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Characterization of Cortisol Secretion Rate in Secondary Adrenal Insufficiency

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Context: In secondary adrenal insufficiency (SAI), chronic deficiency of adrenocorticotropin (ACTH) is believed to result in secondary changes in adrenocortical function, causing an altered dose-response relationship between ACTH concentration and cortisol secretion rate (CSR).

Objective: We sought to characterize maximal cortisol secretion rate (CSR_{max}) and free cortisol half-life in patients with SAI, compare results with those of age-matched healthy controls, and examine the influence of predictor variables on ACTH-stimulated cortisol concentrations.

Design: CSR_{max} was estimated from $ACTH_{1\text{-}24}$ (250 μg)–stimulated cortisol time-concentration data. Estimates for CSR_{max} and free cortisol half-life were obtained for both dexamethasone (DEX) and placebo pretreatment conditions for all subjects.

Setting: Single academic medical center.

Patients: Patients with SAI (n = 10) compared with age-matched healthy controls (n = 21).

Interventions: The order of DEX vs placebo pretreatment was randomized and double-blind. Cortisol concentrations were obtained at baseline and at intervals for 120 minutes after $ACTH_{1\text{-}24}$.

Main Outcome Measures: CSR_{max} and free cortisol half-life were obtained by numerical modeling analysis. Predictors of stimulated cortisol concentrations were evaluated using a multivariate model.

Results: CSR_{max} was significantly ($P < 0.001$) reduced in patients with SAI compared with controls for both placebo (0.17 ± 0.09 vs 0.46 ± 0.14 nM/s) and DEX (0.18 ± 0.13 vs 0.43 ± 0.13 nM/s) conditions. Significant predictors of $ACTH_{1\text{-}24}$–stimulated total cortisol concentrations included CSR_{max}, free cortisol half-life, and baseline total cortisol, corticosteroid-binding globulin, and albumin concentrations (all $P < 0.05$).

Conclusions: Our finding of significantly decreased CSR_{max} confirms that SAI is associated with alterations in the CSR-ACTH dose-response curve. Decreased CSR_{max} contributes importantly to the laboratory diagnosis of SAI.

Freeform/Key Words: hydrocortisone, numerical analysis, computer-assisted, cosyntropin, adrenal insufficiency, metabolic clearance rate, multivariable analysis

Abbreviations: ACTH, adrenocorticotropin; AI, adrenal insufficiency; CBG, corticosteroid-binding globulin; CPR, cortisol production rate; CSR, cortisol secretion rate; CSR_{max}, maximal cortisol secretion rate; DEX, dexamethasone; SAI, secondary adrenal insufficiency; SD, standard deviation.

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Secondary adrenal insufficiency (SAI) is a common clinical condition related to relative or absolute deficiency of adrenocorticotropin (ACTH) [1]. SAI may coexist with other anterior pituitary hormone deficiencies in the setting of structural pituitary disease [1, 2], whereas isolated ACTH deficiency often occurs following administration of exogenous glucocorticoids or successful cure of endogenous Cushing syndrome [3–5]. The term *tertiary adrenal insufficiency* is also used in the literature in reference to forms of ACTH deficiency resulting from exogenous glucocorticoids or following cure of Cushing syndrome. Clinical manifestations of both secondary and tertiary adrenal insufficiency (AI) are typically related to glucocorticoid deficiency in association with subnormal serum concentrations of total and free cortisol. Gold standard laboratory diagnostic tests for SAI include metyrapone and insulin tolerance tests, for which integrated activation of the hypothalamic-pituitary-adrenal axis is necessary for generation of a cortisol secretory response. Component tests for SAI, such as high- and low-dose cosyntropin ($ACTH_{1\text{-}24}$) stimulation tests, assess only the adrenal cortisol response to exogenous ACTH stimulation but are commonly used in clinical practice because of superior clinical utility and validated, albeit imperfect, diagnostic performance [1, 6, 7].

Two related but distinct mechanisms contribute to the pathophysiology of cortisol deficiency in SAI. The first is related to the decreased feed-forward drive of ACTH-dependent cortisol secretion. A nonlinear (sigmoidal) dose-response curve characterizes the feed-forward relationship between ACTH concentration and cortisol secretion rate (CSR) [8–10]. This relationship predicts that lower ACTH concentrations will be associated with lower CSR and, consequently, lower concentrations of total and free cortisol. A second mechanism contributing to cortisol deficiency in SAI reflects an acquired change in adrenocortical function such that at any given ACTH concentration, CSR is decreased in patients with SAI compared with healthy controls [1, 4, 11, 12].

