for storage until assay. At the end of each session, saliva samples 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 min (Weinstein et al., 2019). Assay sensitivity metrics are available at <https://www.icpsr.umich.edu/web/NACDA/studies/36901/data-documentation>. The unit of cortisol measurement was nanomoles per litre (nmol/L).

The first salivary cortisol sample was collected immediately upon arrival to the laboratory, followed by a second sample collected after completion of practice trials of both stress tasks while participants were seated and just prior to administration of the stress tasks, in line with prior literature suggesting the importance of a habituation period in order to avoid elevated cortisol baseline levels (Goodman et al., 2017). A third sample was obtained immediately following the 6 min recovery period of the second cognitive stressor. A fourth sample was taken to reflect a timepoint of approximately 24 min after the onset of the first stress task. A final sample was collected after a 30 min rest period, and therefore approximately 54 min after the onset of the first stress task (Love et al., 2010).

## 2.5 | Heart rate variability

Throughout the administration of the cognitive stress tasks, participants' cardiovascular reactivity was also collected from continuous measurement of electrocardiogram (ECG) data for assessment of HRV. Beat-to-beat ECG waveforms were calculated to derive indices of HRV defined as variability in the interval between consecutive R waves. Data collected from ECG was analysed with a specified 300 s epoch duration and compartmentalised into 7 epochs. The first two epochs (HRV 1 and HRV 2) captured HRV during an 11 min seated baseline period after completion of practice trials on both stress tasks, and prior to administration of the first stress task. Following, two epochs captured HRV during each of the cognitive stress tasks (HRV 3 and HRV 5) and two during their corresponding recovery periods (HRV 4 and HRV 6), with a final epoch after the second recovery period (HRV 7). During each epoch, the root mean square of successive differences (RMSSD) between heartbeats was calculated. Root mean square of successive differences is a reliable and commonly-used marker of HRV (Hill & Siebenbrock, 2009; Malik et al., 1996) and provides a time-domain measure of the vagally mediated changes reflected in HRV (Shaffer & Ginsberg, 2017).

## 2.6 | Data analysis

Of the  $N = 863$  invited to participate, a total of  $N = 779$  individuals completed the stress protocol, of which  $N = 701$  had complete cortisol data available across all 5 samples and data on lag time between awakening and first cortisol sample. To be considered eligible for analysis, participants also needed to have HRV data available for HRV calculations (e.g., spanning both baseline and stress periods). This retained a sample of  $N = 480$  for analyses. Prior to analysis, outliers were reviewed following guidelines from previous cortisol and HRV research (Pulopulos et al., 2018) such that values  $\pm 3$  SD were removed. Additionally, participants with missing data for relevant covariates ( $n = 52$ ) were removed. This retained a final sample size of  $N = 386$ . This is higher than the required sample size of  $N = 304$  (estimating  $\alpha = 0.05$ ) to detect medium-sized effects ( $f^2 = 0.15$ ) with a conserved power of 80% (completed using G\*Power; Faul et al., 2007). Prior to analysis, cortisol and RMSSD values were assessed and found to be not normally distributed and therefore were transformed using the natural log.

We first used LGMM to examine if individual trajectories of cortisol existed across participants. Subsequently, a number of metrics were calculated to define cortisol and RMSSD changes during the stress tasks. The relationship between cortisol and RMSSD metrics was then tested within subgroups identified via LGMM using partial Pearson's correlation.

### 2.6.1 | Latent growth mixture modelling

Latent growth mixture modelling was completed using the R version 4.0.2 *lcm* package (Proust-Lima et al., 2017). To evaluate 1 to 6

class solutions with and without the inclusion of covariates, 12 models were run. In order to tune hyperparameters, an automatic grid search of 50 random initial values was employed (Proust-Lima et al., 2017). Splines were used as the link function as they are useful for assessing non-linear relationships (Perperoglou et al., 2019). Final model estimates were selected that generated the best-log likelihood estimate following 100 iterations (Proust-Lima et al., 2017). Final class selection was decided when the addition of a class failed to improve fit indices. The highest posterior probability of class membership was used to assign individuals to a trajectory.

To ensure selection of the optimal model based on fit of  $n = 1-6$  classes, we followed recommended guidelines which suggests assessment of multiple criteria including reduction of log-likelihood, Akaike Information Criteria (AIC), Bayesian Information Criteria (BIC), sample-adjusted BIC (SABIC), and entropy (higher values reflecting better separation among classes) (Nguena Nguetack et al., 2020; Shmueli, 2010; van de Schoot et al., 2017). While both AIC and BIC penalise for the number of free parameters, BIC considers sample size and implements a stronger penalisation (Klijin et al., 2017). In accordance with current guidelines, the model with the lowest BIC was selected based on expert consensus that it is the favoured approach (van de Schoot et al., 2017) and because the research question of the present study was exploratory rather than predictive in nature (Shmueli, 2010). Additionally, model comparisons considered theoretical interpretability, parsimony, and sample size of the smallest class (>5% is recommended) (Nguena Nguetack et al., 2020).

