Dehydroepiandrosterone Sulfate in the Assessment of the Hypothalamic-Pituitary-Adrenal Axis

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Context: The role of dehydroepiandrosterone-sulfate (DHEA-S) in assessing the integrity of the hypothalamic-pituitary-adrenal (HPA) axis in patients with suspected insufficiency is uncertain.

Objective: The objective of the study was to prospectively evaluate the diagnostic value of DHEA-S on HPA function in consecutive patients with suspected HPA insufficiency with and without pituitary lesions at a tertiary referral center.

Design and Patients: In 70 consecutive patients, insulin tolerance test was accompanied by measurement of basal DHEA-S. Assessment of HPA axis was based on peak cortisol response in insulin tolerance test (normal $\geq$ 550 nmol/liter). To account for the age and gender dependency of DHEA-S, a z-score was calculated using age- and gender-specific reference values of the assay.

Results: Individuals with HPA insufficiency had significantly lower z-scores than those with normal HPA function (-1.66 *vs.* -0.62, $P < 0.0001$). In individuals up to 30 yr of age, a z-score of -2.0 had 100% sensitivity and specificity regarding HPA function [area under receiver operating characteristics (ROC) curve 1.00], whereas z-scores proved less useful in older individuals. In individuals with pituitary macroadenoma, a z-score below -2.0 had 100% specificity to predict HPA insufficiency (area under ROC curve 0.82). In the absence of a pituitary adenoma, the diagnostic value of the z-score was reduced (area under ROC curve 0.71).

Conclusions: Individuals with HPA insufficiency have lower z-scores for DHEA-S than those with normal HPA function. There is evidence that a z-score could be of diagnostic value in assessing HPA integrity, especially in younger patients and patients with pituitary macroadenoma, but further studies are needed to consolidate these findings. **(*J Clin Endocrinol Metab* 93: 539–542, 2008)**

The diagnosis of an insufficiency of the hypothalamic-pituitary-adrenal (HPA) axis remains challenging in clinical practice. Basal serum cortisol levels, although easy to measure, were found to be of limited value (1, 2), and limitations were also reported for the short ACTH test (3–5). The insulin tolerance test (ITT) is therefore still considered the gold standard in assessing the integrity of the HPA axis. However, the ITT is cumbersome, requires experienced staff and close supervision, and has several contraindications. Dehydroepiandrosterone and its sulfate (DHEA-S) are androgen precursors that are produced by the adrenal cortex. Their secretion is, at least partially, controlled by ACTH (6). DHEA-S is secreted in a large plasma pool and therefore exhibits a longer half-life and fewer circadian variations, compared with dehydroepiandrosterone (6, 7). DHEA-S levels are influenced by several factors, most importantly age and gender (6, 8), and several studies reported low DHEA-S levels in patients with documented hypopituitarism and HPA insufficiency (9, 10). In particular, in patients with a newly diagnosed large pituitary tumor, DHEA-S levels appeared to be a reliable prognostic parameter for the diagnosis of HPA insufficiency (3). In contrast, comparably little is known on the prognostic value of DHEA-S in assessing HPA function in a mixed population of individuals with and without a pituitary lesion.

The aim of the present study was to prospectively evaluate the

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2007-1780 Received August 8, 2007. Accepted October 30, 2007.
First Published Online November 6, 2007

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Abbreviations: DHEA-S, Dehydroepiandrosterone-sulfate; HPA, hypothalamic-pituitary-adrenal; ITT, insulin tolerance test; ROC, receiver operating characteristic.

diagnostic value of DHEA-S adjusted for age and gender in assessing the integrity of the HPA axis in consecutive patients with suspicion of HPA insufficiency at a tertiary referral center.

