Aromatase inhibitors in pediatrics

Jan M. Wit, Matti Hero and Susan B. Nunez

Abstract | Aromatase, an enzyme located in the endoplasmic reticulum of estrogen-producing cells, catalyzes the rate-limiting step in the conversion of androgens to estrogens in many tissues. The clinical features of patients with defects in *CYP19A1*, the gene encoding aromatase, have revealed a major role for this enzyme in epiphyseal plate closure, which has promoted interest in the use of inhibitors of aromatase to improve adult height. The availability of the selective aromatase inhibitors letrozole and anastrozole —currently approved as adjuvant therapy for breast cancer—have stimulated off-label use of aromatase inhibitors in pediatrics for the following conditions: hyperestrogenism, such as aromatase excess syndrome, Peutz–Jeghers syndrome, McCune–Albright syndrome and functional follicular ovarian cysts; hyperandrogenism, for example, testotoxicosis (also known as familial male-limited precocious puberty) and congenital adrenal hyperplasia; pubertal gynecomastia; and short stature and/or pubertal delay in boys. Current data suggest that aromatase inhibitors are probably effective in the treatment of patients with aromatase excess syndrome or testotoxicosis, partially effective in Peutz–Jeghers and McCune–Albright syndrome, but probably ineffective in gynecomastia. Insufficient data are available in patients with congenital adrenal hyperplasia or functional ovarian cysts. Although aromatase inhibitors appear effective in increasing adult height of boys with short stature and/or pubertal delay, safety concerns, including vertebral deformities, a decrease in serum HDL cholesterol levels and increase of erythrocytosis, are reasons for caution.

Wit, J. M. *et al. Nat. Rev. Endocrinol.* **8**, 135–147 (2012); published online 25 October 2011; doi:10.1038/nrendo.2011.161

Introduction

Aromatase inhibitors were originally developed for the treatment of estrogen-receptor-positive breast cancer,[1] and these drugs have been used in clinical trials for other conditions in children and adolescents since the 1980s. However, interest has increased in the past decade owing to a potential effect of these agents on adult height. Novel insight into the use of aromatase inhibitors for this indication was based on the discovery of tall (>+2 SDS) patients with defects in the genes encoding aromatase and the estrogen receptor.[2–7] The potential of aromatase inhibitors to increase adult height in boys with short stature and/or pubertal delay was further underscored by the results of four placebo-controlled clinical trials.[8–11] Although it has been emphasized that their use should be considered experimental,[12–18] aromatase inhibitors are often prescribed off-label to short boys, although actual data on the frequency of this practice are lacking.

After short introductions to the physiology of aromatase and chemical characteristics of aromatase inhibitors, this Review will focus on the efficacy and safety of aromatase inhibitors in pediatrics. The US public health grading system was used to grade the evidence and strength of recommendations.[19] Although this Review is not a practice guideline, we aimed to adhere to modified appraisal of guidelines research and evaluation (AGREE) criteria.[20]

Competing interests
The authors declare no competing interests.

Physiology of aromatase

The hemoprotein-containing aromatase enzyme is a complex formed by two proteins, cytochrome P450 19A1 (also known as aromatase) and the NADPH–cytochrome p450 reductase.[14,21] The protein aromatase is encoded by the *CYP19A1* gene, which is located on chromosome 15q21.2. The entire gene spans more than 123 kb of DNA, but only the 30-kb-long 3'-region encodes aromatase, whereas a large 93-kb-long 5'-flanking region serves as the regulatory unit of the gene. Tissue-specific expression of aromatase results from the interplay of organ-specific enhancers and promoters,[22,23] which probably explains the large variation in protein expression in various tissues. Aromatase catalyzes the rate-limiting step in the conversion of testosterone to estradiol and androstenedione to estrone, but estrogens are also substrates for aromatase.[14]

