Inhaled corticosteroids (ICS) were first introduced for the treatment of asthma in 1972. Their efficaciousness and cost-effectiveness is well established (1, 2). Their role in controlling symptoms, reducing exacerbations, improving lung functions and quality of life is undisputed (3, 4). Maintenance therapy with ICS has become the accepted standard. Concerns about side effects have been raised, but were considered minor when set against the risk of inadequately controlled asthma (5–7).

Suppression of the hypothalamic-pituitary-adrenal axis (HPA) has been regarded as a ‘benign physiological response’ to exogenous corticosteroids (8–10). According to the latest Clinical Evidence database, adrenal suppression associated with the use of ICS is thought to be rare. In a recent UK survey, however, 2% of all paediatricians and adult endocrinologists mailed, reported at least one case of adrenal crisis associated with ICS (11). This frequency was higher than expected. 28 of the 33 cases identified were children. Most of these were on fluticasone metered dose inhaler (MDI) with a spacer on very high doses of 500–2000 l g/day. In another case series a dose of 400 l g budesonide given with a MDI and a nebulizer for 1 yr was enough to cause symptomatic adrenal suppression (12).

It is generally thought that adrenal crisis can easily be prevented by avoiding ICS doses that
are higher than those licensed for children (11). However, the clinical presentation of adrenal insufficiency varies with the degree and the rate of loss of adrenal function, as well as with the degree of stress the child is exposed to. It also varies depending on whether mineralocorticoid production is preserved or not (13). HPA suppression should therefore not be regarded as one single clinical entity, but is more appropriately compared to an iceberg with clinically recognizable adrenal crisis as the tip and subclinical HPA suppression as the greater submerged part of the iceberg. The objective of this review was to describe the clinical and biochemical features of HPA suppression, alert to potential pitfalls and identify the most appropriate test for diagnosing HPA suppression.

**Clinical presentation of hypothalamic-pituitary-adrenal axis suppression**

Acute primary adrenal insufficiency or adrenal crisis is easily recognized by the typical symptoms and signs of vomiting and diarrhoea, dehydration, fever or hypothermia, shock, confusion and coma (14). Laboratory features include hypoglycaemia, metabolic acidosis, hyperkalaemia, hyponatraemia and eosinophilia. In chronic secondary adrenal insufficiency (e.g. HPA suppression caused by ICS) the integrity of the renin-angiotensin-aldosterone system remains intact. Hypovolaemia and adrenal crisis are therefore rare. The clinical presentation of chronic adrenal insufficiency, viz. lassitude, weakness, dizziness, poor growth or weight loss, is non-specific (12, 15). These signs can thus easily be overlooked or misinterpreted. Orthostatic hypotension is a well-known sign of both primary and secondary adrenal insufficiency (15). However, universally accepted definitions of orthostatic hypotension in the paediatric age group are not available. Paediatricians caring for asthmatic children on ICS are therefore unlikely to be actively looking for this sign on a routine basis. Hypoglycaemia of adrenal suppression may also remain unrecognized, because it may occur as early as 1–2 h after a meal rich in carbohydrates at higher levels of glycaemia (3.4–4.5 mmol/l) when compared with normal subjects (14). As mineralocorticoid function is preserved in secondary adrenal insufficiency, hyperkalaemia will be absent. Hyponatraemia alone is the result of water retention caused by secretion of arginine vasopressin (AVP) which is secreted to maintain the blood pressure (13).

If an asthmatic child is compliant with his/her therapy, ICS substitute his/her endogenously produced cortisol, masking the suppression of the HPA. If the child is exposed to stress, such as an infection, injury, burn or surgery, the cortisol requirement outstrips the supply of the exogenous steroid, precipitating acute adrenal insufficiency. Stopping a high dose of ICS abruptly will have the same effect. In any other situation the child would appear clinically well or would present with insidious symptoms as described above. Only an accurate biochemical assessment of the entire HPA can thus confidently establish the diagnosis of HPA suppression. The question is how best is this done? To answer this question, normal cortisol production and regulation needs to be understood (Fig. 1).

