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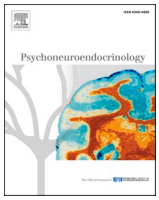

Linking allostatic load, heart rate variability and brain functional networks and structures in healthy men

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ABSTRACT

Introduction: The allostatic load index (ALI) measures the cumulative physiological burden exerted on the body by chronic stress known as allostatic load (AL). The relationship between ALI and heart rate variability (HRV), as well as the brain moderation effect on this relationship in healthy individuals, is underexplored.
Methods: In this cross-sectional study of 88 healthy men (21–40 years) from Medellín, Colombia, we calculated two ALIs composed of four and seven biomarkers (ALI-4 and ALI-7) using a quartile-based risk summation method. Functional and structural neuroimaging metrics were derived from magnetic resonance imaging. Multiple linear regression models were used to evaluate the associations between the ALIs and HRV metrics derived from a 24-hour Holter. Exploratory interaction models tested whether the functional connectivity strength (FCS) of default mode (DMN), salience (SN) and control subnetworks (CEN), their cortical thickness as well as the volume of subcortical structures, moderated the ALI–HRV association.
Results: ALI-7 was positively associated with the LF/HF ratio ($\beta = 0.09$, $p = 0.004$, 95 % CI = 0.03–0.15). Exploratory interactions suggested that ALI-7's association with the standard deviation of the NN intervals (SDNN) was moderated by the FCS in the posterior DMN and by cortical thickness in the anterior SN. However, none of these interactions remained significant after false discovery rate correction.
Conclusion: In healthy men, higher ALI was associated with reduced HRV as indicated by higher LF/HF ratio. Larger studies, including women, are needed to confirm the predictive value of ALI-7 and to elucidate the brain moderation effect on the AL-HRV relationship.

1. Introduction

Allostatic load (AL) refers to the cumulative physiological burden exerted on the body by chronic exposure to stressors and the consequent need to maintain internal equilibrium (McEwen and Stellar, 1993). Elevated AL has been associated with increased risk of physical and mental disorders, as well as with higher risk of all-cause mortality (Guidi et al., 2021; Parker et al., 2022). A widely accepted method for quantifying AL is the allostatic load index (ALI), which aggregates biomarkers representing multiple physiological systems involved in the stress response (McCrory et al., 2023; Seeman et al., 2001). However, operationalizing ALI remains challenging due to heterogeneity in biomarker selection and calculation methods, limiting its widespread application in clinical contexts (Carbone et al., 2022). A recent meta-analysis of 13 predominantly prospective cohorts, including 67,126 individuals and assessing 40 biomarkers across 12 physiological systems, proposed an ALI composed of five biomarkers as a predictor of all-cause mortality risk (McCrory et al., 2023). However, the authors emphasized the need

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to replicate these findings in other populations and to incorporate additional, well-selected biomarkers to enhance its prognostic value.

Heart rate variability (HRV), the fluctuation in the time intervals between consecutive heartbeats, is a well-established biomarker that reflects autonomic nervous system dynamics. It integrates signals from the intrinsic cardiac nervous system and the central nervous system (CNS) through sympathetic and parasympathetic cardiac innervation (Shaffer and Ginsberg, 2017). Low HRV has consistently been associated with increased risk of morbidity and of all-cause mortality (Agorastos et al., 2023; Hoshi et al., 2021; Jarczok et al., 2022; Ortiz-Guzmán et al., 2023; Zhou et al., 2016). Moreover, HRV is considered a psychophysiological marker of emotional self-regulation and has been linked to several psychiatric conditions associated with chronic stress (Agorastos et al., 2023; Bandelow et al., 2017; Chesnut et al., 2021; Holzman and Bridgett, 2017).

In healthy populations, HRV has been extensively used to assess acute stress responses (Corrigan et al., 2021; Immanuel et al., 2023; H.-G. Kim et al., 2018). In contrast, its relationship with chronic stress remains insufficiently explored. A recent systematic review found insufficient evidence supporting HRV as an indicator of chronic stress in healthy first responders and tactical personnel (Corrigan et al., 2021). Only two studies have examined the association between HRV and chronic stress, operationalized as ALI, in apparently healthy individuals (Seeman et al., 2010; Viljoen and Claassen, 2017). Both reported a negative correlation between ALI and HRV metrics. However, these studies did not adequately ascertain or describe participants' health status and did not account for relevant confounding variables such as age and sex (Laborde et al., 2017). Hormonal fluctuations across the menstrual cycle significantly affect emotion processing, stress response and HRV; thus, studies in women should ideally account for cycle phase (Espin et al., 2019; Schmalenberger et al., 2019). In addition, these studies relied on short-term, laboratory-based HRV recordings. Such recordings provide only a limited view of autonomic function, as opposed to 24-hour HRV monitoring, which offers a more comprehensive assessment of autonomic regulation across a diverse array of behavioral and physiological states encountered in daily life (Shaffer and Ginsberg, 2017; Sloan et al., 2007; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

The regulation of HRV has traditionally been attributed to the Central Autonomic Network (CAN), which is a distributed network of interconnected brain structures extending across the telencephalon, diencephalon, and brainstem. The CAN encompasses key regions such as the medial prefrontal cortex (mPFC), anterior cingulate cortex (ACC), insula, amygdala, hypothalamus, periaqueductal gray, dorsal vagal motor nucleus, nucleus of the solitary tract and ventrolateral medulla (Benarroch, 1993). This network integrates exteroceptive, interoceptive and humoral inputs to coordinate psychophysiological responses essential for stress regulation, including HRV (Lamotte et al., 2021).

