# Guidelines on the use of urine steroid profiling during or after glucocorticoid treatment and in conjunction with suppression and stimulation testing

## Background

ACTH acts on the adrenal cortex in a short term way to stimulate cortisol release and in a long term way to promote adrenocortical growth.

Glucocorticoid treatment 'suppresses' the adrenal cortex via diminution of ACTH levels by direct action on the pituitary and via suppression of hypothalamic CRH production: there are no known direct actions on the adrenal cortex. Recovery of adrenocortical function after short term use, as in an overnight dexamethasone suppression test, takes place within hours. Long term glucocorticoid use, as in steroid replacement treatment or exposure to endogenous cortisol excess, (primary (ACTH-independent) Cushing's disease), causes adrenocortical atrophy. Abrupt cessation of treatment would lead to an Addisonian crisis, so it is necessary, if withdrawing treatment, to 'tail down' the dose to allow recovery of adrenocortical function. Surgical removal of a cortisol-secreting adrenocortical tumour must be followed by cortisol replacement therapy. Clinical opinion is divided on whether full recovery after long term suppression is possible. One published study shows that capacity for cortisol synthesis recovers over a year or less, while recovery of adrenal androgen production may take up to 4 years.

The clinical circumstances in which testing may be considered in conjunction with urine steroid profiling are reviewed below.

## 1. Congenital adrenal hyperplasia

### 1.1 Newborns

Firstly, 'hydrocortisone' *is* cortisol, so its use will result in the appearance of cortisol metabolites in urine. If treatment is being given, it is therefore impossible to answer the fundamental question 'can this baby synthesise cortisol?' Acute treatment results in markedly altered ratios of cortisol metabolites, which we can readily detect. Sometimes when we pronounce that cortisol has been given, this is denied. For some cases, further enquiry reveals that hydrocortisone was indeed given, but not adequately recorded. In others, it is consistently denied, so it appears that an acute stress response can mimic recent hydrocortisone treatment. If the sample has been collected soon after treatment was started, there is a chance that full adrenal suppression will not yet have resulted, so that diagnostic steroid markers may remain, but it is clearly difficult to guess at the time course of events based on one sample. We use the 3β-hydroxy-5-ene steroids (DHA and pregnenolone metabolites) as markers of suppression. Thus, if CAH markers are absent but 3βhydroxy-5-ene steroids are present, we can exclude CAH, but if 3β-hydroxy-5-ene steroids are absent, then CAH cannot be reliably excluded.

Given the above considerations, urine samples from babies thought to have CAH should, whenever possible, be collected before any steroid is given. This can be in the form of a wet nappy, which can be posted to us provided it is in a rigid container. A portion of wet wadding removed from the nappy and placed in a 60 mL or 25 mL sample container is also sufficient. If glucocorticoid treatment has been established over a period, long term stimulation to 'wake' the adrenals (as in *Procedure 4)* is the only approach that can be guaranteed to provide a definitive answer. However, babies with 21-hydroxylase deficiency who are receiving maintenance doses of hydrocortisone generally continue to excrete enough definitive steroid markers. There is less data available on differentiating the rarer forms of CAH while on treatment, but we have found that 3β-hydroxysteroid dehydrogenase deficiency on treatment closely mimics 21-hydroxylase deficiency, with a paradoxically greater increase of 17-hydroxyprogesterone than 17-hydroxypregnenolone metabolites. This probably reflects action of the peripheral enzyme HSD3B1 on the more limited amounts of precursor generated during treatment.

If the urine steroid profile is not definitive in a sample collected during treatment, reduction of treatment and repeat collection could be tried, but may not be informative. *Procedure 4* is the one safe and certain way to determine what steroids the adrenal cortex is capable of making. This replaces the glucocorticoid with dexamethasone at equivalent dose with simultaneous administration of intramuscular synacthen depot. Dexamethasomne does not interfere with our analysis (nor with serum steroid analysis) and the synacthen circumvents the HPA axis suppression. Babies on long term glucocorticoid treatment do not usually show a response before Days 4 or 5 of stimulation and the pattern of adrenal steroid secretion becomes clear by Day 7. We have found this to be the only way to identify 3β-hydroxysteroid dehydrogenase deficiency with certainty in treated babies.

