Heart rate fragmentation: A novel analytic approach to early allostatic load detection among healthy adults

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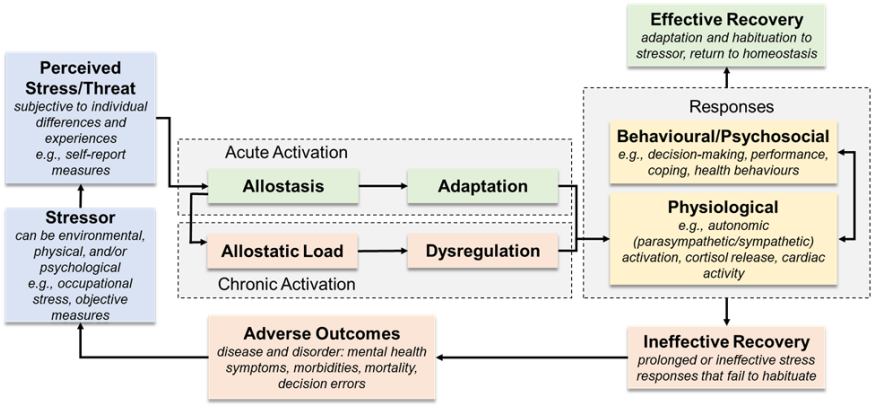
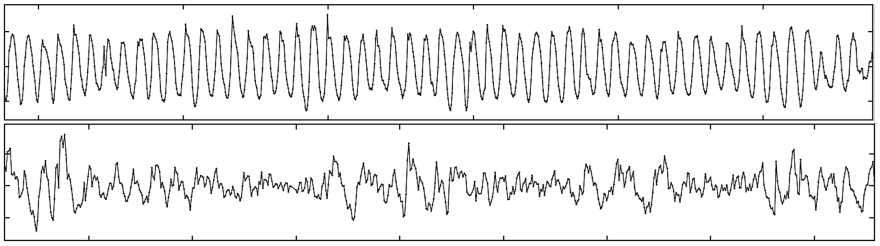
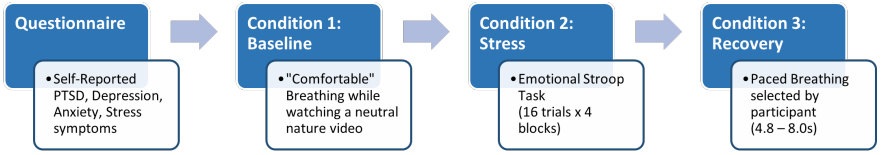
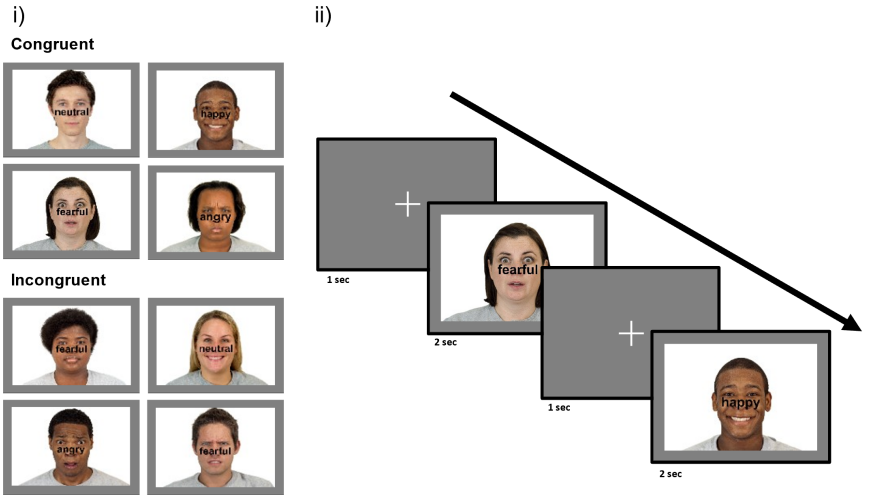
Abstract

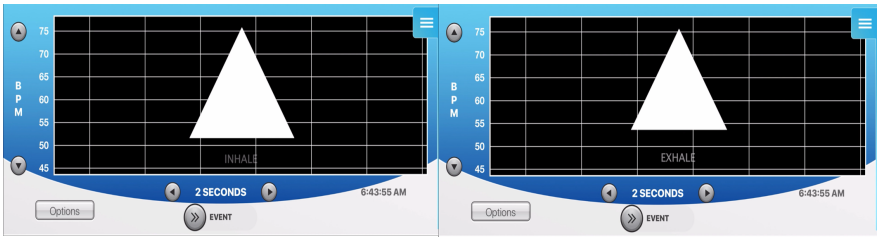
The current study explores an emerging cardiac metric, heart rate fragmentation (HRF), as a novel biomarker for allostatic load (AL). HRF may better address the limitations of existing cardiac biomarkers (e.g., confounds and interpretation consistency) in applied research settings, with nonclinical samples. The study's objectives were: 1) can HRF represent response to psychological stress and 2) can resting HRF be used as a measure of predicting subclinical mental health symptoms. One hundred and fifty-six (n = 156; 75% female) undergraduate students were fitted with a chest band to monitor cardiovascular activity, and completed online demographic and psychosocial surveys in which they were grouped as healthy or displaying probable mental health symptoms (pMH; n = 94, 60.25%) based on respective inventory thresholds for depression, anxiety, and posttraumatic stress disorder. Cardiovascular activity was measured capturing the three R's of cardiac vagal control: a resting baseline, a reactive acute stressor task, and a paced breathing recovery. Results supported the first hypothesis, in that that HRF significantly differentiated between each RRR condition ($p < 0.001$). While healthy and pMH individuals did not significantly differ within individual conditions, exploratory analyses revealed healthy individuals displayed significantly larger change in HRF reactivity between conditions (p's < 0.001) in comparison to pMH, which displayed a more blunted pattern. Overall, this study establishes associations between HRF and mental health, and serves as a promising new biomarker that may identify AL in samples that may be otherwise considered "healthy", while addressing the limitations of prior biomarkers in non-clinical studies.