We included several covariates in our LGMM modelling based on already-established associations between these variables and individual differences in cortisol reactivity (Caballero et al., 2019; O'Neal et al., 2016; Silvia et al., 2014; Sin et al., 2016; Zorn et al., 2017): age, gender, number of minutes since awakening to first cortisol sample, number of hours since last meal to session start time, total number of Metabolic Equivalent of Task (MET) minutes per week (a physical activity marker), Body Mass Index (BMI), and sex hormones ('Yes/No,' whether currently using any of the following: contraceptives; androgens and anabolic steroids; oestrogens; gonadotropins; progestins; sex hormone combinations; gonadotropin-releasing hormone and analogues). Information regarding these covariates was collected from all participants prior to the in-lab visit, except for time since awakening and time since last meal. Including these variables in the LGMM model as covariates allowed us to examine the presence of individual variability in cortisol response not explained by these factors.

### 2.6.2 | Cortisol metrics

Following the calculations from other published work (Coyle et al., 2020; Pulopulos et al., 2018), we calculated two cortisol metrics for each individual to quantify cortisol response to stress, which were subsequently used to correlate with HRV metrics within each subgroup defined by LGMM. First, aligned with prior work, 'Δ stress-induced cortisol' was defined as the difference in cortisol from

baseline levels to maximum cortisol levels after stress tasks (Bibbey et al., 2013; Coyle et al., 2020; Pulpulos et al., 2018). Here we used cortisol sample 2 as the baseline measure, as doing so allowed for a habituation period recommended by the literature in order to avoid the confound of elevated cortisol baseline levels upon arrival (Goodman et al., 2017). Second, following the approach of Pulpulos et al. (2018), 'Δ post-stress cortisol' was calculated as the difference in cortisol from maximum levels after stress tasks to the last cortisol sample (54 min after onset of the first stress task). In both calculations, the maximum cortisol value was each individual's peak cortisol value across samples 3 and 4. This allowed for more precision in capturing variability in interindividual differences in peak cortisol after stress across a 24–30 min window after first stressor onset.

### 2.6.3 | Heart rate variability metrics

To quantify HRV response to stress, we followed the method of previous work assessing adults response to laboratory stress tasks whereby HRV Reactivity is defined as the change in HRV from baseline to stress tasks (Bibbey et al., 2013; Coyle et al., 2020; Pulpulos et al., 2018). Aligned with previous work that utilises more than one cognitive stress task (Bibbey et al., 2013; Coyle et al., 2020), HRV Reactivity was calculated as a difference score between average of the baseline measures taken prior to engagement in the stress tasks (HRV 1 and HRV 2) and average of the stress measures (HRV 3 and HRV 5).

## 3 | RESULTS

### 3.1 | Participants

Participant demographic information is listed in Table 1. Gender differences were found in cortisol metrics, such that Δ stress-induced cortisol and Δ post-stress cortisol were higher in men ( $M = 0.16$ ,  $SD = 0.33$ ;  $M = 0.18$ ,  $SD = 0.36$ ) compared to women ( $M = 0.05$ ,  $SD = 0.31$ ;  $M = 0.11$ ,  $SD = 0.33$ ),  $t(384) = -3.37$ ,  $p < 0.01$ , 95% CI  $[-0.17, -0.05]$ , Cohen's  $d = 0.32$ , and  $t(384) = -2.18$ ,  $p = 0.03$ , 95% CI  $[-0.14, -0.01]$ , Cohen's  $d = 0.34$ , respectively. In addition, individuals with lower BMI experienced a greater decrease in Δ post-stress cortisol ( $r(384) = -0.12$ ,  $p = 0.02$ ), 95% CI  $[-0.22, -0.02]$ . Finally, Δ stress-induced cortisol was lower in individuals who used sex hormones ( $M = 0.01$ ,  $SD = 0.23$ ) than for those who did not ( $M = 0.11$ ,  $SD = 0.33$ ),  $t(384) = -2.34$ ,  $p = 0.02$ , 95% CI  $[-0.18, -0.02]$ , Cohen's  $d = 0.32$ . Age and number of minutes since awakening to first cortisol sample were unrelated to cortisol metrics ( $p > 0.13$ ).

