Adrenals
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This year’s search of ‘adrenal’ and ‘steroidogenesis’ in PubMed yielded more than 6,000 hits from which we have selected the following collection of articles. Whenever possible we have avoided subjects which have been discussed in detail in the Yearbook 2008, unless progress in the field has been incremental [1]. Some emerging themes include modulators of steroidogenesis as a cause of variant phenotypes or new monogenic disorders; peripheral circadian regulation within the adrenal itself; DHEA and adrenarche; and potential cancer therapies developed from studying the molecular pathophysiology of adrenal tumor tissues and cells.

Mechanism of the year
Sulfation defects cause adrenal hyperandrogenism

Inactivating PAPSS2 mutations in a patient with premature pubarche
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Background: Dehydroepiandrosterone (DHEA) sulfotransferase, known as SULT2A1, converts the androgen precursor DHEA to its inactive sulfate ester, DHEAS. This conversion is important in preventing the DHEA being converted to more potent androgens. Normal SULT2A1 catalytic activity requires the sulfate donor, 3’-phosphoadenosine-5’-phosphosulfate (PAPS), which is generated by PAPS synthase 2 (PAPSS2). Defects in these systems could result in impaired DHEA sulfation, resulting in increased conversion of DHEA to androgens.

Methods: Mutational analysis of PAPSS2 in a girl with premature pubarche, hyperandrogenic anovulation, very low DHEAS levels, and increased androgen levels.

Results: Compound heterozygous mutations in the gene encoding human PAPSS2 were identified in this individual. These mutations were found to be inactivated following coincubation of human SULT2A1 with wild-type or mutant PAPSS2 proteins.

Conclusion: PAPSS2 deficiency is a novel monogenic adrenocortical cause of androgen excess.

Although cytochrome P450 and hydroxysteroid dehydrogenase enzymes play a central role in classic steroidogenic pathways, it is becoming increasingly clear that many other cofactors and modulators of steroidogenic activity also play a key role in regulating this process. Disruption of some of these factors has been shown to result in human disease (e.g. P450 oxidoreductase deficiency; variations in hexose-6-phosphate dehydrogenase) [2, 3]. In this recent report, Wiebke Arlt and colleagues hypothesized that the high DHEA levels and undetectable DHEAS levels found in a girl with premature pubarche, hyperandrogenism, and PCOS/secondary amenorrhea could be due to a defect in part of the sulfation pathway. Sulfation of DHEA into the inactive compound DHEAS is important in reducing circulating levels of DHEA and preventing its conversion into more potent androgens such as testosterone. Sulfation of DHEA is primarily regulated by DHEA sulfotransferase (SULT2A1) in the liver and adrenal, which requires the sulfate donor 3’-phospho-adenosine-5’-phosphosulfate (PAPS) for catalytic activity (fig. 1). PAPS is generated by two isoforms of PAPS synthase, PAPSS1 and PAPSS2. Mutational analysis revealed compound (double) heterozygous changes in PAPSS2 in this girl, which
disrupted SULT2A1 activity. Therefore, PAPSS2 deficiency represents a novel regulatory defect of steroidogenesis and a new monogenic cause of adrenal hyperandrogenism. This defect should be considered in children with low or undetectable levels of DHEAS, but with high normal or elevated DHEA and androgens. Of note, loss of PAPSS2 activity has previously been described in one kindred as a cause of spondyloepimetaphyseal dysplasia, Pakistani type (OMIM 603005) [4]. An isoform of PAPSS2 likely plays a key role in proteoglycan sulfation in growth-plate chondrocytes. The girl reported here had advanced bone age, short stature, some vertebral anomalies and shortened tubular bones, which probably reflected a combination of excess androgen exposure together with an underlying bony dysplasia. It remains to be seen how prevalent this condition is, how variable the bony aspects will be, and whether milder disruptions of PAPSS2 or related sulfation factors might be found in more common causes of hyperandrogenism and polycystic ovarian syndrome. On the other hand, DHEAS is a substrate for an activating DHEAS (steroid) sulfatase (fig. 1). This enzyme is expressed in several tissues including adipose tissue and its disruption has been associated with X-linked ichthyosis. However, it is quite possible that other clinical and biochemical phenotypes can be associated with disturbances in sulfation systems. You are welcome to guess the phenotypes of these and find such cases.

**Fig. 1. Lower panel.** Sulfation of DHEA into the inactive compound DHEAS is important in reducing circulating levels of DHEA and preventing its conversion into more potent androgens such as testosterone. This process is primarily regulated by DHEA sulfotransferase (SULT2A1), which requires interaction with the sulfate donor 3′-phospho-adenosine-5′-phosphosulfate (PAPS) for catalytic activity. **Upper panel.** PAPS synthase 2 (PAPSS2) plays a crucial role in generating PAPS in several tissues. Defects in PAPPS2 have now been shown to cause premature pubarche and hyperandrogenism due to defective DHEA sulfation.
Programming of hypertension: associations of plasma aldosterone in adult men and women with birthweight, cortisol, and blood pressure

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Background: Low birth weight is associated with higher blood pressure in adulthood. Several animal models suggest that this effect may be due to chronic activation of the hypothalamic-pituitary-adrenal or renin-angiotensin-aldosterone axes. In humans, low birth weight has been shown to be associated with elevated plasma cortisol, but associations between birth weight and aldosterone have not been reported.

Methods: Aldosterone was measured in serum samples from 106 women and 205 men from the Hertfordshire cohort (aged 67–78 years) for whom birth weight was recorded. Participants underwent an overnight low-dose (0.25 mg) dexamethasone suppression test and a low-dose (1 µg) ACTH (corticotropin) stimulation test. Genotyping of the –344 C/T polymorphism of the CYP11B2 gene encoding aldosterone synthase was also performed.

Results: Median aldosterone was 6.22 (range 0.15–38.74) ng/dl. This value was higher in men than women (p < 0.0001). Higher aldosterone levels after both dexamethasone and ACTH stimulation were associated with higher blood pressure (r = 0.20, p = 0.001; r = 0.33, p<0.0001, respectively) and with lower birth weight (r = –0.16, p = 0.008; r = –0.21, p = 0.001, respectively). Adjusting for age, gender, obesity, and genotype did not affect the significance of these results.

Conclusion: These findings suggest that, in humans, factors in early life that restrict fetal growth and program activation of the hypothalamic-pituitary-adrenal axis result in increased mineralocorticoid as well as glucocorticoid activity.

