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Primary vs secondary adrenal insufficiency: ACTH-stimulated aldosterone diagnostic cut-off values by tandem mass spectrometry

Smita Baid Abraham*, Brent S. Abel*, Ninet Sinaii†, Elizabeth Saverino*, Matthew Wade*, Lynnette K. Nieman*

*The Program in Reproductive and Adult Endocrinology, The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA

†Biostatistics and Clinical Epidemiology Service, Clinical Center, National Institutes of Health, Bethesda, MD, USA

Summary

Objectives—To validate the diagnostic utility of Cortrosyn™ stimulated aldosterone in the differentiation of primary (PAI) and secondary adrenal insufficiency (SAI) and to evaluate the effect of urine sodium levels and posture on test performance.

Design—Cross-sectional study.

Methods—Healthy volunteers (HV; $n = 46$) and patients with PAI ($n = 26$) and SAI ($n = 29$) participated in the study. Testing included cortisol and aldosterone (by liquid-chromatography tandem mass spectrometry) measurements at baseline and 30 and 60 min after 250 μ g Cortrosyn™. Plasma corticotropin (ACTH), renin activity (PRA) and urine spot sodium as a proxy for 24-h urine sodium excretion were measured at baseline. The effect of a sitting or semifowlers posture was evaluated in healthy volunteers.

Results—A Cortrosyn™-stimulated aldosterone level of 5 ng/dl (0.14 nmol/l) had 88% sensitivity and positive predictive value and 89.7% specificity and negative predictive value for distinguishing PAI from SAI. Spot urine sodium levels showed a strong correlation with peak aldosterone levels ($r = -0.55$, $P = 0.02$, $n = 18$) in the SAI but not PAI or HV groups. Posture did not have a significant effect on results.

Conclusions—Once diagnosed with adrenal insufficiency, a stimulated aldosterone value of 5 ng/dl (0.14 nmol/l) works well to differentiate PAI from SAI. However, clinicians should be aware of the possible effect of total body sodium as reflected by spot urine sodium levels on aldosterone results. A 24-h urine sodium measurement may be helpful in interpretation.

Correspondence: Lynnette K. Nieman, CRC, Rm 1–3140, MSC 1109, 10 Center Drive, Bethesda, MD 20892, USA. Tel.: 301 496 5800; Fax: 301 402 0884; niemanl@nih.gov.

Declaration of interest

The authors have nothing to declare.

Introduction

Measurement and interpretation of plasma corticotropin (ACTH) levels are recommended to distinguish between primary (PAI) and secondary adrenal insufficiency (SAI). A high normal or elevated ACTH concentration is consistent with PAI, while a low normal or undetectable level suggests SAI.^{1,2} However, intranasal, inhaled and injectable glucocorticoids³ as well as other agents may reduce ACTH levels.⁴ When the ACTH level is indeterminate, another diagnostic criterion would be useful.

Several investigators have studied the aldosterone response to exogenous ACTH. While the renin-angiotensin system and plasma potassium levels are the primary regulators of aldosterone production,⁵ ACTH has an acute stimulatory effect.^{6–8} In 1974, Dluhy *et al.* reported that after intramuscular Cortrosyn™ (Amphastar Pharmaceuticals, Rancho Cucamonga, CA, USA) (250 µg), aldosterone levels increased to >5 ng/dl (0.14 nmol/l) in six patients with SAI and 12 healthy subjects, but failed to reach this threshold in five PAI subjects.⁷ Basal aldosterone levels varied widely in healthy volunteers, possibly because time of day, posture and diet were not controlled.

Recently, liquid-chromatography tandem mass spectrometry (LC-MS/MS) has been shown to be more accurate than previous techniques for measurement of steroids⁹ Holst *et al.*¹⁰ reported the use of intravenous (IV) 250 µg Cortrosyn™-stimulated steroid profiles, measured by LC-MS/MS, to determine the cause of adrenal insufficiency. Peak aldosterone levels less than 6 ng/dl (0.17 nmol/l) had 100% sensitivity for discriminating PAI ($n = 7$) from normal status ($n = 10$) and SAI ($n = 2$), and an 84% and 83% specificity for determining PAI from normal status and SAI, respectively. Use of a three-steroid profile (aldosterone, 11-deoxycortisol and DHEA) had a 100% sensitivity and specificity for determining PAI from normal and 100% sensitivity and 83% specificity determining PAI from SAI. Subjects were tested in the morning in a seated position, and diet was not controlled.

