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7.1.2 Childhood endocrinology

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Introduction

Between embryonic life and the end of growth, the endocrine milieu undergoes profound changes that need to be known to understand the common paediatric endocrine disorders, to interpret results of hormone assays in newborns, infants, children, and adolescents properly, and to take advantage of some periods of life that are 'windows of opportunity' for some diagnoses. In this section, we will review these changes from the perspective of a practising paediatric endocrinologist. The main focus is on the 'classical' endocrine axes—growth hormone-releasing hormone (GHRH)/somatostatin–growth hormone–insulin-like growth factor, gonadotropin-releasing hormone–luteinizing hormone/ follicle-stimulating hormone (FSH)–gonadal hormones, TRH–TSH–thyroid hormones, corticotropin-releasing factor–adrenocorticotropin (ACTH)–adrenal steroids. Glucose and mineral metabolism will also be briefly discussed, as will childhood obesity. The increasing role of DNA-based diagnosis in paediatric endocrinology will also be highlighted.

The growth hormone axis

In contrast to neonates with a primary deficiency in insulin-like growth factor 1 (IGF-1) (1) or in its receptor (2), in whom severe growth retardation begins before birth, those with congenital growth hormone deficiency have normal birth length, suggesting that growth hormone does not play a significant role in the control of fetal growth. However, its concentrations in cord blood are elevated, with a mean of about 15 µg/l in term newborns. Growth hormone release from the pituitary results from an interplay between stimulation of synthesis and secretion by GHRH and inhibition of secretion by somatostatin. Whether endogenous ghrelin is involved in the physiological control of growth hormone release in children and adolescents remains to be demonstrated (3). The hypothalamic mechanisms that control growth hormone release are, in turn, modulated in a classical endocrine feedback loop by IGF-1, which decreases growth hormone output. The mechanisms underlying the high basal levels of growth hormone in newborns are not completely understood, but are thought to involve both increased pituitary responsiveness to GHRH and decreased inhibitory feedback by IGF-1 (4). The basal plasma concentrations of growth hormone decrease over the first few days of life, but remain higher in the first year of life than thereafter (5). Thus, the newborn period is the only period of life when an undetectable basal level of growth hormone, especially if it is measured in cord blood, may be used as an argument in favour of growth hormone deficiency.

If a diagnosis of growth hormone deficiency is considered in a newborn, it may be possible to retrieve cord blood (which is stored for a few days after birth in many maternity units); if cord blood is no longer available, it is preferable to measure growth hormone levels on a 'critical sample' (see below) drawn at the time of spontaneous hypoglycaemia. Because birth size is within normal limits in congenital growth hormone deficiency, hypoglycaemia may be the only presenting symptom in the neonatal period. The degree of suspicion becomes higher if there is evidence for deficiency of other pituitary hormones. These include: (1) cholestatic jaundice, which is very often present in newborns with ACTH deficiency, because cortisol is necessary for choleresis (6); (2) prolonged unconjugated hyperbilirubinaemia, which may be suggestive of hypothyroidism; (3) in newborn males, micropenis and cryptorchidism, which suggest gonadotropin deficiency (see below).

During later infancy and childhood, the profile of plasma growth hormone concentrations is characterized by low or undetectable levels most of the time, with peaks that, until puberty, occur mostly during the night. Thus, the determination of plasma growth hormone concentrations on a random sample obtained during the day is only indicated (together with plasma prolactin and IGF-1 levels) for the diagnosis of growth hormone hypersecretion; this exceedingly rare condition in the paediatric age group presents with gigantism, often associated with acromegaloid features and usually results from a pituitary tumour synthesizing both growth hormone and prolactin (7). For the much more commonly considered diagnosis of growth hormone deficiency in a child with an unexplained and sustained deceleration of linear growth, a random daytime growth hormone sample is useless and a variety of provocative tests have been designed (see Section ...).

