Summary of biochemical, clinical and molecular findings of steroid disorders which can be investigated by urine steroid profiling

Introduction

Steroids in serum and urine comprise a handful of hormonally active compounds and hundreds of precursors and metabolites of similar structure. This provides an analytical challenge, which has been met by use of multicomponent methods based on high resolution chromatography and mass spectrometry. Urinary steroid profiling by gas chromatography-mass spectrometry (GC-MS) has been instrumental in the identification of all the newly recognised inborn errors of steroid metabolism in recent decades.

Panelling of hormonal steroids and their pathway intermediates in blood (serum or plasma) and saliva by liquid chromatography-tandem mass spectrometry (LC-MS/MS) promises to revolutionise clinical investigation. Until very recently, immunoassay has been the mainstay of this, but cross reaction by steroids in unusual excess can severely compromise immunoassay specificity; further, immunoassays are not readily available for many useful markers. Our emerging experience is that serum steroid panelling and urinary steroid profiling provide similar information, although factors such as differing metabolic clearance rates for different steroids and timing of the serum sample alter the relationships between levels in the two matrices. The serum precursors of many of the less usual urinary steroids that have been characterised in the newborn period and in inborn errors of steroid metabolism have not been established. This reflects the much sparser structural information that can be gleaned from LC-MS/MS spectra than is available for GC-MS spectra.

The clinical effects of steroid–related disorders arise from deficiency or excess of the actions of hormonal steroids. For example, aldosterone functions to fine tune sodium loss into the urine. Deficient aldosterone synthesis results in an inability to retain sodium (salt wasting), while excess aldosterone results in sodium and water retention, leading to hypertension. Defects in the pathway of steroid hormone response may mimic a steroid hormone deficiency. These can be due to either defective steroid hormone receptors or further downstream. For example, in primary pseudohypoaldosteronism, absolute aldosterone deficiency is mimicked by mutations affecting either the mineralocorticoid receptor or the protein that forms the aldosterone-responsive sodium channel in the distal convoluted tubules of the kidney.

For those steroid hormones for which production rate is controlled by feedback inhibition, a defect in its synthetic pathway will result in excess generation of steroid metabolic intermediates. For example, a defect in the conversion of corticosterone to aldosterone results in excess corticosterone production via hyperstimulation of the renin-angiotensin system. An excess intermediate may show hormonal activity: for example, a defect in conversion of 11-deoxycorticosterone (DOC) to corticosterone results in excess of DOC, which although itself a weak mineralocorticoid, can lead to hypertension if its concentrations build significantly.

Excess production of the major steroid hormones can also arise. This may be due to abnormal secretion of a trophic hormone by a tumour or to some other derangement of control, or there may be direct uncontrolled autonomous secretion from a tumour of the

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adrenals or gonads. For example, excessive aldosterone production may be secondary to an increase in renin, as a result of renal artery stenosis or more rarely a reninoma, or it may be due to autonomous unilateral or bilateral adrenal gland secretion.

In the following sections, findings in blood and urine are summarised before discussion of each disorder.

Defects of cortisol synthesis/action (congenital adrenal hyperplasia)

Cortisol secretion is controlled by the hypothalamic-pituitary-adrenal (HPA) axis. Cortisol feeds back at two points to inhibit both hypothalamic corticotrophin-releasing hormone (CRH) secretion and pituitary adrenocorticotrophic hormone (ACTH) secretion. Thus, a lack of feedback as a result of diminished cortisol concentration or defective cortisol response results in an increase of both CRH and ACTH and enhancement of adrenal activity. ACTH stimulates both adrenal steroid synthesis and growth, so chronic ACTH excess results in bilateral adrenal enlargement.

For inborn conditions, this develops *in utero*, since the fetus has a functioning HPA axis from around 8-12 weeks gestation, giving rise to the term *congenital adrenal hyperplasia*, (CAH). 'CAH' is often used as shorthand for *21-hydroxylase deficiency*, because this is the commonest form of the disorder, representing some 95% of all steroid metabolic defects identified. However, 'CAH' should be used to denote any of the 5 defects in the pathway of conversion of cholesterol to cortisol, as listed below. Four further disorders that cause adrenal hyperactivity because they impair cortisol synthesis or cortisol response or enhance metabolic clearance are listed after these. These may also be associated with ACTH excess and consequent adrenocortical hyperplasia.

Cholesterol to pregnenolone conversion defects: lipoid adrenal hyperplasia due to 20,22-lyase (*CYP11A1*) defect or StAR protein defect (*STARD1*)

Absence of all steroids in blood and urine.

Both adrenal glands and gonads are affected. The adrenal glands are characteristically enlarged and filled with lipid droplets when visualised by microscopy. It was long assumed that the primary defect was of side chain cleavage of cholesterol by 20,22-lyase. It later became clear that these are in the minority and in most cases there is a defect in StAR (Steroid Acute Regulatory) protein, which is synthesised in response to ACTH stimulation and is required for cholesterol transport into the mitochondrion.

Absence of mineralocorticoid results in salt wasting, of glucocorticoid results in hypoglycaemia and of sex steroids results in genetic males appearing phenotypically female. Spontaneous puberty occurs in XX individuals, with cycles that are anovulatory. Successful pregnancy has been achieved with induction of ovulation by clomiphene and use of progesterone cover. Puberty does not occur in XY individuals.

3β-Hydroxysteroid dehydrogenase (*HSD3B2*) deficiency

Increased pregnenolone, 17α-hydroxypregnenolone and dehydroepiandrosterone (DHEA) in serum and their metabolites in urine. Low/absent androgen, corticosterone and cortisol in serum and metabolites in urine.

In newborns, high levels of urinary pregnenolone and DHEA metabolites together with low levels of cortisol metabolites are suggestive of the disorder. A similar pattern is also

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found in preterm, otherwise healthy, infants. The disorder is confirmed biochemically if the pregnenolone and DHEA metabolites persist beyond 3 months post age at term (40 weeks), when they would normally have declined; pregnanetriol (the major metabolite of 17-hydroxypregnenolone) emerges as the steroid in greatest amount at this age. After the time of puberty, DHEA becomes more prominent.

This defect affects steroid synthesis in both gonads and adrenals. When severe, the production of all steroid hormones is nearly abolished. Deficiency of testosterone in the male results in incomplete masculinisation of the external genitalia of the newborn (XY DSD); paradoxically, the female newborn has virilised external genitalia (XX DSD), as a result of excess DHEA giving rise to testosterone via peripheral conversion by 3βHSD 1 and perhaps also through direct androgen receptor binding of DHEA. Deficiency of cortisol results in hypoglycaemia and of aldosterone in salt wasting.

