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CHANGES IN STEROID CONCENTRATIONS WITH THE TIMING OF CORTICOTROPIN STIMULATION TESTING IN PARTICIPANTS WITH ADRENAL SUFFICIENCY

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Abstract

Objective—To determine whether the time of day at which corticotropin stimulation testing is performed influences the steroid concentrations observed in persons with normal adrenal function.

Methods—In this retrospective, secondary analysis, participants with normal adrenal function were studied to determine whether the time of corticotropin stimulation testing influenced results. Participants consisted of 2 groups: healthy volunteers who were not suspected of having adrenal insufficiency and patients being tested for adrenal insufficiency as part of their standard of care who were subsequently shown to have normal adrenal function on the basis of a peak cortisol value of at least 20 µg/dL. A high-dose corticotropin stimulation test was performed in all participants. Baseline, peak, and delta steroid concentrations were documented after corticotropin injection. Steroid concentrations were measured by tandem mass spectrometry. Multivariate analyses adjusted for patient age, sex, and baseline steroid concentrations.

Results—With progression through the day for the time of testing, the baseline cortisol concentration decreased, while the peak and delta cortisol concentration increased (*P* values: <.001, .007, .007, respectively). For 11-deoxycortisol, the baseline decreased, while peak and delta values increased with later testing (*P* values: .017, .012, .02, respectively). Peak aldosterone concentrations increased according to their baseline values (*P*<.001), but were unaffected by time. Peak and delta dehydroepiandrosterone concentrations increased with time (*P* = .015 and .021, respectively). Referring to the various criteria for adequate steroid responses to corticotropin available in the literature, the time-related differences in this small group of patients were insufficient to draw different conclusions about results of testing.

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DISCLOSURE

The authors have no multiplicity of interest to disclose.

Conclusions—Cortisol, 11-deoxycortisol, and dehydroepiandrosterone values were most influenced by testing times. In patients with borderline adrenal function who are tested at different times of the day, the modest differences we observed may be sufficient to affect conclusions about whether adrenal insufficiency is present.

INTRODUCTION

There is a diurnal rhythm in cortisol secretion, with peak cortisol concentrations being seen in the early morning hours and low cortisol levels being characteristic of the evening hours (1,2). A similar pattern is seen with 11-deoxycortisol (3). This pattern seems to reflect greater amplitude of corticotropin (ACTH) secretory pulses in the morning hours (3,4). Secretion of dehydroepiandrosterone (DHEA), which shares its secretagogue ACTH with cortisol, is also highest in the morning and declines as the day progresses (5). Aldosterone concentrations similarly seem to be highest at the time of awakening and lower during the evening (6). Aldosterone's diurnal rhythm seems to be influenced both by ACTH and plasma renin activity (7).

A deficient steroid hormone response to ACTH injection is an indication of adrenal insufficiency (AI) (8). Time of day is generally not accounted for when interpreting the results of steroid stimulation by ACTH. Measurement of peak serum cortisol levels after synthetic ACTH administration is considered a standard test for diagnosing AI (8–10). Cortisol values less than 18 to 20 µg/dL are seen in both primary and chronic secondary AI (9–20). A criterion based on a cortisol increment greater than 7 µg/dL has also been proposed (21), although this is not commonly used. Aldosterone levels after ACTH stimulation can serve to distinguish between primary and secondary AI (18,22,23). Despite the infrequent application of this aldosterone-based test and criteria for adequacy that are not well-established, it is the reference test quoted in the third edition of the *Endocrine and Metabolic Testing Manual* (24). Basal aldosterone levels can also be used, along with plasma renin activity, to detect AI (15). Other steroid hormones including 11-deoxycortisol (13,19,23) and DHEA (23,25) can also be helpful in discriminating normal adrenal function from AI.

We have previously shown (23) that, in comparison with cortisol, ACTH-stimulated steroid profiles measured by tandem mass spectrometry (MS/MS) and composed of up to 3 steroids can also be helpful in evaluating adrenal function. A stimulated aldosterone peak of 6 ng/dL was an excellent cut-off for distinguishing primary AI from secondary AI. A combined 3-steroid profile of aldosterone, 11-deoxycortisol, and DHEA yielded a test with 100% accuracy for distinguishing primary AI from normal status.