Subjects and Methods

Design and participants

The study started on January 1, 2003, and ended on December 31, 2006. Consecutive adult individuals with a suspicion of HPA insufficiency and without contraindications to ITT were included in the study. Primary adrenal insufficiency was ruled out based on basal ACTH levels and absence of clinical features. Each ITT was accompanied by a basal measurement of DHEA-S. All procedures were carried out in accordance with the local ethical guidelines, and informed consent was obtained for each test. Testing was conducted in an ambulatory setting. Patients arrived at 0800 h after an overnight fast. Glucocorticoid medication was stopped at least 24 h before the ITT. After measurement of a basal glucose value, the test was started by iv injection of 0.1–0.2 U/kg body weight insulin (Insulin Actrapid HM; Novo Nordisk A/S, Copenhagen, Denmark) to induce hypoglycemia. The patients remained supine and were constantly supervised by an experienced nurse. Testing was performed under ECG surveillance. Samples were drawn for glucose every 15 min for 2 h and as needed due to hypoglycemic symptoms. Samples were drawn for cortisol at −15, 0, 30, 45, 90, and 120′.

Test quality was defined as adequate if a plasma glucose of 2.2 mmol/liter or less was achieved and hypoglycemic symptoms (perspiration, feelings of hunger, drowsiness) were present. Function of the HPA axis was defined as normal if a cortisol response of 550 nmol/liter or greater (peak level) was achieved (11, 12).

Analytical methods

Glucose was measured by the hexokinase method (HemoCue B-glucose analyzer; HemoCue, Ángelholm, Sweden). Cortisol was measured by competitive immunoassay (Roche Modular E170; F. Hoffmann-La Roche, Diagnostics Division, Basel, Switzerland). Serum DHEA-S was measured by competitive chemiluminescence immunoassay (Immulite; Diagnostic Products Corp., Los Angeles, CA).

Statistical analysis

After checking for normality, continuous outcomes were compared using Student's *t* test. Pearson χ^2/Fisher's exact tests were applied for comparing categorical outcomes. A $P < 0.05$ signified statistical significance. To account for the dependency of DHEA-S on age and gender, a z-score was calculated using reference curves that were developed by fitting two separate half-normal distributions to the age and gender-specific reference ranges provided by the assay manufacturer (13). The z-score expresses how far in units of the population SD a measured value is from the mean of a population with corresponding age and gender. A z-score of −1 indicates that the measured value is 1 SD below the mean of a reference population of the same age and gender. The diagnostic value of the z-score in predicting the function of HPA axis was assessed by performing receiver operating characteristics (ROC) analyzes. To optimize comparability with earlier reports (3), three age groups were prespecified: 30 yr or younger, 31–50 yr, and 51 yr or older. In addition, separate analyses were performed according to presence or absence of pituitary adenoma. All statistical analyses were done with Stata 9.1 (Stata Corp., College Station, TX).

Results

Between 2003 and 2006, a total of 74 ITTs were performed to assess the integrity of the HPA axis. In four cases the tests did not fulfill the prespecified quality criteria, and these patients were excluded from the analysis. Consequently, the present analysis was based on 70 ITTs. Baseline characteristics and indications for ITT are presented in Table 1. Whereas 35 patients had a pituitary adenoma (31 macroadenomas, four microadenomas), there were no differences in the indications for ITT between the two groups and throughout different age groups with, in particular, a similar proportion of pituitary adenoma. A total of 40 subjects revealed an insufficient HPA axis, defined as a peak cortisol of less than 550 nmol/liter in response to the ITT, whereas the HPA axis was normal in 30 subjects. Age ranged from 14 to 70 yr, and subjects with an insufficient HPA axis were

TABLE 1. Patient characteristics

	HPA axis insufficient (n = 40)	HPA axis normal (n = 30)
Gender (female/male)	17/23	16/14
Age (yr) (mean ± SD)	47.7 ± 12.0	39.6 ± 13.0[a]
Body mass index (kg/m^2) (mean ± SD)	26.3 ± 6.1	25.1 ± 5.2
DHEA-S (μg/ml) (median, interquartile range)	0.47, 0.24–0.71	1.25, 0.96–1.80[a]
z-score (mean ± SD)	−1.66 ± 1.53	−0.62 ± 1.08[a]
Indications for ITT		
Pituitary adenoma	22	13
Macroadenoma	21	10
Microadenoma	1	3
Other sellar tumor	4	
Other pituitary disease	1	
Traumatic brain injury		2
Biochemical insufficiency of other pituitary axis	3	6
Clinical features and inconclusive cortisol levels[b]	10	9
Fatigue, impaired physical performance	4	5
Hypoglycemia[c]	3	
Other	3	4

[a] $P < 0.01$ for difference between individuals with insufficient and individuals with sufficient HPA axis.

