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Sex Differences in Basal Cortisol Levels Across Body Fluid Compartments in a Cross-sectional Study of Healthy Adults

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Abstract

Context: Many studies have moved toward saliva and peripheral blood sampling for studying cortisol, even in relation to disorders of the brain. However, the degree to which peripheral cortisol reflects central cortisol levels has yet to be comprehensively described. Data describing the effect that biological characteristics such as age and sex have on cortisol levels across compartments is also limited.

Objective: To assess the relationships of cortisol levels across cerebrospinal fluid (CSF), saliva, and plasma (total and free) compartments and describe the effects of age and sex on these relationships.

Design: Multisite cross-sectional observation study.

Setting: Samples collected in academic outpatient settings in 2001-2004.

Patients or Other Participants: Healthy community volunteers (n = 157) of both sexes, aged 20-85 years.

Interventions: None.

Main Outcome Measures: This study was a secondary analysis of data collected from a previously published study.

Results: CSF cortisol correlated more strongly with plasma ($r = 0.49$, $P < .0001$) than with saliva cortisol levels. Sex but not age was a significant modifier of these relationships. CSF cortisol levels trended higher with older age in men ($R^2 = 0.31$, $P < .001$) but not women. Age-related cortisol binding globulin trends differed by sex but did not correlate with sex differences in cortisol levels in any compartment.

Conclusion: Variability in the correlations between central and peripheral cortisol discourages the use of peripheral cortisol as a direct surrogate for central cortisol measures. Further investigation of how mechanistic drivers interact with biological factors such as sex will be necessary to fully understand the dynamics of cortisol regulation across fluid compartments.

Key Words: cortisol, cerebrospinal fluid, sex differences, aging

Cortisol has long been hypothesized as a modulator in psychiatric and neurological disorders, notably with elevated levels found in depression [1, 2] and dementia [3]; however, despite decades of investigation, its role in the pathophysiology of these disorders remains elusive. One barrier to defining the role of cortisol in disorders of the central nervous system is the heterogeneity of body fluids in which cortisol is measured. A handful of studies have analyzed cerebrospinal fluid (CSF) cortisol levels, but numbers remain small due to the relatively more invasive nature of CSF sampling. As a result, many studies have moved toward sampling peripheral body fluids including saliva and blood for studying cortisol, even in relation to disorders of the brain. However, the degree to which peripheral cortisol reflects central cortisol levels in CSF has yet to be comprehensively described.

Cortisol homeostasis between body fluid compartments has the potential to be influenced by a variety of different physiological mechanisms, including passive diffusion, active transport, and binding to the carrier protein, cortisol binding globulin (CBG). In plasma, less than 6% of total plasma cortisol is unbound and biologically active, with 80% to 90% of cortisol bound by CBG, rendering it biologically inactive [4]. Cortisol crosses from the bloodstream to saliva and CSF compartments through a combination of passive diffusion and active transport through a variety of different transporters [5, 6]. We hypothesized that all these factors may be regulated in different ways in different parts of the body [7], resulting in cortisol homeostasis across compartments that may not correlate as expected. While some studies have investigated the correlation of cortisol measures across some body fluid

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compartments [8-11], the limited scope of these study populations limits the generalizability of previous findings, and the effect of basic biological factors like age and sex on these relationships remains unclear.

With the more invasive nature of CSF sampling, the benefits of peripheral fluid sampling for examining cortisol in clinical studies are clear. However, given the complexity of cortisol dynamics across compartments, as well as some evidence of differential cortisol levels across compartments, we must define these dynamics if we are to attribute any clinical significance of peripheral cortisol when it comes to central nervous system (CNS) pathophysiology. To our knowledge, there are no studies to date that demonstrate a correlation of CSF cortisol with peripheral compartments across age and sex. In this study we measured cortisol in CSF, saliva, and plasma (total and calculated free), as well as CBG, in healthy individuals of both sexes across a wide range of ages and describe the relationships among cortisol levels across compartments in the context of these biological characteristics.

Materials and Methods

Participants

Key inclusion criteria

This manuscript is a secondary analysis of data from study participants who agreed to data and specimen banking as a part of participation in previously published studies [12, 13]. Study sites included VA Puget Sound (Seattle, WA); University of California, San Diego (San Diego, CA); Oregon Health and Science University (Portland, OR); Indiana University (Indianapolis, IN); and University of Pennsylvania (Philadelphia, PA). Briefly, all participants were adults in good health who underwent an evaluation that consisted of medical history, physical and neurologic examinations, and laboratory tests including complete blood count, serum electrolytes, blood urea nitrogen, creatinine, glucose, vitamin B12, and thyroid-stimulating hormone. All results for samples included in this study were within normal limits, including Mini-Mental State Examination scores ≥ 26 and Clinical Dementia Rating scores of 0. Sex was ascertained by self-report. All female subjects of childbearing potential underwent a urine pregnancy test prior to lumbar puncture; no subject had a positive pregnancy test.

Key exclusion criteria

Participant signs or symptoms suggesting cognitive decline or neurologic disease; history of chronic major psychiatric disorder or neurocognitive disorder; unstable medical illness; regular tobacco use; substance use or heavy alcohol use; or use of medications including antidepressants, anxiolytics, antipsychotics, hormonal contraceptives, and other medications known to affect hypothalamic-pituitary-adrenal axis function were excluded.

