Comparison of low dose and standard dose adrenocorticotropic stimulation tests in healthy Nigerians.

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Summary
Hypothalamo-pituitary-adrenal (HPA) axis dysfunction is a potentially life-threatening condition. It is of paramount importance that safe, reliable diagnostic tests be available to identify patients at risk for adrenal insufficiency. The 250 μg Adrenocorticotropic hormone (ACTH) stimulation test is commonly used to assess adrenocortical function. The 250 μg dose is supraphysiological, therefore several investigators, over the years, have used 1 μg ACTH stimulation test to assess adrenocortical function. The aim of the study was to compare the response of healthy adult Nigerian subjects to the 250 μg and 1 μg ACTH tests. Ten healthy subjects, five males and five females, aged between 20-60 years, (mean, 38.7 years) participated in this study. They all had normal medical histories and physical examinations, were nonsmokers, and had never received any type of glucocorticoid therapy. Serum chemistries, full blood counts, erythrocyte sedimentation rate, were all within normal limits. Both low dose ACTH test and standard dose ACTH test were performed on the 10 subjects in a randomized order on different days. There was no statistically significant difference in mean serum cortisol levels between the two test doses at 30 minutes (928.4 vs 929.8 nmol/L). There was a strong correlation between 30-minute cortisol responses to 1 μg and 250 μg ACTH stimulation tests, \( r=0.999; p<0.001 \). In agreement with other published data, our study confirms that 1 μg ACTH stimulates adrenocortical secretion in normal subjects in the period 30 minutes post injection comparable to 250 μg ACTH testing.

Keywords: Cortisol; adrenocorticotropic hormone (ACTH); hypothalamic-pituitary-adrenal (HPA); low dose ACTH Nigerians.

Résumé
Le dysfonctionnement de l’axe hypothalamo-hypophysoïde est potentiellement une condition dangereuse pour la vie. Il est d’une importance capital que les diagnostiques tests justes et fiables soient disponibles pour identifier les patients à risque d’insuffisance adrénales. Le test de stimulation de l’hormone adrenocorticotrope est généralement utilisé pour déterminer les fonctions adrenocorticales. La dose de 250 μg est supra physiologique, cependant plusieurs investigateurs, pendant des années, ont utilisées la dose de 1 μg d’ACTH pour déterminer la fonction adrenocortique. Le but de l’étude était de comparer la réaction des sujets Nigériens adultes et bien portants aux tests de 250 μg et 1 μg ACTH. Dix sujets bien portants, cinq mâles et cinq femelles âgés entre 20-60 ans, (moyenne, 38.7 ans) ont participé à cette étude, ils avaient tous un passé médical normal et des examens physiques normaux, ils étaient non fumeurs et n’avaient jamais reçu un quelconque type de thérapie glucocorticoids. Le sérum chimique, le taux de sang, le taux de sédimentation de l’erythrocyte étaient tous dans les limites normales. La faible dose du test ACTH et la dose standard du test ACTH étaient faites sur les 10 sujets des jours différents. Il y avait statistiquement pas de différence significative sur la moyenne du niveau de sérum cortisol entre les deux doses tests à 30 minutes (928.4 Vs 929.8 nmol/L). Il y avait une forte corrélation entre la réaction à 30 minutes de cortisol au test de stimulation ACTH 1 μg et 250 μg, \( R=0.999; p<0.001 \). En accord avec les données publiées, notre étude confirme que 1 μg d’ACTH de sécrétion stimule adrenocortical sur les sujets normaux dans une période de 30 minutes après l’injection est comparable à 250 μg d’ACTH testé.

Introduction
Hypothalamo-pituitary-adrenal (HPA) axis dysfunction is a potentially life-threatening condition. It is of paramount importance that safe, reliable diagnostic tests be available to identify patients at risk for adrenal insufficiency. Assessment of the adrenal gland functional reserve with the short synacthen (synthetic adrenocorticotrophic hormone (ACTH)) test has long been accepted as the most reliable diagnostic screening procedure in patients with a clinical picture suggestive of adrenocortical hypofunction or in high-risk populations [1]. Classically, the recommended test is carried out with 250 μg synthetic ACTH 1–24, taking blood samples
for serum cortisol at baseline, 30 and 60 min after intravenous (i.v) or intramuscular (i.m) stimulation. Recent evidence, however, suggests that intravenous administration of 250 μg ACTH 1–24 could be a provocative pharmacological rather than a physiological stimulus [2,3,4]. Trials with a reduced number of healthy individuals have shown that 1, 5, 10 and 250 μg ACTH 1–24 i.v. are also able to produce similar serum cortisol peaks [2-7]. In addition, Dickstein et al. [2] evaluating patients on long-term glucocorticoid therapy, found low sensitivity of the standard ACTH testing at identifying secondary adrenal failure when contrasting it to 1 μg ACTH.