Furthermore, recent findings suggest that large-scale resting-state functional networks (RSN), including the default mode network (DMN), salience network (SN), and central executive network (CEN), also play a modulatory role on HRV (Ruffle et al., 2021; Wei and Wu, 2021). The functional connectivity of DMN, SN and CEN changes dynamically and modulates HRV during acute stress in healthy individuals (Chand et al., 2020). However, the influence of these networks on HRV under conditions of chronic stress has not been previously reported, likely due to the methodological challenges in accurately quantifying chronic stress through standardized indices such as the ALI. Finally, evidence from both human and non-human primate studies converges to support the existence of an allostatic-interoceptive network (AIN) (Kleckner et al., 2017). This network integrates interoceptive signals with higher-order neural processes to orchestrate allostatic regulation (Kleckner et al., 2017; Santamaría-García et al., 2025). The system includes regions within the CAN, as well as the DMN and SN, and it regulates psychophysiological outputs, such as HRV (Santamaría-García et al., 2025). No study has yet directly investigated whether HRV is influenced by the interaction between brain structure, functional networks and AL.

Taken together, the limited evidence suggests that HRV could serve as an indicator of the physiological burden of chronic stress when measured through an ALI. However, studies in healthy individuals are scarce, often constrained by insufficient control of confounding variables, and there is a need to improve the measurement of both AL and HRV to strengthen such an association. Because HRV is regulated by a distributed network of brain regions involved in allostasis, its relationship with chronic stress likely reflects both peripheral and central influences. To address these gaps, we focused on healthy men, developed an ALI, assessed its association with 24-hour ambulatory HRV, and examined whether brain functional and structural characteristics influence this relationship. We hypothesized that greater ALI would relate to lower HRV, and that brain functional networks and structures would modulate this association.

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 current version 2024 (WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants – WMA – The World Medical Association), 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 at the University of Antioquia (Approval Act 007 of May 11, 2017). Individuals who participated in this research signed an informed consent (Approval Act 007 of May 11, 2017).

2.2. Study population selection

This cross-sectional study included healthy adult men aged 21–40 years. Because we did not have the resources to control for woman cycle phase through repeated hormonal assessments or cycle tracking, we restricted our sample to men. This decision minimized potential confounding effects and allowed us to investigate the association between AL, HRV, and brain measures under more homogeneous physiological conditions. Exclusion criteria included diagnosed physical or mental illness, routine medication use, drug abuse assessed with the Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST) version 1.1.66, use of cardiac devices or prostheses, left-handedness, and contraindications for magnetic resonance imaging (MRI). All participants underwent a standardized physical examination and completed all study procedures within a four-week period, including biological sample collection, 24-hour Holter monitoring, and both structural and resting-state functional magnetic resonance imaging (rs-fMRI).

2.3. Biomarker selection and allostatic load index construction

The selection of biomarkers was guided by both physiological and conceptual principles that define AL as a multisystem construct (Seeman et al., 1997), as well as by the most robust empirical evidence supporting the contribution of individual biomarkers to composite AL indices (McCrory et al., 2023). Consistent with this framework, we selected biomarkers to build two versions of ALI based on the five-biomarker version proposed by McCrory *et al.* Both ALIs excluded resting heart rate (RHR), given the well-established physiological and mathematical dependency between HR and HRV (Sacha, 2013). The first ALI consisted of four biomarkers (ALI-4): waist-to-height ratio (WHtR, anthropometric domain), glycated hemoglobin (HbA1c, metabolic domain), high-density lipoprotein cholesterol (HDL, metabolic domain),

high-sensitivity C-reactive protein (hs-CRP, immunological domain). The second ALI consisted of the biomarkers included in the ALI-4 plus systolic and diastolic blood pressure (SBP and DBP, cardiovascular domain), and dehydroepiandrosterone sulfate (DHEA-S, endocrine domain). The SBP and DBP were added based on substantial evidence linking AL with blood pressure regulation and the specific prognostic relevance of DBP for morbidity and mortality in healthy individuals (Mocayar Marón et al., 2019). DHEA-S was included as an indicator of neuroendocrine system function, a core domain in the allostatic model of chronic stress. Notably, DHEA-S was among the nine biomarkers most consistently associated with adverse clinical outcomes and was shown to increase the mortality odds ratio when added to the five-biomarker ALI in the meta-analysis by McCrory et al. (2023). Thus, the expanded version of the index, referred to as ALI-7, included the following seven biomarkers in five physiological domains: WHtR (anthropometric), HbA1c and HDL (metabolic), hs-CRP (immunological), SBP and DBP (cardiovascular), and DHEA-S (endocrine).

2.4. Clinical and laboratory assessments

Blood pressure was measured three consecutive times using a calibrated digital blood pressure monitor (Sejoy®, model BSP-13, Hangzhou - China), with participants seated and their left arm supported at heart level. Then, the mean of the three readings was recorded. Waist and hip circumferences were measured twice with a flexible anthropometric tape, averages were calculated and used for subsequent calculations. Height was measured using a stadiometer, and weight was obtained using a calibrated electronic scale. Body mass index (BMI), waist-to-hip ratio (WHR), and waist-to-height ratio (WHtR) were calculated based on these measurements. The biochemical measurements were performed during fasting on fresh serum obtained from peripheral venous blood samples and processed in a certified clinical laboratory. Hs-CRP, HDL, HbA1c and DHEA-S were quantified using Dimension® Flex® reagent cartridges (DF34, DF48B, and DF105A) and the Architect 8K27 platform, respectively. Participants were instructed to abstain from caffeine, nicotine, and high-intensity exercise for a minimum of one hour prior to all clinical and laboratory assessments.