Total sodium balance, potassium, plasma renin activity and posture significantly affect basal aldosterone levels,^{5,11} but the possible effect(s) of these factors on Cortrosyn™-stimulated aldosterone have not been systematically evaluated.

To validate the use of Cortrosyn™-stimulated aldosterone (stimulated aldosterone) levels measured by LC-MS/MS to determine the aetiology of adrenal insufficiency, we compared responses in a large group of healthy volunteers (HV), PAI and SAI subjects. We also compared the utility of stimulated aldosterone vs morning ACTH levels. Urine spot sodium (urine Na⁺), serum potassium (K⁺) and plasma renin activity (PRA) levels were measured to determine their effects on stimulated aldosterone in all subjects, and the effect of posture on stimulated aldosterone was evaluated in the healthy volunteer group.

Patients and methods

The Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development approved the study protocol (NCT00156767). All subjects provided written informed consent.

Healthy volunteers

Healthy adults were recruited from three age groups (<40 years, 40–55 years and >55 years, evenly split by gender) using community flyers, from October 2005 to July 2009. Exclusion criteria included uncontrolled acute or chronic illness, abnormal cell blood count or electrolytes, pregnancy, lactation, recent use of imidazole or glucocorticoid medications, mineralocorticoid antagonists, or potassium supplements, chronic use of nonsteroidal anti-inflammatory drugs and the presence of signs or symptoms of adrenal insufficiency (e.g. unintentional weight loss, nausea, excessive fatigue, low blood pressure, etc.). Well-controlled chronic conditions (e.g. hypertension) were allowed. Healthy volunteers (HV1) recruited between October 2005 and April 2006 underwent a 1- μ g Cortrosyn™ stimulation test immediately followed by a 250- μ g Cortrosyn™ stimulation test; their cortisol responses have been reported.¹² Only those who had serum aldosterone levels as part of their testing (December 2005 onward) are reported here.

A second group of healthy volunteers (HV2), enrolled from February 2008 to July 2009 completed a 250- μ g Cortrosyn™ stimulation test only. The aforementioned inclusion and exclusion criteria applied. All volunteers received monetary compensation for their time.

Known adrenal insufficiency

Subjects were recruited via patient contact, community flyers, letters to local endocrinologists and primary care physicians, and an announcement on the website of the National Adrenal Disorders Foundation (NADF) and were enrolled from February 2006 to July 2012. Individuals were evaluated in the general endocrine clinic (tertiary care clinic, Clinical Center, NIH, Bethesda, MD). Those who were highly suspicious for or with known PAI or SAI were invited to participate. The exclusion criteria for healthy volunteers were applied to these patients with the exception of signs or symptoms of adrenal insufficiency.

High ACTH values, and/or positive 21-hydroxylase antibodies, and/or clinical or genetic evidence of an autoimmune polyglandular syndrome confirmed autoimmunity as the aetiology of disease in all PAI patients. None had history of bilateral adrenalectomy, infectious disease, haemorrhage or infiltrative disorders. SAI was determined by low ACTH values and/or history of pituitary disease or exogenous steroid use.

Aetiology of SAI was supraphysiologic doses of oral or injectable glucocorticoids ($n = 16$), pituitary surgery or radiation ($n = 7$), Sheehan's syndrome ($n = 1$), Hand-Schuller-Christian disease ($n = 1$), isolated ACTH deficiency or multiple pituitary hormonal deficiencies of uncertain aetiology ($n = 4$).

All adrenally insufficient patients were taking or were started on physiologic replacement doses of glucocorticoid (10–12 mg/m² of hydrocortisone or prednisone equivalent). All PAI patients were taking fludrocortisone with the exception of one patient who managed with a high salt diet and salt tablets. Sixteen patients were taking 100 mcg of fludrocortisone/day, and the remaining were on a range of doses from 50 to 200 mcg/day.