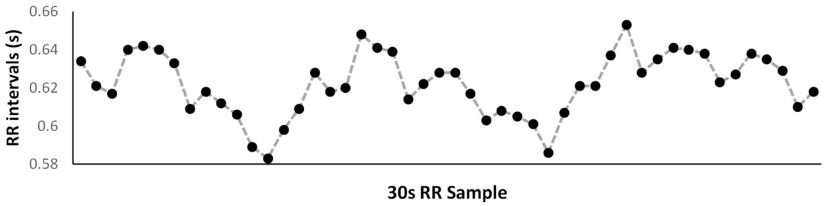


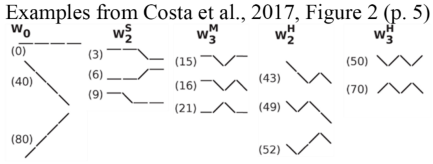


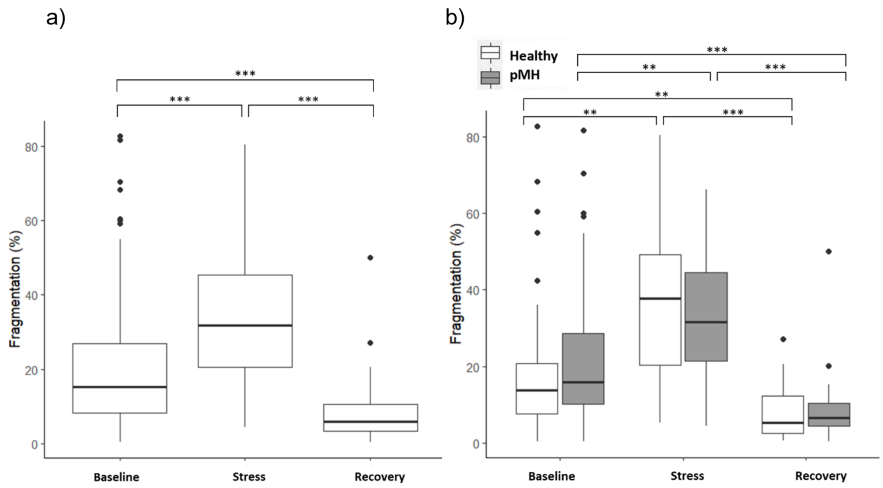








Process	**Example**
$\boldsymbol{RR\ intervals\ (s) = RR_1, \dots, RR_i}$	0.634, 0.621, 0.617, 0.64, 0.642, 0.64, 0.633, 0.609, 0.618, 0.612, 0.606, 0.589, 0.583, 0.598, 0.609, 0.628, 0.618, 0.62, 0.648, 0.641, 0.639, 0.614, 0.622, 0.628, 0.628, 0.617, 0.603, 0.608, 0.605, 0.601, 0.586, 0.607, 0.621, 0.621, 0.637, 0.653, 0.628, 0.635, 0.641, 0.64, 0.638, 0.623, 0.627, 0.638, 0.635, 0.629, 0.61, 0.618
$\boldsymbol{\Delta RR's = (RR_2 - RR_1), \dots, (RR_{i+1} - RR_i)}$	-0.013, -0.004, 0.023, 0.002, -0.002, -0.007, -0.024, 0.009, -0.006, -0.006, -0.017, -0.006, 0.015, 0.011, 0.019, -0.01, 0.002, 0.028, -0.007, -0.002, -0.025, 0.008, 0.006, 0, -0.011, -0.014, 0.005, -0.003, -0.004, -0.015, 0.021, 0.014, 0, 0.016, 0.016, -0.025, 0.007, 0.006, -.001, -0.002, -0.015, 0.004, 0.011, -0.003, -0.006, -0.019, 0.008
Symbolic Mapping of ΔRR "letters" where: $\boldsymbol{Deceleration = 5ms \leq \Delta RR = 1}$ $\boldsymbol{No\ change = -5ms < \Delta RR < 5ms = 0}$ $\boldsymbol{Acceleration = \Delta RR \leq -5ms = -1}$	-1, 0, 1, 0, 0, -1, -1, 1, -1, -1, -1, -1, 1, 1, 1, -1, 0, 1, -1, 0, -1, 1, 1, 0, 1, -1, 1, 0, 0, -1, 1, 1, 0, 1, 1, -1, 1, 1, 1, 0, 0, -1, 0, 1, 0, -1, -1, 0
Grouping letter symbols into rolling 4-letter "words" by number and type of inflection points Word categorization: $\boldsymbol{RR\ Word = w_j^t}$ *where:* *t = the type of inflection points in the word* *H = only hard* *S = only soft* *M = soft and hard* *j = the number of inflection points*	First 10 "words" and their pattern category -1 0 1 0 w_3^S 0 1 0 0 w_2^S 1 0 0 -1 w_2^S 0 0 -1 -1 w_1^S 0 -1 -1 1 w_3^S -1 -1 1 -1 w_2^H -1 1 -1 -1 w_2^H 1 -1 -1 -1 w_1^H -1 -1 -1 -1 w^0 -1 -1 -1 1 w_1^H
Schematic representations of words *"/" = HR acceleration* *"\" = HR deceleration* *"—" = no change*	Examples from Costa et al., 2017, Figure 2 (p. 5) 



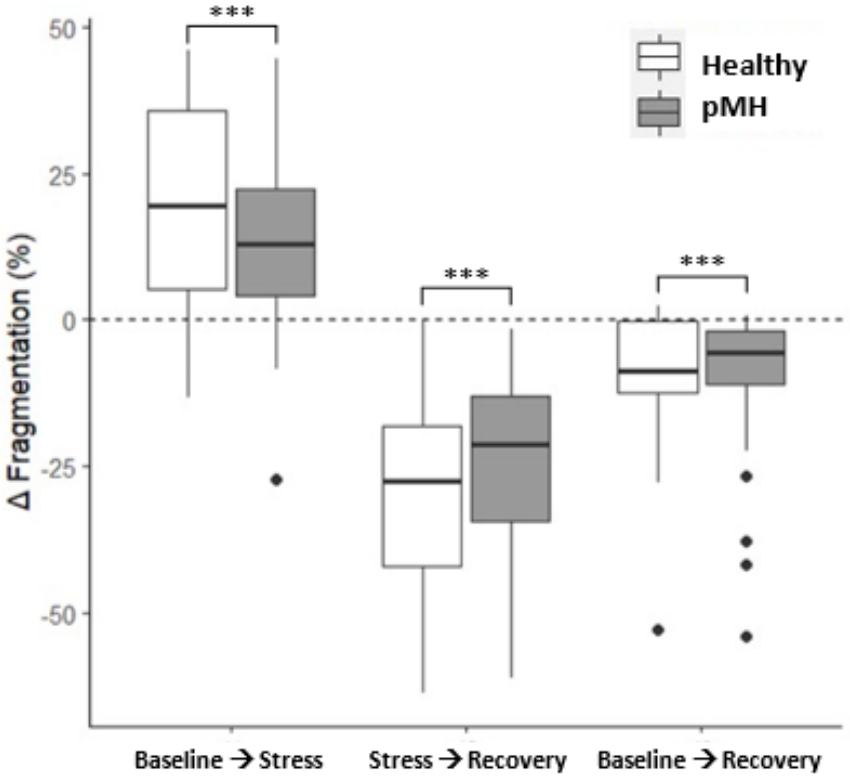


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Table 1 HRF_JFC_JPA.docx available at https://authorea.com/users/696692/articles/685088-heart-rate-fragmentation-a-novel-analytic-approach-to-early-allostatic-load-detection-among-healthy-adults

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Table 2 HRF_JFC_JPA.docx available at https://authorea.com/users/696692/articles/685088-

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Heart rate fragmentation: A novel analytic approach to early allostatic load detection among healthy adults.

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Abstract

The current study explores an emerging cardiac metric, heart rate fragmentation (HRF), as a novel biomarker for allostatic load (AL). HRF may better address the limitations of existing cardiac biomarkers (e.g., confounds and interpretation consistency) in applied research settings, with nonclinical samples. The study's objectives were: 1) can HRF represent response to psychological stress and 2) can resting HRF be used as a measure of predicting subclinical mental health symptoms. One hundred and fifty-six (n = 156; 75% female) undergraduate students were fitted with a chest band to monitor cardiovascular activity, and completed online demographic and psychosocial surveys in which they were grouped as healthy or displaying probable mental health symptoms (pMH; n = 94, 60.25%) based on respective inventory thresholds for depression, anxiety, and posttraumatic stress disorder. Cardiovascular activity was measured capturing the three R's of cardiac vagal control: a *resting* baseline, a *reactive* acute stressor task, and a paced breathing *recovery*. Results supported the first hypothesis, in that that HRF significantly differentiated between each RRR condition ($p < 0.001$). While healthy and pMH individuals did not significantly differ within individual conditions, exploratory analyses revealed healthy individuals displayed significantly larger change in HRF reactivity between conditions (p's < 0.001) in comparison to pMH, which displayed a more blunted pattern. Overall, this study establishes associations between HRF and mental health, and serves as a promising new biomarker that may identify AL in samples that may be otherwise considered "healthy", while addressing the limitations of prior biomarkers in non-clinical studies.

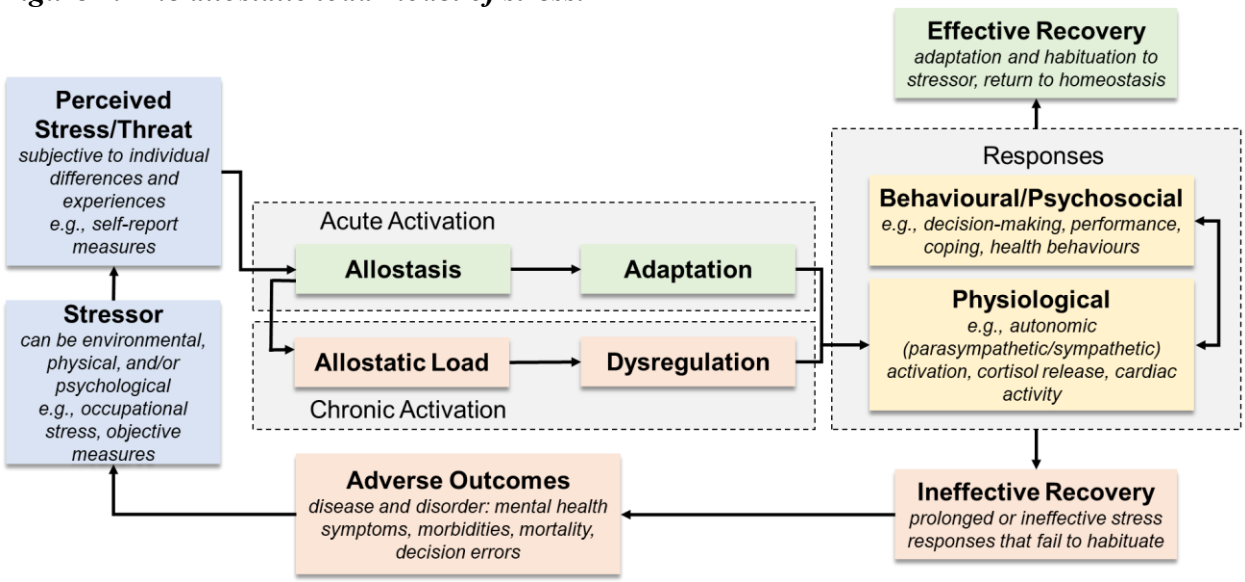
1 Introduction

According to the allostatic load (AL) model of stress, repeated physiological overactivation of the stress response creates 'wear and tear' on the body, resulting in system-wide dysregulation and an inability to adaptively respond to new challenges (McEwen & Stellar, 1993). Over time, elevated allostatic load leads to health disorders and diseases (Juster, McEwen, & Lupien, 2010). For example, physical issues such as cardiometabolic diseases are common (e.g., hypertension, coronary heart disease, diabetes), as are impaired immune responses, slower healing, and severity of viral illness (e.g., H1N1, SARS, and COVID-19) (O'Connor, Thayer & Vedhara, 2020; Glaser et al., 1994, 2000, 2005; Dantzer, 2009; Marsland et al., 2017; Tisoncik et al., 2012; Vasileva & Badawi, 2019; Ye et al., 2020). The rewiring of neuroendocrine and autonomic nervous system (ANS) function associated with AL are also associated with mental health symptoms (e.g., anxiety, depression, posttraumatic stress injury or disorder-PTSD). Mental health symptoms are associated with dysregulations in circulating cortisol that is linked to higher levels of inflammation. In turn, inflammatory states are associated with the exacerbation of mental health symptoms, burnout, and lower cognitive control (Valkanova et al., 2013; Rief et al., 2001; Toker et al., 2005; Shields et al., 2015).

