Inducing puberty

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Abstract

Puberty is the result of increasing pulsatile secretion of the hypothalamic gonadotropin releasing hormone (GnRH), which stimulates the release of gonadotropins and in turn gonadal activity.

In general in females, development of secondary sex characteristics due to the activity of the gonadal axis, i.e., the growth of breasts, is the result of exposure to estrogens, while in boys testicular growth is dependent on gonadotropins and virilization on androgens.

Hypogonadotropic hypogonadism is a rare disease. More common is the clinical picture of delayed puberty, often associated with a delay of growth and more often familial occurring. Especially, boys are referred because of the delay of growth and puberty. A short course (3–6 months) of androgens may help these boys to overcome the psychosocial repercussions, and during this period an increase in the velocity of height growth and some virilization will occur.

Hypogonadotropic hypogonadism may present in a congenital form caused by developmental disorders, some of which are related to a genetic disorder, or secondary to hypothalamic–pituitary dysfunction due to, among others, a cerebral tumor.

In hypogonadotropic hypogonadism puberty can be initiated by the use of pulsatile GnRH, gonadotropins, and sex steroids. Sex steroids will induce development of the secondary sex characteristics alone, while combined administration of gonadotropins and GnRH may induce gonadal development including fertility.

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Introduction

Pubertal development is the result of increasing release of GnRH by the hypothalamus, which in turn increasingly stimulates the pituitary to release both gonadotropins LH and FSH. The gonadotropins stimulate the gonads, ovary, and testis, to develop and produce the sex steroids estrogens and androgens respectively. At the onset of puberty, the first endocrine change is the occurrence of an LH increase only during the night (1). With the progression of puberty, the LH pulse pattern shows an increase in frequency and amplitude with a clear day/night rhythm (2). In adulthood, the day/night rhythm has disappeared. The pulsatile pattern of LH reflects the pulsatile release of GnRH. For FSH, a pulsatile secretion pattern in blood is hard to detect, presumably due to the longer half-life of 4–6 h of FSH compared with 20–30 min for LH (3, 4). In boys, the nocturnal LH increase is associated with a concomitant increase in testosterone, while in girls the highest estradiol increase occurs during the day as a result of delayed response of the ovaries (2, 5, 6).

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The GnRH test is often used as a diagnostic tool in the evaluation of delayed puberty. However, the response of gonadotropins to an acute challenge of GnRH can be difficult to interpret, since in the pre-pubertal state the pituitary, although intact, will not or hardly respond, similar to the situation in hypogonadotropic hypogonadism (7). Repetitive administration of GnRH intravenously does increase gonadotropin levels in patients in a pre-pubertal and hypogonadotropic state. Therefore, this diagnostic test is not able to differentiate between the two conditions. In fact, the response to the GnRH test reflects the measure of previous stimulation by GnRH.

The GnRH agonist test has been described to be an alternative test in the diagnosis of hypogonadotropic hypogonadism (8). However, for both the native GnRH and the GnRH agonist tests, the authors describe significantly higher levels of LH and FSH levels in delayed puberty compared with hypogonadotropic patients. We therefore must conclude that there still is no reliable test for differentiation in the pre-pubertal state between normal, but delayed puberty and hypogonadotropic hypogonadism.

However, clinical aspects such as the family history, height growth, and skeletal age can make a given diagnosis more probable. A patient with delayed puberty

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mostly has a pre-pubertal dip, i.e., a decrease in height growth velocity preceding the pubertal growth spurt, while hypogonadotropic patients tend to continue their growth with pre-pubertal height growth velocity. In addition, in delayed puberty, skeletal age will be retarded, but conforms to the biological physical status, while in hypogonadotropic hypogonadism, skeletal age will start to delay from the age of onset of puberty and this delay increases, but finally remains not in conformity with the biological, pre-pubertal status of the patient.

Pathophysiology of hypogonadotropic hypogonadism

Timing of puberty is the result of both genetic constitution and environmental influences. Well known is the familial nature of delayed puberty, whereby mothers exhibit a late menarche and/or fathers always belong to the shortest adolescents during their first years in high school, but reach a final height within the normal range. Chronic systemic diseases such as Crohn's disease and asthma are often associated with delayed puberty. It appears that under these circumstances the body does not expend energy on growth, puberty, and fertility. Cure will result in the catchup of growth and progression of the developmental process.

Delayed puberty is defined as the start of puberty at a chronological age older than +2 S.D. of average maturers: from the Dutch data, for boys, when testicular growth (testes volume 4 ml or more) has not started at the age of 14 years, and for girls, when breast development is not present at the age of 13 years or menarche did not occur at the age of 15 years. Throughout Europe, there are only slight differences (9).

