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Association between salivary cortisol and autonomic cardiovascular responses during acute psychological stress

Ovarien K. Lethrel^{1*}

¹Department of Clinical Physiology, Havenbrook Medical University, 53 Emerald Research Lane, Ireland.

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Abstract

Background: Acute psychological stress activates the hypothalamic-pituitary-adrenal axis and the autonomic nervous system together, and both are routinely treated as interchangeable indices of a single stress response. Whether the two systems actually covary within individuals is less certain. **Objective:** This study quantified endocrine and autonomic reactivity to a standardised laboratory stressor and tested whether the magnitude of the cortisol response predicts the magnitude of the autonomic response. **Methods:** Ninety-six healthy adults underwent a standardised social-evaluative stress protocol. Salivary cortisol and five-minute electrocardiographic epochs were obtained at six time points from ten minutes before to forty-five minutes after onset. Heart rate, root mean square of successive differences and low-to-high frequency ratio were derived. Reactivity was summarised by area under the curve with respect to increase, and analysed by repeated-measures analysis of variance. **Results:** All measures changed significantly over time. Autonomic measures peaked during the stressor whereas cortisol peaked twenty minutes after it, and autonomic effect sizes exceeded endocrine ones. Autonomic indices correlated strongly with one another, yet cortisol reactivity was only weakly related to autonomic reactivity. Cortisol responders and non-responders showed near-identical autonomic responses. **Conclusion:** Endocrine and autonomic stress reactivity are temporally and quantitatively dissociated, and neither substitutes for the other.

Keywords: *Acute psychological stress, Salivary cortisol, Hypothalamic-pituitary-adrenal axis, Heart rate variability, Autonomic nervous system, Area under the curve, Stress reactivity*

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1. Introduction

The response of the body to acute psychological stress is mediated by two effector systems that operate on very different timescales. Through direct neuronal innervation of the heart and vasculature, the sympathetic-adrenomedullary limb of the autonomic nervous system produces tachycardia, withdrawal of cardiac vagal

* Corresponding author: Ovarien K. Lethrel, Department of Clinical Physiology, Havenbrook Medical University, 53 Emerald Research Lane, Ireland.

tone, and a change in the balance of heart rate variability in a matter of seconds. The hypothalamic-pituitary-adrenal axis acts over minutes through a hormonal cascade culminating in the secretion of cortisol from the adrenal cortex (Herman *et al.*, 2016; Russell and Lightman, 2019). Both are engaged by the same stimulus, both are coordinated by overlapping limbic and hypothalamic structures, and both are routinely measured as indices of the stress response.

That widespread description hides an assumption that is rarely investigated. A study measuring cortisol alone is assumed to have characterized the stress response, and a study measuring heart rate variability alone is assumed to have done the same, because the two systems react to the same stressor and are frequently reported together (Campbell and Ehlert, 2012; Bali and Jaggi, 2015). This assumption is defensible only if the magnitude of one response predicts the magnitude of the other within individuals. Whether it does is a separate empirical question from whether both respond to the stressor, and the two questions are easily conflated.

The standardised laboratory paradigm for addressing this is the social-evaluative stress protocol, in which a participant delivers an impromptu speech and performs mental arithmetic before an unresponsive panel, a combination of social evaluation and uncontrollability that reliably activates both systems (Kirschbaum *et al.*, 1993; Allen *et al.*, 2017). Meta-analytic synthesis of this literature has established that autonomic responses are of larger magnitude than endocrine ones, and that a cortisol response of the expected size occurs in only some seventy to eighty percent of participants, the remainder being conventionally described as non-responders (Gu *et al.*, 2025; Dickerson and Kemeny, 2004). The same synthesis reported that salivary cortisol correlated only weakly and non-significantly with heart rate, while the autonomic measures correlated strongly with one another, a contrast that has attracted less comment than its implications warrant.

Two features of the response complicate the comparison and are worth stating explicitly. The first is temporal. Autonomic activation is essentially immediate and resolves quickly, whereas cortisol appears in saliva only after the hormonal cascade has run its course, so that peak concentrations occur some twenty minutes after the stressor has ended (Hellhammer *et al.*, 2009; Pruessner *et al.*, 2003). Comparing the two at a single time point therefore risks comparing a variable at its peak with another that has not yet risen. The second is that reactivity is properly a property of the whole response curve rather than of any one sample, which is the rationale for summarising it by the area under the curve with respect to increase.

