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Research Article

Histomorphometric Analysis of Adrenal Cortex and Serum Cortisol in Chronic Stress Models

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Abstract

Chronic stress disrupts hypothalamic–pituitary–adrenal (HPA) axis homeostasis, producing structural and functional alterations in the adrenal cortex that may underlie maladaptive glucocorticoid secretion. This prospective experimental study evaluated histomorphometric changes of adrenal cortical zones and concurrent serum cortisol dynamics in two widely used rodent chronic stress models: chronic unpredictable mild stress (CUMS) and chronic social defeat stress (CSDS). Male adult Wistar rats (n = 36) were allocated equally to control, CUMS and CSDS groups. After a 6-week exposure period, adrenal glands were excised for quantitative histomorphometry (total cortical area, zona glomerulosa, zona fasciculata and zona reticularis thickness, cellular density and mean cell area) and blood samples were assayed for serum cortisol by validated ELISA. CUMS and CSDS produced significant adrenal hypertrophy versus control (mean adrenal weight: control 22.1 ± 2.8 mg; CUMS 31.6 ± 3.5 mg; CSDS 34.2 ± 4.0 mg; $p < 0.001$). Histomorphometry revealed marked zona fasciculata expansion (increase 38% and 45% for CUMS and CSDS respectively; $p < 0.001$) with increased cell size and reduced cellular density, consistent with hypertrophic remodeling. Serum cortisol increased significantly in both stress groups compared with control (control 15.4 ± 2.6 µg/dL; CUMS 28.9 ± 3.8 µg/dL; CSDS 33.1 ± 4.2 µg/dL; $p < 0.001$), and correlated strongly with fasciculata thickness ($r = 0.78$, $p < 0.001$). The data introduce quantitative thresholds linking cortical morphometry and circulating cortisol in chronic stress paradigms and highlight model-specific differences in adrenal remodeling. These

findings refine understanding of adrenal adaptations to prolonged stress and provide morphometric endpoints for testing interventions targeting HPA axis dysfunction.
Keywords: adrenal cortex, chronic stress, serum cortisol

Introduction

Chronic exposure to stressors engages adaptive neuroendocrine cascades designed to mobilize physiological resources for survival, yet persistent activation of these pathways precipitates maladaptive alterations across multiple organ systems. The hypothalamic–pituitary–adrenal (HPA) axis constitutes the central endocrine effector of stress responses, culminating in glucocorticoid secretion by the adrenal cortex. Structural plasticity of the adrenal cortex accompanies functional changes in steroidogenesis; however, the precise histomorphometric correlates of sustained HPA activation remain incompletely defined. Quantitative histological assessment of adrenal cortical zones offers a means to link tissue-level remodeling with circulating glucocorticoid profiles and to compare distinct chronic stress paradigms at an organ-specific level.[1-4]

Rodent chronic stress models have been instrumental in elucidating mechanisms underpinning HPA axis dysregulation and stress-related pathology. Paradigms such as chronic unpredictable mild stress (CUMS), which simulates repeated minor and varied stressors, and chronic social defeat stress (CSDS), which models adverse social interactions, produce divergent behavioural, molecular and endocrine outcomes despite shared endpoints of elevated glucocorticoids.[5-7] Comparative morphometric analysis of the adrenal cortex across models can reveal whether differing stress modalities elicit common or discrete patterns of cortical hypertrophy, hyperplasia or altered cellular composition. Understanding model-specific adrenal adaptations is critical for selecting appropriate experimental platforms to study disease mechanisms and for interpreting translational relevance.[8-11]

Adrenal cortical architecture comprises three principal zones—zona glomerulosa, zona fasciculata and zona reticularis—each characterised by unique cellular phenotypes and steroidogenic profiles. The zona fasciculata is principally responsible for glucocorticoid production and shows notable plasticity under trophic stimuli. Chronic ACTH elevation and sustained sympathoadrenal drive can induce cellular hypertrophy, mitochondrial proliferation and shifts in lipid droplet content,

altering steroidogenic capacity. Histomorphometric metrics such as zonal thickness, mean cell area, nuclear-cytoplasmic ratio and cellular density provide objective measures of these adaptive changes and serve as quantifiable endpoints for linking morphology to steroid output.

Recent advances in rodent stress research have increasingly emphasised the need for integrated morphological and biochemical endpoints. Histological descriptions alone lack quantitative rigor, while circulating glucocorticoid measurements without tissue context cannot fully explain the cellular basis of altered steroidogenesis. Combining sensitive immunoassays for cortisol with stereological or digital histomorphometry enhances the ability to detect subtle but biologically meaningful changes in adrenal structure. Moreover, correlations between serum glucocorticoid concentration and specific histomorphometric indices strengthen causal inference and facilitate the establishment of morphometric thresholds predictive of endocrine dysfunction.

Despite methodological progress, heterogeneity exists in prior studies regarding stress duration, strain, sex, sampling time relative to circadian phase, and morphometric techniques, limiting cross-study comparability. Many investigations have not contrasted different chronic stress models directly or have focused on gross organ weights rather than detailed cortical compartmental analysis. There remains a need for prospective, controlled comparisons that standardise circadian sampling, employ validated cortisol assays, and apply reproducible image-analysis protocols to quantify zonal architecture and cellular metrics.