Ethics Approval

The study was approved by the Human Subjects Committee/Institutional Review Boards of all participating institutions, and all participants provided written informed consent prior to any study procedures.

Study Protocol

The present study is a secondary analysis of data from study participants who agreed to data and specimen banking as a part of participation in previously published studies [12, 13]. Briefly, saliva collection, blood draws, and lumbar punctures were performed between 0800 and 1100 hours after an overnight fast, with saliva collected first and lumbar punctures performed last. All samples from a given subject were collected within 30 minutes. Saliva, blood, and CSF were collected as previously described [12, 13].

Biospecimen Stabilization and Storage

Biospecimens were previously collected and placed on wet (saliva, blood) or dry (CSF) ice immediately after collection and transferred to long-term storage at −70 °C prior to analysis [12, 13]. Samples from study sites outside of Seattle were shipped to VA Puget Sound on dry ice; all samples arrived frozen in good condition. The time between collection to assay ranged from 1 month to 3 years for all samples.

Chemicals and Reagents

Cortisol from all samples was measured previously with ImmuChem™ Cortisol Coated Tube RIA Kits according to manufacturer instructions (MP Biomedicals, LLC, Santa Ana, CA). The detection limit was 10 ng/mL (0.01 ng/mL per assay tube), and the intra-assay coefficient of variance was 5.6%. Cortisol-binding globulin (CBG) was measured by radioimmunoassay in a 1:25 dilution of plasma (BioSource Europe S.A., Nivelles, Belgium). The detection limit was 6.25 µg/mL (0.25 µg/mL per assay tube), and the intra-assay coefficient of variance was 2.0%. Free plasma cortisol was estimated from CBG and total plasma cortisol levels by the method of Coolens et al [14]. The validity of this method has been demonstrated under a variety of physiological and pathological conditions that affect total cortisol concentrations.

Data and Statistical Analysis

The correlation among the 4 cortisol compartments was assessed using Pearson correlation coefficients with adjustment for multiple tests using the Benjamini-Hochberg correction. Effect modification in the association between CSF cortisol and the other 3 compartments by age or sex was assessed with linear regression of CSF cortisol (the dependent variable) with cortisol compartment by age or sex interaction terms as the independent variables. Linear regression was used to assess the association between cortisol outcome (the dependent variable) and age, modeled as a 3-degree restricted cubic spline to allow for nonlinear associations between age and cortisol outcome. To determine if the association between cortisol and age differed by sex, a sex-by-age interaction term was added to the model and tested for significance. If there was no age by sex interaction, the difference in cortisol outcome by sex was assessed as a main effect in the regression models described earlier. Hypothesis testing for main effects and interactions was carried out using F-tests. Pairwise comparisons by sex were assessed using simultaneous inference, with *P*-values adjusted for multiple comparisons using the single-step method. Results are presented as mean cortisol and SE and mean differences in cortisol by sex, with SEs and 95% confidence intervals (CI) at selected ages. R^2 was computed for the total sample, with men and women in separate models

Table 1. Participant demographics and clinical characteristics

	F (n = 72)	M (n = 85)	Total (n = 157)	*P*-value[a]
Age at LP				.47
Mean (SD)	51.4 (19.3)	49.1 (21.1)	50.2 (20.3)	
Range	20.0-85.0	21.0-88.0	20.0-88.0	
Race (%)				.30
White	63 (88)	76 (89)	139 (89)	
Black	6 (8)	2 (2)	8 (5)	
Asian or Pacific Islander	2 (3)	4 (5)	6 (4)	
Other	1 (1)	3 (4)	4 (3)	
Ethnicity (%)				.68
Hispanic	3 (4)	3 (4)	6 (4)	
Not Hispanic	69 (96)	82 (96)	151 (96)	
BMI				.083
Mean (SD)	25.0 (4.4)	26.1 (3.2)	25.6 (3.8)	
Range	18.0-41.0	20.0-36.0	18.0-41.0	
Education, years				.12
Mean (SD)	15.9 (2.3)	16.6 (2.7)	16.3 (2.5)	
Range	12.0-22.0	10.0-27.0	10.0-27.0	
College graduate (%)	44 (61)	63 (74)	107 (68)	.089

Abbreviation: BMI, body mass index; LP, lumbar puncture.
[a]Based on the 2-sample *t*-test for continuous variables, Fisher's exact test for categorical variables.

of cortisol outcome on age, with bootstrapped CI. Finally, linear regression was used to assess the association between CSF or saliva cortisol (the dependent variable) and plasma CBG and whether this association was modified by sex or age using interaction terms. Sensitivity analyses were carried out using log transformations for cortisol outcomes that were strongly nonnormal. Analyses were carried out using R 4.2.1 and packages tidyverse, rms, emmeans, multcomp, GGally, and boot [15].

Results

Study Population

The study population was comprised of 157 healthy volunteers, aged 20-88 years, 54% male, and 88.5% White. Study visits took place between October 2001 and August 2004. A summary of demographic characteristics of participants is presented in Table 1.

