HEART RATE VARIABILITY AS A BIOMARKER OF CHRONIC STRESS IN HEALTHY MEN

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Evaluation

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Abstract

This doctoral thesis delved into the relationship between allostatic load (AL) and heart rate variability (HRV) in healthy men, with a particular focus on HRV as a biomarker of chronic stress. Our research, which emphasizes a healthy population sample, significantly contributes to the field of preventive medicine.

After establishing the theoretical framework, we conducted a scoping review to summarize the current evidence on the relationship between AL and HRV, confirming the gap in the topic. Subsequently, we conducted a cross-sectional study with healthy adult men from Medellín, Colombia. We developed an allostatic load index (ALI) and extracted HRV metrics from 24-hour Holter monitoring. Multiple linear regression models were used to evaluate the relationship between ALI and HRV. Additionally, we used magnetic resonance imaging to explore how ALI interacts with brain connectivity and structure in relation to HRV.

Our study included 88 men aged 21-40, with 70% from middle socioeconomic backgrounds. We developed a new ALI using seven parameters (waist to height ratio -WHtR-, high density cholesterol -HDL-, glycosylated hemoglobin, HbA1C, c reactive protein -CRP-, systolic and diastolic blood pressure, and dehydroepiandrosterone sulfate -DHEAS-). The ALI was correlated with sympathovagal quotient -SQ- after adjusting for confounders ($\beta = 0.093$, $p = 0.004$, CI = 0.03-0.15). In the exploratory MLR analyses of the interaction between ALI-7, resting-state brain networks, and structures of the central autonomic network, several interactions had negative correlations with HRV.

These findings suggest that the SQ is an indicator of AL, highlighting its potential as a biomarker for preventing, diagnosing, and managing chronic stress. Our exploratory analysis indicates that HRV reflects the interplay of central and peripheral physiological processes related to chronic stress. This finding underscores the need for validation in future research, which could further contribute to the field of preventive medicine.

CHAPTER 1: INTRODUCTION AND OBJECTIVES

INTRODUCTION

Stress is a necessary physiological response to maintain allostasis and survival (De Kloet et al., 2005). However, excessive stress, both acute and chronic, can become maladaptive and threaten physical and mental health (McEwen, 2007). Continuous exposure to stressors is associated with a higher risk of cardiovascular, neurological, mental, and autoimmune diseases (Catale et al., 2022; Chesnut et al., 2021; Ilchmann-Diounou & Menard, 2020; Osborne et al., 2020). Additionally, chronic stress deteriorates daily functioning and reduces quality of life (Ribeiro et al., 2018). Despite this evidence, measuring stress has been difficult due to the lack of a consensual definition and its multisystemic nature, especially in chronic stress (Crosswell & Lockwood, 2020; Wulsin et al., 2022).

Most chronic stressors are psychosocial (Everly & Lating, 2019) and are evaluated using scales or questionnaires that measure exposure to stressors, individual perception of stress, and its impact on mental and physical health (Epel et al., 2018). However, these instruments present limitations in the clinical context, as most have not been validated and lack consensual recommendations for their application (Crosswell & Lockwood, 2020; Wulsin et al., 2022). Additionally, they do not capture the multisystemic response to stress or its impact on physical and mental health (Cohen et al., 2019; Epel et al., 2018).

In summary, stress includes cognitive, behavioral, physiological, and biochemical reactions, and its comprehensive evaluation should include elements of all these components (Cohen et al., 2019; Crosswell & Lockwood, 2020; Epel et al., 2018; Everly & Lating, 2019).

The identification of biomarkers related to chronic stress is an important line of research. These investigations focus on pathways activated by stressors, but specific biomarkers have not yet been defined, nor the most suitable ones to measure chronic stress (Noushad et al., 2021). In 1993, McEwen and Stellar introduced the concept of allostatic load, which describes the wear and tear on the body due to chronic stress, predisposing it to disease (McEwen & Stellar, 1993). This model has been used as a conceptual framework for empirical studies of chronic stress in humans (Carbone et al., 2022; Guidi et al., 2021; Parker et al., 2022). The first operationalization of allostatic load was proposed by Seeman et al.,

with an allostatic load index (ALI) that included ten variables from different physiological systems (Seeman et al., 1997). Since then, other ALIs have been proposed, combining different medical variables. However, there is no consensus on the most suitable index to measure chronic stress due to uncertainty in selecting and the ideal number of biomarkers, as well as how to calculate the index (Guidi et al., 2021; Mauss & Jarczok, 2021; Carbone et al. 2022). Nevertheless, a recent meta-analysis including individuals from multiple cohorts worldwide suggests that an ALI of five biomarkers can predict mortality and other clinically relevant outcomes, such as handgrip strength, walking speed, and self-rated health status (McCrory et al., 2023).

One of the primary target organs of the stress response is the heart, which increases heart rate through the sympathetic nervous system (SNS). The interaction between the influences of the SNS and the parasympathetic nervous system (PNS) generates the heart rate variability (HRV). It is currently known that HRV mainly depends on the PNS's regulatory effect on the heart through the vagus nerve (Palma & Benarroch, 2014). In healthy individuals, reduced HRV is associated with altered psychophysiological mechanisms related to chronic stress, while those with greater emotional and cognitive self-regulation capacity exhibit higher HRV (Holzman & Bridgett, 2017). Additionally, some studies have found a significant negative correlation between HRV and various psychological measures of chronic stress, such as exposure to stressors and individual perception of stress (Kim et al., 2018; Mauss & Jarczok, 2021), and also with symptoms of anxiety and depression (Chesnut et al., 2021).

Research using neuroimaging techniques supports the neurobiological connection between stress and HRV (Ding et al., 2020; Koenig et al., 2021; Mulcahy et al., 2019). Human studies have shown a positive correlation between HRV and the activation of certain brain structures related to processing emotional and affective information involved in the stress response (Thayer et al., 2012). The structures consistently associated with HRV control are the prefrontal cortex, anterior cingulate cortex, insula, amygdala, periaqueductal gray, pons, and medulla oblongata, collectively known as the central autonomic network (CAN) (Sklerov et al., 2019). Notably, the amygdala and ventromedial prefrontal cortex (vmPFC) play a predominant role among these areas (Thayer et al., 2012). This evidence suggests that HRV

can be a good indicator of autonomic, cognitive, and emotional regulation in healthy individuals and patients with affective disorders (Mulcahy et al., 2019).

In addition to neural control, various peripheral non-neural physiological factors influence HRV. Circulating hormones such as adrenaline and noradrenaline, thyroid hormones, and cortisol; blood pressure; ions like sodium, potassium, magnesium; certain cytokines; body temperature; and respiratory parameters like blood O2 and CO2 levels all influence HRV (Brusseau et al., 2022; Ramesh et al., 2022; Sammito et al., 2024). These peripheral factors interact with neural control to modulate HRV. Indeed, HRV has been negatively correlated with other serum biomarkers related to the biological response to chronic stress (Boschiero & Ilich, 2022; Sloan et al., 2007).

In summary, HRV is considered a biomarker of autonomic regulation and could indicate the functioning of psychophysiological mechanisms related to chronic stress (Mulcahy et al., 2019; Quadt et al., 2022; Ruffle et al., 2021).

Despite several lines of research suggesting HRV's role as a biomarker of acute stress, the evidence on its utility for chronic stress remains scarce and controversial (Corrigan et al., 2021; Solano-Atehortua et al., 2024). Specifically, the following issues require further investigation:

- Most studies focus on acute stress, measuring HRV for a few minutes under experimental conditions in a laboratory (Corrigan et al., 2021; Kim et al., 2018; Laborde et al., 2017), contrasting with clinical guidelines recommending 24-hour recordings for measuring and interpreting HRV (Catai et al., 2020; Shaffer & Ginsberg, 2017; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).
- According to the scoping review (Solano-Atehortua et al., 2024), only two studies have evaluated the relationship between HRV and chronic stress using a multisystemic approach within the allostatic load framework. Again, these studies used short-duration laboratory recordings instead of long-duration ambulatory recordings recommended for the clinical context.
- Few studies on HRV and stress adequately control for confounding factors such as body mass index (BMI), sedentary behavior, sleep quality, diet, alcohol consumption,

smoking, and medication use (Catai et al., 2020; Da Estrela et al., 2021; Föhr et al., 2016; Hayano & Yuda, 2019; Laborde et al., 2017; Ralevski et al., 2019; Strüven et al., 2021; Zaffalon Júnior et al., 2018).

- The relationship between HRV and stress varies by sex. Men typically show lower HRV with high stress levels, but this relationship is less clear in women. Hormonal fluctuations during the menstrual cycle also affect HRV, so studies in women should adjust analyses according to the cycle phase (Jarczok et al., 2018; Kvadsheim et al., 2022; Schmalenberger et al., 2019).

To address some of these issues, this research focused on three key points. First, the development of an ALI that includes multiple biological systems involved in the response to chronic stress. Second, we evaluated the relationship between ALI and HRV to seek evidence of HRV as an indicator of chronic stress. Finally, we assessed the role of functional and structural brain parameters derived from magnetic resonance imaging (MRI) in the relationship between allostatic load and HRV to support and better understand the relationship between chronic stress, allostatic load and HRV.

Considering that HRV can be easily, non-invasively, and cost-effectively measured, and given the various options for managing chronic stress such as physical activity, biofeedback, mindfulness, and yoga (Fogaça et al., 2021; Mizzi et al., 2022; Subhani et al., 2018), accurate HRV measurement will be very useful in defining preventive medicine strategies. Specifically, it could help reduce the incidence of cardiovascular (Gao et al., 2022; Osborne et al., 2020), autoimmune (Ilchmann-Diounou & Menard, 2020), mental (Chesnut et al., 2021; Hickey et al., 2021; Lever-van Milligen et al., 2020), and neurological disorders (Catale et al., 2022; Keynejad et al., 2019; Mohammadi et al., 2022).

Additionally, identifying brain structures and networks involved in the stress response and HRV control, and understanding their interaction with peripheral physiological mechanisms, besides supporting the biological plausibility of this relationship, could contribute to designing neuromodulation protocols aimed at modulating both allostatic load and HRV. These interventions include non-invasive electrical or magnetic stimulation, neurofeedback, audiovisual brainwave modulation, among other techniques that are already available in

clinical practice and have shown efficacy in treating various neuropsychiatric disorders (Cirillo et al., 2019; Mather & Thayer, 2018; Subhani et al., 2018).

Finally, HRV could also become a therapeutic target, considering its modulation has been related to effects on other systems of the body, including the central nervous system (Mather & Thayer, 2018).

In summary, establishing HRV as a biomarker of chronic stress would be a significant advance in diagnosis, prediction, and treatment (Hickey et al., 2021; Magal et al., 2022). It could also be used as a therapeutic target for clinical conditions related to chronic stress (Corrigan et al., 2021; Föhr et al., 2016).

OBJECTIVES

General objective:

Evaluate the association between chronic stress and HRV in healthy men.

Specific objectives:

1. Describe the sociodemographic, anthropometric, biochemical, physiological, and psychological characteristics related to chronic stress in the studied population.
2. Develop an ALI as a multisystemic measurement tool for chronic stress.
3. Establish the correlation between the developed ALI and HRV.
4. Explore the interaction between functional and structural parameters of brain areas involved in the stress response with the ALI and its effect on HRV.

CHAPTER 2: THEORETICAL FRAMEWORK

STRESS

Concepts and definitions

Although there is no universally accepted definition of stress, given its application to the field of health, we will adopt the definition proposed by Everly and Lating (Everly & Lating, 2019), who define it as a "physiological response that serves as a mediating mechanism linking any stressor to its effect on the target organ." It is important to highlight from this definition that stress is assumed as a response or reaction, independent of the stimulus that generates it. The term stressor refers to the stimulus that triggers the stress response. Generally, most stressors are psychosocial, but according to the Everly and Lating model, there are also biogenic stressors, which refer to stimuli that do not require cognitive or emotional processing to generate the physiological stress response, such as the consumption of substances that trigger sympathomimetic physiological mechanisms of stress like caffeine or theobromine.

The nervous system is fundamental in the stress response. The brain determines what is threatening, as well as the reactive behavioral and physiological responses. These responses are adaptive, but when they occur excessively or are not regulated, they become pathological, leading to dysfunction of target organs (McEwen, 2017). In a first cognitive-affective domain, environmental events are interpreted; if they are interpreted as threats, they become stressors. The next step is the activation of brain structures of the limbic system. The hypothalamus integrates stimuli from the limbic system and the prefrontal cortex, giving rise to somatic and visceral efferents in response to emotionally charged stimuli. These structures generate the stress response through at least three main efferent pathways: the neural, neuroendocrine, and endocrine pathways (Everly & Lating, 2019). The neural pathway refers to the activation of the central noradrenergic system from the locus coeruleus in the brainstem and the activation of the ANS, especially the sympathetic nervous system. This generates the fight-or-flight response. If the stressor is prolonged, the adrenergic adreno-medullary axis is activated, allowing the release of catecholamines, especially adrenaline from the adrenal medulla, which perpetuates the systemic adrenergic response. Additionally, with the persistence of the stressor, the endocrine system is activated, involving several endocrine

axes, with the hypothalamic-pituitary-adrenal (HPA) axis being of special importance. This leads to the secretion of cortisol from the adrenal cortex. This cortisol initiates a negative feedback process at various levels, including the pituitary, hypothalamus, hippocampus, and frontal cortex, to control the multisystemic response that this hormone exerts in the body due to the presence of multiple receptors in different organs and tissues (Everly & Lating, 2019).

Despite the understanding of these mediating mechanisms between cognitive-affective processes and physical dysfunction or disease, the stress response is highly variable between individuals, even in the same individual at different times. A determining factor in the intensity of the stress response and its impact on health is *coping*. Coping refers to the efforts, both cognitive and behavioral, aimed at managing external and internal demands, as well as the conflicts between them, which can exceed a person's resources (Everly and Lating, 2019). From this perspective, coping can be understood as cognitive or environmental strategies designed to mitigate the stress response (Everly and Lating, 2019). In this model, coping occurs immediately following the physiological stress response and target organ activation, as an attempt to re-stablish homeostasis. However, coping can occur before a stressful event, known as anticipatory coping (Neupert et al., 2022). Coping strategies can be adaptive or maladaptive. Adaptive strategies can lead to successful coping, reduce organ activation, stress response and promote long-term health, while maladaptive strategies may offer short-term relief but harm long-term health (Owen et al., 2022). The most effective adaptive mechanisms are physical exercise, relaxation techniques, healthy nutrition, social support, time management and planning, problem solving; and the most common maladaptive mechanisms are substance abuse, avoidant behaviors, overeating or neglecting food, social isolation (Bondarchuk et al., 2024). The selection of a coping strategy is influenced by various factors, including past experiences, individual personality traits, and the behaviors modeled by family members (Bondarchuk et al., 2024). Coping theory significantly influences the effectiveness of stress management interventions by providing a framework for understanding how individuals respond to stressors. The *transactional model of stress and coping* emphasizes the dynamic interaction between stressors and coping strategies, which can enhance resilience and adaptive responses. These concepts and models can be applied in clinical practice related to stress management (Theodoratou and Argyrides, 2024).

In the clinical context, acute stress refers to the adaptive response due to short-term exposure to stressors, while chronic stress refers to exposure to threatening or challenging circumstances that disrupt daily life and continue for a prolonged period (a minimum of one month) (Crosswell & Lockwood, 2020).

Allostasis and allostatic load

The term allostasis was introduced by Sterling and Eyer in 1988 (Sterling & Eyer, 1989) to propose a physiological principle that refers to the variation and adaptation of all internal parameters according to environmental demands to achieve long-term stability, or as the authors put it: "stability through change." This allostatic model is a conceptual framework that explains the social and psychological modulation of human physiology and pathology, where the nervous system in general, and the brain in particular, play a fundamental role (Carbone et al., 2022). Through allostasis, the organism's stability is achieved by producing mediators (such as cortisol) that operate within a range to promote adaptation. This operational range of physiological systems or mediators is greater in health than in disease and greater in youth than in the elderly (McEwen & Stellar, 1993).

The development of chronic stress theory continued with the works of McEwen and Stellar, who introduced the concept of allostatic load, defining it as "a state of the organism that predisposes to disease, resulting from the tension produced by the repeated ups and downs of physiological responses, elevated activity of the involved physiological systems, metabolic changes, and the impact of wear and tear on different organs and tissues" (McEwen & Stellar, 1993). The allostatic load theory proposes a progression that begins with exposure to chronic stress and triggers biological changes that, over time, lead to negative health outcomes. The theoretical model proposes the following biological processes and events (Carbone et al., 2022; Marin et al., 2011): long-term exposure to chronic stress can cause changes in the neuroendocrine, hormonal (cortisol, dehydroepiandrosterone sulfate - DHEAS, epinephrine, and norepinephrine), and immune systems (interleukin 6 -IL-6- and tumor necrosis factor alpha). Collectively, these biomarkers are referred to as primary mediators. The long-term effect of primary mediators can cause changes at the cellular level,

known as primary outcomes. Over time, this can lead to subclinical changes known as secondary outcomes, such as blood pressure, abdominal fat deposition, cholesterol levels, glucose levels, fibrinogen, albumin, c reactive protein (CRP), etc. Finally, changes in secondary outcomes lead to the development of tertiary outcomes, including physical diseases (e.g., cardiovascular) and mental disorders (e.g., depression, anxiety). This final stage where the disease appears is known as allostatic overload and occurs when environmental challenges exceed an individual's capacity to cope.

Stress measurement

Due to the lack of a universal definition of stress, its measurement has been subject to much debate. However, most agree that objective biological measurements should be included alongside subjective psychological evaluations. Both components need to be assessed since neither alone fully represents the phenomenon of stress in humans (Epel et al., 2018). There is a wide range of instruments and techniques for assessing stress, and their selection should be based on the research question and the population being studied (Crosswell & Lockwood, 2020). For instance, sometimes it may be more important to measure exposure to stressors, in other cases, the focus might be on individual perception of stress, or the focus could be on measuring biological markers.