Disruption of the Ang II type 1 receptor promotes longevity in mice

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J Clin Invest 2009;119:524–530

Background: The renin-angiotensin system is a key regulator in the pathogenesis of hypertension and many cardiac and renal diseases in humans. It is likely that angiotensin II (Ang II) regulates this effect through Ang II type 1 receptors (AT1 and AT2) to influence immune responses, inflammation, cell growth and proliferation.

Methods: Generation and characterization of a mouse model with targeted disruption of the Agtr1a gene (encoding AT1A).

Results: Agtr1a−/− mice showed marked prolongation of life span compared to wild-types. They developed less cardiac and vascular injury. Multiple organs from these mice displayed less oxidative damage, increased mitochondria and upregulation of the prosurvival genes nicotinamide phosphoribosyltransferase (Nampt) and sirtuin 3 (Sirt3) in the kidney. In cultured tubular epithelial cells, Ang II downregulated Sirt3 mRNA. This effect was inhibited by an AT1 antagonist.

Conclusion: Disruption of AT1 promotes longevity in mice, possibly through the reduction of oxidative stress and overexpression of prosurvival genes. The Ang II/AT1 pathway may be targeted to influence lifespan in mammals.

The association between low birth weight and increased blood pressure and mortality in later life is well established, and a trend towards higher cortisol in children and adults who were smaller at birth has been reported in some studies [5–7]. However, few data exist on the relation between birth weight and the angiotensin-aldosterone axis. In a detailed study of the Hertfordshire cohort (originally investigated by David Barker), John Connell and colleagues show that lower birth weight is associated with higher aldosterone between 67 and 78 years of age, and that aldosterone concentrations were associated with blood pressure measurements. Unfortunately, no measurements of plasma renin activity or aldosterone:renin ratios were reported, and the recorded blood pressures were sur-
prisingly high, potentially reflecting a stress-mediated response. Nevertheless, the fact that recorded aldosterone is higher in later life in individuals born with low birth weight is an important observation. Indeed, the mouse model reported by Benigni et al. shows that disruption of the angiotensin 1A receptor in mice results in quite markedly increased longevity. This is in line with a mouse model of a gain-of-function mutation of AT1 showing progressive cardiac fibrosis with increased expression of collagen. These studies offer a rationale for exploring the possibility that AT1 antagonists will prolong life in humans. Indeed, silent activation of the renin-angiotensin system could be a significant factor in programmed excess morbidity and mortality associated with low birth weight.

Extraadrenal 21-hydroxylation by CYP2C19 and CYP3A4: effect on 21-hydroxylase deficiency

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Background: CYP21A2 gene mutations cause adrenal 21-hydroxylase (P450c21) deficiency (21OHD). CYP21A2 mutations generally correlate well with the 21OHD phenotype, but some children with severe CYP21A2 mutations have residual 21-hydroxylase activity. It is known that progesterone can be 21-hydroxylated by several hepatic P450 enzymes, but the physiological significance of these in modifying 21OHD is not known. Therefore, this study aimed to: (a) determine whether CYP2C19 and CYP3A4 can 21-hydroxylate progesterone and 17-hydroxyprogesterone (17OHP); (b) investigate the potential influence of a common P450 oxidoreductase (POR) variant A503V on these activities, and (c) examine any correlation between CYP2C19 variants and phenotype in patients with 21OHD.

Methods: Human P450c21, CYP2C19, and CYP3A4 were co-expressed in bacteria with wild-type or A503V POR. The 21-hydroxylation of radiolabeled progesterone and 17OHP was assessed, and the maximum velocity (Vmax) and Michaelis constant (Km) of the reactions were calculated. CYP2C19 was genotyped in those 21OHD patients where the genotype predicted a severe form of congenital adrenal hyperplasia but in whom the clinical features were milder than expected.

Results: The Vmax/Km for 21-hydroxylation of progesterone by CYP2C19 and CYP3A4 were 17 and 10%, respectively, compared to P450c21. Neither CYP2C19 nor CYP3A4 could 21-hydroxylate 17OHP. The A503V variant of POR did not affect this Km. The CYP2C19 ‘ultrametabolizer’ allele CYP2C19*17 was homozygous in 1 of 5 patients with a 21OHD phenotype that was milder than predicted by the CYP21A2 genotype.

Conclusion: CYP2C19 and CYP3A4 can 21-hydroxylate progesterone but not 17OHP, potentially compensating for mineralocorticoid deficiency, but not for glucocorticoid deficiency. Extraadrenal 21-hydroxylation is likely to reflect the actions of multiple enzymes.

Genotype-phenotype relationships for 21-hydroxylase deficiency are reported to be fairly good, with the severity of the clinical condition usually tracking with the milder defect in compound (double) heterozygote cases. However, it is apparent that some children and adults who are predicted to have severe disease from their genotype status have surprisingly mild biochemical features, and others, with a marked defect in fasciculata glucocorticoid biosynthesis, have normal or mild electrolyte disturbance. The roles of extraadrenal steroid modification is a major area of research, and in this paper Walter Miller and coworkers hypothesize that extraadrenal 21-hydroxylation might play a role in decreasing the severity of CAH in this group of children. Detailed studies of CYP2C19 and CYP3A4 show that these enzymes are able to 21-hydroxylate progesterone but not 17OHP. This effect might reduce the severity of salt loss in some of these patients, especially as mineralocorticoids are normally produced and are active in quantitatively lower concentrations than glucocorticoids in humans. Furthermore, 1 in 5 of patients studied with a milder than expected phenotype was found to be homozygous for the ‘ultrametabolizer’ CYP2C19 allele. This study focused on two candidate enzymes and also a common variant of P450 oxidoreductase, which was not shown to have a modifying effect [8]. It is possible that other enzymes could play a role in these pathways and that the physiological effects of such changes might be more pronounced in vivo. This paper therefore highlights the importance of extraadrenal steroid modification, which might not only influence patient phenotype but might also influence individual responses to glucocorticoid and mineralocorticoid treatment.
**New hope: biology-based options for treating adrenal carcinoma?**

**Expression of insulin-like growth factor-II and its receptor in pediatric and adult adrenocortical tumors**

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**Background:** Adrenocortical tumors are heterogeneous in nature and their underlying pathogenesis is not well understood. However, studies of adult adrenocortical carcinomas (ACC) have shown that IGF-II overexpression is a frequent feature.