At puberty, daytime spontaneous pulses of growth hormone appear (8). In the investigation of adolescents with very tall stature in whom the diagnosis of growth hormone hypersecretion is considered, these spontaneous peaks should not be misinterpreted as 'paradoxical responses' to oral glucose or to TRH, which are seen in acromegaly and in other pathological conditions (9). There is also a marked increase in the amplitude of the night-time pulses during puberty, so that the total growth hormone production rate becomes up to fourfold higher than in the prepubertal period (10) and higher than in young adults. On the other hand, in growth hormone-deficient adolescents, a normal pubertal height spurt can be obtained without increasing the dose of exogenous growth hormone (11); therefore, the marked increase in endogenous growth hormone secretion in normal adolescents does not appear to be required for normal linear growth and probably serves another role. The fact that young adults males with childhood-onset growth hormone deficiency treated with a constant dose of growth hormone throughout puberty have decreased bone mass (12) suggests that the increase in endogenous growth hormone in normal adolescents may be important for the acquisition of peak bone mass; it may also be essential for the development of muscle mass. It is important to realize that, aside from its role in the stimulation of linear growth, growth hormone has important metabolic functions (chiefly the maintenance of a normal body composition and of a normal lipid profile) that have led to the use of growth hormone as replacement therapy in adults with growth hormone deficiency (13). However, the persistence of growth hormone deficiency should be established once adult height has been attained: the wide availability of biosynthetic growth hormone since the mid-1980s

has led to a relaxation of the criteria for the diagnosis of growth hormone deficiency in children and adolescents, so that some clinics now report that this diagnosis is not confirmed upon re-evaluation in the majority of subjects (14). If the diagnosis is confirmed, adolescents who decline continuing growth hormone therapy should be monitored during the transition period between epiphyseal fusion and young adulthood, and physical activity should be promoted. Indeed, it is striking that no study has compared the benefits of continued growth hormone substitution with those that can be derived from a programme of structured physical exercise.

Growth hormone exerts most of its peripheral growth-promoting actions through IGF-1, which is synthesized primarily in hepatocytes. In contrast to plasma growth hormone, IGF-1 concentrations are relatively stable over a 24-h period. However, these concentrations are also developmentally regulated with a normal range that extends to the lower sensitivity of most assays in the preschool age group, limiting its diagnostic usefulness in that period. Mean plasma IGF-1 increases progressively during childhood and increases sharply at adolescence to a mean peak that is much higher than in the young adult; as is the case for many other hormonal parameters during puberty, IGF-1 levels are better correlated with pubertal stage than with chronological age (15). Thus, the functioning of the growth hormone axis during adolescence is somewhat reminiscent of the acromegalic state.

Most IGF-1 circulates as part of a ternary complex that comprises IGF-1 itself, an acid-labile subunit (ALS, also synthesized by hepatocytes) and an IGF-binding protein (IGFBP). The major IGFBP in plasma is IGFBP-3, a protein synthesized by the endothelial cells of the liver. Like plasma IGF-1, plasma IGFBP-3 is stable over 24 h and has a very wide range of normal values. Although it was initially purported to be a better test for the diagnosis of growth hormone deficiency in childhood than plasma IGF-1 itself, there is no consensus on the 'added value' of IGFBP-3 (16). Probably because of redundancy between the six IGFBPs, deficiency in one of the IGFBPs is likely to be clinically silent and, indeed, no such human case has been described. By contrast, isolated deficiency of ALS has recently been described in children with relatively mild growth failure and pubertal delay (17).

The growth hormone-binding protein (GHBP), in humans, corresponds to the extracellular domain of the growth hormone receptor (GHR), which is shed from the membrane by proteolytic cleavage. Plasma GHBP is therefore considered to reflect the growth hormone sensitivity of the individual. Depending on the nature and location of the mutation in GHR, children with congenital growth hormone insensitivity will have very low, normal, or even high GHBP levels. Using plasma GHBP to screen children with unexplained growth failure for possible partial growth hormone insensitivity has been advocated (18).

An important concept in the physiological control of the growth hormone-dependent peptides described above (IGF-1, IGFBP-3, ALS, GHBP) is that they are also strongly influenced by the energy balance, the body mass index, and/or the body composition of the individual: thus, a diet deficient in either calories or protein, or excessive energy expenditure (for example, intensive exercise) will result in decreased plasma IGF-1 (19); conversely, plasma IGF-1 is slightly increased in children with exogenous obesity, in spite of low growth hormone secretion (20). On the other hand, plasma GHBP is negatively correlated with body mass index. Therefore, the proper interpretation of the plasma concentration of IGF-1

or of the other growth hormone-dependent peptides requires knowledge of the intake and absorption of nutrients, of the energy expenditure, and of the body composition of the subject.