**The hypothalamic-pituitary-adrenal axis**

The central nervous system (CNS) responds to distinct circadian stimuli by activating the hypothalamus to secrete corticotropin-releasing hormone (CRH) and AVP (16). Other activators of the hypothalamus include neurosecretory and limbic signals as well as cytokines (interleukin-1, interleukin-6, tumour necrosis factor-α; 17). CRH and AVP act additively or synergistically on corticotrophs in the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH acutely increases cortisol synthesis and release within 2–3 min (16). When ACTH levels fall, cortisol production decreases rapidly. Most of the required cortisol is produced on demand. Relatively little cortisol is stored in the adrenal for future use. Glucocorticoid-negative feedback occurs at hypothalamic and pituitary level, and possibly at higher centres (13). A short feedback loop to the adrenal has also been described, but its significance is not certain (18, 19).

Basal cortisol levels fluctuate with ACTH pulses which are secreted in an ultradian and
circadian pattern. Levels are low at midnight (00.00–03.00 hours). Pulses start at 04.00 hours and peak at 08.00 hours. Episodic increases also occur at meal times (13, 20). Negative feedback control ensures that the plasma cortisol concentration is kept at the appropriate level at all times. Stress, however, may override both the feedback control and the circadian rhythm.

Inhaled corticosteroid inhibit cortisol production at hypothalamic, pituitary and possibly at adrenal level. Overall daily cortisol production is thus reduced. In the assessment of HPA function investigators have therefore predominantly resorted to basal adrenal function tests (Table 1). Dynamic adrenal function tests, which test the integrity of the axis or a part thereof, were less commonly employed.

## Basal adrenal function tests

### Plasma cortisol

The measured total cortisol level consists of two components, i.e. a bound and free fraction. It is only the free cortisol that is biologically active and is regulated by the hypothalamus and the pituitary gland (21). It is bound to corticosteroid-binding globulin (CBG or transcortin) and to a lesser extent to albumin. Changes in the concentration of CBG can influence the measured total cortisol level. In case of CBG excess, such as in hyperoestrogenism, hyperthyroidism and hereditary CBG excess, plasma free cortisol is maintained at normal levels, but total cortisol levels are increased. The converse is true if CBG is low, as in familial CBG deficiency, hypothyroidism or protein deficiency (21).

Plasma cortisol levels also fluctuate significantly during the course of the day, approaching zero at midnight (20). Apart from the circadian rhythm, variables influencing cortisol production are season, puberty, exercise, cigarette smoking, physical and emotional stresses (13, 22). Psychological stressors precipitating ACTH and cortisol surges may be relatively minor such as the anticipation of a venipuncture, athletic competition or a mental task (13, 21). For physiological and methodological reasons single plasma cortisol levels are therefore unlikely to provide sufficient information on the function of the HPA. Their only value is to confirm frank adrenal insufficiency (23, 24).

### Urinary free cortisol

Chromatographic methods are cumbersome, not readily available and expensive. When urinary

### Table 1. Adrenal function tests utilized in the allergy literature

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Basal</th>
<th>Dynamic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>08.00 hours cortisol</td>
<td>ITT*</td>
</tr>
<tr>
<td></td>
<td>ACTH</td>
<td>Metyrapone test</td>
</tr>
<tr>
<td></td>
<td>DHEAS†</td>
<td>CRH stimulation test</td>
</tr>
<tr>
<td></td>
<td>Cortisol pattern</td>
<td>ACTH stimulation test</td>
</tr>
<tr>
<td>Urine</td>
<td>Free cortisol</td>
<td>Cortisol metabolites</td>
</tr>
<tr>
<td></td>
<td>Cortisol metabolites</td>
<td>Androgen metabolites</td>
</tr>
<tr>
<td>Saliva</td>
<td>Free cortisol</td>
<td></td>
</tr>
</tbody>
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*Insulin tolerance test.
†Adrenocorticotropic hormone.
‡Dehydroepiandrosterone sulphate.
§Corticotropin-releasing hormone.