Affected patients on glucocorticoid treatment paradoxically excrete more 17-hydroxyprogesterone than 17-hydroxypregnenolone metabolites, presumably due to a relatively enhanced effect of peripheral 3βHSD 1 on a diminished amount of precursor. If no urine has been collected before treatment is commenced, then the disorder can only be definitively identified biochemically by substitution of dexamethasone for the usual hydrocortisone (cortisol) and administration of depot synacthen over several days: 17-hydroxypregnenolone will progressively increase relative to 17-hydroxyprogesterone.

Puberty proceeds in both XX and XY individuals.

17α-Hydroxylase (*CYP17A1*) deficiency

Increased serum progesterone, 11-deoxycorticosterone (DOC) and corticosterone and their urinary metabolites. Absent androgens and cortisol in serum and their metabolites in urine; in the newborn, additionally, high levels of urinary pregnenolone metabolites with absence of DHEA metabolites. If corticosterone is quantified by LC-MS/MS, it is important that it is chromatographically resolved from its isobar, 11-deoxycortisol.

The 17α-hydroxylase enzyme combines both hydroxylase and 17,20 lyase activities. By site-directed mutagenesis, some separation of function within the protein has been recognised. Since 17α-hydroxylation precedes side chain cleavage, the biochemical consequences of a severe 17α-hydroxylase deficiency are the same whether side chain cleavage is impaired or not. No conversion of C21 to C19 steroids is possible, resulting in abolition of production of androgens and oestrogens. Cortisol and cortisol precursors are also absent. Genetic males are phenotypically female. Neither gender develops secondary sexual characteristics. Deficiency of cortisol production does not result in glucocorticoid deficiency, because corticosterone, a mild glucocorticoid, which is present in excess in this disorder substitutes. Newly ascertained untreated patients show levels of urinary corticosterone metabolites within a narrow range that is about 5x the normal values for cortisol metabolites: this must reflect the relatively lower glucocorticoid potency of corticosterone. Stimulation of this remaining pathway results in excess of DOC, which has mineralocorticoid activity around 1/20 that of aldosterone and so leads to hypertension.

Genetic males with partial deficiencies show varying degrees of genital ambiguity. At puberty, they may show high gonadotrophin levels, some penile and pubic hair development, gynaecomastia and paradoxical increases in serum 17-hydroxyprogesterone and urinary 17-hydroxyprogesterone metabolites. The urine metabolites do not include increase of 11-oxopregnanetriol (see explanation under *21-*

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hydroxylase deficiency, below), showing that the origin is testicular, evidently under LH hyperstimulation. The gynaecomastia is presumably due to the limited production of androgen being disproportionately converted to oestrogen, a situation paralleled in *cytochrome b5A deficiency* and *17β-hydroxysteroid dehydrogenase deficiency* (below). Functional assays have shown such cases to have significant presence of both 17α-hydroxylase and 17,20 lyase activities, but the disproportionate testicular production of 17-hydroxyprogesterone may lead these, erroneously, to be interpreted as having a predominantly lyase defect.

Mutations of *CYP17A1* affecting only lyase activity have been claimed in the literature. However, all reported patients who were subjected to a synacthen stimulation test showed an impaired cortisol response, indicating that there is also diminished 17α-hydroxylase activity. Specific lyase defects may also be due to *cytochrome P450 oxidoreductase deficiency* or to *cytochrome b5 deficiency due to CYB5A gene mutations* (each described below).

21-Hydroxylase (CYP21A2) deficiency

Increased serum 17-hydroxyprogesterone, 21-deoxycortisol and androgens and their metabolites in urine. In the severe (*classic*) form, cortisol production is very low but immunoassays may produce falsely normal to high serum levels due to cross reaction with excess cortisol precursors. Newer versions of these may perform better, eg: Roche Gen 2. Cortisol metabolites are detectable in urine, but at very low levels. They are paradoxically increased above normal in 24h collections from patients with a partial (*non classic*) defect: this may be due to excess 21-deoxycortisol competing with cortisol for the glucocorticoid receptor, thus attenuating negative feedback. In the newborn, numerous additional metabolites of 17α-hydroxyprogesterone and 21-deoxycortisol are present, several of which are better diagnostic markers than the classical markers that predominate later.

Increased androgen levels result in virilisation of the external genitalia in newborn girls. Two presentations of the classic form are distinguishable: the *simple virilising* and the *salt wasting* forms. No biochemical distinction is possible, but they must differ in the ability to make aldosterone. Since the normal production rate of this steroid is very low in comparison to that of cortisol, small differences in 21-hydroxylase activity close to zero may be crucial. In boys, the defect is missed at birth unless newborn screening is in use. If they have the salt wasting form, they present with dehydration etc. at around 18 days of life. Boys with the simple virilising form are first found with early appearance of pubic hair and penile enlargement at 2-4y of age. If they are not treated, the continuing androgen excess leads to sexual precocity and increased growth, but they have short final height as a result of epiphyseal closure. Those with the simple virilising form may also show high renin levels and so benefit from mineralocorticoid supplementation. Undertreated males may develop benign testicular masses.

Partial deficiency in females most usually presents with signs of androgen excess at around age 7 years but later presentation should always be considered. In unselected women with hirsutism, it is relatively uncommon, with a frequency of perhaps 1 in 200. Urinary steroid profiling enables unequivocal detection of a clinically significant enzyme deficit without the need for synacthen stimulation. When there are signs of androgen excess and serum 17-hydroxyprogesterone and androstenedione are found to be raised, partial deficiency may be differentiated from a gonadal tumour by analysis in serum of 21-deoxycortisol (although this is rarely, if ever, available) or in urine of its metabolite, 11-oxopregnanetriol. Increases specifically signal an adrenocortical source and lack of increases a gonadal one. This reflects activity of 11β-hydroxylase, which is required for

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21-deoxycortisol production and is only present in the adrenals but not in the gonads. When 21-deoxycortisol is quantified by LC-MS/MS, it is important that it is chromatographically resolved from its isobars, corticosterone and 11-deoxycortisol

11β-Hydroxylase (*CYP11B1*) deficiency

Increased serum 11-deoxycortisol, DOC, 17-hydroxyprogesterone and androgens and their urinary metabolites. Very low serum cortisol and urinary cortisol metabolites. Immunoassay of 11-deoxycortisol is especially unreliable in this disorder and should not be used. If 11-deoxycortisol is quantified by LC-MS/MS, it is important that it is chromatographically resolved from its isobars, corticosterone and 21-deoxycortisol. Cortisol by immunoassay may appear be at low/normal/high levels, depending on the extent of cross-reactivity with precursors. Please note, a similar metabolome is encountered when patients are on treatment with drugs which inhibit 11β-hydroxylase activity, including metyrapone and azole antifungals such as ketoconazole and posaconazole. Increased 11-deoxycortisol is also found in association with many adrenocortical carcinomas as well as in those patients in disease remission being treated with mitotane.

Increased androgen levels result in virilisation of the external genitalia in newborn girls. Increased production of DOC, a mild mineralocorticoid, results in hypertension. In boys, the defect may be missed at birth but be signalled later by early appearance of pubic hair and penile enlargement. If untreated, the androgen excess results in sexual precocity and increased growth, but short final height as a result of premature epiphyseal closure.