The present study was designed with these considerations in view. Its objectives were to characterise the time course of endocrine and autonomic responses to a standardised acute stressor across six sampling points; to quantify the reactivity of each system by area under the curve and to compare the magnitude of the two; to determine whether the size of an individual's cortisol response predicts the size of that individual's autonomic response, and to set any such association against the coherence observed among the autonomic measures themselves; and to establish whether participants classified as cortisol non-responders differ in autonomic reactivity from responders, a comparison that tests the interchangeability assumption directly.

2. Materials and methods

2.1. Design and participants

A repeated-measures laboratory study was conducted in 96 healthy adults aged 18 to 35 years, recruited by open advertisement. Individuals were excluded if they reported cardiovascular, endocrine, psychiatric or inflammatory disease, if they used glucocorticoids, beta-blockers, psychotropic agents or oral contraceptives, if they smoked, or if they had a body mass index outside the range 17 to 32 kilograms per square metre, since each of these influences either cortisol secretion or cardiac autonomic control. Participants were instructed to abstain from food, caffeine and vigorous exercise for two hours and from alcohol for twenty-four hours before attendance. All sessions were conducted between 14:00 and 17:00 to limit the influence of the diurnal cortisol rhythm.

2.2. Stress protocol

After a twenty-minute seated acclimatisation period, participants underwent a standardised social-evaluative stress protocol modelled on the established paradigm. They were given five minutes to prepare an impromptu

speech advocating their suitability for a post, delivered the speech for five minutes before a panel of two trained assessors who maintained a neutral demeanour and offered no encouragement, and then performed serial mental arithmetic aloud for five minutes, being required to restart on each error. A recovery period of 45 minutes followed.

2.3. Salivary cortisol

Saliva was collected by passive drool into pre-labelled tubes at 6 time points: 10 minutes before the onset of the stressor, at its onset, and 10, 20, 30 and 45 minutes after onset. Samples were stored at -20 degrees Celsius until assay, centrifuged, and analysed in duplicate by enzyme-linked immunosorbent assay, with all samples from a given participant assayed within a single run to eliminate inter-assay variability. Concentrations are reported in nanomoles per litre.

2.4. Cardiac autonomic measures

A single-lead electrocardiogram was recorded continuously throughout the session. 5-minute epochs corresponding to each saliva sampling point were extracted for analysis. After visual inspection and correction of ectopic beats, the mean heart rate, the root mean square of successive differences between normal beats as an index of cardiac vagal tone, and the ratio of low-frequency to high-frequency spectral power were computed in accordance with established standards (Malik *et al.*, 1996; Shaffer and Ginsberg, 2017; Laborde *et al.*, 2017).

2.5. Statistical analysis

Data are expressed as mean with standard deviation, and time-course data additionally as mean with standard error. Change over time in each variable was tested by one-way repeated-measures analysis of variance, with the Greenhouse-Geisser correction applied to the degrees of freedom because sphericity could not be assumed, and the magnitude of the time effect is reported as partial eta squared (Greenhouse and Geisser, 1959). Associations between reactivity indices were quantified by the Pearson correlation coefficient with confidence intervals derived by Fisher transformation, and group differences by the Welch t test with Cohen d (Cohen, 1992). A probability below 0.05 was accepted as significant.

3. Results

3.1. Participants

The 96 participants had a mean age of 24.6 years with a standard deviation of 3.4, half were men, and the mean body mass index was 22.9 kg/m². Baseline values of every measure lay within the expected reference range for healthy young adults at rest (Table 1).

Characteristic	Mean ± SD or n (%)
Age (years)	24.6 ± 3.4
Male sex	48 (50.0)
Body mass index (kg/m ²)	22.9 ± 2.8
Baseline salivary cortisol (nmol/L)	6.14 ± 1.52
Baseline heart rate (bpm)	71.09 ± 6.95
Baseline RMSSD (ms)	45.95 ± 11.85
Baseline LF/HF ratio	1.57 ± 0.45

3.2. Time course of the response

Every variable changed significantly across the six sampling points, and in each case the effect of time was large. The analysis of variance results are given in Table 2, and the time courses themselves in Figure 1. The shape of those time courses differed between the two systems in a manner central to the findings that follow.