Salivary free cortisol

Measuring plasma free cortisol is technically difficult. In other body fluids such as saliva and urine, its measurement is relatively easy. Salivary free cortisol was seen as an ideal test of adrenal function in children, as a painful venipuncture could be avoided. However, the physiological limitations of total plasma cortisol apply. The radio competition method, measuring salivary free cortisol concentrations, was found to be unreliable [intra-class correlation coefficient (R) of 0.18], performing worse than the plasma cortisol level (R = 0.54; 25, 26). The normal salivary cortisol range in children when measured by radioimmunoassay is lower than in adults (27). The lower limit is close to zero, making the interpretation of these values in the presence of HPA suppression rather difficult. Salivary cortisol is more useful when cortisol excess and the loss of the circadian rhythm need to be demonstrated as in Cushing’s syndrome.

### Total cortisol excretion

To overcome the limitations of single measurements of cortisol ways of integrating a number of cortisol measurements over time were sought. Measuring cortisol excretion over a 24-h period is non-invasive and relatively easy. Virtually all cortisol is converted to its metabolites before it is excreted (21, 28). Cortisol metabolites are measured by gas chromatography mass spectrometry (GC-MS). In the case of HPA suppression, a lower daily total cortisol production is expected. This is, however, only true for frank adrenal insufficiency (21). When the HPA is only partially suppressed, patients with pathology cannot be distinguished from those with a normally functioning axis on the basis of cortisol excretion (13, 21).

### Urinary free cortisol

Chromatographic methods are cumbersome, not readily available and expensive. When urinary
free cortisol (UFC) determinations by immunoassays were developed, it soon became the most frequently used measurement of HPA function in asthmatic children on ICS. Normally, however, only <1% of free cortisol is excreted in the urine (29). This will increase rapidly if the binding capacity of CBG is exceeded at a serum cortisol level of 690 nmol/l (21) which is above the reference range quoted by most laboratories (22). This test is thus suitable to diagnose conditions of cortisol excess, i.e. Cushing’s syndrome. In normal children and those with HPA suppression, CBG will not be saturated and concentrations of UFC will be very low. Consequently, it would be very difficult to distinguish between normality and adrenal insufficiency. The precision of the immunoassays is also unacceptable at low concentrations of UFC (30).

The usefulness of any test is determined by the type of assay being used. When the cortisol production rates in subjects given ICS of four different commercially available immunoassays were compared, they ranged widely from 25% suppression to 100% stimulation (depending on the assay; 30). Placebo, on the other hand, did not account for any intra-assay variability; neither did cortisol levels determined by these immunoassays of patients on ICS correlate with cortisol metabolite production as measured by GC-MS. This may have been due to interference by budesonide metabolites (in the case of Abbot TDX assay), the lack of specificity and the poor precision at low concentrations of urinary cortisol. UFC measurements when established by immunoassays in the setting of adrenal insufficiency are therefore not only invalid; in the presence of ICS they are also unreliable.

Plasma ACTH level

In adults plasma ACTH levels fall during the waking hours from 20–80 pg/ml (4.4–17.8 pmol/l) at 08.00 hours to 5 pg/ml (1.1 pmol/l) at midnight (13). In children morning values are generally lower ranging from <5 to 20 pg/ml (<1.1 to 4.5 pmol/l; 31). These concentrations are maintained for a short period only as plasma half-life of ACTH is very short (<10 min; 21). Single ACTH levels taken from children with HPA suppression can therefore be expected to be undetectable or in the lower range of normal. At this concentration the precision of the assays is generally poorer and the reading thus less reliable (32). Under normal unstressed conditions single ACTH concentrations are therefore not useful to detect secondary adrenal suppression as caused by ICS. Under stress an ACTH rise to triple the normal physiological amount would exclude HPA suppression (33, 34). This, however, requires dynamic testing (vide infra).