In men, most estrogen is synthesized in peripheral tissues through local aromatization of circulating androgens. These androgens are produced mainly by the adrenal glands,[13,24] whereas the testes form only very small amounts. The principal site of aromatization is adipose tissue (stromal cells), but aromatase activity can also be found in many other tissues, such as the brain (hypothalamus, limbic system and cerebral cortex), breast, placenta, liver, muscle, bone (osteoblasts and chondrocytes), testis (Leydig cells and germ cells), vasculature (smooth muscle cells) and skin (fibroblasts and hair follicles).[17,25] Estrogen that is synthesized in peripheral tissues is assumed to act only locally.[13,24,26]

Department of Pediatrics, J6S, Leiden University Medical Center, Albinusdreef 2, 2333ZA, P. O. Box 9600, 2300RC Leiden, The Netherlands (**J. M. Wit**). Children's Hospital, Helsinki University Central Hospital, Stenbäckinkatu 11, P. O. Box 281, 00029 Helsinki, Finland (**M. Hero**). Section of Pediatric Endocrinology, Dell Children's Medical Center of Central Texas, 4900 Mueller Boulevard, Austin, TX 78723, USA (**S. B. Nunez**).

Correspondence to:
J. M. Wit
j.m.wit@lumc.nl

Key points

- Aromatase is expressed in many tissues and converts androgens to estrogens in a tissue-specific fashion
- The third-generation aromatase inhibitors anastrozole and letrozole suppress estrogen production by 97–99% and are highly selective
- Animal experiments have shown that the role of estrogen in growth regulation is different from that in humans, but have highlighted possible adverse effects of aromatase inhibitor use
- Contrary to theoretical expectations, aromatase inhibitors appear ineffective in the treatment of pubertal gynecomastia
- Evidence from controlled and uncontrolled studies in boys with short stature and/or pubertal delay suggests a positive effect of aromatase inhibitors on adult height, but more follow-up data are needed
- The use of aromatase inhibitors in prepubertal boys is not advised because of an association with vertebral deformities

Table 1 | Aromatase inhibitors

Compound	Type	Suppression of estrogen production [%]
First generation		
Aminoglutethimide (250 mg four times daily)	Nonsteroidal	90.6
Testolactone (10 mg/kg four times daily)	Steroidal	<90.0
Second generation		
Fadrozole (2 mg twice daily)	Nonsteroidal	92.6
Formestane (Intramuscular injection of 250 mg every 2 weeks)	Steroidal	91.9
Third generation		
Anastrozole (1 mg per day)	Nonsteroidal	97.3 (E1 81, E2 84.9, E1S 93.5*) Residual E2 10.1‡
Letrozole (2.5 mg per day)	Nonsteroidal	>99.1 (E1 84.3, E2 87.8, E1S 98.0*) Residual E2 5.9‡
Vorozole (2.5 mg per day)	Nonsteroidal	89 (E1 64, E2 80)§
Exemestane (25 mg per day)	Steroidal	98 (E2 62‖)

Data derived from Geffner *et al.*,[17] unless stated otherwise. *Data from Geisler *et al.*[35] ‡Data from Dixon *et al.*[150] §Data from De Jong *et al.*[151] ‖Data from Mauras *et al.*[29] Abbreviations: E1, estrone, E2, estradiol; E1S, estrone sulfate.

Administration of supraphysiologic amounts of estrogen has long been known to increase prepubertal growth, accelerate epiphyseal fusion and reduce adult height.[27] However, the central role of local estrogens in regulating longitudinal growth was shown by the discovery of a man with a mutation resulting in complete estrogen resistance[2] and several men and women with an aromatase defect.[3–7] All individuals showed normal prepubertal growth and onset of secondary sexual characteristics, delayed closure of the epiphyseal growth plates and tall adult stature, but only in patients with aromatase deficiency did estrogen therapy lead to fusion of the epiphyseal plates.

