Classic congenital adrenal hyperplasia and puberty

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Abstract

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency of one of the five enzymes required for synthesis of cortisol in the adrenal cortex. The most common form of the disease is classic 21-hydroxylase deficiency, which is characterized by decreased synthesis of glucocorticoids and often mineralocorticoids, adrenal hyperandrogenism and impaired development and function of the adrenal medulla. The clinical management of classic 21-hydroxylase deficiency is often suboptimal, and patients are at risk of developing in tandem iatrogenic hypercortisolism and/or hyperandrogenism. Limitations of current medical therapy include the inability to control hyperandrogenism without employing supraphysiologic doses of glucocorticoid, hyperresponsiveness of the hypertrophied adrenal glands to adrenocorticotrophic hormone (ACTH) and difficulty in suppressing ACTH secretion from the anterior pituitary. Puberty imposes increased difficulty in attaining adrenocortical suppression despite optimal substitution therapy and adherence to medical treatment. Alterations in the endocrine milieu at puberty may influence cortisol pharmacokinetics and, consequently, the handling of hydrocortisone used as replacement therapy. Recent studies have demonstrated a significant increase in cortisol clearance at puberty and a shorter half-life of free cortisol in pubertal females compared with males. Furthermore, children with classic CAH have elevated fasting serum insulin concentrations and insulin resistance. The latter may further enhance adrenal and/or ovarian androgen secretion, decrease the therapeutic efficacy of glucocorticoids and contribute to later development of the metabolic syndrome and its complications.

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders resulting from deficiency of one of the five enzymes required for synthesis of cortisol in the adrenal cortex. The most frequent form of the disease is steroid 21-hydroxylase deficiency, which accounts for 90–95% of all cases of CAH (1–4). Deletions or mutations of the cytochrome P450 21-hydroxylase gene result in decreased synthesis of glucocorticoids and often mineralocorticoids. The impaired glucocorticoid feedback inhibition at the hypothalamic and anterior pituitary levels leads to increased secretion of corticotropin-releasing hormone (CRH) and adrenocorticotrophic hormone (ACTH) respectively, adrenal hyperplasia, and increased production of adrenal androgens and steroid precursors prior to the enzymatic defect (1–5). The clinical spectrum of 21-hydroxylase deficiency is quite broad, ranging from the most severe to mild forms, depending on the degree of 21-hydroxylase activity. Accordingly, three main clinical phenotypes have been described: classic salt-wasting, classic simple virilizing and nonclassic (1, 3–5).

In addition to impaired adrenocortical function, classic CAH is characterized by compromised adrenomedullary function. The latter is due to developmental defects in the formation of the adrenal medulla, which lead to

depletion of epinephrine stores and decreased production of metanephrine, the *O*-methylated metabolite of epinephrine (6). Patients with classic CAH have significantly lower plasma and urinary epinephrine, and plasma total and free metanephrine concentrations than normal subjects (6). The degree of adrenomedullary hypofunction in classic CAH correlates strongly with the degree of adrenocortical impairment and the expected 21-hydroxylase activity based on genotype (6, 7).

Current treatment of classic CAH aims to provide adequate glucocorticoid and, when necessary, mineralocorticoid substitution to prevent adrenal crises and to suppress the excessive secretion of CRH and ACTH, thereby reducing circulating concentrations of adrenal androgens and steroid precursors. Achieving and maintaining adrenal androgen suppression is far more challenging than preventing adrenal crises, and in a fair number of patients it has proven impossible to control hyperandrogenism without employing supraphysiologic doses of glucocorticoid. Iatrogenic Cushing's syndrome and hyperandrogenism may develop in tandem, representing the main problems encountered in the clinical management of these patients (1–5).

The limitations of standard medical therapy with glucocorticoid and/or mineralocorticoid substitution include: (i) inability to replicate physiologic cortisol

production with exogenous administration of glucocorticoid; (ii) hyperresponsiveness of the hypertrophied adrenal glands to ACTH and increased androgen production following a small ACTH challenge in the event of escape from pituitary suppression (8); (iii) difficulty in suppressing ACTH secretion from the anterior pituitary due to the decreased sensitivity to glucocorticoid feedback inhibition, as well as the fact that glucocorticoid feedback is only one of the mechanisms governing ACTH secretion (8, 9); (iv) resistance to replacement therapy, given that the increased concentrations of androgens and steroid precursors compete with the exogenously administered glucocorticoids and mineralocorticoids for the same receptors, placing patients with classic CAH at greater risk of stress-induced salt-losing crises than their healthy or Addisonian counterparts (8, 10, 11).