Although the adrenal gland is intrinsically normal in SAI, chronic ACTH deficiency appears to result in secondary changes in adrenocortical function that include diminished response to endogenous and/or exogenous ACTH [1, 4, 11, 12]. This acquired diminution in cortisol secretory response to ACTH depends upon the duration and severity of ACTH deficiency [3–5] and is also reversible following normalization of ACTH concentration over an intermediate time frame [11, 12]. This principle is intuitive to the practicing endocrinologist because it is the rationale for assessing the cortisol response to exogenous ACTH in the "short" $ACTH_{1\text{-}24}$ stimulation test commonly performed in the laboratory evaluation of SAI. These considerations suggest that chronic ACTH deficiency results in a reversible shift in the CSR-ACTH dose-response relationship, including diminished CSR at maximal concentrations of ACTH.

Despite extensive literature consistently demonstrating subnormal cortisol concentrations at comparable concentrations of ACTH in SAI [1, 4, 6, 7], there is a paucity of quantitative data demonstrating and characterizing abnormalities in cortisol secretion or production rates in SAI (for additional clarification of cortisol secretion and production rates, see the Supplemental Data). For example, Paisley *et al.* [13] measured cortisol production rate (CPR) using the stable isotope dilution method in 10 patients with SAI. Although controls were not included in this study, they reported that the distribution of 24-hour CPRs in SAI patients fell within the reported reference range for healthy control subjects. In this context, it is important to distinguish between cortisol *production rate* and *concentration* because the relationship between CPR and cortisol concentration is nonlinear and time dependent [8–10, 14]. Even under steady-state conditions, the relationship between CPR and total cortisol concentration is affected by other variables, including cortisol clearance rate, distribution volume, and corticosteroid-binding globulin (CBG) and albumin concentrations [15, 16].

Stable isotope dilution methods are considered the gold standard for determination of rates of cortisol production and clearance. However, use of this methodology is restricted to a research setting owing to requirements for infusion of stable isotope-labeled cortisol, specialized laboratory analytical tools, and steady-state conditions [17–20]. An alternative approach uses numerical modeling and analysis to obtain rates of free cortisol appearance (secretion) and elimination under nonsteady-state conditions (see Supplemental Data) [8–10,

14]. In the current study, we sought to apply this numerical analytic methodology to characterize and compare the CSR in patients with chronic SAI with that of age-matched controls under conditions of maximal ACTH stimulation. We hypothesized that maximal cortisol secretion rates (CSR_{max}) are significantly decreased in patients with chronic SAI compared with age-matched healthy controls. In addition, because cosyntropin stimulation tests are commonly used in the laboratory diagnosis of SAI, a second objective of this study was to evaluate the relative influence of various predictor variables on stimulated cortisol concentrations. We hypothesized that CSR_{max} would prove to be the strongest predictor of stimulated cortisol concentrations in both controls and patients with SAI.

1. Materials and Methods

A. Study Participants

This prospective study was conducted at the University of New Mexico and was approved by the university's Human Research Review Committee. The study was conducted in accordance with the principles described in the Declaration of Helsinki, and all subjects provided written informed consent before participation. The results for CSR_{max} and free cortisol half-life estimates in healthy control subjects (n = 21) were previously reported [8]. Subjects with CAI (n = 10) were recruited concurrently with control subjects. All patients with SAI had an established clinical diagnosis of chronic SAI; they included patients with tertiary AI due to exogenous prednisone therapy for treatment of nonendocrine conditions (n = 5) and patients with an established diagnosis of hypopituitarism with multiple anterior pituitary hormone deficiencies following surgical resection of pituitary macroadenoma (n = 5). Exclusion criteria included age <18 years or >75 years, pregnancy, uncontrolled type 2 diabetes mellitus, alcohol or drug dependence, body mass index >35 kg/m^2, untreated hypothyroidism, congestive heart failure, angina, liver failure, renal failure, regular narcotic administration, acute SAI, or total cortisol concentration >550 nmol/L obtained 60 minutes after $ACTH_{1\text{-}24}$. Additional information regarding replacement therapy for patients with hypopituitarism and tertiary AI is included in Supplemental Table 1.

