Research paper

Intramuscular administration of $ACTH_{1-24}$ *vs.* 24-hour blood sampling in the assessment of adrenocortical function

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ABSTRACT

The standard intravenous short Synacthen® test (SSST) has long been accepted as one of the most reliable diagnostic tests of adrenocortical insufficiency. Intramuscular (i.m.) administration of ACTH obviates the need of venous cannulation and can be used as an alternative to the intravenous test. Nevertheless, reports of correlation between cortisol response to i.m. $ACTH_{1-24}$ and 24-hr average cortisol concentration are scarce. We studied this relation in 64 nonobese healthy men. Blood samples for serial cortisol measurements were collected hourly over 24 hrs. The following day, blood samples were collected at baseline and at 30 and 60 min after intramuscular (IM) administration of 250 μg of $ACTH_{1-24}$. All healthy men reached 24-hr serum cortisol peak values (C_{max}) between 0600h and 1000h. Following i.m. $ACTH_{1-24}$, cortisol levels significantly increased at both 30 ($C_{30}ACTH$) and 60 ($C_{60}ACTH$) minutes, when compared to baseline values. $C_{30}ACTH$ and $C_{60}ACTH$ significantly correlated with C_{max} and with the 24-hr time-integrated cortisol concentration (AUC_{0-24}). Morning mean cortisol was calculated as the average of serum concentrations measured between 0600h and 1000h ($C_{av}6-10$) and correlated very well with AUC_{0-24}. In conclusion, we comfirmed that i.m. administration of $ACTH_{1-24}$, followed by a single blood sampling at 60 min for cortisol measurement represents a valid, convenient and cost- effective screening test of adrenal function.

Key words: Adrenocorticotropin, Cortisol, Diagnostic use, Intramuscular injection

INTRODUCTION

Several tests are currently available to assess the integrity of the hypothalamic-pituitary-adrenal (HPA) axis. However, the reliability and accuracy of such tests in case of suspected adrenal insuffi-

ciency remains a matter of debate. Because of the circadian rhythmicity and pulsatility of cortisol concentration, a single determination of serum levels of this hormone at 0800h reflects the HPA axis activity in a representative, albeit gross fashion, since it does not provide reliable information on adrenocortical function during the rest of the day[1-5]. On the other hand, 24-hr serum cortisol, which overcomes this limitation, has the inconvenience of requiring overnight hospitalization[6]. The cortisol response to insulin-induced hypoglycemia is one of the most valuable tests of adrenal function; yet, it is cumbersome, unpleasant and potentially dangerous[7].

The standard short Synacthen® test (SSST) is peformed by administering 250 µg/1.73 m^2 body surface area of synthetic adrenocorticotropic hormone (ACTH$_{1-24}$) intravenously (i.v.) or intramuscularly (i.m.). Both tests have long been accepted as reliable screening procedures in patients with a compromised adrenocortical reserve[8-12]. When performed in patients with adrenal insufficiency at any time of the day, the SSST can be diagnostic[13,14]. A normal response to SSST is defined as a peak serum cortisol of ≥440 or ≥500 nmol/L after i.m or i.v. ACTH$_{1-24}$ administration, respectively[1,15-19]. However, normal subjects show a ten-fold difference in 24-hr urinary free cortisol measurments pre- and post-Synachten®[20]. This finding, as well as actual measurements of ACTH$_{1-24}$ concentrations, indicates that a Synacthen® dose of 250 µg is pharmacologic. Thus, we sought to study the quantitative concordance between serial serum cortisol measurements and SSST in healthy subjects.

SUBJECTS AND METHODS

Subjects

Sixty four nonobese healthy men (mean ± SD; age: 24±5.4 yrs; BMI: 22.6±2.3 kg/m^2) were included in the study. The subjects belonged to the placebo group of a study on the effect of budesonide and other inhaled corticosteroids on the HPA axis conducted by Schering-Plough in the Netherlands. Patients who had history of major physical or psychiatric illness, food or drug allergy, smoking or alcohol abuse, or tested positive for hepatitis B, C or HIV were excluded from the study. None of the enrolled subjects had clinical or biochemical evidence of hepatic and renal disease, or had been taking any drug known to alter cortisol binding globulin (CBG) concentrations or induce hepatic enzymes for at least one month prior to the study. The study was approved by the Medical Ethics Committee of the "Stichting Beoordeling Ethiek Bio-Medisch Onderzoek", Assen, The Netherlands.