In addition to the above, clinical observations suggest that puberty imposes increased difficulty in attaining adrenocortical suppression despite optimal substitution therapy and adherence to medical treatment. Alterations in the endocrine milieu at puberty may influence cortisol pharmacokinetics and, consequently, the handling of hydrocortisone used as replacement therapy.

Alterations in cortisol pharmacokinetics at puberty

In a prospective, cross-sectional study carried out at the London Centre for Paediatric Endocrinology, London, UK, we determined the pharmacokinetic parameters of total and free cortisol in 40 subjects with classic salt-wasting CAH (14 prepubertal (M: 5; F: 9), 20 pubertal (M: 7; F: 13) and 6 postpubertal (M: 2; F: 4)) (12). We showed that the clearance (CL) of total and free cortisol was significantly higher in the pubertal than the prepubertal and postpubertal patients. The volume of distribution (V) of total and free cortisol was significantly higher in the pubertal and postpubertal than in prepubertal patients. No difference in the half-life ($t_{1/2}$) of total or free cortisol was noted between groups. The latter was thought to be due to the concomitant rise in cortisol clearance and volume of distribution, both important determinants of the elimination of a drug from the body and, hence, its half-life ($t_{1/2} = 0.693 \times V/CL$). Comparison of the pharmacokinetic parameters of free cortisol between males and females in each separate group of patients (prepubertal, pubertal and postpubertal) revealed a significantly shorter half-life of free cortisol in pubertal females than in pubertal males (12).

The net effect of these changes in cortisol pharmacokinetics, if the administration schedule of hydrocortisone remains unchanged, will be a loss of control of the hypothalamic-pituitary-adrenal (HPA) axis, inadequate suppression of the adrenal cortex and excessive production of adrenal androgens and steroid precursors. Both hypocortisolism and hyperandrogenism

may operate as independent factors to amplify the loss of control, while the increased ACTH secretion may further enhance hypocortisolism by increasing the metabolic clearance rate of cortisol (13).

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The primary site of cortisol metabolism in humans is the liver, and a number of cytosolic and microsomal enzymes, including cytochrome P450, 5 α /5 β -reductase, 3 α /3 β -oxidoreductase and 11 β -hydroxysteroid dehydrogenase, play an important role in the hepatic metabolism of cortisol (14–16). The major routes of hepatic metabolism involve A-ring and side-chain reduction followed *in vivo* by conjugation with glucuronic acid and sulfate (17). The inactive glucuronide and sulfate metabolites are excreted by the kidneys, whereas only less than 1% of cortisol is excreted unchanged in the urine. Therefore, the metabolic clearance of cortisol is influenced primarily by factors altering hepatic clearance and to a much lesser extent by factors affecting renal excretion.

The increased cortisol clearance at puberty is most likely due to alterations in the activity of 11 β -hydroxysteroid dehydrogenase (11 β -HSD). 11 β -HSD plays a key role in the hepatic metabolism of cortisol by catalyzing the interconversion of active cortisol to its hormonally inactive metabolite, cortisone. Two isoforms of 11 β -HSD have been identified. Type 1 (11 β -HSD1) isoform is a NADP(H)-dependent enzyme highly expressed in the liver, gonads, adipose tissue and central nervous system tissues, where the reaction direction is predominantly 11 β -reduction, converting cortisone to cortisol and potentially increasing active glucocorticoid levels. Type 2 (11 β -HSD2) isoform is a NAD-dependent dehydrogenase expressed in the mineralocorticoid target tissues, kidney and colon, which catalyzes the conversion of cortisol to cortisone, thus protecting the mineralocorticoid receptor from illicit occupancy by cortisol (18–20).

Both *in vivo* and *in vitro* studies have demonstrated a decrease in the activity of 11 β -HSD1 in association with elevations in growth hormone (GH) and insulin-like growth factor (IGF)-I concentrations (21–24). In adult subjects with hypopituitarism, administration of GH in substitution doses leads to a significant, dose-independent, persistent decrease in the activity of 11 β -HSD1, as evidenced by a decrease in the urinary cortisol to cortisone metabolite ratio (Fm/Em), which represents an index of the overall 11 β -HSD activity, with no concurrent alterations in the urinary free cortisol to free cortisone ratio (FF/FE), an index of 11 β -HSD2 activity (22, 24, 25). In acromegalic patients, withdrawal from medical therapy results in a significant rise in GH and IGF-I concentrations and a concomitant decrease in the activity of 11 β -HSD1, while complete removal of the pituitary tumor by