The standard dose ACTH stimulation test is a diagnostic test for patients suspected to have chronic adrenal insufficiency. This test involves determining serum cortisol responses immediately before, 30 and 60 minutes after intravenous administration of 250 μg of ACTH. This test directly measures only the functional integrity of the adrenal glands. It also provides an indirect assessment of hypothalamic and pituitary function because the adrenal glands depend on endogenous ACTH for its tropic effect [1]. When ACTH production is impaired by pituitary or hypothalamic disease, the adrenal glands lose the capacity to respond to exogenous stimulation because of adrenal atrophy [1]. A rise in serum cortisol concentration after 30 or 60 minutes to a peak of 18 to 20 μg/dL (500 to 550 nmol/L) or more is considered a normal response to high-dose ACTH stimulation test [2, 3].

It excludes the diagnosis of primary adrenal insufficiency. However, if secondary adrenal insufficiency is of recent onset, the adrenal gland will not have atrophied, and will still be capable of responding normally to ACTH stimulation [2,3]. In cases where a low-dose ACTH test or an insulin-induced hypoglycaemia may be required to confirm diagnosis [8].

The LDACTH stimulation test provides a more sensitive index of adrenocortical responsiveness because it results in physiologic plasma ACTH concentrations. It is performed by measuring serum cortisol concentration immediately before and 30 minutes after intravenous injection of ACTH in a dose of 1 μg [9]. This dose stimulates maximal adrenocortical secretion up to 30 minutes post injection.

In normal subjects it results in a peak plasma ACTH concentration about twice that of insulin-induced hypoglycaemia [3]. A value of 18 μg/dL (500 nmol/L) or more at any time during the test is indicative of normal adrenal function. The advantage of the LDACTH test is that it can detect partial adrenal insufficiency that may be missed by the standard high-dose test [8]. It is also preferred in patients with secondary or tertiary adrenal insufficiency.

Various studies have evaluated the basal cortisol, 30 minute and 60 minute cortisol response to the 250 μg short synacthen test (SST) [2,7,10,11]. It is well recognized that the 250 μg dose used in the conventional SST is a supraphysiological dose originally designed as a test for primary adrenal failure. Several groups have reduced this dose considerably and demonstrated that the cortisol response to 1 μg is equivalent to that obtained with 250 μg in normal subjects [2, 7, 10, 11]. The same result was demonstrated in people with pituitary disease. Low dose short synacthen test (LDSST) can replace the SST as demonstrated in these studies [2,7,10,11].

There are few studies in Nigeria that have examined adrenocortical function in healthy Nigerian subjects. These studies used the 250 μg ACTH in testing the adrenal gland. No study in Nigeria has utilized the 1 μg ACTH test. Adadevoh [12,13] measured plasma cortisol levels in normal Nigerians and concluded that plasma cortisol in Nigerians is comparable to those obtained in Caucasians and similar to those reported in east Africans. He used 26 normal Nigerian subjects as control in his study and compared their plasma cortisol levels to those obtained after insulin induced hypoglycaemic stress.

The purpose of this study was to compare the response of normal Nigerian subject to the 250 μg and 1 μg ACTH tests.

Materials and methods
Ten healthy subjects, five males and five females, aged between 20-60 years, (mean, 38.7 years) participated in this study. The subjects were randomly selected. The subjects all had normal medical histories and physical examinations, were nonsmokers, and had never received any type of glucocorticoid therapy. No subject had any contraindications to the performance of ACTH testing. These contraindications, though not absolute contraindications, include diabetes mellitus, hypertension, heart failure and pregnancy. They all have normal blood pressure. Serum chemistries, fasting plasma glucose, full blood counts, erythrocyte sedimentation rate, were all within normal limits. Both low dose ACTH test and standard dose ACTH test were performed on the 10 subjects on different days. The subjects were randomized to either 1 μg ACTH test or 250 μg ACTH at 48 hours interval. Standard requirement
of the ethics committee of the Lagos University teaching Hospital was complied with.