Correlation Among 4 Compartments

All correlations among peripheral compartments were positive and approximately linear (Fig. 1A, $P < .001$). The correlation coefficient between CSF and saliva was weakest ($r = 0.28$) while the other correlations were 0.41 or greater. Focusing on the association between CSF cortisol and the other compartments, age was not a significant modifier of these relationships (age by cortisol compartment interaction $P \geq .28$, data not shown). There was some evidence of effect modification due to sex, with the association between CSF cortisol and saliva or free plasma being slightly stronger for females than males (Fig. 1B, sex by cortisol compartment interaction $P \leq .056$).

Age-related Changes in Cortisol Levels by Compartment and Sex

CSF cortisol generally increased with age ($R^2 = 0.13$, 95% CI [0.05, 0.23], $P < .001$). Sex was a significant modifier of the relationship between CSF cortisol and age (sex-by-age interaction $P = .010$); with the age-related increase in CSF cortisol being due entirely to that seen in men ($R^2 = 0.31$, 95% CI [0.15, 0.45], $P < .001$); an age-related increase in CSF cortisol was not observed in women ($R^2 = 0.01$, 95% CI [0.00, 0.12], $P = .67$) (Fig. 2A). Consequently, men had mean CSF cortisol levels higher by 14 ± 5 µg/dL than women at age 70 (95% CI [2, 26], $P = .012$), with differences of 7 µg/dL or less at ages 50 and 30 ($P \geq .39$).

In contrast to CSF cortisol, saliva cortisol decreased slightly with age ($R^2 = 0.05$, 95% CI [0.00, 0.12], $P = .021$) with this decrease more apparent for women ($R^2 = 0.10$, 95% CI [0.01, 0.24], $P = .049$) than men ($R^2 = 0.03$, 95% CI [0.00, 0.17], $P = .27$, Fig. 2B). However, sex was not a significant effect modifier for the relationship between age and saliva cortisol (sex-by-age interaction $P = .34$). Similar to the CSF results, men had higher mean saliva cortisol levels than women at age 70 (22 ± 8 µg/dL, 95% CI [2, 43], $P = .026$) but mean differences of 8 µg/dL at ages 30 and 50 ($P \geq .72$). As models of saliva cortisol produced heterogeneous errors due to data skewness, sensitivity analyses using log-transformed saliva cortisol as the dependent variable were carried out, with similar findings (data not shown).

No correlation was found between free or total plasma cortisol and age ($R^2 \leq 0.02$, $P \geq .28$, Fig. 2C and 2D, Supplementary Table S1) [16]. Sex was not found to be a significant modifier in the relationship between free plasma or total plasma cortisol and age (age by sex interaction $P \geq .13$). However, free plasma cortisol, averaged across age, was higher in men than women by 17 ± 5 µg/dL ($P < .001$).

Mean cortisol levels by compartment, sex, and age, as well as reference ranges by compartment are reported in Supplementary