Aromatase inhibitors

The effect of aromatase inhibitors on growth appears to be mediated primarily via reduced estrogen production within the epiphyseal chondrocytes, but a decrease of circulating estrogens also affects growth hormone (GH) and insulin-like growth factor 1 (IGF-1) secretion.[28] The current view is that, in men as well as women, estrogen does not participate in the regulation of linear growth before puberty but plays a major part in inducing the pubertal growth spurt (at low levels) and in epiphyseal maturation and closure (at high levels).

Pharmacology

Two types of aromatase inhibitors—nonsteroidal and steroidal—exist, which can be divided into three generations (Table 1).[1] The first steroidal aromatase inhibitor (testolactone) was used in various pediatric studies but had disadvantages compared with newer agents, because it required a dosage of four times per day. Second-generation inhibitors (formestane, fadrozole) were soon replaced by third-generation aromatase inhibitors: the nonsteroidal anastrozole and letrozole and the steroidal exemestane (Figure 1), which can be taken orally once daily (Table 1).

A theoretical advantage of steroidal over nonsteroidal aromatase inhibitors is that covalent binding of the drug to the active site of the enzyme irreversibly inhibits aromatase action.[12,13] However, exemestane has not been used in pediatrics, except for one pharmacokinetic study.[29] Nonsteroidal aromatase inhibitors form a reversible bond with the enzyme.[12] Anastrozole is a very selective inhibitor of aromatase, in contrast to letrozole, which slightly decreases basal and ACTH-stimulated cortisol synthesis.[30–32] Anastrozole is rapidly absorbed (maximum after 1 h) and slowly eliminated (terminal half-life of 46.8 h) after oral dosing.[33] This drug had no negative metabolic effects over a period of 10 weeks in young healthy men.[34] Letrozole is also rapidly absorbed but has a longer half-life (2–4 days) and suppresses aromatase activity slightly more than anastrozole (Table 1),[35] which is illustrated by the higher plasma testosterone and gonadotropin levels found in patients treated with letrozole compared with anastrozole.[8–10]

Animal experiments

Knockout models for estrogen receptors, androgen receptor and aromatase have elucidated the role of sex steroids in multiple physiologic processes, including growth, but have also shown important differences between mouse and man and even between different rodent species. The effects of estrogen receptor α and β inactivation vary with sex and age of the mice.[36] Moreover, male and female aromatase knockout (ArKO) mice appeared phenotypically normal at birth,[37] but adult appendicular growth was significantly retarded in male but not in female ArKO mice.[38] Both sexes displayed osteoporosis.[39] Female ArKO mice had underdeveloped uteri and ovaries and were sterile due to anovulation.[37,40] Male mice were fertile, but to a lesser extent than wild-type littermates.[39] Increased abdominal fat deposition and insulin resistance was present in both sexes.[41]

Studies on the effect of gonadectomy, chemical castration with gonadotropin-releasing hormone (GnRH)

analogues[42] or use of aromatase inhibitors[43–45] in rats have also shown sexual dimorphism. In male rats, the nonsteroidal aromatase inhibitor vorozole decreased body weight and BMD, but femoral length was normal.[43] Exemestane or letrozole treatment of young male rats caused osteopenia and prostatic hyperplasia and reduced body weight, tail length and IGF-1 levels.[44,45] By contrast, in male mice, letrozole increased body weight and tail length gain, the width of the epiphyseal growth plates and GH levels.[46] Anastrozole administration to adult male rats had no effect on the number of Sertoli cells or germ cells, or on the volume of the seminiferous epithelium, tubule lumens or interstitium.[47] In female rats, exemestane increased weight gain and growth plate width, but less prominently than in ovariectomized rats. Trabecular bone was negatively affected, and the ovaries contained multiple cysts and were lighter than those of controls.[48]

Aromatase inhibitor use in pediatrics

Aromatase inhibitors are approved solely for the palliative or adjuvant treatment of postmenopausal women with estrogen-receptor-positive breast cancer;[49,50] however, these drugs have also been used off-label for ovulation induction[51] and several other conditions.[52] In men with low fertility associated with a decreased testosterone:estradiol ratio, for example, in Klinefelter syndrome and obesity-associated hypogonadotropic hypogonadism, aromatase inhibitors have a positive effect.[52,53] In pediatrics, aromatase inhibitors have been used off-label for the treatment of the following four groups of conditions: hyperestrogenism, hyperandrogenism, pubertal gynecomastia, and short stature and/or delayed puberty.