Hypogonadotropic hypogonadism can develop first due to a developmental abnormality or secondly due to an underlying disease interfering with the function of the hypothalamic–pituitary axis such as a cerebral tumor (Table 1). Also, a pubertal arrest may result in hypogonadotropic hypogonadism after some spontaneous development. With respect to pubertal arrest, mutations and polymorphisms in gonadotropin genes and their receptors can be responsible for this phenotype or for complete hypogonadotropic hypogonadism.

Table 1 Causes of hypogonadotropic hypogonadism.

Primary/congenital:
Idiopathic
Mutations of LH β and FSH β-subunits
Kallmann syndrome
GnRH receptor gene mutations
NR0B1 gene mutation
GPR54 gene mutation
Transcription factor genes mutation: *PROP1*, *LHX3*, and *HESX1*, in combination with other pituitary hormone deficiencies
Hypothalamic dysfunction in combination with other syndromes (Prader–Willi syndrome, Laurence–Moon syndrome CHARGE, and others)
Secondary/achieved:
Brain tumor
Irradiation of the brain

The congenital form of hypogonadism may be suspected in the newborn with multiple pituitary deficiencies. Cryptorchidism and a micropenis may be presenting symptoms in boys. The first months can be used to diagnose a GnRH deficiency, since there is a spontaneous GnRH-related gonadotropin increase. Absence of this gonadotropin peak confirms hypogonadotropic hypogonadism (10).

Kallmann syndrome, hypogonadotropic hypogonadism in combination with anosmia, is the result of a genetic disorder. An X-linked form, an autosomal dominant and an autosomal recessive form are identified. A mutation of the *KAL1* gene located on the Xp22.3 region encoding for the protein anosmin has been detected. Anosmin is involved in the neuronal migration and axonal path finding. In the absence of anosmin, migration of the GnRH releasing, as well as of the olfactory neurons, cannot be completed, resulting in hypogonadotropic hypogonadism and anosmia (11). The autosomal form of Kallmann syndrome is the result of a mutation of a gene encoding the fibroblastic growth factor receptor 1 located on the short arm of chromosome 8 (12). For the autosomal recessive form, no genetic mutation has been identified yet.

Downstream to the GnRH neurons, receptor abnormalities may be responsible for partial to complete forms of hypogonadotropic hypogonadism. Inactivating mutations of the GnRH receptors result in the absent to low pulsatile levels of gonadotropins in combination with low sex steroid levels. These patients show an inadequate response to exogenous pulsatile GnRH.

The *NR0B1* gene is involved in the development and function of the adrenal gland, as well as the hypothalamic–pituitary axis related to gonadotropin secretion. Mutations of the *NR0B1* gene may present with an X-linked congenital adrenal hypoplasia and lack of pubertal development in boys. With *NR0B1* gene mutation, heterozygous girls may have delayed puberty (13).

GPR54 is described as a novel regulator of the central control of puberty. The *GPR54* gene encodes for a G-protein-coupled receptor. Patients with mutations of the *GPR54* gene have hypogonadotropic hypogonadism, while they do respond to exogenous pulsatile GnRH stimulation. In affected mice, GnRH levels at the hypothalamic level are normal. Therefore, it appears that *GPR54* is not involved in the production, but in the release of GnRH (14).

GnRH and gonadotropin deficiencies can be part of a hypothalamic and/or pituitary developmental defect. *PROP1* is one of the transcription factors involved in the early differentiation of gonadotropic, somatotropic, lactotropic, and thyrotropic cells. A *PROP1* mutation will present with deficiencies for gonadotropins, growth

hormone, prolactin, and thyroid-stimulating hormone (TSH). The gene defect inherits in an autosomal recessive trait. Heterozygous carriers are not affected (15). In *PROP1* gene defects, an enlarged anterior pituitary may precede pituitary hypoplasia later on. These patients have a normal posterior pituitary lobe (16, 17).

Patients with mutations of the transcription factor *LHX3* have deficiencies for all pituitary hormones except for POMC and show either an enlarged or a hypoplastic pituitary (18).

The septic–optic dysplasia, a midline defect, can be associated with mutations of the homeobox gene *HESX1*. These patients are deficient for gonadotropins, growth hormone, prolactin, TSH, and POMC. Other features of the syndrome are an impaired vision, pendular nystagmus, and absence of the septum pellucidum (19).

Hypothalamic dysfunction can be part of a syndrome. For instance in Prader–Willi syndrome, the insufficient secretion of GH, POMC, and gonadotropins are presumed to be the result of hypothalamic dysfunction (20–22).