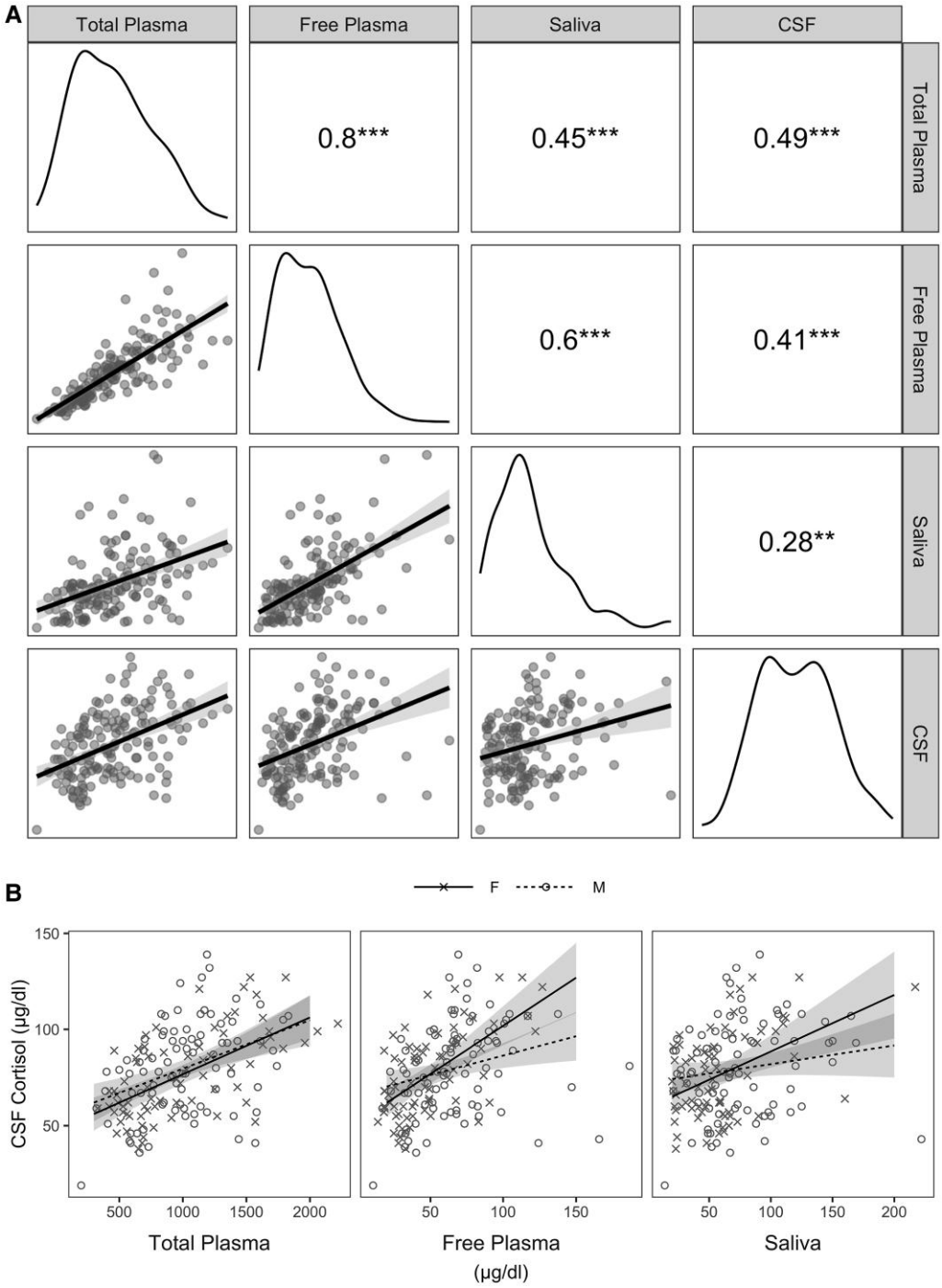

Figure 1. (A) Matrix plot of correlations by compartment. Lower diagonal panels show scatter plots of cortisol levels with linear regression fits and 95% confidence intervals. Upper diagonal panels show Pearson correlation coefficients: ***$P < .0001$, **$P < .001$. Diagonal panels show density plots of each measure. (B) Correlation between CSF and peripheral cortisol compartment (total plasma, free plasma, or saliva) by sex. Fitted lines shown from regression models of CSF on cortisol compartment by sex interaction.

Abbreviation: CSF, cerebral spinal fluid.

Table S1 [16]. Raw cortisol levels in each compartment are shown for each participant in Supplementary Fig. S1 [16].

Plasma CBG and Cortisol in Other Compartments

Because most cortisol in plasma is bound to CBG, plasma CBG levels could theoretically impact cortisol dynamics between plasma and other compartments. To assess the influence of plasma CBG levels on cortisol in other compartments, we examined the correlation between plasma CBG levels and other compartments (Fig. 3A). The correlation between plasma CBG and saliva cortisol was negative and weak ($R^2 = 0.03$, $P = .036$). An even weaker correlation was found between plasma CBG and CSF cortisol ($R^2 = 0.01$, $P = .18$). Furthermore, no significant sex modification or age modification ($P \geq .26$, data not shown) was seen for either relationship.

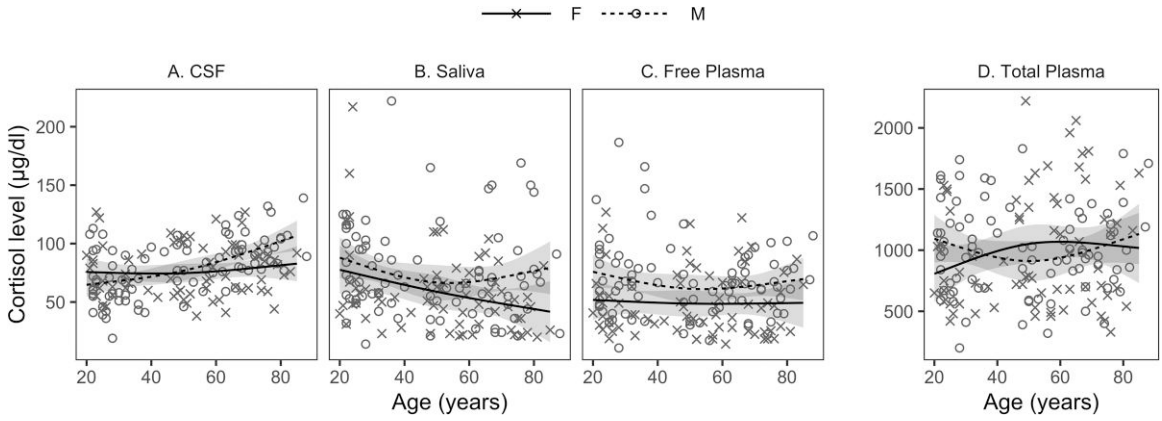

Figure 2. Age-related changes in cortisol levels by sex measured in cerebral spinal fluid, saliva, and free and total plasma. Fitted lines are shown from regression models of cortisol outcome on age, sex, and age by sex interaction with age modeled as a 3-degree restricted cubic spline, with shaded bands indicating 95% confidence intervals.