**Methods:** Expression of IGF-II and its receptor (IGF-IR) was analyzed by quantitative real-time PCR in 57 adrenocortical tumors (37 adenomas and 20 carcinomas; 23 from children, 34 from adults). The effects of a selective IGF-IR kinase inhibitor (NVP-AEW541) on cell proliferation and apoptosis were assessed in NCI H295 cells as well as in a new cell line established from a pediatric adrenocortical adenoma.

**Results:** Overexpression of IGF-II transcripts was seen in both pediatric ACC and adenomas, and in adult ACC (ACC, 270.5 ± 130.2 vs. adenoma, 16.1 ± 13.3; p = 0.0001). IGF-IR expression was significantly higher in pediatric ACC than adenomas (ACC, 9.1 ± 3.1 vs. adenoma, 2.6 ± 0.3; p = 0.0001), whereas its expression was similar in adult ACC and adenomas. Univariate analysis showed that IGF-IR expression was a predictor of metastases in pediatric ACC (hazard ratio 1.84; 95% confidence interval 1.28–2.66; p = 0.01). The IGF-IR kinase inhibitor, NVP-AEW541, blocked cell proliferation in a time- and dose-dependent manner in both cell lines, primarily through a significant increase in apoptosis.

**Conclusion:** IGF-IR overexpression appears to be a biomarker of pediatric adrenocortical carcinomas compared to adenomas. A selective IGF-IR kinase inhibitor has antitumor effects in both adult and pediatric adrenocortical tumor cell lines. IGF-IR inhibitors might represent a promising therapy for human adrenocortical carcinoma in the future.

**Preclinical targeting of the type I insulin-like growth factor receptor in adrenocortical carcinoma**


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**Background:** Adrenocortical carcinoma (ACC) is a rare and lethal malignancy. Current drug treatment is largely empirical and ineffective. A more effective approach might be to direct new treatments at molecular targets known to be critical in the pathophysiology of ACC.

**Methods:** Human adrenal tumors and ACC cell lines were profiled by DNA microarray analysis to assess activated IGF signaling. The efficacy of two IGF receptor (IGF-1R) antagonists (NVP-AEW541 and IMC-A12), alone and in combination with mitotane, was assessed in ACC cell lines that display or lack activated IGF signaling and in ACC xenograft tumors in athymic nude mice.

**Results:** Transcriptional profiling data of human adrenal tumors revealed IGF2 as the single highest upregulated transcript in most carcinomas. The majority of ACC cell lines tested showed constitutive IGF ligand production and activation of downstream effector pathways. Both of the IGF-1R antagonists tested produced significant dose-dependent growth inhibition in ACC cell lines. This growth inhibition was enhanced when mitotane was used in combination with the IGF-1R antagonists. IGF inhibition also markedly reduced ACC xenograft tumor growth greater than that observed with mitotane treatment alone. Combination therapy with mitotane suppressed tumor growth even further.

**Conclusion:** IGF signaling plays a key role in the pathophysiology of ACC. IGF-1R antagonists provide a rational targeted approach to the treatment of ACC and could form the basis of future clinical trials. See also:
Inhibition of adrenocortical carcinoma cells proliferation by SF-1 inverse agonists
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The T cell factor/β-catenin antagonist PKF115-584 inhibits proliferation of adrenocortical carcinoma cells
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Adrenocortical carcinoma (ACC) can be a difficult tumor to treat and classification and prognostic indices are poor. In recent years, several papers have shown distinct molecular events in these tumors such as overexpression of steroidogenic factor-1 (SF-1), disturbances in inhibin A and β-catenin/WNT-4 pathways, and upregulation of insulin-like growth factor signaling [9–11]. These events are often somatic (occurring just within the tumor tissue), and may provide a ‘second hit’ on top of loss of heterozygosity of a tumor suppressor such as TP53, or dysregulation of imprinting mechanisms in the case of IGF II. Several studies this year are starting to use this information to develop potential novel therapeutic approaches to the treatment of adrenocortical carcinoma. The papers by Almeida et al. and Barlaskar et al. focus on targeting the type 1 insulin-like growth factor receptor and show increased apoptosis of tumor cells in culture and regression of ACC xenograft tumors in nude mice. Furthermore, data from Enzo Lalli’s group have shown that inverse agonists of SF-1 or disruption of β-catenin signaling can reduce the growth of adrenal tumor cell lines. Taken together these data suggest that several new therapeutic options may be on the horizon. The difficulty will be targeting these treatments to adrenal or metastatic adrenal carcinoma tissue as, of course, IGF-, β-catenin- and SF-1-mediated pathways are widespread in many different endocrine and metabolic tissues. Nevertheless, these reports do provide good examples of how studying rare events and molecular mechanisms may ultimately translate back into more effective patient treatment.

New concerns: under pressure

Blood pressure in pediatric patients with Cushing syndrome
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Background: Hypertension (HTN) has been reported in up to 50% of children with Cushing syndrome (CS) but its course, consequences, and relation to different causes of CS are not well documented.

Methods: Blood pressure (BP) was measured in pediatric patients with CS before and after transphenoidal surgery (TSS; 86 children with Cushing disease, CD) or adrenalectomy (ADX; 27 children with ACTH-independent CS, AICS) to identify the prevalence of residual HTN and consequences of it.

Results: Patients with CD and AICS had significant HTN before surgery. Higher BP correlated with cortisol levels. Systolic HTN (SHTN) was more frequent in AICS than in CD (74 vs. 44%, p = 0.0077), but the prevalence of diastolic HTN (DHTN) was similar between groups. Significant decreases in SHTN
were seen immediately post-TSS and ADX and 1 year later. However, 16 and 4% of the patients with CD, and 21 and 5% of the patients with AICS still had SHTN and DHTN, respectively, at the 1-year follow-up. Two patients suffered serious consequences of hypertension: 1 child had multiple infarcts and another had hypertensive encephalopathy.

Conclusion: Residual HTN can be found in a proportion of children treated for CD despite a significant improvement after surgical cure. HTN appears to correlate with the degree of hypercortisolemia. Serious HTN-related side effects are relatively rare but can occur in the perioperative period.