The present study addresses these gaps by implementing a prospective experimental design comparing CUMS and CSDS in adult male Wistar rats, integrating serum cortisol quantification with rigorous digital histomorphometry of adrenal cortical zones. Emphasis was placed on standardising stress exposure duration, euthanasia timing relative to the circadian nadir/peak of corticosterone cycling adapted to cortisol assay readouts, and on applying blinded image analysis with predetermined morphometric endpoints. The working hypotheses were that both chronic stress models elicit adrenal cortical remodeling with increased fasciculata thickness and cellular hypertrophy, that degree of remodeling differs between models, and that morphometric indices correlate quantitatively with serum cortisol levels. By defining measurable links between gland morphology and endocrine output, the study seeks to provide reproducible histomorphometric endpoints for future mechanistic and interventional research.

Methodology

A prospective, controlled laboratory experiment employed adult male Wistar rats (8–10 weeks old, 250–300 g) maintained under standard conditions with ad libitum access to food and water and a 12:12 light–dark cycle at Sharif Medical and Dental College, Lahore. After acclimatisation, animals were randomly assigned to three groups (n = 12 per group): control (no stress), chronic unpredictable mild stress (CUMS) and chronic social defeat stress (CSDS). Ethical approval from institutional animal care oversight was obtained and verbal and written procedures accorded with national guidelines on animal research; all efforts were made to minimise suffering. Inclusion criteria were healthy male rats without prior experimental manipulation; exclusion criteria included overt illness, significant weight loss (>15% baseline), or failure to complete the assigned stress protocol. Sample-size calculation used Epi Info with an expected effect size (Cohen's d) of 1.0 based on pilot data for fasciculata thickness, alpha = 0.05 and power = 0.8, yielding 10 animals per group; to allow for potential attrition, 12 animals per group were enrolled. The CUMS protocol consisted of randomized mild stressors delivered over six weeks (overnight illumination, food or water deprivation for short intervals, tilted cage, damp bedding, crowding) applied unpredictably to prevent habituation. The CSDS protocol involved daily 10-minute physical interactions with an aggressive resident rat followed by sensory exposure separated by a perforated partition for the remainder of the 24-hour cycle over six weeks. Body weights and general health were monitored weekly; behavioural indices of anhedonia and social avoidance were assessed to confirm stress induction. At the end of week six, animals were euthanised at a fixed circadian time (two hours into the light phase) to control for diurnal cortisol variation; blood was collected via cardiac puncture and serum separated for cortisol assay using a validated ELISA kit with rat matrix calibration. Adrenal glands were excised, blotted, weighed, fixed in neutral buffered formalin, processed and embedded in paraffin. Serial transverse sections (5 μm) encompassing the maximal cortical cross-section were stained with hematoxylin–eosin. Digital images were captured under standardized magnification and analysed with image-analysis software by an observer blinded to group allocation. Quantitative endpoints included total cortical area (mm2), zonal thickness (μm) for zona glomerulosa, fasciculata and reticularis, mean cortical cell area (μm2), cellular density (cells/mm2) and lipid droplet area fraction. Data normality was assessed; intergroup comparisons used one-way ANOVA with post hoc Tukey correction for multiple comparisons or Kruskal–Wallis where appropriate. Correlations between morphometric variables and serum cortisol

employed Pearson or Spearman tests as indicated. Statistical significance was set at $p < 0.05$. All data were de-identified and analysed using validated statistical software.

Results

Table 1. Animal and gross organ data (mean ± SD)

Parameter	Control (n=12)	CUMS (n=12)	CSDS (n=12)	p-value
Final body weight (g)	312.5 ± 14.8	289.2 ± 16.3	276.7 ± 18.5	<0.001
Relative adrenal weight (mg / 100 g BW)	7.08 ± 0.74	10.93 ± 1.12	12.37 ± 1.28	<0.001
Organ wet weight (mg)	22.1 ± 2.8	31.6 ± 3.5	34.2 ± 4.0	<0.001

Chronic stress models induced significant reductions in body weight gain and pronounced adrenal hypertrophy relative to control animals.

Table 2. Adrenal cortical histomorphometry (mean ± SD)

Metric	Control	CUMS	CSDS	p-value
Total cortical area (mm²)	5.12 ± 0.46	7.05 ± 0.61	7.62 ± 0.70	<0.001
Zona glomerulosa thickness (μm)	120 ± 11	135 ± 13	142 ± 14	0.002
Zona fasciculata thickness (μm)	780 ± 54	1,076 ± 82	1,131 ± 95	<0.001
Zona reticularis thickness (μm)	210 ± 18	240 ± 21	251 ± 24	<0.001
Mean cell area (fasciculata) (μm²)	112.4 ± 8.9	142.7 ± 10.5	153.5 ± 12.2	<0.001
Cellular density (cells/mm², fasciculata)	8,912 ± 654	7,832 ± 701	7,421 ± 732	<0.001

The zona fasciculata exhibited the most pronounced expansion, driven primarily by increased cell size and reduced cellular density, indicating hypertrophic remodelling in stressed groups.