Hyperestrogenism

Aromatase excess syndrome

Aromatase excess syndrome is a rare, dominantly transmitted syndrome caused by the transposition of a constitutively active cryptic promoter in front of the aromatase gene[54,55] or a mutation in the promoter.[56–58] The clinical picture is characterized by prepubertal or peripubertal gynecomastia, hypogonadotropic hypogonadism and compromised adult height in men, and precocious thelarche, macromastia, enlarged uterus and menstrual irregularities in women.[56–58] Treatment with anastrozole was effective and increased the initially reduced testicular volume to normal size, promoted virilization and normalized serum estrone and testosterone levels in three cases.[57,58]

Peutz–Jeghers syndrome

Peutz–Jeghers syndrome is a rare, autosomal dominant disorder characterized by multiple gastrointestinal hamartomatous polyps, mucocutaneous pigmentation, increased predisposition to neoplasms, gynecomastia and advanced bone age due to estrogen-producing large-cell-calcifying Sertoli cell tumors. In an affected 7-year-old boy, treatment with testolactone reduced breast base diameter and bone age advance.[59] In three other patients, treatment with anastrozole decreased estradiol levels, gynecomastia, growth and skeletal maturation, as well as Sertoli cell markers (inhibin A, inhibin B and anti-Müllerian hormone).[60,61]

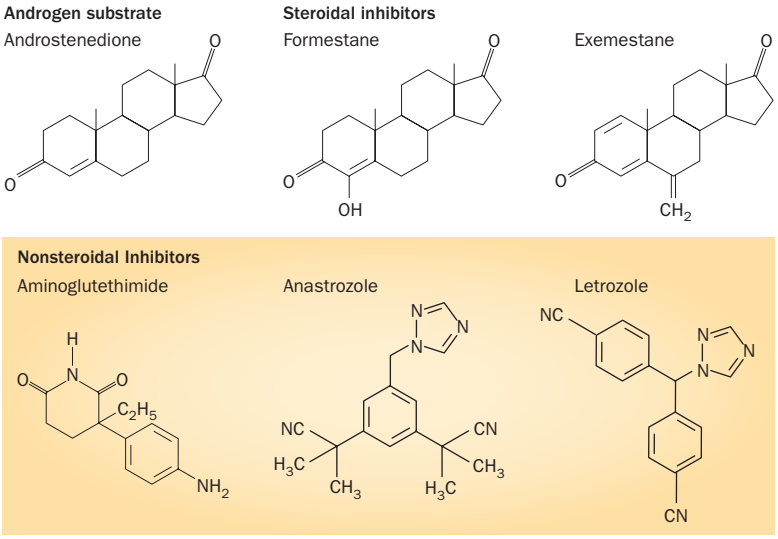

Figure 1 | Chemical structure of currently available aromatase inhibitors.

McCune–Albright syndrome

McCune–Albright syndrome is a rare disorder classically defined by the triad of peripheral precocious puberty, polyostotic fibrous dysplasia and café-au-lait pigmentation. These symptoms are caused by a postzygotic activating missense mutation (Cys or His to Arg^{201}) in exon 8 of the *GNAS1* gene, which encodes the α-subunit of the stimulatory G protein that regulates the coupling of hormones and receptors to adenylyl cyclase.[62] This mutation results in a constitutive ligand-free activation of cellular function in a mosaic distribution, leading to a high variability of organ involvement and degree of severity.[63] Affected ovarian tissues form large estrogen-secreting cysts.[64]