With respect to HRV, individuals who used sex hormones exhibited lower HRV Reactivity ( $M = -0.07$ ,  $SD = 0.09$ ) compared to individuals who did not ( $M = -0.04$ ,  $SD = .12$ ),  $t(384) = -1.69$ ,  $p = .046$ , 95% CI  $[-0.07, 0.01]$ , Cohen's  $d = 0.12$ . Additionally, number of minutes since awakening to first cortisol sample was negatively correlated with HRV Reactivity ( $r(384) = -0.11$ ,  $p = .03$ ), 95% CI  $[-0.21, -0.01]$ , such that less time elapsed was associated with greater HRV Reactivity. Gender, age, and BMI were unrelated to

TABLE 1 Sample demographics (N = 386).

	Mean (SD)
Age in years	49.04 (12.14)
Minutes since awakening to first cortisol sample	196.41 (60.95)
Hours since last meal to session start time	0.97 (0.91)
Total MET minutes per week	1463.54 (2069.36)
Body Mass index (kg/m <sup>2</sup> )	30.58 (7.47)
	n (%)
Gender (female)	208 (53.9%)
Race	
White	281 (72.80%)
Black and/or African American	22 (5.70%)
Native American or Alaska Native Aleutian Islander/Eskimo	6 (1.55%)
Asian	5 (1.30%)
Native Hawaiian or Pacific Islander	1 (0.26%)
Other	19 (4.92%)
Not reported/data missing	52 (13.47%)
Sex hormones (yes)	350 (9.3%)
Antihypertensive medication (yes)	253 (33.9%)

Note: Data on use of antihypertensive medication was missing for  $n = 3$  participants.

HRV Reactivity ( $p = 0.47$ ;  $p = 0.07$ ;  $p = 0.16$  respectively). Finally, number of hours since last meal to session start time ( $p$ 's  $> 0.06$ ), total number of MET minutes per week ( $p$ 's  $> 0.43$ ), and use of antihypertensive medication ( $p$ 's  $> 0.17$ ) were unrelated to any HRV or cortisol metrics.

### 3.2 | Differential patterns of cortisol responding

Examination of individual trajectories of cortisol across participants via LGMM revealed a 3-group solution. Table 2 presents all fit metrics (with and without the inclusion of covariates) for solutions estimating 1 through 6 classes. As model complexity increased to contain three classes, the log-likelihood, AIC, BIC, and SABIC values dropped. The 3-group solution with the inclusion of covariates showed the lowest BIC (11,028.63), retained acceptable latent group sizes ( $>5\%$ ), and was clinically interpretable. The 3-group solution was assessed in relation to the 4-group, 5-group, and 6-group solutions that yielded lower AIC and SABIC. Although the AIC and SABIC values dropped, the change was slight. Moreover, the addition of classes beyond three yielded models with higher BIC values and which all incorporated one or more class sizes that were relatively small ( $<5\%$ ). Thus, the 3-group model was selected for further examination in accordance with recommended guidelines of weighing BIC more heavily than AIC and retaining a smallest class of  $>5\%$  (Nguena Nguetack et al., 2020; van de Schoot et al., 2017).

Table 3 details final model specifications for the 3-group solution, which controlled for all covariates. The 3-group solution included a 'prototypical' subgroup based on expected rise and fall of cortisol over the testing session ( $N = 309$ , 80% of the sample), a 'decline'

subgroup that started high and declined throughout the testing session ( $N = 28$ , 7% of the sample), and a 'rise' subgroup that started low and increased throughout the testing session ( $N = 49$ , 13% of the sample) as shown in Figure 1. We also evaluated latent classes feeding outliers back into the model ( $n = 32$ ) to allow for the possibility that individual variability in extreme psychophysiological responses would alter results. Results remained unchanged such that a 3-group model quantified by similar distributions emerged as the best fit. Thus, in keeping with conventions of other published work (Pulopulos et al., 2018) we excluded outliers and report results accordingly.

In post-hoc analyses, we examined significant differences in cortisol across time within each of the subgroups. In the 'prototypical' group, cortisol was significantly higher at the timepoint immediately following the second cognitive stress task (Cort 3) with respect to the baseline (Cort 2) ( $t(308) = -2.73$ ,  $p < 0.01$ ), and higher with respect to the fourth (Cort 4)  $t(308) = 3.05$ ,  $p < 0.01$  and final timepoint (Cort 5) ( $t(308) = 4.59$ ,  $p < 0.01$ ). In the 'decline' group, cortisol was significantly lower at each subsequent timepoint following arrival

TABLE 3 Final latent growth mixture modelling model ( $N = 386$ ).