B. Study Protocol and Laboratory Investigations

Subjects were pretreated in double-blind fashion and randomized order with either 1 mg of DEX or placebo at 2300 hours as previously described [8]. The median time interval between DEX and placebo studies was 14 days (interquartile range: 14, 21 days). Usual glucocorticoid replacement was withheld after 1400 hours. At 0800 hours on the following morning, fasting baseline samples were obtained for free and total cortisol, CBG, and albumin concentrations followed by intravenous administration of 250 μg of cosyntropin ($ACTH_{1\text{-}24}$). Total cortisol level was sampled at 5, 10, 15, 20, 30, 45, 60, and 120 minutes after $ACTH_{1\text{-}24}$. Free cortisol concentrations were measured at 0 (baseline) and 60 minutes after $ACTH_{1\text{-}24}$.

C. Assay Methods

Total serum cortisol level was measured using a chemiluminescent immunoassay (Immulite 1000; Siemens Healthcare Diagnostics, Deerfield, IL), with an interassay coefficient of variation of 7.9%. Plasma free cortisol concentration was measured by equilibrium dialysis followed by liquid chromatography tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA), with assay characteristics as previously reported [8]. CBG and albumin assay methods and characteristics were as previously described [8].

D. Numerical Estimation of CSR_{max} and Free Cortisol Half-life

A schematic representation and overview of the compartmental cortisol model used for numerical solution of cortisol rate parameters is shown in the Supplemental Data. Additional

definitions and details of the model, differential equations, and solution algorithm were as reported previously [14].

E. Statistical Analysis of Clinical and Numerical Analytic Data

Descriptive statistics included means and standard deviations or medians (interquartile range). Univariate differences between DEX and placebo were analyzed using paired comparison methods (paired *t* test and Wilcoxon signed rank test). Differences between control and SAI groups were analyzed by unpaired comparisons (*t* test and Wilcoxon rank sum test). Multivariable analysis of predictors of $ACTH_{1\text{-}24}$–stimulated total and free cortisol concentrations was by mixed, repeated measures analysis of covariance models. These predictor variables included CSR_{max}, free cortisol half-life, and concentrations of CBG, albumin, and baseline cortisol. An optimal model was obtained by backward elimination of nonsignificant effects. Results are reported as standardized β (STB) and corresponding *P* values. Main analyses were performed using PROC MIXED (SAS 9.4).

2. Results

A. Study Population

Patients with SAI (n = 10) included six females and four males. The mean age of was 52.9 ± 17.3 years. Demographics were similar to those of control subjects (n = 21) [8] with respect to age ($P = 0.27$) and sex balance ($P = 0.69$). Mean body mass index in subjects with SAI was 29.6 ± 6.4 kg/m^2, which was similar to that of control subjects ($P = 0.92$) [8].

B. CBG and Albumin Concentrations

CBG concentrations for patients with SAI were similar ($P = 0.21$) for both placebo (554 ± 267 nM) and DEX (596 ± 138 nM) studies and were not significantly different from those previously reported for our control subjects [8] for both placebo ($P = 0.84$) and DEX ($P = 0.36$) conditions. Although CBG concentrations were generally higher in women (635 ± 312 nM) than in men (433 ± 136 nM), the difference between CBG concentrations by sex was not statistically significant ($P = 0.20$), possibly because of the small sample size. Albumin concentrations in patients with SAI were also similar ($P = 0.83$) for placebo (546 ± 66 μM) and DEX (554 ± 76 μM) studies and were not significantly different from those previously reported for control subjects ($P = 0.29$ for placebo and $P = 0.79$ for DEX conditions) [8].

C. Total Serum Cortisol Concentrations (0 to 120 Minutes After $ACTH_{1\text{-}24}$)

The total cortisol concentration time series response to $ACTH_{1\text{-}24}$ for control and patients with SAI is shown in Fig. 1. Total cortisol concentrations were significantly decreased in patients with SAI compared with controls for all time points and for both placebo (solid line) and DEX (dashed line) pretreatment conditions ($P < 0.001$ for all SAI vs control comparisons). Within both SAI and control subjects, cortisol concentrations were also significantly decreased by DEX at early time points (0 to 30 minutes). In paired analysis, total cortisol increased significantly at each consecutive time point for both SAI and control groups and for both DEX and placebo pretreatment conditions ($P < 0.01$ for all comparisons).