Procedures

Staff performed a complete history and physical examination. All medications were recorded.

All testing was started between 8 and 10 AM. All subjects were seated or in the semifowlers position for 20–30 min before testing and were not subject to Na⁺ or fluid restrictions, and, if applicable, glucocorticoid and fludrocortisone replacement doses were held until test completion.

In all groups, a chemistry panel (serum Na⁺, potassium, glucose, BUN and creatinine) and plasma renin activity (reference range: Na-depleted, upright, 18–39 years, 2.9–24.0 ng/ml/h, 0.81–6.67 ng/l/s; >40 years, 2.9–10.8, 0.81–3.0 ng/l/s; Na-replete, upright 18–39 years 0.6–4.3, 0.17–1.19 ng/l/s; >40 years, 0.6–3.0, 0.17–0.83 ng/l/s; radioimmunoassay, Mayo Medical Labs, Rochester, MN, [Mayo]) were measured at time 0. Prior to February 2008, fasting, morning plasma ACTH levels were measured if needed for clinical management of PAI and SAI patients. After this date, HV2, PAI and SAI subjects had plasma ACTH levels (chemiluminescence immunoassay, Department of Laboratory Medicine, NIH, Bethesda, MD; reference range 0–46 pg/ml [10 pmol/l]) measured at time 0 and spot urine Na⁺ levels measured that morning.

In HV1 subjects, 1 µg ACTH 1–24 (CortrosynTM; Amphastar Pharmaceuticals) was administered intravenously, followed by 10 ml saline. Serum total cortisol (competitive chemiluminescent enzyme immunoassay, Department of Laboratory Medicine, NIH, Bethesda, MD) and plasma aldosterone concentration (PAC; assay details below) were drawn just before (time 0) CortrosynTM injection and 30 and 60 min later. A 250-µg CortrosynTM dose was injected immediately after the 60-min blood draw with the same steroid measurements drawn 30 and 60 min later. HV2, PAI and SAI subjects only underwent the 250 µg CortrosynTM stimulation test with cortisol and aldosterone measurements at baseline (time 0) and 30 and 60 min after CortrosynTM injection.

Sixteen HV2 subjects underwent two tests, once in a seated position and once in the semifowlers position (resting at a 45-degree angle).

Aldosterone assay

PAC was measured by radioimmunoassay (RIA) from February 2005 to August 2007, Mayo; and tandem mass spectrometry (LC-MS/MS; Mayo) afterwards. The amount of serum extracted for the LC-MS/MS assay was 0.5 ml. The intra-assay coefficients of variation (CVs) were 9.3%, 3.1% and 2.5% at 37, 212 and 1319 ng/dl (1.0, 5.9 and 36.5 nmol/l), respectively. Interassay CVs were 16.7%, 14.9%, 8.1% and 7.3% at 4.4, 18, 87 and 400 ng/dl (0.12, 0.50, 2.4 and 11 nmol/l), respectively.

Statistics

PAC measured by RIA (PAI, $n = 3$; SAI, $n = 5$; HV1, $n = 16$) were converted to LC-MS/MS values using the following calculation: $y \text{ (MS)} = 0.91 \times (\text{RIA}) - 0.62$ (provided by Mayo). Peak cortisol and peak PAC were defined as the highest value at any time point. Delta

aldosterone was calculated by subtracting baseline PAC from the peak; it was used to compute percent change from baseline.

Median peak aldosterone values and baseline and peak cortisol values were not statistically significantly different between the HV1 and HV2 group; these results were combined (HV) for the analyses.

No significant differences in plasma renin activity, baseline or peak PACs between the sitting and semifowler position tests were identified. Thus, the earlier test date (regardless of posture) results were used for the HV2 subjects.

Simple descriptive statistics [mean $\pm$ standard deviation or median (interquartile range)] and frequency distributions were applied based on type and distribution of values. To compare continuous data, analysis of variance (anova) or nonparametric Kruskal-Wallis tests or the *t*-test or Wilcoxon rank sum test were used depending on the number of comparisons. Categorical data were compared by Fisher's exact test. Correlation analyses were performed using Spearman's rho. The potential confounding effects of age, gender and oral contraceptive (OCP) use were tested, and none were identified. Sensitivity, specificity, positive predictive value and negative predictive values of basal ACTH were computed based on the upper reference range (46 pg/ml; 10 pmol/l). Receiver operating characteristic (ROC) determined the optimal cut-off for peak PAC. Data were log-transformed as needed. A *P*-value <0.05 was considered statistically significant, and Bonferroni-corrected *P*-values for multiple comparisons are reported. Data were analysed using SAS v. 9.2 (SAS Institute, Inc, Cary, NC, USA).