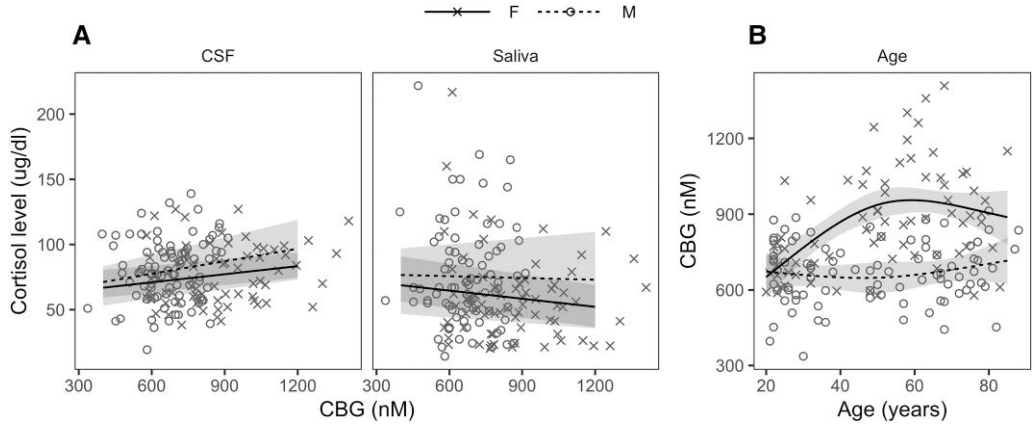

Figure 3. Associations with CBG. (A) Cerebral spinal fluid and saliva cortisol levels by CBG and sex. Fitted lines are shown from regression models of cortisol outcome on age, sex, and age-by-sex interaction with age modeled as a 3-degree restricted cubic spline, with shaded bands indicating 95% confidence intervals. (B) CBG levels by age and sex with fitted lines from regression models of CBG on age and sex. Fitted lines are shown from regression models of cortisol outcome on age, sex, and age by sex interaction with age modeled as a 3-degree restricted cubic spline, with shaded bands indicating 95% confidence intervals.

Abbreviation: CBG, cortisol binding globulin.

The association between plasma CBG and age differed by sex (age by sex interaction $P < .001$, Fig. 3B), with age-related increases in CBG seen in women ($R^2 = 0.25$, $P < .001$) but not in men ($R^2 = 0.02$, $P = .56$). Furthermore, the association between CBG and age in women was not linear, with CBG levels plateauing after age 50. Consequently, CBG was found to be higher in women than men, especially after age 50 ($P < .001$). While plasma cortisol was calculated directly from measured total plasma cortisol and CBG, the higher levels of free plasma cortisol seen in men vs women was independent of CBG levels. In a regression model of free plasma on sex, with CBG as a covariate, the higher levels of free plasma cortisol in men vs women were maintained. Furthermore, in regression models of CSF or saliva cortisol in women on age with CBG as a covariate, the association between either cortisol compartment and age was unchanged, suggesting that age-related trends in CBG in women did not influence age-related trends in other compartments (data not shown).

Discussion

This study is the first to our knowledge to examine the relationships in cortisol concentrations simultaneously among total and free plasma, saliva, and CSF compartments in a healthy adult population of both sexes across the adult lifespan. It provides important insights on how cortisol levels from different compartments are intercorrelated as well as how cortisol concentrations vary with biological characteristics of age and sex. A small handful of previous studies have examined the relationships between CSF and plasma or serum cortisol, although previous studies were limited either by small sample size [10] or study populations with narrow generalizability, including 1 sex only [8], older adults only [9-11], or participants with comorbid health conditions [8, 9, 11, 17]. Here we discuss our findings in the context of existing literature.

CSF Cortisol Is Moderately Correlated With Peripheral Cortisol Measures

In the present study, we found a moderate positive correlation between CSF and plasma cortisol across the adult lifespan, whereas the correlation between CSF and saliva cortisol was weaker. The correlations did not vary by age but did vary by sex, with positive correlations between CSF cortisol and

plasma or saliva cortisol that were stronger in women than in men. These findings demonstrate the importance of considering the level of correlation and the role that sex and age differences play in the levels of correlation across compartments when using either plasma or salivary cortisol as a surrogate for CSF cortisol.

The Relationships Among Cortisol, Age, and Sex Differ Across Compartments

CSF

This study is the first to our knowledge to report CSF cortisol levels in healthy adults of both sexes and an age range spanning 7 decades. Previous studies of participants undergoing treatment for various medical conditions have suggested that CSF cortisol increases with age [8, 9, 17]; however, age-related CSF cortisol trends in healthy individuals were previously unknown and the effect of sex was not considered in the analyses. In the present study, we found that CSF cortisol was positively correlated with increasing age in men but not in women, with men having higher CSF cortisol than women at older but not younger ages. Given that there are no comparable studies of CSF cortisol across age and sex in healthy individuals, these previous studies merit mention to provide context but cannot be directly compared to results of the present study.

Saliva

There is no clear consensus when it comes to the effects of age and sex on saliva cortisol in healthy adults. Findings in the present study demonstrating decreased saliva cortisol with increased age conflict with 1 large study resulting from the Midlife Development in the United States survey dataset demonstrating that saliva cortisol increases with age [18, 19], another study revealing age-related changes by decade without a clear directional trend [20], and another that found no age-related changes in saliva cortisol [21]. Some of these discrepancies may be explained by the timing of saliva sampling, as discussed further later. Though in the present study sex was not a significant modifier of age-related saliva cortisol trends, we did find that saliva cortisol was generally higher in men than women across age, in agreement with the Midlife Development in the United States study [18, 19], with differences that were more apparent at older ages. In contrast, Nyberg et al reported higher average saliva cortisol in women than men when stratifying by sex [20] but did not specifically explore the modifying effect of sex on the association between cortisol and age as illustrated in our study. Furthermore, the remote tribal population examined by Nyberg and colleagues is likely not generalizable but rather what the authors frame as "a benchmark against which to reference cortisol levels from industrialized populations" [20].