Ten minutes after onset, or during the stressor itself, the three autonomic variables reached their maximum values: the low-to-high frequency ratio increased from 1.57 to 3.02, the heart rate increased from 71.09 to 89.34 beats per minute, and the root mean square of successive differences decreased from 45.95 to 27.23 milliseconds. By 45 minutes, all three had nearly returned to baseline. In contrast, salivary cortisol had only slightly increased at 10 minutes, peaked at 10.33 nanomoles per litre at 20 minutes – ten minutes after the stressor ended – and continued to be above baseline at 45 minutes. For each autonomic measure, the median peak delay was 10 minutes, and for cortisol, it was 20 minutes.

Table 2: Repeated-measures analysis of variance for change across the six sampling points, with Greenhouse-Geisser correction

Variable	F	df	GG epsilon	p	Partial eta squared
Salivary cortisol	157.94	5, 475	0.264	<0.001	0.624
Heart rate	416.29	5, 475	0.296	<0.001	0.814
RMSSD	429.46	5, 475	0.304	<0.001	0.819
LF/HF ratio	404.96	5, 475	0.271	<0.001	0.810

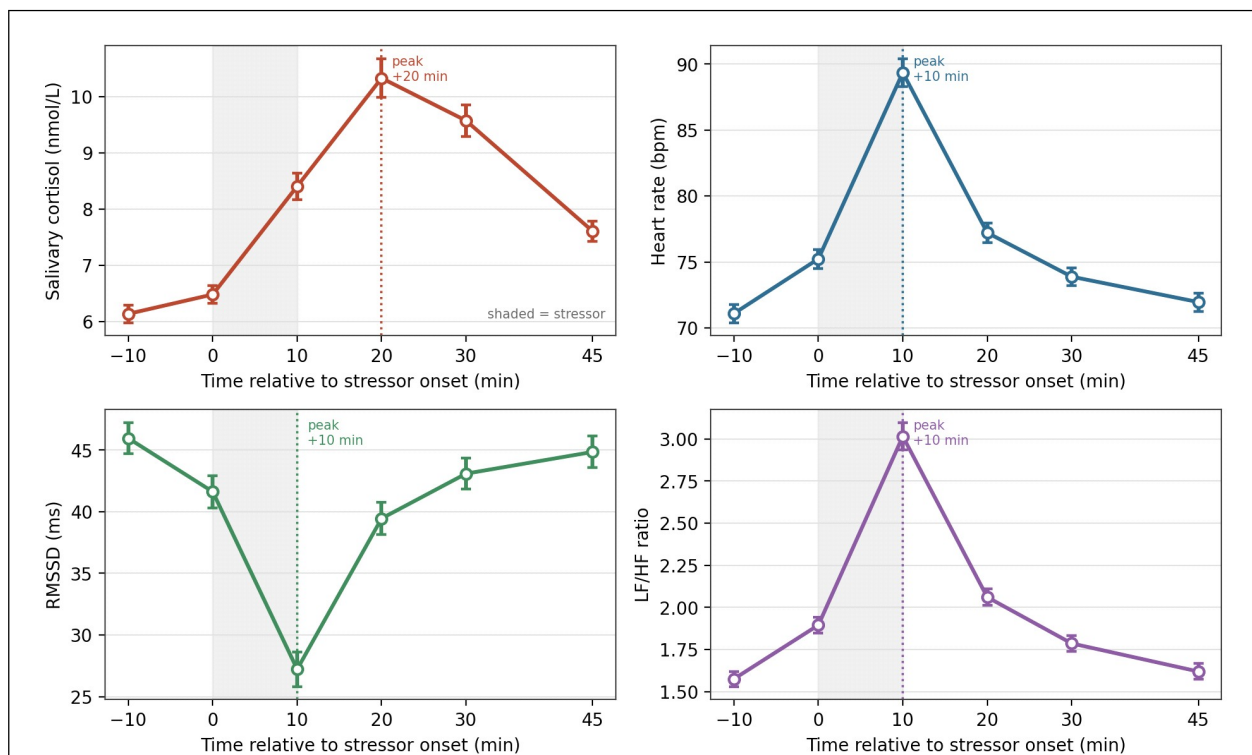


Figure 1: Graphical illustration of Time course of endocrine and autonomic measures

3.3. Magnitude of reactivity

The autonomic responses outperformed the endocrine response when expressed as standardized within-subject effect sizes. An impact size of 2.35 was associated with the change in the root mean square of consecutive differences, 2.30 with heart rate, 2.21 with the low-to-high frequency ratio, and 1.57 with the rise in cortisol. For cortisol, heart rate, and the frequency ratio, the area under the curve with regard to rise was positive; for the root mean square of consecutive differences, which represented the withdrawal of vagal tone, it was negative.