Apparent cortisone reductase deficiency and cortisone reductase (*HSD11B1*) deficiency

These are due to deficiency of hexose-6-phosphate dehydrogenase (*H6PD*) and 11-hydroxysteroid dehydrogenase I, respectively. Serum steroid levels, including of cortisol, are *normal.* In urine there is a decreased ratio of cortisol/cortisone metabolites and an overall increase of adrenal steroid metabolites. Deficiency of hexose-6-phosphate dehydrogenase produces this metabolome because it is essential for the activity of 11-hydroxysteroid dehydrogenase I, being required to regenerate NADPH, its hydrogen donor, in the endoplasmic reticulum.

Both defects of cortisone 11-reduction result in an increase in the cortisol clearance rate. For cortisol levels in the blood to be maintained, the adrenals are chronically hyperstimulated. As a consequence, adrenal androgen production is also increased, resulting in hirsutism in both men and women and menstrual irregularity and infertility in women. Menstrual cyclicity and fertility are readily restored by low-dose dexamethasone treatment, which emphasises the deleterious effects of adrenal androgen excess.

Cytochrome P450 oxidoreductase (*POR*) deficiency (associated with Antley-Bixler syndrome)

Increased corticosterone and 17-hydroxyprogesterone in serum and of the corresponding metabolites in urine. In the newborn, a striking relative lack of urinary steroids with additional hydroxyl groups. In a pregnancy with an affected fetus, low maternal oestriol, high ratios of urinary 5α/5β-reduced steroids, especially androsterone/aetiocholanolone and of the 5α/5β epimers of 17-hydroxypregnanolone, together with increased *epiallo*pregnanediol.

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Cytochrome P450 oxidoreductase has a role in supplying electrons to the steroid hydroxylases, which are cytochrome P450 enzymes. Deficiency may thus impair any of these enzymes, but particularly affects 17α-hydroxylase and 21-hydroxylase, thus producing a biochemical profile that resembles a mixture of the two disorders. However, relative enzyme deficits are variable, presumably because different mutations differentially affect interaction of POR with the various cytochrome P450s. The male at birth shows incomplete masculinisation of the external genitalia as a result of deficient androgen production, while paradoxically, the female newborn has virilised external genitalia, which do not further virilise after birth. This has been hypothesised to be the result of androgen synthesis by an alternative ('backdoor') pathway via the 5α-epimer of 17-hydroxypregnanolone which is only important in the fetus. There are cranial and other bone malformations, which may be due to cholesterol deficiency and accumulation of toxic sterol intermediates.

7-Dehydrocholesterol reductase (*DHCR7*) deficiency, (associated with Smith Lemli Opitz Syndrome, SLOS)

This enzyme catalyses the last step in the cholesterol synthesis pathway. Deficiency leads to increase of serum 7-dehydrocholesterol. This is metabolised to 7- and 8-dehydro forms of the usual steroids in both pregnancy and the newborn, with 8-dehydro oestriol and 7-dehydropregnanediol useful markers in urine from pregnant women and 7- or 8- dehydro-16α-hydoxypregnenolone in newborns. Clinical problems include mental disability and dysmorphia, including syndactyly, probably resulting from cholesterol deficiency and accumulation of toxic sterol intermediates. Affected patients have slightly elevated ACTH but normal synacthen response.

Glucocorticoid receptor (*NR3C1*) defects

Serum cortisol is elevated but shows normal circadian variation. Urine cortisol, corticosterone and adrenal androgen metabolite levels are elevated.

As with cortisone reductase deficiency (above), the adrenals are chronically hyperstimulated, leading to androgen overproduction and hirsutism in women and also to DOC overproduction, resulting in hypertension. Since cortisol feedback control operates via the glucocorticoid receptor, a decreased affinity for cortisol results in the concentration of cortisol in the blood being automatically appropriately increased.

Mild glucocorticoid resistance might be predicted to have a high prevalence, since it would be automatically compensated by upregulation of the HPA axis. It would be expected to cause mild adrenal androgen excess. Studies aiming to identify this as a cause of precocious adrenarche (see *precocious puberty/virilisation in children,* below) and *hirsutism in women* (below), groups that tend to show increases of urine cortisol metabolites, have not generally given positive results, even finding that there is increased glucocorticoid sensitivity in some hirsute women. However, studies may have been compromised by use of the synthetic glucocorticoid, dexamethasone, in place of cortisol, since this steroid may not truly reflect altered receptor affinity for cortisol.

Defects of sex hormone synthesis/action

Inborn defects that change production of testosterone result in effects on sexual differentiation, now collectively classified as disorders (or differences) of sex development (DSDs). Deficiency *in utero* results in incomplete masculisation in males but no phenotypic effect in females, while excess causes virilisation of females but no phenotypic effect in males.

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All five forms of CAH (see section above) are associated with DSD. Aromatase deficiency is the only disorder causing only a metabolic block in oestradiol synthesis, but it results in accumulation of its immediate precursor, testosterone. Effects at puberty depend on interaction with the hypothalamic-pituitary-gonadal (HPG) axis, with deficiencies resulting in hyperstimulation due to diminished feedback inhibition. Defects that result in low production of both androgen and oestrogen generally result in oestrogenisation in males. This probably reflects the much lower normal physiological levels of oestradiol than testosterone + dihydrotestosterone, so the balance tilts in favour of oestrogen. Most such defects have minimal effects on puberty of affected females.

Cytochrome b5 deficiency due to a *CYB5A* mutation, causing apparent 17,20 lyase deficiency

Steroid findings are dependent on stage of life. In the newborn, urinary DHA metabolites are paradoxically normal but pregnenolone metabolites are elevated, giving a clue to impaired side chain cleavage. Cortisol metabolites are normal. Serum testosterone and androstenedione are low and show minimal response to hCG; serum cortisol is normal and responds normally to synacthen. In childhood, serum testosterone is low but detectable, while androstenedione is undetectable. The urine steroid profile during childhood has no abnormal features. After the time of puberty, the androgens remain low but in genetic males, 17-hydroxyprogesterone is markedly increased. This is testicular in origin, which parallels the situation of males with partial 17-hydroxylase deficiency (see *17α-Hydroxylase deficiency* above): there are high levels of 17-hydroxyprogesterone metabolites in urine, but not of 21-deoxycortisol in blood nor its urinary metabolite 11-oxopregnanetriol.

The 17,20 lyase activity of 17α-hydroxylase requires electron transfer from NADPH via cytochrome P450 oxidoreductase (POR, see section on POR deficiency above), an electron donor enzyme. Electron transfer from POR is promoted by the allosteric co-factor cytochrome b5A (CYB5A). This cytochrome was formerly regarded as a permissive factor, but is clearly essential, since all the described mutations feature very low androgen levels and expression of a mutant gene in a vector led to very low enzyme activity. Cytochrome b5A has a role, in conjunction with cytochrome b5 reductase, in clearance of methaemoglobin. Deficiency therefore results in methaemoglobinaemia. This is usually not clinically apparent but readily established by methaemoglobin assay, a readily available test.