2.5. Electrocardiographic recording and analysis

HRV data were obtained through 24-hour ambulatory Holter electrocardiogram (ECG) monitoring. The Custo Flash 510 V device was placed on participants between 7:00 and 10:00 a.m. and removed at approximately the same time the following day, ensuring a minimum of 21 h of continuous ECG recording. The monitor recorded data across three channels at a sampling frequency of 400 Hz (2.5 ms $\pm$ 0.1 % per channel), with a quantification amplitude of 5.6 μV/Bit $\pm$ 1 % at 10 bits, a frequency response range of 0.05–45 Hz, and an input impedance $\geq$ 10 MΩ, filtered at 50 Hz with 80 dB attenuation. Participants were instructed to follow their normal daily routines during the recording period, but to refrain from engaging in physical exercise. They were instructed to record their daily activities.

ECG recordings were processed using the custo diagnostic Holter ECG module and the ANS Diagnostics module. Artifact detection and removal were performed as follows: First, in an automated step, the software detected R-peaks and flagged RR intervals that could not be attributed to sinus rhythm, including artifacts, signal loss, non-sinus beats, and pathological pauses. These intervals were automatically excluded from HRV computation. Additionally, the software generated a supplementary file of uncertain or ambiguous beats for manual review. In a second step, an experienced cardiologist–electrophysiologist visually inspected this file to ensure accurate exclusion. During this stage, the expert used a template-based beat morphology classification available in the full Holter ECG analysis module to visually compare beats and ensure accurate retention of sinus beats for HRV analysis. After this verification, only clean normal-to-normal (NN) intervals were retained. Individuals with clinically significant non-sinus rhythms or arrhythmias incompatible with reliable HRV analysis were excluded.

HRV was analyzed in successive 5-minute windows across the entire recording period and summarized for daytime, nighttime, and all-day periods. Only the all-day data were used for further statistical analysis. HRV computations were based on the native RR-interval series obtained from the Holter's 400 Hz acquisition, and no additional resampling or interpolation was applied. Spectral power was calculated within three predefined frequency bands: very low frequency (VLF, 0.003–0.04 Hz), low frequency (LF, 0.04–0.15 Hz), and high frequency (HF, 0.15–0.50 Hz). Within the HF band, the lower portion (approximately 0.15–0.40 Hz) reflects physiological respiratory sinus arrhythmia (RSA), whereas the upper end of the band (0.40–0.50 Hz) was considered non-physiological and predominantly interpreted as artifacts or extrasystoles.

The following HRV parameters were extracted: (1) heart rate (beats·min^{-1}, bpm); (2) natural logarithm of VLF (ln VLF), LF (ln LF), and HF (ln HF) spectral power (ln (ms^2)); (3) sympathovagal quotient (SQ), defined as the natural logarithm of the LF to HF ratio (ln (LF/HF), dimensionless); (4) standard deviation of NN intervals (SDNN, ms); (5) root mean square of successive differences between normal heartbeats (RMSSD, ms); (6) logarithm of respiratory sinus arrhythmia (logRSA, log (ms)). LogRSA and ln VLF were excluded because RSA indices are strongly influenced by respiratory rate and tidal volume, which were not measured, and VLF power has low physiological specificity and limited interpretability in long-term recordings (Shaffer and Ginsberg, 2017; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Higher HF is considered a marker of greater parasympathetic (vagal) tone, whereas elevated LF indicates mixed autonomic activation and baroreceptor influence on blood pressure. The LF/HF is an indicator of the interaction between autonomic innervation and diverse physiological and contextual situations. SDNN reflects the total variability of the heartbeat interval series, encompassing both short-term fluctuations (e.g., beat-to-beat, respiratory-linked) and longer-term oscillations (e.g., circadian rhythms, baroreflex and other autonomic modulation, activity-related influences). Finally, RMSSD is considered an index of parasympathetic modulation.(Shaffer and Ginsberg, 2017)

2.6. Neuroimaging acquisition and processing

MRI data were acquired using a Philips Ingenia 3 T scanner (Philips Healthcare, Best, The Netherlands) equipped with a 16-channel phased-array rigid head coil. Detailed acquisition parameters and processing procedures have been previously described in Miranda-Angulo et al. (2024).

Functional and structural brain parameters were derived from T1-weighted and rs-fMRI data. A total of 400 cortical regions were defined using the Schaefer parcellation scheme and assigned to one of 17 large-scale RSN according to the Yeo functional atlas (Yeo et al., 2011). The two limbic networks were excluded from analyses due to the low signal-to-noise ratio typically observed in orbital frontal regions. Additionally, 53 subcortical structures were segmented using FreeSurfer, yielding a total of 453 brain regions included in structural analyses.

Resting-state functional connectivity strength (FCS) of each RSN was quantified using the node strength (Stg) metric derived from graph theory, which quantifies the total weight of connectivity of a given cortical node with all other cortical nodes. Stg was computed with the Brain Connectivity Toolbox as the sum of link weights associated with each node (Rubinov and Sporns, 2010). The average Stg for each RSN was calculated by dividing the total weight by the number of connected nodes within that network (Fornito et al., 2016).

Structural parameters, including cortical thickness and subcortical volumes, were obtained using FreeSurfer's validated automated segmentation pipeline. Cortical thickness was defined as the distance between the pial surface and the gray-white matter boundary and

estimated via FreeSurfer's standard recon-all process, which includes Talairach transformation, intensity normalization, skull stripping, and surface reconstruction with topological correction. Volumetric measures were adjusted for total intracranial volume (ICV) to control for head size.