**Testing Protocol**
Both tests were performed in a random order and single blinded. The low dose 1μg ACTH test and the 250μg ACTH test were carried out between 08.00 hour and 09.00 hour. The subjects had an intravenous cannula inserted into a cubital vein and kept patent with heparinized saline. To prepare 1μg of ACTH solution, 1mL of ACTH solution was drawn from an ampoule containing 250μg/mL of ACTH. This was diluted with 24mL of normal saline to yield a concentration of 1μg/mL. 1mL of this solution, containing 1μg ACTH, was used as bolus low-dose injection. After resting for 30 minutes, 1μg of 1-24 ACTH or 250μg of 1-24 ACTH [Alliance Pharmaceuticals Ltd, Chippingham, Wiltshire SN15 2BB] was injected as a bolus. Blood samples were taken at 0 minute and 30 minutes after the injection colorimetric method for quantitative determination of cortisol concentration in serum.

**Statistical evaluation of data**
Results are expressed as means ± standard error of mean (SEM). Calculations and analysis were done using the SPSS 10.0 software. The serum cortisol values were transformed to logarithm before analysis. Statistical comparisons were made using the Student's t test for quantitative variables. The level of statistical significance was taken as p<0.05.

**Results**
Serum cortisol response values to both dosages of ACTH for both groups are as shown in Table 1. There were no statistical differences between basal cortisol and stimulated cortisol values using the LD and SD ACTH tests.

Both low and high ACTH group reached serum cortisol levels greater than 500nmol/L at 30

**Table 1:** Comparison of cortisol value in response to LD and SD ACTH test (Mean ± SEM), (Range)

<table>
<thead>
<tr>
<th>Type of Value</th>
<th>Mean±SEM Concentration (nmol/L)</th>
<th>(Range) Serum</th>
<th>Cortisol P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 minute</td>
<td>291.70±37.47 (187.61-579.39)</td>
<td>296.62±38.66 (171.06-568.35)</td>
<td>0.501</td>
</tr>
<tr>
<td>30 minutes</td>
<td>928.40±92.24 (510.42-1338.12)</td>
<td>929.84±89.39 (529.14-1340.63)</td>
<td>0.777</td>
</tr>
<tr>
<td>Increment</td>
<td>636.70±104.06 (96.57-1013.35)</td>
<td>633.22±102.24 (108.67-1013.19)</td>
<td>0.664</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>250μg ACTH</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
</table>

**Table 2:** Comparison of cortisol value in response to LD and SD ACTH test in male and female subjects (Mean±SEM).

<table>
<thead>
<tr>
<th>Type of value</th>
<th>Mean±SEM serum cortisol concentration (nmol/L)</th>
<th>p-value</th>
<th>250μg ACTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg ACTH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>281.9±34.8 (301.6±71.1)</td>
<td>0.810</td>
<td>299.1±40.1</td>
</tr>
<tr>
<td>Female</td>
<td>301.6±71.1</td>
<td></td>
<td>294.1±71.6</td>
</tr>
<tr>
<td>30 Minutes</td>
<td>1081.53±121.7 (775.3±188.4)</td>
<td>0.097</td>
<td>1078.6±117.8</td>
</tr>
<tr>
<td>Male</td>
<td>1081.53±121.7</td>
<td></td>
<td>781.1±104.9</td>
</tr>
<tr>
<td>Female</td>
<td>775.3±188.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

of ACTH for serum cortisol assay. All the samples were separated and were stored at -20°C until assayed.

**Laboratory determination of serum cortisol level**
Serum cortisol levels were determined by an Enzyme Linked Immunosorbent Assay (ELISA) technique using the Diagnostic automation Inc. cortisol assay method. It is a competitive immunoenzymatic

minutes. There were no significant difference between the two test doses at 30 minutes (928.40±92.24 vs 929.84±89.39nmol/L respectively, P = 0.777). There were no statistical differences in the serum cortisol increment and percentage increment on contrasting 1μg and 250μg tests at 30 minute.

The serum cortisol values in response to 1μg and 250μg ACTH tests in males and females were
also compared. There were no statistical differences in the 1µg and 250µg ACTH test on comparing the serum cortisol values of males and females. This is shown in table 2. The mean cortisol values in both males and females for both low dose and standard dose ACTH test at 30 minutes were 1081.5±121.7 vs 775.3±188.4nmol/L, p=0.097 and 1078.6±117.8 vs 781.1±104.9nmol/L, p=0.096 respectively.