Precocious puberty is the most common endocrinologic manifestation of McCune–Albright syndrome and is more frequently diagnosed in girls than in boys. In girls, precocious puberty is caused by the estrogen-producing ovarian cysts, independent of gonadotropin action.[64] Girls with McCune–Albright syndrome frequently present with sudden onset of painless vaginal bleeding due to withdrawal of estrogen's effect from the resolving ovarian cyst, as early as the first year of life.[65] Breast development can be slow or develop rapidly. Although prolonged intervals of quiescence occur between vaginal bleeding episodes, some girls with McCune–Albright syndrome experience progression of precocious puberty with frequent menstrual bleeding, associated development of other secondary sexual characteristics, acceleration of linear growth and advancement of skeletal maturation leading to early closure of the epiphyseal growth plates and compromised adult height.[65]

Treatment with testolactone initially appeared beneficial,[66] resulting in a decrease in estradiol level, mean ovarian volume and cessation of menses, as well as a decreased rate of bone maturation, in three girls who were menstruating regularly. However, the response to

treatment beyond 6 months was mixed, indicating progressive decline in efficacy or escape from therapeutic effects after 2–4 years and/or poor compliance.[67]

Also, the effect of fadrozole on mean serum levels of estrone and estradiol, ovarian volume, frequency of menses or rates of bone age advance was disappointing in a study of 16 girls.[68] Fadrozole caused a dose-dependent inhibition of cortisol and aldosterone biosynthesis, which returned to normal following its discontinuation, except in one patient.[68]

Letrozole was used in an open-label therapeutic trial comprising nine girls with precocious puberty as part of the McCune–Albright syndrome.[69] Response to treatment after the initial 6 months and after long-term treatment up to 36 months showed partial efficacy; mean serum estradiol and ovarian volume fell markedly in the first 6 months, but increased toward or above the pretreatment level by 24 months due to recurrence or increase in size of the ovarian cysts in some girls. Skeletal maturation and growth velocity were significantly decreased at 36 months. Cessation of menses was incomplete in three of the nine girls, but the frequency of bleeding decreased. The other six girls had complete cessation of menses in the 12–36 months of letrozole therapy. Adverse effects included an increased ovarian volume and cyst enlargement, and one girl experienced ovarian torsion.[69] By contrast, anastrozole treatment over 1 year was ineffective in achieving the therapeutic targets.[70]

The limited efficacy of aromatase inhibitors in patients with McCune–Albright syndrome is probably owing to the inherent heterogeneity in the tissues involved in this syndrome and due to the underlying genetic abnormality, that is, the resulting constitutive activating effect of the genetic mutation might be too aggressive to be counteracted by these medications.

Functional follicular ovarian cysts

Increased estrogen secretion from ovarian cysts can cause peripheral precocious puberty. These cysts are usually self-limiting and resolve spontaneously; however, in some cases these cysts can persist, and, rarely, surgical intervention is required. Anastrozole has been reported to be successful in treating a girl who presented with unilateral enlargement of the ovary and a recurrent autonomous ovarian cyst, without adverse effects.[71]

Hyperandrogenism

Testotoxicosis

Testotoxicosis, also known as familial male-limited precocious puberty, is caused by an activating mutation in the luteinizing hormone (LH) receptor, which is associated with increased testosterone production from early childhood onwards, independent of gonadotropin regulation. This increase leads to early pubertal development, advanced skeletal maturation and early closure of the epiphyseal growth plates, thus resulting in short adult height.[72]

Testolactone combined with spironolactone decreased growth velocity and skeletal maturation over 6 months,[73] but no change occurred in the predicted adult height (PAH).[74] However, in a follow-up report in 10 boys treated with testolactone for at least 6 years, who received the GnRH analogue deslorelin following onset of central precocious puberty, growth rate normalized within 1 year and remained normal during the next 5 years of treatment. The rate of bone maturation normalized during the second year of treatment and remained normal thereafter. PAH increased by 13 cm after 6 years of therapy.[75]