The AL model recently celebrated its 30th anniversary. Over the decades, the literature has evolved along with scientific discoveries that support the exploration of new biomarkers for calculating AL in research and clinical settings in order to better detect health risk and prevent adverse health trajectories across the lifespan. Importantly, recent work calls for a shift in focus to include examining AL among non-clinical samples in field settings with more accurate and valid biomarkers (Juster & Misiak, 2023). The early detection of allostatic load paves the way

for prevention and early intervention efforts to reduce adverse health symptoms and the repeated reliance on clinical care.

Figure 1. The allostatic load model of stress.



Adapted from McEwen's 1998 allostatic load model.

1.1 Allostatic load model of stress and cardiovascular activity

Broadly, dysregulated cardiovascular reactivity is often associated with several biopsychosocial health risks, including obesity, addiction, depression symptoms, and reduced cognitive ability (Carroll et al., 2017). As demonstrated by the literature on AL, stress, health, and autonomic nervous system (ANS) efficacy are often indexed by heart rate variability (HRV) (Laborde et al., 2017; Thayer et al., 2012). Dysregulated resting HRV values are significantly associated with cardiovascular risk and PTSI symptoms (e.g., anxiety, depression, and PTSD) as measured by indicators such as physiological hyperarousal and elevated heart rate (Billman, 2013; Shaffer & Ginsberg 2017; Williams et al., 2017; Violanti et al., 2007; Thayer & Friedman, 2004; Menning, Seifert, & Maercker, 2008; Liu et al., 2016).

1.2 Pitfalls of heart rate variability measures

Biomarkers are advantageous for use in psychological research, as they add a layer of objective measures to subjective observations, providing insights for understanding mechanisms of disease and possible strategies for intervention and research. When identifying diagnostic biomarkers as effective for predicting disease, it is necessary for the marker to be both sensitive (correctly identify individuals with disease–few false negatives) and specific (correctly identify negative individuals, few false positives) (Swift et al., 2020). There are three main confounding factors of HRV measures discussed in this study: context of use and interpretation, age, and respiration.

Many HRV metrics are high in sensitivity and specificity when measured in clinical settings, but confounding factors reduce HRV's predictive capabilities beyond extremely constrained contexts, even between experimental studies (Laborde et al., 2017). When seeking accurate HRV measures, considerations such as recording method, sampling frequency, the removal of artifacts, and the context in which HRV data is collected is crucial for interpretation. Specifically, individual factors such as body position, movement, recent physical activity, and other factors significantly impact ANS regulation and associated HRV measurements (Shaffer & Ginsberg, 2017). If many factors impact HRV measures, increasing its sensitivity, it ultimately limits HRV's specificity and predictive value beyond the environment, population, and context it is measured in.

Several standard measures of HRV display paradoxical relationships with age. For example, despite the deterioration of cardiac parasympathetic function that occurs with aging, HRV observably improves in the elderly starting around age 75 (Shaffer & Ginsberg 2017; Hayano et al., 2020; Arakaki et al., 2023). Such paradoxical relationships suggest that HRV does not accurately represent vagal tone or ANS function across individuals and thus the metrics may

overestimate its accuracy as a proxy of ANS activation and modulation (Heathers & Goodwin, 2017; Hayano & Yuda, 2019).

Respiration is a particularly frequent confounding factor in HRV measurement. Research has found that respiration impacts HRV outcomes, with specific implications for SDNN (standard deviation of N to N intervals), RMSSD (root mean square successive differences), and RSA (respiratory sinus arrythmia), respectively (Shaffer & Ginsberg, 2017). SDNN and RMSSD are respectively long and short-term time-domain-based HRV measures to index vagal tone. SDNN is considered the gold standard for 24-hour medical recordings, but impractical for application in naturalistic occupational environments. RMSSD does not reflect increases in HRV during deep breathing, making it a poor indicator of parasympathetic reactivity (Ali et al., 2023). Another popular HRV measure respiratory sinus arrythmia (RSA) does account for respiration, as healthier RSA functioning involves coupling between breathing and heartrate. However, RSA is still impacted by the other aforementioned factors (Hayano & Yuda, 2019). However, RSA does not necessarily represent vagally driven HRV modulation. Not only are RSA and HR regulated by different vagal motor neurons, respiratory parameters' effects on RSA act independently of cardiac vagal activity; "healthy" sinus rhythms (gradual HR changes) that are vagally driven can still occur when HR and respiration are not coupled (Hayano et al., 1994, 2019). While sinus rhythm regulation decreases with aging and organic heart disease, high-risk groups can also display paradoxical increases in HRV. Furthermore, respiratory sinus arrhythmias are difficult to identify from electrocardiogram (ECG) recordings alone (Costa et al., 2017), requiring an added layer of interpretation that can be difficult for transferability from clinical to naturalistic settings.

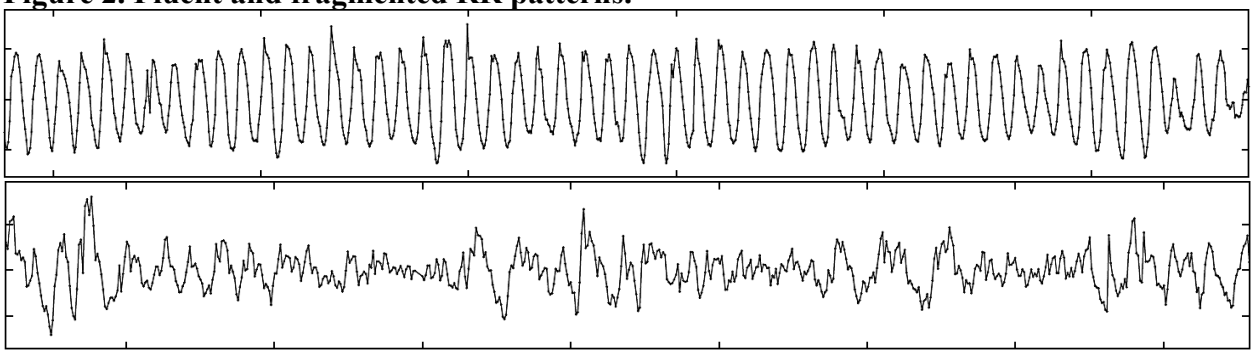
Despite a growing body of literature critiquing the use of HRV to index ANS activity (Costa et al., 2017; Hayano & Yuda, 2019; Hayano et al., 2020; Ali et al., 2023), the number of

publications using HRV to represent psychological constructs (e.g., cognition, neurological health and stress) has sharply increased in the last 2 decades (Arakaki et al., 2023); thus increasing the need for the exploration of more accurate biomarkers in field settings. One proposed approach to identifying novel cardiac biomarkers is to shift from focusing solely on linear time-domain metrics, to instead assessing dynamic HR pattern signatures. From this, an emerging metric of short-term cardiovascular function, called heart rate fragmentation, has been developed by Costa and colleagues (Costa et al., 2017).

1.3 Heart rate fragmentation

Heart rate fragmentation (HRF) is defined as increased density of HR acceleration sign changes, with higher fluctuations or fragmentation indicating the breakdown of regulatory control networks involved in heart rate dynamics (Costa et al., 2017). HRF is considered to be less impacted by the limitations of HRV metrics reviewed above. HRF relies on the framework model that healthy, adaptive HR control requires hierarchal contribution between the ANS (PNS) and electrophysiologic cardiovascular components. Higher integrity of these networks allows for more gradual (fluent) changes in HR, whereas dysfunction and breakdown of physiological coupling results in more erratic (fragmented) sinus rhythms. Breakdown of system components of cardiovascular control networks result in high frequency fluctuations in HR that compete or exceed the vagal system's shortest term modulatory response. This breakdown of physiologic coupling can be visually identified in RR or NN recordings as abrupt changes in HR acceleration signs (see Figure 2).

Figure 2. Fluent and fragmented RR patterns.



Examples of fluent (top) and fragmented (bottom) RR recording samples from the current study.