## 1.2 Children/Adults

Not uncommonly, a diagnosis of CAH in a patient who has been on long term glucocorticoid treatment is called into question. If the identification of the disorder was not based on a urinary steroid profile and only on serum steroid immunoassay, it may be erroneous – we have seen several examples over the years of one form of CAH being identified as another, or CAH being identified when it was not present. Patients may be non-compliant with treatment and deny it; alternatively, they may show no excess of marker steroid (usually 17-hydroxyprogesterone in 21-hydroxylase deficiency) because they are overtreated. A single urinary steroid profile while on the usual treatment regime can be the first investigation. Overtreatment can completely obscure 21-hydroxylase deficiency, so if no markers are relatively increased, it is useful to tail off treatment and check again. In partial ('late onset') CAH, the profile shows a combination of increased markers and cortisol metabolites. This is invariably evident in a baseline sample: synacthen testing is not required. Partial CAH can usually be distinguished from a hydrocortisone-treated severe CAH, but it is helpful if the request form notes whether treatment is being given. We use metabolites of other adrenal steroids such as DHA and corticosterone to judge whether hydrocortisone treatment is causing HPA axis suppression.

If short term synacthen testing is planned (as part of a CAH screening protocol, but, as noted above, this is not needed for diagnosis), or to determine if hydrocortisone support is required, then collection of urine samples as in *Procedure 3* can be carried out. Collection of urine at 30 or 60 min after synacthen injection never shows a response: the

time lag between increase in blood and the metabolites in urine is around 3 h *(ref 1)*, and this protocol takes this into account. Please note that this procedure is unlikely to provide more information than a baseline sample, but we are happy to receive sets of samples if required.

## 2. Adrenocortical insufficiency

Deficient adrenocortical function may be *primary* due to defects of adrenal ACTH response (e.g. ACTH resistance, autoimmune adrenal destruction), or *secondary* due to lack of ACTH (e.g. pituitary insufficiency, suppression by glucocorticoid treatment). All feature a lack of steroid secretion. Plasma ACTH quantification enables primary and secondary causes to be distinguished. For secondary forms, *Procedure 4* can be used to determine whether the adrenal cortex is capable of making cortisol, but, given the number of variables, could not determine whether there is an adequate ACTH response. Standard short synacthen testing remains the best first investigation.

## 3. Endogenous cortisol excess (Cushing's syndrome)

Use of urinary steroid profiling in conjunction with glucocorticoid suppression testing in suspected Cushing's syndrome is probably not helpful, but primary Cushing's (adrenocortical tumour) would be revealed by a long dexamethasone suppression test (*Procedure 1*). A urine steroid profile at baseline will reveal any unusual steroid production associated with a tumour. Cushing's gives rise to distinctive changes in relative proportions of cortisol and other metabolites. This metabolome is common to other causes of acute cortisol increase (acute hydrocortisone treatment, synacthen stimulation, stress). The degree of abnormality may give useful information on the time course and severity of the cortisol increase and also on whether a primary or secondary form is likely. The changes typical of adults are not always seen in pre-pubertal children with Cushing's, so more care in interpretation of findings is required.

## 4. Glucocorticoid resistance

High levels of steroid excretion coupled with clinical evidence of androgen and/or mineralocorticoid excess may be due to glucocorticoid resistance. This has proved difficult to characterise. Use of dexamethasone for glucocorticoid suppression testing may be inappropriate since this steroid, in contrast to cortisol, has poor penetration of the blood-brain barrier, lacks binding to cortisol binding globulin (CBG) and has a different three dimensional structure such that, although it binds type 2 (glucocorticoid) receptors with high affinity, it does not bind Type 1 (mineralocorticoid) receptors. It is also difficult to prepare a dose low enough to detect subtle decrease of glucocorticoid affinity. For these reasons, we have developed a low dose prednisolone suppression test (*ref 2*) – see *Procedure 2*. This cannot be used in conjunction with cortisol immunoassay because of extensive cross reaction, but GC-MS of the urine metabolites readily distinguishes them, thus enabling checks on compliance and metabolism. For patients with apparently high levels of steroid excretion derived from analysis of an untimed sample, an appropriate next step would be a further analysis using a 24h urine collection. If this confirms high steroid levels, then this procedure could be used next.