Trajectory intercept	Coef	SE	Wald	p-value
1 'Prototypical'	0	-	-	-
2 'Decline'	2.84	0.44	6.42	<0.001
3 'Rise'	-3.38	0.31	-10.76	<0.001

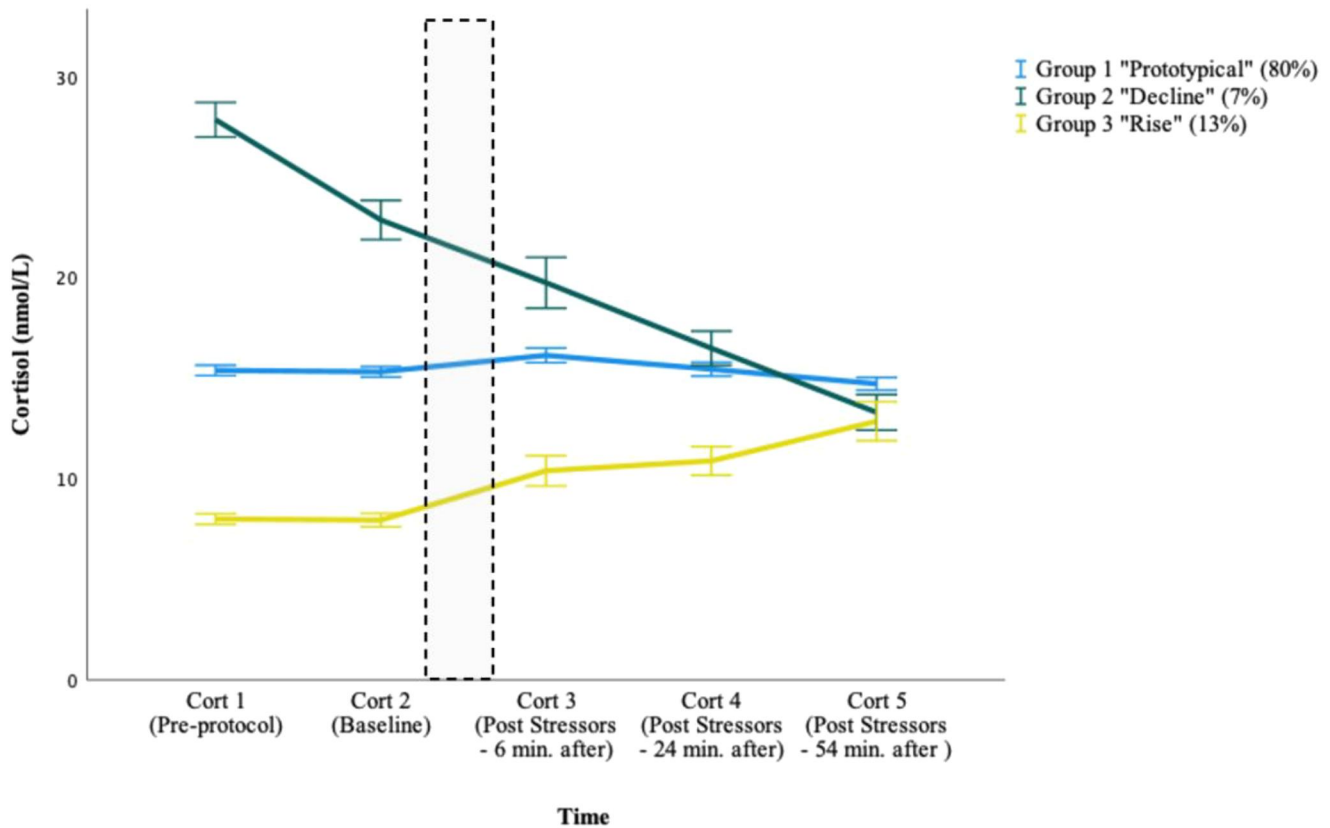
Abbreviations: Coef, coefficient; SE, standard error; Wald, Wald Statistic.

TABLE 2 Latent growth mixture modelling (LGMM) fit metrics ( $N = 386$ ).