Later studies using the potent antiandrogen bicalutamide and anastrozole or letrozole in four boys had a clear effect on growth velocity and skeletal maturation and preserved or increased PAH,[18,76,77] as did the combination of anastrozole and cyproterone acetate.[78] The results of a phase II study on the combination of anastrozole and bicalutamide ($n = 14$) showed that, after 1 year, the mean height velocity decreased by 1.6 cm per year, the mean bone age or chronological age ratio decreased from 2.1 to 1.0, and aggressive behavior was reduced by 50%.[79] Gynecomastia, breast tenderness and central precocious puberty were the most common treatment-related adverse events.[79]

Congenital adrenal hyperplasia

Long-term studies on growth in children with the most common form of congenital adrenal hyperplasia, steroid 21-hydroxylase deficiency, who are treated with corticosteroids and mineralocorticoids, have shown that average adult height is decreased, presumably caused by a combination of androgen excess (undertreatment) and corticosteroid excess (overtreatment).[80–82] In an effort to improve these results, a combination of testolactone, the antiandrogen flutamide, fludrocortisone and a low dose of hydrocortisone was compared with standard treatment with hydrocortisone and fludrocortisone. A trend toward increased PAH was observed but did not reach statistical significance.[83] In 2004, patients in the experimental group were switched to letrozole,[14] but no results have been reported to date.

Other causes of hyperandrogenism

Theoretically, aromatase inhibitors could be useful in conditions treated by the use of high doses of androgens, as these drugs prevent the consequences of androgen to estrogen conversion. One possible example is the treatment of men with hypogonadism caused by the partial androgen insensitivity syndrome. In theory, the combination of high-dose testosterone in combination with an aromatase inhibitor might give better results than treatment with testosterone alone,[84] but no reports on this approach exist thus far.

Pubertal gynecomastia

Gynecomastia is a frequent phenomenon in boys during puberty and is thought to result from an imbalance of the stimulatory effects of estrogen relative to the inhibitory effects of androgen in breast tissue.[14,85,86] In most boys, this imbalance resolves spontaneously, although it persists for more than 2 years in 25% of boys.[14,87]

In an open-label study of 42 boys with gynecomastia (mean age 13.0 years), treatment with anastrozole for

6 months reduced breast area and volume by 63% and 57%, respectively.[33] However, in the only randomized, placebo-controlled study,[88] anastrozole treatment of 80 boys with gynecomastia (mean age 14.6 years) for at least 6 months had no effect on the percentage of boys achieving >50% reduction in breast volume (38.5% versus 31.4% for anastrozole and placebo, respectively), although a clear effect was seen on the serum testosterone:estradiol ratio. The difference in apparent efficacy between the uncontrolled and controlled study could be due to the difference in mean age—in boys with early gynecomastia, the breast development would have largely resolved without treatment. A retrospective uncontrolled study has suggested that an estrogen receptor modifier (tamoxifen or raloxifene) might be more efficacious than aromatase inhibitors.[89]

Short stature and/or delayed puberty

Based on the observations of unfused epiphyseal growth plates in adult men with tall stature due to a disruptive mutation in the aromatase[3–7] or estrogen receptor gene,[2] it was postulated that blocking endogenous estrogen synthesis (or bioactivity) might result in retardation of bone maturation, thereby prolonging the time for growth and increasing adult height.[12–18,90,91] A GnRH analogue alone has minimal efficacy for this indication[92] but can increase adult height by 5 cm in combination with GH,[93,94] albeit at the expense of a decreased BMD.[94,95] A retrospective study on seven pubertal boys with the estrogen receptor antagonist tamoxifen suggested that this agent might delay skeletal maturation and increase PAH,[96] but no controlled studies have been performed.