The most common secondary cause of hypogonadotropic hypogonadism is a cerebral tumor. In childhood, the tumor in question is known as a craniopharyngeoma, originating from Rathke's pouch. Growth failure in combination with headache, polydipsia and polyuria, and other symptoms can be the result of pituitary hormone deficiencies. Depending on the size of the tumor, surgery with radiation or radiation alone are the therapeutic approaches. Other brain tumors that may interfere with hypothalamic function are astrocytomas, gliomas, histiocytosis X, germinomas, and prolactinomas.

Radiation of brain areas in the treatment of cancer may also damage hypothalamic–pituitary function and result in delayed puberty or hypothalamic dysfunction (23). The onset of pituitary dysfunction may become clear, many years after the moment of radiation.

Induction of puberty

Treatment in order to induce pubertal development is indicated in patients who will not undergo spontaneous development, either due to a central defect or a gonadal defect in the gonadal axis. In patients with delayed puberty, treatment will be required only temporarily in order to overcome deficiencies in the period previous to the natural start of pubertal development. Dependent on the cause, one may treat with GnRH in order to mimic the normal physiological process, with gonadotropins and sex steroids. The management of these three options will be discussed separately.

GnRH

GnRH is a decapeptide. With the availability since 1970, an era of research on gonadotropins secretion has been started (24). GnRH stimulates the release of both LH and FSH. In adults, the gonadotropin response seems to increase quantitatively with the GnRH dose. The dose–response relationships between FSH and LH are different. Consequently, the FSH/LH ratio changes at different GnRH doses (25). Throughout puberty, the LH and FSH response increases with the progression of puberty. In girls, the highest FSH basal and peak levels are obtained only in stage 2 of puberty (26).

GnRH is released by GnRH-producing neurons into the portal system. In peripheral blood, GnRH is hard to detect. Clarke and Cummins (27) demonstrated simultaneous episodic fluctuations of GnRH in portal blood and LH in peripheral blood in sheep. As LH is secreted in a pulsatile pattern, it is assumed that this pattern is the result of GnRH release. Further evidence to support this is found in the observation that in the human, the pituitary can only be stimulated appropriately when GnRH is administered in a pulsatile fashion.

Continuous stimulation of the pituitary by GnRH first induces an increase followed by a drop due to pituitary desensitization. This last phenomenon is used in the treatment of patients, whereby suppression of gonadotropin secretion is desired. Replacement of amino acids at the sixth position, where the major site of metabolic degradation is located, changes the affinity of binding to the receptor, as well as the bioactivity of the GnRH agonist. An increase in receptor affinity and biopotency will easily induce desensitization after an initial stimulation and will finally result in the suppression of gonadotropin levels (28).

Patients with a GnRH deficiency, the isolated form, in Kallmann syndrome and in combination with other hypothalamic deficiencies, can be treated with GnRH infused in a pulsatile fashion as the most physiological approach. A physiological treatment includes pulses with an interval of 90–120 min. i.v. infusion will result in the most effective pulsatile stimulation and therefore pulsatile release of gonadotropin, while in the case of s.c. administration, the gonadotropin levels are more flattened, and can also result in adequate gonadal stimulation.

For hypogonadotropic girls, GnRH treatment is not the first choice, since sex steroids will result in adequate development of sex characteristics. For diagnostic purpose, pulsatile GnRH will provide information on the possibilities to induce ovulation, which can be applied when fertility is desired (29).

In male hypogonadotropic hypogonadism, GnRH will result in a complete development with testicular growth including spermatogenesis and virilization. This in contrast to sex steroids initiating virilization only, without testicular development (30). When spermatogenesis has been achieved, human chorionic gonadotropins (hCG) treatment once or twice a week applied subcutaneously will maintain this development in the long term.

Gonadotropins

Both gonadotropins LH and FSH, the human chorionic gonadotropins, as well as TSH are glycoproteins. These hormones consist of an α- and β-subunit. The α-subunits appear to have a similar amino acid sequence, and the β-subunits have a characteristic structure for each hormone. Synthesis and release of LH and FSH by the pituitary has been demonstrated to take place as early as at 70–100 days of gestation (31).

LH and FSH plasma levels increase to very high levels at about 20–25 weeks of gestation followed by a decline. At birth, the levels are low. During the first months, there is a transient peak in girls, longer than in boys, which is followed by a complete suppression of gonadotropin secretion. At the onset of puberty, the central axis reawakens and the first endocrine change is an increase in nocturnal LH. With the progression of puberty LH and FSH increase. This increase reflects the increase in GnRH stimulation. Especially, the LH pulsatile pattern gives information on the changes of GnRH release. For LH, the pulses increase in number as well as in amplitude (1, 2, 30). For FSH, the pulses are difficult to identify probably due to its longer half-life.