# Classes	Log-likelihood	AIC	BIC	SABIC	Entropy	1	2	3	4	5	6
Without covariates											
1	-5521.08	11,058.17	11,089.81	11,064.43	1.00	100.00					
2	-5490.47	11,002.94	11,046.45	11,011.55	0.66	84.72	15.28				
3	<b>-5477.49</b>	<b>10,982.98</b>	<b>11,038.36</b>	<b>10,993.94</b>	<b>0.72</b>	<b>80.05</b>	<b>12.95</b>	<b>6.99</b>			
4	-5471.27	10,976.55	11,043.80	10,989.86	0.75	79.79	6.99	1.81	11.40		
5	-5468.20	10,976.39	11,055.51	10,992.05	0.67	43.52	7.77	1.55	37.56	9.59	
6	-5466.49	10,978.97	11,069.96	10,996.98	0.73	40.67	8.29	1.55	38.08	1.55	9.84
With covariates											
1	-5497.71	11,025.42	11,084.76	11,037.17	1.00	100.00					
2	-5464.89	10,965.78	11,036.99	10,979.87	0.68	15.28	84.72				
3	<b>-5451.78</b>	<b>10,945.56</b>	<b>11,028.63</b>	<b>10,962.00</b>	<b>0.73</b>	<b>80.05</b>	<b>12.69</b>	<b>7.25</b>			
4	-5446.08	10,940.15	11,035.09	10,958.94	0.76	78.50	12.69	1.81	6.99		
5	-5442.48	10,938.97	11,045.77	10,960.11	0.71	47.41	34.46	6.22	1.81	10.10	
6	-5438.97	10,937.95	11,056.62	10,961.44	0.72	20.73	11.66	0.78	10.10	2.59	54.15

Note: Bolded lines reflect final model selected.

Abbreviations: AIC, Akaike Information Criteria; BIC, Bayesian Information Criteria; SABIC, sample-size-adjusted BIC.



**FIGURE 1** Three cortisol trajectories identified in latent growth mixture modelling (LGMM) analysis: a 'prototypical' group characterised by expected rise and fall in cortisol in response to and recovery from stress ( $n = 309$ ), a 'decline' group ( $n = 28$ ) that remained significantly lower after stress compared to baseline, and a 'rise' group ( $n = 49$ ) that remained significantly heightened in cortisol after stress compared to baseline. Shaded area depicts when cognitive stress tasks occurred. Error bars reflect one standard error above/below the mean.

(Cort 1) ( $p$ 's  $< 0.01$ ). Finally, within the 'rise' group, cortisol at the timepoint immediately following the second cognitive stress task was significantly higher than baseline ( $t(48) = -3.47, p < 0.01$ ), and continued rising such that it was significantly higher at the final timepoint compared to immediately following the second cognitive stress task ( $t(48) = -2.93, p < 0.01$ ).

Finally, because we controlled for demographic differences in the LGMM analysis, we note that subgroups identified via LGMM did not differ with respect to age ( $p = 0.73$ ), gender ( $p = 0.13$ ), number of minutes since awakening to first cortisol sample ( $p = 0.16$ ), number of hours since last meal to session start time ( $p = 0.42$ ), total number of MET minutes per week ( $p = 0.76$ ), BMI ( $p = 0.19$ ), nor use of sex hormones ( $p = 0.70$ ).

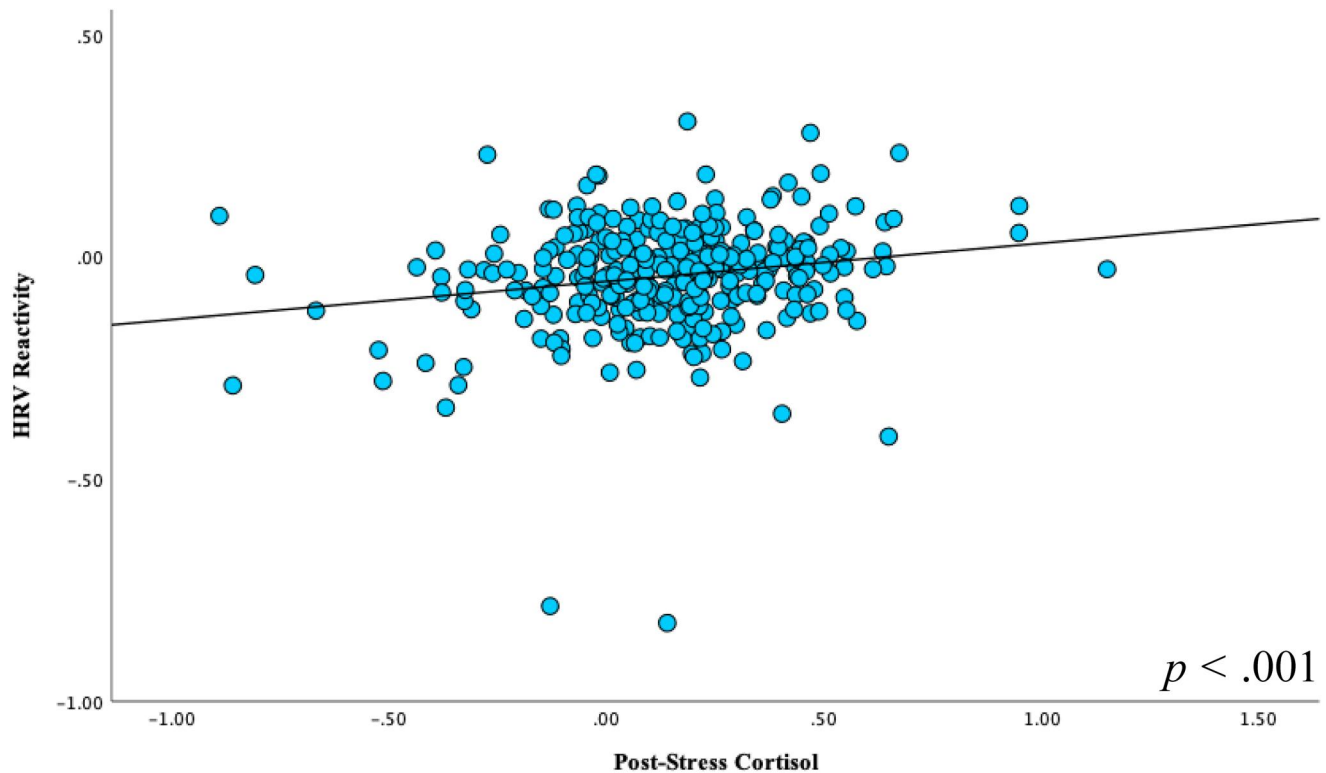
### 3.3 | Relationship between cortisol and heart rate variability within subgroups