Generally, stress measurement has been grouped into three main approaches (Cohen et al., 2016): 1) the environmental approach, focusing on the measurement of stressors, 2) the psychological approach, focusing on the subjective interpretation of the stressor event (stress perception), and 3) the biological approach, aimed at measuring the activation of different physiological systems. In this last approach, a series of biomarkers associated with the chronic stress response have been identified. These are objective biological indicators of physiological processes involved in the pathway between stress and disease or serve as markers of these processes (Crosswell & Lockwood, 2020).

Despite having several biomarkers related to chronic stress, none is specific to it (Crosswell & Lockwood, 2020). In this context, a widely used and accepted approach to measure the effect of chronic stress on health is the allostatic load model described previously, as it allows

for a more comprehensive and integrated evaluation of the stress response in humans. The first to use this model in chronic stress research were Seeman et al., who developed an allostatic load index (ALI) (Seeman et al., 1997) and validated it in studies, including longitudinal studies (Merkin et al., 2014; Seeman et al., 2001; Upchurch et al., 2015). This index provides an assessment of the cumulative effect of stress on health and uses ten biological parameters to evaluate the functioning of the Hypothalamic-Pituitary-Adrenal (HPA) axis, (24-hour urinary cortisol, dehydroepiandrosterone sulfate -DHEA-S-), the sympathetic nervous system (24-hour urinary epinephrine and norepinephrine), the cardiovascular system (diastolic and systolic blood pressure, waist-to-hip ratio), and metabolic processes (glycosylated hemoglobin -HbA1c-, total/high density cholesterol ratio -TC/HDL-, and HDL cholesterol). This original ALI was associated with poorer physical and cognitive performance and predicted greater decline in these functions, as well as an increased risk of cardiovascular disease incidence (Seeman et al., 2001). Additionally, a recent systematic review and meta-analysis reported a correlation between high ALI and a greater risk of dying from any cause (22%) and from cardiovascular diseases (31%) (Parker et al., 2022). After Seeman's original version, other ALIs have been proposed, combining different medical variables. An interesting version, given its ease of measurement and application, is the simplified index with five variables, including diastolic blood pressure, HbA1c, LDL cholesterol, abdominal circumference, and HRV, developed by Maus et al., which has shown a significant relationship with stress levels in various populations (Mauss et al., 2016; Mauss et al., 2015) and with general stress perception (Mauss & Jarczok, 2021). However, there is still no consensus on which ALI is the most suitable as an indicator of chronic stress. This lack of consensus is mainly due to uncertainty in selecting the ideal number of variables to include in the index and how to calculate the risk score (Guidi et al., 2021; Mauss, Li, et al., 2015; Mauss & Jarczok, 2021). Additionally, to constitute a more complete and possibly more specific evaluation of chronic stress and to facilitate the interpretation of findings, different studies should examine the relationship between ALI and clinimetric assessments, ideally with validated instruments (Guidi et al., 2021).

HEART RATE VARIABILITY

Heart rate variability measurement

When analyzed beat by beat, the heart rate in healthy individuals varies considerably, primarily depending on the parasympathetic nervous system (PNS) regulatory effect on the heart (Palma & Benarroch, 2014). At rest, the PNS activity predominates over the SNS activity, resulting in a much lower heart rate than the intrinsic rate of the sinoatrial node. Vagal stimulation results in an immediate response that typically occurs within the cardiac cycle in which it occurs and affects only one or two heartbeats after its initiation; after the cessation of vagal stimulation, the heart rate quickly returns to its previous level (Shaffer et al., 2014). Conversely, vagal blockade generates a rapid increase in heart rate (HR). On the other hand, the effect of the SNS on the sinoatrial node is more delayed compared to the PNS. In conclusion, sudden changes in HR (increase or decrease) are mainly mediated by the PNS. HRV is operationally defined as the change in the time interval between adjacent heartbeats (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). This can be assessed using various analytical approaches, the most commonly used being frequency domain analysis (spectral analysis of the area under the curve) and time domain analysis. Initially, in a continuous electrocardiographic (ECG) recording, each QRS complex is detected, and the intervals called NN (normal to normal) are identified, meaning all intervals between adjacent QRS complexes resulting from sinoatrial node depolarization.

Time domain analyses calculate the heart rate at any time or the intervals between successive normal QRS complexes. Time domain measurements can be obtained directly from R-R intervals or differences between adjacent R-R intervals. The most recommended include the standard deviation of NN intervals (SDNN) and the root mean square of successive differences between heartbeats (RMSSD) (Shaffer & Ginsberg, 2017).

SDNN is used as an estimate of overall HRV. It is more accurate when calculated over 24 hours than over shorter periods, as the former encompasses both short-term high-frequency variations and lower-frequency components observed over a longer period (Task Force of

the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

RMSSD reflects beat-to-beat variation in heart rate and is the primary time-domain measure used to estimate changes mediated by vagal tone, which can be measured in both long and short recordings (Laborde et al., 2017; Shaffer & Ginsberg, 2017).

Frequency domain measures use spectral analysis of the area under the curve to separate HRV into its rhythmic components operating within different frequency ranges (Shaffer & Ginsberg, 2017). The main advantage of spectral analysis is that it provides information on both the frequency and amplitude of specific HRV rhythms, allowing for the quantification of various oscillations over a given period in the recording. Four components are recognized in an HRV spectrum: high-frequency (HF) component, low-frequency (LF) component, very low-frequency (VLF) component, and ultra-low-frequency (ULF) component.

The HF component represents the area under the curve in the frequency range between 0.15 and 0.4 Hz. Generally, the HF area under the curve represents changes in heart rate related to respiration and is widely accepted as a measure of respiratory sinus arrhythmia (RSA) and the parasympathetic contribution to HRV. The HF component is generally correlated with RMSSD (Shaffer & Ginsberg, 2017).

The LF component ranges from 0.04 to 0.15 Hz. The origin of the LF power has generated much controversy. Initially, it was considered a measure of sympathetic activity with a small contribution from the parasympathetic nervous system. However, current evidence opposes the use of LF as a sympathetic marker, and it is now considered a reflection of the combination of sympathetic and parasympathetic activity and the baroreflex (Laborde et al., 2017; Shaffer & Ginsberg, 2017).

The LF/HF ratio was previously considered the sympatho-vagal balance, with an increase in this ratio indicating a predominance of sympathetic activity and a decrease indicating a predominance of parasympathetic activity. However, given the controversy in interpreting

the LF component and the non-reciprocal and non-linear relationship between sympathetic and parasympathetic activity, the LF/HF ratio should be interpreted with caution, especially in short-duration recordings (Shaffer & Ginsberg, 2017).

The VLF component represents the area under the curve in a range between 0.04 and 0.003 Hz. Some studies suggest that the VLF rhythm is intrinsically generated by the heart and that the amplitude and frequency of these oscillations are modulated by sympathetic efferent activity (Lee & Becker, 2019; Shaffer et al., 2014). An increase in VLF area under the curve at rest could then reflect greater sympathetic activity. The ULF component falls below 0.0033 Hz (5.6 min) and is only visible in long-term recordings (24 hours). The clinical relevance of these lower frequency rhythms is currently unknown (Shaffer et al., 2014).

It is important to remember that the duration of the ECG recording significantly affects HRV measures, both in frequency and time domain parameters, and has significant implications for their interpretation (Hayano & Yuda, 2019). For psychophysiological research, some experts recommend focusing on studying vagal tone, ideally measuring RMSSD in the time domain and HF in the frequency domain. The measurement of additional parameters would be made according to the research question. Regarding this, studies suggest that RMSSD is less affected by respiratory influences, making it preferable over HF in case the experimental task involves respiratory changes (Lee & Becker, 2019).

In recent years, the use of wearable devices for HRV measurement has become increasingly widespread due to their ease of long-term use in naturalistic settings (Immanuel et al., 2023). Many of these devices utilize photoplethysmography, a low-cost, non-invasive optical technique that detects changes in blood volume in peripheral tissues to derive pulse rate variability (PRV) (Allen, 2007). Through PRV, it is possible to estimate the same HRV parameters in both frequency and time domains (Pietilä, J. et al. 2018). However, while PRV can serve as a reliable proxy for HRV in healthy individuals, it may not always provide the same degree of precision, particularly under circumstances where blood flow is inconsistent. For instance, cold exposure has been shown to reduce the correlation between HRV and PRV, with the degree of variance depending on the site of PRV measurement (distal vs. central

areas) (Mejia-Mejia et al., 2020). While HRV remains a direct indicator of ANS influences on the heart, PRV may offer additional insight into ANS responses at peripheral sites, particularly under conditions of acute and chronic stress (Mejia-Mejia et al., 2020). Although both HRV and PRV can be monitored over extended periods in ambulatory settings, PRV measurement facilitates the assessment of ANS activity across a broader range of daily activities. This is substantiated by some studies using wearables devices for measuring HRV in several populations (Chrousos et al., 2022; Mason et al., 2024; Natarajan et al., 2020; Singstad et al., 2021). Nonetheless, for accurate interpretation of PRV as a surrogate for HRV, it is critical to account for local physiological conditions and external environmental factors (Hu et al., 2024).

Neural control of the HRV

Heart rate is regulated by structures distributed throughout the neuroaxis. The intrinsic cardiac nervous system is a complex neuronal network composed of ganglionic plexuses embedded in epicardial fat and the heart wall. This system's function is controlled by extrinsic influences mediated by the vagus nerve and sympathetic nerves. The sympathetic innervation of the heart originates in the intermediolateral cell columns of the spinal cord. Cardiac sympathetic preganglionic neurons are cholinergic and send small myelinated axons that synapse with noradrenergic neurons in the superior, middle cervical ganglia, and cervicothoracic ganglia. Sympathetic activation increases the automatism of the sinoatrial node (SA), atrioventricular node (AV), excitability of the His-Purkinje system, contraction strength during systole, and relaxation speed of the cardiac muscle during diastole (Palma & Benarroch, 2014). Cardiac vagal preganglionic neurons are located in the nucleus ambiguus (NA) and the dorsal motor nucleus of the vagus (DMNV). Most vagal nerve fibers innervate the atrium, SA node, and AV node. The main parasympathetic effects (through cholinergic neurons in the cardiac ganglia) are the inhibition of SA node pacemaker activity (decreased heart rate), reduction of AV conduction, and decreased excitability of the His-Purkinje system. These effects are mediated by M2 muscarinic receptors, which are coupled to G-protein transduction pathways (SA node hyperpolarization). Under resting conditions, vagal tone predominates over the sympathetic system in SA node automatism (Shaffer et al., 2014).

At the brainstem level, the rostral ventrolateral medulla (RVLM) contains glutamatergic sympathoexcitatory neurons that tonically activate sympathetic preganglionic neurons in the intermediolateral column (spinal cord) and serve as a common effector of descending and reflex pathways controlling cardiac function. RVLM neurons are activated by psychological stress, pain, hypoxia, hypovolemia, and hypoglycemia, both directly and through descending impulses from the forebrain. The NA contains most of the cardioinhibitory vagal motor neurons controlling SA node automatism and AV node conduction. These NA neurons are activated by glutamatergic afferents from the nucleus tractus solitarius (NTS) and inhibited by local GABAergic neurons and signals from the ventral respiratory group activated during inspiration. Neurons in the DMNV contribute to the control of heart rate, AV conduction, and contractility to a lesser extent. The NTS is the first relay station for visceral afferent information. The caudal portion of the NTS receives afferents from baroreceptors, cardiac receptors, chemoreceptors, and pulmonary receptors, primarily via vagal and glossopharyngeal afferents, and is the first central relay for all bulbar reflexes, including cardiac reflexes that control blood pressure and heart rate (Dampney, 2016).

Brain regions and networks involved in the regulation of stress response and HRV

Some forebrain areas form a network that initiates integrated autonomic, neuroendocrine, and behavioral responses (stress response) to emotionally relevant or stressful stimuli. Key areas involved in this response include regions of the prefrontal cortex (PFC), insular cortex, anterior cingulate cortex (ACC), central nucleus of the amygdala (CeA), and various hypothalamic nuclei. These regions project to brainstem and medullary nuclei that control cardiac function; these projections are direct or indirect through the periaqueductal gray (PAG) (Quadt et al., 2022).

Cardiovascular afferent information is transmitted by dorsal horn (lamina I) or NTS neurons to cortical areas via the thalamus. Visceral afferent input can also reach the thalamus, hypothalamus, and amygdala via the parabrachial nucleus of the pons, and from catecholaminergic neurons of the A1/C1 group in the ventrolateral medulla. The hypothalamus contributes to autonomic heart control primarily from its paraventricular and

dorsomedial nuclei. These send sympathoexcitatory signals to the RVLM and intermediolateral columns of the spinal cord (Palma & Benarroch, 2014).

The hypothalamus also modulates cardiovagal responses through its effects on the NTS and NA. The dorsomedial hypothalamic nucleus may contribute to reduced HRV in anxiety-like states (Palma & Benarroch, 2014).

In the insula, the posterior insular cortex (IC) receives thalamic afferents transmitting convergent pain, temperature, and visceral sensitivity information, providing a primary interoceptive representation. The middle IC integrates this information with afferents from sensory cortical areas, the ACC, and the amygdala, then transmits it to the anterior IC, which represents the awareness of the internal bodily state and is a key component of emotional experience (Zaki et al., 2012). Activation of the left IC has been observed during vagal modulation of HRV (Napadow et al., 2008).

The rostral (or ventral) ACC includes a pregenual and subgenual region with strong connections to the CeA, hypothalamus, and parabrachial nucleus, playing an important role in emotional responses and behaviors. The ventral ACC (subgenual) has projections to parasympathetic nuclei and is associated with vagal modulation of HRV (Lane et al., 2009). The rostral ACC is part of the default mode network, active when attention is not focused on external stimuli but on internal cognitive processes such as memory retrieval, future thinking, and mind-wandering. The dorsal ACC, along with the anterior insular cortex, is a central component of the salience network, mainly involved during the transition from resting state (default network) to tasks requiring cognitive control (Critchley, 2009). Activation of the dorsal ACC during these tasks is associated with increased sympathetic drive, which increases heart rate (and decreases HRV).

The amygdala assigns emotional valence to sensory stimuli and is involved in fear conditioning mechanisms. The medial CeA subdivision projects to the hypothalamus and brainstem, triggering autonomic, endocrine, and motor manifestations of fear responses. Sympathoexcitatory responses involve excitatory connections with the RVLM and inhibition

of barosensitive neurons in the NTS. Functional neuroimaging studies consistently found coactivation of the lateral and medial amygdalae with HRV changes during rest and emotional content tasks (Lane et al., 2009).

In the PFC, the orbitofrontal and ventromedial PFC areas exert an inhibitory effect on the amygdala through GABAergic neurons in the lateral CeA and intercalated nuclei (Motzkin et al., 2015). These prefrontal influences are considered the basis of emotional regulation mechanisms, including fear extinction (Kraynak et al., 2018). Thus, besides promoting vagal stimulation, these prefrontal areas can tonically inhibit sympathoexcitatory responses initiated in the amygdala.

Research on the relationship between HRV and brain structures, functions, and networks has increased in recent years. Initial functional magnetic resonance imaging (fMRI) studies confirmed the existence of a central autonomic network (CAN) that includes these described structures and the relationship between functional changes within and between these brain areas and HRV changes during rest and specific cognitive tasks (Napadow et al., 2008). This relationship is age-dependent, as HRV reduction with age is accompanied by changes in brain functional connectivity (Kumral et al., 2019). High HRV is associated with greater functional connectivity between the ACC, basal ganglia, thalamus, amygdala, and midbrain; and between the amygdala and the ACC, basal ganglia, anterior insula, and dorsolateral PFC (Chang et al., 2013a). Within frequency domains, HF HRV fluctuations correlate with the functional connectivity strength of the amygdala and ACC with the thalamus and brainstem. Conversely, LF HRV fluctuations correlate with greater connectivity between the amygdala and ACC with the occipitoparietal cortex. HRV positively correlates with connectivity between the medial PFC and ACC, pregenual ACC, and anterior insula, and negatively with connectivity between the medial PFC and brainstem. At rest, increased HRV is associated with stronger connectivity between the right amygdala and medial PFC. These findings support the central role of the PFC in HRV control, generally through its modulatory effect on other CAN regions and specifically through inhibitory control over the amygdala. Recent research also suggests that brain areas involved in executive functions (mainly within the PFC) have inhibitory effects on habitual and reactive responses encoded within subcortical

structures (amygdala and basal ganglia to hypothalamus PAG and brainstem) (Mulcahy et al., 2019). However, the brain networks related to the autonomic nervous system, particularly those involved in HRV control, are much more complex than described so far. Recent studies have shown that although conventional structural and functional maps identify regions jointly modulated by the sympathetic and parasympathetic systems, only graph theory analysis techniques can discriminate between them, revealing that autonomic systems are mediated by widely distributed network interactions far more complex than previously described (Ruffle et al., 2021). Graph theory has provided a more advanced description of these networks. The most recent and comprehensive study of brain autonomic regulation of HRV (autonomic connectome) included 518 individuals and constructed an autonomic connectome with multiple MRI modalities (volumetry, DTI, and resting-state fMRI). This connectome was initially constructed with each neuroimaging modality individually, then a multimodal structural and functional model was generated. Unlike previous studies, this study discriminates the relationship between HRV and sympathetic and parasympathetic networks and found that these are mediated by high-level distributed interactions in the central nervous system, offering a new multidimensional generative network representation of the autonomic system. It is assumed that HRV could serve as a marker of autonomic function in the CNS (Ruffle et al., 2021). This new theoretical framework of the interaction between the autonomic connectome and HRV could be used for research related to chronic stress. It is important to clarify that despite these significant advances, the cerebral control of HRV in humans is not fully understood.

Theoretical models on the interaction between the nervous system, stress and HRV

Given the central role of the brain in the perception and response to stress and the evidence from various approaches (from psychology, neuroscience, neuropsychiatry) linking these phenomena to different autonomic functions and especially HRV, different authors have developed conceptual models to build a theory to frame the findings so far and future research. The most relevant models for our research are mentioned here. These models are more complementary than exclusive.