In more extreme examples, high fragmentation can result in acceleration changes can happen at every beat, resulting in a "sawtooth" pattern. These patterns may act as identifiable dynamic signatures, that may be further emphasized by converting them into symbolic representations, which deemphasize the magnitude of the time domain (Costa et al., 2017). Thus, HRF can be applied as a mathematical representation of AL while addressing various limitations of other cardiac biomarkers, like movement and respiration artifacts.

Unlike HRV metrics, higher HRF frequency is significantly associated with coronary artery disease and adverse cardiovascular event incidents (Costa et al., 2017, 2018; Omoto et al., 2021), type 2 diabetes (Galdino et al., 2023), and higher mortality (Lensen et al., 2020). Higher resting HRF is negatively associated with cognitive performance on the Cognitive Abilities Screening Instrument (CASI), processing speed (digit symbol coding), and working memory (digit span) (Costa et al., 2021). However, while HRF has been increasingly associated with the identification of physiological stress outcomes among clinical samples, the application of HRF measurement in psychological research on stress and mental health remains a gap in the literature (see Table 1).

Table 1. Current themes in heart rate fragmentation research.

Citation *Sample*	**Main Finding(s)**
Costa et al., 2017 *Cardiovascular disease (CAD) (n = 271) and healthy patients (n = 202)*	• HRF increases with age in healthy and patients with CAD • HRF is higher in CAD patients than healthy patients • Older healthy patients have significantly more soft inflections, whereas older CAD patients have more hard inflection points • HRF outperforms short-term HRV indices (e.g., pNNx, RMSSD, SDSD, HF), and nonlinear measures (sample entropy, detrended fluctuation analysis short term exponent) distinguishing healthy and CAD patients
Costa et al., 2018 Multiethnic study of atherosclerosis (*MESA) Cohort (n = 1963)*	• Increased HRF was significantly associated with increased adverse cardiovascular events (CVE) • Traditional HRV (e.g., total spectral power, LF/HF ratio) and fractal indices were not associated with CVE or death
Hayano & Yuda, 2019 *Literature Review*	• Critically reviewed HRF as a phenomenon which confounds other HRV metrics, although the exact mechanisms for HRF are unclear • Suggests contribution sinoatrial degeneration or disorganization of the sinus node
Hayano et al., 2020 *ALLSTAR Holster ECG Database (3917 24-h RR data)*	• 24-h HRF is observed in childhood (0–20), increases after 75-years, but largely impacts individual HF differences at age 60–90
Lensen et al., 2020 *Cardiology clinic patients (n = 2893)*	• Increased HRF along with other risk factors are independently associated with poorer survival and 2-year mortality
Costa et al., 2021 *MESA cohort (n = 1897)*	• Increased HRF during sleep was associated with decreased cognitive performance (cognitive abilities screening instrument) and processing speed (digit symbol encoding)
da Silva et al., 2021 *Wistar rats* (n = 18)*	• PIP and w_3 HRF increased while w_1 decreased after pharmacological autonomic blockade. Blockade decreased w^H and increased w^S. • Suggests cardiac sympathetic and parasympathetic influences similarly decrease HRF, while parasympathetic control increases hard inflection points, pointing towards cardiac autonomic control in HRF
Omoto et al., 2021 *Myocardial Infarction (MI) (n = 18) and sham operation (n = 20) rats**	• MI operation increases HRF and cardiac function at both 4- and 12-weeks following operation

Galdino et al., 2023 *Type 2 diabetes mellitus (T2DM)* (n = 82) and healthy* patients (n = 82)*	• T2DM Patients had higher PIP, w^S w^M and w_3, and lower w^H and w_1 than healthy participants • Words grouped by inflection type (i.e., soft, mixed, hard) are more closely related to HRV measures (RMSSD and HF indices)
Arakaki et al., 2023 *Literature Review*	• The concept of HRF discussed primarily in respect to Costa and colleagues' findings, future studies needed to clarify the role of HRF versus contributions to HRV

(*) = all male sample, PNNx = , RMSSD = root mean square of successive differences, SDSD = standard deviation of successive differences, HF = high frequency spectrum, LF/HF = low frequency to high frequency ratio, PIP = percentage of inflection points, w^S, w^M, w^H = soft, mixed, hard inflection points respectively, w_1,w_2, w_3 = 1, 2, 3 grouped inflection points respectively

1.4 The Present Study

The current study explores an emerging cardiac metric heart rate fragmentation (HRF) as a novel biomarker for allostatic load (AL) that better addresses the limitations of existing cardiac biomarkers (e.g., confounds and interpretation consistency) in an applied research setting, with a nonclinical sample. The study's objectives were: 1) can HRF represent response to psychological stress and 2) can resting HRF be used as a measure of predicting subclinical mental health symptoms. Building upon prior literature, the specific hypotheses were:

1) Based recommendations to assess the "three R's" of cardiac vagal control: Resting, reactivity, and recovery to assess different levels of adaptability to stress (Laborde et al., 2018), we predict that acute psychological stress will be associated with an increase in the frequency of HRF in comparison to rest and recovery states,.
2) Individuals with mental health symptoms will exhibit higher HRF overall in comparison to individuals with mild or no mental health symptoms, in alignment with the AL model.
3) Exploratory analyses will assess HRF reactivity to stress in healthy versus individuals with probable mental health symptoms (pMH).

2 Method

2.1 Participants

A total of one hundred and fifty-six (n = 156) undergraduate psychology students were recruited from the University of Toronto. All participants provided informed consent in accordance with study procedures approved by the University of Toronto Research Ethics Board. Exclusion criteria were minors (under age 18), clinically diagnosed mental health, cardiovascular, or immune conditions. Refer to Table 2 for complete demographic data.

Table 2. Demographic and descriptive summary (n = 156).

			N (% of 156)	M(SD)
Age				20.18 (2.59)
Sex	*Male*		36 (23.08%)	
	Female		118 (75.64%)	
	Other/Unspecified		2 (1.28%)	
Race	*Asian (East)*		35 (22.44%)	
	Asian (South)		38 (24.36%)	
	Black/African American		13 (8.33%)	
	White/Caucasian		32 (20.51%)	
	Hispanic		3 (1.92%)	
	Indigenous		1 (0.64%)	
	Other/Unspecified		11 (7.05%)	
	Multiracial		23 (14.74%)	
Cardiovascular Measures	*Baseline HRF %*			20.00% (16.30%)
	Stress HRF %			33.61% (17.32%)
	Recovery HRF %			8.09% (7.72%)
Psychosocial Measures	*PCL-5*			31.08(17.03)
	DASS21-D			7.38(5.54)
	DASS21-A			6.84(4.87)
	≥ threshold score	*PTSD*	69 (44.23%)	
		Depression	66 (42.30%)	
		Anxiety	74 (47.43%)	
		pMH	94 (60.25%)	

M = mean, SD = standard deviation, participants who indicated more than one racial background were categorized as multiracial.

2.2 Procedure

All participants completed the experiment in-person on a computer located in the laboratory testing space. Following providing informed consent, participants were fitted with an HR chest band to monitor cardiovascular activity. Participants completed a series of online self-reported demographic and psychosocial surveys. Following the psychosocial survey, participants were given the opportunity to resolve any physical discomfort (e.g., use the bathroom, drink some water) before the cardiovascular measures began. All condition start times were recorded. Consistent with literature recommendations for HRV experimental structure that encompasses the three R's of cardiac vagal control (i.e., rest, reactivity, recovery) (Laborde et al., 2017, 2018; Juster et al., 2010, 2023), cardiovascular recordings were extracted across three conditions:

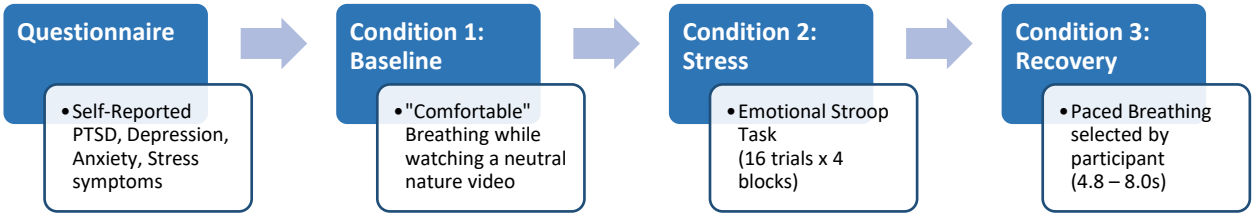
- ***Baseline:*** participants watched a 7-minute neutral nature video to collect "resting" cardiovascular activity, in which they were simply instructed to breathe at a comfortable pace (i.e., spontaneous breathing).
- ***Stress:*** A Face-Word version of an emotional Stroop task (Ovaysikia et al., 2011; Haas et al., 2006) was presented to the participants as an acute stressor. The Stroop task has been shown to significantly elicit sympathetic arousal (Ovaysikia et al., 2011; Haas et al., 2006). Participants were presented with male and female faces with happy, neutral, angry, or fearful facial expressions. Facial expressions were superimposed with the emotional word at a 90-degree angle over facial image that were either congruent (facial expression and word described same emotion) or incongruent (facial expression and word described a different emotion). Participants were instructed to press buttons to report the emotion displayed to them as quickly as possible. Prior to each block, participants were presented with written instructions on

the screen to either report the facial expression, or the written word. Each trial consisted of a 1 second fixation cross, followed by the stimuli image for 2 seconds in which the participant must respond within, followed by the next trial (Refer to Figure 3). Four condition blocks were congruent-face, congruent-word, incongruent-face, incongruent-word, with 16 randomized trials per block. Conditions instructions were alternated for participants to report either the facial expression or superimposed word. The 4 blocks take a maximum of 192 seconds (3.2 minutes), with the entire task (including instructions) taking approximately 5 minutes to complete. See Figure 3b for paradigm example paradigm.