Results

Subjects and cortisol response to Cortrosyn™

Enrollment details and demographic data are presented in Table 1. Only race was statistically different between groups.

Comparing HV1 and HV2, median peak cortisol (median [IQR]: 28.0 µg/dl [23.1, 28.7] vs 26.0 [23.1, 29.5]; 769.7 nmol/l [637, 792] vs 706 [637, 814]) values were not significantly different; however, baseline values (6.9 µg/dl [5.3, 8.5] vs 9.3 [6.7, 11.7], *P* = 0.019; 190 nmol/l [146, 234] vs 257 [185, 32]) were significantly different. When two subjects on OCPs were excluded from the HV2 group, the result was no longer significant. All subjects in the HV1 and HV2 groups had peak cortisol levels of > 18 µg/dl except one HV2 subject whose peak value was 17.6 µg/dl (Fig. 1a). Peak cortisol values were seen at 60 min in all HV1 subjects and in 29/30 HV2 subjects. All PAI and SAI patients failed the 250 µg Cortrosyn™ stimulation test with a peak cortisol value of <18 µg/dl (497 nmol/l) except one SAI patient who had a peak value of 20 µg/dl (552 nmol/l). However, this patient failed an insulin tolerance test given within 2 days, with a peak cortisol value of 16.4 µg/dl (452 nmol/l).

Plasma ACTH values

In the PAI group, 15/21 patients with available data had elevated ACTH levels consistent with PAI (Table 2). Six patients had ACTH values within the reference range (0–46 pg/ml;

10 pmol/l) (12.4, 12.3, 25.1, 27.8 and 33.2 [$n = 2$] pg/ml; 2.7, 2.7, 5.5, 6.1, 7.3 [$n = 2$] pmol/l). Two of the six patients had exposure to supraphysiologic levels of glucocorticoids. In the others, it is not clear why the ACTH levels were lower than anticipated. One patient reported using ciclofenide nasal inhaler at the recommended dose, but had stopped it 3 days prior to testing, and the second patient had no other history to explain a low ACTH level. Both confirmed taking physiologic replacement doses of hydrocortisone. The third patient was taking prednisone 5 mg and was on cyclosporine and mercaptopurine for the treatment of autoimmune liver disease. This patient had a repeat ACTH level the next morning, that was also low. In the fourth patient, the aetiology of AI came into question so that she was excluded from analyses involving diagnostic test performance. All six patients were women between the ages of 18 and 59 years with duration of illness ranging from 4 to 32 years. These patients were confirmed to have PAI by previously documented elevated ACTH levels, positive adrenal antibody titres or medical history consistent with the disease. Furthermore, after learning of their low ACTH levels, each patient was contacted and asked again about any glucocorticoid use (including inhalers, injections and topical steroid creams), over the counter and prescription medications. All six patients denied use of medications known to affect ACTH levels.

In the SAI group, 19/26 had ACTH values within the normal range; of these, seven were frankly suppressed (<5 pg/ml). Three patients had low values at 5.3, 5.7 and 5.8 pg/ml (1.2, 1.3, 1.3 pmol/l). Four patients had supra-normal ACTH values (ULN 46 pg/ml, 10 pmol/l). Two of the four patients had values around 50 pg/ml (11 pmol/l). One had a history of radiation therapy for a growth hormone secreting pituitary tumour 40 years prior and the other had a 15-year history of partial hypophysectomy for prolactinoma. The third patient had an ACTH value of 101 pg/ml (22.2 pmol/l) a few weeks after receiving intravenous glucocorticoids during a hospital admission. The fourth patient received injectable and intravenous steroids throughout the previous year after having unilateral adrenalectomy for Cushing's syndrome. The patient received a 100-mg hydrocortisone injection 1 month prior and a 50-mg hydrocortisone injection 1 week prior to testing.