Hypertension is well established as a presenting feature of Cushing syndrome in children and is extremely important to control in the perioperative period. Although blood pressure usually falls following treatment of the primary cause, this paper shows that a significant proportion of children treated for Cushing disease or ACTH-independent Cushing syndrome have residual hypertension at follow-up 1 year after surgery. This is a difficult group of children to perform well-controlled follow-up studies with, for example, approximately one third of those treated for Cushing disease were still on steroid replacement therapy at the time of evaluation and 1 of the patients was probably in the early stages of disease recurrence. Furthermore, calculating hypertension based on the 95th percentile adjusted for height, age and gender is complicated by the fact that many of these children had an elevated BMI and their growth patterns are disrupted following their disease. The duration of Cushing or hypertension before diagnosis may be an important prognostic factor, which remains unknown in this cohort, underscoring the importance of early diagnosis of patients with hypercortisolemia and hypertension to prevent late sequelae. Nevertheless, this report does show that residual hypertension may be found in a significant proportion of children treated for Cushing syndrome. As this may be in part due to vascular remodeling or other programmed events, long-term follow-up of blood pressure and morbidity relating to hypertension is essential [12]. A more detailed study of circadian blood pressure in adults who had been treated for Cushing syndrome in childhood or adolescence would be useful in order to get a true reflection of how significant a long-term problem this might be.

Concepts revised: the adrenal’s got rhythm

Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production
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Proc Natl Acad Sci USA 2008;105:20970–20975

Background: Adrenal production of glucocorticoids (GC) shows a robust daily oscillation. This circadian rhythm is thought to be controlled by the suprachiasmatic nucleus of the hypothalamus and mediated by the hypothalamic-pituitary-adrenal axis.

Methods: Peripheral clock mechanisms were studied in the adrenal gland itself as well as the effects of an adrenal-specific knockdown of the canonical clock protein BMAL1 in mice.

Results: The adrenal gland has its own peripheral clock that is tightly linked to steroidogenesis by the steroidogenic acute regulatory protein. Mice with adrenal-specific deletion of BMAL1 showed that the adrenal clock machinery is required for circadian GC production. Furthermore, circadian behavioral patterns were disrupted in these animals and altered expression of Period1 was seen in several peripheral organs.

Conclusion: The adrenal has a peripheral clock that plays an essential role in harmonizing mammalian circadian systems by generating a robust circadian GC rhythm.

Circadian rhythms are essential in the hypothalamic pituitary adrenal (HPA) axis. Our traditional view is that the suprachiasmatic nucleus (SCN) of the hypothalamus influences CRF and ACTH release resulting in a peak of GC in the morning and subsequent fall in GC concentrations throughout the
day. However, studies more than 30 years ago showed that GC rhythm could be maintained in hypothysectomized rats given continuous ACTH infusions, suggesting that an inherent circadian rhythm of GC synthesis and release from the adrenal gland could exist [13]. The identification of several molecular and cellular components of oscillatory systems (such as \textit{Clock} and \textit{Period}) has revolutionized the study of circadian biology. With this has come the realization that many peripheral tissues have their own circadian rhythms. Several recent papers have addressed the role of a potential peripheral clock mechanism in the adrenal gland, which may be regulated through ACTH-independent mechanisms such as melatonin signaling or splanchnic innervation [14–16]. This study by Son and colleagues goes one step further by generating and characterizing a mouse model with an adrenal-specific knockdown of the clock protein, BMAL1. Using this model, the authors have clearly shown that the adrenal gland has its own clock mechanism regulating steroidogenesis, which is mediated by StAR and disrupted following adrenal specific deletion of BMAL1. The fact that altered patterns of circadian clock proteins were seen in other organs confirms that the adrenal gland itself plays a direct role in regulating the circadian rhythms of other peripheral tissues [17]. This work does not detract from the fact that the hypothalamus plays a key role in synchronizing these systems but the adrenal gland itself is now emerging as an increasingly important regulator of systems chronobiology. Another peripheral clock was reported to tick in the liver, as discussed in the Editors’ Choice [p. 240].

**Important for clinical practice: reproductive issues in CAH**

Clinical and molecular characterization of a cohort of 161 unrelated women with nonclassical congenital adrenal hyperplasia due to 21-hydroxylase deficiency and 330 family members


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**Background:** Non-classical congenital adrenal hyperplasia (NC-CAH) due to partial 21OH deficiency is one of the most prevalent autosomal recessive diseases in humans. This study aimed to investigate genotype/phenotype relationship in probands and family members.

**Methods:** The study group consisted of 161 unrelated women with NC-CAH (diagnosed on account of late-onset symptoms, mainly hirsutism, and a post-ACTH 17-hydroxyprogesterone (17OHP) >10 ng/ml) and 330 of their relatives. Genotyping of CYP21A2 was performed in 124 of the probands.

**Results:** The most frequent mutation found was V281L. One severe mutation was found in 63.7% of probands and, surprisingly, two severe mutations were found in four probands. Basal and post-ACTH 17OHP concentrations were significantly higher in probands carrying at least one severe mutation than in those with two mild mutations (p < 0.01), but clinical signs, basal testosterone and androstenedione were not significantly different. Among the 330 family members, 51 were homozygotes or compound heterozygotes for CYP21A2 changes. Of these, 42 were clinically asymptomatic. A total of 242 were heterozygotes and androstenedione were not significantly different. Among the 330 family members, 51 were homozygotes or compound heterozygotes for CYP21A2 changes. Of these, 42 were clinically asymptomatic. A total of 242 were heterozygotes and 37 carried no CYP21A2 changes (unaffected). Post-ACTH 21-deoxycortisol (21dF) was significantly higher in heterozygotes than in unaffected, but an overlap existed. In 12 (5%) heterozygotes, post-ACTH 21dF was <0.55 ng/ml, the cutoff value usually accepted for detecting heterozygosity.

**Conclusion:** NC-CAH has variable expression even within a family, suggesting that modifier factors may modulate phenotype. Post-ACTH 21dF cannot detect heterozygous subjects in all cases. Considering the high frequency of heterozygotes in the general population, it is essential to genotype the partner(s) of the patients with one severe mutation in order to offer genetic counseling.

Nonclassic CAH (NC-CAH) is one of the most common recessively inherited conditions in humans. Extensive studies by New [18] have shown a prevalence of this condition of approximately 1 in 100 of
Adrenals

Reassessing fecundity in women with classical congenital adrenal hyperplasia (CAH): normal pregnancy rate but reduced fertility rate

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Background: Although fertility in women with classical congenital adrenal hyperplasia (CAH) is reported to be low, the true pregnancy rate for women trying to conceive with this condition is not known.