Plasma

Similarly, there is no clear consensus when it comes to the effects of age and sex on plasma cortisol in healthy adults [17, 22-25]. We offer 2 of many possible explanations for these discrepancies. First, 1 study that sampled 24-hour mean total plasma cortisol found that age-related increases in total plasma cortisol were only evident in late-evening cortisol levels and not at other times of the day [24], demonstrating that diurnal fluctuations in cortisol have particularly large effects on cortisol levels measured in plasma [26] and perhaps 1 plausible explanation for why we did not observe age-related increases in plasma cortisol in the present study. Second, sex effects on plasma cortisol trends likely differ depending on whether free or total plasma cortisol is examined due to sex differences in CBG, as described later. Pooled 24-hour mean total plasma cortisol data from 7 independent research groups demonstrated an increase in total plasma cortisol with age in both sexes, with a near-statistically significant age by sex interaction with larger increases with age seen in women than men [25]. In the present study, we found that men exhibit higher plasma cortisol than women across the adult lifespan, especially at older ages; however, sex differences were only evident in measurements of free but not total plasma cortisol.

Plasma CBG Level Differs by Sex But Has a Negligible Effect on Cortisol Levels in Other Compartments

The effects of biological and demographic factors on plasma CBG we saw in the present study were complex, with sex differences playing a clear role likely due to the hepatic stimulation of CBG synthesis by estrogens as previously reviewed [27]. Given that most plasma cortisol is bound to CBG and only unbound cortisol is free to diffuse across capillary boundaries [27], we hypothesized that plasma CBG levels may indirectly affect cortisol levels in saliva and CSF by influencing free plasma cortisol levels available for diffusion or transport to other compartments. In the present study, we observed an increase in plasma CBG with age in women, resulting in lower free plasma cortisol in women compared to men. However, plasma CBG was not strongly correlated with either CSF or saliva cortisol. While plasma CBG may play an important role in the regulation of biologically active cortisol in blood, our findings suggest that its role in cortisol regulation across other compartments may be negligible.

Importance of Sample Collection Timing

In summary, the relationships between cortisol across compartments and demographic factors are complex, and existing data are often conflicting and challenging to compare across studies. Some of these inconsistencies may be due to a lack of standardization in the timing of cortisol collection. Cortisol levels fluctuate widely throughout the course of a day and are highly responsive to sample collection time relative to an individual's sleep-wake cycle. Given the diurnal cortisol rhythm, the timing of sample collection likely accounts for much of the widely variable results seen from study to study and even variability within the same study. While the present study examined samples collected midmorning only as did several other studies cited earlier [9, 21, 28], others examined samples collected in the afternoon [8] or at multiple timepoints throughout the day [10, 18-20, 24-26, 29] or did not specify the time of sample collection [17].

Diurnal cortisol fluctuations appear to heavily influence saliva cortisol [30] and plasma cortisol [26], whereas CSF cortisol appears to maintain more stable levels throughout the day [31]. Notably, the most consistent trends in cortisol were seen in the plasma cortisol studies done using evening sample collection [25, 28], likely due to the cortisol awakening response and the midmorning acrophase contributing to steep cortisol slopes where small differences in the timing of samples can result in high variability of morning cortisol concentrations.

Although diurnal fluctuations in CSF cortisol are generally considered small, especially compared to peripheral cortisol, to our knowledge this has not been previously systematically studied. Saliva may be uniquely suited for standardized collection times given the ability to self-collect saliva samples at home, improving the accuracy of sample collection relative to each participant's diurnal rhythm.

Following the dates of our sample collection, substantial efforts to move toward standardized collection guidelines have been made, such as those outlined by the International Society of Psychoneuroendocrinology for the cortisol awakening response [30]. Such standardized collection times will facilitate cross-study comparison. Whether these standards can be feasibly applied in sample collection that requires in-person study visits such as for CSF or blood collection remains to be seen.

Cortisol Is Differentially Regulated Across Compartments and Further Research Is Needed to Elucidate Our Mechanistic Understanding of Cross-compartment Cortisol Dynamics

The present study focused on observational correlations of cortisol across compartments and the effect of basic demographic and biological factors on these relationships. While mechanistic explanations for our findings are beyond the scope of our study, several areas of cortisol regulation merit mention. Our findings corroborate previous conclusions that plasma CBG likely plays a relatively small role in the regulation of cortisol dynamics across compartments. Another study suggests that protein transporters may also play a limited role in the transport of glucocorticoids from the bloodstream to other compartments, though efflux pumps appear to play a significant role in the protection of certain targets (such as the brain) from high plasma cortisol levels [5]. In fact, enzymes appear to play a more dominant role in cortisol regulation by regulation of cortisol metabolism and homeostasis [5, 6, 27]. One example is the corticosteroid 11β-dehydrogenase-mediated conversion of cortisol to cortisone, rendering cortisol partially inactive [27]. With high expression in parotid glands [27] and many parts of the CNS [32], 11β-dehydrogenase has a marked influence on the dynamic regulation of saliva and CSF cortisol worth exploring in future studies.