**Relationship between 30 minute Serum cortisol response to 250µg and 1µg ACTH tests**

Figure 1 shows the relationship between serum cortisol levels in the two ACTH groups. The correlation coefficient (r) between 30 minute cortisol response to 250µg and 1µg ACTH tests was 0.999, p<0.001.

![Graph showing the relationship between 30-minute cortisol response to 250µg and 1µg ACTH tests](image)

**Fig. 1:** Relationship between 30-minute cortisol response to 250µg and 1µg ACTH tests in ten (5 males, 5 females) healthy subjects. The correlation coefficient was 0.999. Ref-female, Green-male.

**Discussion**

The adrenal glands play an important role in the body’s ability to cope with stresses such as infections, hypotension and trauma including surgery [9,14]. Adrenocortical function can be assessed using various dynamic testing methods. These include ACTH stimulation test, which could be short or prolonged; insulin tolerance test and metyrapone test.

The Insulin tolerance test (ITT) is widely regarded as the gold standard dynamic test of HPA axis function and it is considered to simulate physiological stresses [1,15]. Unfortunately, this test is costly, unpleasant, and contraindicated in certain patients (e.g. those with cerebrovascular or cardiovascular disease), this thus limits its clinical use [1]. The metyrapone test, which relies on intact pituitary feedback mechanisms, is also a sensitive test of HPA axis function [5,16,17-19], but its requires access to an 11-deoxycorticisol assay and may precipitate acute adrenal insufficiency [1,20]. ACTH stimulation tests provide an indirect assessment of hypothalamic and pituitary function, relying on the detection of adrenocortical atrophy secondary to ACTH deficiency. Despite this, the potential advantages of safety and convenience continue to make ACTH testing an attractive method for screening patients with suspected secondary hypoadrenalism.

ACTH stimulation test can be done using the standard dose (SD) or low dose (LD). SD is the standard test for assessment of adrenocortical failure [9,21,22]. Assessment of the adrenal gland’s functional reserve with the short cosyntropin or synacthen® (synthetic adrenocorticotropic (ACTH)) test has long been accepted as the most reliable diagnostic screening procedure in patients with a clinical picture suggestive of chronic adrenocortical hypofunction or in high-risk populations [1]. Intravenous administration of 250µg ACTH has been shown to be a provocative pharmacological rather than a physiological stimulus [2,4,23]. Dickstein et al. evaluating patients on long-term glucocorticoid therapy, found low sensitivity of the standard ACTH testing at identifying adrenocortical failure when contrasting it to 1µg ACTH [2]. The mean basal cortisol levels were comparable in both the low dose and the standard dose tests. The 30-minute serum cortisol levels and the increment in cortisol level were also comparable in both LD and SD ACTH test.

The serum cortisol levels for LD ACTH test correlated strongly with that of the SD ACTH test. The LD ACTH test adequately stimulated the adrenal gland and produced normal cortisol response after 30 min in the healthy controls. This is in keeping with result of the study done by Dickstein G and his co-workers where 130 normal individuals had normal response to LD ACTH test [7]. Two groups of 10 normal individuals were studied by Dickstein et al [2]. They showed that there was no difference in cortisol levels after intravenous injection of 250mcg or 1mcg of ACTH at 30 minutes [2]. Similar results were obtained in children using 1mcg ACTH [24]. Strong evidence thus abound that 1mcg ACTH will stimulate the adrenal glands maximally for 30 minutes post injection.
Errors in administration of the correct dose of ACTH is of concern [23]. Of concern is the issue of incomplete injection and binding of ACTH to plastic surfaces. This is obviated by preparing fresh solution of 250μg ACTH before each test [2,5,26]. In agreement with other published data, our study confirms that 1μg ACTH stimulates adrenocortical secretion in normal subjects in the period 30 minutes post injection comparable to 250μg ACTH testing. There is a very high and significant positive correlation between serum levels of cortisol following adrenocortical stimulation by using the 1μg ACTH test and 250μg ACTH test. Many investigators now propose that 1μg ACTH testing may replace the 250μg ACTH testing in the evaluation of suspected hypoadrenalism [5,6,8,11,27,28]. However, some studies do not support this view [24,29].

We recommend that the 1μg ACTH test can thus be used to stimulate the adrenal gland instead of using the 250μg ACTH test.

References
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