According to Porges' polyvagal theory (Porges, 2007), vagal nerves contain specialized subsystems that regulate adaptive responses. According to this theory, unmyelinated vagal fibers, originating from the DMNV complex, participate in regulating the "freezing response," causing immobilization and passive avoidance. On the other hand, myelinated fibers, phylogenetically more recent, originating in the NA, are involved in inhibitory control or "vagal brake," allowing emotional self-regulation and inhibition of sympathetic flow. As humans, we are not limited to basic behavioral responses of fight, flight, or freeze; we can self-regulate and initiate prosocial behaviors when exposed to stressors. The theory suggests that the capacity for emotional and behavioral self-regulation depends on proper autonomic nervous system functioning, specifically the vagal system. This implies that standardized assessment of vagal tone could serve as a potential marker of self-regulation capacity (Shaffer et al., 2014) and, therefore, as a biomarker of top-down regulation of cognitive, emotional, and behavioral processes.

In 2000, Thayer and Lane (Thayer & Lane, 2000) proposed a model called neurovisceral integration, integrating the autonomic nervous system, cognitive and affective systems into a structural and functional network in the nervous system, playing a fundamental role in emotional regulation. This network behaves as an integrated system necessary for cognitive, affective, and autonomic control and for regulating neuroendocrine and behavioral responses. According to this model, brain inhibitory processes, through negative feedback systems, play a necessary role in interrupting ongoing behavior and redistributing resources to other tasks (Thayer & Lane, 2000). When these negative feedback mechanisms fail (defective inhibitory systems), sympathetic activation occurs, observed in chronic stress states and their related conditions, such as anxiety disorders. This network and its neural structures have been associated with HRV (Shaffer et al., 2014), and low HRV is associated with psychopathological and pathophysiological states related to stress (Thayer & Lane, 2009).

Brosschot, Verkuil, and Thayer, continuing along the same lines of neurovisceral integration, proposed a model called the generalized unsafety theory of stress (Brosschot et al., 2017). This theory posits that the stress response is a default response, not generated but disinhibited. Inhibition is tonically provided by the prefrontal cortex as long as safety is perceived, and

this inhibition is reflected in high HRV. Thus, chronic stress responses are due to the perception of generalized unsafety, which may or may not depend on exposure to stressors. For survival, the disinhibition of the threat response must be as immediate and rapid as possible. That is why it is a predetermined response, "always ready," only to be inhibited when there is certainty of safety. In this context, low resting HRV ("vagal withdrawal") is an index of chronically disinhibited stress response; and it is important to mention that HRV can be persistently low even when stressors are not present (Brosschot et al., 2017).

In addition to neural control, several non-neural peripheral factors influence HRV. Stress hormones, such as adrenaline and cortisol, as well as thyroid hormones, can also reduce HRV (Brusseau et al., 2022). Baroreceptors in the arterial pressure system regulate HRV through parasympathetic activity (Lamotte et al., 2021). Glucose levels, as well as lipid profiles and chronic inflammatory markers, body temperature, and blood levels of O2 and CO2, also influence HRV (Sammito et al., 2024). Finally, HRV tends to decrease with age and shows differences between men and women (Koenig & Thayer, 2016; Kvadsheim et al., 2022). All these peripheral factors interact with neural control to modulate HRV, and their impact can vary according to individual and contextual conditions.

EMPIRICAL EVIDENCE ON THE RELATIONSHIP BETWEEN STRESS AND HRV

HRV as a biomarker of emotional and cognitive self-regulation

Cognitive neuroscience evidence supports the proposition that HRV can be used as a biomarker for self-regulation (the ability to regulate behavioral, cognitive, and emotional processes). However, research results on the relationship between self-regulation and HRV are heterogeneous (Arakaki et al., 2023). A meta-analysis of 123 studies that examined the relationships between HRV parameters and various aspects of self-regulation (executive functioning, emotional regulation, effortful control) found that higher HRV is associated with better self-regulation. The effect size was small but statistically significant. It is important to note that the meta-analysis also reported that this correlation was stronger in older individuals compared to younger ones. This correlation persisted across all evaluated components

(cognitive, behavioral, and emotional self-regulation), suggesting that HRV may serve as a global biomarker of self-regulation rather than a specific component. The authors conclude that more research is needed to establish this relationship, particularly considering mediating and confounding factors (Holzman & Bridgett, 2017).

Heart rate variability (HRV) has also been employed to measure *physiological coherence*, which refers to the degree of synchronization between various oscillatory systems within the body (McCraty and Childre, 2010). Elevated physiological coherence has been correlated with a positive psychological state and linked to enhanced social, cognitive, and physical performance, greater emotional stability, and numerous health benefits (Sarabia-Cobo, 2015). Empirical evidence suggests that physiological coherence, measured through HRV, significantly increases during breathing techniques used in HRV biofeedback exercises aimed at managing stress responses. However, that further research is necessary to validate its use as a reliable marker of acute stress (Mejia-Mejia et al., 2018). While physiological coherence is a valuable tool for assessing autonomic balance, its use for diagnosing and treating chronic stress requires more research.

Relationship between stress biomarkers and HRV

HRV has also been associated with biomarkers that have been clearly linked to chronic stress. A study involving 757 healthy adults revealed that all HRV indices inversely relate to levels of IL-6 and CRP. In a multivariate model that included factors such as gender, race, age, smoking, physical activity, SBP, and BMI, this relationship with inflammatory markers remained significant (Sloan et al., 2007). A more recent study with 40 healthy individuals aged 21 to 56 found significant correlations between reduced parasympathetic vagal tone (evaluated by RMSSD and pNN50) and higher salivary cortisol levels and lower DHEA levels (Mazgelyte et al., 2021). Similarly, reduced HRV is also associated with poor recovery from cardiovascular, endocrine, and immunological markers after stress and increased tendency towards systemic inflammation and HPA axis activation (Mulcahy et al., 2019). Some anthropometric parameters have also been associated with HRV. A study involving 43 healthy university students found a significant correlation between some HRV parameters

(LF and the SQ -LF/HF index-) and higher body fat percentage, intramuscular adipose tissue, and lower skeletal and bone muscle mass (Liew et al., 2013).

Relationship between psychological stress measurements and HRV

Regarding the relationship between HRV and stress scales, there are also knowledge gaps. A meta-analysis published in 2018 summarizes studies examining the relationship between HRV and psychological stress measurement (Kim et al., 2018). This study indicates that, generally, low HRV is significantly associated with higher stress levels in various settings (work, student, university, medical). The most frequently reported HRV variation factor was low parasympathetic activity. However, this review included 21 studies, and only two of them included validated stress scales applicable to the general population, and these two studies recorded HRV only for 5 minutes. Only four studies included 24-hour Holter recordings, two of which used scales to measure work stress, one evaluated stress in a medical emergency setting, and another measured exposure to stressful events. Another meta-analysis also published in 2018 focused on the relationship between work stress and HRV during work and included ten articles meeting their criteria (Järvelin-pasanen et al., 2018). The most common methods for assessing work stress were the Job Content Questionnaire (JCQ) and the Effort-Reward Imbalance (ERI) questionnaire. HRV was evaluated using 24-hour Holter ECG. The main finding was that high occupational stress was associated with reduced HRV, specifically with reduced parasympathetic activation. Reduced parasympathetic activation was observed as decreases in RMSSD and HF and increases in the LF/HF ratio. There was heterogeneity among the studies regarding the methodology for evaluating stress and HRV, limiting the comparability of the results (Järvelin-pasanen et al., 2018).

Allostatic load, HRV and morbidity and mortality

Research on allostatic load has produced several proposals for an Allostatic Load Index (ALI), ranging from the original, which includes ten medical variables—some of which are difficult to measure routinely in clinical practice—to more recent simplified versions like that of Mauss et al., which includes HRV among its variables. Despite these efforts, there is still no consensus on the most suitable ALI as an indicator of chronic stress. This lack of consensus primarily stems from uncertainty in selecting the ideal number and type of

variables to include in the index and the method for calculating the risk score (Carbone et al., 2022).

HRV, measured by RMSSD, is included in Mauss's ALI. However, as noted in their latest study, RMSSD did not negatively correlate with the Perceived Stress Scale (PSS); instead, they found a controversial positive correlation (Mauss et al., 2021). Despite this, previous evidence leads these authors to recommend using vagally mediated HRV parameters as a neurophysiological variable for calculating allostatic load. Some researchers also suggest incorporating clinimetric instruments, such as scales or other psychological measures, to assess stress (Guidi et al., 2021).

It is important to remember that, similar to the ALI, numerous studies have found associations between low HRV and an increased risk of various physical and mental disorders (Agorastos et al., 2023; Chesnut et al., 2021), as well as higher mortality rates in both patient and healthy populations (X. Gao et al., 2022; M. Jarczok et al., 2022)

CHAPTER 3: HEART RATE VARIABILITY AS A BIOMARKER OF ALLOSTATIC LOAD: A SCOPING REVIEW

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Abstract

Objective: This study aims to characterize the evidence for a relationship between allostatic load (AL) measured as a multisystemic index and heart rate variability (HRV) in healthy individuals.

Introduction: AL is a term used to describe the cumulative physiological burden that results from chronic stress. It is best quantified using a multisystemic assessment approach, which integrates multiple stress biomarkers. Elevated AL is correlated with an increased risk of adverse physical and mental health outcomes. While HRV has been proposed as a stress biomarker, its relationship with AL in healthy individuals remains unclear.

Inclusion criteria: This review included human studies that examined the relationship between AL with a multisystemic assessment and HRV, specifically focusing on participants without diagnosed physical or mental disorders. No restrictions were placed based on age or sex.

Methods: The review followed the Joanna Briggs Institute methodology and adhered to the PRISMA extension for scoping review guidelines. Databases searched included PubMed, Embase, Google Scholar, Epistemonikos, and LILACS. Two independent reviewers selected the studies and extracted the data.

Results: Two studies met the inclusion criteria. Both studies reported a negative relationship between AL and HRV. However, the studies differed in their definitions of the participant's health status, methods of measuring AL and HRV, and methods to test the AL-HRV relationship.

Conclusion: Current evidence is insufficient to establish a definitive relationship between AL and HRV in healthy individuals. Limited evidence suggests a negative relationship between these two, indicating that HRV may serve as a potential biomarker for chronic stress. Future studies should use standardized measurements of both AL and HRV in healthy populations to further elucidate this relationship and its clinical implications.

Keywords: Heart rate variability, allostatic load, chronic stress, biomarker, healthy.

1. Introduction

Allostatic load (AL) refers to the wear and tear of the body due to chronic stress (McEwen, 2007). It has been widely accepted as a framework for research on the relationship between chronic stress and human health-disease processes (McEwen, 2017). AL is operationalized through the allostatic load index (ALI), a multisystemic approach that assigns an AL score to individuals based on stress-related biomarkers (Seeman et al., 1997). Higher AL scores are associated with a greater risk of physical and mental disorders (Guidi et al., 2021) and mortality (Parker et al., 2022).

Heart rate variability (HRV) is the fluctuation in the duration of heartbeat intervals (Shaffer et al., 2014). Reduced HRV indicates dysfunction of chronic stress-related psychophysiological mechanisms (Holzman & Bridgett, 2017). It serves as a marker of autonomic regulation, an index of psychophysiological well-being (Mather & Thayer, 2018), and an all-cause mortality risk marker (Jarczok et al., 2022).

While there is evidence of the relationship between stress biomarkers and HRV (Boschiero & Ilich, 2022; Sloan et al., 2007), and between the acute stress response and HRV in healthy populations (Corrigan et al., 2021; Immanuel et al., 2023; Kim et al., 2018), little research has been conducted regarding HRV and its relationship with the multisystemic model of chronic stress, specifically within the AL framework.

A recent systematic review summarized the evidence of the relationship between occupational stressors and HRV in frontline workers and tactical operators (Corrigan et al., 2021). The authors found that acute exposure to occupational stressors was correlated with reduced HRV. In contrast, they did not find enough evidence to establish HRV as a marker of chronic exposure to stressors in this population. It is essential to underline that the studies in this review operationalize AL through individual biomarkers or psychometric tools and did not apply a multisystemic approach. In addition, the health status of participants was not clearly defined.

The main challenges in evaluating the relationship between AL and HRV originate from the heterogeneity in their measurement methods. Electrocardiographic recording duration and context, HRV metric selection, and interpretation in relation to stress differ among studies (Chesnut et al., 2021; Immanuel et al., 2023; Kim et al., 2018; Thielmann et al., 2021). In addition, the biomarker selection and calculation methods for the AL scoring across studies are inconsistent (Carbone et al., 2022; McCrory et al., 2023).

This scoping review systematically characterizes current research on the relation between AL measured in a multisystemic way and HRV in healthy individuals. Given this goal and the expected scarcity of research and knowledge on this relationship, a scoping review is deemed more appropriate than other evidence synthesis approaches.

Evaluating the relationship between AL and HRV in healthy individuals could provide insights into the reliable and standardized use of HRV as a biomarker of chronic stress in preventive medicine. Moreover, considering the numerous disorders related to chronic stress and the widespread use of wearable devices that can measure HRV in naturalistic situations,

establishing HRV as an AL biomarker could significantly impact public health (McEwen, 2022).

Review question:

What is the empirical evidence regarding the relationship between AL and HRV in healthy individuals?

2. Methods

This scoping review followed the methodology outlined by the Joanna Briggs Institute (JBI) for scoping reviews (Peters et al., 2020) and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis extension for Scoping Reviews (PRISMA-ScR) (Tricco et al., 2018).

2.1. Protocol and registration

This review protocol was registered and is publicly available on the Open Science Framework (https://osf.io/n7ka8/). While there were no deviations from the original protocol, updates were made to the review concept, databases, and inclusion criteria (https://osf.io/n7ka8/). Initially, the Web of Science and ProQuest databases were planned for inclusion but were excluded due to limited access.

2.2. Eligibility criteria

2.2.1. Participants:

This review included studies with participants who had no diagnosed physical or mental disorders, without restrictions based on age or sex.

2.2.2. Concept:

Studies exploring the relationship between AL operationalized through a multisystemic assessment and HRV in healthy individuals were included. Studies that evaluated individual biomarkers or psychological measures of AL were excluded.

2.2.3. Context:

Studies including healthy individuals from any population, setting, or context were considered. Studies based on sex, geographic area, culture, ethnicity, or race were not excluded.

2.2.4. Types of studies:

Quantitative, qualitative, and mixed-methods study designs were considered for inclusion, along with systematic reviews, text, and opinion papers. Due to resource limitations for translations from other languages, only English, Spanish, and Portuguese studies were searched. The inclusion criteria spanned from 1997 to February 2024, chosen because the first proposal for operationalizing the AL concept in a multisystemic way was presented in 1997 (Seeman et al., 1997).

2.3. Information sources

The search strategy aimed to locate published and unpublished primary studies and reviews. Databases searched included PubMed, Embase, Google Scholar, LILACS, and Epistemonikos. Reference lists of included studies were screened for additional studies. The initial search was conducted on May 5, 2023, and the last on February 05, 2024. Corresponding authors of included studies were contacted by e-mail to inquire about needed information or possible unpublished research and data.

2.4. Search strategy

An initial limited search of MEDLINE was conducted to identify relevant articles. Text words in titles, abstracts, and index terms were used to develop a comprehensive search strategy. This strategy, including all identified keywords and index terms, was adapted for each information source. See **Supplementary Table 1** for details on the search strategy and the total number of records gathered from each database.

2.5. Study selection

Following the search, all identified citations were collated and uploaded to the online systematic review platform Rayyan (Qatar Computing Research Institute, Doha, Qatar)

(Ouzzani et al., 2016), and duplicates were removed. Two independent reviewers, the first (JMSA) and the last (ALMA) authors screened the titles and abstracts to ensure they were relevant to the inclusion criteria. The full text of selected citations was then assessed in detail to verify the inclusion criteria. Reasons for excluding studies that did not meet the criteria were recorded and reported (**Supplementary Table 2**). Disagreements between reviewers were resolved through discussion. Critical appraisal of selected studies was not conducted, as this scoping review aimed to provide an overview of the literature.

2.6. Data extraction

JMSA and ALMA independently extracted data from the included papers using a data extraction tool (**Supplementary Table 3**) developed based on the JBI guideline (Peters et al., 2020). The tool was refined following piloting with a few studies and then applied to the included studies. Data extracted included participant details, concepts, context, and critical findings relevant to the review question. This encompassed information such as HRV measurement methods, specific AL biomarkers and number of biomarkers used, methods for calculating AL score, and the primary results regarding the relationship between AL and HRV. Any disagreements between reviewers were resolved through discussion.

2.7. Synthesis of results

The extracted data was presented in a table format, aligning with the aims of this scoping review. The table format was pre-defined in the review protocol. A narrative summary with relevant tabulated results is provided to address the aim and review question.

3. Results

3.1. Study inclusion

Initially, 1662 reference articles were identified, and 1057 remained after removing duplicates. Fifty-one articles underwent full-text review after title and abstract screening. Finally, two studies met the inclusion criteria (Figure 1). Detailed exclusion reasons are presented in Supplementary Table 2.