This was a retrospective, secondary analysis to determine whether the time of day at which ACTH stimulation testing is performed influences the steroid concentrations achieved in participants with normal adrenal function. We speculated that given the marked circadian rhythm in adrenal steroids, it was possible that different steroid concentrations might be achieved at different times of the day. If this were the case, we wished to determine whether the differences were sufficient to be clinically significant and to cause different conclusions

to be drawn about the test results. We were also interested in examining whether considering the timing of testing might be helpful in cases of borderline cortisol responses to ACTH.

METHODS

Participants and Protocol

The results of ACTH testing in 2 groups of participants were analyzed. The 2 groups consisted of healthy volunteers who were not suspected of having AI and patients from Georgetown University endocrine clinic being tested for AI as part of their standard of care who were subsequently shown to have normal adrenal function on the basis of a peak cortisol value of at least 20 µg/dL. None of the standard-of-care patients were suspected of having recent-onset pituitary dysfunction. An analysis of these patients, plus additional patients found to have adrenal insufficiency, has already been reported (23), but time of day was not included in the analyses.

The institutional review board approved the study protocol. Healthy volunteers had no chronic medical problems. Patients were being evaluated for possible AI. Those who were pregnant or breastfeeding were excluded from participation. After providing written informed consent, participants underwent a standard high-dose ACTH stimulation test performed on an outpatient basis in the Georgetown University Clinical Research Unit. Participants were in a supine position and rested for at least 10 minutes before testing began. They remained supine for the duration of testing. The times at which testing began and was completed were documented. A 23-gauge indwelling catheter was inserted and a baseline blood sample was drawn. After intravenous injection of 250-mcg of Cortrosyn, blood samples were drawn at 30 and 60 minutes. Blood pressure and heart rate were measured before and after testing. A medication history was taken, and no participants were taking steroid-containing medications, including oral contraceptives and spironolactone. Information regarding dietary salt intake was not collected.

Laboratory Measurements

At all 3 time points, 4 steroids were analyzed by MS/MS as previously described (26–28). Serum samples were deproteinized and injected onto the column after centrifugation. Chromatographic separation was performed on a reverse phase C18 (27) or C8 (28) analytic column. Samples were washed online and eluted using a methanol/water gradient and introduced into the mass spectrometer. The assay protocol used separates the total ion chromatogram into 4 sections to optimize parameters for each individual analyte (27). Atmospheric pressure photoionization was used to measure 3 of the steroids in the positive ion mode; aldosterone was measured in the negative mode. The method is based on isotope dilution and is specific for the steroid of interest. Aldosterone was analyzed after adding an aliquot of cortisol to prevent precipitation of aldosterone with cortisol-binding globulin. There was far greater sensitivity and specificity for aldosterone when it was measured in the negative mode. The API-5000 mass spectrometer (Applied Biosystems, Foster City, CA) was used for all assays. Assay accuracy was evaluated in 2 ways. The assays were compared with MS/MS assays performed at the Mayo Clinic: correlation coefficients ranged from 0.908 to 0.999 (27). Recovery studies were also performed and yielded mean recoveries of

the analytes of 90% to 110% (27). Cortisol was measured on the DPC Immulite 1000 (Diagnostic Products Corporation, Los Angeles, California). This cortisol assay is a solid-phase competitive chemiluminescent enzyme immunoassay. The analytic sensitivity of the assay was 0.2 µg/dL; the intra-assay coefficient of variation was 4.5%.

Designation of Steroid Concentrations and Adrenal Status

The baseline steroid concentration was the value obtained at 0-minute before ACTH injection. The peak steroid level was defined as the maximum value after intravenous ACTH injection, regardless of whether this was the 30-minute or 60-minute value. Delta values were defined as the difference between the peak value after ACTH injection and the baseline. Patients who received standard of care and were suspected of having AI who had peak cortisol values of 20 µg/dL or above as measured by either MS/MS or immunoassay were classified as having normal adrenal function. Gold-standard testing such as an insulin tolerance test or metyrapone test was not performed.

Statistical Analysis

Regression models, Pearson correlation coefficients, and Pearson partial correlation coefficients were used to explore the relationship between time of day and various steroid levels. A regression model with time, age, sex (male vs female), and patient group (healthy participants vs patients receiving standard of care) was examined. The correlation coefficient associated with this model was a “partial” correlation coefficient, ie, partialing out the effect of the other 3 variables. For peak and delta hormone levels, the model adjusted for age, sex, participant category (healthy participant vs patients receiving standard of care), and respective baseline hormone levels. The *P* value associated with the correlation coefficient was the same as the *P* value for the time effect in the regression model.