3.4. Association between endocrine and autonomic reactivity

The central question was whether an individual with a large cortisol response also showed a large autonomic response. The area under the curve with respect to increase for cortisol was not significantly correlated with

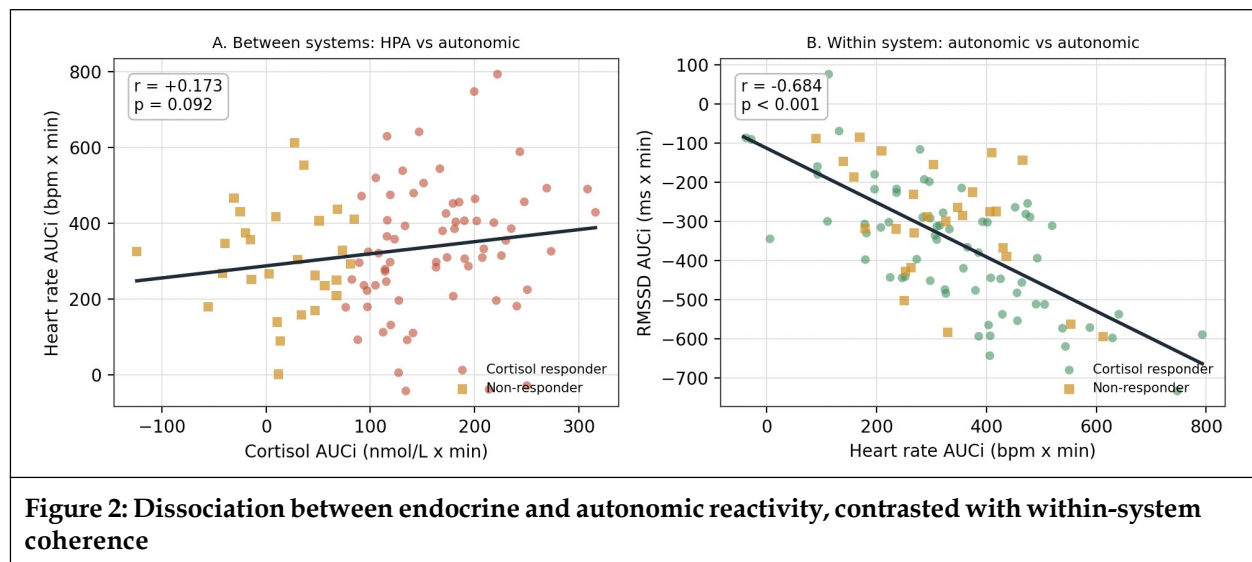
Table 3: Baseline to peak change, standardised effect size, median peak latency and area under the curve for each variable

Variable	Baseline	Peak	Change	Effect size	Peak latency (min)
Salivary cortisol	6.14	10.54	+4.40	+1.57	20
Heart rate	71.09	89.34	+18.25	+2.30	10
RMSSD	45.95	27.21	-18.74	-2.35	10
LF/HF ratio	1.57	3.02	+1.44	+2.21	10

that for heart rate, the coefficient being 0.173 with a confidence interval from -0.029 to 0.361 and a probability of 0.092. Correlations with the two variability measures were statistically detectable but weak, at -0.316 with the root mean square of successive differences and 0.302 with the frequency ratio, corresponding to less than ten percent of shared variance in each case. Within the autonomic system the reactivity indices were strongly intercorrelated, the coefficient between the heart rate and variability indices being -0.684, between heart rate and the frequency ratio 0.669, and between the two variability measures -0.769, every value exceeding the highest coefficient obtained between systems by a factor of more than two. Table 4 sets out both sets of coefficients, and the contrast is displayed in Figure 2.