We used a Bonferroni correction for multiple comparisons to test the relationship between cortisol and HRV within subgroups. The relationship between HRV Reactivity and  $\Delta$  stress-induced cortisol as well as  $\Delta$  post-stress cortisol was tested for each of the three subgroups, resulting in six tests total. Effects were considered significant

if they surpassed  $\alpha = 0.008$  ( $\alpha = 0.05/6 = 0.008$ ). Each of the relationships between cortisol and HRV was tested within each of the three subgroups. Significant relationships are presented in Figure 2. Additionally, in testing the relationship between HRV Reactivity and cortisol within subgroups we controlled for use of antihypertensive medication (1 = yes) as such medication could impact HRV.

Results showed a significant relationship between cortisol and HRV in the 'prototypical' subgroup only ( $n = 309$ ). For this group,  $\Delta$  post-stress cortisol was positively correlated with HRV Reactivity ( $r(306) = 0.19, p < 0.001, 95\% \text{ CI } [0.19, 0.68]$ ), indicating that a greater increase in HRV during stress was associated with a greater decrease in cortisol after stress from its maximum. Findings reflected a small effect size and indicate that, with frequent repeated sampling, 95% of the calculated means fall between 0.19 and 0.68. Notably,  $\Delta$  post-stress cortisol and HRV Reactivity were unrelated to one another in the 'decline' subgroup ( $n = 28$ ) ( $p = 0.551$ ) and the 'rise' subgroup ( $n = 49$ ) ( $p = 0.271$ ). Further,  $\Delta$  stress-induced cortisol was unrelated with HRV Reactivity across all three subgroups ( $p$ 's  $> 0.05$ ).

In exploratory analyses we examined whether combining those in the 'decline' and 'rise' subgroups ( $N = 77$ ) would assist in boosting power to detect differences. We still found no relationship between  $\Delta$  stress-induced and HRV Reactivity ( $r(75) = 0.20, p = 0.089$ ) nor  $\Delta$  post-stress cortisol and HRV Reactivity ( $r(75) = -0.13, p = 0.269$ ) in



**FIGURE 2** A significant relationship emerged between  $\Delta$  post-stress cortisol and heart rate variability (HRV) Reactivity for individuals defined by a 'prototypical' cortisol trajectory, while controlling for known covariates.

the combined 'atypical' group. Finally, we examined the relationship between cortisol and additional cardiovascular metrics (e.g., blood pressure and heart rate) implicated in the biological stress response and tested for differences between groups with regard to mental and physical health (please see Supplementary Material S1).

#### 4 | DISCUSSION

This investigation examined whether differential patterns of cortisol exist during acute stressors and subsequently tested whether an association existed between cortisol and HRV within subgroups qualified by differential patterns of cortisol responding. Several important results emerged: first, we found evidence of differential cortisol trajectories over the course of a 90 min stress protocol: three groups emerged with respect to their cortisol release qualified as a 'prototypical' group that exhibited the expected rise, peak, and fall of cortisol in response to stressors (i.e., peaking within 24–30 min of stress exposure and then falling towards baseline levels post-stress), a 'decline' group that started high and steadily declined, and a 'rise' group that started low, increased, and then continued to rise. Second, we found an association between cortisol and HRV that differed based on cortisol trajectories. In the 'prototypical' group, cortisol and HRV Reactivity were correlated. Specifically, higher HRV during the stress protocol was associated with a greater decline in cortisol after stressors compared to peak stress task-induced levels. This

relationship was limited to specific timepoints, such that  $\Delta$  stress-induced cortisol was not associated with HRV. Finally, this relationship only emerged in individuals who exhibited a 'prototypical' cortisol response to stress and was absent in those whose trajectories deviated from this response (i.e., "rise" and "decline" groups). Together, these findings indicate that a relationship between cortisol and HRV changes in response to stress may exist, but might be constrained to a 'prototypical' cortisol response, while deviations from this pattern might be associated with a disruption in the relationship with HRV.