Diagnostic test performance of plasma ACTH and peak PAC

An ACTH greater than the upper limit of normal (ULN) had a sensitivity of 75.0%, specificity of 84.6%, positive predictive value of 79% and negative predictive value of 81% for distinguishing PAI from SAI patients. Excluding AI patients with atypical ACTH values for their disease brings the sensitivity, positive and negative predictive value of an ACTH level to 100% and specificity to 84% to distinguish PAI from SAI.

Peak PAC occurred at the 30-min time point in 22/29 SAI, 5/16 HV1 and 16/30 HV2 subjects. The remaining peak PACs occurred at 60 min except for one HV patient whose peak PAC was at time 0. Four PAI subjects had peak PAC values of >4 ng/dl, 0.11 nmol/l (6.9–8.3 ng/dl; 0.19–0.23 nmol/l), while all of the remaining were <4 ng/dl (Fig. 1b). Using ROC analysis, a peak PAC value <5.0 ng/dl (0.14 nmol/l) was identified as most optimal to distinguish PAI from SAI patients with a sensitivity and positive predictive value of 88.0% and specificity and negative predictive value of 89.7%. ROC analyses excluding patients that had aldosterone values measured by RIA did not change this conclusion.

Use of ACTH levels and peak PAC as a combined variable diagnostic test to distinguish PAI from SAI was also considered. Compared to peak PAC alone, the sensitivity and specificity of this combined variable was low at 61.9% and 19.2%, respectively.

Electrolyte, PRA and PAC relationships

Relevant laboratory findings are provided in Table 2. Urine Na⁺, baseline, peak, delta and percent change aldosterone levels were similar in SAI patients with pituitary disease and those with exogenous steroid use.

As expected, plasma K⁺ showed a strong correlation with PRA in PAI patients ($r_s = 0.62$, $P = 0.003$, $n = 21$), but not with PAC. In SAI patients, urine Na⁺ showed a strong negative correlation ($r_s = -0.55$, $P = 0.018$, $n = 18$) with peak PAC, delta PAC ($r_s = -0.57$, $P = 0.01$) and percent change PAC ($r_s = -0.51$, $P = 0.03$; Fig. 2). There were no other significant correlations between urine Na⁺ or plasma K⁺ and PRA, baseline or peak PAC, delta or percent change aldosterone in any of the groups.

In all subjects combined, age showed a negative correlation with baseline aldosterone ($r_s = -0.26$, $P = 0.008$). Five women (2 HV and 3 PAI) were taking OCPs at the time of testing and had a median PRA of 2.8 ng/ml/h [1.0, 2.8] vs 0.7 [0.60, 1.3] (0.78 ng/l/s [0.28, 0.78] vs 0.19 [0.17, 0.36] in those not on OCPs, $P = 0.012$).

Regarding other medications that could potentially affect PAC, one PAI, one SAI and one HV subjects were taking an ACE-inhibitor/angiotensin-receptor blocker; three SAI subjects were taking diuretics, and four PAI, two SAI and three HV subjects were on beta-blocker therapy. These patients' values were not outliers. Analyses considering the use of these interfering medications (ACE-I, ARBs, diuretics, beta-blockers and OCP) on plasma renin activity (PRA), potassium, urine sodium, ACTH, and baseline and peak cortisol and aldosterone levels did not alter our conclusions.

Discussion

A stimulated peak PAC of <5 ng/dl (0–14 nmol/l) (stimulated PAC) 30 or 60 min after 250 µg CortrosynTM accurately distinguished between PAI and SAI in 88% of the patients. This value is similar to the stimulated aldosterone level of <6 ng/dl (0.17 nmol/l) reported by Holst and colleagues (also using LC-MS/MS assay) and by Dluhy *et al.* (5 ng/dl [0.14 nmol/l], by RIA) to differentiate between the two types of adrenal insufficiency. Peak PAC and plasma ACTH levels had similar diagnostic performance, suggesting that either can be used to determine the aetiology of adrenal insufficiency. Thus, by studying a larger population of adrenal insufficiency patients, this work confirms and extends previous reports.