Dehydroepiandrosterone sulphate and urinary androgen metabolites

Plasma levels of dehydroepiandrosterone sulphate (DHEAS), the most abundant adrenal androgen, as well as urinary androgen metabolites (androsterone and aetiocholanolone), have been used to assess adrenal function in asthmatic children (35–37). DHEAS was thought to be an ideal candidate for HPA assessment because it has a longer half-life and is subjected to minor diurnal fluctuations compared with cortisol. However, levels rise during adrenarche (22), and decrease during other disease states such as fasting, severe illness, anorexia nervosa and ageing (13). The same is true for the urinary androgen metabolites (38). It is also clear that adrenal androgen production is not under the sole control of ACTH. Other factors, e.g. insulin-like growth factor 1 (IGF 1) or a proposed cortical androgen-stimulating hormone, may be instrumental in stimulating adrenal androgen production (13, 39). Plasma DHEAS or androgen metabolite excretion is therefore unlikely to be useful in the assessment of HPA function. Furthermore, neither has been compared with gold standard adrenal function tests. On critical analysis of the published DHEAS study, the pre-test and post-test odds were found to be the same (35), confirming that this test is not helpful in the diagnosis of HPA suppression.

Plasma cortisol profile

The cortisol production rate is classically measured by isotope dilution methods requiring the injection of labelled cortisol isotope, followed by a series of blood samples or a 24-h urine collection (13). Most researchers assessing the HPA suppressive effects of ICS have; however, resorted to the indirect measure of cortisol secretion, i.e. plasma cortisol concentration profiles taken over a period of time, varying from 4 to 24 h. This should not be equated to the cortisol production rate, because the plasma cortisol level at any particular moment is a function of its secretion, its distribution in body fluids and its elimination (32, 40). Deconvolution techniques are the best mathematical methods currently available to correct for the clearance of a measured hormone from the plasma (40). This and other techniques can also
be used to measure the pulsatility of cortisol (32, 40, 41). Most research studies performed in patients on ICS have only established mean and integrated cortisol concentrations (42, 43) or the cortisol area vs. time curve (AUC; 44). These parameters alone may not be adequate to fully describe the secretory dynamics of cortisol production.

The allergy literature has emphasized the importance of finding statistically significant differences in cortisol secretion rate between subjects on and those that not on ICS. Control groups, however, cannot provide for a surrogate reference range, because their numbers are inappropriately small or the subjects are the wrong age. Reference values for healthy boys and girls have now been established (20). These have shown a marked interindividual variability in secretory pattern. On the other hand, the cortisol rhythm of an individual remains relatively constant, provided it is not modified by plasma cortisol-binding, stress or a disease (Cushing’s syndrome, liver or thyroid disease; 45). The suppressive effects of exogenous steroids on the cortisol pattern should therefore only be compared with the child’s own normal rhythm and not to that of others. This was carried out in some studies investigating healthy volunteers, asthmatic adults and children. In healthy volunteers the AUC was reduced (46–48). Studies in asthmatic children, however, yielded conflicting results with some studies showing a decrease and others no significant change in AUC (48–51). Whenever a significant reduction in basal cortisol secretion is demonstrated, this is said to be due to the ‘systemic effect’ of the administered ICS, implying that cortisol production is suppressed by the HPA negative feedback mechanism. A significant difference in the production rate and the pulsatility of cortisol does, however, not automatically mean that the particular child will be unable to respond appropriately to stress. The insulin tolerance test (ITT) on the other hand, quantifies the response to stress (vide infra).

The peak cortisol level obtained during the ITT correlated poorly with the integrated plasma cortisol obtained by constant blood withdrawal \((r = 0.31; 52)\). This correlation was only marginally better than that for the basal cortisol level \((r = 0.25)\). It is therefore unlikely that a plasma cortisol profile can ever predict whether the HPA can respond to stress (the only clinically relevant outcome). Cortisol profiles, however, may be useful when equi-systemic effects of various ICS are evaluated or the bioactivity of the ICS given by various delivery devices is determined (53).

### Dynamic adrenal function tests

#### Insulin tolerance test

Only dynamic testing can unequivocally identify patients with HPA suppression. Gold standard adrenal function tests either induce stress, e.g. the ITT, or simulate stress, i.e. the metyrapone test. In the ITT hypoglycaemia is produced by injecting insulin. This leads to an increased secretion of cortisol, growth hormone, prolactin and catecholamines (22). The ITT therefore tests the entire stress system and not only HPA function. Studies have shown that the conventional ITT is not as sensitive as the metyrapone test (33, 54). If ACTH as well as cortisol is measured during the test, milder forms of HPA dysfunction will be diagnosed (33, 55). Mortalities during the performance of this test have been described (56). Some units have therefore abandoned the ITT and have replaced it by alternative tests.