[b] Patients with suspicion of HPA insufficiency based on personal history, symptoms, and signs, but without documented lesion to pituitary or sellar region, primary adrenal insufficiency was excluded, cortisol levels were between 200 and 500 nmol/liter.

[c] Other causes for hypoglycemia were excluded before testing.

generally older than those with a normal HPA axis ($P < 0.01$). In contrast, no differences in gender or body mass index were found between the two groups. Levels of DHEA-S ranged from 0.3 to 4.8 μg/liter and were significantly lower in patients with insufficient compared with individuals with normal HPA function (median 0.47 *vs.* 1.25 μg/ml, $P < 0.01$). Similarly, the mean z-score adjusted for age and gender was lower in individuals with HPA insufficiency (-1.66 *vs.* -0.62 for insufficient and normal HPA axis, respectively, $P < 0.01$).

Figure 1 shows the distribution of the age- and gender-specific z-scores of DHEA-S for the three prespecified age groups and individuals with and without pituitary adenoma by HPA axis function. In individuals up to 30 yr old with insufficient HPA axis, the mean z-score was significantly lower than in individuals with normal HPA function (-4.03 *vs.* -0.38, $P < 0.0001$). A z-score of -2.0 (*i.e.* 2 SD below the mean of a population with corresponding age and gender) perfectly predicted the outcome of the ITT (sensitivity and specificity of 100%, area under the ROC curve of 1) (Fig. 2A).

In the age group between 31 and 50 yr, similar z-scores were found in individuals with normal and insufficient HPA axis (-0.88 and -0.72, $P = 0.75$) (Fig. 1), and the prognostic value of the z-score was low (area under the ROC curve 0.57) (Fig. 2A). In individuals older than 50 yr, the z-score was again significantly lower in individuals with an insufficient HPA axis than in those with a normal HPA function (-1.62 *vs.* 0.56, $P = 0.02$) (Fig. 1). The area under the ROC curve of the corresponding ROC curve was 0.77, and a cutoff value for the z-score at -1.42 correctly predicted the outcome of the ITT in 70% of the cases.

Separate analysis of individuals with a pituitary macroadenoma revealed a significantly lower z-score if HPA axis was insufficient than if it was normal (-1.73 *vs.* -0.61, $P = 0.003$) (Fig. 1). The area under the corresponding ROC curve was 0.82 (Fig. 2B) and a z-score below -2.0 had a specificity of 100%, whereas a z-score above 0.5 had a sensitivity of 100% in predicting outcome of ITT. The z-score had a lower prognostic value in individuals without a pituitary adenoma (area under the ROC curve 0.71) (Fig. 2B). A specificity of 100% was reached with a z-score below -2.5, whereas sensitivity was 100% with a z-score above 2.2.

Discussion

The findings of the present study in individuals with suspected insufficiency of the HPA axis are 2-fold: 1) the age- and gender-specific z-score for DHEA-S is lower in individuals with HPA insufficiency than in those with a normal HPA function; and 2) there is some evidence that a z-score for DHEA-S is of prognostic value in predicting HPA function in younger individuals as well as in patients with a pituitary macroadenoma.