Methods

Patients were admitted to the hospital two days prior to the study and standard anthropometric measurements were obtained by a single trained observer. An indwelling venous catheter was inserted at least 12 hours before sampling. Patients were permitted normal ambulatory activity, but strenuous physical exercise was avoided. Standard hospital meals were given at 0800h, 1230h and 1730h.

On the first day of the study, baseline investigations were performed at 0800h, and blood samples for measurements of serum cortisol were collected hourly over 24 hours. The night before the SSST, all subjects remained supine from midnight to 0700h. On the second day of the study, 250 µg of ACTH$_{1-24}$ were injected i.m. at 1100h, and blood was obtained at baseline and at 30 and 60 minutes after ACTH$_{1-24}$ administration. Plasma was separated by centrifugation immediately after blood collection and stored at –20° C until assayed.

Cortisol assay

Serum total cortisol was measured using the Coat-A-Count radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). This is a solid phase assay with a sensitivity of 5.5 (nmol/L). The intra-assay coefficients of variation (CVs) were 5.7% and 2.6% at serum concentrations of 28.0 and 550.0 (nmol/L), respectively. The inter-assay CVs were 6.3% and 4.5% at serum concentrations of 140.0 and 275.0 (nmol/L), respectively.

Data analyses

Non-normally distributed data were logarithmically transformed prior to statistical analysis. Time-integrated cortisol concentration was calculated as the area under the curve of serum concentrations over 24 hours (AUC$_{0-24}$), using the trapezoid method. Cortisol peak was calculated as the highest se-

rum concentration measured in 24 hours (C_{max}). Morning mean cortisol was calculated as the average of serum concentrations measured between 600h and 1000h ($C_{av}6\text{-}10$).

One-way analysis of variance (ANOVA) with the Student-Newman-Keuls (SNK) post hoc correction was used to compare cortisol responses to $ACTH_{1\text{-}24}$ at 30 ($C_{30}ACTH$) and 60 ($C_{60}ACTH$) min vs. baseline values. Correlations between C_{max} or $AUC_{0\text{-}24}$ and $C_{30}ACTH$ or $C_{60}ACTH$ were tested by linear regression and calculation of Pearson's correlation coefficient. All reported values are expressed as mean ± SD, unless otherwise specified. Statistical differences are denoted by a Bonferroni-corrected P value of < 0.05.

RESULTS

All subjects reached 24-hr cortisol peak values (C_{max}, 427.6 ± 60.7 nmol/L) between 0600h and 1000h. C_{max} was attained between 0800h and 0900h in 58 subjects (90.7%).

$C_{30}ACTH$ and $C_{60}ACTH$ were both significantly increased when compared to baseline values (515.9 ± 66.2 and 584.9 ± 69.0 vs. 242.8 ± 85.5 nmol/L, respectively, $P < 0.001$). Peak cortisol responses to $ACTH_{1\text{-}24}$ were observed at 60 minutes in 61 subjects (95.3%) and at 30 min in the remaining 3 (4.7%). All the subjects achieved a peak cortisol response of ≥440(nmol/L).

Both $C_{30}ACTH$ and $C_{60}ACTH$ showed correlation with C_{max} ($r = 0.454$ and 0.459 respectively, $P < 0.001$) (Figure 1, A and B) and $AUC_{0\text{-}24}$ ($r = 0.549$ and 0.537, respectively, $P < 0.001$) (Figure 1, C and D). Additionally, we found positive correlations between $C_{av}6\text{-}10$ and $AUC_{0\text{-}24}$ ($r = 0.709$, $P < 0.0001$) (Figure 2A), $C_{30}ACTH$ ($r = 0.249$, $P < 0.05$) (Figure 2B) and $C_{60}ACTH$ ($r = 0.226$, $P = 0.07$) (Figure 2C).

