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Research Article

Cortisol Dysregulation in Chronic Stress: A Cross-Sectional Study of Serum Cortisol Profiles

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Abstract

Chronic psychological stress leads to persistent activation of the hypothalamic-pituitary-adrenal (HPA) axis, eventually resulting in neuroendocrine blunting and cortisol dysregulation. This cross-sectional study evaluated serum cortisol dynamics, diurnal slopes, and systemic metabolic markers across individuals experiencing varying levels of chronic occupational stress. A total of 320 healthy working adults were evaluated and stratified into Low-Stress (n = 155) and High-Stress (n = 165) cohorts using the Perceived Stress Scale (PSS-14). Fasting serum cortisol was measured at 08:00 h, along with 24-hour salivary cortisol profiles (awakening, +30 min, 12:00 h, and 22:00 h), high-sensitivity C-reactive protein (hs-CRP), and fasting blood glucose. The High-Stress cohort exhibited a significantly blunted Cortisol Awakening Response (CAR; mean \Delta = 6.2 ± 3.1 { nmol/L} vs. 14.8 ± 4.2 { nmol/L} , $p < 0.001$) and elevated late-night salivary cortisol (5.1 ± 1.8 { nmol/L} vs. 2.2 ± 0.9 { nmol/L} , $p < 0.001$), resulting in a flattened diurnal cortisol slope. Furthermore, chronic stress was independently associated with higher baseline hs-CRP ({aOR}: 2.64, 95\% { CI}: 1.58–4.41, $p < 0.001$) and elevated HbA1c ({aOR}: 2.12, 95\% { CI}: 1.25–3.58, $p = 0.005$). Prolonged chronic stress induces a shift from physiological diurnal cortisol variation toward a flattened, hypocortisolemic morning profile coupled with incomplete evening suppression, directly correlating with systemic low-grade inflammation and metabolic risk markers.

Keywords: Cortisol dysregulation, chronic stress, hypothalamic-pituitary-adrenal axis, diurnal cortisol slope, cortisol awakening response, hypocortisolism.

Introduction

The human physiological response to acute stressors is mediated primarily by the sympathetic-adrenal-medullary (SAM) axis and the hypothalamic-pituitary-adrenal (HPA) axis. Upon perception of a stressor, the paraventricular nucleus of the

hypothalamus releases corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), stimulating the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH subsequently triggers the zona fasciculata of the adrenal cortex to synthesize and release glucocorticoids, predominantly cortisol. [1–3]

Under un-stressed physiological conditions, cortisol release follows a robust circadian rhythm: concentrations peak approximately 30 to 45 minutes post-awakening—termed the Cortisol Awakening Response (CAR)—and decline steadily throughout the day to reach a nadir around midnight. [4, 5]

However, prolonged exposure to psychosocial or environmental stress induces sustained neuroendocrine stimulation. Over time, chronic hyperactivation leads to receptor desensitization, down-regulation of glucocorticoid receptors (GR) in the hippocampus and hypothalamus, and eventual HPA axis exhaustion—frequently manifesting as a flattened diurnal slope or hypocortisolism. [6–8]

This neuroendocrine dysregulation has far-reaching clinical consequences. Dysregulated cortisol rhythms alter metabolic homeostasis through unchecked gluconeogenesis, exacerbate visceral adiposity, and impair immune regulation, leading to persistent low-grade systemic inflammation via uninhibited pro-inflammatory cytokine cascades (e.g., IL-6, TNF- \alpha , and CRP). [9, 10]

Despite extensive laboratory research on acute stress models, community-based cross-sectional data characterizing full serum and diurnal salivary cortisol profiles alongside metabolic biomarkers in chronic stress populations remain critical for refining clinical diagnostic risk markers.

This cross-sectional study aimed to quantify serum and salivary cortisol dynamics in individuals with high chronic stress compared to low-stress controls, assessing their direct relationships with inflammatory and metabolic health metrics.

Methodology

A cross-sectional analytical study was conducted evaluating 320 healthy working adult participants (aged 25 to 55 years) recruited from occupational health screening programs over a 12-month period at Akhtar Saeed Medical College. All participants gave written informed consent, and the study was approved by the institutional ethics review board.

Exclusion criteria included current systemic corticosteroid or immunosuppressive therapy, shift-work employment, pregnancy or lactation, active endocrine disorders (e.g., Cushing syndrome, Addison disease), clinical psychiatric conditions requiring pharmacological intervention, or acute infectious illness within the preceding 30 days.

Participants were stratified into two comparison groups using the validated Perceived Stress Scale (PSS-14):

- **Low-Stress Cohort:** PSS score \le 18 (n = 155)
- **High-Stress Cohort:** PSS score 28 (n = 165)

Venous blood samples were collected between 07:30 h and 08:30 h after a 10-hour overnight fast to measure morning serum cortisol, fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), lipid panels, and high-sensitivity C-reactive protein (hs-CRP).