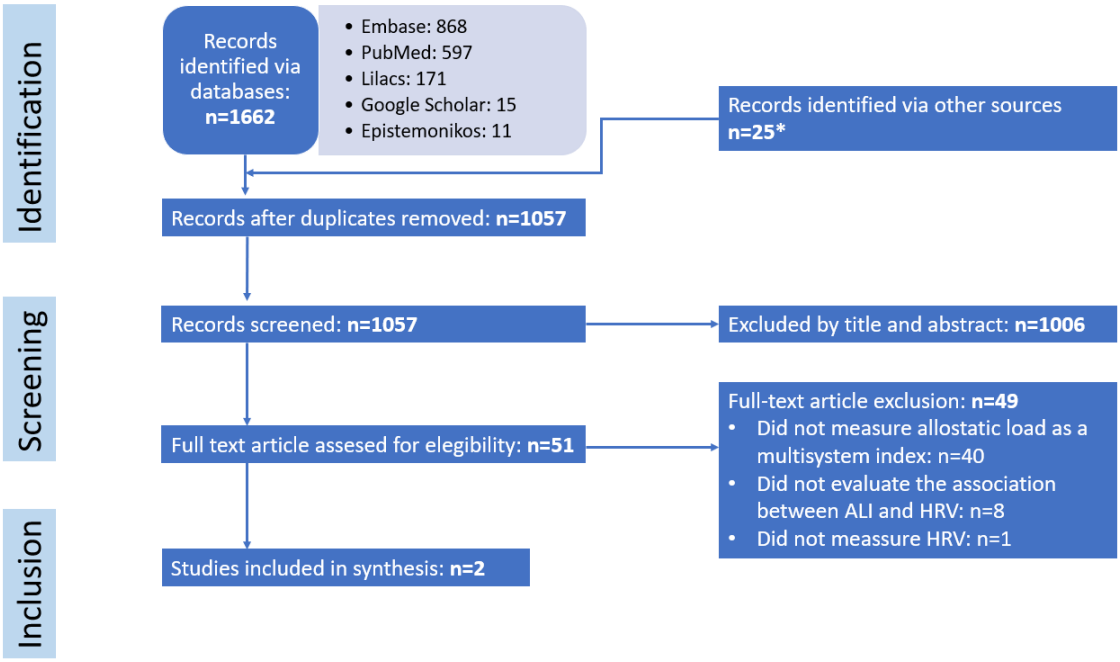

Fig. 1. Flow chart of the studies selection. *These are the references that were retrieved from the papers during the full-text screening process.

3.2. Characteristics of the included studies

The first study, published in 2010, analyzed data from 782 individuals from the USA included in the Coronary Artery Risk Development in Young Adults Study (CARDIA) (Seeman et al., 2010). The second study, published in 2017, analyzed data from 27 men from South Africa (Viljoen & Claassen, 2017). Table 1 presents the characteristics of the two included studies, including the variables relevant to this review.

3.3. Review findings and synthesis

This review found two studies that investigated the correlation between AL and HRV in healthy individuals. Both studies recorded electrocardiograms (ECG) to derive HRV metrics. Seeman et al. (2010) included the high-frequency band (HF) and low-frequency band (LF) in the frequency domain analysis, while Viljoen and Claassen (2017) used total HRV, HF, and LF metrics in the frequency domain, as well as the root mean square of successive differences (RMSSD) and the standard deviation of all normal-to-normal intervals (SDNN) metrics in the time domain analysis, and Poincare (SD1 and SD2) metrics in the nonlinear HRV analysis. Viljoen and Claassen (2017) used RMSSD, HF, and SD1 as indicators of

vagal activity. Both studies found a statistically significant negative relationship between AL and HRV. Table 1 provides more information on each study's main characteristics and results.

Table 1. Characteristics and main results of the included studies

Author, year of publication, country	Type of source	Aims	Study design	Population	Health status	Sample size	Age range	Sex	ECG recording environment	ECG recording position	ECG recording duration	HRV metrics	Number of AL biomarkers	AL biomarkers used	Statistical method for AL assesment	Statistical method for testing AL and HRV relationship	Results
Seeman et al. 2010, USA	Research paper	1. To test and compare alternative models of AL 2. To evaluate the factorial invariance of the final AL model across sex and ethnicity.	Cross-Sectional	Black (54.7%) and white (45.3%) from the CARDIA Cohort (Year 15 exam): Birmingham, Chicago, Minneapolis, and Oakland	The health status of participants when measuring AL biomarkers and HRV is unclear	782	32-47	Males (42.1%) and females(57. 9%)	Controlled conditions (Laboratory)	Sitting	10 minutes	HF LF	18	HR, HF, LF, SBP, DBP, waist circumference, , glucose, insulin, HDL, LDL, TGC, , interleukin-6, CRP, fibrinogen, , 12-hour urinary norepinephrine and epinephrine, salivary cortisol AM rise and PM decline	Structural equation modeling was used to generate alternative models of AL	Factor loadings and path coefficients	1. The best fit to the data was a meta-factor model of AL as an aggregate measure of six biological systems represented as latent subfactors (blood pressure, metabolic, inflammatory, HRV, sympathetic nervous system, and hypothalamic-pituitary-adrenal axis). 2. Metabolic, inflammation, and BP subfactors showed the highest factor loadings and path coefficients to the AL meta-factor. 3.HRV was the fourth-highest factor loading (-0.33, $p<0.05$) and had showed an inverse correlation with the AL meta factor, showing the fourth-highest factor loading (-0.33, $p<0.05$). 4. The meta-factor model showed minimal variance across sex and ethnicity.
Viljoen, Claassen. 2017, South Africa	Research paper	1. To compare AL and HRV as disease risk indicators 2. To evaluate the feasibility of HRV inclusion into an ALI	Cross-Sectional	Armed protection subjects employed in Pretoria	Employees who were not taking medications that affect HRV and "passed" a routine evaluation by the company's medical consultant. No further information was provided on the clinical status of the participants.	27	28-57	Male	Controlled conditions (Laboratory)	Supine and standing	4 periods of 5 minutes each (20 minutes total)	HF SDNN RMSSD SD1 and SD2	13	SBP, DBP, WTH, BMI, HDL, LDL, TC, aldosterone, albumin, CRP, fasting blood glucose, HbA1c, overnight (12-hour) urinary cortisol excretion.	The ALI score was determined by adding the number of parameters falling within the highest risk quartile, except for HDL, which is considered high risk if it falls within the lowest quartile. The risk quartiles were determined based on normal clinical ranges for biochemical parameters. Normal ranges for anthropometric and blood pressure measurements were determined using international guidelines.	Spearman´s rank correlation coefficients	1. Negative correlations between the ALI and SDNN for all periods and positions (r=-0.65 for Ph0, -0.53 for Ph1, -0.56 for Ph2, and -0.48 for Ph3; all $p<0.01$). 2. Negative correlation between AL and vagal measures of HRV for the supine position (r=-0.59, -0.58, -0.59 for RMSSD, HF, SD1, respectively) and the 10 minutes after standing up (r=-0.41, -0.41, -0.40 for RMSSD, HF, SD1, respectively). No correlation between ALI and vagal response to orthostatic stress (first 5 minutes after standing up). 3.Total and vagal measures of HRV are comparable to ALI as health risk indicators.

AL, allostatic load; ALI, allostatic load index; ECG, electrocardiogram; SBP, systolic blood pressure; DBP, diastolic blood pressure; HRV, heart rate variability; HF, high-frequency band; LF, low-frequency band; RMSSD, root mean square of differences between successive R-R intervals; SDNN, standard deviation of all normal R-R intervals; SD1, standard deviation of the immediate or short-term heart rate variability; SD2, standard deviation of the long-term heart rate variability; BMI, body mass index; WTH, waist-to-hip ratio; TC, total cholesterol; HDL, high-density cholesterol; LDL, low-density cholesterol; TGC, triglycerides; CRP, c-reactive protein; HbA1c, glycated hemoglobin.

4. Discussion

This scoping review found two studies that evaluated the relationship between AL and HRV in healthy individuals. Both studies reported a negative relationship but employed different AL calculating methods and statistical approaches to test this relationship.

4.1. There is insufficient evidence of the relationship between AL and HRV in healthy individuals.

As hypothesized, the evidence of the relationship between AL and HRV in healthy people is scarce. This finding aligns with a recent systematic review by Corrigan et al., who also found insufficient evidence for HRV to measure the physiological status of individuals under occupational chronic stress (Corrigan et al., 2021). It is essential to note some differences between Corrigan's review and our study. Corrigan et al. (2021) focused on occupational stress, while our study did not limit the stress type or context. Additionally, their study population was limited to first responders and tactical operators, while our study focused on individuals in the general population without specifying their occupational status. Corrigan et al. (2021) did not clearly define the health status of their participants, which may have introduced variability in their findings. In contrast, our study targeted healthy individuals as the population of interest, ensuring a homogeneous sample concerning health status. Additionally, Corrigan et al. did not restrict the methods used to evaluate AL, including studies that assessed AL through psychological tools or with individual biomarkers, potentially limiting its validity as a measure of AL. In our review, we focused on studies that utilized a multisystemic approach to measure AL, which provides a comprehensive assessment of chronic stress. These differences are important when interpreting and applying results and designing future research on this topic.

The lack of research on the link between AL and HRV in healthy individuals experiencing chronic stress may be in part attributed to the challenges in defining operational parameters for AL (Carbone et al., 2022; Mauss & Jarczok, 2021). This is supported by the fact that the main reason the papers were excluded from our review was the absence of an ALI or other multisystemic assessment in the published studies (Supplementary Table 2).

4.2. The evidence suggests a negative relationship between AL and HRV

Seeman's study utilized a meta-factor model of AL, which included HRV as a latent subfactor, and found a significant negative path coefficient between the AL and the HRV subfactor measured with the HRV metrics HF and LF. Viljoen and Claassen's study (Viljoen and Claassen, 2017) calculated an AL score using the classical highest-risk quartile and found a statistically significant negative Spearman correlation between ALI and total HRV. In addition, they also found a correlation between ALI and vagal measures of HRV using frequency, time, and nonlinear domain analysis. These findings are particularly interesting as they mirror observations in acute experimental and real-life stress scenarios, where a negative correlation between acute stress and HRV has been noted (Corrigan et al., 2021; Immanuel et al., 2023; Kim et al., 2018; Thielmann et al., 2021). However, Corrigan et al.'s systematic review reveals that studies conducted on populations exposed to repeated stressors have reported varied results in HRV measurements, including increases, decreases, and no changes, and they suggest that experiencing repeated stressors may result in a more complex HRV response than observed in acute stressor settings, emphasizing the need for further research on the use of HRV as an appropriate AL monitoring tool (Corrigan et al., 2021).

4.3. Studies apply different methods for measuring AL and HRV as well as for testing their relationship

The two studies reviewed have critical methodological differences, making them incomparable. The main differences in the approaches for measuring and analyzing AL and HRV are the following. Variations in AL biomarkers selection and score calculation: both studies used serum, cardiovascular, metabolic, inflammatory, and neuroendocrine biomarkers. Seeman et al. (2010) also included markers of autonomic nervous system function (HRV and 12-hour overnight urine norepinephrine); however, they included more parameters for the metabolic and inflammation systems, resulting in differences in the exhaustiveness of assessment across the AL biological systems. In Seeman et al.´s study, HRV was one of six latent biological subfactors of the AL meta-factor using a structural equation modeling. Viljoen and Claassen also included more metabolic parameters than the other AL domains; they used a classical highest-risk quartile method

to calculate the AL score. Different approaches to generate the AL score have been proposed, with the most used being the sum of the number of parameters falling into the highest risk quartile (Carbone et al., 2022). However, a standardized operational definition of AL has been a persistent issue, hindering research and clinical applications. While substantial progress has recently been made in this direction, further research is required to establish the best optimal set of AL biomarkers as well as the method of calculating the AL score that can be used across different populations and clinical settings (Carbone et al., 2022; McCrory et al., 2023).

HRV metrics: Viljoen and Claassen measured HRV using frequency domain (HF), time domain (SDNN and RMSSD), and nonlinear analyses (Poincare SD1 and SD2), while Seeman et al. used only frequency domain (HF and LF) analysis. The most appropriate HRV metric to measure stress has not been established. The vagal metrics support the most significant evidence of the relationship between stress and HRV (Laborde et al., 2017). For this reason, when conducting psychophysiology research, it is recommended to include metrics indicative of cardiovagal function. However, to avoid potential measurement bias, it is recommended to include several measurements of this component, such as one in the time domain and another in the frequency domain (Laborde et al., 2017). The physiologic origins of the nonlinear HRV metrics are not clear, and more research is required to make recommendations for their use in psychophysiology (Shaffer & Ginsberg, 2017).

ECG recording duration and position: In the study by Seeman et al. The ECG recording was performed for 10 minutes while the patient rested in a sitting position. For their part, Viljoen and Claassen did it in four periods of five minutes each, the first with the patient in a supine position followed by three consecutive periods in a standing position. Several guidelines have been published for measuring HRV in medical research (Laborde et al., 2017; Quintana et al., 2016; Shaffer & Ginsberg, 2017; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). It is important to note that long-term recordings (24 hours), short-term recordings (5 minutes), and ultra-short recordings (<5 minutes) rely on different physiological mechanisms that regulate HRV. Therefore, their values and applications are not interchangeable (Shaffer et al., 2014). For clinical contexts, short-term recordings may lose their predictive capacity in comparison to 24-hour recordings

(Shaffer & Ginsberg, 2017). However, in psychophysiological research, a five-minute recording under controlled conditions may be preferable to facilitate comparison between studies (Laborde et al., 2017). As a result, the duration and analysis method of HRV measures will depend on the objective and research question. Given that evaluating the individual's autonomic and medical status is crucial for interpreting the relationship between stress and HRV (Kim et al., 2018) and that longer records have more predictive value for clinical outcomes (Shaffer & Ginsberg, 2017), using 24-hour recordings in real-life outpatient settings could be more appropriate for evaluating HRV as a potential biomarker of chronic stress. At this point, it is worth mentioning that wearable devices are currently being used to measure HRV in natural contexts. Many of these devices use photoplethysmography to measure pulse rate variability (PRV) as a measure of HRV. However, although these devices can indirectly measure HRV and have been used in studies with different populations (Chrousos et al., 2022; Mason et al., 2024; Natarajan et al., 2020; Singstad et al., 2021), for an adequate interpretation of this measure it is important to take into account the physiological circulatory conditions and external environmental factors such as temperature (Mejia-Mejia et al., 2020).

Other critical methodological concerns: The results are limited to the age range of these studies (28 - 57 years). This is crucial as both AL and HRV vary with age (Koenig et al., 2021; Upchurch et al., 2015). Viljoen and Claassen included only men in their study, which is an essential issue since the regulation of HRV is different for men and women. For example, compared to men, women have a more significant parasympathetic component of HRV (Koenig & Thayer, 2016). Additionally, the AL also differs between the sexes (McCrory et al., 2020). The study by Seeman et al. (2010) included white and black Americans, while Viljoen and Claassen did not specify the race/ethnicity of participants. Describing race or ethnicity is essential when analyzing HRV and AL, given that these variables and their relationship vary according to different ethnicities (Hill et al., 2015; Tavares et al., 2022). The participant´s health status was not clearly specified in either study. Both studies were conducted with cross-sectional analysis, which limits the ability to establish a causal relationship between the AL score and the progressive physiological accumulation of wear and tear due to chronic stress (Seeman et al., 2010). Finally, these studies did not consider critical confounding factors in the relationship between AL and HRV, such as physical activity, smoking, socioeconomic status, medications, and educational level (Laborde et al., 2017; McCrory et al., 2023).

4.4. Limitations of this review

The study's limitations include being unable to search some databases, such as Web of Science and ProQuest. However, it is unlikely that additional sources meeting the inclusion criteria would be found in these databases, as leading journals on the topic are indexed in the databases included in this review. Furthermore, authors and experts were contacted, and no additional studies on the AL-HRV relationship were identified. Another limitation is the exclusion of studies that evaluated the relationship between HRV and specific biomarkers related to chronic stress response. The decision was made to limit the search to studies that evaluated AL objectively and in a multisystemic way (i.e., through ALI or a multisystemic AL construct) according to the conceptual framework of AL and our review question.

4.5. Implications for practice and research and next steps

Although HRV has been proposed as a chronic stress biomarker, and some researchers have included it in the operational biomarkers of AL for both healthy people and patients (Mauss & Jarczok, 2021), the following are important issues that require addressing: 1) More studies in healthy populations are necessary to understand the relationship between AL and HRV. This is very relevant for preventive medicine, considering the large number of clinical conditions related to chronic stress for which we could implement primary or secondary prevention measures. 2) It is essential to clearly define the study population, particularly its health status, and describe the methods used to measure HRV and AL. 3) Standardized recommendations are necessary for HRV measurement in chronic stress research, including ECG recording duration and context and HRV metric selection. 4) Determining the optimal number and specific AL biomarkers, ensuring a multisystemic assessment for application in different healthy populations, is crucial. 5) Longitudinal studies, including relevant clinical outcomes in healthy populations, are needed to define the role of HRV as a biomarker of chronic stress and its clinical usefulness.

5. Conclusion

There is currently insufficient research to establish the nature of the relationship between AL and HRV in healthy individuals. The limited evidence shows a negative relationship between these two, which suggests that HRV could be a chronic stress biomarker for

healthy populations. Uncovering evidence in this area could significantly impact public health, especially in preventive medicine, given the many disorders linked to chronic stress and the widespread use of wearable devices that can measure HRV in real-life situations. However, research advances in this field require an operational, standardized, and valid measurement of AL and HRV, properly defining healthy individuals in different populations. Furthermore, longitudinal rather than cross-sectional studies in healthy populations will be more informative to understand the complex relationship between AL and HRV.

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Author contributions:

Juan Marcos Solano Atehortua: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft, writing-review and editing, project administration, supervision. **Yedselt V. Ospina-Serrano**: resources, investigation. **Juan D. Caicedo-Jaramillo**: resources, investigation, writing -review and editing. **Juan C. Calderón**: resources, writing -review and editing. **Jaime Gallo-Villegas**: resources, writing-review and editing. **Ana L. Miranda-Angulo**: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-review and editing, project administration, supervision and funding acquisition.