Aside from these advances in the biochemical tools available to investigate growth disorders, imaging is important. While magnetic resonance imaging has become the gold standard for diagnosing hypothalamic-pituitary malformations and tumours, it obviously cannot be used for screening, for which a standard X-ray of the sella turcica is still useful: it may show a small sella in congenital hypopituitarism (except in patients with PROP1 mutations, who present with a large sella) and it will identify most craniopharyngiomas, because the vast majority of these tumours are calcified or resulted in parasellar bone destruction. When a diagnosis of 'idiopathic' growth hormone deficiency is made on stringent clinical and biochemical criteria, magnetic resonance imaging reveals in up to 80–90% of patients the triad of ectopic posterior pituitary, small anterior pituitary and thin or interrupted pituitary stalk (21). A normal hypothalamic-pituitary anatomy on magnetic resonance imaging should lead to questioning whether the diagnosis of growth hormone deficiency is indeed correct, or to the search for a rare genetic disorder such as a mutation in the *GHI* gene or an inactivating mutation of the pituitary transcription factor PIT-1 (22). In patients with the triad, the likelihood that other anterior pituitary hormone deficiencies will develop and that growth hormone deficiency will persist into adulthood is higher, but it is not 100% (23). The triad has been observed in hypopituitary neonates, suggesting that the deficient pituitary function results from a defect in hypothalamic-pituitary connection during embryogenesis rather than to obstetrical trauma, as previously believed. In most cases, the condition is sporadic and unexplained, but a few single gene disorders have been described (see Section).

Gonadotropin-releasing hormone—gonadotropins—gonadal hormones

Recent studies have shown that differentiation of the bipotential gonad of the early embryo into an ovary is an active process (24), but the hormones produced by the fetal ovary do not play an active role in the sexual differentiation of the internal and external genitalia. This requires the production of müllerian inhibiting hormone and testosterone, respectively, by the fetal testis (see Section ...). Therefore, in the absence of an embryonic testis, the phenotype will be female regardless of karyotype. Testosterone secretion by the testis begins before there is any significant gonadotropin production by the embryonic pituitary and, in humans, is primarily driven by placental human chorionic gonadotropin (hCG) (acting through the luteinizing hormone/hCG receptor on Leydig cells). During the second and third trimester, fetal testosterone production becomes dependent upon normal gonadotropin output by the fetal pituitary. Hence, congenital hypopituitarism may lead to arrested testicular descent and penile growth, but does not affect the differentiation of the male genitalia. Only primary testicular defects or defects in testosterone metabolism or action, if present before the 13th week of gestation will result in abnormal differentiation (ranging from hypospadias to a completely female appearance of the external genitalia).

The activity of the hypothalamic-pituitary-gonadal axis between birth and adulthood goes through several phases: after a very early peak in plasma testosterone to a mean of 11.5 nmol/l (range 5.9–20.3)

at 20–24 h of life in boys (25), there is a high level of activity in neonates and infants in both sexes followed by a quiescent period between ages 1–2 years and 10–12 years, at which time the progressive increase in gonadotropins and gonadal steroids brings about the appearance and progression of secondary sexual characteristics.

The marked and transient activation of the pituitary–gonadal axis in the first few months of life has important diagnostic implications: in boys, it results in plasma testosterone levels at 6–8 weeks of age that are similar to those seen in mid-pubertal boys. A single basal testosterone obtained at that critical period can be used to determine: (1) whether the whole hypothalamic–pituitary–testicular axis is intact or disrupted, for example, in newborns with micropenis or other evidence of hypopituitarism; and (2) whether intra-abdominal testes are present in boys with nonpalpable gonads (26). If that ‘critical sample’ is not obtained, the older boy with nonpalpable testes can still be assessed until 3–4 years of age by a single measurement of plasma FSH: if FSH is very elevated, a diagnosis of bilateral anorchia is very likely. Beyond that age, this diagnosis will require a determination of the plasma concentration of müllerian inhibiting hormone (27), an assay that is not yet routinely available in many clinical laboratories, or a stimulation test with hCG, which may yield false positive results (that is, no testosterone rise in a boy who, nevertheless, has intra-abdominal testes) (28). Interestingly, genetic males with partial androgen insensitivity have a normal postnatal testosterone surge, but that may be accompanied by a high luteinizing hormone (29), while patients with complete androgen insensitivity have, for unknown reasons, almost always a complete lack of the postnatal surge in either testosterone or in luteinizing hormone (30). Finally, the postnatal testosterone surge appears to occur at the same time and has the same amplitude in otherwise healthy premature boys.