To the best of our knowledge, this is the first study assessing the diagnostic value of DHEA-S on HPA function using an age- and gender-specific z-score in a mixed population of individuals with and without a pituitary lesion. Half of the patients in our study had a pituitary tumor, but the incidence of adenoma was comparable within each age group. Together with the fact that indications for testing were generally comparable throughout age-groups, confounding by indication is unlikely to have occurred. An earlier report in a similar number of patients comparing DHEA-S with ITT was limited to patients with large pituitary adenomas exclusively (3). The authors of this study reported an excellent diagnostic performance of DHEA-S with an area under the ROC curve of almost 1. In accordance with this report, we found a z-score of DHEA-S to have an acceptable prognostic value in the presence of a pituitary macroadenoma, although in our study, the area under the ROC curve was lower. Potential explanations for these differential findings are 3-fold: first, in contrast to the study of Nasrallah and Arafah (3), the patients in the present study were all tested after pituitary surgery. Noteworthy, it was shown before that HPA function can be restored after surgical treatment of macroadenoma (14). Second, Nasrallah and Arafah used a lower cutoff value for cortisol to define HPA integrity (510 *vs.* 550 nmol/liter). We additionally performed a sensitivity analysis using the lower value and found results unaffected (data not shown). Third, we found some evidence that a

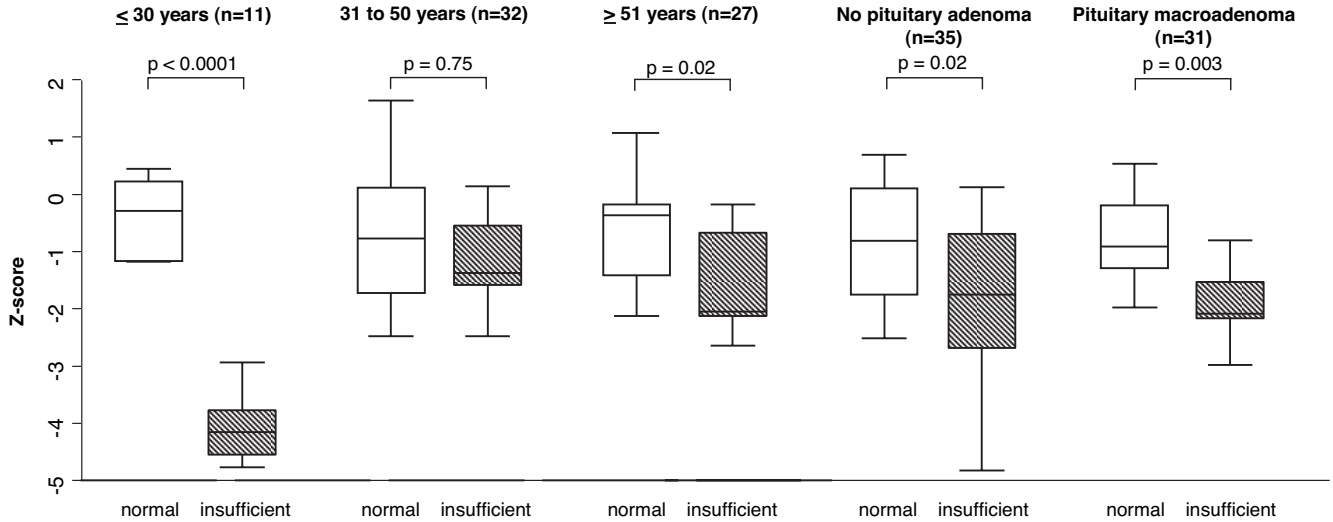

FIG. 1. Box plots of age- and gender-specific z-scores of DHEA-S for three age groups (≤30 yr, 30–50 yr, ≥51 yr) and individuals with pituitary macroadenoma and without pituitary adenoma according to integrity of the HPA axis in ITT; individuals with pituitary microadenoma (n = 4) were excluded from the analysis; insufficient denotes peak level of cortisol in ITT less than 550 nmol/liter; normal denotes peak level of 550 nmol/liter or greater.