Affected males show undervirilisation at birth and varying degree of virilisation at puberty and also gynaecomastia, a situation again paralleled by partial 17α-hydroxylase deficiency. A single reported adult female with a *CYB5A* mutation was phenotypically normal and able to carry a normal pregnancy.

17β-Hydroxysteroid dehydrogenase (*HSD17B3*) deficiency

Increased serum ratio of androstenedione/testosterone. In prepubertal subjects, hCG stimulation is required to show the abnormality. No definitive changes are usually seen in urine metabolites before the time of puberty. Thereafter, there is often an increase of testosterone + androstenedione metabolites as a result of lack of androgen feedback on the HPG axis, with a particular relative increase of androsterone. Please note though, these findings are not universally encountered in the steroid profile in affected children, and molecular testing should now be considered as the gold standard for diagnosis.

Affected males most commonly have a female phenotype at birth but show some

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masculinisation at puberty. Affected females are asymptomatic.

The Type 1 enzyme (**HSD17B1**) is required for conversion of oestrone to oestradiol, and thus a deficiency of this might have similar effects to 17α-hydroxylase deficiency on sexual differentiation in females.

5α-Reductase 2 (*SRD5A2*) deficiency

Increased serum ratio of testosterone/dihydrotestosterone. In prepubertal subjects, hCG stimulation is required to show the abnormality. Increased ratio of $5\beta/5\alpha$ reduced urinary metabolites of androgens, corticosterone and cortisol, with cortisol metabolites (the *tetrahydocortisols*) being the most diagnostic. Diagnosis by urine steroid profiling does not require hCG stimulation and has been shown to be more reliable that serum testosterone/DHT measurements.

The condition is exactly mimicked by exposure to the 5α-reductase 2 inhibitor, finasteride and also by severe hypothyroidism and very low BMI (e.g. in anorexia). Treatment with thyroid hormone reverses this. Severe wasting syndromes at birth have, rarely, produced a similar picture. The broader spectrum 5α-reductase inhibitor dutasteride causes more profound decrease of the 5α-reduced androgen metabolite, androsterone, than does finasteride, indicating that this steroid is not only derived by reduction by 5α-reductase 2. This may explain why the serum ratio of testosterone/dihydrotestosterone is less reliable for diagnosis than urine steroid ratios, especially near birth: some dihydrotestosterone probably results from action of another 5α-reductase, likely 5α-reductase 1, which is known to be more expressed in the newborn.

In healthy newborns, none of the $5\beta/5\alpha$ reduced pairs of urinary steroid metabolites are detectable at birth. Tetrahydrocortisols can usually be distinguished by high-sensitivity selected ion monitoring GC-MS by about 21 days post full term. Unfortunately, in 5α-reductase 2 deficiency the increase is delayed (so this, paradoxically, provides an indication that the disorder is present) and it may be up to 3 months before a clear diagnosis can be made. Another clue at this stage is that the normal post-partum progression of cortisol metabolites from consisting of only cortisone (11-oxo) metabolites at birth to increasing proportions of cortisol (11-hydroxy) metabolites is delayed. If patients have already been gonadectomised, the serum testosterone/dihydrotestosterone ratio can no longer be utilised but urinary tetrahydrocortisols can still be used for diagnosis.

The heterozygous condition is characterised by mild elevations of the $5\beta/5\alpha$ reduced pairs of urinary steroids, but this is not diagnostically useful. This contrasts with most inborn errors of steroid metabolism, in which heterozygotes are indistinguishable from normal.

The existence of this defect in isolated communities in the Dominican Republic first pointed to the importance of dihydrotestosterone (DHT) as a potent androgen. Boys are born with incomplete masculinisation, but show significant virilisation at puberty, suggesting that DHT is more important for development of the external genitalia *in utero* but testosterone is more important later, when increases are driven by LH as a result of maturation of the HPG axis, enhanced by attenuation of androgen-mediated feedback inhibition. Affected females are asymptomatic.

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3α-hydroxysteroid dehydrogenase (*AKR1C1-4*) deficiency

Male DSD due to mutations in genes for 3α-hydroxysteroid dehydrogenase types 2 and 4 has been claimed in one report, but no further cases have been published. One of the families in the study had an earlier steroid profile providing strong biochemical evidence for a 17,20-lyase deficiency. The later findings have been taken as evidence for the importance of the 'back door' pathway of androgen synthesis. However, this does not appear to explain the very low levels of androgen synthesis shown by urine profile, since the usual route (i.e.: the 'front door' pathway) does not require this activity.

Androgen receptor (*AR*) defect (androgen insensitivity syndrome, AIS, testicular feminisation)

Serum and urine androgen levels are normal or elevated in the male.

Absence of androgen effect results in a genetic male being phenotypically female. Sexual hair does not develop. Since in the male, testosterone can be converted to oestrogen when it is unopposed by androgen, there is development of breasts and female body habitus.

Aromatase (*CYP19A1*) deficiency

In the pregnant woman carrying a fetus with aromatase deficiency, serum and urine oestriol is very low and androgen levels are high. Urine steroid levels are normal in the child until puberty, but there may be signs of androgen excess. This suggests that oestrogen has a role in feedback inhibition of the HPG axis before puberty. After the time of puberty, serum and urine androgens and 17-hydroxyprogesterone are high.

Female fetuses are virilised *in utero* and the mother is also virilised, as a result of the failure of the placenta to convert androgens arising from the fetus into oestrogens. A differential for this combination is pregnancy-induced luteoma, where a gonadotrophin-responsive mass in the mother is activated by high human placental gonadotrophin levels; oestriol production would be expected to be normal in luteoma. Regression of maternal virilisation takes place post-partum in both conditions.

In both genders, there is failure of epiphysial closure of the long bones, so that growth continues into adulthood, leading to tall stature. There is androgen and 17-hydroxyprogesterone excess as a result of increase of gonadotrophins. Puberty takes place in the male, but in the female, development of a female body habitus does not progress as a result of lack of influence of oestrogen. These observations demonstrate that steroid-induced bone maturation and feedback control of pituitary gonadotrophin production, previously ascribed to androgen in the male, are in fact significantly dependent on oestrogen. Oestrogen administration to normal adult males results in profound suppression of testosterone production.

Oestrogen receptor (*ERα*) defect (oestrogen insensitivity syndrome, EIS)

In serum after puberty, very high oestrogens and increases of androgens, progesterone and 17-hydroxyprogesterone.

As for aromatase deficiency, there is failure of epiphysial closure of the long bones, so that growth continues into adulthood, leading to tall stature. Females have normal pubic hair but no female secondary sexual characteristics. Normal pubertal development has been recorded in one male but delay associated with cryptorchidism in another.