## 5. Glucocorticoid treatment

For patients receiving glucocorticoid treatment, it is difficult to judge what level of suppression a given dose will cause. The wide serum cortisol normal range provides evidence that glucocorticoid response is very variable in the normal population, so it would be unwise to conclude that a patient on a 'low' dose of glucocorticoid is not fully suppressed, nor that a patient on a 'high' dose is fully suppressed.

Dexamethasone is much more potent that cortisol, so that a so–called 'low dose' can effectively cause complete adrenocortical suppression.

It is also difficult to predict how the adrenals will recover following cessation of treatment. Unsurprisingly, recovery after full suppression tends to be slower than after partial suppression and slower after long-term compared with short-term treatment. For evaluation of adrenal function of patients treated long term, the common practice of stopping treatment for 1 or 2 days followed by synacthen stimulation cannot be recommended. At least a week is likely to be required for some recovery of adrenocortical function to be seen.

If a glucocorticoid other than hydrocortisone or cortisone acetate is being given, the effects on adrenocortical function can be gauged by a steroid profile of a 24 hour collection: diminished levels of adrenocortical steroids show that there is suppression of the HPA axis. This provides a clear result without risk of interference by the glucocorticoid, and without needing to identify the glucocorticoid in question. If prednisolone is being given, the metabolites are readily seen by our method and indeed the total amounts of these can be used to estimate the daily dose of treatment. Some steroids such as triamcinolone can have unexpectedly long term effects. The more potent topical preparations, such as clobetasol propionate, can cause profound adrenocortical suppression, especially if used on broken skin. Inhaled glucocorticoids (such as beclomethasone diproprionate) above a certain threshold dose can 'break through' and cause adrenocortical suppression. Some synthetic 'progestogens' such as medroxyprogesterone acetate (Depot Provera) are also potent glucocorticoids. Most synthetic glucocorticoids cannot be detected on a urinary steroid profile by GC-MS. LC-MS/MS has potential for identifying them in serum or other body fluids; serum dexamethasone analysis is available in our laboratory.

## 6. Suspicion of glucocorticoid administration

If a patient is thought to be taking glucocorticoids unwittingly or surreptitiously, a urine profile can be helpful. Prednisolone interferes with some (but not all) cortisol immunoassays, so a patient with Cushing's, high serum 'cortisol' and low ACTH may conceivably be taking this steroid. Many 'herbal' preparations contain synthetic steroids. These include Chinese creams for eczema (one commonly used preparation, *'Pyong Ping'*, contains dexamethasone), African skin-lightening creams, which frequently contain dexamethasone or clobetasol and pills from the Indian subcontinent that look like dried vegetable material, which may contain prednisolone. The latter, when given for respiratory problems may bring about dramatic improvement in the short term, but this is reversed as the Cushingoid effects take hold.

Please note that *'?taking steroids'* is not sufficient information on a request form. If anabolic androgenic steroid abuse is suspected, it is more appropriate to get these specifically screened for. We recommend contacting the Drug Control Centre, King's College Waterloo Campus of this is being considered. A urine steroid profile of a postpubertal male taking anabolic steroids may show low levels of androstenedione metabolites, but since testosterone is often taken as well, the absence of such a decrease does not exclude anabolic steroid abuse.

## 7. Procedures

Please note that analysis of multiple samples will be charged as one test. If non 24h samples are collected on a series of days, the same clock times should be used as far as possible. For the short synacthen test, is important to record the times of injection and of sample collection and to record the volume of each sample. This enables us to calculate rates of steroid excretion per hour. Sending laboratories are seldom set up to be able to handle this concept, so it is important to specify to the laboratory that this information should be recorded and transmitted.