There is a marked early postnatal increase in plasma FSH in normal girls, so that during the first month of life, plasma FSH cannot be used for the diagnosis of gonadal dysgenesis in girls with Turner's syndrome (31). However, plasma FSH in older infants with Turner's syndrome is markedly higher than in normal girls until about 3–4 years of age (32). A normal plasma FSH level at that age in a girl with Turner's syndrome therefore suggests the presence of functional ovaries, which may be further documented by a pelvic ultrasound; in this situation, spontaneous thelarche, and even menarche and fertility, can occur (mostly in patients with mosaic karyotypes or with structural anomalies of the X chromosome). Such information is important for appropriate counselling of the parents and of the children.

The mid-childhood pause in gonadotropin secretion is observed in both sexes and occurs regardless of the presence or absence of functional gonads (28, 32). It is therefore likely that the intrinsic pulsatile secretory capacity of the hypothalamic gonadotropin-releasing hormone-producing neurons (33) is inhibited by the central nervous system (CNS), although the chemical nature of these CNS inhibitors has remained elusive. However, gonadotropin secretion is not completely absent: with the newer sensitive immunoradiometric assays for FSH and luteinizing hormone, low levels of gonadotropins can be measured in the plasma of prepubertal children. In spite of the development of these sensitive assays, making a diagnosis of hypogonadotrophic hypogonadism in boys with cryptorchidism, with or without micropenis or anosmia, remains very difficult to establish during mid-childhood.

In contrast, normal prepubertal girls have a more robust rise in FSH after gonadotropin-releasing hormone stimulation than boys; a normal FSH response strongly suggests that spontaneous puberty will develop (34).

On the other hand, while the testicular production of testosterone is completely abolished in boys between about 6 months of life and puberty, resulting in undetectable testosterone levels, there appears to be a basal secretion of oestradiol by the prepubertal ovary as measured by ultrasensitive oestradiol assays (35). This may, in part, account for the high frequency of isolated premature thelarche or of true precocious puberty in girls. Starting at birth, bones mature faster in girls than in boys, so that the bone maturation of a 2-year-old girl is similar to that of a 3-year-old boy (36). During mid-childhood, the maturation of long bones actually proceeds at a similar rate in both sexes (37), which is hard to reconcile with the concept of active ovaries and of completely quiescent testes.

Another important concept related to sexual dimorphism is that testosterone secretion by the testes is driven by luteinizing hormone, whereas oestradiol production by the ovary also requires FSH; thus, tumours secreting hCG, a luteinizing hormone-like molecule (38), and germline (39) or somatic (40) activating mutations in the luteinizing hormone receptor gene induce sexual precocity in boys, but not in girls. Puberty is heralded by a reamplification of the pulsatile secretion of gonadotropins, mostly luteinizing hormone, which initially occurs during the night. This reamplification probably results in part from a decreased sensitivity of the gonadostat to the negative feedback effect of the low circulating concentrations of sex steroids (41). As a consequence of increased nocturnal luteinizing hormone output, basal plasma testosterone levels increase in boys, first during the early morning hours (42), before becoming detectable throughout the day. In girls, plasma oestradiol levels also increase progressively, but because oestradiol secretion may be intermittent and because most currently available assays are not very sensitive, obvious clinical signs of oestrogenization can be observed with undetectable plasma oestradiol.

In parallel with the changes in basal gonadotropin secretion, the pituitary response to exogenous gonadotropin-releasing hormone matures from a predominantly FSH to a predominantly luteinizing hormone response. This is particularly striking in girls, in whom, as mentioned above, the FSH predominance of the response to gonadotropin-releasing hormone is more pronounced in the prepubertal period. In addition, the magnitude of the luteinizing hormone response to gonadotropin-releasing hormone increases markedly. While these changes in the luteinizing hormone and FSH responses to gonadotropin-releasing hormone are useful in documenting the central nature of the process in cases of sexual precocity, they are less useful in the assessment of delayed puberty. Most cases of congenital hypogonadotrophic hypogonadism (with anosmia, an association known as Kallmann syndrome, or without anosmia) have a hypothalamic origin with a normal pituitary response to gonadotropin-releasing hormone. This is found in some cases of unequivocal hypogonadism while the luteinizing hormone response to a single bolus of gonadotropin-releasing hormone may be blunted or absent in others: the exceptional occurrence of an inactivating mutation in the gonadotropin-releasing hormone receptor should be considered in this setting, but a blunted response is more often the reflection of ‘disuse atrophy’ after prolonged lack of stimulation by endogenous gonadotropin-releasing hormone. Indeed, long-term