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Supplements

Supplementary Table 1. Search terms and results of each database

Database	Search terms	Results
Embase	(allostasis:ab,ti OR 'allostatic load':ab,ti OR 'allostatic overload':ab,ti) AND 'heart rate variability':ab,ti OR 'cardiac vagal control':ab,ti OR 'cardiac vagal tone':ab,ti	868
PubMed	((("allostasis"[Title/Abstract] OR "allostatic load"[Title/Abstract] OR "allostatic overload"[Title/Abstract]) AND "heart rate variability"[Title/Abstract]) OR "cardiac vagal control"[Title/Abstract] OR "cardiac vagal tone"[Title/Abstract]) AND (1999/1/1:2024/2/5[pdat])	597
LILACS	(allostasis) OR (allostatic load) OR (allostatic overload) AND (heart rate variability) OR (cardiac vagal control) OR (cardiac vagal tone)	171
Google Schollar	"allostasis" "allostatic load" "allostatic overload" AND "heart rate variability" "cardiac vagal control" "cardiac vagal tone"	15
Epistemonikos	(title:((title:(allostasis) OR abstract:(allostasis)) OR (title:(allostatic load) OR abstract:(allostatic load)) OR (title:(allostatic overload) OR abstract:(allostatic overload)) AND (title:(heart rate variability) OR abstract:(heart rate variability)) OR (title:(cardiac vagal control) OR abstract:(cardiac vagal control)) OR (title:(cardiac vagal tone) OR abstract:(cardiac vagal tone))) OR abstract:((title:(allostasis) OR abstract:(allostasis)) OR (title:(allostatic load) OR abstract:(allostatic load)) OR (title:(allostatic overload) OR abstract:(allostatic overload)) AND (title:(heart rate variability) OR abstract:(heart rate variability)) OR (title:(cardiac vagal control) OR abstract:(cardiac vagal control)) OR (title:(cardiac vagal tone) OR abstract:(cardiac vagal tone))))	11
Total		**1662**

Supplementary Table 2. Reasons for exclusion

Author	Title	Selection	Reasons for exclusion
Viljoen	Allostatic load and HRV as health risk indicators	**Included**	
Seeman	Modeling multi-system biological risk in young adults- the Coronary Artery Risk Development in Young Adults Study (CARDIA)	**Included**	
Causadias	Culture and Biology Interplay: An Introduction	Excluded	Measures ICA and HRV but not the association between them
Corrigan	Monitoring Responses to Basic Military Training with Heart Rate Variability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Corrigan	Monitoring stress and allostatic load in first responders and tactical operators using heart rate variability- a systematic review	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Corrigan	Soldier performance management_ The utility of heart rate variability to evaluate allostatic load	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Friedman	An autonomic flexibility–neurovisceral integration model of anxiety and cardiac vagal tone	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Jiryis	Resting-state heart rate variability (HRV) mediates the association between perceived chronic stress and ambiguity avoidance	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Ketheesan	Stress allostatic load and mental health in Indigenous Australians	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Macartney	Overnight sleeping heart rate variability of Army recruits during a 12-week basic military training course	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Mauss	A streamlined approach for assessing the Allostatic Load Index in industrial employees	Excluded	Measures ICA and HRV but not the association between them
Mauss	The streamlined Allostatic Load Index a replication of study results	Excluded	Measures ICA and HRV but not the association between them
Mauss	The streamlined allostatic load index is associated with perceived stress in life findings from the MIDUS study	Excluded	Measures ICA and HRV but not the association between them
Milosevic	Research Methodology for Real-Time Stress Assessment of Nurses	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Porges	Vagal tone- a physiologic marker of stress vulnerability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Thayer	Beyond Heart Rate Variability Vagal Regulation of Allostatic Systems	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Viljoen	Cynicism as subscale of burnout	Excluded	Measures ICA and HRV but not the association between them
Visnovcova	Alterations in Vagal-Immune Pathway in Long-Lasting Mental Stress	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Zalli	Shorter telomeres with high telomerase activity are associated with raised allostatic load and impoverished psychosocial resources	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Andrade	Heart rate and cardiac autonomic responses to concomitant deep breathing, hand grip exercise, and circulatory occlusion in healthy young adult men and women	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Balzarotti	Cardiac Vagal Control as a Marker of Emotion Regulation in Healthy Adults- A Review	Excluded	Measures ICA and HRV but not the association between them
Beckie	A Systematic Review of Allostatic Load, Health, and Health Disparities	Excluded	Measures ICA and HRV but not the association between them
Kim	Stress and Heart Rate Variability- A Meta-Analysis and Review of the Literature	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Low	The effects of Hong Kong employees workplace stress on heart rate variability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Martin	Low cardiac vagal control is associated with genetic liability for elevated triglycerides and risky health behaviors	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Matuz	Enhanced cardiac vagal tone in mental fatigue_ Analysis of heart rate variability in Time-on-Task, recovery, and reactivity	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Mohammadi	The persistent effect of acute psychosocial stress on heart rate variability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Petrowski	Stress load of emergency service- effects on the CAR and HRV of HEMS emergency physicians on different working days (N=20)	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Pluim	Correlation of heart rate variability with cardiac functional and metabolic variables in cyclists with training induced left ventricular hypertrophy	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Pulopulos	Association between changes in heart rate variability during the anticipation of a stressful situation and the stress-induced cortisol response	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Ramos	Lower cardiac vagal tone in non-obese healthy men with unfavorable anthropometric characteristics	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Rash	Maternal cortisol during pregnancy is related to infant cardiac vagal control	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Rhudy	Are Cardiometabolic Markers of Allostatic Load Associated With Pronociceptive Processes in Native Americans- A Structural Equation Modeling Analysis From the Oklahoma Study of Native American Pain Risk	Excluded	Measures ICA and HRV but not the association between them
Schmid	Associations between being overweight, variability in heart rate, and well-being in the young men	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Smeets	Autonomic and hypothalamic–pituitary–adrenal stress resilience- Impact of cardiac vagal tone	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Steptoe	Stress responsivity and socioeconomic status- a mechanism for increased cardiovascular disease risk	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Su	Heart Rate Variability Feature Selection using Random Forest for Mental Stress Quantification	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Thayer	A meta-analysis of heart rate variability and neuroimaging studies- Implications for heart rate variability as a marker of stress and health	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Tran	Heart Rate Variability Measurement to Assess Acute Work-Content-Related Stress of Workers in Industrial Manufacturing Environment—A Systematic Scoping Review	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Tung	Cardiac Vagal Control in Response to Acute Stress during Pregnancy- Associations with Life Stress and Emotional Support	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Valensi	Insulin- and glucagon-like peptide-1-induced changes in heart rate and vagosympathetic activity- why they matter	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Zhang	Exploring the Associations Between Perceived Stress and Physiological Stress Using Heart Rate Variability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Dina Tell	Heart Rate Variability and Inflammatory Stress Response in Young African American Men	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Nelson	Psychobiological markers of allostatic load in depressed and nondepressed mothers and their adolescent offspring	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Frasch	Heart Rate Variability Code - Does It Exist and Can We Hack It	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Gruenewald	Allostatic load and frailty in older adults	Excluded	Does not measure HRV
Kemp	From Psychological Moments to Mortality- A Multidisciplinary Synthesis on Heart Rate Variability Spanning the Continuum of Time	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Rios	Heart Rate Variability and Allostasis in Individuals with Depression and Anxiety Symptoms	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Streeter	Effects of yoga on the autonomic nervous system, gamma-aminobutyric-acid, and allostasis in epilepsy, depression, and post-traumatic stress disorder	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Young	Heart-rate variability- a biomarker to study the influence of nutrition on physiological and psychological health	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Kim	Short Term Analysis of Long Term Patterns of Heart Rate Variability in Subjects under Mental Stress	Excluded	The measure does not account for the allostatic load as a multisystemic construct
	Retrieved from references of the selected papers		
George	Assessing the effect of long term physical training and classification of training status using HRV and HRR of female police recruits.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Grant	The difference between exercise-induced autonomic and fitness changes measured after 12 and 20 weeks of medium-to-high intensity military training.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Huovinen	Relationship between heart rate variability and the serum testosterone-to-cortisol ratio during military service.	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Jouanin	Analysis of heart rate variability after a ranger training course.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Jouanin	Short half-life hypnotics preserve physical fitness and altitude tolerance during military mountainous training.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Nikolova	Psychophysiological assessment of stress and screening of health risk in peacekeeping operations.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Head	Prior mental fatigue impairs marksmanship decision performance	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Clemente-Suarez	Psychophysiological response to acute-high-stress combat situations in professional soldiers.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Brisinda	Real-time imaging of stress-induced cardiac autonomic adaptation during realistic force-on-force police scenarios	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Diaz-manzano	Higher use of techniques studied and performance in melee combat produce a higher psychophysiological stress response.	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Giessing	Effects of coping-related traits and psychophysiological stress responses on police recruits' shooting behavior in reality-based scenarios	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Adams	Ambulatory blood pressure and Holter monitoring of emergency physicians before, during, and after a night shift	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Clemente-Suarez	Psychophysiological response and fine motor skills in high-altitude parachute jumps	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Clemente-Suarez	Psychophysiological response in an automatic parachute jump	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Gnam	On the relationship between physical activity, physical fitness, and stress reactivity to a real-life mental stressor	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Hansen	Relationship between neuroticism, threat of shock and heart rate variability reactivity	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Souza	Resting vagal control and resilience as predictors of cardiovascular allostasis in peacekeepers	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Tornero-Aguilera	Use of psychophysiological portable devices to analyse stress response in different experienced soldiers	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Sandvik	Physical fitness and psychological hardiness as predictors of parasympathetic control in response to stress: a Norwegian police simulator training study	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Sanchez-Molina	Assessment of psychophysiological response and specific fine motor skills in combat units	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Hynynen	Cardiac autonomic responses to standing up and cognitive task in overtrained athletes	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Michael S	Submaximal exercise intensity modulates acute post-exercise heart rate variability	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Andrew ME	Police work stressors and cardiac vagal control	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Matuz	Enhanced cardiac vagal tone in mental fatigue: analysis of heart rate variability in time-on-task, recovery, and reactivity	Excluded	The measure does not account for the allostatic load as a multisystemic construct
Sevre	Reduced autonomic activity during stepwise exposure to high altitude	Excluded	The measure does not account for the allostatic load as a multisystemic construct

Supplementary table 3. Data extraction formulary

Item	Content (include page number)
Author	
Year of publication	
Title	
Journal	
Type of source (journal, thesis, poster, etc)	
Language	
Sample size	
Gender included	
Race	

Age range
Type of population (employees, sports, etc.)
Health status
The environment of ECG measurement
Duration ECG measurement
HRV metrics
Number of AL biomarkers
Method for AL score calculation
Method to test HRV and AL relationship
Clinical outcomes (Y/N)
Comments

CHAPTER 4: ALLOSTATIC LOAD AND ITS INTERACTION WITH BRAIN STRUCTURES AND NETWORKS IS ASSOCIATED WITH HEART RATE VARIABILITY.

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Abstract

Introduction: Heart rate variability (HRV) has been suggested as a potential marker of allostatic load (AL). However, there is limited available evidence to support this assumption, and there is no empirical data on the link between AL and ambulatory-measured HRV. This study examined the relationship between an allostatic load index (ALI) and 24-hour HRV metrics in healthy men. Additionally, we sought to investigate how structural and functional central nervous system parameters interact with AL to influence HRV.

Methods: This cross-sectional study included healthy adult men from Medellín, Colombia. Exclusion criteria were the presence of diagnosed medical or mental disorders, symptoms of physical or mental illnesses, and routine medication use at the time of the

study. The ALI was developed using the quartile risk summation method. Data from 24-hour Holter monitoring was used to extract HRV metrics (sympathovagal quotient, SQ; the root mean square of the successive difference, RMSSD; the standard deviation of the NN intervals, SDNN; the low-frequency band, LF; and high-frequency band, HF). We evaluated the ALIs and HRV relationship using multiple linear regression (MLR) models. Additionally, we obtained measurements of brain connectivity and structure using magnetic resonance imaging., and investigated how ALI interacts with the resting-state functional connectivity strengths of three major brain networks (default, salience, and control), the cortical thickness of brain structures within these networks, and the volume of subcortical areas in their influence on HRV.

Results: 88 men aged between 21 and 40 years were included. 70% were middle socioeconomic. A new ALI composed of seven parameters (waist-to-height ratio, WtHR; high-density lipoprotein HDL; glycated hemoglobin, HbA1C; high-sensitivity C-reactive protein, hs-CRP; systolic blood pressure; diastolic blood pressure; and dehydroepiandrosterone sulfate) was correlated with the SQ after adjusting for potential confounders (β=0.093, p=0.004, IC=0.03-0.15). In the exploratory MLR analyses of the interaction between ALI-7 and resting-state brain networks and structures, several interactions had negative correlations with HRV (SDNN and HF).

Conclusion: These findings suggest that the SQ is an indicator of AL, highlighting its potential as a biomarker for preventing, diagnosing, and managing chronic stress. Our exploratory analysis indicates that HRV reflects the interplay of central and peripheral physiological processes related to chronic stress. This finding will need validation in future research.

Keywords: Chronic stress, allostatic load, allostatic load index, sympatovagal quotient, heart rate variability, central autonomic network.

1. Introduction

Allostatic load (AL) reflects the cumulative wear and tear on the body due to chronic stress, making individuals more susceptible to disease (McEwen & Stellar, 1993). An allostatic load index (ALI) is a well-accepted approach for measuring stress, incorporating multiple biomarkers that represent various physiological systems involved in the stress response (Seeman et al., 2001). Elevated AL levels are linked to a higher risk

of physical and mental disorders as well as increased mortality (Guidi et al., 2021; Parker et al., 2022). Nonetheless, the measurement of AL faces challenges such as inconsistency in selecting biomarkers and methods for calculating the ALI, which restricts its application in clinical practice (Carbone et al., 2022). A recent comprehensive meta-analysis has attempted to establish a consensus and has been published, endorsing the use of an ALI comprising five biomarkers associated with mortality risk (McCrory et al., 2023). This recent publication highlights the need to reproduce these findings in other populations.

Heart rate variability (HRV) refers to the fluctuation in the duration of intervals between heartbeats (Shaffer et al., 2014). It integrates the intrinsic heart nervous system with central nervous system (CNS) regulation and the sympathetic and parasympathetic innervation of the heart (Shaffer & Ginsberg, 2017). HRV has also been proposed as a marker of neurophysiological processes of emotional self-regulation (Holzman & Bridgett, 2017). Additionally, there is evidence of the relationship between biomarkers related to the stress response and HRV (Boschiero & Ilich, 2022; Sloan et al., 2007). In patients and healthy populations, HRV has been considered a marker of acute stress (Chesnut et al., 2021; Corrigan et al., 2021; Immanuel et al., 2023; Kim et al., 2018). Consistent with this, low HRV has been associated with a higher risk of morbidity and all-cause mortality (Jarczok et al., 2022).

Based on the above information and its ability to represent the interaction between central and peripheral physiological processes, HRV has been suggested as a potential indicator of mental well-being, chronic stress, and related psychiatric disorders (Agorastos et al., 2023; Bandelow et al., 2017; Chesnut et al., 2021). However, a systematic review published in 2021 reported insufficient empirical evidence to support the use of HRV as an indicator of chronic stress (Corrigan et al., 2021). A more recent scoping review (Solano-Atehortua et al., 2024) reported only two studies that assessed the relationship between chronic stress measured in the AL framework and HRV. The main concern with these two studies is that they focused on measuring HRV in experimental laboratory settings using short-duration recordings with the patient at rest rather than employing long-term ambulatory recording in natural and clinical contexts, as recommended for clinical practice (Shaffer & Ginsberg, 2017; Task Force of the European Society of

Cardiology and the North American Society of Pacing and Electrophysiology. Circulation., 1996)

The Central Autonomic Network (CAN) is a pivotal group of brain structures that regulate the body's autonomic function. This network integrates signals from the central and peripheral nervous systems to maintain balance in the body and coordinate the stress response (Lamotte et al., 2021). The CAN, which includes the anterior cingulate cortex (ACC), prefrontal cortex, amygdala, hypothalamus, insula, nucleus accumbens, and brainstem nuclei, among other structures (Quadt et al., 2022), plays a crucial role in balancing sympathetic and parasympathetic activity, a key factor in generating and regulating HRV. Resting-state networks (RSNs) have also been shown to modulate both chronic stress and HRV (Chang et al., 2013; Valenza et al., 2019). Despite these findings, no studies have evaluated the interaction between ALI, the CAN structures, and the RSNs to modulate HRV.

This study examines the relationship between AL and HRV. In addition, we explored how the AL interacts with the structural and functional parameters of the CNS to modulate HRV.

2. Methods

2.1. Ethical Considerations

This study was conceived and designed within the framework of international agreements on research involving human subjects. Specifically, it adheres to the Declaration of Helsinki of the World Medical Association, in its seventh revision (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), and the International Ethical Guidelines for Health-Related Research Involving Humans (CIOMS-WHO Guidelines) 2016-2017. The project was approved by the Bioethics Committee of the Faculty of Medicine of the University of Antioquia (Approval Act 007 of May 11, 2017) and by all participating institutions. Individuals who participated in this research signed an informed consent form after a detailed explanation and clarification of any doubts (Approval Act 007 of May 11, 2017).

2.2. Study population selection

This cross-sectional study included healthy adult men aged 21 to 40 years. Exclusion criteria were the presence of diagnosed medical illnesses or mental disorders, symptoms of physical or mental illnesses, drug abuse, routine medication use at the time of the study, use of cardiac devices or prostheses, and contraindications for magnetic resonance imaging. Participants were recruited through printed notices and e-mail at the University of Antioquia, radio and television announcements, and personal recommendations among research group members. All subjects underwent physical examination to obtain anthropometric and blood pressure measurements. Biological samples, 24-hour Holter, and structural and resting-state functional magnetic resonance imaging (rsfMRI) data were collected within 4 weeks. All measurements performed on the volunteers were completed within a maximum period of 4 weeks from study enrollment.

2.3. Health parameters

2.3.1. Anthropometric and blood pressure measurements

Blood pressure was measured three consecutive times using a calibrated sphygmomanometer with the participant seated and left arm supported. The average of the three readings was calculated. Waist and hip circumference were measured twice using an anthropometric measuring tape, and the averages were calculated. Height was measured using a stadiometer, and weight was measured using a calibrated electronic scale. Body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated accordingly.