#### Metyrapone test

The metyrapone test is a true test of feedback control. Metyrapone inhibits the 11 \(\beta\)-hydroxylase enzyme which converts 11-desoxycortisol to cortisol (22). In the absence of cortisol, ACTH secretion is increased. There are three versions of this test (standard, short overnight, intravenous). Studies have shown that the conventional ITT is not as sensitive as the metyrapone test (33, 54). If ACTH as well as cortisol is measured during the test, milder forms of HPA dysfunction will be diagnosed (33, 55). Mortalities during the performance of this test have been described (56). Some units have therefore abandoned the ITT and have replaced it by alternative tests.

#### ACTH stimulation tests

Unfortunately, metyrapone is not registered or has been deregistered for commercial reasons in several countries. This may be one of the reasons why some units have turned to alternative tests, in particular to the ACTH stimulation test.

The standard-dose ACTH stimulation test (250 \(\mu g\) or 250 \(\mu g/1.73 m^2\) or 250 \(\mu g/m^2\)) is classically used to identify patients with primary
adrenal insufficiency. In long-standing and severe secondary adrenal insufficiency (as precipitated by exogenous steroid administration), loss of ACTH action on the adrenals results in atrophy (21). The ACTH stimulation test was therefore advocated as a suitable ‘screening test’ in this setting (58). Adrenal atrophy, however, does not occur if HPA suppression is partial or of recent onset. The adrenal glands will still respond to the supra-physiological dose of 250 µg (21, 59, 60).

Low-dose ACTH stimulation tests utilizing a more ‘physiological’ dose of ACTH have been developed. They are considered to be safe, easy to perform and sensitive. However, anaphylaxis to ACTH has been described (61, 62). Moreover, several versions exist – one utilizing a standard dose of 1 µg, others utilizing a variable dose corrected for body surface area (1 µg or 0.5 µg/1.73 m² or 0.5 µg/m²). There is still no consensus on which version should be used in clinical practice. A universally acceptable pass criterion, which varies between 415 and 600 nmol/l, has also not been defined (63, 64). Some investigators consider an incremental plasma cortisol rise of > 200 nmol/l to be an adequate response, but this may not be detected if frequent sampling is not performed (65). The performance of the 1 µg test was most frequently evaluated. When compared with ITT or metyrapone (ACTH levels not measured), its sensitivity ranges between 50% and 100% depending on the pass criterion (63, 64). Summary receiver operating characteristic (ROC) curves were generated from all studies (of paediatric and adult patients) which provided sensitivity and specificity data for the 1 µg and the 250 µg tests. The performance of the two tests for secondary adrenal insufficiency did not differ significantly when summary ROC curves of the 1 µg and the 250 µg tests were compared (66). The 1 µg test therefore does not seem to offer any advantage over the 250 µg test in diagnosing secondary adrenal insufficiency.

The 0.5 µg/m² and the 250 µg/m² tests, performed in children with secondary adrenal insufficiency, were compared with the overnight metyrapone test in one study (ACTH levels not measured; 67). The 0.5 µg/m² test was found to be more sensitive and specific (at a cortisol cut-off of 546 nmol/l the sensitivity was 100% and the specificity 89%) than the 250 µg/m² test (at a cortisol cut-off of 839 nmol/l the sensitivity was 82% and the specificity 78%). Unfortunately, 95% confidence intervals were not calculated, but the AUC for the 0.5 µg/m² test was greater than for 250 µg/m² test. The results of this study are promising. It would nevertheless be premature to recommend this version of the low-dose test before these results are confirmed in a separate study and consensus among endocrinologists is reached.