Methods: Pregnancy rate (calculated as a proportion achieving pregnancy of those who had attempted conception) and fertility (expressed as live birth rate (LBR) per total population) were calculated in a cohort of 106 women with classical CAH followed in a multidisciplinary service (81 salt-losing (SL) and 25 non-salt-losing (NSL) forms).

Results: Twenty-five (23.6%) women with CAH considered motherhood (16/25 NSL-CAH vs. 9/81 SL-CAH). Of these, 23 had actively tried to conceive and 21 (91.3%) achieved 34 pregnancies. The pregnancy rate was no different from that in the normal population (95%). Pregnancy rates were similar in the SL (88.9%) and NSL (92.9%) subgroups, and optimized glucocorticoid and mineralocorticoid regimen during times of fertility monitoring resulted in spontaneous conception in nearly all of the recent cases. However, the fertility rate was 0.25 live births per woman compared to 1.8 in the UK population (p < 0.001).

Conclusion: This study shows a normal pregnancy rate (91.3%) for women with classical SL and NSL CAH. The fertility rate, however, remains much lower than in general population.

See also:

Fertility and pregnancy outcome in women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency

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Several studies have reported low fertility in women with classic CAH, which has been suggested to reflect poor control or hyperandrogenism/PCOS in many cases. This single center study of fecundity in more than 100 women with CAH considered fertility and pregnancy rates separately. The number of live births per person was considerably below that of women in the general population. However, the pregnancy rate of those trying to get pregnant was around 90%. Similar findings were reported by Hagenfeldt and co-workers. For those women who did not conceive spontaneously within 6 months on routine steroid replacement, follicular phase (day 2–8 of the cycle) progesterone was measured and the dose of prednisolone was increased in three equally divided doses until progesterone was suppressed to less than 2 nmol/l. This approach may have helped optimize fertility by pre-
venting a transient rise in progesterone having an adverse effect on the follicular phase endometrium. The single daily dose of fludrocortisone was also increased if necessary in order to suppress plasma renin activity into the normal range, as poor control of the aldosterone pathway might also give rise to progesterone excess. Whilst these findings are encouraging, the converse side of this study is the high proportion of women (especially those with SL CAH) who did not seek to get pregnant. As the authors state, this probably reflects complex factors including ‘less frequent partnerships’ and possibly ‘gender dysphoria and the after effects of genital surgery’.

Gonadal function, first cases of pregnancy, and child delivery in a woman with lipid congenital adrenal hyperplasia

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Background: Mutations in the gene encoding steroidogenic acute regulatory protein (StAR) classically cause lipid congenital adrenal hyperplasia (LCAH). In this condition, reduced adrenal and gonadal steroid synthesis results in adrenal failure, impaired androgenization in 46,XY individuals, and altered ovarian steroidogenesis in 46,XX girls/women. Pregnancy in LCAH has not yet been reported.

Methods: Data were obtained regarding the gonadal function, pubertal development, and support of pregnancy in a female with a p.L275P mutation in the StAR gene.

Results: The patient (46,XX) presented with LCAH at 4.5 months of age and had developed and grown normally on glucocorticoid and mineralocorticoid replacement. Puberty started at the age of 11 7/12 years and progressed normally with menarche at 14 2/12 years. Regular anovulatory cycles ensued. Following clomiphene stimulation, pregnancy was achieved at 25 years of age but this resulted in a spontaneous abortion at 6 weeks gestation. The second pregnancy (with clomiphene stimulation) occurred a year later. Progesterone therapy was added on the 17th day of the cycle to protect the pregnancy and 2 normal weight boys (dizygotic twins) were born at 30 weeks. Two years later, again with clomiphene stimulation and progesterone treatment, she had a further successful singleton pregnancy and delivered a normal weight female baby at 36 weeks.

Conclusion: Despite dysfunctional StAR, pregnancy is possible in 46,XX women with LCAH using an appropriate therapeutic strategy.

StAR plays an important role in adrenal and gonadal steroidogenesis by regulating the influx of cholesterol into the mitochondrial membrane. The dynamics of this process are complex, as StAR-independent mechanisms exist [20]. Defects in StAR have been proposed to affect steroidogenesis through a ‘two-hit mechanism’. In the first hit, impaired StAR activity results in a reduction in steroid production by the adrenal gland or gonads. This results in elevated ACTH or gonadotropin stimulation of the cell which increases lipid uptake and causes accumulation of cholesterol in the cytoplasm. This second hit results in cell toxicity [21]. Girls (46,XX) with classic LCAH usually present with adrenal failure in infancy but show breast development and uterine growth at puberty, possibly because the second hit is less severe in the ovary as gonadotropin stimulation is cyclical, only one follicle is recruited each month, and low-level steroidogenesis may occur [21]. However, cycles are generally believed to be anovulatory, so this report of the induction of 3 pregnancies using clomiphene in a woman with a disruptive StAR mutation offers hope for patients with this rare condition. It is not surprising that progesterone therapy was needed to prevent early fetal loss as the maternal corpus luteum, which also expresses the StAR protein, is the major source of progesterone during the first trimester in humans. Normally, the fetally derived placenta takes over progesterone production in the 2nd and 3rd trimesters and StAR is thought not to play a significant role in placental steroidogenesis. Thus, in both the pregnancies reported here progesterone treatment could be withdrawn at between 17 and 25 weeks gestation. Reports such as this therefore provide interesting insight into the basic regulatory mechanisms of steroidogenesis in humans and important information when counseling families and girls with classic and non-classic forms of LCAH [22].
Effects of dehydroepiandrosterone therapy on pubic hair growth and psychological wellbeing in adolescent girls and young women with central adrenal insufficiency: a double-blind, randomized, placebo-controlled phase III trial

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Background: The potential benefits of oral dehydroepiandrosterone (DHEA) for hair growth and psychological wellbeing in young females with central adrenal insufficiency are unknown.

Methods: A cohort of 23 young females (mean age 18 range 13–25 year) was enrolled in a double-blind randomized placebo-controlled trial of DHEA treatment. All women had: ACTH deficiency plus two or more additional pituitary deficiencies; pubertal stage greater than B2, and a confirmed serum DHEA of <400 ng/ml. Any patients who had had recent tumor (<1 year remission), high-dose (>30 Gy) cranial irradiation, hypothalamic obesity, amaurosis, psychiatric disorders, or poorly controlled hormone replacement were excluded. Patients were randomized to receive 25 mg HPLC-purified DHEA/day (n = 11) or placebo (n = 12) orally for 12 months after stratification into a nontumor (n = 7) and a tumor group (n = 16). Pubic hair stage was assessed at 0, 6, and 12 months (primary endpoint), and psychometric evaluation (Symptom Check-List-90-R and the Centre for Epidemiological Studies-Depression Scale) was performed at 0 and 12 months (secondary endpoint). Androgen levels and safety parameters were measured at 0, 6, and 12 months; 24-hour androgen urinary excretion rates were calculated at 0 and 12 months.