In additional analyses we examined the relationship between cortisol and other cardiovascular measures (e.g., blood pressure and heart rate) (see Supplementary Material S1). In doing so we demonstrated that greater rise in blood pressure during the stress task was related to greater decrease in cortisol at recovery but that this relationship again only existed in individuals within the prototypical group. This suggests that the stress task was physiologically impactful in these individuals. That these relationships were absent in the atypical cortisol groups further points to the possibility that the relationship between biological metrics of the stress response system may not be coordinated in individuals who fail to exhibit a prototypical cortisol response.

In addition, we also demonstrated that HRV Reactivity was negatively correlated with HR Reactivity, in the prototypical and rise groups, suggesting that HRV metrics reported herein were vagally mediated.



Understanding interindividual differences in cortisol responding is critical given links of atypical (blunted or heightened) responding and the onset and progression of physical and mental health disorders (Coyle et al., 2020; Henckens et al., 2016; Jones & Gwennin, 2021; McEwen, 2008). Findings reported herein add to the literature on interindividual differences in cortisol responding. Importantly, we found differential cortisol response trajectories even when controlling for many variables that are known to influence cortisol response, such as age, gender, time since waking, time since last meal, fitness, and use of sex hormones, demonstrating that variability in cortisol response occurs beyond influence of such factors. While variability in magnitude of cortisol secretion is well-researched, differential trajectories have received little attention (Felt et al., 2017). Here, we found evidence for three distinct trajectories of cortisol response to stress aligned with limited prior work conducted in smaller samples also examining trajectories. For example, Van Ryzin et al. (2009) used group-based modelling to examine diurnal salivary cortisol at rest in children ( $N = 106$ ) and found evidence for three distinct trajectories, qualified by a 'normative' pattern of change and two 'atypical' patterns (i.e., a blunted and a heightened cortisol response). These authors compared group-based modelling to other analytic approaches and concluded that trajectory analysis is particularly appropriate for detecting changes in heterogeneous (vs. homogeneous) samples which may be especially relevant for psychiatric populations, who often present with heterogeneous psychopathology (Van Ryzin et al., 2009). Notably, this referenced study examined only resting cortisol and did not evaluate acute stress responses. Studying cortisol responses to a prolonged acute stress task, Admon et al. (2017) found three cortisol trajectories in a sample of healthy women ( $N = 79$ ), qualified by 'hyper-response,' 'moderate-response,' and 'mild-response' groups. These authors found that the trajectories were associated with differential patterns of affective response to stress such that the 'moderate-response' group demonstrated less  $\Delta$  stress-induced negative affect compared to the 'hyper-response' and 'mild-response' groups (Admon et al., 2017). Thus, our findings extend the findings of multiple cortisol trajectories from prior work to a larger and more diverse sample (i.e., across genders). Notably we point out that the trajectories identified here differed in their starting cortisol levels at the baseline time period prior to the acute stressors. That these differences emerged at the first timepoint despite controlling for covariates (e.g., time since awakening), indicates that there are undetermined variables yet to be explored that may be related to interindividual differences in basal cortisol levels. The use of growth curve modelling for cortisol trajectories is gaining traction. Recent models have been developed using an advanced modelling approach that is stochastic and which take into account more precise changes in known pharmacokinetics that influence cortisol concentrations (Miller et al., 2018). These changes are time-dependent and are also influenced by starting basal levels. In accordance, it is becoming increasingly recognized that more common approaches to quantifying cortisol, such as using an area under the curve approach, may

be insufficient for capturing important variance in the cortisol response.