Cortisol concentrations at several clinically relevant time points (*i.e.,* 0, 30, and 60 minutes after $ACTH_{1\text{-}24}$) are illustrated in greater detail in Fig. 2. Total cortisol concentrations were significantly decreased in patients with SAI compared with concentrations in controls ($P < 0.01$ for all three time points and for both placebo and DEX pretreatment conditions). In a subgroup analysis comparing patients with hypopituitarism and tertiary AI, baseline total cortisol concentrations for the placebo pretreatment study were generally lower in patients

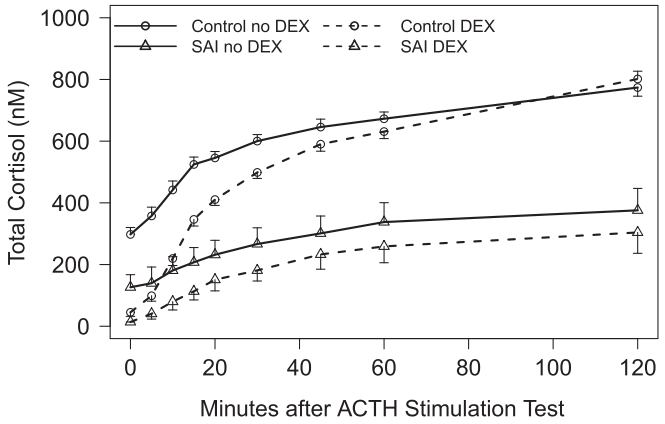

Figure 1. Total cortisol concentrations for SAI (△) and control subjects (○) for both DEX (dashed line) and placebo (solid line) pretreatment conditions at time points 0 to 120 minutes after $ACTH_{1\text{-}24}$ (250 μg). Data are mean ± standard error of the mean.

with hypopituitarism (93 ± 91 nM) than in patients with tertiary AI (159 ± 61 nM); however, this was not statistically significant ($P = 0.45$), possibly because of sample size.

D. Free Serum Cortisol Concentrations at Baseline and 60 Minutes After $ACTH_{1\text{-}24}$

For placebo pretreatment, baseline free cortisol concentrations were significantly reduced ($P = 0.046$) in patients with SAI (5.9 ± 8.7 nM) compared with controls (12.9 ± 7.3 nM). For DEX pretreatment, baseline free cortisol concentrations were similarly suppressed ($P = 0.37$)

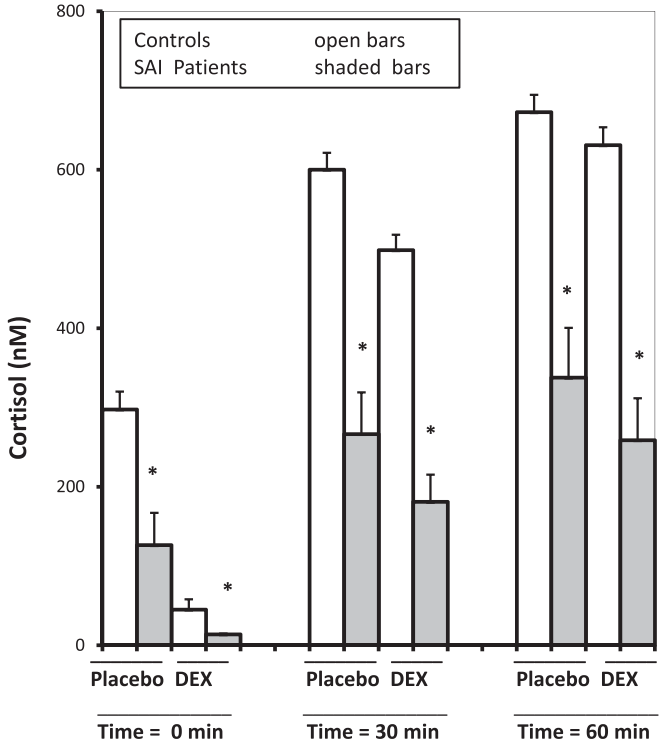

Figure 2. Bar graphs showing mean ± standard error of the mean for total cortisol concentrations for control subjects (open bars) and SAI subjects (shaded bars) for placebo and DEX pretreatment conditions as indicated and at various time intervals (0, 30, 60 minutes) following intravenous administration of $ACTH_{1\text{-}24}$ (250 μg). *$P < 0.05$ for control vs SAI.

for controls (1.9 ± 1.6 nM) and patients with SAI (1.5 ± 0.6 nM). Stimulated free cortisol concentrations 60 minutes after $ACTH_{1\text{-}24}$ were reduced in patients with SAI compared with control subjects for both placebo and DEX pretreatment conditions (both $P < 0.001$). In a subgroup analysis, baseline free cortisol for placebo pretreatment was lower in patients with hypopituitarism (3.4 ± 4.4 nM) than in patients with tertiary AI (8.4 ± 11.7 nM); however, this was not significant ($P = 0.41$), possibly because of sample size.