pulsatile gonadotropin-releasing hormone administration results in complete pubertal development and fertility in most cases of congenital hypogonadotrophic hypogonadism, confirming the hypothalamic nature of the defect. Further evidence supporting an optimistic outlook in these patients stems from the fact that reversal of hypogonadotrophic hypogonadism in adulthood has also been described (43). Variable patterns of inheritance of normosmic and hypo/anosmic hypogonadotrophic hypogonadism have been described, and several mono- or digenic mechanisms are possible (44). Thus, while the differential diagnosis between simple delayed puberty and permanent hypogonadotrophic hypogonadism remains difficult, a careful family history is essential and targeted molecular studies may confirm a diagnosis and clarify the prognosis.

TRH–TSH–thyroid hormones

Given the crucial effect of thyroid hormones on brain development, a brief review of perinatal thyroid hormone physiology is important. From the standpoint of thyroid hormones, the endocrine milieu of the fetus has the following characteristics: (1) it is a low T_3 milieu, because of the presence of the placenta, with its very rich content in type III deiodinase, which transforms the prohormone T_4 into the inactive hormone reverse T_3 (rT_3) and T_3 itself into the inactive T_2 ; the low T_3 milieu is thought to be responsible for the maintenance of a low level of *in utero* thermogenesis; (2) it is a high TSH milieu, because of extrahypothalamic sources of TRH, such as the pancreas and the placenta; (3) it contains large amounts of inactive sulphated iodothyronines (45). In addition, two characteristics of fetal life may explain how the fetal brain can be protected to some extent from the effect of deficient production of thyroxine by the fetal thyroid: (1) brain cells derive most of their active hormone, T_3 , from intracellular deiodination of T_4 , and this intracellular conversion to T_3 is up-regulated in hypothyroidism; (2) limited transplacental passage of T_4 from mother to fetus has been demonstrated during the first trimester (46) and this passage becomes substantial in the third trimester, so that the cord blood T_4 of athyreotic neonates is 20–50% of the mean value of euthyroid neonates (47). Although this may protect the brain of a hypothyroid fetus carried by a euthyroid mother, it may also account for the evidence suggesting a deleterious influence of maternal hypothyroidism on the developmental outcome of the offspring (48, 49). Immediately after birth, presumably partly as a consequence of the precipitous drop in ambient temperature, plasma TSH increases markedly in normal newborns, with a peak in the first 24 h of life. This is followed by a more shallow increase in plasma T_4 , peaking during the second day of life. Thus, screening for congenital hypothyroidism using TSH as the primary method is best delayed until after 24 h of life, otherwise the number of false positive tests would become unacceptably high. On the other hand, congenital hyperthyroidism is often suspected in babies born to mothers with a history of Graves' disease, but is, in fact, exceedingly rare. For a discussion of the management of women with Graves' disease during pregnancy, the reader is referred elsewhere (50). However, it is important to realize that Graves' disease need not be active in the mother: thyroid-stimulating hormone (TSH)-receptor stimulating immunoglobulins, which are the cause of this type of congenital hyperthyroidism, may remain present in maternal plasma for years after the mother has been rendered euthyroid or, more commonly, hypothyroid (as with, for

example, radioactive iodine). On the other hand, asymptomatic neonates born to mothers with inactive Graves' disease and negative TSH-receptor stimulating immunoglobulins, in whom routine neonatal care suffices, often undergo unnecessary biochemical testing.

In premature newborns, the postnatal peaks of TSH and of T_4 occur within the same time frame, but their amplitude is somewhat lower than that observed in term newborns. The prevalence of permanent primary congenital hypothyroidism is not higher in premature or small for gestational age babies. However, these babies have a mean level of total T_4 (and to a less degree, free T_4) that remains below that of term newborns, but with a normal TSH. Lower T_4 is generally associated with higher mortality or with long-term morbidity. This is, in general, considered as a situation akin to that seen in adults with severe nonthyroidal illness. With the possible exception of very premature infants (gestational age below 27 weeks, in whom the apparent benefit from thyroxine may be from the iodine contained in this molecule), randomized, double blind, placebo-controlled studies of T_4 supplementation have not shown a significant benefit in terms of morbidity, mortality, or developmental outcome at 2 years (51). Systematic supplementation of all low birthweight babies is therefore not recommended at this time (52).