In all regression models, the standardized (β) coefficient associated with each predictor is interpreted as the change (increase or decrease, depending on sign) in standard deviations of the hormone variable associated with a change of 1 standard deviation in the predictor. In the case of categorical predictors like sex and participant category, the β coefficient indicates the difference in the hormone variable between the specific value listed first (eg, male) and the alternative value (eg, female). In multiple regression models, the coefficients indicate the change in the response, while keeping all other variables constant.

Additionally, time of day was dichotomized as “before noon” (values up to and including noon) and “afternoon” (all other values). The *t* test or Mann-Whitney *U* test, as applicable, was used to compare steroid levels between time groups.

A *P* value less than .05 was considered significant. All analyses were conducted in SAS 9.1 (SAS Institute Inc, Cary, North Carolina).

RESULTS

Participants

ACTH stimulation tests from 64 participants were analyzed. Of the 64 participants, 21 (33%) were healthy volunteers who all had normal cortisol responses to ACTH stimulation,

both by MS/MS and immunoassay measurements, and 43 (67%) were patients receiving standard-of-care clinical evaluation who were determined to have normal adrenal function on the basis of their cortisol responses to ACTH (reaching a value of at least 20 µg/dL by either MS/MS or immunoassay). All participants were combined into 1 group for subsequent analyses. The mean age in the combined group was 42 years. We observed no adverse events after ACTH injection, other than transient flushing and lightheadedness in a few participants. Vital signs did not change significantly during testing.

Steroid Hormone Responses After ACTH Stimulation

The concentrations of 4 steroids were selected for this analysis because they appeared to best discriminate between adrenal sufficiency and AI (23). Table 1 shows the steroid responses of the combined group reported as mean and standard deviation for the baseline values, peak values, and delta values. There were significant increases in the levels of all 4 steroids after ACTH stimulation (23).

The Figures show the baseline steroid concentrations, peak steroid concentrations, and delta steroid concentrations for cortisol measured by MS/MS (Fig. 1), cortisol measured by immunoassay (Fig. 2), aldosterone measured by MS/MS (Fig. 3), 11-deoxycortisol measured by MS/MS (Fig. 4), and DHEA measured by MS/MS (Fig. 5), respectively displayed according to the time at which the ACTH stimulation test was performed. The influence of time of day differed according to the steroid involved.

The distributions of cortisol concentrations with time are shown in Figures 1 and 2. In a model including age, sex, and patient group (Table 2), baseline cortisol was affected by time only. After including age, sex, patient group, and baseline cortisol in the model, the peak cortisol was associated with baseline cortisol in both the assays used, but was also influenced by time ($P = .007$) only when measured by MS/MS (see Table 2A). In the model, cortisol delta was not influenced by time when it was measured by immunoassay (P value = .10) (see Table 2B), although it was affected by time when measured by MS/MS (P value = .007).

The distributions of aldosterone concentrations are shown in Figure 3. A model including age, sex, patient group, and, for the peak and delta values, baseline aldosterone values, is shown in Table 2C. Baseline aldosterone values were lower in the patients receiving standard of care than in the healthy participant group ($P = .029$). Peak aldosterone values were not influenced by time, but were affected by baseline aldosterone values ($P < .001$). None of the variables in the model affected the aldosterone delta. The distributions of 11-deoxycortisol and DHEA concentrations with time are shown in Figures 4 and 5. The results of the regression model are shown in Table 3.

When steroid concentrations were divided into those obtained when the ACTH stimulation test was started before or at noon, compared with concentrations when testing was conducted in the afternoon, some significant differences were seen (Table 4). Baseline values for cortisol (regardless of which assay was used) and 11-deoxycortisol were lower in the afternoon. The cortisol delta was higher when testing took place in the afternoon, but

only when it was measured by MS/MS. Both aldosterone peak and delta values were higher in the afternoon. The DHEA delta was also significantly higher in the afternoon.

DISCUSSION

The 4 steroids analyzed were differently affected by the time at which stimulation testing was conducted. However, additional factors including participant sex, age, and baseline steroid values also influenced the results and, depending on the particular steroid, had a more profound effect on the results.