E. Parameter Solutions Obtained From Numerical Modeling Analysis (CSR_{max} and Free Cortisol Half-life)

CSR_{max} was significantly reduced ($P < 0.001$) in patients with SAI compared with controls [8] for both placebo (0.17 ± 0.09 vs 0.46 ± 0.14 nM/s) and DEX (0.18 ± 0.13 vs 0.43 ± 0.13 nM/s) conditions (see Fig. 3). In our study design, the order of DEX and placebo pretreatment was randomized, and we observed no order effect by which CSR_{max} or free cortisol half-life differed between the initial and subsequent cosyntropin study or in relation to DEX vs placebo pretreatment condition ($P > 0.4$ for all comparisons). As shown in Fig. 4, free cortisol half-life was not significantly different between patients with SAI and controls for either placebo condition (1.7 ± 1.3 vs 2.3 ± 1.3 minutes; $P = 0.19$) or DEX pretreatment (1.4 ± 1.7 vs 2.0 ± 1.0 minutes; $P = 0.28$). In a subgroup analysis comparing AI subjects with hypopituitarism with those with tertiary AI, there were no significant differences in parameter solutions for CSR_{max} ($P = 0.26$) or free cortisol half-life ($P = 0.51$). Similarly, there were no sex differences in parameter solutions for CSR_{max} or free cortisol half-life (both $P > 0.24$).

F. Goodness of Fit (R^2) and Reproducibility (Coefficient of Variation) of CSR_{max} and Free Cortisol Half-life Estimates

R^2 values provide an estimate of goodness of fit between predicted and measured total cortisol concentrations. For subjects with SAI, R^2 values were similar (Wilcoxon $P = 0.35$) for placebo (85.7% ± 18.4%) and DEX (93.8% ± 3.5%) conditions, and were similar to R^2 values obtained in control subjects [8].

G. Multivariable Analysis of Predictors of Stimulated Total and Free Cortisol Concentrations

Results of the multivariable analysis are expressed in STB units and shown in Table 1. STB can be a regression coefficient, or effect size, that has been standardized to be unitless. This

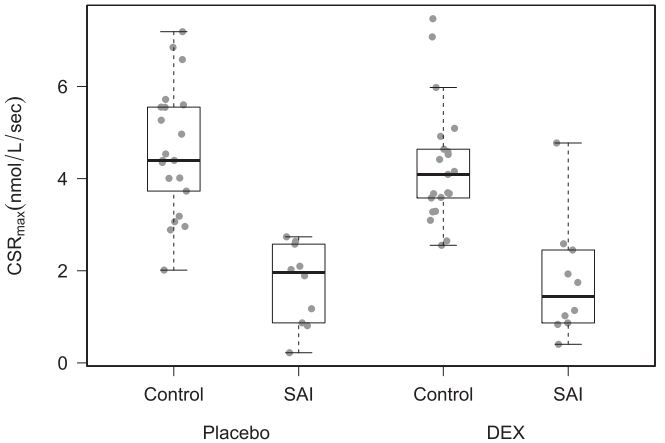

Figure 3. Box plot showing distribution of CSR_{max} for SAI and control subjects for both DEX and placebo pretreatment conditions. ● indicates CSR_{max} values for individual subjects. The solid lines represent the 25th, 50th, and 75th percentiles. Whiskers are drawn to the most extreme values that are not outliers, where outliers are defined as any value beyond 1.5 × IQR and IQR is the interquartile range (length of the box).