Such findings also add to prior work in a sample of healthy adults which found greater HRV during preparation for a stressor was associated with less  $\Delta$  stress-induced cortisol (Pulopulos et al., 2018). Our results show that this pattern may also extend to one whereby higher HRV during stress is related to decline in cortisol after stress. Evidence here of a significant relationship between cortisol and HRV across stress (during and after) may be related to the fact that this analysis was sufficiently powered to detect such effects, while prior work was conducted in comparatively smaller samples ( $N = 213$ , Murdock et al., 2017;  $N = 171$ , Pulopulos et al., 2018). By employing a data-driven approach to study how cortisol was differentially changing in response to stress across individuals and demonstrating significant effects within some—but not all—groups, suggests that prior studies that failed to find such associations may benefit from considering 'prototypical' versus 'atypical' cortisol response patterns. Notably, greater HRV during stress (an adaptive response) being related to less cortisol after stress (also an adaptive response) in the 'prototypical' group offers empirical evidence in support of literature theorising an inhibitory role of vagal activity on the regulation of the HPA-Axis stress response (Thayer & Sternberg, 2006). Such a relationship may be related to the return of the body to homeostasis, as evidenced by a decline in cortisol after stress. If true, this suggests that targeting the interplay between cortisol and HRV may be an advantageous therapeutic for governing prototypical response to stress. Future work is needed to test whether there is a mechanistic link between HRV biofeedback training and changes in cortisol.

Importantly, the relationships between cortisol and HRV that emerged in these groups are correlational and do not imply causation. While these results point to the possibility that a compensatory relationship between HRV during stress with cortisol after stress causes 'prototypical' cortisol responding, another equally likely possibility is that exhibiting a 'prototypical' cortisol response facilitates a relationship between HRV during stress and cortisol after stress. Additional work is needed in samples who do not follow a 'prototypical' cortisol stress response to understand how cortisol relates to HRV in these individuals. Furthermore, results of the present study do not allow for inferences as to whether the two 'atypical' response patterns are associated with dysregulation of the HPA-Axis or otherwise reflective of maladaptive responding linked with negative health outcomes. Here, we failed to find differences in some mental and physical health metrics among our groups (see Supplementary Material S1). Though notably the atypical groups constituted smaller sample sizes and therefore may lack power to detect effects. Thus, future work is necessary to uncover whether such response profiles hold any deleterious health implications or alternatively represent healthy variability in cortisol responding in larger samples.

In addition to the above effects, we also found evidence of demographic differences in cortisol and HRV response to stress. Effects reported here, specifically that cortisol was lower in individuals using sex hormones and that men had greater cortisol compared to women, aligns with previous work (Foley & Kirschbaum, 2010; Zorn

et al., 2017). Additionally, the association between higher BMI and greater  $\Delta$  post-stress cortisol supports previous work showing links between higher BMI and greater cortisol output (Jackson et al., 2017). Finally, we also found that individuals who had a fewer number of minutes since awakening to first cortisol sample exhibited greater HRV Reactivity, as did those who did not use sex hormones (compared to those who did). Notably, in trajectory analyses we added these covariates, controlling for them in the model; yet, different trajectories of cortisol were still evident in our sample. This suggests that there are unaccounted factors other than age, gender, sex hormones, BMI, and time of awakening relative to first cortisol sample that define individuals into different stress response patterns.

The present study is not without limitations. Of note, the protocol herein lacks a clear anticipatory stress phase and thus does not allow for an investigation of HRV during anticipatory stress, but which has been demonstrated to relate in temporally distinct ways to cortisol stress responding (Pulopulos et al., 2018). Additionally, participants were largely racially homogenous (over 80% white) and more work is needed to examine 'prototypical' response to stress in racially diverse populations (Sin et al., 2016). It is also important to contextualise individuals' biological stress response in this sample to relative age given that this sample was an older demographic. Notably, HRV tends to decrease with age (Umetani et al., 1998). The mean age in the present sample was 49 and thus findings herein may look different in younger adults.

## 5 | CONCLUSIONS

The present study demonstrated differential trajectories of cortisol responding during a laboratory stress protocol in a large sample of adults. Results show relevance for studying individual differences in the biological response to stress through cortisol, such that individuals may qualify by either 'prototypical' or 'atypical' responding. Such qualifications matter, in that we found that cortisol and HRV were related to one another in individuals with prototypical cortisol response, while these variables were unrelated to one another in individuals who deviated from this response. This suggests that trajectory differences in cortisol response to stress may be meaningful and that such trajectories may be qualified by a functional relationship between cortisol and HRV. Results may provide an avenue for clarifying our understanding of individual differences in the biological stress response, a necessary precursor to advancing therapeutics aimed at prevention and treatment of maladaptive stress responses and associated deleterious health outcomes.

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## CONFLICT OF INTEREST STATEMENT

The authors state that there are no conflicts of interest associated with the research.

## DATA AVAILABILITY STATEMENT

Publicly available data from the MIDUS study were used for this research and can be accessed here: <https://www.midus.wisc.edu>.

## INFORMED CONSENT

Harvard University, Georgetown University, the University of California at Los Angeles, the University of Wisconsin institutional review boards, and the University of California at Los Angeles authorised the MIDUS Refresher. All MIDUS refresher participants provided written informed consent.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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