2.7. Assessment of potential confounders

2.7.1. Psychological stress and mental health status

Exposure to stressors and perceived stress were assessed using three validated instruments. The Daily Hassles Scale (DHS) interview was administered to evaluate exposure to daily stressors over the past month (Hannan et al., 2015). The Revised Life Stressor Checklist (LSC-R) was used to identify stressful and potentially traumatic events across the lifespan (LSC-R total score) and the persons subjective rating of their impact during the previous year (LSC-R perception) (Humphreys et al., 2011). Additionally, the 10-item Perceived Stress Scale (PSS) was applied to measure perceived stress in the past month (Campo-Arias et al., 2014). These instruments do not include diagnostic cut-off scores; rather, higher scores indicate greater exposure to stressors or higher perceived stress. To assess anxiety symptoms, two screening instruments were used. The State-Trait Anxiety Inventory (STAI) evaluates both state (STAI-S) and trait (STAI-T) anxiety, with scores ranging from 20 to 80. Established cut-off points of $\geq$ 41 for STAI-S and $\geq$ 44 for STAI-T were applied to indicate clinically relevant symptoms (Guillén-Riquelme and Buela-Casal, 2011). Depressive symptoms were assessed using the Zung Self-Rating Depression Scale, with scores $\geq$ 50 indicating the presence of depression. Cut-off scores for the Zung scale were calculated by multiplying raw scores by 1.25 (Dunstan and Scott, 2019). All instruments were administered in their Spanish-language versions, which are validated for the Colombian population (Campo-Arias et al., 2005, 2014; Guillén-Riquelme and Buela-Casal, 2011; Humphreys et al., 2011). Sleep quality was evaluated by asking participants to rate their sleep using one of three categories: good, average, or poor. Sleep quantity was assessed by self-reporting the number of sleep hours most days using three categories: less than 4 h, between 4 and 6 h, and more than 6 h of sleep per night.

2.7.2. Assessment of physical activity and nutritional habits

Physical activity habits were evaluated using the short Spanish version of the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003), which classifies individuals into three categories of physical activity: light, moderate, and vigorous. Dietary intake over the previous 12 months was assessed through a digital adaptation of a self-administered, semi-quantitative food frequency questionnaire (FFQ) that was locally developed and validated (Monsalve Álvarez and González Zapata, 2011). Nutritional estimates of total energy and macronutrient intake were derived from the FFQ using a custom-built R script developed in-house.

2.8. Statistical analysis

2.8.1. Sample size and power analysis

Based on a previous study reporting correlations between ALI and RMSSD ranging from 0.296 to 0.6 (Viljoen and Claassen, 2017), a minimum sample size of 85 participants was estimated to detect a correlation of 0.3 with 80 % power and a two-tailed alpha of 0.05. This calculation was performed using Epidat software, version 4.2.

2.8.2. Descriptive analysis

Categorical variables were described as frequencies and percentages, while continuous variables were summarized using means and standard deviations or medians and interquartile ranges, depending on their distribution. Normal distribution was evaluated using the Shapiro-Wilk test, histograms, and Q-Q graphs.

2.8.3. ALI score calculation

The ALI-4 and ALI-7 scores were computed using the traditional summation method based on within-sample quartile distributions (Carbone et al., 2022). For each biomarker, we first calculated quartiles from the distribution of values in our sample. Then, a biomarker was coded as "high risk" when the participant's value fell within the high-risk quartile, defined as follows: Upper quartile ($\geq$75th percentile) for biomarkers where higher values indicate greater physiological risk: WHtR, SBP, DBP, HbA1c, and hs-CRP. Lower quartile ($\leq$25th percentile) for biomarkers where lower values indicate greater physiological risk: HDL and DHEA-S. Thus, the terms "upper quartile" and "lower quartile" refer specifically to the 75th–100th percentile range and the 0–25th percentile range of the sample distribution, respectively. Finally, each biomarker was assigned a score of one if it fell within its respective high-risk quartile, and zero otherwise. The total ALI score represents the cumulative number of biomarkers in high-risk ranges, with higher scores reflecting greater multisystem physiological burden.

2.8.4. Relationship between ALI and HRV

Simple linear regression (SLR) was used to examine the relationship between ALI (ALI-4 and ALI-7) and HRV metrics (ln (LF/HF) ratio, ln HF, ln LF, RMSSD, and SDNN). Multivariate linear regression (MLR) was then performed for those HRV metrics showing significant associations in the SLR, with ALI as the main independent variable. SDNN and RMSSD were log-transformed to meet normality assumptions. Confounding variables were selected based on biological plausibility, previous literature (Laborde et al., 2017; Shaffer and Ginsberg, 2017), and backward stepwise selection in STATA® version 16.0 (StataCorp LLC, Texas, USA). The final models included age, physical activity, dietary cholesterol intake, and the LSC-R perception score as confounder variables.

2.8.5. Sensitivity analysis

To assess the robustness of the ALI-7 and HRV association, we repeated the MLR analyses by testing alternative confounding variables (sleep quantity, sleep quality, habitual diet, stress scales, STAI scale, and Zung depression scale score).

2.8.6. Interaction between ALI with functional connectivity and brain structures to modulate HRV

HRV metrics were used as dependent variables (SDNN, RMSSD, ln HF, and the ln (LF/HF ratio)). The independent, interacting variables were: FCS from subnetworks of the CEN (Control A–C), DMN (DMN A–C), and SN (Salience A–B) (Yeo et al., 2011), the average cortical thickness for regions corresponding to the above subnetworks, and the volumes of bilateral subcortical structures within the CAN or reported to be correlated with HRV (hippocampus, anterior cingulate cortex, nucleus accumbens, amygdala, caudate, thalamus, and cerebellum). All independent variables were mean-centered, and interaction terms were computed as the product of the centered variables. MLR models were adjusted for age, mean arterial pressure (MAP), and LSC-R perception score. MLR model assumptions were verified using the Shapiro-Wilk test (normality of residuals), variance inflation factor (multicollinearity, VIF<5), Durbin-Watson statistic (autocorrelation, d=1.5–2.5), and Breusch-Pagan test (homoscedasticity). Model fit was assessed via the Akaike Information Criterion (AIC), R^2, and adjusted R^2. Statistical significance was set at $p \leq 0.05$. False Discovery Rate (FDR) correction using the Benjamini-Hochberg method was applied to account for multiple comparisons. All the interaction analyses were performed in STATA® version 16.0 (StataCorp LLC, Texas, USA).