2.3.2. Biochemical parameters

Fresh serum obtained from peripheral blood was used for all measurements. C-reactive protein (CRP), high-density lipoprotein (HDL), total cholesterol (TC), glycated hemoglobin (HbA1c), and dehydroepiandrosterone sulfate (DHEAS) were measured in a specialized clinical laboratory using Dimension® Flex ® DF34, DF48B, DF27, and DF105A reagent cartridges and Architect 8K27, respectively.

2.3.3. Electrocardiographic recording and analyses

Electrocardiographic (ECG) recordings were performed using a Custo Flash 510V model monitor installed in volunteers between 7 am and 10 am, and it was removed the following day around the same time to ensure a minimum ECG recording of 21 hours. The monitor performed recordings in three channels every 2.5 ms ± 0.1% per channel, with a quantification amplitude of 5.6 μV/Bit ± 1% of 10 bit, a response frequency range of 0.05-45 Hz, and a resistance ≥ 10 MΩ filtered at 50 Hz 80 dB. Volunteers were asked to perform their usual life activities during recording but were instructed to avoid physical exercise. They were also asked to log their daily activities. HRV time- and frequency-domain metric calculations were performed using the ANS diagnostic module of the Custo diagnostics Holter ECG (Customed Inc., Germany). The HRV metrics used for the analyses were SDNN, the root mean square of successive differences between normal heartbeats (RMSSD), the natural logarithms of LF and HF (ln LF and ln HF), and SQ as ln (LF/HF).

2.3.4. Neuroimaging processing and analyses

Scanning was performed using an Ingenia 3T Philips MRI scanner with a 16-channel phased-array rigid head coil. The neuroimaging acquisition was done as previously described (Miranda-Angulo AL. et al. 2024). We measured the resting-state functional connectivity strength (FCS) of RSNs using the node strength connectivity metric (Stg) derived from the graph theory approach. This metric provides information about the weight of the correlation between a node and the rest of the cortex. Using the Brain Connectivity Toolbox, Stg was computed as the sum of the link weights connected to a node (Rubinov et al., O. 2010). Afterward, the average Stg was calculated by dividing it by the number of connected nodes in each network (Fornito et al., 2016). Volumetric parameters and cortical thickness were measured using the segmentation of 453 regions with the freely available and extensively validated FreeSurfer software based on the basic recon-all Freesurfer routine (400 regions based on Schaefer's cortical parcellation and 53 subcortical regions). The volume measurements were normalized using each subject's intracranial volume (ICV). We used default parameters for cortical thickness to create a 3-dimensional cortical surface model. The steps include automated Talairach transformation, intensity normalization, and nonbrain tissue removal. Hemispheres are separated, and the cerebellum and brain stem are excluded. A tessellation of the gray and white matter boundary and topology correction follows this. Cortical thickness was

calculated as the distance between the white and pial surface, and surface deformation enabled the detection of tissue boundaries. Cortical thickness of 400 regions based on Schaefer's cortical parcellation to Yeo 17 networks were extracted.

2.3.5. Stress, depression, and anxiety evaluation

We assessed exposure to stressors and perceived stress using the following three scales: The Daily Hassles Scale (DHS) interview to assess exposure to daily stressors in the last month (Hannan et al., 2015). The Revised Checklist of Stressful Life Events (LSC-R) was used to identify stressful and potentially traumatic events throughout life and their impact in the last year, calculating scores for perception, severity, and number of post-traumatic stress disorder symptoms (Humphreys et al., 2011). We also applied the 10-item Perceived Stress Scale (PSS) to evaluate perceived stress in the last month (Campo-Arias et al., 2014). These three scales do not have cut-off points, but higher scores indicate greater exposure to stressors or higher perceived stress according to the scale. To assess anxiety symptoms, we used the following two screening scales: The State-Trait Anxiety Inventory (STAI) (Spielberger et al, 1970), which detects anxiety as a state (STAI-S) and trait (STAI-T), with scores ranging from 20 to 80 points, using 41 as the cut-off point for STAI-S and 44 for STAI-T (Guillén-Riquelme & Buela-Casal, 2011). Finally, we used the Zung Depression Scale (Zung, 1965) to evaluate the presence of depressive symptoms. Scores range from 20 to 80 points, with a score equal to or higher than 50 indicating depression (Campo-Arias et al., 2005). These instruments are validated in Spanish and for the Colombian population.

2.3.6. Physical activity, habitual diet, and sleep patterns

The short Spanish version of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003) was used to assess physical activity habits. This questionnaire categorizes physical activity into three levels: light, moderate, and vigorous (Craig et al., 2003). Usual dietary intake over twelve months was assessed using an electronic adaptation of a self-administered semi-quantitative food frequency questionnaire (FFQ) developed locally (Monsalve-Álvarez and González-Zapata, 2011). The determination of nutritional energy and macronutrient content from this questionnaire was performed using an in-house developed R script. To evaluate sleep quality, participants were asked to rate their sleep in one of three categories: good, average, and bad. For sleep quantity

assessment, participants were asked to define the amount of sleep they had each night recently on most days in one of three categories: less than 4 hours, between 4 and 6 hours, and more than 6 hours.

2.4. Statistic al analysis

2.4.1. Sample size and power analysis

A published study found a correlation between ALI and RMSSD between 0.296 and 0.6 (Viljoen & Claassen, 2017). Assuming a correlation of 0.3, a type 1 error of 0.05, and a power of 80%, a sample of 85 individuals is required. This was calculated using Epidat software version 4.2.

2.4.2. Descriptive analyses

The variables were analyzed according to their nature (quantitative or qualitative). Qualitative variables were presented as absolute values and percentages in the descriptive analyses. Based on their distribution, quantitative variables were grouped using central tendency and dispersion measurements (means with standard deviations or medians with interquartile ranges). To conduct multivariate linear regression analyses (MLR), SDNN and RMSSD were transformed using natural logarithms in the SPSS software version 25.

2.4.3. Development of ALI

Parameters were selected based on physiological and conceptual criteria of AL as a multisystemic construct (Seeman et al., 2001), along with the most robust and most recent scientific evidence of each parameter's ability to mediate AL within an ICA (McCrory et al., 2023). Based on the 5 biomarkers recommended by McCrory et al. (WHtR, HbA1C, CRP, HDL, HR), considering that our objective was to evaluate the relationship of ALI with HRV, we decided to exclude resting HR from the ICA parameters. In this way, to these 4 initial biomarkers, ICT, HbA1C, HDL, and CRP, we decided to add parameters of the cardiovascular system, i.e. systolic and diastolic blood pressure, considering the robust evidence of the relationship between AL and blood pressure (Chiger et al., 2022; Feres et al., 2019), and the relationship of the latter with relevant clinical outcomes with morbidity and mortality in healthy men (Volpe et al., 2019). Finally, to measure the neuroendocrine system, another fundamental system in the theory of chronic stress and AL, and also because DHEA-S was one of the 9 biomarkers related to clinical outcomes

in the McCrory metanalysis, we included the DHEA-S as another ALI biomarker. Thus, our developed ALI comprises the following 7 parameters (ALI-7): WHtR, HbA1C, HDL, CRP, SP, DP, and DHEA-S. The ALI scores were calculated using a summation method involving several steps. First, the distribution of biomarker values for the population was determined. Second, the risk quartile for each biomarker was calculated, with the upper quartile defined as the risk quartile for WHtR, SBP, DBP, HDL, HbA1C, and CRP, and the lower quartile defined as the risk quartile for HDL and DHEA-S. Third, the total score of the ALI was then calculated for each individual by assigning one point for each biomarker falling within the risk quartile, resulting in a total ALI score. This score reflects the number of biomarkers in the highest risk levels.

2.4.4. Evaluation of the relationship between ALI and HRV

First, simple linear regression analyses (SLR) were conducted between the developed ALI (ALI-7) and each HRV metric SQ, HF, LF, RMSSD, and SDNN. Then, we performed MLR analyses using ALI as the independent variable and the HRV metrics that showed statistically significant results in the SLR as dependent variables. We chose the confounding variables based on biological plausibility, published evidence, and data analysis using the stepwise backward method in Stata software. The following confounding variables were included: age, physical activity, daily cholesterol consumption, and the LSC-R perception score.

2.4.5. Evaluation of the relationship between ALI, the structural and functional parameters of brain regions, and HRV

We used RLM analyses to evaluate the effect of the interaction between ALI and resting-state functional connectivity and brain structures on HRV. For the analysis of interactions between ALI and functional connectivity of brain networks, the independent variables were ALI and the resting-state FCS of the subnetworks belonging to the executive control network (Control A, Control B, and Control C), default mode network, DMN, (DMN A, DMN B, and DMN C), and salience network (Salience A and Salience B). The dependent variables were SQ, SDNN, RMSSD, and HF, and the interaction term was ALI multiplied by the FCS of each subnetwork. To assess the interactions between ALI and cortical structures, the independent variables used in the functional analyses were ALI and the average cortical thickness of the brain areas belonging to the same subnetworks used for

the functional analyses. The dependent variables were SDNN, SQ, and HF. The interaction term was ALI multiplied by the cortical thickness of each subnetwork. Finally, to evaluate interactions between ALI and subcortical structures, the independent variables were ALI and the volume of subcortical structures belonging to the CAN (hippocampus, anterior cingulate cortex, accumbens, and amygdala) in both sides of the brain. The dependent variables were SDNN and SQ and the interaction term was ALI multiplied by the subcortical structure volume. In all the RLM models, interaction variables were centered by subtracting their respective means, and the interaction term was obtained by multiplying the centered variables. Confounding variables included age, mean arterial pressure, and LSC-R stress perception score. These confounders were selected using the stepwise backward selection in Stata version 16, considering biological plausibility and literature evidence (Laborde et al., 2017; Shaffer & Ginsberg, 2017).

2.4.6. Other statistical considerations

For all multiple linear regressions (MLR), we checked the assumptions of normality of residuals using the Shapiro-Wilk test ($p>0.05$), absence of multicollinearity by calculating the variance inflation factor ($VIF<5.0$), absence of autocorrelation with the Durbin-Watson test ($d=1.5–2.5$), and homoscedasticity with the Breusch-Pagan test ($p>0.05$). The quality of the models was verified using the Akaike Information Criterion (AIC), R^2, and adjusted R^2. Statistical significance was defined for all analyses by a p-value less than or equal to 0.05. All MLR analyses were performed using the STATA software, version 16. Additionally, to verify the robustness of the relationship found between the ALI-7 and HRV, we conducted a sensitivity analysis testing the model with other confounding variables. Finally, we compared the ALI-7 to an ALI (ALI-4) composed of four out of the five biomarkers recommended by McCrory et al. (excluding the FC for the reasons already stated) with respect to its relationship with the HRV.

3. Results

3.1 Descriptive Data

Out of 402 volunteers who completed the online pre-selection questionnaire, 299 were excluded because they did not meet the inclusion criteria. Of the remaining 103 subjects, 15 were withdrawn for various reasons, leaving 88 participants for analyses

(Supplementary Figure 1). Table 1 describes the study population's demographic, psychological, physiological, biochemical, and anthropometric characteristics**.**

The median age was 30 years; most participants had a bachelor's degree or were pursuing one, and most belonged to a middle socioeconomic level. Anthropometric and cardiovascular parameters were within normal ranges for their age and sex. Although participants were screened for mental disorder diagnoses and psychiatric medication use during the pre-selection process, some scored above the cutoff points for these scales: Zung scale (n=15, 17.04%), STAI-T (n=14, 15.9%), STAI-S (n=3, 3.4%), and both Zung and STAI (n=13, 14.8%).

Table 1. Demographic and clinical characteristics of study population

Variables	n=88
Sociodemographic	
Age (years), median (IQR)	30 (25.3-34.0)
Level of education, total (%)	
Bachelor	2 (2.3)
Undergraduate	68 (77.3)
Postgraduate	18 20.5)
Socioeconomic status, total (%)	
Low	17(19.3)
Middle	61(69.3)
High	10(11.3)
Anthropometrics	
BMI (kg/m^2), mean (SD)	24.2 (±2.6)
WHR (cm), median (IQR)	0.86 (0.8-0.9)
WHTR (cm), mean (SD)	0.48 (±0.04)
Cardiovascular	
Heart rate (beats/min), mean (SD)	70.1 (±7.8)
SBP (mmHg), mean (SD)	114.3 (±9.4)
DBP (mmHg), mean (SD)	70.3 (±7.0)
SDNN (ms), median (IQR)	84.3 (71.6-98.6)
RMSSD (ms), median (IQR)	55 (45.4-73.7)
Ln LF (ln ms^2), mean (SD)	7.5 (±0.5)
Ln HF(ln ms^2), mean (SD)	6.6 (±0.8)
SQ (Ln (LF/HF), mean (SD)	0.9 (±0.4)
Biochemical	
HbA1c (%), median (IQR)	5.3 (5.1-5.6)
HDL (mg/dL), median (IQR)	46.0 (40.1-52.1)

TC (mg/dL), mean (SD)	180.5 (±34)
TC/HDL (mg/dL), median (IQR)	3.8 (3.1-4.6)
hs-CRP (mg/L) median (IQR)	1.0 (0.1-1.4)
DHEA-S (µg/dL), mean (SD)	313 (±104)
Habitual diet	
Caloric intake (Kcal), median (IQR)	2350 (1775-2888)
Protein (gr), median (IQR)	83.6 (64.8-102.2)
Total fats (gr), median (IQR)	79.8 (58.9-103.6)
Carbohydrates (gr), median (IQR)	304.3 (224.8-396.3)
Fiber intake (gr), median (IQR)	17.9 (12.6-25.1)
Stress, anxiety and depression scales	
Perceived stress scale, mean (SD)	20 (±9)
DHS - Frequency, mean (SD)	22.4 (±11.5)
DHS - Severity, median (IQR)	40 (23-63)
LSC-R - Affirmative, median (IQR)	6 (4-9)
LSC-R - Perception, median (IQR)	9 (6-15)
Zung scale score >50, total (%)	15 (17.0)
STAI-T score >44, total (%)	14 (15.9)
STAI-S score >41, total (%)	3 (3.4)
Hours of sleep per day, total (%)	
≤6 hours	52 (59)
>6 hours	36 (41)

Values are expressed in percentages (%), mean (SD, standard deviation), or median (IQR, interquartile range). BMI, body mass index; WHR, waist to hip ratio; WHTR, waist to height ratio; HbA1c, glycated hemoglobin; HDL, high density lipoprotein; TC, total cholesterol; hs-CRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; SDNN, standard deviation of the NN intervals; RMSSD, root mean square of successive differences between normal heartbeats; Ln LF, natural logarithm of low frequency power; Ln HF, natural logarithm high frequency power; SQ, natural logarithm of the LF /HF ratio; DHEA-S, dehydroepiandrosterone sulfate; DHS, daily hassles scale; LSC-R, life stressot checklist; STAI-T, trait subscale of the STAI (State-Trait Anxiety Inventory); STAI-S, state subscale of the STAI.

3.2 ALI is positively related to the SQ

The ALI had a minimum score of 0 and a maximum of 5, with a median of 2 and an interquartile range of 1-3 (Supplementary table 1).

1-3 (Supplementary Table 1). In bivariate analyses, the ALI-7 was correlated with all HRV metrics (**Supplementary Table 2**). However, in multivariate analyses adjusted for

confounding variables, ALI-7 was related only to the SQ (β=0.093, 95% CI=0.03 - 0.15, *p*=0.004) (**Table 2**).

3.3. Sensitivity analyses

The correlation between the ALI-7 and SQ remained despite adjusting for the other confounding variables. The alternative ALI-4 was not correlated with any HRV parameter in the MLR analysis (**Supplementary table 3**)

Table 2. Multivariate linear regression between ALI and SQ

Dependent variable	Independent variables	β	*p*-value	95% CI	Model		
					Prob. F	R^2	Adjusted R^2
SQ	ALI	0.093	0.004	0.03 to 0.15	<0.01	0.27	0.22
	Age	0.011	0.148	-0.004 to 0.026			
	Chol_mg	0.0002	0.032	$2.3e^{-5}$ to $5.1e^{-4}$			
	LSC-R	-0.01	0.013	-0.018 to -0.002			
	PA						
	Low	0.078	0.41	-0.11 to 0.266			
	Moderate	-0.14	0.14	-0.33 to 0.049			

SQ, the natural logarithm of the LF/HF ratio; ALI, allostatic load index; PA, physical activity; Chol_mg, cholesterol consumption per day in milligrams; LSC-R_per, life stress checklist revised perception score.

3.4 Interaction between ALI and resting-state default mode network FCF is related to HRV

We found a significant interaction between the Default Mode Network B FCF and the ALI-7 using HF as the dependent variable (β= = -0.028, 95% CI= -0.057 to -0.0004, *p*=0.047) and between the Default Mode Network C FCF and the ALI-7 using SDNN as the dependent variable (β= -0.008, 95% CI= -0.015 to -0.001, *p*=0.022), with both interaction terms showing a negative correlation with HRV (**Table 3 and Supplementary Figure 2**).

Table 3. MLR of the interaction between ALI and FCS of Default B and Default C brain networks

Dependent variable	Interaction model	Independent variables	β	*p*-value	95% CI	Model		
						Prob. F	R^2	Adjusted R^2
HF	ALI*FCS_DMNB	ALI	-0.417	0.047	-0.185 to 0.103	<0.001	0.4	0.36
		FCS_DMNB	-0.019	0.272	-0.052 to 0.015			
		ALI*FCS_DMNB	-0.028	0.047	-0.057 to -0.004			
		Age	-0.952	<0.001	-0.125 to -0.065			
		MAP	-0.013	0.302	-0.038 to 0.012			
		LSC_per	0.002	0.018	-0.014 to 0.018			
SDNN	ALI*FCS_DMNC	ALI	-0.004	0.863	-0.0537 to 0.045	<0.001	0.4	0.30
		FCS_DMNC	0,003	0.525	-0.006 to 0.012			
		ALI*FCS_DMNC	-0.008	0.022	-0.015 to -0.001			
		Age	-0.025	<0.001	-0.035 to -0.015			
		MAP	-0.006	0.155	-0.015 to -0.002			
		LSC_per	0.003	0.249	-0.008 to 0.003			

HF, natural logarithm high-frequency power; SDNN, standard deviation of the NN intervals; ALI, allostatic load index; MAP, medial arterial pressure; LSC-R_per, life stress checklist revised perception score; FCS, functional connectivity strength; DMNB, default mode network B; DMNC, default mode network C.