Assuming free diffusion of cortisol across compartments to justify the use of cortisol measures from an easily accessible compartment, such as saliva, to approximate cortisol levels in another is tempting but can lead to inaccurate assumptions. Here we have demonstrated that in practice this is a poor approximation, with a moderate correlation between CSF and plasma cortisol and a weak correlation between CSF and saliva cortisol. However, this does not necessarily invalidate cortisol measures in peripheral compartments in the investigation of diseases of the CNS. Caution should be exercised when interpreting the clinical significance of peripheral cortisol measures in the context of CNS disorders, insofar as the direct effects of cortisol on CNS pathology. This nuance underscores an unmet need to move from an organ-level conceptualization of disease toward a systems-level understanding of pathophysiology. Rather than viewing cortisol levels in 1 compartment as a reflection of cortisol in another compartment, and arriving at conclusions based on these assumptions, studies must focus on investigating the regulation and roles of cortisol in each compartment in the context of disease pathophysiology. Further research is necessary to fully elucidate our understanding of cortisol regulation across central and peripheral compartments.

Sex was a noteworthy covariate across the analyses carried out for this study, with sex being a significant modifier in the relationships between CSF and peripheral cortisol levels, the relationship between CSF cortisol and age, and the relationship between CBG levels and age. Future studies should aim to further clarify how the relationships between CSF and peripheral cortisol differ in men vs women. Evidence of sex differences in stress exposure is mounting, with rodent models consistently demonstrating an increased susceptibility to chronic stress in males compared to females [33] and a recent longitudinal study of cognitively normal older adult humans in which higher perceived stress was found to be associated with steeper rates of cognitive decline in men compared to women [34]. While the protective effect of estrogens has been proposed as a leading hypothesis to explain this sex difference, the sex differences in CSF cortisol seen in advancing age suggest that the role of glucocorticoid homeostasis may be important to continue exploring in this context as well. Investigation of polymorphisms in cortisol regulatory genes and posttranscriptional changes in gene expression may lend insight into the mechanisms behind the influence of demographic factors on cortisol regulation. Given the complex relationships among sex and other environmental influences in the context of cortisol, future study populations should be expanded to include participants of diverse racial, socioeconomic, and educational backgrounds to ensure adequate power when examining the influences of environmental factors. More work should be done to increase our understanding of how biological factors influence the response to various stress exposure paradigms.

Finally, advanced analytical techniques such as mass spectrometry would yield more precise measurements of cortisol concentrations than the antibody-based assays employed in the present study.

In summary, our study demonstrated that cortisol levels do not just vary by compartment—they also vary by biological characteristics of age and sex. These findings highlight the importance of careful study design in understanding the mixed findings in the literature between cortisol concentrations and functional changes.

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Disclosures

The authors have nothing to disclose.