3. Results

3.1. Descriptive data

Out of 402 volunteers who completed the online pre-selection

questionnaire, 299 met exclusion criteria. Of the remaining 103 subjects, 15 were withdrawn for various reasons, leaving 88 participants for analysis (Supplementary Figure 1). The median age was 30 years, most participants had an undergraduate degree or were pursuing one, and the majority 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 selection process, some scored above the cutoff points for these scales: Zung scale (n = 15, 17 %), STAI-T (n = 14, 16 %), STAI-S (n = 3, 3.4 %), and both Zung and STAI (n = 13, 15 %). All demographic, psychological, physiological, biochemical, and anthropometric characteristics of the study population are described in Table 1.

The minimum score was 0 for both ALIs, and the maximum was 3 and 5 for ALI-4 and ALI-7, respectively (Supplementary Table 1). There was a similar contribution of each biomarker to the ALIs; however, the HbA1C was the most frequent and the HDL the least frequent (Supplementary Table 2)

3.2. Relationship between ALI and the LF/HF ratio

In SLR analyses, ALI-7 correlated with all evaluated HRV metrics except ln LF, whereas ALI-4 correlated with all except the ln (LF/HF) ratio (Supplementary Table 3). In the MLR analysis, adjusted for age, the LSC-R scale, physical activity and cholesterol consumption, ALI-7 was positively correlated only with the ln (LF/HF) ratio (β=0.09, 95 % CI=0.03–0.15, p = <0.01) (Table 2). The alternative ALI-4 did not correlate with any HRV parameter in the MLR analysis (data not shown).

3.3. Senstitivity analyses

The correlation between the ALI-7 and the ln(LF/HF) ratio remained significant despite adjusting for alternative confounding variables such as sleep quality, nutritional status, stress levels, STAI scale and Zung depression scale (data not shown).

3.4. The moderation effect of brain functional and structural parameters on the association between ALI and HRV metrics

Initially, we found a significant moderation effect of FCS of the DMN-C and cortical thickness of SN-A on the association between ALI-7 and SDNN (β = −0.008, 95 % CI = −0.02 to −0.001, p = 0.03; β = −0.47, 95 % CI = −0.92 to −0,003 p = 0.04, respectively). However, none of these interactions remained statistically significant after FDR correction (Supplementary Table 4). The brain areas present in each network are described in Supplementary Table 5. There was no significant moderation effect of any subcortical structure on the ALI-7 and HRV association (data not shown).

4. Discussion

The findings of this study indicate a positive association between ALI-7 and 24-hour ambulatory LF/HF ratio in healthy adult males. In contrast, exploratory interaction analyses involving ALI-7, functional connectivity, and structural brain parameters with HRV did not yield statistically significant results after correction for multiple comparisons. These findings suggest that, while some trends were observed, the interaction effects lack robust statistical support and should be interpreted with caution.

4.1. ALI-7 is associated with 24-hour LF/HF ratio

ALI-7 was positively correlated with the LF/HF ratio, suggesting that individuals with higher AL exhibit reduced HRV. The physiological interpretation of the LF/HF ratio remains debated, mainly due to the uncertainty about the physiological origin of the LF component (Billman, 2013). Furthermore, in 24-hour ambulatory recordings the LF/HF ratio is not only the result of the nonlinear interaction between the sympathetic and parasympathetic nervous systems on the heart. The LF/HF ratio incorporates influences from thermoregulation, metabolism, locomotor activity, sleep–wake patterns, and hormonal rhythms as well as contextual factors including posture, measurement duration, physical activity, circadian timing, and behavioral state (Shaffer and Ginsberg, 2017). Despite this controversy, higher LF/HF ratio values are generally considered an indicator of lower HRV (Brusseau et al., 2022; Rubio et al., 2025; Shiga et al., 2021; Zhang et al., 2025). Importantly,

Table 1
Demographic and clinical characteristics of study population.