Results: Four of the placebo group dropped out (tumor recurrence; development of type 1 diabetes; change of residence, n = 2), and 1 of the DHEA group dropped out (recurrent anxiety attacks). DHEA substitution normalized DHEA sulfate morning serum levels (2 h after administration; p < 0.006) and 24-hour urinary metabolite levels (androstanediol glucuronide; p < 0.0001). Placebo had no effect. Morning serum levels of androstenedione increased in the DHEA group (p < 0.02) but did not normalize. The DHEA group exhibited significant pubic hair growth (from Tanner stage I–III to II–V; mean +1.5 stages), whereas the placebo group did not (p = 0.0046). Eight of the 10 Symptom Check-List-90-R scores improved in the DHEA group compared to the placebo group, including those for anxiety, depression, interpersonal sensitivity and global severity index (p < 0.048). DHEA was tolerated well.

Conclusion: Daily replacement with 25 mg DHEA orally improved pubic hair growth and psychological wellbeing in a cohort of adolescent girls with hypothalamic-pituitary adrenal insufficiency (‘central’).

There is currently much debate about the potential benefits of DHEA replacement in women with adrenal disorders. DHEA is produced by the zona reticularis of the adrenal gland following adrenarche and is a major pathway to the generation of androgens in post-pubertal women, which have been implicated in improving libido, cognitive function and mood. Several controlled studies have now been performed in women with Addison’s disease and positive benefits of DHEA have been shown in some cases [23]. However, DHEA treatment may also be associated with potential side effects such as hirsutism or adverse lipid profiles, so further detailed properly performed studies in different age groups and conditions are needed [24, 25]. This double-blind randomized placebo-controlled trial of DHEA treatment by Binder and colleagues assessed the benefits of 1 year of DHEA replacement in adolescent girls and young women with secondary adrenal insufficiency. This was a well-structured study but it is complicated by relatively small numbers, heterogeneity in the underlying diagnosis, and relatively high drop out rate for unrelated reasons (e.g. 4 of the 12 girls randomized to receive the placebo did not complete the study). Nevertheless, the authors do report improved hair growth in the treatment group during this period and improved questionnaire-based reports of psychological wellbeing. DHEA probably plays an important role as a neurosteroid, facilitating brain maturation, mood and memory. DHEA treatment is now being recommended on an individual basis for many adult women with primary adrenal insufficiency [26] but relatively few data are available for DHEA replacement in late childhood or adolescence. It would be ideal if several more well-struc-
tured and suitably powered studies of DHEA replacement in adolescents could be undertaken before this treatment is offered widely. However, it is quite possible that DHEA treatments will be given to children on an individual basis if randomized control data are not available soon.

New mechanisms: family preferences

MRAP and MRAP2 are bidirectional regulators of the melanocortin receptor family

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Background: The melanocortin receptor (MCR) family consists of five G protein-coupled receptors (MC1R-MC5R) with diverse physiologic roles. MC2R (the ACTH receptor) plays a critical role in the hypothalamic-pituitary-adrenal axis. MC3R and MC4R play an important role in regulating energy homeostasis and MC4R mutations are the single most common cause of monogenic obesity currently known. MRAP is an MC2R accessory protein responsible for adrenal MC2R trafficking and function. However, the role of MRAP with other MCRs has not been established.

Methods: This study aimed to identify potential MRAP homologs and to investigate whether MRAP could influence the function of other MCR family members.

Results: A unique homolog of MRAP expressed in brain and the adrenal gland was identified (MRAP2). MRAP and MRAP2 were found to interact with all 5 MCRs. This interaction increased MC2R surface expression and signaling. In contrast, MRAP and MRAP2 reduced MC1R, MC3R, MC4R, and MC5R responsiveness to [Nle4,D-Phe7]α-melanocyte-stimulating hormone (NDP-MSH).

Conclusion: MRAP and MRAP2 are unique bidirectional regulators of the MCR family.

MRAP was first identified in 2005 as the cause of familial glucocorticoid deficiency type II and has been shown to play a key role in trafficking the ACTH receptor (MC2R) to the surface of the adrenal cell to enable interaction with the peptide ligand and initiation of downstream signaling pathways [27, 28]. Currently, there are 5 members of the MCR family. These receptors show unique tissue expression patterns throughout endocrine and metabolic systems but the role of MRAP or its homologs in regulating these other melanocortin receptors is unknown. Here, MRAP and a newly identified homolog termed MRAP2 were confirmed as having major facilitative influences on MC2R surface expression but interacted negatively with other members of the MCR family. Much attention is focusing on the regulation of MC4R as it plays such a central role in regulating appetite, energy homeostasis and obesity. This study supports a role for MRAP and MRAP2 in the regulation of the central melanocortin system, as MRAP/MRAP2 reduced signaling of the MC3R- and MC4R-dependent pathways. This bidirectional regulation of MC2R and MC3R/MC4R may reflect for example a physiologic advantage of switching off appetite at times when a maximal adrenal response is required, such as acute stress. Whether the central effects of MRAP and its homologs could be targeted to alter MC4R expression and influence appetite and weight gain remains to be seen.
Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse
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Background: Hypothalamic-pituitary-adrenal (HPA) function in the rat can be influenced by maternal care through epigenetic programming of glucocorticoid receptor expression. In humans, childhood abuse alters HPA stress responses and increases the risk of suicide.

Methods: Epigenetic regulation of a neuron-specific glucocorticoid receptor (NR3C1) promoter was compared between postmortem hippocampus obtained from: (1) suicide victims with a history of childhood abuse; (2) those from either suicide victims with no childhood abuse, or (3) controls.

Results: Decreased levels of glucocorticoid receptor mRNA were found in the abused suicide group, as well as decreased mRNA transcripts bearing the glucocorticoid receptor 1F splice variant and increased cytosine methylation of an NR3C1 promoter. Decreased NGFI-A transcription factor binding and NGFI-A-inducible gene transcription was seen using patch-methylated NR3C1 promoter constructs that mimicked the methylation state in samples from abused suicide victims.