The relative dose (in $\mu\text{g/kg}$ per day) of thyroxine needed to return plasma TSH to normal in hypothyroid infants decreases exponentially during the first year of life from about 10 to about 5 $\mu\text{g/kg}$ per day (53). It then decreases in a more or less linear fashion until the 2 $\mu\text{g/kg}$ per day typically needed by hypothyroid adults is reached. In absolute terms, the 50 μg of thyroxine needed by a term newborn correspond to about 15% of the neonatal intrathyroidal iodine pool, whereas the 150 μg needed by an adult correspond to only 1% of the mature intrathyroidal iodine pool (54). Consistent with this concept of a higher iodine turnover in the thyroid gland throughout infancy, childhood, and adolescence, the normal range of plasma T_3 extends to considerably higher values than in adults and a high T_3 level compared with adult normal ranges (54) should not be taken as evidence of hyperthyroidism (for which the main diagnostic criterion should be a plasma TSH below the normal range or even undetectable). Although there is evidence for a further transient increase in iodine turnover at puberty, this is not reflected in consistent alterations in the plasma concentrations of TSH or of thyroid hormones. Relative to body surface area, thyroid gland size estimated by ultrasound doubles at puberty in both sexes (55); thus, the concept of a 'pubertal goitre' with a female predominance merely reflects the lesser growth of the tracheal cartilage in adolescent girls.

The principal regulator of thyroid growth and function throughout life is TSH. TSH secretion itself is under the stimulatory control of hypothalamic thyroid-releasing hormone (TRH). TRH is a pure 'releasing factor' in that it has little effect on TSH synthesis, while stimulating the release of already synthesized TSH. This contrasts with GHRH, which stimulates both the synthesis and the release of growth hormone. Thus, in children with hypopituitarism, the injection of a single bolus of GHRH in general results in a blunted growth hormone response, whereas that of TRH induces an ample and sustained TSH response (typically, plasma TSH 90 min after TRH will be still higher than at 10–20 min: this is often called a 'hypothalamic profile'). This demonstrates that pituitary thyrotroph cells remain capable of TSH synthesis, but not release, when there is no stimulation by endogenous TRH. Together with the neuroradiological findings described above, TRH testing demonstrates

that hypopituitarism in children does not result from a primary pituitary abnormality, but rather from chronic understimulation by the endogenous hypothalamic hypophysiotrophic factors. This contrasts with the situation in adult-onset hypopituitarism, which most often results from a destruction of the pituitary by tumour or haemorrhage. However, the decision to treat hypopituitary children with thyroxine stems primarily from clinical factors and from sequential measurements of plasma free T_4 , and some authors have therefore advocated that the TRH test should be abandoned (56).

Corticotropin-releasing factor–ACTH–adrenal steroids

The adrenal cortex of the fetus is characterized anatomically by a large fetal zone and the relative weight of the adrenal gland is much larger than after birth; functionally, the fetal adrenal cortex has a low level of activity of the enzyme 3- β -hydroxysteroid dehydrogenase, resulting in the production of large amounts of dehydroepiandrosterone; this compound is then sulphated to dehydroepiandrosterone sulphate, which has a considerably longer plasma half-life. It is also noteworthy that dehydroepiandrosterone is the fetal adrenal androgen that, after being hydroxylated at position 16 in the fetal adrenal and liver, serves as the substrate for oestriol production by the placenta. Oestriol, in turn, stimulates prostaglandin production by the amniotic membranes and thereby contributes to the onset of labour. The clinical consequences of this are that: (1) a low maternal plasma or urinary oestriol (in the presence of normal plasma levels of chorionic somatomammotropin, to rule out placental insufficiency) strongly suggests adrenal hypoplasia or ACTH deficiency in the fetus; these rare, but life-threatening conditions (57) are now potentially detectable prenatally in programmes using the 'triple test' (hCG, α -fetoprotein, oestriol) to screen for pregnant women carrying a fetus with trisomy 21; and (2) both adrenal hypoplasia and ACTH deficiency (but not congenital adrenal hyperplasia resulting from 21-hydroxylase deficiency, a situation in which high amounts of dehydroepiandrosterone are, in fact, produced by the fetal adrenal) are often associated with delayed or absent spontaneous labour (58).