Variables	n = 88
Sociodemographic	
Age (years), median (IQR)	30 (25−34)
Level of education, n (%)	
Bachelor	2 (2.27)
Undergraduate	68 (77.27)
Postgraduate	18 (20.45)
Socioeconomic status, n (%)	
Low	17 (19.31)
Middle	61 (69.31)
High	10 (11.36)
Anthropometrics	
BMI (kg/m^2), mean (SD)	24.15 (±2.60)
WHR, median (IQR)	0.86 (0.83–0.89)
WHtR, mean (SD)	0.48 (±0.04)
Cardiovascular	
HR (beats/min), mean (SD)	70 (±7)
SBP (mmHg), mean (SD)	114 (±9.43)
DBP (mmHg), mean (SD)	70 (±7)
SDNN (ms), median (IQR)	84.29 (71.58–98.63)
RMSSD (ms), median (IQR)	54.5 (44.70–72.50)
ln LF (ln ms^2), mean (SD)	7.50 (±0.53)
ln HF(ln ms^2), mean (SD)	6.64 (±0.88)
ln (LF/HF), mean (SD)	0.88 (±0.39)
Biochemical	
HbA1c (%), median (IQR)	5.35 (5.12–5.61)
Total cholesterol (mg/dL)	180 (±34)
HDL (mg/dL), median (IQR)	46 (40.13–52.10)
hs-CRP (mg/L) median (IQR)	1 (0.08–1.33)
DHEA-S (μg/dL), mean (SD)	313 (±104)
Habitual diet	
Calories (Kcal), median (IQR)	2350 (1775–2888)
Protein (gr), median (IQR)	84 (64.84–102.24)
Total fats (gr), median (IQR)	80 (58.91–103.63)
Cholesterol (mg), median (IQR)	408 (303−693)
Carbohydrates (gr), median (IQR)	304 (224.80–396.28)
Fiber (gr), median (IQR)	18 (12.63–25.12)
Stress, anxiety and depression scales	
Perceived stress scale, mean (SD)	20.22 (±9)
DHS - Frequency, mean (SD)	22.42 (±11.54)
DHS - Severity, median (IQR)	40 (23−63)
LSC-R - Total score, median (IQR)	6 (4−9)
LSC-R – Perception score, median (IQR)	9 (6−15)
Zung scale score > 50, n (%)	15 (17)
STAI-T score > 44, n (%)	14 (16)
STAI-S score > 41, n (%)	3 (3.40)
Hours of sleep per day, n (%)	
≤ 6 h	52 (59)
> 6 h	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; hs-CRP, high sensitivity C-reactive protein; HR, mean heart rate from 24-h Holter; 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 of high frequency power; ln(LF/HF), natural logarithm of the LF/HF ratio; DHEA-S, dehydroepiandrosterone sulfate; DHS, daily hassles scale; LSC-R, life stressor checklist revised; STAI-T, trait subscale of the STAI (State-Trait Anxiety Inventory); STAI-S, state subscale of the STAI.

Table 2
Multivariate linear regression between HRV and ALI-7.

Dependent variable	Independent variables				Model		
		β	*P*-value	95 % CI	Prob. F	R^2	Adjusted R^2
ln (LF/HF)	ALI-7	0.09	< 0.01	0.03–0.15	< 0.01	0.27	0.22
	Age	0.01	0.15	0.40E-02–0.02			
	Chol_mg	0.27E-03	0.02	0.30E-04–0.52E-03			
	LSC-R_per	-0.01	0.01	-0.01 to −0.002			
	PA						
	1	0.10	0.30	-0.09–0.28			
	2	-0.13	0.18	-0.33–0,049			
ln SDNN	ALI_7	-0.22	0.32	-0.67–0.02	< 0.01	0.33	0.28
	Age	-0.02	0.00	-0.03 to −0.01			
	Chol_mg	-0.57E-04	0.50	-0.22E-04–0.11E-03			
	LSC_per	-0.24E-02	0.39	-0.81E-02–0.32E-02			
	PA						
	1	-0.08	0.21	-0.21–0.04			
	2	-0.07	0.29	-0.06–0.20			
ln RMSSD	ALI_7	-0.04	0.15	-0.10–0.01	< 0.01	0.31	0.26
	Age	-0.03	0.00	-0.04 to −0.01			
	Chol_mg	-0.10E-03	0.36	-0.33E-03–0.12E-03			
	LSC-R_per	-0.28E-03	0.94	-0.77E-02–0.72E-02			
	PA						
	1	-0.11	0.21	-0.27–0.06			
	2	0.10	0.28	-0.08–0.27			
ln HF	ALI_7	-0.11	0.10	-0.23–0.02	< 0.01	0.42	0.38
	Age	-0.09	0.00	-0.11 to −0.05			
	Chol_mg	-0.27E-03	0.26	-0.76E-03–0.21E-03			
	LSC-R_per	0.54E-02	0.50	-0.01–0.02			
	PA						
	1	-0.11	0.55	-0.48–0.26			
	2	0.37	0.05	-0.01–0.75			
ln LF	ALI_7	-0.01	0.90	-0.09–0.08	< 0.01	0.23	0.18
	Age	-0.04	0.00	-0.06 to −0.02			
	Chol_mg	-0.11E-03	0.49	-0.46E-03–0.22E-03			
	LSC-R_per	-0.38E-02	0.51	-0.01–0.76E-02			
	PA						
	1	-0.02	0.86	-0.28–0.23			
	2	0.18	0.17	-0.08–0.45			

ln (LF/HF), the natural logarithm of the LF/HF ratio; ln SDNN, natural logarithm of standard deviation of the NN intervals; ln RMSSD, natural logarithm of root mean square of successive differences between normal heartbeats; ln LF, natural logarithm of low frequency power; ln HF, natural logarithm of high frequency power; ALI, allostatic load index; PA, physical activity; Chol_mg, cholesterol consumption per day in milligrams; LSC-R_per, perception score of the life stress checklist revised scale.

meta-analytic and large-cohort studies indicate that men typically have lower HF power and higher LF/HF ratios compared with women (Koenig and Thayer, 2016; Voss et al., 2015). These differences arise from hormonal, cardiovascular, and autonomic regulatory factors among others. Therefore, restricting our sample to men reduces confounding due to sex related variability in autonomic markers, leading to a more internally consistent interpretation of LF/HF while appropriately limiting generalization to male populations.

In the adjusted regression model, ALI-7 and covariates explained approximately 22 % of the variance in HRV (adjusted $R^2 = 0.22$). While modest in absolute terms, this level of explanatory power is notable given the complexity of HRV and aligns with expectations for multifactorial physiological phenomena. Importantly, our aim was not to maximize prediction of HRV from a broad range of epidemiological or biological factors, but rather to examine its specific association with AL while accounting for key confounders. For example, studies modeling HRV with multiple demographic and physiological predictors have reported adjusted R^2 values ranging from 0.30 to over 0.90 depending on the HRV parameter (Kim et al., 2023). In contrast, a study specifically evaluating the association between AL and HRV by Viljoen *et al.* reported negative correlations corresponding to R^2 values between 0.18 and 0.45 (r = –0.43 to –0.67), without adjustment for confounders (Viljoen and Claassen, 2017). Thus, our findings support the relevance of ALI-7 as a meaningful correlate of HRV, even within a parsimonious model framework.