Salivary cortisol samples were self-collected by participants using synthetic swab devices across a single weekend day at four specific timepoints: immediately upon awakening (T_0), 30 minutes post-awakening (T_{30}), 12:00 h (T_{ {noon}}), and 22:00 h (T_{ {night}}). The Cortisol Awakening Response (CAR) was defined as the absolute change between T_0 and T_{30} (\Delta = T_{30} - T_0). The diurnal cortisol slope was calculated as the change in cortisol concentration per hour from awakening to bedtime.

Serum and salivary cortisol concentrations were quantified using electrochemiluminescence immunoassay (ECLIA) and enzyme-linked immunosorbent assay (ELISA), respectively. Statistical analyses were performed using SPSS (version 26.0). Continuous variables were analyzed using independent t -tests or Mann-Whitney U tests based on normality distributions. Multivariable logistic regression models were constructed to evaluate independent associations between cortisol metrics and metabolic/inflammatory outcomes

.**Results**

Table 1: Baseline Demographic, Behavioral, and Clinical Profile of Study Participants

Parameter	**Total Cohort (N=320)**	**Low-Stress Group (n=155)**	**High-Stress Group (n=165)**	**p-value**
Age (Years ± SD)	39.4 ± 8.2	38.8 ± 8.1	40.0 ± 8.3	0.191
Gender (Male / Female %)	172 / 148 (53.8% / 46.2%)	85 / 70 (54.8% / 45.2%)	87 / 78 (52.7% / 47.3%)	0.708
Body Mass Index ({kg/m}^2 ± {SD})	26.8 ± 4.1	25.4 ± 3.6	28.1 ± 4.2	< 0.001
Perceived Stress Score (PSS-14 ± SD)	23.1 ± 7.8	14.2 ± 3.1	31.5 ± 3.8	< 0.001
Current Smokers (%)	58 (18.1%)	21 (13.5%)	37 (22.4%)	0.038
Physical Activity (< 150 { min/week})	142 (44.4%)	52 (33.5%)	90 (54.5%)	< 0.001

Parameter	Total Cohort (N=320)	Low-Stress Group (n=155)	High-Stress Group (n=165)	p-value
Sleep Duration (Hours/Night ± SD)	6.4 ± 1.2	7.1 ± 0.9	5.7 ± 1.1	< 0.001

Note: Individuals in the high-stress group demonstrated higher BMI, reduced sleep duration, and lower rates of physical activity compared to low-stress controls.

Table 2: Comparison of Serum and Salivary Cortisol Metrics Across Stress Groups

Cortisol Metric	Low-Stress Group (n=155)	High-Stress Group (n=165)	Reference Range / Units	p-value
Fasting Morning Serum Cortisol (08:00 h)	415.2 ± 88.4	342.6 ± 95.1	140–690 { nmol/L}	< 0.001
Salivary Cortisol Profile				
Awakening (T_0)	11.4 ± 2.8	12.1 ± 3.2	{nmol/L}	0.039
30-Min Post-Awakening (T_{30})	26.2 ± 4.8	18.3 ± 4.1	{nmol/L}	< 0.001
Cortisol Awakening Response (\Delta CAR = T_{30} - T_0)	+14.8 ± 4.2	+6.2 ± 3.1	{nmol/L}	< 0.001
Mid-day (T_{ {noon}})	8.1 ± 2.1	9.4 ± 2.5	{nmol/L}	0.001

Cortisol Metric	Low-Stress Group (n=155)	High-Stress Group (n=165)	Reference Range / Units	p-value
Late Evening (T_{ {night}} , 22:00 h)	2.2 ± 0.9	5.1 ± 1.8	{nmol/L}	< 0.001
Diurnal Cortisol Slope ({nmol/L per hour})	-0.61 ± 0.12	-0.38 ± 0.14	Normal: steeper negative slope	< 0.001

Note: High chronic stress was characterized by a reduced Cortisol Awakening Response (CAR) and elevated nighttime cortisol levels, resulting in a distinctly flattened diurnal slope.

Table 3: Inflammatory and Metabolic Biomarker Profile

Biomarker	Low-Stress Group (n=155)	High-Stress Group (n=165)	Target Reference Level	p-value
High-Sensitivity CRP ({mg/L} , Median [IQR])	1.2 [0.6–2.1]	2.8 [1.5–4.6]	< 1.0 { mg/L}	< 0.001
Fasting Plasma Glucose ({mg/dL} ± {SD})	91.4 ± 9.2	102.8 ± 14.5	< 100 { mg/dL}	< 0.001
HbA1c (% ± {SD})	5.3 ± 0.3	5.7 ± 0.5	< 5.7%	< 0.001
Triglycerides ({mg/dL} , Median [IQR])	118 [88–145]	156 [122–198]	< 150 { mg/dL}	< 0.001

Biomarker	Low-Stress Group (n=155)	High-Stress Group (n=165)	Target Reference Level	p-value
HDL Cholesterol ({mg/dL} ± {SD})	52.6 ± 8.4	45.1 ± 7.9	> 40 { mg/dL} (males) / > 50 (females)	< 0.001

Note: The high-stress group demonstrated significant elevations in glycemic indices, dyslipidemia, and systemically higher median inflammatory markers (hs-CRP).