In the first few days after birth, plasma dehydroepiandrosterone sulfate decreases rapidly, but its levels in cord blood or before 3–4 days of life can be used to document the presence of an adrenal gland and of normal corticotrophic function. This is particularly helpful because plasma cortisol levels are often low in the normal neonate and remain so for most of the first year of life (59). Like dehydroepiandrosterone sulfate, plasma 17-hydroxyprogesterone, the metabolite commonly used to diagnose 21-hydroxylase deficiency, is high in cord plasma from normal newborns: if a diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency is considered (as in a newborn with ambiguous genitalia and no palpable gonads), it is best to wait until the third day of life before drawing blood for 17-hydroxyprogesterone assay. Probably because the glomerular filtration rate is low at birth and increases during the first few postnatal days, salt loss seldom occurs before the end of the first week of life; therefore, waiting until the 3rd day of life to draw a plasma sample for 17-hydroxyprogesterone (the results of which can be obtained within 24 h) does not entail an unacceptable risk. Plasma 17-hydroxyprogesterone levels are considerably higher in premature babies and in stressed infants until at

least 6 months of age and this has to be taken into account in order not to over diagnose congenital adrenal hyperplasia (60, 61).

The cortisol production rate increases throughout childhood and adolescence, but remains relatively constant (at 7–10 mg/m² per day) when expressed as a function of body surface area. In contrast, the aldosterone production rate remains constant in a given child over time (59). At about 6–8 years of age, in both sexes, plasma dehydroepiandrosterone sulphate begins to increase again from the undetectable levels characteristic of the younger child. This process, called adrenarche (the clinical correlate of which is the later appearance of pubic hair or pubarche), is thought to play a permissive role in the development of gonadal puberty (although children with Addison's disease undergo gonadal puberty at a normal age) (62). The mechanisms underlying the onset of adrenarche remain unknown (63): while a pituitary hormone distinct from ACTH has long been postulated, its existence has never been conclusively established; local intra-adrenal mechanisms, such as maturation of some steroidogenic enzymes, possibly under the influence of the rising plasma IGF-1 concentrations during mid-childhood, have also been postulated (64).

Pituitary ACTH release is primarily under the control of hypothalamic corticotropin-releasing hormone, and to some extent of vasopressin. Corticotropin-releasing hormone (CRH) is most often used in the evaluation of pituitary-dependent Cushing's disease. In hypopituitary children, an ample ACTH response to CRH has been observed (65). This, together with the TSH response to TRH and neuroradiological findings described above, demonstrate again that the defect is hypothalamic in origin. CRH testing has also been used in the assessment of recovery of the hypothalamic–pituitary–adrenal axis after suppression from the use of exogenous glucocorticoids (66). However, this frequent and complex problem is still most often evaluated by serial determinations of early morning plasma cortisol. Once the 08.00 h cortisol is back to normal (about 200 nmol/l), a stimulation test with ACTH is performed to document the patient's capacity to withstand stress. The low dose version of this test has gained wide acceptance in this context but the paediatric literature remains scarce and 'grey zone results' (i.e. peak cortisol levels of 400 to 550 nmol/l) are frequent and of uncertain significance. A low plasma DHEAS has been suggested as a screening test for adrenal suppression in children (67). Empirically, it is probably safe to 'cover' with stress doses of glucocorticoids in case of major stress, such as surgery under general anaesthesia, for up to 12 months after suppression of the axis has been documented or is likely to have occurred.

Glucose homeostasis

Glucose homeostasis during infancy, childhood, and puberty has characteristics specific of each period, and these have to be kept in mind in the investigation of spontaneous hypoglycaemia. In contrast to what occurs in older children or in adults, plasma glucose in infants declines after a few hours of fasting: this probably reflects lower hepatic glycogen stores in young children. Children with ketotic hypoglycaemia are thought to represent the tail end of the normal distribution for the decrease in glucose with fasting (68, 69). However, before such a benign diagnosis can be made, it is essential to:

- ♦ document that the hypoglycaemic episode is, indeed, associated with ketosis—the first voided urine after the hypoglycaemic episode should therefore be assayed for the presence of ketone

bodies; if these are absent, either hyperinsulinism or a defect in the B oxidation of fatty acids should be suspected. If hyperinsulinism is suspected, plasma ammonium should be measured: in the 'hyperinsulinism-hyperammonaemia syndrome', which is due to activating mutations in glutamate dehydrogenase (70), plasma ammonium is elevated regardless of feeding or fasting and serves as a useful diagnostic clue

- ♦ document that the normal hormonal adaptation to fasting hypoglycaemia has occurred, that is, suppression of insulin secretion and increase in the counter-regulatory hormones, growth hormone and cortisol; these hormonal determinations should be carried out on plasma obtained before the correction of the hypoglycaemia; this is the 'critical sample' alluded to above. With modern techniques, these hormones can be measured on microlitre amounts of plasma, and one can use the samples left over after the routine biochemical determinations (electrolytes, urea, calcium) that are usually requested at the same time as the blood glucose have been carried out

Consistent with the concept that ketotic hypoglycaemia is an exaggeration of normal physiology, it is a benign, self-limited condition: typically, the phenomenon becomes manifest in toddlers (after an unusually long period of fasting) and disappears by mid-childhood.