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Aromatase excess syndrome (*CYP19A1* mutations)

In serum after puberty, excess oestrogens, with oestrone predominating over oestradiol; androgens are either low or normal.

There is overexpression of aromatase, associated with autosomal dominant mutations or duplications affecting *CYP19A1*, the gene for aromatase. This leads to high rates of peripheral conversion of androgens to oestrogens. There is sexual precocity in both genders, with accelerated bone maturation and short final height. The male develops a feminised appearance, including gynaecomastia and has small testes and micropenis.

Precocious puberty/virilisation in children

Increased secretion of androgens by the adrenals may result in early growth of pubic and axillary hair without breast development in girls and in sexual hair growth without testicular enlargement in boys. This may be due to partial virilising forms of CAH or, rarely, to an androgen-secreting tumour. A much more common cause is *premature adrenarche*, which is conceived as a premature increase in adrenal androgen synthesis due to early maturation of the zona reticularis of the adrenal cortex. Affected patients do not usually progress early to true puberty, but may show advance of height and bone age. True precocious puberty can occur as a result of early maturation of the HPG axis. This can be idiopathic or caused by certain brain lesions, such as a hamartoma.

Premature adrenarche and premature puberty both result in increased urinary testosterone/androstenedione metabolites. If there is increase of DHEA metabolites, this provides positive evidence for adrenarche, but equally, a lack of increase does not negate adrenarche, because the rate of conversion of DHEA to androstenedione shows great inter-individual variability. This is presumably consequent on variable 3β-hydroxysteroid dehydrogenase activity.

Isolated growth of pubic hair in the first years of life also has an idiopathic presentation: there are no increases of serum androgens or urinary androgen metabolites and the hair growth tends to diminish with time. Although this has been described in the literature only for boys, leading to suggestions that it is initiated by the *post-partum* androgen surge, we find it occurs in girls with equal frequency, suggesting an alternative aetiology.

Hirsutism in women

Excess testosterone in serum is a common finding, but concentrations may be normal in association with low sex hormone-binding globulin, which results in increased free steroid. Serum testosterone values in women obtained by immunoassay may be unreliable, so obtaining a value by LC-MS is always advisable to confirm immunoassay findings.

Partial forms of virilising CAH rarely occur. These are readily distinguished by urine steroid profiling. Polycystic ovary syndrome (PCOS) is very common in this group. The polycystic ovary secretes excess androgens, but there is also evidence in many patients for increased adrenal secretion of androgens and cortisol. This may be due to chronic adrenocortical hyperstimulation secondary to glucocorticoid resistance (see *glucocorticoid receptor (NR3C1) defects* above) or to enhanced cortisol clearance due to a relative increase of cortisol 11-dehydrogenation, as indicated by increase of the ratio of urine cortisone/cortisol metabolites. Others have reported increased 5α/5β steroid ratios in hirsute women, but we have never observed this. They have concluded that

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increased 5α-reduction activity may enhance cortisol clearance. Precocious adrenarche (above) may have similar origins in enhanced cortisol clearance: girls with precocious adrenarche tend to develop PCOS later.

Steroid sulphotransferase (*SULT2A1*) converts DHEA to DHEA sulphate. Deficiency of this enyme has been postulated to result in DHEA excess and corresponding androgenisation, but no case studies have been reported. This enzyme requires a 3'-phosphoadenosine-5'-phosphosulphate (PAPS) cofactor, generated by PAPS synthase (*PAPSS1 & 2*) and a *PAPSS2* defect in a virilised girl has been reported.

Defects of mineralocorticoid synthesis/action

Listed below are those disorders, other than the three salt-wasting forms of CAH (described above). A defect in aldosterone production or action results in excess stimulation of precursor production via the renin-angiotensin system. Since serum electrolytes in the fetus are regulated by the maternal system, affected newborns initially show normal serum electrolytes and normal steroid profiles. Thereafter, serum sodium shows a progressive decrease over 7 days or so: this coincides with rapid decrease of urinary pregnenolone and DHEA metabolites, steroids which would normally remain at high levels during this period. This appears to be secondary to a 'drive' to make aldosterone and is probably mediated by angiotensin 2, since this hormone has been shown to induce apoptosis in the foetal zone of the adrenal cortex in cultured foetal adrenal explants. Steroid profiles characteristic of each condition also develop over this time course. Since these are dependent on renin-angiotensin system activation, it follows that if serum sodium concentrations are maintained by treatment with salt, the profiles normalise and differentiation of the disorders becomes impossible.

Aldosterone synthase (*CYP11B2*) deficiency (corticosterone methyl oxidase (CMO) defect)

Increased corticosterone and low or normal aldosterone levels in serum, along with increased urinary corticosterone metabolites. A type I CMO defect (lack of 18 hydroxylation) can be distinguished from a type 2 defect (lack of 18 oxidation) by relative absence or relative excess respectively of 18-hydroxylated corticosterone metabolites.

Absence of aldosterone synthesis results in salt-wasting in the newborn period. A consequent increase of renin stimulates the production of DOC and corticosterone. The DOC substitutes to some extent for aldosterone. Beyond early childhood, the condition is ameliorated, probably because patients have an increased salt appetite, so can compensate for their increased salt loss, but steroid profiles are usually still diagnostic.

Pseudohypoaldosteronism (PHA)

All forms of PHA that lead to salt-wasting show increased corticosterone and aldosterone in serum and their metabolites in urine. *Type 1* PHA has two genetically determined forms, one of which is severe (autosomal recessive, more common, presenting in the first week of life) due to an apical sodium channel (*SCNN1A, B or G*) defect, also called *Type 1B*, while the other is milder (autosomal dominant, less common, presenting later) due to a mineralocorticoid receptor (*NR3C2*) defect, also called *Type 1A*.

Secondary PHA Type 1 (named *Type 3* by some authors) arises as a normal physiological response to any source of uncontrolled sodium loss. This most commonly originates from a urinary tract infection or urinary tract abnormality with or without

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associated infection. Rarer causes include severe exudative eczema, in which electrolytes are lost in the exudate and severe enteropathy. There is similarly increased aldosterone production. If PHA is secondary to urinary tract infection, cholesterol is diagnostically increased in the urinary steroid profile. It probably originates from epithelial cell debris excreted in urine. Clinical consequences of PHA are similar to those of aldosterone synthase defect. If there is evidence of salt wasting, but the urine steroid profile in a sample collected before any treatment is neither consistent with a defect of aldosterone synthesis nor of PHA, then *cerebral salt wasting* should be considered. This is characterised by a lack of an appropriate renal response to the salt loss.

Please note, *Type 2* PHA (Gordon's syndrome) causes hypertension and hyperkalaema, *but not salt wasting*. It is due to defects of lysine-deficient protein kinase (*WNK1 & WNK4*).