To date, only two studies have investigated the relationship between AL and HRV (Seeman et al., 2010; Viljoen and Claassen, 2017). Seeman *et al.* developed an 18-biomarker AL model and reported a negative association between AL and HRV components, specifically HF and LF (Seeman et al., 2010). Similarly, Viljoen and Claassen employed a 13-biomarker ALI and observed a significant negative correlation between ALI and SDNN, RMSSD, and HF (Viljoen and Claassen, 2017). Our study differs from these in several key aspects. First, the biomarker composition and calculation methods of the ALI differed across studies. Seeman *et al.* developed a meta-factor model of AL using 18 biomarkers spanning six physiological systems. Although Viljoen and Claassen used the traditional high-risk quartile method that we used in our study, they included 13 biomarkers whereas we included a more concise set of seven biomarkers. Given that our biomarkers were carefully selected based on theoretical models of stress response and supported by empirical evidence linking them to clinically relevant outcomes, and considering the observed association with the LF/HF ratio, we propose that ALI-7 represents a more practical and scalable tool for routine implementation and future research compared to the broader indices proposed by Seeman *et al.* and Viljoen and Claassen. Second, previous research used HRV metrics calculated from short ECG recordings, in contrast, we used 24-hour ambulatory Holter recordings, which capture physiological fluctuations across different activity states, improving predictive value for clinical outcomes (Hayano and Yuda, 2022; Shaffer and Ginsberg, 2017; Zschocke et al., 2022). Third, we accounted for key confounding variables such as physical activity, smoking, socioeconomic status, medication use, and education level, which are essential in HRV

research (Laborde et al., 2017). By adjusting for these factors, our findings are less prone to bias and can be interpreted with greater confidence as reflecting the independent association of interest. Finally, we found a positive association between ALI and the LF/HF ratio, which, to our knowledge, has not been previously reported. Taken together, the evidence consistently indicates that higher AL is associated with lower HRV, regardless of the specific HRV metric.

Low HRV is a well-established marker of increased risk for all-cause mortality (Jarczok et al., 2022) and has been associated with stress-related cardiovascular and psychiatric disorders (Agorastos et al., 2023; Hoshi et al., 2021). Specifically, higher LF/HF ratio has been linked to elevated psychological stress and the presence of stress-related psychiatric conditions (Schneider and Schwerdtfeger, 2020), whereas lower LF/HF ratio values have been associated with greater psychological well-being (Shiga et al., 2021). Our findings are consistent with this literature, demonstrating that increased LF/HF ratio is associated with higher AL, a marker of chronic stress, which in turn is known to elevate the risk for both physical and mental health disorders and mortality (Agorastos et al., 2023; Hoshi et al., 2021; Jarczok et al., 2022; Ortiz-Guzmán et al., 2023; Zhou et al., 2016).

Our comparative analysis between ALI-4 and ALI-7 revealed that only ALI-7 was significantly associated with HRV. This suggests that ALI-7 may provide a more reliable measure of the physiological burden influencing autonomic function. One explanation is that ALI-7 incorporates additional biomarkers more directly linked to cardiovascular (DBP and SBP) and neuroendocrine pathways (DHEA-S) that influence autonomic regulation. In addition, by expanding the scope of physiological systems represented, ALI-7 may better capture the multisystemic effects of chronic stress and enhance the biological specificity of the index.

Taken together, our findings support the notion that chronic stress and the cumulative physiological burden, as measured by the ALI-7, are associated with an increased 24-hour LF/HF ratio. Chronic exposure to stressors promotes sustained activation of the hypothalamic–pituitary–adrenal axis (e.g., decreased DHEA-S) and the sympathetic-adrenal-medullary system (e.g., elevated blood pressure). This neuroendocrine imbalance contributes to metabolic disturbances (e.g., increased HbA1C, decreased HDL, unfavorable anthropometric indicators such as WHR and WHtR) and low-grade inflammation (e.g., elevated hs-CRP). Together, these pathways promote autonomic imbalance characterized by sympathetic predominance and reduced vagal activity, which are well-recognized mechanisms underlying decreased HRV (Agorastos et al., 2023; Shaffer and Ginsberg, 2017; Sloan et al., 2007). This framework provides a physiological explanation for the observed association between higher ALI and reduced HRV.

4.2. *The moderation effect of brain functional and structural parameters on the association between ALI and HRV metrics did not reach statistical significance after correction for multiple comparisons*

Prior to correction for multiple comparisons, we observed a potential moderating effect of brain functional and structural parameters on the relationship between ALI-7 and SDNN. In these cases, the relationship between ALI-7 and HRV became significant only when the brain functional and structural parameters were introduced as moderators. In addition, in all cases, the coefficient of the interaction term was negative. These findings suggest that the effect of ALI-7 on SDNN changes linearly in response to variations in both functional (FCS of the DMN-C) and structural (cortical thickness of the SN-A) brain parameters. Also, individuals with elevated ALI-7 and increased FCS in DMN-C or cortical thickness in SN-A showed lower SDNN. This possibly reflects a detrimental combined effect of chronic stress burden and functional and structural brain network adaptations on autonomic regulation. Although these interaction effects did not remain statistically significant after FDR correction, they are consistent with current neurobiological models of allostatic and autonomic regulation and should be interpreted as hypothesis-generating.

Notably, the observed interaction patterns align with the AIN model, which posits that the DMN and SN are central for integrating interoceptive signals (such as those indexed by ALI biomarkers) and generating predictive responses to maintain allostasis, including autonomic regulation. Specifically, within this framework, key nodes of the AIN include the anterior insula and medial frontal cortex (core components of the SN-A) and the parahippocampal cortex (part of the DMN-C), which together contribute to the integration of peripheral physiological signals with central processing to regulate homeostatic and stress-related responses (Kleckner et al., 2017; Santamaría-García et al., 2025).