Randomized controlled trials

Three trials have been performed in boys with various combinations of short stature and delayed puberty (Table 2), and one study was done in GH-deficient boys.[8–11] In a Finnish study, 23 boys with delayed puberty (mean age 15.1 years) were randomly allocated to two treatment groups: 1 year of letrozole (2.5 mg once daily) or placebo. Both groups also received testosterone injections for 6 months and were evaluated 18 months after initiation of therapy.[8] A third, nonrandomized group received no treatment. PAH increased significantly more in letrozole-treated boys than in placebo-treated boys (5.1 cm versus 0.3 cm), owing to a significant reduction in bone maturation with letrozole treatment (Table 2). The nonrandomized, untreated controls gained approximately 2 cm. Serum estradiol levels were significantly suppressed, and serum testosterone, LH, follicle-stimulating hormone (FSH) and inhibin B levels were increased. In a follow-up study,[97] near-adult height of the letrozole-treated group was 6.9 cm more than the placebo group and only 1.3 cm lower than target height, a mean increment of 0.6 SDS. In placebo-treated boys, near-adult height was 4.8 cm below target height, consistent with previous reports on spontaneous growth (or growth after low-dose testosterone therapy).[98–103]

Some additional observations can be made from this study. First, almost all participants had entered into puberty at the start of the study, were not extremely short and had a PAH in the normal range, so most clinicians would offer reassurance or a short course of androgens, with a generally good outcome. Furthermore, the results might have been affected by selection bias at the start of treatment (boys receiving letrozole tended to be taller, have a higher PAH and taller parents than placebo-treated controls) and in the final analysis (the difference between the groups with respect to PAH at the start of treatment was larger in the 17 patients followed until near-adult height than in the 22 patients initially included). Moreover, the results obtained with a combination treatment (letrozole plus testosterone) cannot automatically be extrapolated to letrozole alone, and no adult height data were reported for the untreated boys. Finally, the real adult height might be considerably higher than the near-adult height, as the range of bone ages at

Table 2 | Letrozole vs placebo in boys with short stature and/or delayed puberty

Characteristics	Wickman *et al.*[8,97]*	Hero *et al.*[9,104]	Salehpour *et al.*[11]
Population (at baseline)			
Diagnosis	Delayed puberty	ISS	CDGP
Treatment	Letrozole vs placebo (+ 6 months testosterone)	Letrozole vs placebo	Letrozole vs placebo
Duration (months)	12	24	24
n	10 vs 10	16 vs 14	31 vs 30
Age	15.1	11.0	13.5
Bone age	13.1 vs 12.6	9.1 vs 8.9	12.1 vs 11.7
Height (cm)	155.3 vs 151.9	128.5 vs 127.5	NA
Height SDS	–1.8 vs –2.0	–2.3 vs –2.4	–2.9 vs –2.9
Pubertal stage (G)	2 (2–3)	1 (1–3)	1
Target height (cm)	176.5 vs 175.3	175.5 vs 177.2	174.6 vs 176.5
Target height SDS	–0.4 vs –0.5	–0.5 vs –0.3	NA
PAH (cm)	176.5 vs 174.9	167.0 vs 165.8	167.6 vs 171.9
PAH SDS	–0.3 vs –0.8	–1.8 vs –2.0	NA
Evaluation			
Time after start (months)	18	24	24
Growth velocity (cm per yr)	7.6 vs 7.9	5.3 vs 5.2	NA
Height SDS change	NA	NA	+0.5 vs 0.0
Δ bone age	0.9 vs 1.7 per 1.5 years	1.2 vs 2.1 per 2 years	1.1 vs 0.5 per 2 years
PAH (cm)	182.1 vs 175.2	172.9 vs 166.9	173.7 vs 173.3
Δ PAH (cm)	5.1 vs 0.3	5.9 vs 1.1	6.1 vs 1.4
Follow-up			
Age (years)	19.9 vs 18.2	16.9 vs 17.3	NA
Bone age (years)	16.9 vs 16.7	15.8 vs 16.6	NA
Height (cm)	175.8 vs 169.1	159.1 vs 161.1	NA
PAH (cm)	NA	166.5 vs 162.4	NA