DAX-1 (*NROB1*) defect

Major deletions can result in salt-wasting. The urinary steroid profile shows only cortisol metabolites, without the relative increases of corticosterone metabolites expected in the causes listed above. See also the section on *congenital adrenal hypoplasia* below.

Glucocorticoid remediable hyperaldosteronism (GRA)

Increase of serum and urinary aldosterone and 18-hydroxycortisol. Analysis of urinary 18-hydroxycortisol by GC-MS is difficult due to poor recovery, but quantification by LC-MS/MS in both media is possible (although rarely, if ever, available).

GRA diagnosis is best achieved by molecular testing; GRA is due to formation of a chimeric gene involving an unequal crossing-over at meiosis in which the 5' regulatory region of the 11β-hydroxylase gene, *CYP11B1*, is joined to the coding region of the aldosterone synthase gene, *CYP11B2* (with which it shares 90% sequence homology). This leads to ACTH-dependent expression in the zona fasiculata of the adrenal cortex, resulting in aberrant synthesis of aldosterone and 18-hydroxycortisol. Hypertension due to aldosterone excess may be ameliorated by glucocorticoid treatment, since it diminishes ACTH secretion.

Corticosteroid 11-dehydrogenase (*HSD11B2*) deficiency / Apparent mineralocorticoid excess

Serum steroid levels, including of cortisol, are *normal.* In urine, there is an increased ratio of urinary cortisol/cortisone metabolites, of free cortisol/free cortisone and decreased adrenal steroid metabolites as a result of downregulation of the HPA axis to compensate for a decreased cortisol clearance rate. The urine metabolite pattern invariably shows an increase of 5α- v. 5β-reduced tetrahydrocortisol.

Mineralocorticoid receptors in the distal convoluted tubule of the kidney have similar affinity for cortisol and aldosterone. They are, however, normally protected from exposure to cortisol by effectively complete conversion to cortisone by 11-dehydrogenase, which is also localised in the tubule. Enzyme deficiency therefore exposes the receptors to high levels of cortisol, resulting in severe hypertension and hypokalaemia.

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Other disorders directly related to altered steroid production

Steroid sulphatase (*STS*) deficiency (placental sulphatase deficiency)

This disorder may be detected by chance in pregnancy when oestriol as quantified, e.g. in the *triple test* for Down's syndrome. It is by far the most common cause of a very low value when the fetus is apparently healthy. Oestriol is mostly formed in pregnancy from sulphated precursors that arise from the fetus. Within the placenta, these are first desulphated and then further metabolised. Steroid sulphate levels are greatly increased in maternal urine and oestriol is low in maternal serum and urine. Prenatal diagnosis is useful to differentiate this benign cause of low oestriol from the metabolic causes *cholesterol to pregnenolone conversion defect*, *17α-hydroxylase deficiency* (but the latter prenatal diagnosis has never been reported), together with *congenital adrenal hypoplasia.* This last is never associated with oestriol levels as low as in steroid sulphatase deficiency.

The defect causes *X-linked ichthyosis,* (the skin has fish-like scales), probably resulting from diminished epidermal shedding consequent on accumulation of cholesterol sulphate in the dermis. Affected individuals show increased serum cholesterol sulphate. There are no differences in urine steroid metabolites.

Congenital adrenal hypoplasia

This may be *primary,* due to a failure of adrenal development or *secondary,* due to absence of ACTH stimulation, due in turn to either defective pituitary production of ACTH or to a defect of ACTH response. All adrenal steroids in serum and urine may be low or absent in the primary form, while angiotensin-dependent aldosterone production is intact in the secondary form. Isolated ACTH resistance is also known as *FGD* (Familial Glucocorticoid Deficiency).

Primary failure may be either X-linked, due to a DAX-1 defect (Dosage sensitive sex reversal, due to *NROB1* mutation), or autosomal recessive, due to an SF-1 defect (steroidogenic factor 1, due to *NR5A1* mutation).

ACTH resistance (which is autosomal recessive in the various forms), may be due to a defect of the ACTH receptor (*MC2R*, Melanocortin Type 2 or MC2-R), also called FGD 2, or to an MRAP (*MRAP*, Melanocortin Receptor Accessory Protein) defect, also called FGD 1. MRAP transports the ACTH receptor to the cell membrane and activates it. Another cause, Allgrove syndrome, comprises alacrimia, achalasia and adrenocortical insufficiency plus neurological disorders (*AAAS*, Triple A syndrome).

There is deficiency of cortisol in both forms, which results in hypoglycaemia and of aldosterone in the primary form, which results in salt-wasting. Some individuals with primary adrenal failure tend not to spontaneously enter puberty, suggesting that adrenal androgen production has a priming role, but other functions of the affected gene(s) may be involved.

Addison's disease

This is due to progressive destruction of the adrenal cortex, most commonly by an autoimmune processes, but also by tuberculosis. Adrenocortical steroid levels may be normal as a result of trophic hormone (angiotensin 2 and ACTH) hyperstimulation, but they decline when destruction is at an advanced stage. Autoantibodies to 21-hydroxylase enzyme are common in the autoimmune form and may cause a decrease in its activity

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that is detectable in the urine steroid profile.

Adrenocortical insufficiency is also a component of two multisystem disorders. These are adrenoleucodystrophy, a disorder of peroxisomal fatty acid β-oxidation, caused by a defect of a peroxisomal membrane transporter protein (ATPase binding cassette, **ABCD1**) and Wolman disease, a disorder of liposomal acid lipase (**LIPA**), which leads to adrenal calcification.

Cushing's syndrome

High levels of cortisol in blood and saliva and of free cortisol and cortisol metabolites in urine. The normal circadian rhythmicity of cortisol is lost in both blood and saliva. The pattern of urine metabolites is distinctive although not specific, with increases of 5β- vs. 5α-reduced metabolites, of cortisol (11-hydroxy) vs. cortisone (11-oxo) metabolites, of cortisol vs. androgen metabolites and with a relative increase of free cortisol. The faster the increase of cortisol production, the more marked these changes are. Other sources of acute cortisol increase, such as stress, hydrocortisone administration and synacthen injection produce similar changes. This urine steroid pattern is also commonly found in older patients, but without marked increases in cortisol metabolite levels and so may reflect short term stress in this age group. In contrast, chronic, stable, cortisol hypersecretion, such as is associated with a slow-growing adrenocortical adenoma or glucocorticoid resistance, is usually associated with a normal urine metabolite pattern. This presumably is a result of induction of the hepatic catabolic enzymes. In such circumstances, the urine free cortisol assay could also give a normal value.