Evaluation of plasma ACTH levels has been the standard diagnostic tool used to differentiate between PAI and SAI. However, as seen in this study, external factors can affect ACTH levels, reducing its diagnostic accuracy. Four of six PAI patients with normal ACTH values had no easy explanation. They reported taking physiologic replacement doses of hydrocortisone or prednisone and no other exposure to oral, topical, inhaled or injectable

glucocorticoids. While our subjects denied their use, several nonglucocorticoid medications, for example opiates, can decrease ACTH levels¹³ Serotonin antagonists and GABAergic agents such as valproic acid and somatostatin analogues can inhibit ACTH production.⁴ Clinicians should be aware of this possibility.

Plasma ACTH levels can be affected by the time of the blood draw and handling of the sample. In this study, ACTH levels were measured before the morning physiologic dose of glucocorticoid, when ACTH levels in properly replaced PAI patients should be above normal. Plasma for ACTH should be drawn into a chilled tube and placed in ice water immediately. If not, degradation of this peptide hormone can occur¹⁴ This cannot be excluded as a cause of unexpectedly low values in the current study.

In contrast, four patients with SAI had elevated ACTH levels. We believe that both patients with minimally elevated values had partial ACTH insufficiency due to surgery and radiation. Both had peak PAC >5 ng/dl (0.14 nmol/l) supporting the diagnosis of SAI. In two patients with ACTH >100 pg/ml (22.2 pmol/l), we hypothesize that the HPA axis was recovering from corticotrope suppression causing increased ACTH production in an effort to stimulate atrophied adrenal gland(s). Two case reports describe a similar increase in ACTH levels during HPA recovery in patients with hypophysitis^{15,16} In these two patients, aldosterone levels remained below 4 ng/dl making PAI a possible diagnosis, if only cortisol and aldosterone were considered. There are two other possible explanations. First, although acute stimulation of the adrenal by ACTH results in a rise in aldosterone, chronic stimulation by ACTH results in pre-infusion suppressed aldosterone levels⁸ If these two patients had chronically elevated ACTH levels, this might explain their lack of aldosterone response to an acute infusion of ACTH (Cortrosyn™). Second, liberal dietary intake of sodium might have suppressed aldosterone levels, as their urine Na⁺ levels were 169 and 211 mmol/l. Thus, in patients suspected of SAI, spoturine Na⁺ levels may help identify patients in whom stimulated PAC will be misleading.

In the SAI group, spot urine Na⁺ levels showed a strong and significant correlation with peak, delta and percent change PAC. This relationship was not seen in the other groups. In the PAI group, no correlation might be expected because destruction of the zona glomerulosa and lack of aldosterone production are inherent parts of the disease. The wide range in urine Na⁺ and PRA levels mitigates against the possibility that the fludrocortisone doses used suppressed PRA and prevented maximal PAC stimulation (Table 2).

The lack of a relationship between urine Na⁺ and stimulated aldosterone in the HV population is curious. The range of spot urine Na⁺ values in the HV population was broad, and 24-h urine Na⁺ levels, which may more accurately represent total body sodium balance, were not measured. PAC decreases in response to sodium loading and increases in response to sodium deprivation.^{5,11} In a small study of healthy volunteers, the most important determinant of PAC response to Cortrosyn™ stimulation was Na⁺ balance. On a 4 g Na/day diet, 24-h urine Na⁺ levels showed wide variability, which decreased when subjects on a 2 g vs 8 g Na⁺ diet were compared. The magnitude of rise in PAC in response to Cortrosyn™ was significantly dampened by the high salt diet.¹⁷ Based on these data, we speculate that the HV population was not taking a very high sodium diet.

The limitations of this study include lack of 24-h urine Na⁺ excretion and dietary sodium intake to corroborate the spot urine sodium levels and not further validating this data in a new group of patients suspected for adrenal insufficiency. However, the data presented provide validation of the 250 µg CortrosynTM-stimulated PAC as a diagnostic tool to differentiate PAI from SAI, using a stimulated PAC of 5 ng/dl (0.14 nmol/l). This conclusion is further supported by the fact that exclusion of patients on interfering medications as well as patients whose initial aldosterone levels were measured by RIA did not alter our conclusions or affect the optimal cut-off. Our results also suggest that measurement of urine spot Na⁺ levels might guide the interpretation of stimulated PAC results, although future studies are needed to confirm this proposal.