Aside from their greater tendency to fasting hypoglycaemia, young children are also more sensitive to insulin than adolescents or adults. This is especially marked in children with hypopituitarism, so much so that deaths occurring after stimulation tests with insulin-induced hypoglycaemia or even with glucagon to investigate growth hormone reserve have been reported in children (71). Since these reports, the 'insulin tolerance test', which remains much used in adults, has become used less often in paediatric centres.

With puberty, insulin sensitivity decreases markedly. This has obvious implications in the management of diabetes mellitus. Another implication is seen in children with hypoglycaemia due to hypopituitarism, in whom the tolerance to fasting typically improves with age: this probably represents a combination of increased glycogenic storage capacity of the liver and of decreased peripheral insulin sensitivity. Finally, pubertal insulin resistance combined with the growing rates of obesity has resulted in the diagnosis of glucose intolerance on oral glucose tolerance testing in 21% of obese adolescents (72). However, the practical implication of making this diagnosis at an early age is questionable, since the first steps in management (diet and lifestyle modifications) will be the same regardless of glucose tolerance.

Mineral metabolism

The plasma concentrations of calcium, but especially of phosphorus and of alkaline phosphatase, vary considerably during growth. This is a reflection of the bone remodelling that is most pronounced in infancy and during puberty. Accordingly, plasma phosphorus and alkaline phosphatase are high during the first 3 years of life, decrease during the period of slower growth between 3 and 10 years of age, and increase again thereafter to values well above the normal adult range. Thus, before a diagnosis of vitamin D deficiency is considered, interpretation of biochemical values should be based on comparison with normal ranges appropriate for age or, better still, for pubertal stage (73).

In the investigation of hypo- or hypercalcaemia, the concept of the critical sample applies again. Indeed, PTH secretion is exquisitely sensitive to variations in plasma calcium concentrations: thus, a low PTH level in the face of hypocalcaemia suggests hypoparathyroidism, whereas a detectable PTH level in the face of hypercalcaemia suggests hyperparathyroidism. The importance of tracking the samples on which abnormal biochemical values have been measured cannot be overemphasized.

Childhood obesity

Worldwide, obesity has been increasing in all age groups in the last decades, becoming a major public health problem and a source of endless frustration for patients, families, and clinicians. The differential diagnosis of the cause of obesity is made easier in children than in adults by observing linear growth. Children with exogenous obesity have increased height velocity (20), while those with excess weight gain from treatable endocrinopathies, such as hypothyroidism and hypercortisolism have decreased linear growth. Thus, in a child who is gaining weight excessively, but growing normally in height, the determination of plasma TSH or cortisol is useless and may, in fact, be misleading (74). While mutations in the melanocortin receptor type 4 have been recently identified in up to 2.4% of obese children (75), this molecular diagnosis has no therapeutic implications at this point. Although the fundamental physiological importance of leptin has been well established, the clinical relevance of leptin assays for diagnosis and of recombinant leptin for treatment is very limited. Exogenous obesity is associated with leptin resistance, not deficiency, and so is obesity after neurosurgery for craniopharyngioma or associated with the Prader-Willi syndrome. Leptin deficiency appears to be exceedingly rare. Outside of research settings, it seems reasonable to recommend measurement of plasma leptin only in the unusual situation of severe obesity beginning in an infant born to nonobese parents, especially if they are consanguineous, or in massively obese adolescents who fail to go into puberty, and who do not have evidence of Prader-Willi syndrome or of other syndromes characterized by obesity and hypogonadism (such as the Lawrence-Moon-Biedl syndrome).

Conclusion

The practitioner investigating a paediatric patient for a possible endocrine abnormality should keep in mind the conceptual and temporal framework outlined in this section to select the most appropriate time point and hormonal parameter to analyse given the clinical signs and symptoms; indeed, abnormalities in endocrine investigations are very much dependent on the stage of maturity of the child and thus of the developmental stage of the endocrine system under investigation.

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