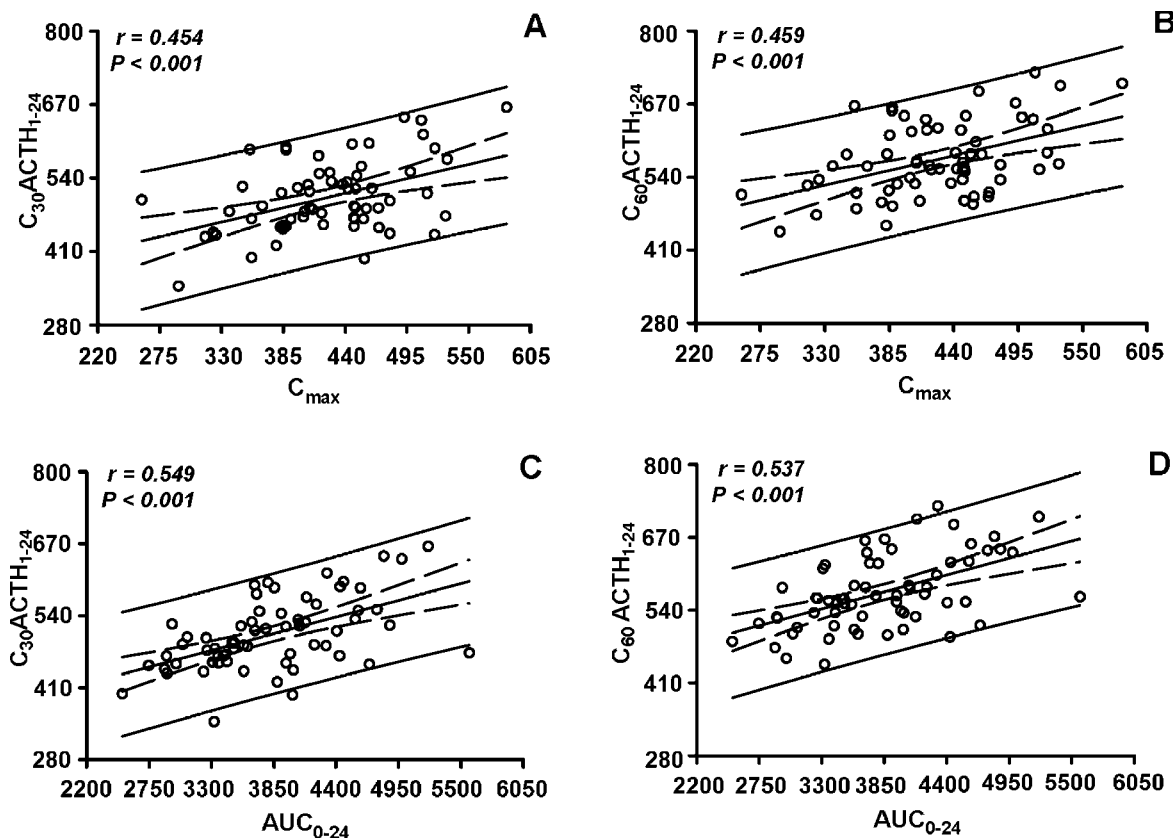**Figure 1.** Scatterplots of cortisol responses to i.m. $ACTH_{1\text{-}24}$ at 30 ($C_{30}ACTH_{1\text{-}24}$) and 60 ($C_{60}ACTH_{1\text{-}24}$) min vs. 24-hr serum cortisol peak (C_{max}) (A and B, respectively) and 24-hr time-integrated cortisol concentration ($AUC_{0\text{-}24}$) (C and D, respectively). Regression lines are shown with their 95% confidence intervals. Cortisol concentrations are in nmol/L, AUC are in nmol/L x h.
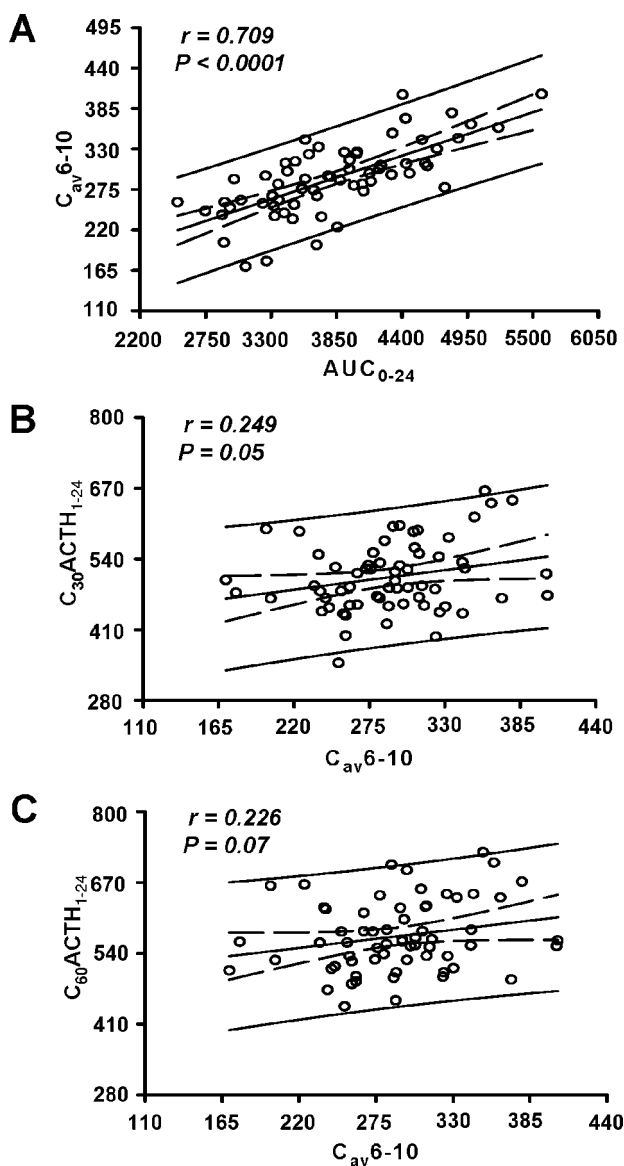
A