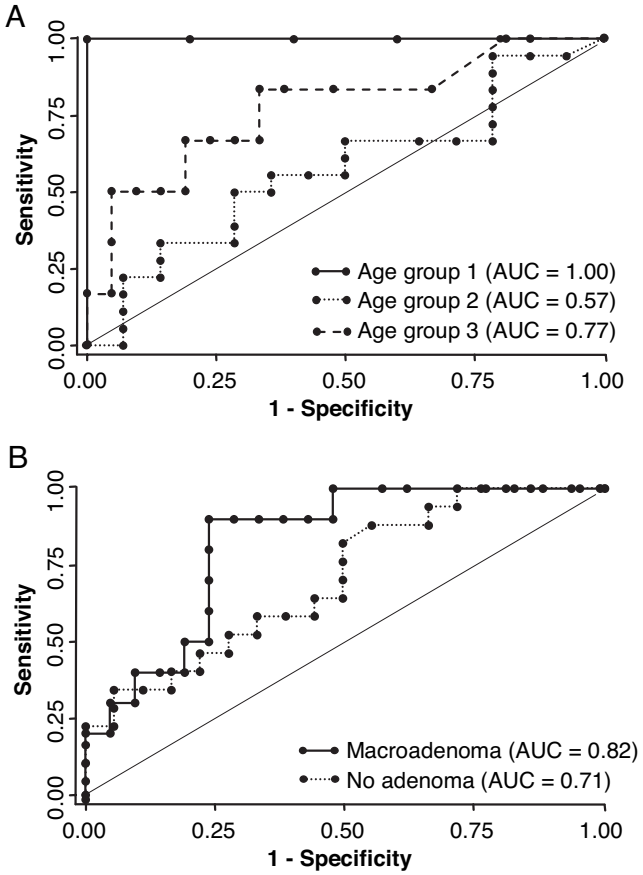

FIG. 2. ROC curves for z-scores of DHEA-S according to age group (group 1, ≤30 yr; group 2, 31–50 yr; group 3, ≥51 yr) (A) and according to presence or absence of pituitary macroadenoma (B); the curves are drawn based on the results of the ITT as the reference. AUC, Area under the ROC curve.

z-score for DHEA-S has an advantage in predicting HPA integrity in younger individuals. Patients with macroadenoma and insufficient HPA axis were older in the present study than in the report by Nasrallah and Arafah (3), and this may thereby have weakened the prognostic value of DHEA-S in our population.

The age dependency in the diagnostic value of a single biochemical measure on pituitary function is not a unique finding. It was shown previously that the diagnostic reliability of IGF-I in predicting the integrity of the GH axis is best in young individuals (15). Higher absolute levels as found in younger individuals might improve the discriminatory power of DHEA-S. Furthermore, it could be hypothesized that the regulatory influence of nonpituitary factors on DHEA-S secretion (6) may increase with age, thereby further weakening its diagnostic value.

A strength of the present study is the prospective design that allowed us to simultaneously compare DHEA-S with the ITT, the gold standard. The calculation of an age- and gender-specific z-score accounted for the dependency of DHEA-S on these two parameters. The inclusion of patients with and without pituitary lesions enabled us to assess the value of DHEA-S in a population reflecting daily clinical practice at our endocrine division. Nevertheless, we acknowledge several limitations of our study. First, although in view of the complexity of the ITT, a sample size of 70 individuals can be regarded a considerable number, the statistical power of subgroup analyses is clearly limited. Therefore, we feel that especially our findings related to the subgroup of younger patients have to be considered with particular care. Second, we used an assay that has a comparably high lower limit of detectability when compared with other reports (3). However, the cutoff levels that were found to be of diagnostic value were clearly above the lower limit of the assay.

In conclusion, individuals with and without pituitary adenoma suffering from HPA insufficiency have lower z-scores for DHEA-S than those with normal HPA function. We found evidence that a z-score could be of diagnostic value in assessing HPA integrity, especially in younger patients as well as patients with pituitary macroadenoma, but further studies are needed to consolidate these findings.

Acknowledgments

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C. S. is a PROSPER fellow supported by the Swiss National Science Foundation (Grant 3233B0-115212).

Disclosure Information: All authors have nothing to declare.

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