Cushing's syndrome arises from excess cortisol exposure. If the source is endogenous, cortisol levels are high in blood and urine, and the normal circadian rhythmicity of cortisol is lost. This may be *primary*, resulting from autonomous overproduction by an adrenal tumour (see next section) or *secondary*, as a result of ACTH excess, or *tertiary* as a result of CRH excess. ACTH excess may arise from the pituitary, (referred to as Cushing's *disease*) or from a tumour elsewhere (referred to as *ectopic ACTH syndrome*). Another rarer form, which may be regarded as secondary, is *food-dependent Cushing's syndrome,* in which gastric inhibitory polypeptide (GIP) receptors are abnormally expressed in adrenocortical tissue. A post-prandial increase of GIP thus stimulates cortisol secretion. Chronic hyperstimulation may also result in bilateral nodular adrenal hyperplasia. Receptors for other hormones (vasopressin, serotonin etc.) may be similarly abnormally expressed.

Cushing's syndrome may alternatively be *iatrogenic*, arising from treatment with glucocorticoids (by inhaled, topical or oral routes). These may be prescribed, secretly self-administered or present (and usually undeclared) in 'herbal' medicine preparations. Use of prednisolone may confuse investigations because it cross-reacts in some (but not all) cortisol immunoassays; distinction is possible by LC-MS/MS cortisol measurement or urine steroid profiling. Most other synthetic glucocorticoids cannot be detected by urinary steroid profiling, but their use would be indicated by suppression of endogenous steroids.

Autonomous steroid production

Adrenocortical tumours

Adrenocortical tumours usually produce very distinctive patterns of steroid increase which are readily detected by urine steroid profiling. Most are sporadic, but genetically

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determined forms of adrenal tumours exist: **Li-Fraumeni syndrome** due to lack of a tumour suppressor, *tumour protein 53* (*TP53*), **Beckwith-Wiedemann syndrome** (caused by changes on chromosome **11p15.5**) and **Lynch syndrome** (caused by alterations in several genes involved in DNA mismatch repair). In addition, **McCune Albright syndrome** causing multinodular hyperplasia, is due to a mutated G-protein signalling molecule (*GNAS*), which prevents down-regulation of cyclic AMP signalling. Finally, primary pigmented nodular adrenocortical hyperplasia is usually associated with the **Carney complex** due to mutation of a tumour-suppressor gene (*PRKAR1A*). Infants with McCune Albright syndrome may show signs of Cushing's at birth and have high urine levels of the characteristic DHA and pregnenolone metabolites as well as cortisol metabolites.

In adults, adenoma and carcinoma are nearly always distinguishable by urinary steroid profiling. Steroid-producing adenomas may generate excess cortisol, leading to increased urinary cortisol metabolites. Another common pattern is relative increase of 11-hydroxylated androstenedione metabolites.

In adrenocortical carcinoma (ACC), findings are very heterogeneous. Clinical evidence of steroid overproduction (e.g., Cushing's syndrome, androgen excess in females) is typically only evident in <50% of cases. These will be mirrored in urine, with increases of androgen or cortisol metabolites. Much more rarely, there is excess production of 11-deoxycorticosterone, resulting in mineralocorticoid hypertension and hypokalaemia, but with suppressed renin and aldosterone measurements in blood. Even rarer, there may be oestrogen production, leading to gynaecomastia in the male. We have seen too few of the latter to be able to classify their steroid profiles, but we find that other steroids are simultaneously secreted in excess.

For the majority of ACC cases, the patterns found resemble those of one or more partial steroid enzyme deficiencies. We typically see high levels of urinary metabolites of intermediates in the glucocorticoid and mineralocorticoid pathways; often there are no signs of steroid hormone excess clinically. We find that metabolites of 11-deoxycortisol, DHEA, pregnenolone and 17-hydroxypregnenolone are often useful ACC markers. The commonly used single steroid assays in serum may not necessarily show any corresponding excesses. Relative increases of unusual urinary steroids, not readily ascribed to a particular enzyme deficiency, are also common and form excellent markers, both diagnostically and for monitoring. If steroid sulphates are among the steroids that are increased in urine, there may also be large increases of serum and urine cortisol sulphate. Whether this is detected by cortisol assays will depend on the method used. Steroid patterns may have prognostic use, but this is still under study. Steroid levels are not correlated with tumour size: even though steroid concentrations are frequently much increased, the production rate per weight of tumour tissue is generally much lower than by the normal adrenal cortex.

In children, distinction of steroid patterns in adrenocortical adenoma and carcinoma is not so well established, since histological classification is less certain. A peak of incidence of virilising tumours occurs between 18 months to 4 years. The most common pattern features high levels of DHEA and DHEA metabolites and, less commonly, there are increases of 11-hydroxylated androstenedione metabolites. The latter may be associated with a benign form, as in adults, but the required observational data are lacking. Mixtures of the two patterns also occur.

For reasons that are not clear, DHEA sulphate in blood and urine are poorly correlated across the spectrum of disorders in which it is increased. This does not appear to reflect shortcomings in the analytical methods. It may relate to variable rates of renal clearance,

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since the metabolic clearance rate of this steroid is especially slow.

Conn's adenoma describes an aldosterone-secreting tumour, which usually has a distinctive yellow appearance and different origin from other adrenocortical adenomas. Many are now thought to be associated with one of a number of different single gene mutations, with the most common being that of the potassium channel GIRK4 (coded by *KCNJ5*). Conn's adenomas do not usually show distinctive urine steroid profiles, but may secrete other steroids, including cortisol and 18-hydroxycortisol. The classical picture of a single discrete mass that can be differentiated from multinodular adrenocortical hyperplasia is probably never true: histologically, zona glomerulosa hyperplasia and subcapsular foci are also seen.

Gonadal tumours

Gonadal tumours may secrete androgens, oestrogens, pregnenolone, progesterone or 17-hydroxyprogesterone. If the latter is secreted, urinary metabolites do not include those of 21-deoxycortisol, enabling distinction from CAH due to 21-hydroxylase deficiency and other adrenal causes of 17-hydroxyprogesterone excess. If serum testosterone is increased and the source is an adrenal tumour, there are invariably large increases of androstenedione, DHEA and DHEA metabolites in urine. If the source is gonadal, there may be no increases of urinary androgen metabolites. A convenient way to think of this is that gonadal testosterone production is much more 'efficient' than adrenal production. Gonadal steroid-secreting tumours are often benign and responsive to gonadotrophins. Abnormal development of gonadal tissue (as in ovarian hyperthecosis) may give rise to a similar pattern of steroids. Some may develop in postmenopausal women, as a result of gonadotrophin increase. Both ovarian and extra ovarian masses may be difficult to visualise on tomographic scanning: steroid suppression by GnRH analogue therapy provides positive evidence for their presence.