When examining cortisol concentrations with time of day as the sole independent variable (data not shown), their relationship was such that the negative association between time and cortisol baseline was compensated by a positive association between cortisol delta and time, leading to a cortisol peak that was unaffected by time. When other variables including baseline cortisol values were considered, time affected both the peak and delta values measured by MS/MS. The only additional influence on peak concentrations was baseline cortisol concentration. There were too few patients with borderline values to determine whether these time-related changes could be sufficient to encroach upon the criteria for assigning adrenal sufficiency of a peak greater than 20 µg/dL (9–20). None of these time-related changes appeared sufficient to encroach on criteria for assigning adrenal sufficiency of a delta of greater than 7 µg/dL (21).

With respect to aldosterone concentrations, we could not replicate earlier reports of baseline concentrations being affected by time of day (6). However, both peak and delta concentrations increased slightly during later testing. These time-related differences were sufficient to result in a mean morning delta value of 5.9 ng/dL, compared with a mean delta value of 9.1 ng/dL in the afternoon ($P = .014$). The morning and afternoon peaks were 10.6 ng/dL and 14.1 ng/dL, respectively ($P = .003$) (Table 4). It is possible that our failure to show an effect of time of day on basal aldosterone levels was because of our inability to account for interpatient variability.

After studying 12 healthy patients and 11 patients with untreated AI, Dluhy et al (18) proposed testing criteria based on aldosterone concentrations. In this study, their 5 patients with primary adrenal insufficiency had a maximum delta of 1 ng/dL and a maximum peak of 4 ng/dL. They suggested that healthy patients had minimal increments or deltas of 4 ng/dL and minimum peak concentrations of 5 ng/dL. The delta of at least 4 ng/dL is also the criteria quoted in the third edition of the *Endocrine and Metabolic Testing Manual* (24), with the Dluhy study (18) given as the reference. However, the aldosterone assay used at this time (29) is unlikely to have been used subsequently. Manglik et al used a similar peak value greater than 5.4 ng/dL as indicating adrenal sufficiency (22). Their study used a different, commercially available assay. If aldosterone peaks or deltas of about 4–5 ng/dL are used as cut-offs, values lower than this may be more likely encountered with morning testing, based on the mean delta being lower than during afternoon testing (5.9 vs 9.1 ng/dL).

Based on our own data, we previously determined that having a peak aldosterone value greater than 6 ng/dL excludes primary AI, but that having peak aldosterone values less than 6 ng/dL does not diagnose primary AI because some healthy subjects had such values (23). That is, aldosterone criteria are more useful for excluding rather than confirming a diagnosis of primary AI. In this present study of 64 participants with normal adrenal function, we again cannot confirm that peak or delta aldosterone concentrations less than 4 to 6 ng/dL suggest AI. Figure 3 shows that irrespective of the time at which testing took place, some healthy participants had both peak and delta values less than 5 ng/dL throughout the course of the day.

It is interesting that baseline aldosterone values were affected by whether the participant was a healthy volunteer or a patient receiving the standard of care whose testing excluded AI. Values were lower in patients receiving standard of care (Table 2C). It is possible that a decrement in baseline aldosterone concentrations is an early or isolated sign of developing AI, which is not yet reflected in other steroids. Some studies have suggested that deficient aldosterone secretory response may be seen without deficient cortisol responses in AI of certain etiologies (30,31).

Similar to the case with cortisol, in multivariate analyses, 11-deoxycortisol baseline values declined with advancing time of day, but both the peak and delta 11-deoxycortisol values increased with increasing time of day. Both peaks and deltas were positively correlated with baseline values. Interestingly, these values also increased with advancing age, a phenomenon not previously reported.

DHEA concentrations during ACTH stimulation testing appeared to be subject to influence by 2 variables. Both the peak and delta values increased with later times of testing. In addition, the values declined with advancing age. The age-sensitivity of DHEA values is well documented (32) and this perhaps limits the usefulness of this steroid as an indicator of adrenal insufficiency, as it would involve additional effort to account for patient age when interpreting test results.

There are clearly several limitations of our study. Chief among these is the fact that this is a retrospective, secondary analysis of a study conducted to examine steroids concentrations measured by MS/MS. A second limitation is that we do not have data for the same individuals tested at different times of day; rather, each of our patients was tested at a single time of day. Thus, our data are likely to be quite variable because of the inherent difference between individuals.