3.5 Interaction between ALI and cortical thickness of default mode, salience, and control networks has a negative correlation with HRV

ALI-7 interacted with the average thickness of networks control B (β= -0.51, 95% CI= -0.970 to -0.050, *p*=0.030), DMN A (β= -0.473, 95% CI= -0.923 to -0.022, *p*=0.040), and salience A (β= -0.478, 95% CI= -0.927 to -0.029, *p*=0.037) when SDNN was the dependent variable. Also, ALI-7 showed an interaction with control B (β= -154, 95% CI= --2.875 to -0.204, *p*=0.024) and DMN A (β= -1.35, 95% CI= -2.669 to -0.046, *p*=0.043) when HF was the dependent variable. All β coefficients of these interaction terms were negative **(Table 4 and Supplementary Figure 2).**

Table 4. MLR of the interaction between ALI and cortical thickness of Salience A, Control B, and Default A brain networks

Dependent variable	Interaction model	Independent variables	β	*p*-value	95% CI	Model		
						Prob. F	R^2	Adjusted R^2
HF	ALI*ContB_th	ALI	-0.028	0.702	-2.875 to -0.204	<0.001	0.41	0.37
		ContB_th	-0.214	0.821	-2.093 to 1.66			
		ALI*ContB_th	-1.539	0.024	-2.875 to -0.204			
		Age	-0.948	<0.001	-0.127 to -0.063			
		MAP	-0.012	0.358	-0.037 to 0.0134			
		LSC_per	0.006	0.502	-0.011 to 0.022			
HF	ALI*DMNA_th	ALI	-0.041	0.579	-0.187 to 0.1052	<0.001	0.40	0.36
		DMNA_th	-0.168	0.852	-1.944 to 1.609			
		ALI*DMNA_th	-1.357	0.043	-2.669 to -0.046			
		Age	-0.092	<0.001	-0.123 to -0.060			
		MAP	-0.013	0.318	-0.038 to 0.012			
		LSC_per	0.004	0.602	-0.012 to 0.021			
SDNN	ALI*SalA_th	ALI	0.018	0.482	-0.033 to 0.069	<0.001	0.33	0.29
		SalA_th	-0.308	0.317	-0.916 to 0.3001			
		ALI*SalA_th	-0.478	0.037	-0.927 to -0.029			
		Age	-0.029	0.037	-0.040 to -0.018			
		MAP	-0.005	0.263	-0.0134 to 0.004			
		LSC_per	-0.002	0.476	-0.008 to 0.004			
SDNN	ALI*ContB_th	ALI	0.007	0.775	-0.042 to 0.057	<0.001	0.33	0.28
		ContB_th	0.106	0.745	-0.542 to 0.754			
		ALI*ContB_th	-0.51	0.030	-0.970 to -0.050			
		Age	-0.027	<0.001	-0.038 to -0.016			
		MAP	-0.005	0.264	-0.014 to 0.004			
		LSC_per	-0.002	0.464	-0.008 to 0.004			
SDNN	ALI*DMNA_th	ALI	0.003	0.911	-0.047 to 0.053	<0.001	0.33	0.28
		DMNA_th	0.076	0.805	-0.534 to 0.686			
		ALI*DMNA_th	-0.473	0.040	-0.923 to -0.022			
		Age	-0.026	<0.001	-0.037 to -0.015			
		MAP	-0.005	0.244	-0.014 to 0.004			
		LSC_per	-0.002	0.389	-0.008to 0.003			

HF, natural logarithm high-frequency power; SDNN, standard deviation of the NN intervals; ALI, allostatic load index; MAP, medial arterial pressure; LSC-R_per, life stress checklist revised perception score; ContB_th, mean cortical thickness of the control B network; DMNA_th, mean cortical thickness of the default mode network A; SalA_th, mean cortical thickness of the salience A network.

3.6 Interaction between ALI and volume of subcortical structures is negatively correlated with HRV

When SDNN was the dependent variable, ALI-7 interacted with the volumes of the left nucleus accumbens (β= -11447, 95% CI= -21372 to -1521, *p*=0.024), left amygdala (β= 10832, 95% CI= -21251 to -413, *p*=0.042), and ACC (β= -5086, 95% CI= -10135 to -36.666, *p*=0.048) with the ALI. Additionally, ALI interacted with the nucleus accumbens (β= -30474, 95% CI= -59328 to -1619, *p*=0.039) volume when HF was the dependent variable. All β coefficients of the interaction terms between the ALI and subcortical volumes were negative (**Table 5, Supplementary Figure 2**).

Table 5. MLR of the interaction between ALI and volumes of subcortical brain structures

Dependent variable	Interaction model	Independent variables	β	*p*-value	95% CI	Model		
						Prob. F	R^2	Adjusted R^2
HF	ALI*L_Accumb	ALI	-0,082	0.27	-0.230 to 0.065	<0.001	0.4	0.37
		L_Accumbens	29327,24	0.11	-6783 to 65438			
		ALI*L_Accumb	-30474	0.039	-59328 to 1619			
		Age	-0,086	<0.001	-0.116 to -0.056			
		MAP	-0,014	0.26	-0.0390 to 0.011			
		LSC_per	0,003	0.746	-0.013 to 0.019			
SDNN	ALI*L_Accumb	ALI	-0,011	0.664	-0.062 to 0.040	<0.001	0.3	0.29
		L_Accumbens	7734,96	0.219	-4686 to 20156			
		ALI*L_Accumb	-11447	0.024	-21372 to -1521			
		Age	-0,025	<0.001	-0.035 to -0.014			
		MAP	-0,006	0.206	-0.014 to 0.003			
		LSC_per	-0,003	0.291	-0.008 to 0.003			
SDNN	ALI*L_Amygdala	ALI	-0,004	0.864	-0.055 to 0.046	<0.001	0.3	0.3
		L_Amydala	11381.17	0.117	-2912 to 25675			
		ALI*L_Amygdala	-10832	0.042	-21251 to -413			
		Age	-0,026	<0.001	-0.036 to -0.015			
		MAP	-0,006	0.187	-0.014 to 0.003			
		LSC_per	-0,003	0.262	-0.009 to 0.002			
SDNN	ALI*ACC	ALI	-0,005	0.841	-0.056 to 0.045	<0.001	0.3	0.28
		ACC	2603.57	0.463	-4416 to 9623			
		ALI*ACC	-5086	0.048	-10135 to -36.666			
		Age	-0,025	<0.001	-0.036 to -0.015			
		MAP	-0,006	0.181	-0.014 to 0.023			
		LSC_per	-0,003	0.257	-0.009 to 0.002			

HF, natural logarithm high-frequency power; SDNN, standard deviation of the NN intervals; ALI, allostatic load index; MAP, medial arterial pressure; LSC-R_per, life stress checklist revised perception score;.L_Accumb, volume of the left nucleus accumbens; L_Amydala, volume of the left amygdala; ACC, anterior cingulate cortex.

4. Discussion

In this research, we developed the first ALI to measure chronic stress using a multisystemic approach in a Colombian population. This index can be used for future research and, eventually, in clinical practice. To our knowledge, no other study has simultaneously evaluated the three components in a single individual: AL (via the ALI), HRV (with 24-hour Holter monitoring), and the functional and structural parameters of the CNS. Unlike most studies evaluating HRV and AL, we accounted for confounding factors related to HRV in all analyses.

4.1 The developed ALI improves the current proposal for multisystemic measurement of chronic stress

An ALI consisting of seven biomarkers is positively correlated with SQ. This ALI included biomarkers from multiple physiological systems according to expert consensus recommendations and a recent meta-analysis that included over 67,000 individuals from

13 different cohorts worldwide, aiming to reach a consensus on the biomarkers and methodology for measuring IAL (Carbone et al., 2022; Mauss & Jarczok, 2021; McCrory et al., 2023). The meta-analysis identified nine biomarkers that individually belong to twelve physiological systems (DHEA-S, LF-HRV, CRP, RHR, PEF, HDL, HbA1C, cystatin C, WHtR) and were reliably related to three relevant health indicators: grip strength, walking speed, and subjective health assessment. Additionally, an index composed of a subset of five biomarkers (WHtR, HbA1C, CRP, RHR, and HDL) was associated with mortality risk better or equal to indices with a larger number of biomarkers (McCrory et al., 2023).

Given the substantial evidence supporting the use of HRV alone as an indicator of morbidity and mortality, we used HRV as a surrogate outcome. Our seven-biomarker ALI differs from McCrory's five-biomarker ALI in that we excluded resting heart rate and added systolic and diastolic blood pressures. We decided to add these parameters, considering the robust evidence of the relationship between AL and blood pressure (Chiger et al., 2022; Feres et al., 2019) and the relationship of the latter with relevant clinical outcomes with morbidity and mortality in healthy men (Volpe et al., 2019). Another difference is that we included DHEAS. We included this biomarker to assess the neuroendocrine system, another fundamental system involved in the stress response and adrenal fatigue (McCrory et al., 2023) because it was one of the 9 biomarkers related to clinical outcomes in the McCrory meta-analysis. It's worth noting that DHEAS has been proposed as a biomarker of chronic stress (Noushad et al., 2021) and has been linked to cardiovascular outcomes (Zhang et al., 2022). Our comparative analysis of ALI-4 and ALI-7 (sensitivity analysis) revealed a positive correlation between the ALI-7 and the SQ, but not between ALI-4, suggesting that ALI-7 better measures AL-related to HRV changes compared to ALI-4.

Another widely discussed aspect is the methodology for calculating the ALI (Carbone et al., 2022). The risk quartiles method, used to calculate the ALI score in our study, presents several significant advantages. The main advantage is that it allows for a clear and direct classification of risk levels based on the population distribution of each biomarker, facilitating the interpretation of the ALI score for each individual, something that is not always easy with other methodologies (Carbone et al., 2022; Mauss & Jarczok, 2021). However, the main disadvantage is that this method can be susceptible to the variability

of the studied population; what is considered a high-risk quartile in one population may not be so in another. This can affect the generalizability of the results and requires calibration of the quartiles specifically for each population before applying the index (Carbone et al., 2022). Despite this disadvantage, this method was used in McCrory et al.'s meta-analysis, suggesting that the advantages of its use outweigh the limitations.

The SQ is an HRV metric in the frequency domain. Although its interpretation is still controversial, it is clear that it contains components of the sympathetic and parasympathetic effects on the heart (Catai et al., 2020). It is associated with various physical and mental health outcomes. A high SQ is linked to disorders like obesity and stress-related mental illnesses (Costa et al., 2019; Schneider & Schwerdtfeger, 2020), while a low SQ is associated with psychological well-being (Shiga et al. 2021).

We identified only two studies (Viljoen & Claassen, 2017; Seeman et al., 2014) in a systematic and exhaustive literature search on the relationship between AL and HRV (Solano-Atehortua et al., 2024). Seeman et al. used an 18-biomarker ALI and a meta-factor model of AL and found a negative path coefficient between AL and the HRV subfactor measured with HF and LF. On the other hand, Viljoen and Claassen calculated a 13-biomarkers ALI using the classic high-risk quartile method and found a significant negative correlation between ALI and SDNN, RMSSD, and HF.

Our study used 24-hour Holter recordings in an ambulatory setting, a more comprehensive approach than the short-duration recordings used in the two mentioned studies. Long-duration recordings better capture physiological variations during daily activities and sleep-wake states (Chesnut et al., 2021; Kim et al., 2018). They are more predictive of clinical outcomes (Shaffer & Ginsberg, 2017) and could be more appropriate for evaluating the relationship between AL and HRV in clinical medicine. Additionally, unlike Viljoen and Seeman's studies, we considered and included confounding factors such as physical activity, smoking, socioeconomic status, medication use, and education level, which are recommended to be adjusted for in HRV research (Laborde et al., 2017).

Despite the differences between previous studies and ours, our findings reinforce the observation that as AL increases, there is an imbalance in the heart's autonomic regulation related to chronic stress (Agorastos et al., 2023).

4.2 Resting-State Default Mode Network FCS and ALI Interact to Modulate HRV

The interaction between the ALI and the resting-state FCS of Default Mode Network B and C negatively influences HF and SDNN. In other words, the combination of higher AL with higher FCS of the default mode network contributes to lower HRV (SDNN and HF). HRV is considered a measurement that reflects the integration of processes in the central nervous system (CNS) and peripheral factors (Agorastos et al., 2023; Shaffer et al., 2014). This could explain why we found that the interaction between ALI (peripheral factors) and the FCS of functional brain networks (CNS processes) is related to HRV, but each independently does not achieve a significant relationship.

No previous studies have evaluated the interaction of AL with FCS of resting-state networks in relation to HRV. However, several studies have reported a relationship between resting-state network connectivity, including the default mode network, and HRV (Matusik et al., 2023). Additionally, there are reports on the relationship between AL and HRV (Corrigan et al., 2021; Viljoen & Claassen, 2017). Moreover, it is important to highlight that resting-state network connectivity, particularly the default mode network, has been related to stress-related disorders. The default mode network plays a crucial role in internal processes such as introspection, reflection, autobiographical memory, and future planning (Luo et al., 2024). Additionally, this network is involved in emotional self-regulation and adaptive behaviors to stress (Winkelman et al, 2017). Recent studies suggest that the phenomenon of *hyperconnectivity*, specifically in the default mode network (DMN), may indicate an overload of the regulatory system (Doucet et al., 2020). This can lead to ineffective emotional regulation and contribute to the onset of anxiety and chronic stress symptoms. Additionally, hyperconnectivity in the DMN has been linked to major depression and post-traumatic stress disorder (PTSD), both of which are related to chronic stress and, consequently, high AL. This high allostatic load, in turn, is linked to autonomic dysregulation and low HRV (Galindo et al., 2018).

Interestingly, when evaluating the interaction between ALI and resting-state connectivity with HRV, a relationship with HF and SDNN emerged that was not found in the regression models adjusted for confounding variables. Physiologically, SQ is analogous to SDNN since both derive from a combination of sympathetic and parasympathetic activity (Shaffer et al., 2014). SDNN is considered the gold standard for cardiovascular risk stratification and is considered a predictor of morbidity and mortality (Shaffer et al.,

2017; Gao et al., 2022; Jarczok et al., 2022). On the other hand, HF (an indicator of cardiac vagal tone) is the most reported parameter correlated with resting-state functional connectivity (Matusik et al., 2023). Additionally, both HRV parameters have been independently related with higher AL (Viljoen & Claassen, 2017).

In summary, our findings support HRV's capability to represent the interaction of central and peripheral physiological processes, highlighting it as an excellent candidate for a comprehensive biomarker of chronic stress.

4.3 Brain structures related to stress interact with ALI to modulate HRV

In this study, we found that HRV parameters, SDNN and HF, are modulated by the interaction of IAL with the cortical thickness of Control Network B, Default Mode Network A, and Salience Network A sub-networks, and with the volumes of subcortical structures such as the left nucleus accumbens, left amygdala, and ACC. This finding suggests that higher chronic stress combined with greater cortical thickness and the volume of the described subcortical structures is related to lower HRV. Cortical thickness in multiple areas has been related to chronic stress (Matusik et al., 2023). Studies have reported positive (Winkelmann et al., 2017), negative (Wei et al., 2018a), or no significant correlation (Fridman et al., 2021). This can be explained by factors such as age, which influences this correlation´s magnitude and direction (positive or negative). However, no studies have reported relationship between the average thickness of networks or sub-networks and HRV.

The negative correlation between the amygdala's and ALI's combined effects on HRV may indicate that a larger amygdala volume is correlated with greater sympathetic system activation, leading to reduced vagal activity and, consequently, lower HRV. Prolonged exposure to chronic stress can also cause amygdala hypertrophy due to its constant hyperactivation (Sakaki et al., 2016; Wei et al., 2018a), generating parasympathetic system inhibition with a consequent decrease in HRV. These findings are supported by existing evidence on the role of amygdala-related neural circuits in HRV modulation and emotional regulation (Wei et al., 2018b).

In combat veterans, the volume of the ACC correlated positively with the magnitude of respiratory sinus arrhythmia (RSA), indicating a possible relationship between ACC size and HRV (Woodward et al. 2008). In our study, we found a negative effect of the

interaction between ACC volume and IAL on HRV. This may reflect the inefficiency of this structure related to central autonomic control in individuals with high AL. However, it is worth noting that in our case, we used long-term SDNN, unlike the cited study that used short-term RSA, parameters that represent different physiological phenomena and should not be interpreted in the same way. Other studies have found a positive relationship between caudal ACC thickness and HF (Winkelmann et al., 2017). These findings, and generally the results of other studies (Matusik et al., 2023), corroborate a relationship between the ACC and HRV.

No published studies have shown a relationship between the nucleus accumbens and HRV. However, our results suggest that a larger nucleus accumbens volume combined with high AL is correlated with a significant decrease in HRV. This finding suggests that individuals with these characteristics may have less efficient autonomic regulation (and consequently lower HRV), possibly related to the accumulation of repeated stress responses and, therefore, high AL and low HRV.

Our findings can be interpreted in the Central Autonomic Network (CAN) context. The CAN includes key structures such as the prefrontal cortex, anterior cingulate cortex, amygdala, nucleus accumbens, and other subcortical and cortical regions that regulate autonomic responses (Quadt et al., 2022). This network integrates autonomic and emotional signals to maintain body homeostasis and respond adequately to stress (Agorastos et al., 2023). Dysfunction or overload of these structures with high AL can lead to inefficient autonomic regulation, which is consistent with our results. Overall, based on the interaction analyses, we can propose that, on one hand, the effect of AL on HRV depends on the functional and structural parameters of the nervous system, and on the other hand, the effect of the nervous system on HRV is modified by IAL. While the neuronal control of HRV is essentially carried out through the ANS, primarily by the PNS, HRV is also influenced by other peripheral physiological factors (cardiovascular, metabolic, etc.).