There are several plausible explanations for why the analyses of the interaction between ALI and brain functional and structural parameters and its effects on HRV metrics did not reach statistical significance. First, our sample consisted of healthy adult males with low ALI-7 scores and normal HRV metrics; as a consequence, this might lead to a floor effect, limiting the variability necessary to detect meaningful moderation effects of brain functional and structural properties on the associations between ALI and HRV (Haqiqatkhah et al., 2024). Second, the sample size, although adequately powered for detecting the association between main effects, may have been underpowered to detect interaction, which might require larger samples (McClelland and Judd, 1993). Third, cross-sectional designs limit the ability to capture dynamic changes in AL, brain function, and HRV over time, changes that may be more detectable in longitudinal studies or randomized controlled trial designs. Finally, brain-body interactions are complex and likely influenced by additional unmeasured variables, such as genetic factors, neuroendocrine mediators, or lifestyle behaviors not fully captured in our models (Bower and Kuhlman, 2023).

In summary, while our findings did not reach statistical significance following correction for multiple comparisons, their alignment with theoretical and empirical models of stress physiology and HRV regulation highlights their potential relevance (Kleckner et al., 2017; Ruffle et al., 2021; Santamaría-García et al., 2025). These preliminary observations support the hypothesis that the interaction between central neurobiological substrates and cumulative physiological burden influences autonomic function, as indexed by HRV metrics, specifically SDNN. Future studies should prioritize the use of robust methodological designs alongside larger sample sizes to more effectively validate and extend these findings.

4.3. *Strengths, limitations, and future directions*

This is the first study that develops ALI using a multisystemic framework in a sample of Colombian healthy men, thereby contributing a novel tool for both clinical and research applications in the region. Additionally, we concurrently evaluated the association between AL, HRV using 24-hour Holter monitoring, and neuroimaging functional and structural parameters in a sample of healthy individuals. Our analytical approach also incorporated relevant confounding factors.

Nonetheless, several limitations must be acknowledged. First, the cross-sectional design precludes causal inferences. Second, the sample consisted of healthy men (for the reasons described above) from a specific region in Colombia, which limits the generalizability of the findings to women, individuals from other geographical origins, or those with different health conditions. Third, the ALI was computed using risk quartiles within this sample, rather than clinical cut-offs, which (although appropriate for studying physiological variation in healthy individuals) limits the comparability of these values across populations or clinical settings. Finally, while several key covariates were included, the absence of validated assessments of sleep introduces a potential source of residual confounding, particularly given the known influence of sleep quality on HRV. The small sample size and the inclusion of only healthy men limited the ability to confirm the moderating effects of the brain's functional and structural parameters tested.

Future studies should aim to establish standardized guidelines for

HRV acquisition and processing, and explore its integration into predictive models of AL, potentially leveraging machine learning techniques to incorporate multidimensional physiological and psychological variables. The widespread use of HRV measurement in naturalistic conditions through wearable devices offers an opportunity for future evaluation of the utility of HRV, particularly the LF/HF ratio, to assess AL. This could further simplify the quantification of chronic stress, aiding in the prevention, diagnosis, and management of stress-related disorders. Additionally, the implementation of methodological designs that allow for the determination of the directionality and predictive validity of the associations between ALI, HRV, and health outcomes are needed. Such studies should also assess the generalizability and clinical utility of the ALI-7 in predicting stress-related outcomes and identifying at-risk individuals in diverse populations. Additionally, our findings suggest the need to test the moderating role of brain functional connectivity and structure in the relationship between peripheral physiological burden and autonomic regulation. Evaluating the interaction between peripheral and central markers of allostasis may advance theoretical models such as the AIN, and inform personalized strategies and neuromodulatory interventions targeting brain networks for modulation of chronic stress and HRV. This should be extended in longitudinal and interventional designs to clarify whether specific brain features amplify or mitigate the impact of chronic stress on autonomic functioning.

5. Conclusion

Higher chronic stress, assessed using an ALI composed of seven biomarkers, was associated with reduced 24-hour HRV measured through the LF/HF ratio in healthy adult men. Although the moderation effect of brain functional connectivity and structural parameters on the association between AL and HRV did not remain statistically significant after correction for multiple comparisons, they are in alignment with established allostatic and autonomic regulation models, highlighting the need for further investigation. Given the well-established association between reduced HRV and increased risk of morbidity and all-cause mortality, our findings suggest that the ALI-7 may serve as a useful tool for identifying individuals at higher risk of morbidity and all-cause mortality due to chronic stress, emphasizing the need for future confirmatory studies.

CRediT authorship contribution statement

Juan M. Solano-Atehortua: Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gabriel Castrillón:** Writing – review & editing, Software, Resources, Methodology, Investigation, Formal analysis, Data curation. **Yedselt V. Ospina-Serrano:** Resources, Investigation, Data curation. **Ana L. Miranda-Angulo:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Jaime Gallo-Villegas:** Writing – review & editing, Resources. **Juan D. Caicedo-Jaramillo:** Resources, Investigation, Data curation. **Hawkins-Caicedo Valentina:** Investigation, Data curation. **Juan C. Calderón:** Writing – review & editing, Resources. **Juan D. Sánchez-López:** Software, Investigation, Formal analysis, Data curation. **Daniel A. Vargas-Tejada:** Investigation, Data curation. **Jazmin X. Suarez-Revelo:** Software, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this study, the authors used Grammarly and ChatGPT to assist and improve English writing. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2026.107759.

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