At the onset of puberty, when LH can be identified only during the night, some FSH increase can be observed during the day. In general, when highly specific assays are used, daytime gonadotropin measurements show undetectable LH and clearly measurable FSH levels.

The early FSH increase is needed for an optimal LH bioactivity in boys, since FSH stimulates the development of Leydig cells, probably by factors produced by Sertoli cells (32). In girls, FSH initiates follicular growth, induces receptors for LH, and increases granulosa cell aromatase enzymes (33).

Based on the interactions between both gonadotropins, a combination of these will be the best treatment option to induce puberty in hypogonadotropic patients. Treatment of hypogonadism may have two purposes: the development of the secondary sex characteristics and fertility. In hypogonadotropic boys, androgen substitution will give an adequate, adult virilization, but the testes remain undeveloped. To induce testicular growth, gonadotropin activity is needed. In girls, an adequate development of the secondary sex characteristics can be obtained with estrogens alone. That is the reason why gonadotropins are not used to induce puberty in girls. They can be applied later on in adulthood to induce ovulation, when these women have a wish for children.

In boys, human chorionic gonadotropins as a single treatment have been used to induce puberty. Furthermore, for decades, hCG has been used for maldescensus testis. Just recently, a meta-analysis on the efficacy and safety of this treatment in cryptorchidism made the authors conclude that considering the efficacy of about 20% in combination with possible side effects, the general use of hCG in the treatment of cryptorchidism cannot be further recommended (34). For the management of induction of puberty, hCG alone induces an increase in androgens associated with a pubertal growth spurt and some virilization (35). In congenital hypogonadotropic hypogonadism, hCG alone is not a feasible treatment with respect to the stimulation of testicular development. However, in men with partial or with a post-pubertal achieved gonadotropin deficiency, hCG treatment may be sufficient to stimulate androgen production, spermatogenensis, and fertility (36).

In general, FSH and LH activity in the combination of hMG/hCG or recombinant FSH/hCG is used in the management of hypogonadotropic males in order to stimulate testicular growth and to induce androgen production. For both treatment options, successful results are described. Gonadotropin treatment in hypogonadotropic males initiates testicular growth, spermatogenesis, and testosterone production. The treatment schedule varies from 1250–5000 IU hCG in combination with 12.5–150 IU hMG three times per week intramuscularly (37–39). The hCG dose is to be adjusted on the testosterone levels, while hMG is mostly added during the hCG treatment and is adjusted in dose in response to clinical signs. Multiple pituitary deficiency, congenital hypogonadotropic hypogonadism, and cryptorchidism appear to be the prognostic factors for successful stimulation. Prior androgen treatment had no adverse effect.

Using pure FSH, similar doses (75–100 IU) are used subcutaneously concomitantly with hCG intramuscularly (40).

Comparing gonadotropin treatment with pulsatile GnRH treatment shows that both are effective therapeutic modalities to achieve spermatogenesis and testosterone production. Controversial data are published on whether or not the results of both treatments are similar.

Schopohl *et al.* (41) observed a better outcome with respect to testicular growth in patients with congenital hypogonadotropic hypogonadism and treated with pulsatile GnRH compared with gonadotropin treatment. In achieved (secondary) hypogonadism, gonadotropin treatment is a good alternative to obtain testicular development. Others describe similar results (40, 42, 43). From our own experience, we managed to induce spermatogenesis in hypogonadotropic patients using pulsatile GnRH with a previous failure of gonadotropin treatment.

From most reports, it is clear that the smaller the testicular volume at the start of the treatment, the lower the final achieved volume at the end of the treatment. Especially, in patients with the congenital form of hypogonadism, it is difficult to achieve a normal testicular volume at the end of the treatment. This is in contrast to patients with an achieved, secondary hypogonadotropic hypogonadism. With respect to the sperm count, although most patients will achieve spermatogenesis, most of them have oligospermia. However, a low sperm concentration does not exclude fertility in these hypogonadotropic men (30).

Side effects of gonadotropin treatment can be the inconvenient way of administration to gynecomastia and the induction of antibodies to hCH as has been described by Thau *et al*. (44). These antibodies cause the patient to no longer respond to hCG treatment.

Sex steroids

When development of the secondary sex characteristics is the ultimate goal, sex steroids can give an adequate outcome.