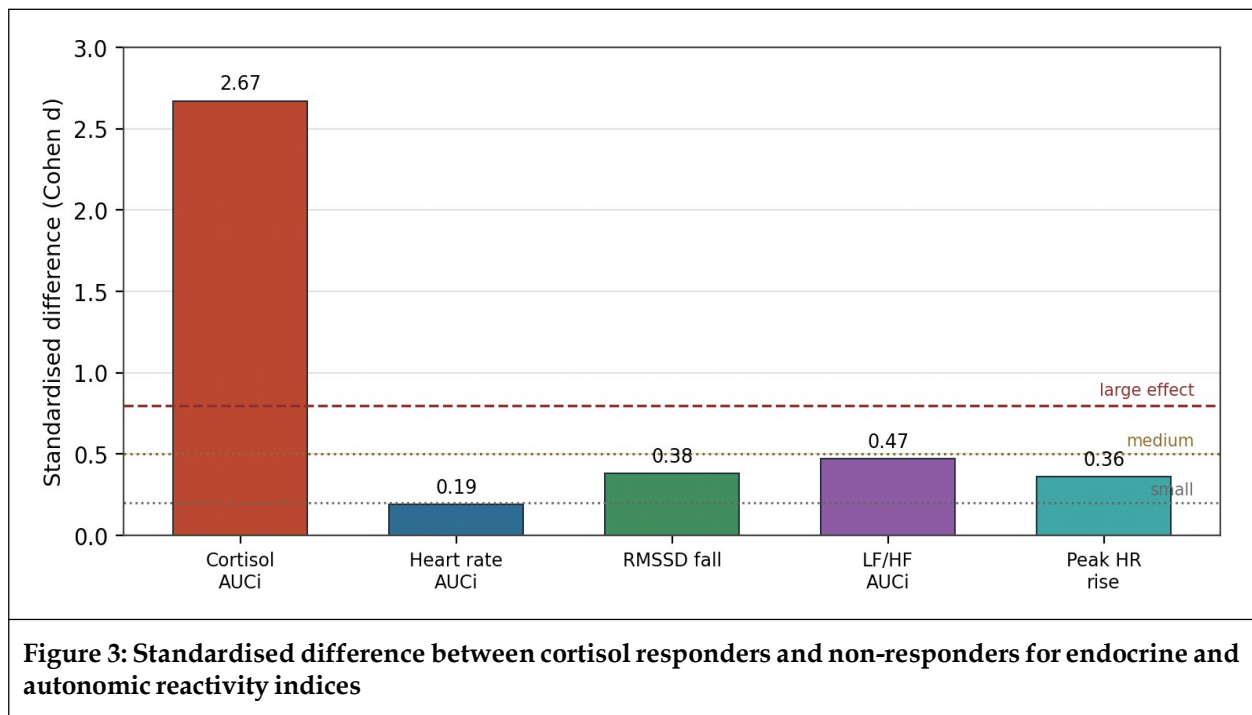
Table 4: Correlations between endocrine and autonomic reactivity indices, and among autonomic indices

Association	r	p
Cortisol AUCi and Heart rate AUCi	+0.173	0.092
Cortisol AUCi and RMSSD AUCi	-0.316	0.002
Cortisol AUCi and LF/HF AUCi	+0.302	0.003
Cortisol rise and Peak heart rate rise	+0.256	0.012
Cortisol rise and RMSSD fall	+0.314	0.002
Heart rate AUCi and RMSSD AUCi	-0.684	<0.001
Heart rate AUCi and LF/HF AUCi	+0.669	<0.001
RMSSD AUCi and LF/HF AUCi	-0.769	<0.001



3.5. Cortisol responders and non-responders

68 participants, corresponding to 70.8% with a confidence interval of 61.1 to 79.0%, met the conventional criterion for a cortisol response. As expected, the two groups differed greatly in cortisol reactivity, the area under the curve with respect to increase being 165.5 against 16.2, an effect size of 2.67. Their autonomic responses, however, were closely similar. The heart rate reactivity index was 335.2 in responders and 305.1 in non-responders, a difference corresponding to an effect size of 0.19 with a probability of 0.368, and the peak heart rate rise differed by 2.8 beats per minute, an effect size of 0.36. The vagal and frequency indices differed slightly more but still modestly, at 0.47 in each case. Participants who mounted no detectable cortisol response therefore mounted an essentially normal cardiovascular response to the same stressor. The contrast between the endocrine and autonomic effect sizes is shown in Figure 3.