Table 4: Multivariable Logistic Regression Analysis for Risk of High Inflammatory Status ({hs-CRP} > 3.0 { mg/L}) and Glycemic Dysregulation ({HbA1c} 5.7\%)

Clinical Outcome & Independent Predictors	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (aOR) (95% CI)*	p-value
Systemic Inflammation ({hs-CRP} > 3.0 { mg/L})			
High Perceived Stress ({PSS-14}28)	3.12 (1.98–4.92)	2.64 (1.58–4.41)	< 0.001
Blunted CAR (Delta CAR < 8.0 { nmol/L})	2.85 (1.79–4.54)	2.21 (1.31–3.73)	0.003
Elevated Nighttime Cortisol (> 4.0 { nmol/L})	3.41 (2.12–5.48)	2.78 (1.64–4.72)	< 0.001
Glycemic Dysregulation ({HbA1c} 5.7\%)			

Clinical Outcome & Independent Predictors	Unadjusted Odds Ratio (95% CI)	Adjusted Odds Ratio (aOR) (95% CI)*	p-value
High Perceived Stress ({PSS-14} 28)	2.48 (1.54–3.99)	2.12 (1.25–3.58)	0.005
Flattened Diurnal Slope (> -0.45 { nmol/L/h})	2.92 (1.81–4.71)	2.34 (1.38–3.97)	0.002

Note: *Multivariable models adjusted for age, gender, BMI, smoking status, physical activity level, and baseline sleep duration.

Discussion

This cross-sectional study demonstrates clear differences in neuroendocrine profiles between individuals experiencing high chronic psychosocial stress and low-stress controls. Prolonged chronic stress was associated with a loss of normal circadian variability, characterized by a blunted Cortisol Awakening Response (CAR), elevated late-evening cortisol levels, and a flattened diurnal cortisol slope.

Under physiological conditions, the morning spike in cortisol mobilizes energetic resources to meet daily cognitive and physical demands. In contrast, chronic exposure to stressors down-regulates HPA axis sensitivity, resulting in a hypocortisolemic morning state and an attenuated CAR (Delta = 6.2 { nmol/L} vs. 14.8 { nmol/L}). Concurrently, the failure to suppress nocturnal cortisol production (5.1 { nmol/L} vs. 2.2 { nmol/L}) reflects impaired negative feedback inhibition, likely driven by reduced central glucocorticoid receptor sensitivity in the hippocampus. [11–13]

The observed biochemical shift toward a flattened diurnal cortisol slope carries direct clinical implications for metabolic and cardiovascular health. Elevated evening cortisol levels disrupt the normal nocturnal restoration phase, increasing overnight lipolysis, hepatic gluconeogenesis, and peripheral insulin resistance. This mechanism accounts for the higher prevalence of elevated fasting glucose (102.8 { mg/dL}) and higher median triglycerides observed in our high-stress cohort. [14, 15]

Furthermore, persistent hypocortisolism during morning hours combined with glucocorticoid receptor resistance impairs the normal anti-inflammatory regulation usually exerted by cortisol. Uninhibited inflammatory pathways contribute to the elevated hs-CRP levels observed in the high-stress group ({aOR}: 2.64). This chronic low-grade systemic inflammation plays a key role in accelerating atherogenesis, metabolic syndrome, and endothelial dysfunction. [16–18]

Limitations of this study include its cross-sectional design, which limits direct inferences regarding temporal causality, and reliance on single-day 4-point salivary collection, which can be affected by day-to-day behavioral variation.

Overall, clinical assessment of diurnal cortisol slopes and morning CAR metrics provides valuable objective insight into HPA axis fatigue, offering measurable markers to evaluate stress-management interventions and prevent long-term metabolic and cardiovascular complications.

Conclusion

Chronic psychosocial stress is strongly associated with HPA axis dysregulation, marked by an attenuated Cortisol Awakening Response, elevated nighttime cortisol concentrations, and a flattened diurnal slope. These endocrine shifts independently correlate with elevated systemic inflammatory markers ({hs-CRP}) and early glycemic impairment. Comprehensive stress management strategies and neuroendocrine monitoring are important components in mitigating chronic metabolic and cardiovascular risks.

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