In girls, the final outcome of the induction of puberty using sex steroids is not different from a spontaneous puberty. Since estrogens induce progressive epiphyseal fusion, one should take into account the actual height and growth potential before starting with estrogens (45). Estrogens can be prescribed in a gradually increasing dose (Table 2) (46). Most commonly used is the synthetic estrogen ethinyl estradiol. It is very cheap and there is a lot of experience in its use. Limited clinical experience is available with the natural estrogen 17β-estradiol. Natural estrogens are preferable to synthetic estrogens because of incomplete metabolization and a greater risk of thromboembolism and arterial hypertension of the latter. In addition to oral 17β-estradiol tablets, transdermal patches are also applicable (47, 48).

Experience in the induction of puberty using oral 17β-estradiol in girls is shown in Fig. 1. The curves present the estradiol levels during substitution with 17β-estradiol in an increasing dose of 5, 10, 15, and 20 μg/kg per day, with a final dose of 2 mg per day. The results are depicted in the reference values of nocturnal and diurnal estradiol levels during puberty, obtained in a healthy population of girls with the various stages of puberty.

After 1 to 2 years of substitution with estrogens, a progesterone should be added to prevent endometrial hyperplasia.

In boys, stimulation of growth and development of the secondary sex characteristics are usually possible to achieve using gradually increasing doses of testosterone derivates. In general, one is advised not to start before a skeletal age of 12 years or a chronological age of 14 years (48). The common route of testosterone administration is in the form of depots of testosterone esters with different half-life times. This way of treatment is effective and relatively cheap and well accepted by the patient (Table 3). This testosterone treatment results in an adequate virilization. However, serum testosterone levels exhibit large fluctuations with high levels immediate after the injection followed by a decrease into the low range. By increasing the dose, testosterone levels remain in the normal adult range for a longer period (36).

Oral testosterone preparations are not preferable for the induction of puberty. To achieve adequate testosterone levels (and therefore virilization), testosterone undecanoate must be taken two to three times per day, which is difficult to sustain for most patients.

For several years, transdermal testosterone patches are available, which should be a good alternative for induction of puberty in boys. However, the clinical experience in boys is sparse. Scrotal patches are too large and result in relatively high doses. Gels with androgens, which can be applied on the skin of the shoulder, are an alternative option. Studies in male adolescents are needed in order to obtain information about doses and related blood levels in the pubertal male.

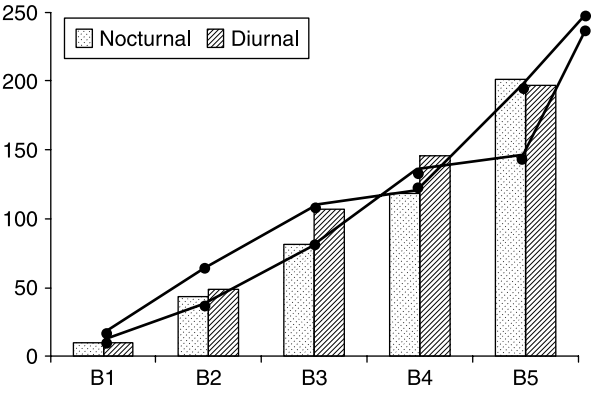

Figure 1 Estradiol blood levels in two patients during induction of puberty with oral 17β-estradiol in doses of 5, 10, 15, and 20 μg/kg per day and a final dose of 2 mg per day. The bars represent reference values of nocturnal and diurnal estradiol levels of healthy girls throughout puberty (adapted from Wennink *et al*. 1990 (5)).

Table 2 Induction of puberty in girls.

Treatment with 17β-estradiol in an increasing dose schedule every 6 months:
5 μg/kg per day p.o.
10 μg/kg per day p.o.
15 μg/kg per day p.o.
20 μg/kg per day p.o.
Adult dose at about 2 mg per day
Treatment with ethinyl estradiol in an increasing dose schedule every 6 months:
0.1 μg/kg per day p.o.
0.2 μg/kg per day p.o.
0.4 μg/kg per day p.o.
0.6 μg/kg per day p.o.
Adult dose is about 30 μg per day. Then a contraceptive pill can be used

Table 3 Induction of puberty in boys.

Induction of puberty in boys using testosterone esters
Increasing dose schedule every 6 months:
25 mg/m^2 per 2 weeks i.m.
50 mg/m^2 per 2 weeks i.m.
75 mg/m^2 per 2 weeks i.m.
100 mg/m^2 per 2 weeks i.m.
Adult dose Sustanon 250 per 3–4 weeks

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