CONCLUSION

Although the time that a high-dose ACTH stimulation test is performed does have an effect on the resultant concentrations of cortisol, aldosterone, 11-deoxycortisol, and DHEA, the effect is only modest and appears unlikely to affect the conclusions drawn from the testing. If a future study examined the issue of time of day, the study design could incorporate repeat testing at different times of day for the same individuals. Perhaps individuals with borderline

responses to ACTH stimulation could be targeted, as it is possible that in this population different test results could be obtained with morning vs afternoon testing.

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Abbreviations

ACTH	corticotropin
AI	adrenal insufficiency
DHEA	dehydroepiandrosterone
MS/MS	tandem mass spectrometry

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Fig. 1. Baseline (*Panel A*), peak (*Panel B*), and “delta” (*Panel C*) cortisol concentrations during corticotropin stimulation testing (measured by tandem mass spectrometry).

Fig. 2.

Baseline (*Panel A*), peak (*Panel B*), and “delta” (*Panel C*) cortisol concentrations during corticotropin stimulation testing (measured by immunoassay).

Fig. 3. Baseline (*Panel A*), peak (*Panel B*), and “delta” (*Panel C*) aldosterone concentrations during corticotropin stimulation testing (measured by tandem mass spectrometry).

Fig. 4. Baseline (*Panel A*), peak (*Panel B*), and “delta” (*Panel C*) 11-deoxycortisol (DOC) concentrations during corticotropin stimulation testing (measured by tandem mass spectrometry).

Fig. 5. Baseline (*Panel A*), peak (*Panel B*), and “delta” (*Panel C*) dehydroepiandrosterone (DHEA) concentrations during corticotropin stimulation testing (measured by tandem mass spectrometry).

Table 1

Steroid Concentrations After Corticotropin Stimulation in All Participants Combined

Steroid concentrations	Mean	SD	Minimum	Maximum
Cortisol baseline (MS/MS), µg/dL	11.27	5.45	2.65	26.8
Cortisol peak (MS/MS), µg/dL	30.58	8.03	18.10	57.60
Cortisol delta (MS/MS), µg/dL	19.31	6.69	7.90	43.20
Cortisol baseline (immunoassay), µg/dL	10.02	4.31	3.41	25.00
Cortisol peak (immunoassay), µg/dL	28.30	6.63	17.00	49.30
Cortisol delta (immunoassay), µg/dL	18.27	6.14	5.70	38.80
Aldosterone baseline (MS/MS), ng/dL	4.85	3.57	0.50	14.90
Aldosterone peak (MS/MS), ng/dL	12.68	7.10	2.80	35.80
Aldosterone delta (MS/MS), ng/dL	7.84	5.51	0.80	29.40
11-deoxycortisol baseline (MS/MS), ng/dL (ng/dL)	77.20	74.76	6.00	380.00
11-deoxycortisol peak (MS/MS), ng/dL	644.89	270.83	52.00	1420.00
11-deoxycortisol delta (MS/MS), ng/dL	575.06	224.62	225.00	1329.00
DHEA baseline (MS/MS), ng/dL	203.77	193.11	5.00	750.00
DHEA peak (MS/MS), ng/dL	589.22	338.08	67.00	1270.00
DHEA delta (MS/MS), ng/dL	378.20	241.00	13.00	893.00

Abbreviations: DHEA, dehydroepiandrosterone; MS/MS, tandem mass spectrometry

Table 2

Correlation of Cortisol Concentrations Measured by Tandem Mass Spectrometry (Table Section “A”), Cortisol Concentrations Measured by Immunoassay (Table Section “B”), and Aldosterone Concentrations Measured by Tandem Mass Spectrometry (Table Section “C”) in a Regression Model Incorporating Age, Sex, Patient Group, and Baseline Steroid as Independent Variables^a

“A” Cortisol by MS/MS	Cortisol baseline (MS/MS)		Cortisol peak (MS/MS)		Cortisol delta (MS/MS)	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Time	−0.45	<.001	0.33	.007	0.40	.007
Baseline cortisol	...	...	0.70	<.001	0.03	.86
Correlation coefficient ^b	−0.433		0.344		0.344	

“B” Cortisol by immunoassay	Cortisol baseline (IA)		Cortisol peak (IA)		Cortisol delta (IA)	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Time	−0.49	<.001	0.23	.10	0.25	.10
Baseline cortisol	...	...	0.54	<.001	−0.12	.43
Correlation coefficient ^b	−0.475		0.211		0.212	

“C” Aldosterone by MS/MS	Aldosterone baseline (MS/MS)		Aldosterone peak (MS/MS)		Aldosterone delta (MS/MS)	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Time	−0.001	.99	0.142	.15	0.183	.62
Patient group (patients receiving standard of care vs healthy volunteers)	−0.30	.029	−0.135	.21	−0.175	.47
Baseline aldosterone	...	...	0.561	<.001	0.074	.59
Correlation coefficient ^b	−0.001		0.148		0.184	

Abbreviations: IA, immunoassay; MS/MS, tandem mass spectrometry.