- ***Recovery:*** Based on the vagal control model of "Resting, Reactivity, and Recovery", participants were instructed to engage in a standard recovery activity (paced breathing) to measure their ability to recover (i.e., a predicted reduction in HRF) from stress when given the opportunity to do so (i.e., following the acute stressor task). Following the baseline but prior to the stressor task, participants went through a series of breathing-pace videos, to identify a recovery breathing pace that was "comfortable" for them. They were instructed to stop breathing with a pace if it was uncomfortable (e.g., if they felt like they were hyperventilating- choose a slower pace; if they are not getting enough air - to choose a faster pace). The breathing paces presented to participants ranged from 4.8 to 8.0 breaths per minute at increasing increments of 0.4 seconds, for a total of 9 possible paces to choose from. Paced breathing has been identified as a technique that facilitates recovery following stress (Lehrer et al., 2020; Andersen, Arpaia & Gustafsberg, 2021). Following the stressor task, participants completed a 10-minute recovery breathing recording in which they

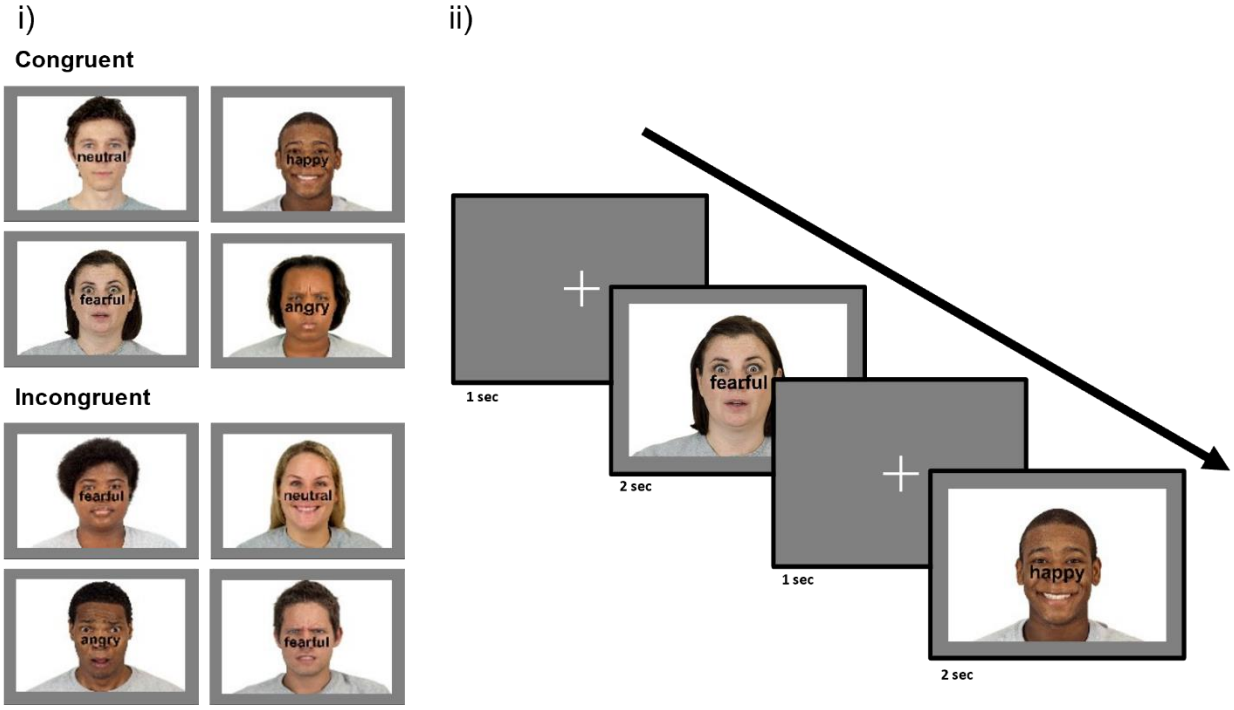
followed a video in which a "breathing triangle" changed in size increased (inhaled) and decreased (exhale) according to the recovery breathing pace they earlier identified as most comfortable (see Figure 3c).

Figure 3a. Study procedure.



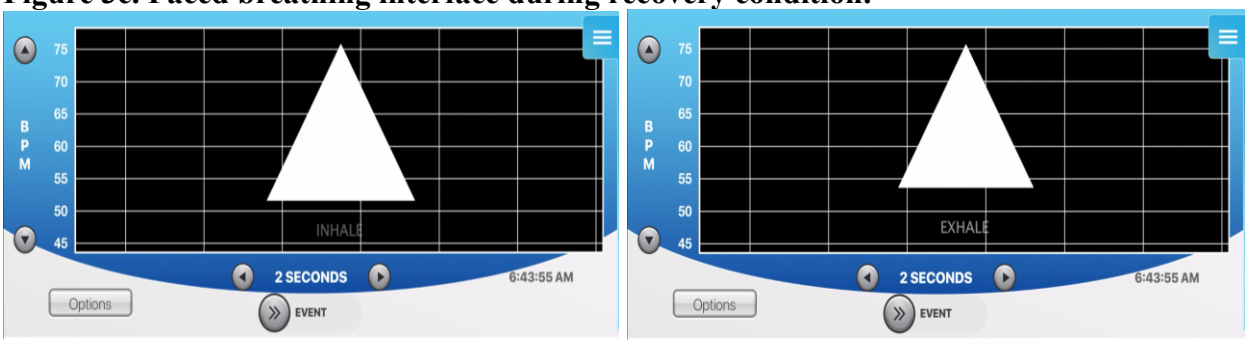
Experiment timeline. Prior to the questionnaire participants provided informed consent and were fitted with HR chest bands.

Figure 3b. Paradigm and examples of stimulus of each condition type within the face-word emotional Stroop.



i) congruent and incongruent stimuli examples and ii) procedure example of two trials within the condition. The stimuli here are not exact depiction of those used in experiment.

Figure 3c. Paced breathing interface during recovery condition.



Participants were given the above interface to follow breathing paces from 4.8-8.0 seconds, in which the triangle increased and decreased in size based on their chosen pace.

2.3 Measures

2.3.1 Psychosocial measures

Posttraumatic Stress Disorder (PTSD) – The PTSD checklist for DSM-5 (PCL-5) is a 20-item self-report questionnaire that assesses the 20 DSM-5 symptoms of PTSD. Participants respond on a 5-point scale ("Not at all" [0], to "Extremely" [4]), on the degree to which they have experienced the present items in the past month. A total score of 31 or greater is considered indicative of probable PTSD across samples (Weathers et al., 2013). The PCL-5 has high internal consistency (Cronbach's $\alpha = 0.96$), as well as convergent validity, correlating with PGQ depression and generalized anxiety disorder scales, and panic somatization disability and functional impairment, in Veterans recruited through VA Healthcare system ($n = 468$, $M_{Age} = 53$, 12% female) (Bovin et al., 2015).

Depression and Anxiety − Participants completed the Depression, Anxiety and Stress Scale-21 (DASS-21), a 21-item self-report questionnaire, a shortened version of the larger 42-item measure (DASS-42) of Lovibond & Lovibond (1995)'s depression, anxiety, and stress. Participants respond on a 4-point scale ("Did not apply to me at all" [0] to "Applied to me very much or most of the time" [3]), on the degree to which they have experienced the present items in the last week. Total scores of each subscale have recommended cut-off thresholds for

symptom severity levels of “Normal”, “Mild”, “Moderate”, “Severe” and “Extremely Severe”. In a large (n = 1794) nonclinical sample, the DASS-21 subscales revealed very similar scores as the full DASS-42, and can be validly used to measures of depression, anxiety, and stress as a more general dimension of psychological distress or negative affectivity. The DASS-21 displays high internal consistency for individual subscales of depression (Cronbach’s $\alpha = 0.88$), anxiety (Cronbach’s $\alpha = 0.90$), stress (Cronbach’s $\alpha = 0.93$), and overall as a total scale (Cronbach’s $\alpha = 0.93$) (Henry & Crawford, 2011).