While our findings support existing theories on the neuroscience of stress and align with empirical evidence, it's crucial to note that our conclusions about the interaction of brain structures and networks with the ALI are based on exploratory analyses and need to be validated in future studies. In particular, the issue of multiple hypothesis tests, considering

the number of relationships assessed in the models, is something that should be considered to confirm these findings in future research.

4.4 Limitations and future directions

Our study has important limitations. First, it is a cross-sectional study, which limits the ability to establish causal relationships for the correlations found. Second, we included only men from a specific area of Colombia, so the results are not generalizable to other population groups. It is worth noting the greater challenges and resources required for HRV and allostatic load research in female populations. Third, the method for calculating the IAL score is based on risk quartiles for each biomarker instead of clinical cut-off points. This generates population-specific values that are not extrapolatable to other populations. We did not use clinical cut-off points because we focused on a healthy population, and very few individuals would show biomarker values that reach those clinical cut-offs. The evaluation of sleep parameters, an important factor related with both HRV and allostatic load, was not conducted with validated instruments, which may introduce measurement bias. Additionally, the methods for measuring structural and functional parameters of brain networks used in this study differ from those in other studies, making direct comparisons challenging. Finally, as mentioned earlier, the findings regarding the interaction between brain structures, connectivity parameters, and ALI are only the results of exploratory analysis. The issue of multiple tests being conducted does not allow for the establishment of these interactions. Despite these limitations, we believe this study significantly contributes to the knowledge of chronic stress, AL, and their relationship with HRV. To progress in this field, we suggest the following: i) Conducting longitudinal studies to establish causal relationships between ALI and HRV. ii) Replicating and validating the ALI-7 in other populations. iii) Incorporating clinical cut-off points in future studies, especially in populations with higher clinical risk levels, to facilitate comparability and clinical applicability of the results. iv) Continuing studies on the relationship between structural and brain connectivity parameters, AL, and HRV, especially in the interactions suggested in this study.

5. Conclusion

Chronic stress, measured through an ALI composed of seven biomarkers, is positively correlated to HRV in healthy adult men. Additionally, HRV parameters in both the time

domain (SDNN) and frequency domain (HF) are modulated by the interaction between the IAL and the FCS of the default mode network, the average cortical thickness of the three major resting-state networks (default mode, salience, and control), and the volumes of subcortical areas involved in the CAN. According to these results, HRV can be considered a measure representing the combination of central and peripheral physiological processes related to chronic stress. Furthermore, the findings suggest that SQ is an indicator of allostatic load, while SDNN and HF behave more like indicators of the interaction between AL and the CAN. Considering that a decrease in HRV is associated with higher morbidity and mortality, can be easily measured in natural environments with available technological tools, and can be modified by multiple therapeutic strategies, our findings are highly relevant for preventing, diagnosing, and managing chronic stress and related disorders. Additional research will help clarify the complex interaction between brain processes and allostatic load and validate the role of HRV as a biomarker of chronic stress in clinical practice.

Conflict of interest: The authors declare that no known financial interests or personal relationships could have influenced the work reported in this paper.

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Author contributions

Juan Marcos Solano-Atehortua: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-original draft, writing-review and editing, visualization. **Gabriel Castrillón**: methodology, software, formal analysis, investigation, data curation, resources, data curation, writing-review and editing supervision. **Jazmin X. Suarez-Revelo**: methodology, software, formal analysis, investigation, data curation. **Juan D. Sánchez-López:** software, formal analysis, investigation, data curation. **Daniel A. Vargas-Tejada**: investigation, data curation. **Valentina Hawkins-Caicedo**: investigation, data curation. **Yedselt V. Ospina-Serrano**: Investigation, resources, data curation. **Juan D. Caicedo-Jaramillo**: Investigation, resources, data curation. **Juan C. Calderón:** resources, writing - review and editing. **Jaime Gallo-Villegas**: resources, writing - review and editing. **Ana L. Miranda-Angulo**: conceptualization, methodology, formal analysis, investigation, resources, data curation, writing-review and editing, visualization, project administration, supervision and funding acquisition.

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Supplements:

Supplementary Table 1 ALI biomarkers distribution in the study population

	WHtR	DBP	SBP	hs-CRP	TC/HDL	HbA1c	DHEA-S	ALI score
Median	0.47	70	114	1.02	3.76	5.3	294.75	2
Minimum	0.39	55	94	0	2.34	4.1	117.8	0
Maximum	0.59	92	143	26	8.08	6.89	656.7	5
P25	0.45	65	107	0.1	3.13	5.12	243.45	1
P50	0.47	70	114	1.02	3.76	5.3	294.75	2
P75	0.51	75	120	1.4	4.58	5.6	368.38	3

WHtR, waist-to-height ratio; HbA1c, glycated hemoglobin; TC/HDL, total cholesterol-high-density lipoprotein ratio; hs-CRP, high sensitivity C-reactive protein; SBP, systolic blood pressure; DBP, diastolic blood pressure; DHEA-S, dehydroepiandrosterone sulfate; ALI, allostatic load index.

Supplementary Table 2: Summary of SLRs using ALI as a predictor and HRV metrics as outcomes

	R2	Adjusted R2	Sig model (p)	Beta	Sig beta (p)
SQ_all	0.12	0.11	0.001	0.1	0.001
ln_HF	0.1	0.09	0.002	-0.22	0.002
RMSSD_ln	0.06	0.05	0.01	-0.08	0.015
SDNN_ln	0.06	0.05	0.02	-0.05	0.024
ln_LF	0.01	0.001	0.28	-0.05	0.28

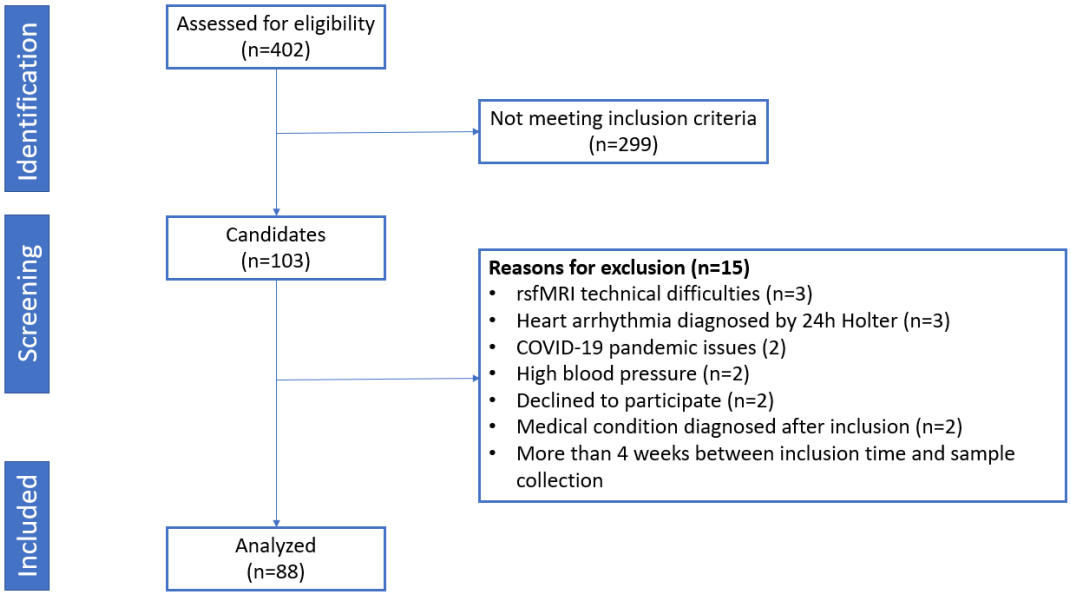
SDNN, standard deviation of the NN intervals; RMSSD, root mean square of successive differences between normal heartbeats; Ln LF, natural logarithm of low frequency power; Ln VLF, natural logarithm of very low frequency power; Ln HF, natural logarithm high frequency power; SQ, natural logarithm of the LF /HF ratio; ALI, allostatic load index.

Table 3. Multivariate linear regression between *SQ* and *ALI_4*

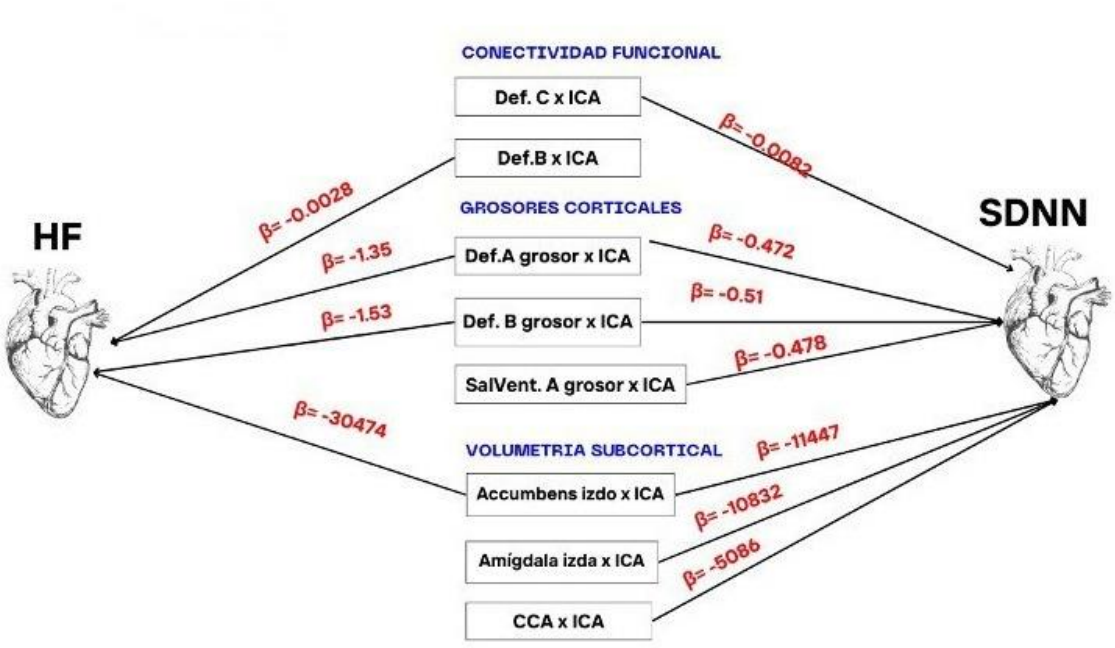
Dependent variable	Independent variables	β	*P*-value	95% CI	Model Prob. F	R^2	Adjusted R^2
SQ	ALI_4	0.058	0.191	-0.029 to 0.147	<0.01	0.21	0.15
	Age	0.015	0.06	-0.0006 to 0.3067			
	Chol_mg	0.0002	0.026	0.00003 to 0.0005			
	LSC-R_per	-0.01	0.017	-0.019 to -0.0019			
	PA						
	1	0.132	0.175	-0.059 to 0.324			
	2	-0.108	0.282	-0.307 to 0.802			

SQ, the natural logarithm of the LF/HF ratio; ALI, allostatic load index; PA, physical activity; Chol_mg, cholesterol consumption per day in milligrams; LSC-R_per, life stress checklist revised perception score.

SUPPLEMENTARY Fig 1



SUPPLEMENTARY Fig 2



CHAPTER 5: GENERAL DISCUSSION AND CONCLUSIONS

This thesis contributes valuable new insights into the relationship between chronic stress, AL, and HRV, as well as some of the biological mechanisms underlying this interaction. Through a combination of theoretical review and original research, we advanced our understanding of how these variables are interrelated, with important implications for clinical medicine and preventive health.

The first part of the research, a scoping review, highlighted that although HRV has been proposed as a stress biomarker (Agorastos et al., 2023; Mulcahy et al., 2019; Quadt et al., 2022), there are still significant gaps in the literature that prevent drawing definitive conclusions, especially for chronic stress. We underscore the need for further studies, particularly in healthy populations, to better define HRV's role as a clinical biomarker of chronic stress. In addition, we emphasize the necessity for standardized recommendations regarding the measurement of HRV and AL, including aspects such as the duration of ECG recordings and the selection of HRV parameters in chronic stress research. Such standardization would facilitate more valid comparisons across studies and populations, with potential applications in preventive medicine.

To address these knowledge gaps, the second research in this thesis explored the relationship between AL (using a multisystemic approach) and HRV, measured over a 24-hour period in healthy men. We also investigated how brain structures and connectivity and AL interact to influence HRV. In this study, we proposed a novel ALI consisting of seven biomarkers, which was found to correlate with the 24-hour SQ, a global frequency domain HRV metric that has been linked to stress-related mental illnesses (Costa et al., 2019; Schneider & Schwerdtfeger, 2020) and psychological well-being (Shiga et al. 2021). This multisystemic ALI includes key biomarkers from physiological systems involved in the stress response, including the cardiovascular, metabolic, and neuroendocrine systems. We built upon the largest consensus of ALI to date (McCrory et al., 2023), while adding SP, DP, and DHEAS to our index, given their well-established roles as risk factors linked to chronic stress, AL and clinical outcomes (Chiger et al., 2022; Feres et al., 2019; Noushad et al., 2021; Zhang et al., 2022). Our ALI showed significant associations with HRV, improving upon the McCrory et al. proposal.

Furthermore, our research revealed that interactions between functional and structural parameters of the central nervous system (CNS) and ALI significantly modulate HRV across both time and frequency domains (SDNN and HF). Specifically, we found that functional connectivity within the default mode network (DMN), the average cortical thickness across major resting-state networks (DMN, salience network, and control network), and the volumes of subcortical regions involved in the central autonomic network (CAN) play critical roles in this modulation. To our knowledge, these findings are novel and support the concept that HRV reflects an integration of central and peripheral processes involved in the chronic stress response. This interaction between AL and brain functional and structural connectivity significantly influences HRV, reinforcing the notion that HRV can serve as a comprehensive biomarker of chronic stress.

Our results underscore the importance of the CAN in modulating HRV and highlight its role in the autonomic dysfunction observed in individuals experiencing chronic stress and related disorders (Lamotte et al., 2021; Quadt et al., 2022). This has major clinical implications, particularly given the availability of neuromodulation techniques that could be leveraged for the preventive and therapeutic management of chronic stress (Cirillo et al., 2019; Mather & Thayer, 2018; Subhani et al., 2018).

Unlike findings related to acute stress, where parasympathetic components are most often associated with the stress response (Fridman et al., 2021; Matusik et al., 2023; Thayer et al., 2012) our study suggests that chronic stress may be more accurately reflected by HRV parameters that include both sympathetic and parasympathetic components (e.g., SQ and SDNN). While our results indicate that 24-hour measurements of SQ, SDNN, and HF are strong candidates for chronic stress biomarkers, additional research is needed to further validate their utility and accuracy in different clinical contexts.

Considering the profound societal and economic burden posed by chronic stress and its associated disorders (Desmond, 2017; Kalia, 2002), identifying reliable biomarkers for assessing and managing these conditions is imperative. Our findings suggest that HRV is a practical and effective biomarker to address these challenges, offering both accessibility and utility through real-world measurements enabled by wearable technologies (Chrousos et al., 2022; Mason et al., 2024; Natarajan et al., 2020; Singstad et al., 2021).

HRV fulfills several criteria of an ideal biomarker for chronic stress. By capturing the dynamic interactions between the sympathetic and parasympathetic systems, it provides a real-time and integrative perspective of autonomic regulation, essential for assessing the cumulative burden of chronic stress. Unlike static biomarkers, HRV reflects temporal variations and systemic responses, making it particularly suited for monitoring physiological dysregulation over time.

Nonetheless, standardizing HRV measurement protocols remains a critical challenge. Key aspects such as recording duration, context (e.g., rest or sleep), and metric selection (e.g., SDNN, RMSSD, LF/HF ratio) must be harmonized to ensure reliability and reproducibility across studies. Moreover, future research should explore HRV's predictive capacity for clinical outcomes across diverse populations, addressing confounding factors such as age, sex, and lifestyle. The integration of machine learning and advanced analytics could further enhance HRV's utility by combining it with other stress-related biomarkers.

Given the widespread impact of chronic stress and the availability of evidence-based interventions (Kejriwal, 2023; Subhani et al., 2018), incorporating HRV into clinical workflows represents a transformative opportunity. Beyond its role in public health and preventive medicine, HRV offers potential in personalized care, enabling the quantification of treatment efficacy and monitoring of stress-related health trajectories. Leveraging HRV as a biomarker could significantly reduce the societal burden of stress-related disorders while advancing precision medicine approaches.

Certain limitations of this study must be considered. First, the cross-sectional nature of our design prevents the inference of causal relationships. Second, the study population consisted only of men from a specific region, limiting the generalizability of the results. Third, the methodology used to calculate the ALI, based on risk quartiles, has drawbacks in terms of applicability to other populations. Despite these limitations, this study provides a solid foundation for future research on the use of HRV as a biomarker of chronic stress and its eventual application in clinical practice.

This research proposes a novel framework with the potential to significantly impact preventive medicine and public health. However, given the limitations outlined and those of other studies in the field, we recommend the following future directions:

6. Replicate the findings in other populations, including women, to validate and generalize these results.
7. Conduct longitudinal studies to better understand the causal relationships between chronic stress, allostatic load, HRV, and relevant clinical outcomes.
8. Continue research to confirm the interactions between allostatic load and brain processes and their effect on HRV, which will allow the development of novel and effective therapeutic strategies.
9. Encourage international collaboration to conduct studies in large populations and obtain more robust and generalizable results.

In conclusion, our findings demonstrate that HRV, modulated by the interaction between AL and structural and functional brain parameters, can serve as an integral biomarker of chronic stress. These results also emphasize the importance of multisystemic approaches in assessing the impact of chronic stress on health and underscore the need for continued investigation into these interactions to establish more effective preventive and therapeutic measures for managing chronic stress.

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Main academic milestones:

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