^a Only those variables showing significant associations are shown in the table.

^b Correlation coefficient for relationship between steroid concentrations and time. Correlation coefficient partials out the effect of age, sex, patient group, and baseline steroid values. P values for the partial correlation coefficients are the same as P values for the time effect in the models. P values were determined by a t test for difference from 0.

Table 3

Correlation of 11-Deoxycortisol (Table Section "A") and Dehydroepiandrosterone Concentrations (Table Section "B") Measured by Tandem Mass Spectrometry With Time in a Model Incorporating Age, Sex, Patient Group, and Baseline Steroid^a

"A"	11-Deoxycortisol baseline (MS/MS)		11-Deoxycortisol peak (MS/MS)		11-Deoxycortisol delta (MS/MS)	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Time	-0.30	.017	0.29	.012	0.32	.02
Age	0.01	.92	0.29	.012	0.31	.017
Sex						
(male vs female)	0.33	.006	0.11	.32	0.10	.43
Baseline 11-deoxycortisol	...	...	0.61	<.0001	0.41	.003
Correlation coefficient ^b	-0.305		0.323		0.312	

"B"	DHEA baseline (MS/MS)		DHEA peak (MS/MS)		DHEA delta (MS/MS)	
	β Coefficient	P value	β Coefficient	P value	β Coefficient	P value
Time	-0.13	.26	0.20	.015	0.28	.021
Age	-0.46	.0003	-0.25	.009	-0.3	.033
Sex						
(male vs female)	0.28	.015	-0.05	.50	-0.06	.61
Baseline DHEA	...	...	0.62	<.0001	0.03	.82
Correlation coefficient ^b	-0.144		0.314		0.297	

Abbreviations: DHEA, dehydroepiandrosterone; MS/MS, tandem mass spectrometry

^a Only those variables showing significant associations are shown in the table.

^b Correlation coefficient for relationship between steroid concentrations and time. Correlation coefficient partials out the effect of age, sex, patient group, and baseline steroid values. P values for the partial correlation coefficients are the same as P values for the time effect in the models. P values were determined by a t test for difference from 0.

Table 4
Comparison of Morning Steroid Concentrations With Afternoon Steroid Concentrations

Steroid concentration during corticotropin stimulation testing	Morning (before and including noon) (n = 26)		Afternoon (n = 38)		P value
	Mean	SD	Mean	SD	
Cortisol baseline (MS/MS), µg/dL	13.72	5.61	9.59	4.70	.002
Cortisol peak (MS/MS), µg/dL	30.67	7.62	30.52	8.41	.94
Cortisol delta (MS/MS), µg/dL	16.95	6.60	20.93	6.34	.018
Cortisol baseline (immunoassay), µg/dL	12.31	4.63	8.46	3.31	.0007
Cortisol peak (immunoassay), µg/dL	29.00	7.45	27.82	6.06	.49
Cortisol delta (immunoassay), µg/dL	16.68	7.49	19.35	4.83	.12
Aldosterone baseline, ng/dL	4.62	3.08	5.00	3.90	.68
Aldosterone peak, ng/dL	10.61	4.42	14.10	8.21	.032
Aldosterone delta, ng/dL	5.99	3.59	9.10	6.24	.014
11-Deoxycortisol baseline, ng/dL	110.62	89.43	54.34	52.83	.007
11-Deoxycortisol peak, ng/dL	639.85	284.05	648.34	265.22	.90
11-Deoxycortisol delta, ng/dL	547.38	202.18	594.00	239.55	.42
DHEA baseline, ng/dL	223.42	236.01	190.32	159.38	.54
DHEA peak, ng/dL	524.12	370.15	633.76	311.49	.21
DHEA delta, ng/dL	294.54	220.14	435.45	240.56	.02

Abbreviations: DHEA, dehydroepiandrosterone; MS/MS, tandem mass spectrometry; SD, standard deviation.

^a Steroid values with significant diurnal variation are highlighted in bold font.