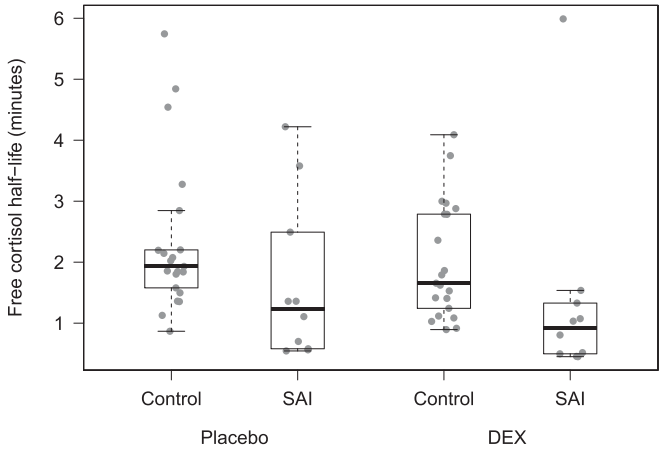

Figure 4. Box plot showing distribution of the free cortisol half-life for SAI and controls for both DEX and placebo pretreatment conditions. ● indicates free cortisol half-life values for individual subjects. The solid lines represent the 25th, 50th, and 75th percentiles. Whiskers are drawn to the most extreme values that are not outliers, where outliers are defined as any value beyond 1.5 × IQR and IQR is the interquartile range (length of the box).

standardization involves division by standard deviations (SDs) obtained from the data. STBs are expressed as the number of SDs of the outcome variable (numerator) for each SD variation in the predictor variable (denominator) in a multivariate context.

As shown in Table 1, several predictor variables, including CSR_{max}, free cortisol half-life, and CBG concentration, had a significant and relatively large magnitude of effect (STB >0.45) on stimulated total cortisol concentrations at both 30 and 60 minutes after $ACTH_{1\text{-}24}$. Other predictor variables, including baseline total cortisol and albumin concentrations, had a significant but smaller magnitude of effect (STB <0.2) on stimulated total cortisol concentrations.

We also evaluated predictor variables for stimulated free cortisol, shown in Table 1. The STB analysis for stimulated free cortisol differed from that for stimulated total cortisol by the

Table 1. Multivariate Analysis of Predictors of Stimulated Cortisol After Cosyntropin

	Total Cortisol		Total Cortisol		Free Cortisol	
	30 Min After $ACTH_{1\text{-}24}$		60 Min After $ACTH_{1\text{-}24}$		60 Min After $ACTH_{1\text{-}24}$	
	STB[a]	*P* Value	STB[a]	*P* Value	STB[a]	*P* Value
Baseline cortisol, nM	0.25	<0.001[b]	0.10	0.13[b]	0.25	<0.001[b]
CSR_{max}	0.51	<0.001[b]		0.003[c]	0.69	<0.001[b]
CSR_{max} (control)			0.69	<0.001		
CSR_{max} (AI)			0.55	<0.001		
CBG, nM		0.03[c]		0.003[c]		0.73[b]
CBG, nM (control)	0.59	<0.001	0.83	<0.001		
CBG, nM (SAI)	0.18	0.09	0.22	0.04		
Free cortisol half-life		0.047[c]	0.47	<0.001[b]	0.45	<0.001[b]
Free cortisol half-life (control)	0.51	<0.001				
Free cortisol half-life (SAI)	0.73	<0.001				
Albumin, nM	0.12	0.003[b]	0.17	<0.001[b]	0.20	0.001[b]

[a]Standardized β is a slope parameter that represents the change in the outcome variable in standard deviation (SD) units per 1 SD unit change in the predictor variable; this is equivalent to an effect size.
[b]*P* value for additive model (controls and SAI patients pooled).
[c]*P* value for control vs SAI interaction term.

lack of influence of CBG concentration and the absence of significant effects on the interaction with SAI vs control subjects.

3. Discussion

Numerical modeling and analytic methods have been used to estimate CSR_{max} and free cortisol half-life in healthy controls [8, 9, 21] and in other clinical conditions [14, 22, 23]. The current study extended the characterization of free cortisol secretion and elimination rate parameters to patients with SAI. Our results support our primary hypothesis that CSR_{max} is significantly reduced in patients with SAI relative to healthy controls. Objectivity in estimation of CSR_{max} and half-life parameters in our study was achieved by blinding the analysis to clinical status (SAI vs control) and pretreatment condition (placebo vs DEX). An additional strength of the study is that our main finding of significantly reduced CSR_{max} in patients with SAI vs controls was replicated under independent experimental conditions (placebo vs DEX) and analysis, which provided an additional measure of validation and statistical power. The rationale for including both DEX and placebo pretreatment conditions was to minimize the potential confounding effect of variable baseline cortisol concentrations on computed cortisol secretion and elimination parameters [8]. As previously observed in control subjects [8], estimates for CSR_{max} and free cortisol half-life were similar for both placebo and DEX studies, indicating that these parameter estimates are independent of baseline cortisol concentration. An additional advantage of applying numerical modeling to estimate free cortisol appearance and elimination rates is that the solution procedure adjusts for individual variations in CBG and albumin concentrations [8, 14] (see Supplemental Data).