2.3.2 Cardiovascular measures

The current study made use of commercially available ambulatory heart rate monitors that were advertised to be able to collect medical grade cardiac measures (e.g., RR intervals) for use in naturalistic settings (Zephyr Biomodule Bioharness 3, Zephyr Performance Systems, Annapolis, MD, USA). Participants were fitted with heart rate (HR) monitors to log cardiac metrics throughout the experiment. Sampling frequency was 1 kilohertz (kHz). cardiac measures were completed in accordance with recommendations from Laborde, Mosely, & Thayer (2017) of “Heart Rate Variability and Cardiac Vagal Tone in Psychophysiological Research – Recommendations for Experiment Planning, Data Analysis, and Data Reporting”. This includes:

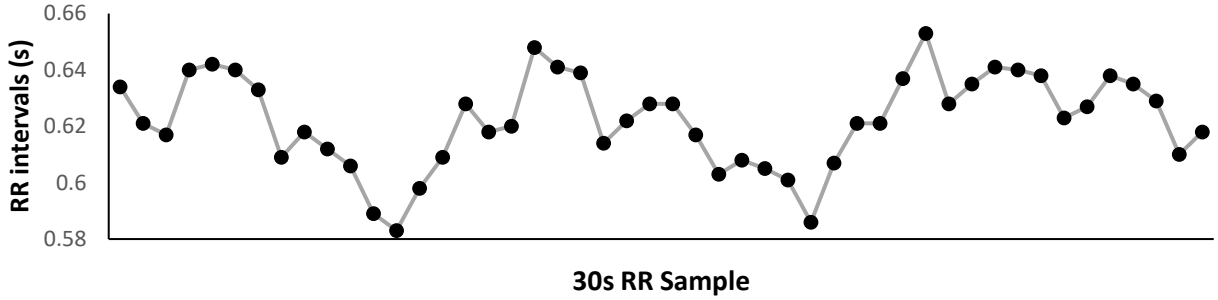
- Instructions to the participant prior to the experiment to maintain a normal sleep routine and no intense physical training or alcohol 24 hours prior to the experiment, and no meal or caffeinated drinks within 2 hours before the experiment.
- Confirmation of comfort prior to recording, and the opportunity to resolve any discomfort prior to cardiovascular recordings (e.g., go to the washroom, relieve themselves, drink water).

- Maintaining a seated body position with the chest extended (but not strained), knees at 90 degrees, feet flat on the floor, and hands on the thighs with palms facing upward – similar to what is recommended for blood-pressure recording.

Heart Rate Fragmentation (HRF) – HRF was symbolically mapped from RR data with MatLab programming we designed in accordance with Costa et al., 2017's method. The symbolic mapping sequence between RR intervals (ΔRR) were as follows: heart rate decelerations and accelerations were mapped "1" and "-1" respectively, and "0" was used to represent intervals that did not change. The series of symbols were then segmented in rolling groups of 4 consecutive ΔRRs to form RR words. Once words were grouped in lengths of four, they were categorized based on the number and type of acceleration inflection points within the word. Transitions from symbol "1" to "-1" or vice versa were considered "hard" inflection points, transitions that included "0" acceleration points were considered "soft", and mixed includes a combination of both hard and soft inflection points. Refer to Figure 4 for stepwise example of forming HRF "words" from RR samples.

A total of 81word combinations are possible. Overall, symbolic mapping further emphasizes RR acceleration signs and focuses on dynamical pattern signatures while deemphasizing the magnitude of those changes. For the purpose of this study, fragmentation was operationalized as the percentage of words that consisted of 2 (w_2^H) or 3 (w_3^H) hard inflection points, reflecting the most extreme fragmentation patterns, in which the HR acceleration sign changes almost every beat.

Figure 4. Example word development from RR intervals to symbolic mapping and schematic representation.

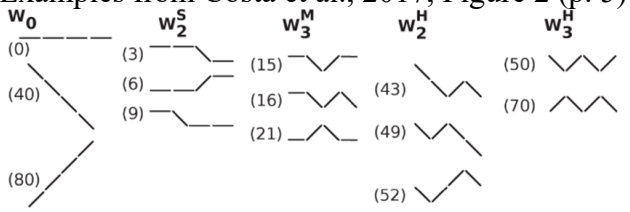


Process	Example
$\boldsymbol{RR\ intervals\ (s) = RR_1, \dots, RR_i}$	0.634, 0.621, 0.617, 0.64, 0.642, 0.64, 0.633, 0.609, 0.618, 0.612, 0.606, 0.589, 0.583, 0.598, 0.609, 0.628, 0.618, 0.62, 0.648, 0.641, 0.639, 0.614, 0.622, 0.628, 0.628, 0.617, 0.603, 0.608, 0.605, 0.601, 0.586, 0.607, 0.621, 0.621, 0.637, 0.653, 0.628, 0.635, 0.641, 0.64, 0.638, 0.623, 0.627, 0.638, 0.635, 0.629, 0.61, 0.618
$\boldsymbol{\Delta RR's = (RR_2 - RR_1), \dots, (RR_{i+1} - RR_i)}$	-0.013, -0.004, 0.023, 0.002, -0.002, -0.007, -0.024, 0.009, -0.006, -0.006, -0.017, -0.006, 0.015, 0.011, 0.019, -0.01, 0.002, 0.028, -0.007, -0.002, -0.025, 0.008, 0.006, 0, -0.011, -0.014, 0.005, -0.003, -0.004, -0.015, 0.021, 0.014, 0, 0.016, 0.016, -0.025, 0.007, 0.006, -.001, -0.002, -0.015, 0.004, 0.011, -0.003, -0.006, -0.019, 0.008
Symbolic Mapping of ΔRR "letters" where: $\boldsymbol{Deceleration = 5ms \leq \Delta RR = 1}$ $\boldsymbol{No\ change = -5ms < \Delta RR < 5ms = 0}$ $\boldsymbol{Acceleration = \Delta RR \leq -5ms = -1}$	-1, 0, 1, 0, 0, -1, -1, 1, -1, -1, -1, -1, 1, 1, 1, -1, 0, 1, -1, 0, -1, 1, 1, 0, 1, -1, 1, 0, 0, -1, 1, 1, 0, 1, 1, -1, 1, 1, 0, 0, -1, 0, 1, 0, -1, -1, 0
Grouping letter symbols into rolling 4-letter "words" by number and type of inflection points Word categorization: $\boldsymbol{RR\ Word = w_j^t}$ *where:* *t = the type of inflection points in the word* *H = only hard* *S = only soft* *M = soft and hard* *j = the number of inflection points*	First 10 "words" and their pattern category -1 0 1 0 w_3^S 0 1 0 0 w_2^S 1 0 0 -1 w_2^S 0 0 -1 -1 w_1^S 0 -1 -1 1 w_3^S -1 -1 1 -1 w_2^H -1 1 -1 -1 w_2^H 1 -1 -1 -1 w_1^H -1 -1 -1 -1 w^0 -1 -1 -1 1 w_1^H

Schematic representations of words

"/" = HR acceleration
"\" = HR deceleration
"—" = no change

Examples from Costa et al., 2017, Figure 2 (p. 5)



2.4 Data Analyses

Participants were grouped as either having a probable mental health (pMH) disorder, or healthy. pMH was operationalized as scoring at or above the threshold for "moderate" or higher levels of symptoms (according to the relevant scale scoring cut-offs) on at least one of the self-reported psychosocial inventories administered online (e.g., depression, anxiety, posttraumatic stress disorder-PTSD for at least one of the psychosocial inventories within the psychosocial questionnaire, as measured by self-report inventories (Weathers et al., 2013; Henry & Crawford, 2011). Individuals scoring "mild" or lower levels of symptoms for all measured psychosocial inventories were grouped as non pMH (i.e., healthy). Of the combined sample collected, 60.65% of participants met criteria to be categorized in the pMH group.

RR intervals during each condition (Baseline, Stress, Recovery) were extracted and pre-processed through Kubios HRV Standard. Due to experimental control of laboratory settings mitigating artifact risk, automatic artifact correction was applied, due to its pre-established reliability for effectively detecting and reducing artifact impact (Lipponen & Tarvainen, 2019). Artifacts were corrected using a low (0.35s) threshold level. RR recordings with ≥5% beat correction were excluded from analyses. The average beat correction percentage of the remaining data overall was 0.28% (SD = 0.62%) and ≤ 0.33% (SD ≤ 0.73%) in each condition. Refer to supplementary Table S1 for complete artifact correction summary of each condition.

Analyses and figures were completed in SPSS Statistic 25 (IBM, Armonk, New York) and R (R Core Team, 2021), with an alpha (α) of 0.05. A mixed model analysis was used to test the primary hypotheses. Fragmentation (HRF) was modeled as a function of probable mental health, the experimental condition, and the interaction between these variables. Fixed effects were mental health severity (pMH or healthy), condition (Baseline, Stress, Recovery) and the interaction of these variables (mental health severity × condition). Eta-squared (η^2) for within study effect sizes (Lakens, 2013). Exploratory analyses to compare HRF reactivity (the change in fragmentation percentage between conditions = ΔHRF) between pMH and healthy individuals were completed using multiple Wilcoxon rank sum tests, with a Bonferroni-corrected alpha (α_{bonf}). Hedges' g correction was used to estimate effect size for smaller sample sizes (Lakens, 2013).