Conclusion: These findings support data from rats and show that parental care in humans can influence epigenetic regulation of hippocampal glucocorticoid receptor expression.

Sequencing the human genome is undoubtedly one of the greatest achievements so far in the history of mankind. This knowledge has led to extremely important and rapid advances in identifying single-gene disorders, and many loci linked to common population diseases are now being identified. However, it is also likely that not all variations in human biology will be explained by variability at the level of the DNA, so other non-genomic processes need to be considered. This is where the current explosion of interest in epigenetics comes in. Epigenetics can be defined as changes in phenotype or gene expression due to mechanisms that do not involve changes in the underlying DNA sequence. These changes can remain through cell division cycles, sometimes for the lifespan of the organism, and may even be transmitted through generations. The exact mechanisms influencing epigenetic regulation are still being investigated but several processes such as DNA methylation, histone modification (e.g. methylation, acetylation) and RNA interference are likely to be involved. Such modifications can ‘silence’ or ‘activate’ expression of key genes with important downstream biological functions. This challenging study from Michael Meaney’s group reported glucocorticoid receptor (GR) expression and regulation in the hippocampus of suicide victims and controls. Using a unique resource of banked brains with detailed history and phenotype, the authors showed decreased levels of GR expression only in those adults committing suicide who had been abused in childhood. GR expression was the focus of this work as studies in rats have shown that poor maternal care is associated with decreased hippocampal GR expression and subsequent hyperactivation of the HPA axis in basal and stressed states [29]. Furthermore, childhood abuse has been reported to be associated with altered HPA activity in some studies in humans, although the data are less consistent [30]. The work presented here deals with a sensitive subject and highlights the need to study gene expression changes and regulation in relevant organs, which is not always easy (e.g. the brain). Nevertheless, epigenetic research is set to develop rapidly in the next decade. Hopefully, those from genetic and epigenetic backgrounds will work together to understand regulatory pathways rather than trying to prove one aspect is more important than the other.

Girls with premature adrenarche have accelerated early childhood growth
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Background: The relation between premature adrenarche (PA) and early prepubertal growth has not been studied in many different populations.
Methods: Anthropometric data between birth and 7 years of age was reviewed retrospectively for 54 girls with PA and 52 control girls. The growth variables were correlated with concentrations of serum insulin-like growth factor (IGF)-1, dehydroepiandrosterone sulfate and insulin at follow-up (median age 7.6 years).

Results: No significant differences in birth weight or length standard deviation scores (SDSs) occurred between the 2 groups studied. Girls with PA demonstrated a significantly greater length SDS increment during the first 2 years of life (median +1.0 SDS; \( p < 0.001 \)). Compared with controls, they were taller (median current height 1.2 vs. 0 SDS; \( p < 0.001 \)) and had gained more weight throughout childhood with differences in weight-for-height becoming significant at a later age. Median serum IGF-1 concentration, adjusted for body mass index and age, was higher in the PA group (24 vs. 19 nmol/l; \( p < 0.031 \)).

Conclusion: In this population of girls, PA was not associated with small birth size but was associated with increased growth. This increased growth was already evident in early childhood and was not explained by weight gain. Enhanced IGF-1 production may be one factor contributing to the prepubertal growth acceleration in PA.

In several studies, babies born small-for-gestational age have been shown to have an increased incidence of premature adrenarche in childhood, and population-based studies have found an inverse relation between birth weight and DHEA-S [31, 32]. This study, from a North European population, has looked at a cohort of girls with premature adrenarche and not found any significant differences in birth weight amongst them compared to a matched control group. This discrepancy deserves more attention. However, one notable finding was the accelerated growth in infancy and early childhood in the PA group, as well as higher serum IGF-1 concentration. The early growth acceleration supports Ken Ong’s findings in a population-based investigation of postnatal growth and DHEA-S [32]. The elevated IGF-1 findings supports data from Silfen et al. [33]. The causes of adrenarche are poorly understood but this study suggests that early events such as growth acceleration and IGF-1 production may be linked with it and could be causative.

Prepubertal healthy children’s urinary androstenediol predicts diaphyseal bone strength in late puberty

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Background: During adrenarche, healthy children secrete increasing amounts of weak androgenic steroids, which can be partly metabolized to potent sex steroids. The relation between adrenarchal androgen release and bone strength in later life is not well documented.

Methods: A cohort of 45 healthy children were studied who had had studies of urine steroid output undertaken at 8 years of age and in whom diaphyseal bone strength was measured at 16 years of age. Prepubertal urinary hormones quantified by gas chromatography-mass spectrometry were: dehydroepiandrosterone, its 16-hydroxylated downstream metabolites, 5-androstene-3,17β-diol (androstenediol), sums of total androgen and glucocorticoid metabolites, cortisol, and 6β-hydroxycortisol. Proximal forearm bone and muscle area measurements were obtained by peripheral quantitative computed tomography.

Results: Of all the prepubertal hormones analyzed, only sex- and age-specific androstenediol levels were significantly associated with pubertal stage-, height-, and muscularity-adjusted diaphyseal bone modeling (periosteal circumference, \( \beta = 0.67 \); \( p = 0.002 \); cortical area, \( \beta = 2.15 \); \( p = 0.02 \)), bone mineral content (\( \beta = 2.2 \); \( p = 0.04 \)), and polar strength strain index (\( \beta = 12.2 \); \( p = 0.002 \)). Androstenediol explained 5–10% of the late-pubertal variability in diaphyseal radius.

Conclusion: Androstenediol is an early predictor of the diaphyseal bone strength in late puberty. This urinary steroid metabolite is produced primarily following peripheral conversion of dehydroepiandrosterone by 17β-hydroxysteroid dehydrogenase.