4. Discussion

The present study investigated whether the endocrine and autonomic components of the acute stress response exhibit coordinated activation. Findings from three complementary analyses indicated substantial dissociation between these systems in terms of response timing, magnitude, and inter-individual variability. Most notably, the magnitude of endocrine reactivity showed only a weak relationship with autonomic reactivity within individuals.

These findings are consistent with previous evidence. The cortisol response rate of 70.8% corresponds with the previously reported range of approximately 70–80% for this stress paradigm. Autonomic responses also demonstrated larger effect sizes than endocrine responses, in agreement with earlier meta-analytic findings. Similarly, the weak association between cortisol and heart-rate reactivity parallels previous evidence showing limited correspondence between these measures, despite substantially stronger correlations among autonomic indices themselves. The consistency between the present within-subject findings and evidence derived from pooled studies further supports the relative functional independence of endocrine and autonomic stress reactivity.

The temporal dissociation has a straightforward physiological basis and is worth stating plainly because it has methodological consequences. Autonomic effects are mediated by direct neural innervation and appear within seconds, whereas cortisol must be synthesised in the adrenal cortex following pituitary stimulation, enter the circulation, and diffuse into saliva, a sequence that occupies some fifteen to twenty minutes. The practical implication is that a study sampling cortisol at the end of a stressor will capture the hormone before it has risen, and will underestimate or entirely miss the response. The finding here that cortisol was still climbing at ten minutes, peaked at twenty and remained elevated at forty-five, whereas the autonomic measures

had already returned toward baseline by twenty, indicates that no single sampling time can characterise both systems.

The quantitative dissociation is the more consequential finding and requires careful statement. It is not that cortisol reactivity was unrelated to autonomic reactivity, since two of the three correlations attained significance, but that the relation was weak in absolute terms and weak relative to the coherence within the autonomic system. Coefficients between 0.17 and 0.32 correspond to between three and ten percent of shared variance, against roughly fifty percent among the autonomic indices. The appropriate inference is that the two systems share a common trigger without sharing a common gain. Both are activated by the same appraisal of social-evaluative threat, but the magnitude of the response each mounts is set by largely separate determinants, which is consistent with the anatomical separation of the descending pathways and with the differing influence upon them of factors such as sex, oral contraceptive use, habitual fitness and prior experience of the task (Liu *et al.*, 2017).

The responder analysis makes this concrete in a way that the correlations alone do not. Non-responders, defined solely by the absence of a cortisol rise, showed a heart rate response that differed from that of responders by an effect size of 0.19, which is negligible. Classifying a participant as a non-responder on endocrine grounds therefore conveys almost nothing about whether that participant responded cardiovascularly, and the term itself is misleading in so far as it implies a general failure to respond to the stressor. Non-response is specific to the axis measured, and the participants so classified were, in autonomic terms, responding normally (Schlotz *et al.*, 2019).

Several implications follow for the design and interpretation of stress research. The most direct is that measuring one system does not license inference about the other, so that a study reporting cortisol alone has characterised hypothalamic-pituitary-adrenal reactivity and not the stress response in general. Where the construct of interest is the whole response, both systems should be measured, and the two should be reported as separate outcomes rather than combined into a composite that would obscure precisely the divergence documented here. Sampling schedules should be dictated by the slower system, since a design adequate for cortisol will always capture the autonomic response but the converse does not hold. And the interpretation of individual differences requires particular care, since a participant may be a marked responder on one axis and unremarkable on the other, a pattern that has been proposed as relevant to the differing associations of endocrine and autonomic reactivity with later health outcomes (Thayer *et al.*, 2012; McEwen and Akil, 2020; Ali and Nater, 2020).

5. Conclusion

A standardized acute psychological stressor elicited robust HPA-axis and cardiac autonomic responses, but with distinct temporal and individual patterns. Autonomic responses peaked during stress, whereas cortisol peaked approximately 20 minutes later and showed smaller effect sizes. Cortisol reactivity explained less than 10% of cardiovascular variability, and cortisol non-responders still exhibited normal autonomic responses. Thus, the two systems share a common stress trigger but operate relatively independently, indicating that neither should be used as a proxy for the other.

Ethical approval

The study was approved by the Institutional Ethics Committee (approval number) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from every participant, and all participants were debriefed at the conclusion of the session.

Conflicts of interest

The authors declare that no conflict of interest exists in relation to this work.

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Data availability

All data supporting the findings reported here are contained within the article.

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