3 Results

3.1 Primary Analyses

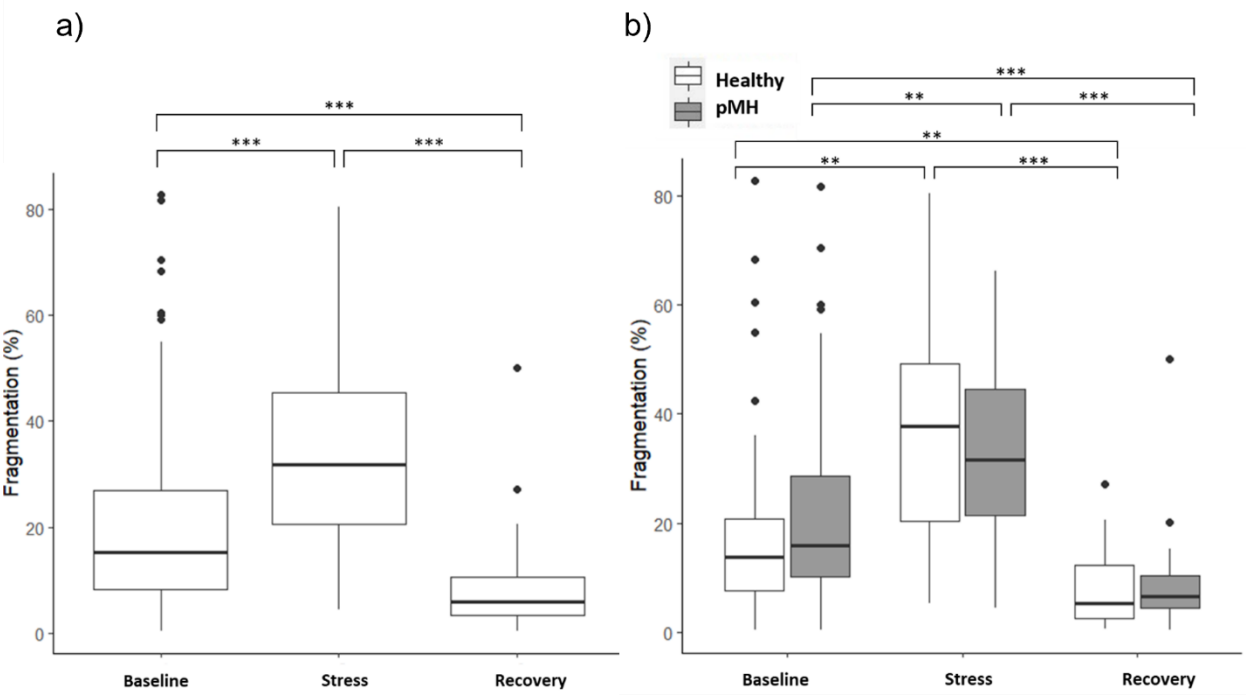
We found support for the first hypothesis; HRF significantly related to the task condition (i.e., baseline, stress, recovery) ($F_{(2,74.13)} = 58.90$, $p < 0.001$). HRF increased and decreased with the presentation and termination of stress, respectively. Hypothesis two was not supported. Specifically HRF was not related to probable mental health symptoms ($F_{(1, 81.31)} = 0.001$, $p > 0.05$), or the interaction between condition and group ($F_{(2, 13)} = 0.88$, $p > 0.05$).

3.1.1 Post-Hoc Analyses

Bonferroni-corrected pairwise comparisons revealed that baseline, stress, and recovery conditions significantly differed from each other (Figure 5a), The stress condition had the highest

amount of fragmentation (M = 34.05, SE = 2.45, CI_{95} [29.10, 39.00]), followed by the baseline condition (M = 19.39, SE = 1.41, CI_{95} [16.59, 22.19]), with the recovery condition having the least amount of fragmentation (M = 8.03, SE = 1.00, CI_{95} [6.03, 10.07]). The percentage of HRF increased from baseline when presented with stress and was significantly lower than baseline or stress conditions during the paced breathing condition; the same dynamic relationship is maintained in pMH and healthy subsamples (Figure 5b). All comparisons had medium to large effect size, suggesting up to 47% of the total variance was accounted for by task (see Table 3a for all pairwise comparison values and 3b for all effect sizes).

Figure 5. Heart rate fragmentation across conditions.



Heart rate fragmentation percentage across conditions of a) the combined sample and b) separated by healthy versus probable mental health (pMH) subsamples. (**) $p < 0.01$, (***) $p < 0.001$.

Table 3a. Estimates and pairwise comparisons statistical values.

		Estimates [a] M(SE), [CI_{95}]	**Pairwise Comparisons** [a] $\Delta M_{(C1-C2)}(SE)^{sig}$, [$CI_{95}$],	
			Condition2	
	Condition1		*Stress*	*Recovery*
Combined	*Baseline*	19.39(1.41), [16.59, 22.19]	-14.66(2.83)***, [-21.60, -7.72]	11.37(1.73)***, [7.18, 15.55]
	Stress	34.05(2.45), [29.10, 39.00]	–	26.02(2.64)***, [19.49, 32.56]
	Recovery	8.03(1.00), [6.03, 10.04]	–	–
Healthy	*Baseline*	17.67(2.28), [13.11, 22.24]	-18.26(4.75)**, [-30.19, -6.33]	9.90(2.73)**, [3.20, 16.49]
	Stress	35.94(4.17), [27.29, 44.58]	–	28.16(4.44)***, [16.85, 39.47]
	Recovery	7.78(1.52), [4.63, 10.92]	–	–
pMH	*Baseline*	21.11(1.68), [17.78, 24.45]	-11.06(3.06)**, [-18.56, -3.55]	12.83(2.22)***, [7.70, 17.97]
	Stress	32.17(2.56), [26.98, 37.35]	–	23.89(2.86)***, [16.81, 30.97]
	Recovery	8.28(1.29), [5.67, 10.89]	–	–

pMH = probable mental health, M = mean, SE = standard error, CI_{95} = confidence interval (95%), C1 = condition 1, C2 = comparison condition 2, [a] = based on estimated marginal means, η^2 = eta-squared*, sig = significance, (*) p < 0.05, (**) p < 0.01, (***), p < 0.001, Bonferroni-adjusted.*

Table 3b. Pairwise comparison effect sizes.

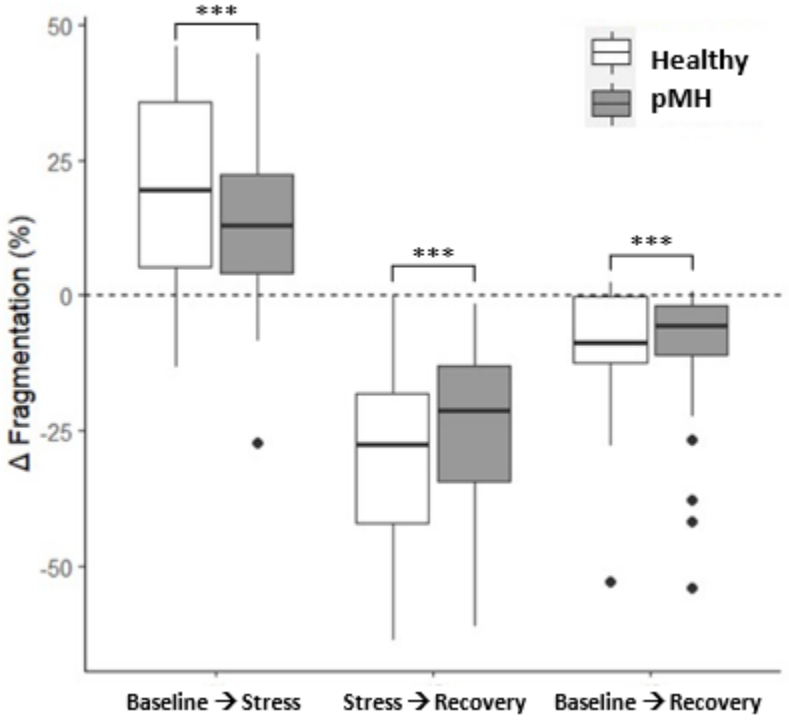
	Task Comparisons	**Sample**	**Effect Statistic**	**Value**
Baseline	*Baseline – Stress*	Combined	η^2	0.13
		pMH	η^2	0.18
		Healthy	η^2	0.11
	Stress – Recovery	Combined	η^2	0.44
		pMH	η^2	0.47
		Healthy	η^2	0.47
	Baseline – Recovery	Combined	η^2	0.15
		pMH	η^2	0.11
		Healthy	η^2	0.21
Change (Δ) in HRF Reactivity	*Baseline – Stress*	pMH vs Healthy	g	0.33
	Stress – Recovery	pMH vs Healthy	g	0.32
	Baseline – Recovery	pMH vs Healthy	g	0.02

η^2 = eta-squared, g = Hedges' g.