The cortisol and the ACTH responses to 1 µg or 1 µg/1.73 m² were found not to fully reproduce the ITT (34, 68). The cortisol levels in these tests also do not rise as high and the ACTH secretion is not sustained as in the ITT. Consequently, the design of a new continuous ACTH infusion test based on a 5 µg intravenous bolus has been suggested (34). Others recommended the 8-h ACTH infusion test (68) which was initially designed to distinguish primary from central adrenal insufficiency (24). Supra-physiological levels of ACTH are maintained to produce supra-physiological levels of cortisol in normal people (13, 22) such is seen during severe stress. This test is therefore inappropriate when subtle changes need to be detected in recent or partial adrenal insufficiency. As a single plasma ACTH level is a much simpler way to differentiate between primary and central adrenal insufficiency, this test is rarely performed today. At this point of time therefore it seems that no version of the ACTH stimulation test can replace gold standard adrenal function tests in the assessment of the HPA.

CRH test

The CRH stimulation test has been suggested as a suitable and safe alternative to the ITT. Different preparations are used in different parts of the world. The synthetic ovine CRH (available in North America) causes a greater and more prolonged cortisol response than human CRH (used predominantly in Europe; 22, 69). This test has been designed to identify the level of the defect in patients with confirmed hypothalamic pituitary defects (13). It was also found to be helpful in distinguishing Cushing’s disease from the ectopic ACTH syndrome (especially when combined with dexamethasone; 22). However, when compared with the ITT (ACTH levels not measured), the human CRH stimulation test had a diagnostic yield of only approximately 60%, no better than a 08.00 hours serum cortisol (with a cut-off value of <230 nmol/l; 70). Even high doses of ovine CRH can only induce submaximal cortisol and ACTH responses compared to those induced by the ITT and the overnight metyrapone test (71). Furthermore, CRH is costly and approximately a third of tested patients experience minor side effects (some manifest transient, but significant hypotension; 22, 71). The dose varies between investigators, some preferring a standard dose, others a dose corrected for body...
weight. Due to its limitations this test is best reserved for patients whose anterior pituitary gland’s ACTH secretory ability needs to be assessed. It is not recommended for diagnosing HPA suppression.

Conclusions

Clinical criteria cannot be used to diagnose HPA suppression in children on ICS. All basal adrenal function tests, including plasma cortisol profiles, are unable to identify which children will not be able to respond to stress. In the clinical setting this could lead to adrenal crisis and at worst to death. There is no evidence to suggest that the degree of physiological adjustment of the HPA to ICS can predict clinically significant HPA suppression. Cortisol profiles should therefore only be used to demonstrate differences in systemic activity of various ICS and delivery devices.

Basal adrenal function tests should also not be regarded as screening tests. Their measurements (particularly UFC) are often unreliable and/or invalid, and unable to detect partial HPA suppression. It is therefore highly unlikely that these tests can ever be ‘sensitive’ as it has been claimed (72–74). Moreover, most of them have not had their diagnostic performances evaluated. When such an evaluation was carried out, the interpretation of the results is limited by methodological quality (75).

Only dynamic adrenal function tests can assess the integrity of the HPA or part thereof. As ICS suppress the axis primarily at pituitary and hypothalamic level, it is essential that the whole axis is assessed. Failure to do so may result in a number of cases being missed. Of the two gold standard adrenal function tests available to identify patients with HPA suppression, the correctly performed overnight metyrapone test (i.e. with ACTH levels) is the safest and the best. The unavailability of metyrapone in some countries can be overcome by obtaining special permission from the respective drug-regulating bodies to utilize this drug for testing purposes.

Whenever new ICS are being developed regulatory bodies such as the Federal Drug Administration (FDA) of the USA should insist on trials evaluating the HPA with a gold standard adrenal function test, before it is declared safe and allowed to be marketed. Allergologists and pharmaceutical companies are encouraged to collaborate with endocrinologists in the planning and execution of these trials.

Many reviews on the benefits and risks of ICS have been published. Results of studies assessing HPA suppression were often found to be conflicting. This is not surprising, as reviewers have ignored whether studies have utilized basal or gold standard adrenal function tests. A re-analysis of the published literature, identifying only those studies that have utilized gold standard adrenal function tests, is necessary. The results might help to establish the lowest safe dose and duration of ICS. If inconclusive, further studies utilizing appropriate tests are needed.

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References