In the present investigation, decreased CSR_{max} was observed in patients with SAI. Our results differ from those of Paisley *et al.* [13], indicating that 24-hour CPRs in patients with SAI were within the reported reference range for healthy controls. The difference in results between the two studies is most likely related to our use of $ACTH_{1\text{-}24}$ stimulation to achieve maximal cortisol secretion rates, whereas Paisley *et al.* [13] assessed CPR under baseline (unstimulated) conditions.

Systematic alterations in free cortisol half-life have been reported in a variety of settings, including decreased free cortisol half-life in obesity [8, 24] and prolonged free cortisol half-life in critical illness, sepsis, septic shock, and chronic liver disease [14, 18, 22, 25, 26]. We observed no difference in free cortisol half-life between SAI and control subjects, consistent with the notion that subnormal cortisol concentrations in SAI are driven by differences in free cortisol secretion rather than elimination. Free cortisol half-life estimates were not different for DEX and placebo conditions, indicating that DEX at the dose used in our study did not affect the free cortisol elimination rate in patients with SAI. This observation is similar to and consistent with previous findings that DEX did not significantly influence free cortisol half-life parameters in healthy control subjects [8].

Our analysis identified multiple predictor variables that significantly influenced concentrations of ACTH-stimulated total cortisol in both SAI and control groups. These include CBG, albumin, and baseline total cortisol concentrations, as well as free cortisol half-life and CSR_{max} (Table 1). The magnitude of effect for individual predictor variables was expressed as the STB value. Our finding that STB values were substantial (all >0.45) for several predictor variables, including CSR_{max}, free cortisol half-life, and CBG concentration (see Table 1), does not support our secondary hypothesis that CSR_{max} is the strongest predictor of stimulated total cortisol concentrations. However, the importance of CSR_{max} in the context of SAI is emphasized by the fact that among the various predictor variables with a relatively large magnitude of effect, only CSR_{max} differed significantly between SAI and control groups.

Baseline total serum cortisol concentration was another predictor of stimulated total cortisol concentrations that, like CSR_{max}, was significantly lower in SAI patients than in control subjects. This finding suggests that the distinction between euadrenal and SAI patients on the basis of $ACTH_{1\text{-}24}$–stimulated total cortisol concentrations is dependent on differences in CSR_{max} and/or baseline cortisol concentrations. We investigated this possibility

further in a simulation analysis, which showed that adjustment for SAI vs control group differences in both CSR_{max} and baseline total cortisol concentration, but not either parameter alone, fully accounted for the observed differences in stimulated total and free cortisol concentrations between the groups (data not shown). Taken together, these observations suggest that differences in CSR_{max} and, to a lesser extent, baseline cortisol concentration are the principal and perhaps only factors by which euadrenal and SAI patients can be discriminated using the standard $ACTH_{1\text{-}24}$ stimulation test.

In consideration of our finding that baseline cortisol concentration had a significant influence on stimulated total cortisol concentrations at 30 minutes but not at 60 minutes after $ACTH_{1\text{-}24}$, it follows that the diagnostic accuracy of the $ACTH_{1\text{-}24}$ stimulation test for SAI may vary depending on the timing of cortisol collection, even under conditions of uniform (*e.g.,* maximal) CSR [1, 7, 27, 28]. This inference has some relevance to the controversy within the literature as to whether 1- and 250-μg $ACTH_{1\text{-}24}$ tests differ in their diagnostic accuracy for SAI [1, 6, 7]. For example, we note that in most studies comparing the diagnostic performance of high- and low-dose $ACTH_{1\text{-}24}$ tests, stimulated cortisol concentrations were obtained 30 and 60 minutes poststimulation for the 1- and 250-μg $ACTH_{1\text{-}24}$ tests, respectively [1, 27, 28]. For comparisons of 1- and 250-μg $ACTH_{1\text{-}24}$ stimulation tests in which stimulated cortisol concentrations are obtained at 30 and 60 minutes, respectively, it is therefore possible that the differential influence of baseline cortisol at early vs late time points, rather than any difference in CSR, accounts for differences in diagnostic performance reported for 1- and 250-μg $ACTH_{1\text{-}24}$ tests, respectively. This conclusion is consistent with previous data demonstrating that cortisol concentrations obtained 30 minutes poststimulation were similar for both 1- and 250-μg doses of $ACTH_{1\text{-}24}$ [27, 28].