$r = 0.709$
$P < 0.0001$

(y-axis: C_{av}6-10; x-axis: AUC_{0-24})

B

$r = 0.249$
$P = 0.05$

(y-axis: $C_{30}ACTH_{1-24}$; x-axis: C_{av}6-10)

C

$r = 0.226$
$P = 0.07$

(y-axis: $C_{60}ACTH_{1-24}$; x-axis: C_{av}6-10)

Figure 2. Scatterplots of morning serum cortisol average between 0600h and 1000h (C_{av}6-10) *vs.* AUC_{0-24} (A), C_{30} $ACTH_{1-24}$ (B) and C_{60} $ACTH_{1-24}$ (C). Regression lines are shown with their 95% confidence intervals. Cortisol concentrations are in nmol/L, AUC are in nmol/L x h.

DISCUSSION

Several medical conditions can alter adrenocortical homeostasis causing abnormal adrenal function. Chronic glucocorticoid treatment, infections, autoimmune adrenalitis, infiltrative or neoplastic diseases, as well as congenital or acquired abnormalities of the HPA axis are among the most common causes of adrenal insufficiency[11]. Unfortunate-

ly, many symptoms related to adrenal failure, such as weight loss, anorexia and fatigue are nonspecific.

Many laboratory tests are currently available to assess the integrity of the HPA axis, each one with its own advantages and limitations[1-5]. The use of synthetic $ACTH_{1-24}$ given i.v. or i.m. at low or high dose (500 ng or 250 µg/1.73 m^2 body surface area, respectively) are among the most frequently used test in clinical practice. The low-dose ACTH test, which may offer high reliability in the diagnosis of mild forms of primary hypoadrenalism, is less sensitive in the diagnosis of secondary hypoadrenalism[21]. Serial measurements of serum cortisol levels over 24 hours provide accurate information about the functional status of the HPA axis, given the ability to reveal pitfalls in the pulsatility and circadian rhythm of cortisol concentration[6]. However, they are costly and often subject to low compliance from the patient.

Cortisol values of ^{3} 440 (nmol/L) in response to i.m. $ACTH_{1-24}$ were detected in all of our study subjects. The large majority reached a cortisol peak response at 60 min, suggesting that blood sampling at 30 min may be unnecessary during i.m. SSST.

These findings, while confirming our previous observations[16], strongly support prior reports of similar cortisol responses with similar cut-off values after i.m. and i.v. $ACTH_{1-24}$ administration[1,19,22]. Furthermore, we showed for the first time that cortisol responses to i.m. $ACTH_{1-24}$ correlates with 24-hr cortisol serum peak (C_{max}) and time-integrated concentration (AUC_{0-24}), which are both reliable indices of HPA axis endogenous activity. Interestingly, we found that morning serum cortisol average between 0600h and 1000h (C_{av}6-10) correlates well with AUC_{0-24}.

Among the limitations of this study, we have to point out that we did not study patients with adrenal disease, but rather attempted to define normal relations between SSST and serial serum cortisol measurements. Another caveat is that we did not compare SSST against the gold standard insulin tolerance test for (ITT) for cortisol.

In conclusion, our results show that i.m. administration of $ACTH_{1-24}$, followed by a single cortisol

measurement at 60 min, is comparable to 24hour cortisol sampling and represents a valid and rapid screening test of adrenal function in healthy men. In combination with this test, the $C_{av}6$-10 may be a more reliable index of cortisol morning peak than the single morning measurement of plasma cortisol. Further validation of these results in patients with adrenal insufficiency is warranted.

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