3.2 Exploratory Analyses

Nonparametric Wilcoxon rank sum tests were completed to compare healthy and pMH groups change in fragmentation between conditions. With a Bonferroni-corrected alpha ($\alpha_{bonf} = 0.05/3 = 0.017$) for multiple testing, pMH individuals displayed significantly different HRF changes between conditions in comparison to the healthy sample. Healthy individuals had significantly larger changes in fragmentation in response to each condition ($w_{baseline \rightarrow stress} = 3545$, $p < 0.001$; $w_{stress \rightarrow recovery} = 0$, < 0.001; $w_{baseline \rightarrow recovery} = 337$, $p < 0.001$) in comparison to pMH, with small effect Hedges' g ($g_{baseline \rightarrow stress} = 0.33$, $g_{stress \rightarrow recovery} = 0.32$, $g_{baseline \rightarrow recovery} = 0.02$). As displayed in Figure 6, healthy individuals display larger fluctuations in HRF between conditions compared to pMH, in which HRF is more stable.

Figure 6. Change (Δ) in HRF between conditions.



pMH = probable mental health, Δ = change/difference score. (***) p < 0.001.

4 Discussion

The present study builds on prior research by 1) establishing HRF's capability to differentiate the three R's of cardiac vagal control (i.e., *resting* baseline, stress *reactivity*, and *recovery*), and 2) finding associations between mental health and HRF outcomes. Both findings have implications for AL theory.

4.1 Psychological baseline stress and fragmentation

The primary hypothesis was supported; HRF changed with the presentation and recovery from an acute stressor, with mild psychological stress sufficient to engage body's regulatory control networks resulting in increased HRF severity, and successfully adapting and recovering with a lower level of HRF once the stress was terminated. Furthermore, the finding aligns with a large body of research demonstrating physiological responses to mild stress (and the task used in this study: i.e., Stroop task) (Ovaysikia et al., 2011; Haas et al., 2006) and the AL model (McEwen 1998a; McEwen, 2002; Lovallo, 2016). Furthermore, the findings support the capability of HRF to represent each independent state within the AL model (resting, reactivity, and recovery). The significant differences between conditions in study supports recommendations to consider all states within the AL model; whereas focusing on a single state may result in an inaccurate interpretation of experimental findings (a problem noted in existing field literature). The results from this study also support potential use of HRF as a "field-ready" biomarker to assess an individual's biological capability to mount a stress response and recover from the stress. HRF changes were sensitive enough to be measured using commercial wearable devices and displayed direct links between allostatic load from everyday stressors, which may identify potentially adverse health trajectories (severe fragmentation) even in a "healthy" sample.

The second hypothesis was not supported; healthy and pMH groups' HRF did not significantly differ from each other across conditions (Baseline, Stress, Recovery). The results suggest that HRF magnitude alone may not be as indicative of mental health as it is for cardiovascular diseases, as observed in cardiovascular measures in non-clinical young-adult samples. One factor that may have contributed toward this was the average age in the sample. Costa et al., (2017) found a positive relationship between age and HRF, however their sample consisted of only participants older than age 25, the majority of which were between the ages of 33–67. Further, a large cross-sectional study found that hard fragmentation increased after birth up to age 20, decreased to a plateau before increasing again after age 75; for 'w_3^H' category words (in which changes from acceleration to deceleration happen every beat) specifically, increases do not occur until after age 40 (Hayano et al., 2020). In this study we may be measuring a period of HRF fluctuation that is sensitive, but less specific in comparison to other age timepoints. Furthermore, there is evidence from large cross sectional age studies of instability in stress-related variables during adolescence and peaking in young adulthood of healthy individuals. Baseline cortisol shows a rapid increase with the onset of puberty that peaks in the early 20s (Miller et al., 2016). Differential HRV measures also show different rates of change across the lifespan; while SDNN linearly change with age, RMSSD and pNN50 have a rapid, quadratic rate of change that slows from the second (age 20–29) to third decade of life, before stabilizing in the sixth decade of life (Umentani et al., 1998). Based on the age-related differences, and the average age of the sample (~20 years), it may be possible that baseline stress levels at this point of time are less related to vagal tone and instead biological changes that happen with physical development, which may be obscuring any differences associated with pMH.

The current study examined HRF in the context of a non-clinical sample and thus the results cannot be generalized to clinical samples. Of note, the outcomes show the presence of a high number of adverse mental health symptoms in a sample of supposedly 'healthy' young individuals who did not self-report a diagnosed mental health condition. Specifically, 60% of the sample surpassed threshold for moderate or greater symptoms, reflecting a concerning trend for the mental health of university undergraduates. The current study presents promising preliminary uses for HRF in detecting increased mental health risk with implications for early intervention or prevention of adverse health risk trajectories.

4.2 Psychological stress reactivity and fragmentation in healthy versus pMH individuals

While base HRF measures in each condition did not reveal significant differences between healthy and pMH participants, exploratory analysis found that significant HRF differences between healthy and pMH participants when comparing change scores between conditions. Healthy individuals displayed larger HRF fluctuations in response to stress in comparison to pMH individuals. These findings have significant implications for prevention and onset of health risk trajectories; while subclinical mental health symptoms may not change the magnitude of baseline HRF, but it might reduce flexibility and response to stressors, and less likely to change out of unhealthy patterns at even a very early age as demonstrated in the current sample with an average age of 20 years-old. Within the AL model, this reduced flexibility may contribute towards ineffective recovery from stress, increasing allostatic load, and subsequent adverse health.

Furthermore, the results from this study support growing research perspectives on stress reactivity in which deviations beyond the normal range of a biological stress response can have

negative consequences for health. Previous research has found both exaggerated and blunted stress reactivity to psychological stress (Juster, McEwen, & Lupien, 2010; Carroll et al., 2017). In relationship to mental health symptoms, blunted cardiovascular reactivity (e.g., blood pressure, heart rate) is also previously associated with anxiety symptoms (Souza et al., 2015), depression scores even controlling for baseline cardiovascular activity (de Rooij et al., 2010; Carroll et al., 2007), and worsening depressive addiction, and bulimia (Carroll et al., 2017; Lovallo et al., 2000, 2006; Brenner & Beauchaine, 2011; Ginty et al., 2014).

The lack of flexibility in stress response reactivity aligns with the AL model, in which mental health symptoms result in ongoing perseverative cognitions, resulting in individuals continuing to respond as if they are still facing a stressor, rather than recovering appropriately when the stressor has terminated (Laborde et al., 2018; Ottaviani, 2018).

4.3 Limitations

While previous research and use of the Stroop task within our own projects provided task completion estimates of 5 minutes, participants within this study on average took a much shorter amount of time to complete the Stroop test than the expected (average 1.5 minutes). While typical HRV measure standards typically require 5 minute-length samples, research has proposed the use of ultra short-term HRV for periods of 10–60 seconds, and 60–240 seconds particularly for use in mobile settings (Salahuddin et al., 2007; Baek et al., 2015; Esco & Flatt, 2014). Further testing is required to determine if less than 5 minutes can truly capture HRF.

We made every effort to use the best ‘medical grade’ ambulatory equipment to measure cardiovascular outcomes. Despite our efforts, commercially available ambulatory equipment still presented some technical challenges in recording (resulting in 10.2% of data excluded – see

supplementary table S1). Other psychophysiological research using the same equipment (Zephyr Bioharness 3) report up to 35% data loss from improper affixation by participants, intermittent device connectivity and excessive noise (Anderson & Farb, 2018). Notably from the current study, the chest bands compatible to the Zephyr Bioharness 3 are not body inclusive, resulting in poor fit and inconsistent electrode contact on average to smaller body frames. However, in anticipation we followed literature recommendations for HR recording, increasing experimental control to mitigate potential issues; in combination with the industry standard for HR processing (Kubios), the total RR sample extracts excluded due to ≥5% artifact concentration was 10.20% (*30/294 samples)*, with only 0.28% average correction (See supplementary Table S1).

4.4 Future Directions

The interest in HRV biomarkers has grown exponentially, regardless of myriad measurement confounds (Arakaki et al., 2023; Mosley & Laborde, 2022; Stephenson et al., 2021; Quintana & Heathers, 2014). Future directions include comparing HRV and HRF metrics in the same sample to test the predictive power of each measure to differentiate conditions and adverse outcomes reliably over time and across study paradigm.

5 Conclusions

The current study builds upon prior research to integrate an emerging cardiovascular measure into the current knowledge of psychological stress and health. At the time of writing this, there was currently no published research associating HRF with mental health variables. The current study observed the significant effect that subclinical levels of mental health can have on cardiovascular reactivity. Furthermore, this program of research serves as an example of the importance of considering the feasibility and utility of different cardiac metrics used in research

based on both the hypotheses and the setting (i.e., field versus laboratory); particularly, as different measures of the same tasks can present very different narrative interpretations. HRF also has implications for identifying health risk and measuring response to interventions and prevention efforts such as paced breathing and biofeedback.

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