Data Availability

Datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

References

1. Mousten IV, Sørensen NV, Christensen RHB, Benros ME. Cerebrospinal fluid biomarkers in patients with unipolar depression compared with healthy control individuals: a systematic review and meta-analysis. *JAMA Psychiatry*. 2022;79(6):571-581.
2. Herbert J. Cortisol and depression: three questions for psychiatry. *Psychol Med*. 2013;43(3):449-469.
3. Ouanes S, Popp J. High cortisol and the risk of dementia and Alzheimer's disease: a review of the literature. *Front Aging Neurosci*. 2019;11:43.
4. Lewis JG, Bagley CJ, Elder PA, Bachmann AW, Torpy DJ. Plasma free cortisol fraction reflects levels of functioning corticosteroid-binding globulin. *Clin Chim Acta*. 2005;359(1-2):189-194.
5. Devine K, Villalobos E, Kyle CJ, *et al.* The ATP-binding cassette proteins ABCB1 and ABCC1 as modulators of glucocorticoid action. *Nat Rev Endocrinol*. 2022;19(2):112-124.
6. Mason BL, Pariante CM, Jamel S, Thomas SA. Central nervous system (CNS) delivery of glucocorticoids is fine-tuned by saturable transporters at the blood-CNS barriers and nonbarrier regions. *Endocrinology*. 2010;151(11):5294-5305.
7. Wilkinson C, Li G, Colasurdo EA, Shofer JB, Peskind ER. Correlations among simultaneous basal measurements of human cerebrospinal fluid, saliva, and total and free plasma cortisol as a function of age and Alzheimer's disease/mild cognitive impairment. Gerontological Society of America Annual Scientific Meeting. 21 Nov 2013, New Orleans, LA.
8. Murakami K, Nakagawa T, Shozu M, Uchide K, Koike K, Inoue M. Changes with aging of steroidal levels in the cerebrospinal fluid of women. *Maturitas*. 1999;33(1):71-80.
9. Johansson P, Johansson JO, Labrie F, *et al.* Mild dementia is associated with increased adrenal secretion of cortisol and precursor sex steroids in women. *Clin Endocrinol (Oxf)*. 2011;75(3):301-308.
10. Triana-Baltzer G, Timmers M, De Boer P, *et al.* Profiling classical neuropsychiatric biomarkers across biological fluids and following continuous lumbar puncture: a guide to sample type and time. *Compr Psychoneuroendocrinol*. 2022;10:100116.
11. Popp J, Wolfsgruber S, Heuser I, *et al.* Cerebrospinal fluid cortisol and clinical disease progression in MCI and dementia of Alzheimer's type. *Neurobiol Aging*. 2015;36(2):601-607.
12. Peskind ER, Li G, Shofer J, *et al.* Age and apolipoprotein E*4 allele effects on cerebrospinal fluid beta-amyloid 42 in adults with normal cognition. *Arch Neurol*. 2006;63(7):936-939.
13. Li G, Cherrier MM, Tsuang DW, *et al.* Salivary cortisol and memory function in human aging. *Neurobiol Aging*. 2006;27(11):1705-1714.
14. Coolens JL, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem*. 1987;26(2):197-202.
15. R: a language and environment for statistical computing. In: Team RC, ed. *R.4.2.1*. R Foundation for Statistical Computing; 2022.
16. Supplemental material placeholder.
17. Guazzo EP, Kirkpatrick PJ, Goodyer IM, Shiers HM, Herbert J. Cortisol, dehydroepiandrosterone (DHEA), and DHEA sulfate in the cerebrospinal fluid of man: relation to blood levels and the effects of age. *J Clin Endocrinol Metab*. 1996;81(11):3951-3960.
18. Karlamangla AS, Friedman EM, Seeman TE, Stawksi RS, Almeida DM. Daytime trajectories of cortisol: demographic and socioeconomic differences–findings from the national study of daily experiences. *Psychoneuroendocrinology*. 2013;38(11):2585-2597.
19. Piazza JR, Charles ST, Stawski RS, Almeida DM. Age and the association between negative affective states and diurnal cortisol. *Psychol Aging*. 2013;28(1):47-56.
20. Nyberg CH. Diurnal cortisol rhythms in Tsimane' Amazonian foragers: new insights into ecological HPA axis research. *Psychoneuroendocrinology*. 2012;37(2):178-190.
21. Pruessner JC, Wolf OT, Hellhammer DH, *et al.* Free cortisol levels after awakening: a reliable biological marker for the assessment of adrenocortical activity. *Life Sci*. 1997;61(26):2539-2549.
22. Tingelstad HC, Filion LG, Martin J, Spivock M, Tang V, Haman F. Levels of circulating cortisol and cytokines in members of the Canadian Armed Forces: associations with age, sex, and anthropometry. *Appl Physiol Nutr Metab*. 2018;43(5):445-452.
23. Halbreich U, Asnis GM, Zumoff B, Nathan RS, Shindledecker R. Effect of age and sex on cortisol secretion in depressives and normals. *Psychiatry Res*. 1984;13(3):221-229.
24. Deuschle M, Gotthardt U, Schweiger U, *et al.* With aging in humans the activity of the hypothalamus-pituitary-adrenal system increases and its diurnal amplitude flattens. *Life Sci*. 1997;61(22):2239-2246.
25. Van Cauter E, Leproult R, Kupfer DJ. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. *J Clin Endocrinol Metab*. 1996;81(7):2468-2473.
26. Upton TJ, Zavala E, Methlie P, *et al.* High-resolution daily profiles of tissue adrenal steroids by portable automated collection. *Sci Transl Med*. 2023;15(701):eadg8464.
27. Perogamvros I, Ray DW, Trainer PJ. Regulation of cortisol bioavailability–effects on hormone measurement and action. *Nat Rev Endocrinol*. 2012;8(12):717-727.
28. Laughlin GA, Barrett-Connor E. Sexual dimorphism in the influence of advanced aging on adrenal hormone levels: the Rancho Bernardo study. *J Clin Endocrinol Metab*. 2000;85(10):3561-3568.
29. Magri F, Locatelli M, Balza G, *et al.* Changes in endocrine circadian rhythms as markers of physiological and pathological brain aging. *Chronobiol Int*. 1997;14(4):385-396.
30. Stalder T, Kirschbaum C, Kudielka BM, *et al.* Assessment of the cortisol awakening response: expert consensus guidelines. *Psychoneuroendocrinology*. 2016;63:414-432.
31. Leverenz JB, Wilkinson CW, Wamble M, *et al.* Effect of chronic high-dose exogenous cortisol on hippocampal neuronal number in aged nonhuman primates. *J Neurosci*. 1999;19(6):2356-2361.
32. Bini J, Bhatt S, Hillmer AT, *et al.* Body mass Index and age effects on brain 11β-hydroxysteroid dehydrogenase type 1: a positron emission tomography study. *Mol Imaging Biol*. 2020;22(4):1124-1131.
33. Luine V, Gomez J, Beck K, Bowman R. Sex differences in chronic stress effects on cognition in rodents. *Pharmacol Biochem Behav*. 2017;152:13-19.
34. Paolillo EW, You M, Gontrum E, *et al.* Sex differences in the relationship between perceived stress and cognitive trajectories. *Am J Geriatr Psychiatry*. 2023;31(6):401-410.