transphenoidal surgery results in a decrease in GH concentrations and a parallel increase in 11β -HSD1 activity (23). *In vitro* studies, performed on cells stably transfected with either the human 11β -HSD1 or 11β -HSD2 complementary DNA and primary cultures of human omental adipose stromal cells expressing only the 11β -HSD1 isozyme, indicate that IGF-I inhibits the activity of 11β -HSD1 in a dose-dependent manner. The effect of GH on the activity of 11β -HSD1 appears to be mediated by IGF-I. Neither GH nor IGF-I has any effect on the activity of type 2 isoform of the enzyme (23). By inhibiting the activity of 11β -HSD1, GH and IGF-I increase the metabolic clearance rate of cortisol (23).

The concept of decreased 11β -HSD1 activity at puberty as a result of the rise in GH and IGF-I concentrations is supported by the fact that pubertal patients with classic CAH had significantly higher urinary excretion of cortisone metabolites [tetrahydrocortisone (THE)] than prepubertal and postpubertal patients (12) (Fig. 1A). A concomitant significant decrease in

the urinary cortisol metabolite to cortisone metabolite ((THF + allo-THF)/THE) ratio, which represents an index of the overall activity of 11β -HSD1, was noted at puberty (12) (Fig. 1B). Furthermore, there was a significant positive correlation between the activity of 11β -HSD1 and the half-life of cortisol (Fig. 2), as well as between IGF-I concentrations and cortisol clearance corrected for body-mass index (BMI) (12) (Fig. 3). The decreased activity of 11β -HSD1 in patients with classic CAH, who have minimal endogenous production of cortisol, will result in decreased conversion of cortisone to cortisol and hypocortisolism (Fig. 4). The latter will further activate the HPA axis and enhance adrenal hyperandrogenism.

In addition to GH and IGF-I, gonadal steroids may influence cortisol metabolism. In rats, a sexually dimorphic pattern in the activity of 11β -HSD1 has been described, with females demonstrating significantly lower hepatic 11β -HSD1 activity and mRNA expression than males (26–29). In male rats, gonadectomy and estradiol treatment lead to a marked decrease in both 11β -HSD1 activity and mRNA expression, while gonadectomy and testosterone replacement have no such effect. On the other hand, in female rats, gonadectomy results in a marked increase in the activity of

Figure 1 (A) Urinary concentrations of cortisone metabolites and (B) urinary cortisol to cortisone metabolite ratio, which reflects the overall activity of 11β -hydroxysteroid dehydrogenase, in prepubertal, pubertal and postpubertal subjects with classic congenital adrenal hyperplasia. Asterisks indicate significant differences between groups. THE: tetrahydrocortisone; THF: tetrahydrocortisol.

Figure 2 Linear correlation between 11β -hydroxysteroid dehydrogenase activity and half-life of total cortisol. THE: tetrahydrocortisone; THF: tetrahydrocortisol.

Figure 3 Linear correlation between insulin-like growth factor (IGF)-I concentrations and total cortisol clearance (CL) corrected for body-mass index (BMI).

Figure 4 Schematic representation of the alterations in GH/IGF-I axis at puberty leading to suboptimal control of classic congenital adrenal hyperplasia despite optimal substitution therapy and adherence to medical treatment. E: cortisone; F: cortisol; GFR: glomerular filtration rate; GH: growth hormone; 3β-HSD: 3β-hydroxysteroid dehydrogenase; 11β-HSD: 11β-hydroxysteroid dehydrogenase; IGF-I: insulin-like growth factor I.

11β-HSD1, which is reversed by estradiol but not testosterone replacement therapy (26). This sexually dimorphic pattern in the hepatic expression of 11β-HSD1 is pituitary-mediated and results from sex-specific differences in the pattern of GH secretion in the rat, with males demonstrating a pulsatile pattern and females displaying a rather continuous pattern of GH secretion (21, 27). Sexual dimorphism in the activity of 11β-HSD1 has been documented in healthy subjects (30) and patients with hypopituitarism receiving optimal replacement therapy (31), with females demonstrating decreased conversion of cortisone to cortisol compared with males. However, recent evidence suggests that the sexually dimorphic pattern in cortisol metabolism in humans is due to decreased A-ring reduction of cortisol in females compared with males rather than decreased reactivation of cortisone to cortisol by 11β-HSD1 (32, 33).