The biological effects of adrenarche are poorly understood but it is possible that the weak androgenic steroids released from this time are converted to more potent androgens or estrogens at extra-
The discovery of new genetic causes of endocrine disorders normally focuses on those most severe and predictable phenotypes. With time, it usually emerges that milder or non-classic variants of these conditions exist. Examples of this include the descriptions of non-classical CAH [see Bidet et al., above], non-classic lipoid congenital adrenal hyperplasia [22], late-onset X-linked adrenal hypoplasia congenita and variants of 17α-hydroxylase deficiency associated with only mild genital changes. This case report by Rubtsov et al. describes a novel mutation in P450scc in a boy with hypospadias who developed adrenal insufficiency at 9 years of age. P450scc deficiency was originally thought to be incompatible with survival in humans as this enzyme is essential for progesterone production by the fetally derived placenta from the second trimester onwards, and subsequent maintenance of pregnancy. However 6 cases of P450scc deficiency have now been described [34]. All cases have presented with severe adrenal failure in early childhood and usually under-androgenized external genitalia in 46,XY individuals. The milder case of P450scc deficiency reported here reminds us of the common link between adrenal and gonadal steroidogenesis. Although hypospadias is common, more severe forms of hypospadias associated with cryptorchidism or a small penis may in some cases reflect an underlying steroidogenic defect and in some of these children there may be a risk of adrenal failure later in life. Trying to define those boys at risk is a challenge but it is important not to dismiss potential signs of adrenal failure when a history of hypospadias or undescended testes is present.
Corticotropin tests for hypothalamic-pituitary-adrenal insufficiency: a meta-analysis

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Background: The diagnostic value of tests to evaluate hypothalamic-pituitary adrenal insufficiency (HPAI) (‘central AI’) is controversial. The aim of this meta-analysis was to compare standard-dose and low-dose corticotropin tests for diagnosing HPAI.

Methods: PubMed (1966–2006) was searched for studies reporting the diagnostic value of standard-dose or low-dose corticotropin tests with patient-level data obtained from original investigators. Studies were included if there were more than 10 patients who were being evaluated because of suspected chronic HPAI. Studies were excluded if they used tests with no accepted reference standard for HPAI (insulin hypoglycemia or metyrapone test), if test subjects were in the intensive care unit, or if only normal healthy subjects were used as controls. Receiver operator characteristic (ROC) curves were constructed using patient-level data from each study. Summary ROC curves were generated from these data sets with adjustments for cortisol assay method and study size. The diagnostic value of each test was measured by calculating area under the ROC curve (AUC) and likelihood ratios.

Results: The meta-analysis included patient-level data from 13 of 23 studies (57%; 679 subjects). The AUC were as follows: low-dose corticotropin test, 0.92 (95% CI 0.89–0.94), and standard-dose corticotropin test, 0.79 (95% CI 0.74–0.84). Among patients with paired data (7 studies, 254 subjects), the diagnostic value of the low-dose corticotropin test was superior to that of the standard-dose test (AUC 0.94 and 0.85, respectively; p < 0.001).

Conclusion: The low-dose corticotropin test was superior to standard-dose testing for diagnosing chronic HPAI. However, the low-dose test has technical limitations.

Stimulation tests to assess adrenal function and to diagnose adrenal insufficiency can be challenging to interpret especially in the diagnosis of central hypothalamo-pituitary insufficiency where chronic under-stimulation of the adrenal gland occurs. In such situations, standard doses of corticotropin stimulation, which are massively supraphysiological, may well result in an adequate cortisol response but may not reflect the normal physiological situation at times of stress. With this in mind a low-dose corticotropin stimulation test has been introduced in the past 30–40 years, but very few large series are available that directly compare these two tests in one individual. This meta-analysis of low and standard dose corticotropin tests reviewed literature since 1966 and gathered information where patient-level data were available. Analysis using ROC curves showed that the low dose corticotropin test was better at diagnosing chronic HPAI than standard dose testing. A low-dose test peak of <440 nmol/l best predicted HPAI, whereas a peak of >600 nmol/l best predicted a normal test result. Unfortunately, it is not clear how many of the subjects primarily included in the analysis were children or at least had been treated in childhood or adolescence. Almost half of the cohort had had pituitary macroadenoma, suggesting that the majority of cases were likely to have been treated and tested in adulthood. It would be useful to know if the characteristics of these tests were different in a younger age group. Indeed, a recent study by Maguire et al. [35] of 31 children and adolescents with suspected central adrenal insufficiency and using human corticotropin-releasing hormone testing as the gold-standard for diagnosis (peak <400 nmol/l) suggested that mild cases of HPAI may be missed using a low-dose stimulation test. However, in this study the cortisol peak to diagnose HPAI was set at <267 nmol/l (10th centile for the normal population), which was considerably lower than in the meta-analysis. Nevertheless, both these papers recommend that the low-dose corticotropin stimulation test is useful in those cases of suspected HPAI where an unstressed basal cortisol taken around 9 a.m. falls within an ‘intermediate’ range (150–390 nmol/l, Kazlauskaite et al., above; 108–381 nmol/l, Maguire et al. [35]). Disadvantages of the low-dose test include: (1) inaccuracies in reconstituting the corticotropin analogue, which requires several dilution steps; (2) adherence of the hormone to plastic tubing, and (3) timing of the cortisol sample (20–30 min after stimulation is recommended by Kazlauskaite et al.). Thus, a greater degree of operator experience and knowledge is required.
Attractive men induce testosterone and cortisol release in women

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Background: Recently, studies have demonstrated that men release testosterone and cortisol in response to brief social interactions with young women. The aim of the current study was to determine whether women show a similar endocrine response to physically and behaviorally attractive men.

Methods: The study cohort consisted of 120 women (70 naturally cycling and 50 using hormonal contraceptives) who were shown one of four 20-min video montages extracted from popular films. The following scenarios were depicted: (1) an attractive man courting a young woman (experimental stimulus); (2) a nature documentary (video clip control); (3) an ‘unattractive’ man courting a woman (male control), and (4) an attractive woman with no men present (female control). Pre- and post-stimulus saliva samples were taken for analysis of testosterone and cortisol by immunoassay.

Results: Naturally cycling women experienced a significant increase in both testosterone and cortisol in response to the experimental stimulus but to none of the control stimuli. Women taking hormonal contraceptives also showed a significant cortisol response to the attractive man.

Conclusion: Women may release adrenal steroid hormones to facilitate courtship interactions with high mate-value men.

The psychology literature is peppered with hypothalamic-pituitary-adrenal axis data of variable quality, but this study is well controlled and interesting. The teleological and physiological significance of the cortisol response is debatable, with one possible effect being ‘to mobilize energy reserves for shared activity and possible sexual intercourse’. It is possible that the ‘participants enjoyment of the video, independent of whatever attraction they felt to the protagonist, could have mediated hormonal release’, although – as the authors state – there is no a priori reason why this should be the case, and there was certainly no response to the interesting selection of control scenarios.