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Fig. 1.

(a) Baseline and peak cortisol values in healthy volunteers (HV - dotted line), and subjects with primary (PAI - long/short dashed line) and secondary (SAI - solid black line) adrenal insufficiency (b) baseline (circles) and CortrosynTM-stimulated peak (triangles) aldosterone levels in healthy volunteers (HV), and subjects with primary (PAI) and secondary (SAI) adrenal insufficiency.

Fig. 2. Correlation between urine spot sodium levels and (a) delta CortrosynTM-stimulated peak and (b) percent change CortrosynTM-stimulated aldosterone levels in subjects with secondary adrenal insufficiency.

Enrolment details and demographic data for patients with primary (PAI) and secondary (SAI) adrenal insufficiency and healthy volunteers (HV)

Table 1.

Diagnosis	PAI	SAI	HV1	HV2	HV combined
Enrolled	<i>n</i> = 29	<i>n</i> = 30	<i>n</i> = 60	<i>n</i> = 30	
Excluded	High creatinine (<i>n</i> = 1) CS exposure (<i>n</i> = 1) No PAC (<i>n</i> = 1)	Low K ⁺ , 2.9 mmol/l (<i>n</i> = 1)	No PAC (<i>n</i> = 42) low K ⁺ , 5.3 mmol/l (<i>n</i> = 1) low Na ⁺ , 126 mmol/l (<i>n</i> = 1)		
Analysable data	<i>n</i> = 26	<i>n</i> = 29	<i>n</i> = 16	<i>n</i> = 30	<i>n</i> = 46
Age (years)	46 ± 18	46 ± 18	56 ± 9	45 ± 13	49 ± 13
BMI (kg/m ²)	25.0 ± 4.5	28.2 ± 6.9	27.2 ± 4.4	25.4 ± 4.1	26.0 ± 4.3
% Women	77	62	56	50	52
Race (% White)*	100	93	80	62	68

HV1, healthy volunteers group 1; HV2, healthy volunteers group 2; HV, combined HV1 and HV2 groups; CS, high-dose systemic corticosteroid; PAC, plasma aldosterone concentration(s).

* *P* < 0.001 among groups; no other between group comparisons were statistically significant.

Table 2.

Electrolyte and plasma ACTH values in all groups (median [IQR])

	HV	PAI	SAI
Plasma renin activity (ng/ml/h)	0.60 [0.60, 0.90] [*] <i>n</i> = 41	2.15 [0.60, 3.70] [‡] <i>n</i> = 26	0.65 [0.60, 1.20] <i>n</i> = 28
Plasma potassium (mmol/l)	4.2 [4.0, 4.4] [‡] <i>n</i> = 45	3.8 [3.7, 4.2] <i>n</i> = 21	3.9 [3.7, 4.1] <i>n</i> = 24
Spot urine sodium (mmol/l)	113 [73.0, 150.5] <i>n</i> = 28 (HV2 only)	98.5 [61.5, 140.5] <i>n</i> = 20	80.0 [45.0, 129.0] <i>n</i> = 18
ACTH (pg/ml)	13.8 [9.4, 21.4] <i>n</i> = 30 (HV2 only)	251.0 [33.2, 938.0] [§] <i>n</i> = 21	8.8 [5.0, 16.1] <i>n</i> = 26

HV, healthy volunteers, represent combined HV1 and HV2 group data; PAI, primary adrenal insufficiency; SAI, secondary adrenal insufficiency, HV2, healthy volunteer group 2; To convert to SI units for cortisol, multiply presented value by 27.5862; for aldosterone, multiply by 0.0277; for PRA, multiply by 0.2778; for ACTH, multiply by 0.2222.

^{*} HV vs PAI, *P* < 0.001;

[‡] PAI vs SAI, *P* = 0.007;

[‡] HV vs PAI, *P* < 0.001, and HV vs SAI, *P* < 0.001;

[§] PAI vs SAI, *P* < 0.001.