Besides their effects on the activity of 11β-HSD1, GH and IGF-I may contribute to the increased clearance of cortisol by increasing its renal excretion. Although cortisol metabolism is principally effected in the liver, a very small proportion of cortisol is excreted unchanged in the urine. Therefore, factors influencing renal clearance at puberty would have an effect, albeit small, on cortisol clearance. The renal clearance of a drug is the net result of three different processes: filtration, secretion and reabsorption. Renal clearance by filtration increases secondary to an increase in the glomerular filtration rate (GFR) (34). Both GH and IGF-I increase renal plasma flow and GFR (35) (Fig. 4). The action of GH is likely to be mediated by IGF-I rather than being direct. IGF-I increases renal GFR via a direct effect on the glomerular vasculature, a decrease in renal glomerular afferent and efferent arteriolar resistances, and an increase in the glomerular ultrafiltration coefficient (36, 37).

The rise in GH and IGF-I concentrations at puberty (38, 39) is associated with a marked fall in insulin sensitivity and a parallel elevation in serum insulin concentrations (40, 41). At the tissue level, insulin reduces

IGF-binding protein-1 (IGFBP-1) concentrations, further enhancing the effects of IGF-I on the activity of 11β-HSD1 (42, 43). Children with classic CAH have significantly higher serum insulin concentrations than those expected to arise as a result of the pubertal process itself, and a greater insulin resistance homeostasis model assessment (HOMA) index than their normal, BMI-matched counterparts (44). This is most likely the result of long-standing adrenomedullary hypofunction, given that catecholamines inhibit the secretion of insulin through β-adrenergic receptors (45). Obesity, intermittent hypercortisolism, and adrenal and/or ovarian hyperandrogenism in the not adequately controlled patients and/or females with polycystic ovarian syndrome (PCOS) may be additional factors that contribute to the elevated insulin concentrations and insulin resistance (46).

Further to increasing the metabolic clearance of cortisol, hormonal changes at puberty may enhance hyperandrogenism by influencing the activity of other enzymes participating in adrenal steroidogenesis. Human studies provide evidence for a decrease in the activity of 3β-HSD and an increase in the activity of 17,20-lyase following administration of GH (47, 48). *In vitro* studies performed on cultured human adrenal fasciculate-reticularis cells suggest that IGF-I and IGF-II enhance adrenal steroidogenesis by enhancing responsiveness to ACTH (49–52). IGF-I and IGF-II enhance mostly androstenedione secretion in a dose-dependent manner, with IGF-II being more potent than IGF-I in that respect (51, 52). The effect of IGF-I and IGF-II on adrenal steroidogenesis is associated with an increase in 17α-hydroxylase and type II 3β-HSD activity and mRNA expression (51, 52) (Fig. 4).

Insulin stimulates primarily adrenal and, to a much lesser extent, ovarian steroidogenesis, and it constitutes an important component of the pathogenetic mechanism of hyperandrogenism (49, 50, 53, 54). *In vitro* studies performed on human adrenocortical cells have shown that physiologic concentrations of insulin

increase 17 α -hydroxylase and 3 β -HSD mRNA levels in the absence of cAMP or ACTH (49–51). Moreover, insulin decreases both 11 β -HSD1 activity and mRNA expression, and antagonizes the effect of glucocorticoids on the enzymatic activity of the 11 β -HSD1 (25). Human studies also support the concept of enhanced ACTH-mediated insulin-induced steroidogenesis (53). These effects of insulin would compound the effects of GH and IGF-I on 11 β -HSD1 activity, and would potentiate hypocortisolism and hyperandrogenism. The latter may be further amplified in hyperinsulinemic states, given that insulin suppresses the synthesis of sex hormone-binding globulin by the liver (55).

Summary

Alterations in the endocrine milieu at puberty are associated with alterations in cortisol pharmacokinetics, which lead to inadequate suppression of the HPA axis and suboptimal control of classic CAH despite optimal substitution therapy and adherence to medical treatment. The rise in GH and IGF-I concentrations may inhibit 11 β -HSD1 activity, resulting in decreased conversion of cortisone to cortisol and hypocortisolism. The increased GH/IGF-I concentrations in association with the elevated insulin concentrations at puberty may enhance adrenal and ovarian hyperandrogenism. Both hypocortisolism and hyperandrogenism will lead to increased secretion of ACTH, which will further amplify hypocortisolism by increasing the metabolic clearance rate of cortisol. Medical therapy of pubertal patients with classic 21-hydroxylase deficiency should be based on the understanding of the above pathophysiologic alterations, and should aim to provide appropriate glucocorticoid substitution, as well as to prevent and/or treat hyperandrogenism and insulin resistance.

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