Multivariable analysis also identified other predictor variables that significantly influenced stimulated cortisol concentrations but did not differ between control and SAI groups. These predictor variables included free cortisol half-life and CBG and albumin concentrations. Variation in these predictor variables would be expected to increase the heterogeneity of stimulated total serum cortisol concentrations in both control and SAI groups, which would contribute to greater overlap between control and SAI populations and decreased diagnostic accuracy. Our finding that the determination of stimulated free cortisol concentrations (Table 1) eliminated the significant influence of CBG concentration as a predictor variable suggests that the measurement of stimulated free cortisol may be superior to the measurement of total cortisol in discriminating between euadrenal and SAI populations. This conclusion is consistent with the report of Burt *et al.* [2], in which discrimination between SAI and euadrenal patients for both 1- and 250-μg $ACTH_{1\text{-}24}$ tests was superior using free rather than total cortisol concentrations. Although measurement of free cortisol may eliminate the significant effect of CBG concentration, the influence of free cortisol half-life and albumin concentration remained significant for both total and free stimulated cortisol concentrations (Table 1).

In consideration of the conclusion that CSR_{max} is the predominant factor driving differences in stimulated cortisol concentrations between controls and patients with SAI, we speculate that changes in CSR_{max} correspond to the trend in ACTH-stimulated cortisol concentrations observed in the natural history of SAI. For example, in consideration of the time course for the development of subnormal cortisol response to $ACTH_{1\text{-}24}$ following suppression of endogenous ACTH [3–5], we reason that an analogous decline in CSR_{max} occurs over a similar time frame during hypothalamic-pituitary-adrenal axis suppression and, similarly, follows a temporal pattern of recovery that parallels ACTH-stimulated cortisol concentrations [4]. These temporal considerations also support the corollary conclusion, not addressed in the present investigation, that CSR_{max} is normal in *acute* SAI [1, 29]. On the basis of previous studies demonstrating that intermediate-duration (*e.g.,* 48 hours) administration of long-acting ACTH is able to increase ACTH-stimulated cortisol concentrations in SAI [4, 11, 12], we reason that the decrease in CSR_{max} observed in SAI is also reversible and follows an analogous time course of recovery over an intermediate duration (*e.g.,* 48 hours) of ACTH exposure.

There are several limitations to the present investigation. First, the selection of patients with SAI was based on clinical diagnosis, and the number of patients with SAI was small.

Future studies using larger sample sizes and gold standard tests of hypothalamic-pituitary-adrenal axis function may provide more complete characterization of the distribution and diagnostic value of CSR_{max} and related parameters in SAI and control subjects. Second, the estimation of CSR_{max} is subject to the bias of cortisol assays. As previously shown, different commercial cortisol assays varied in cortisol concentration [30–32] as well as bias during conditions of $ACTH_{1\text{-}24}$ stimulation [30, 33]. Future studies using more specific cortisol assay methods, such as liquid chromatography mass spectrometry, may provide a more accurate estimation of CSR_{max} [33]. Third, the numerical model developed for estimation of cortisol secretion rates does not distinguish between adrenal and extra-adrenal contributions to cortisol appearance (secretion) rates [34, 35]. Therefore additional studies are required to define the potential contribution of extra-adrenal sources to cortisol appearance and elimination rates [36, 37]. Also, our multivariable analysis depended to some degree upon the distribution and correlation structure of predictor variables; therefore, results may differ in populations having different distributional properties.

Although our investigation was adequately powered to show differences in CSR_{max} between SAI and controls groups, it was not designed or powered to determine whether estimation of CSR_{max} might provide superior sensitivity or specificity compared with concentration-based cut-scores for the diagnosis of SAI. Therefore future studies are required to define the potential role of CSR_{max} and related parameters in the clinical diagnosis of SAI. Because application of numerical modeling and analysis to the standard cosyntropin test may be accomplished using readily available computing technology and with little additional cost, further investigation of cortisol appearance and elimination rates in the pathophysiology and clinical diagnosis of SAI appear to be warranted.

In summary, we have demonstrated significantly decreased CSR_{max} in patients with chronic SAI without significant changes in free cortisol half-life, which confirms our hypothesis that chronic ACTH deficiency results in secondary alterations in the CSR-ACTH dose-response relationship. We conclude that a subnormal CSR response to ACTH, in addition to ACTH deficiency *per se*, contributes importantly to the pathophysiology and laboratory diagnosis of cortisol deficiency in SAI.

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