### 7.1 Procedure 1 - The long 'low-dose' dexamethasone test

This is a standard long version of the test. Our interpretations of steroid profiling results are based on our unpublished findings in adult volunteers undertaking the test. Using sequential daily urine collections, suppression is maximal by Day 3. On this regime, around 5% of urinary cortisol metabolites remain by Day 3 in both men and women, while about 50% of androgen metabolites remain in women and 70% in men. Corticosterone metabolites parallel cortisol metabolites, showing that, while aldosterone production is largely dependent on the renin-angiotensin system, production of its precursor, corticosterone, is largely ACTH-dependent. This can be used for checking for autonomous adrenal steroid production (ie: from an adrenocortical tumour) or to determine the likely contribution of the adrenal to androgen excess.

*Make a 24h urine collection, then start dexamethasone 0.5 mg qds. Make a further 24 h collection on Day 3 (48-72 h of treatment). The sending laboratory should record total volumes and send a 20 mL portion. We report percent reductions of the major groups of metabolites. If this procedure is judged impractical (eg in children) an 'overnight' test will give some indication as to whether there is any autonomous steroid production. However, caution is needed, since metabolic clearance rates of steroids are variable. Clearance of steroid sulphates, such as DHA sulphate, is especially slow. Thus, if for example, an adrenocortical tumour secreting DHA sulphate were suspected, then a relative increase of this steroid in the sample collected the morning after dexamethasone being given at midnight might just reflect slower clearance rather than autonomy.*

### 7.2 Procedure 2 - The prednisolone suppression test

This is intended to look for partial glucocorticoid resistance as a possible cause of chronic hyperadrenalism, which may in turn be responsible for androgen excess in precocious adrenarche and polycystic ovary syndrome. It uses prednisolone in place of dexamethasone at a dose that results in around 50% adrenal suppression in normal adults, so that both glucocorticoid resistance and hypersensitivity can be explored *(ref*

*2).* Prednisolone is used because it is structurally very similar to cortisol so it is likely to be a more accurate probe than dexamethasone for mutations in the glucocorticoid receptor. Being less potent than dexamethasone, an appropriate dose is easier to select. USP readily distinguishes cortisol and prednisolone metabolites, so compliance can be checked.

*Make a urine collection between 0900 and 1800. Take prednisolone 5 mg at midnight the same day. Make a second collection between 0900 and 1800 the following day. The sending laboratory should record total volumes of each collection and send a 20 mL portion. We report percent reduction of total cortisol metabolites.*

## 7.3 Procedure 3 - The short synacthen test

This is the standard short procedure, but with appropriately timed urine collections added. If urine is collected 30 or 60 min after injection, no response is detectable in the urine steroid profile, because at least 2 hours are required before an increase of cortisol in serum results in an increase of cortisol metabolites in urine.

*Collect urine from 2 h before synacthen to the time of dosing (-2 to 0 h sample). Give intravenous synacthen (250 µg for adults or 250 µg/1.75 $m^2$ for children). Collect further urines at 0-2 and 2-4 h. It is recognised that these times cannot be closely adhered to in young patients, but noting the times and urine volumes (as given above) will improve the quality of the results.*

## 7.4 Procedure 4 - The long synacthen test

This procedure is the only safe and certain way to determine which steroids the adrenal cortex is capable of making if a patient is being maintained on steroid replacement. This replaces the glucocorticoid with dexamethasone at equivalent dose with simultaneous administration of intramuscular synacthen depot. Dexamethasone does not interfere with our analysis (nor with serum steroid analysis) and the synacthen circumvents the HPA axis suppression. Babies on long term glucocorticoid treatment do not usually show a response before Days 4 or 5 and this becomes unambiguous by Day 7. We have found this to be the only way to identify 3β-hydroxysteroid dehydrogenase deficiency with certainty in treated babies (see above).

*Change glucocorticoid to dexamethasone at equivalent dose. A commercially available reformulation as a syrup can be used to enable the required amount to be measured out. This is especially useful for children. Give synacthen depot (1 mg or 0.25-0.5 mg in children) intramuscularly on alternative days on 4 occasions. Collect urine on or after Day 7. We are happy to receive sequential collections before Day 7 to chart the change in steroid level and pattern. These can be untimed but collection at around the same time on each occasion is advised.*

References

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