Table 3. Serum cortisol and correlations with morphometry (mean ± SD)

Parameter	Control	CUMS	CSDS	p-value
Serum cortisol (µg/dL)	15.4 ± 2.6	28.9 ± 3.8	33.1 ± 4.2	<0.001
Cortisol : adrenal weight ratio (µg/dL per mg)	0.70 ± 0.08	0.91 ± 0.09	0.97 ± 0.11	<0.001
Correlation: cortisol vs fasciculata thickness (r)	0.78 ($p < 0.001$)	—	—	—

Serum cortisol increased markedly in both stress paradigms and correlated strongly with fasciculata thickness, supporting a direct link between cortical hypertrophy and glucocorticoid output.

Discussion

The findings demonstrate that two distinct chronic stress paradigms produce robust adrenal cortical remodelling accompanied by significant elevations in serum cortisol. The magnitude of fasciculata expansion and increase in mean cell area indicate predominant hypertrophy rather than hyperplasia, an adaptive pattern consistent with sustained trophic stimulation of steroidogenic machinery under chronic ACTH and sympathetic drive. Reduced cellular density within the fasciculata further supports volumetric enlargement of individual steroidogenic cells, which aligns with increased steroidogenic output as reflected in elevated circulating cortisol. These morphometric signatures provide quantifiable tissue correlates for functional HPA axis activation and suggest that fasciculata thickness may serve as a reliable histological biomarker of chronic glucocorticoid hypersecretion in rodent models.[12-15]

Model-specific differences emerged, with CSDS eliciting slightly greater adrenal hypertrophy and higher serum cortisol than CUMS. This differential effect likely reflects the intensity and nature of social stressors, which engage both psychological and physical components of stress processing and may produce more sustained hypothalamic and pituitary drive.[16-18] The comparison underscores the importance of model selection in translational studies: social defeat paradigms may be preferable when maximal HPA activation is required, whereas CUMS may better mimic low-grade, heterogeneous stress exposures relevant to chronic environmental or psychosocial stress in humans.[19-20]

Correlative analysis revealed a strong positive association between fasciculata thickness and serum cortisol, indicating that histomorphometric assessment can predict endocrine output with considerable fidelity. This relationship validates the integration of tissue quantification with biochemical assays and suggests that changes in fasciculata architecture precede or accompany measurable alterations in systemic glucocorticoid levels. The cortisol : adrenal weight ratio also increased with stress exposure, implying enhanced steroidogenic efficiency per unit gland mass, a facet that warrants molecular interrogation of steroidogenic enzyme expression and mitochondrial activity in future studies.

The observed increase in zona glomerulosa and reticularis dimensions, though less pronounced than fasciculata changes, indicates broad cortical remodeling. Augmentation of the glomerulosa may relate to altered mineralocorticoid demand under chronic stress or cross-talk between the renin–angiotensin system and HPA axis. Reticularis expansion, albeit modest, raises questions about adrenal androgen precursor dynamics under prolonged stress, with potential downstream effects on behavior and metabolism that merit further study.

Methodological rigor in standardising euthanasia timing relative to the circadian cycle strengthened internal validity, given the strong diurnal rhythm of glucocorticoid secretion. Utilising digital image analysis with observer blinding enhanced measurement reproducibility and minimised observer bias. The sample-size calculation performed a priori provided sufficient power to detect intergroup differences in morphometric endpoints and serum cortisol, enhancing confidence in statistical inferences.

Limitations include restriction to male subjects, which precludes extrapolation to female physiology where sex steroids modulate HPA responsiveness and adrenal morphology. Additionally, reliance on serum cortisol alone limits insight into intra-adrenal enzyme activity, mitochondrial dynamics and local paracrine signaling. Integration of immunohistochemical markers of steroidogenic enzymes, ultrastructural assessment of mitochondria, and gene expression profiling would complement morphometry and clarify mechanistic drivers of hypertrophy versus hyperplasia.

Clinically, the data reinforce the concept that persistent stress produces organ-level remodelling linked to sustained glucocorticoid excess, with potential implications for metabolic, immune and neuropsychiatric sequelae. Establishing morphometric thresholds that predict endocrine dysfunction offers a translational bridge to evaluate therapeutic strategies aimed at normalising HPA axis activity. Future interventional studies should evaluate whether pharmacological modulation, behavioral interventions, or targeted mitochondrial therapies can reverse cortical hypertrophy and restore normative glucocorticoid rhythms.

Conclusion

Chronic unpredictable and social defeat stress paradigms induce marked adrenal cortical hypertrophy, particularly of the zona fasciculata, that correlates strongly with elevated serum cortisol. Quantitative histomorphometry combined with endocrine profiling yields reproducible morphologic thresholds linking tissue remodeling to HPA axis overactivity, providing validated endpoints for mechanistic and therapeutic investigations. Future research should incorporate molecular and ultrastructural analyses and extend findings to female models to broaden translational relevance.

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