Clinical considerations

Clinical effects of steroid-secreting tumours are those of excess of the steroid hormones produced. If cortisol is secreted, the contralateral adrenal gland will be atrophied as a result of ACTH suppression. Cortisol production from a growing tumour may not become clinically apparent as Cushing's until the production rate from the tumour exceeds the normal daily production rate of cortisol i.e.: until the capacity of the HPA axis to compensate by down regulation has been exhausted. Following surgery, it is necessary to supplement with glucocorticoid in a diminishing dose to enable the remaining gland to recover. Clinical experience suggests that recovery of adrenal function is not always complete, and some practitioners maintain that lifelong steroid supplementation may be necessary. Very rarely, ACTH is not fully suppressed in primary Cushing's syndrome. This may be a result of competition for the glucocorticoid receptor by tumour steroid products, of which 21-deoxycortisol is a prime candidate. When there is no over secretion of any hormonal steroids, the patient may present with loin pain, or a mass may be detected unexpectedly during a tomographic scan for unrelated reasons. Such tumours thus tend to be large and the time course of their growth and the evolution of their steroid production remains unknown.

Following surgery for adrenocortical carcinoma, steroids identified as increased may be used to detect residual tumour and then monitored at intervals to detect recurrence. The same pattern is often preserved on recurrence, but in some cases there are clear differences, probably due to differences in the advancement of the cell clones that proliferate in the secondaries. Some secondaries with histological evidence that they are adrenocortical tumour tissue show no evidence of steroid secretion.

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Mitotane is in universal use for adjuvant therapy, as an adrenolytic agent. This results in profound changes to the urine profile: suppression of 20β-hydroxysteroid dehydrogenase and 5α-reductase type 2 and also induction of CYP3A4 (6β-hydroxylation). These effects can be taken into account when checking in patients on treatment for the reappearance of steroids that were increased before surgery. Since mitotane is invariably used with hydrocortisone (cortisol) at doses that would be expected to cause profound ACTH suppression, any steroid increase found is likely to be autonomous. If cortisol was the major steroid secreted by an adrenocortical carcinoma, it would be necessary to substitute dexamethasone as the replacement glucocorticoid in order to detect resurgence of autonomous cortisol production. Androgen metabolite excretion tends to be very low during mitotane treatment, but women of reproductive age still show evidence of cycling. Mitotane may suppress testicular androgen secretion, but this is not well studied. Androgen substitution may be beneficial.

Effects of steroid treatment

Glucocorticoids

Oral treatment with cortisol or synthetic analogues results in suppression of endogenous cortisol production. Use of inhaled corticosteroids above a threshold dose will cause adrenal suppression. Glucocorticoid-containing skin creams can cause virtually complete adrenal suppression, especially if used extensively on broken skin or if the agent is especially potent (e.g. clobetasol propionate). Use of long-acting depot preparations, such as of triamcinolone, can result in unexpectedly prolonged and profound suppression. If glucocorticoid treatment exceeds normal physiological levels, symptoms of Cushing's syndrome are produced. This may result in total abolition of ACTH-dependent adrenal steroid production. When investigating causes of adrenocortical insufficiency in patients who have been on hydrocortisone (cortisol) treatment, one protocol is to substitute dexamethasone and give depot synacthen (synthetic ACTH). Steroid secretion usually takes several days to show increase. Short withdrawal of treatment prior to investigation is thus uninformative, both because the adrenal will not have recovered from suppression and because this secondary effect cannot be differentiated from a primary adrenal insufficiency.

Androgen treatment and anabolic-androgenic steroid abuse

Androgens suppress gonadotrophin production, resulting in reversible testicular atrophy in the male, with consequent reduction of androgen production and decrease of sperm count. Testosterone predominantly gives rise to the metabolites androsterone and aetiocholanolone in urine. These also arise from androstenedione and DHA, so that testosterone treatment cannot be usefully monitored by urine profiling.

In suspected anabolic-androgenic steroid abuse, if steroids other than testosterone are being taken, the testosterone + androstenedione metabolites in urine will be low. Since testosterone is often taken as well, normal levels of these do not exclude the use of other anabolic steroids. A standard urine steroid profile is therefore of limited use in such cases. Specific targeting of the known urinary metabolites of synthetic androgens by a specialist sports doping laboratory is necessary and is strongly advised. We recommend contacting the Drug Control Centre, King's College Waterloo Campus of this is being considered. Testosterone abuse may also be detected in such laboratories by an increase of testosterone/epitestosterone in serum and by isotope ratio measurements, which can differentiate endogenous from exogenous sources.

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Steroid contraceptives

Numerous reports of metabolic effects of steroid contraceptives are not matched by any detailed systematic studies of effects on serum or urine steroid patterns. We know of none that would be expected to change interpretation of steroid profile results.

Other disorders indirectly associated with changed steroid metabolism

Liver disease

Liver diseases which result in restricted venous flow, leading to portal hypertension, show consistent changes in the urinary steroid profile. These comprise increase of DHEA metabolites, especially androstenetriol and of the cortisol metabolite, α-cortolone. We have found this to be common to cirrhosis due to alcoholism or hepatitis C, congenital hepatic fibrosis, haemochromatosis, liver tumours and Wilson's disease.

Cirrhosis of the liver is associated with increase of circulating oestrogen and decrease of androgen and consequent gynaecomastia in the male. This is normalized after liver transplant.

Both observations may be explained by a failure of normal steroid metabolism in the liver, leading to increase in circulating precursors that are then metabolised differently in the periphery.

Altered thyroid status

Cortisol metabolic clearance rate is higher in hyperthyroidism and lower in hypothyroidism; these are probably the result of enhanced and diminished cortisol 11-oxidation respectively. As noted under *5α-Reductase 2 (SRD5A2) deficiency,* severe hypothyroidism mimics this, with relatively low levels of 5α-reduced urine metabolites.

Epilepsy

Children with epilepsy seem to have a high frequency of presentation with signs of precocious adrenarche. Urine findings generally support precocious adrenarche, showing increased DHEA and DHEA metabolites but with a disproportionate rise in the metabolites relative to DHEA. This suggests that 16α-hydroxylase in adrenals and/or liver is induced by their anticonvulsant treatment.

Porphyria

A decrease of urinary 5α-reduced steroids has been observed in acute intermittent porphyria, with an association with active episodes. A more recent detailed study has not confirmed this, but shown significant decreases in many cortisol metabolites.

Aetiocholanolone fever

Pyrexia has been reported on occasion to be associated with increase of serum free aetiocholanolone and with increase of aetiocholanolone (5β-reduced) relative to androsterone (5α-reduced) in urine. The ratio of urinary metabolites in normal subjects shows wide inter-person variability: we have never been able to demonstrate a marked

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relative increase of aetiocholanolone in patients with pyrexia of unknown origin.

Changes in body mass

Severe weight loss, as is seen in anorexia nervosa, is associated with relative decrease of 5α-reduced compared with 5β-reduced urine steroid metabolites. This is reversed on refeeding. To some extent, the opposite is true for weight increase, although the effects of morbid obesity are surprisingly small. There is a weak correlation between urinary steroid output and body mass. Neither changes of insulin sensitivity nor diabetes result in clear changes in the urinary steroid profile. Extreme insulin resistance and insulin sensitization by use of metformin are two situations in which major change is still not found.

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