*Besides the two randomized groups, 10 patients did not wish to undergo medical intervention (untreated controls). Seven of them could be analyzed for the primary endpoint. PAH increased by 2 cm after 18 months. Abbreviations: CDGP, constitutional delay of growth and puberty; G, Tanner stage (genital); ISS, idiopathic short stature; NA, not available; PAH, predicted adult height; vs, versus.

follow-up was quite wide (15.8–18.0 years). The results of this study might, therefore, have been overstated.

In a second Finnish study, 30 boys with idiopathic short stature (ISS) aged 9.0–14.5 years were randomly allocated to receive either letrozole or placebo for 2 years (Table 2).[9] Most of them (81% and 93%, respectively) were prepubertal at the start, and 44% remained prepubertal after 2 years. Their height at the start was either <−2 SDS or >2 SD below target height, and bone age was <14 years. Letrozole-treated boys showed similar height velocities to those receiving placebo, but bone age advanced less with letrozole therapy. Given that letrozole delayed bone maturation similarly in prepubertal and pubertal boys, regardless of bone age, the investigators concluded that estrogen already plays a part in the maturation process of the growth plate before puberty. Height SDS for bone age improved by 0.7 SD, whereas no change occurred in the placebo group, and the PAH increased by 5.9 cm.[9] However, at follow-up 6 years after the start of the study, the difference in PAH between the letrozole-treated and placebo-treated group was not statistically significant (166.5 cm versus 162.4 cm).[104] A critical comment on this study is that most participants were prepubertal, a patient group to whom most clinicians would be reluctant to give aromatase inhibitors owing to the frequency of vertebral deformities in this population (see below).[104] Furthermore, no near-adult height data are available yet.

In the third study, from Iran, 91 boys with constitutional delay of growth and puberty were randomly allocated to three treatment groups: letrozole, placebo or oxandrolone for 2 years (Table 2).[11] Letrozole increased PAH more than placebo; however, remarkably, letrozole appeared to increase height velocity (difference in height SDS +0.5 versus 0), and bone age advance (1.1 year per 2 years versus 0.5 year per 2 years) was surprisingly slow in both groups. Clarification of these issues and follow-up data until adult height is reached are awaited.

The efficacy of anastrozole co-treatment with GH in GH-deficient males has been investigated in two studies from the USA.[4,105] An open-label pilot study on 20 patients treated for 1 year did not show an effect on PAH.[105] In a later study, 52 male adolescents with GH deficiency were randomly allocated to co-treatment with anastrozole (1 mg) or placebo for 1–3 years.[10] Inclusion criteria were GH deficiency and GH treatment (0.3 mg/kg per week) for at least 6 months before the start of the study. Moreover, the study participants had to be pubertal and have a residual height potential. PAH increased in the anastrozole-treated group (+1.3 cm, +4.5 cm and +6.7 cm after 1, 2 and 3 years, respectively), whereas only 1 cm of PAH gain was observed in the placebo group.[10] The decrease in growth velocity during the course of the study was greater in the placebo group than in the anastrozole group at 36 months.[10,91] No adult height data are available to date.

Retrospective studies

In a retrospective study in 24 male adolescents with various endocrine conditions who exhibited short stature and low PAH (mean age 14.0 years), letrozole treatment for a mean duration of 1 year led to an increase of PAH of 5.5 cm, independent of co-treatment with androgens or GH.[12,32] Two other retrospective studies in boys with delayed puberty also reported an increased PAH.[106,107]

Although bone age progression appeared to decrease in one retrospective study on the effect of aromatase inhibitors (in combination with a GnRH analogue) in girls with a relatively low PAH and rapidly progressing bone age,[12] we believe that aromatase inhibition for growth enhancement in girls with ISS is contraindicated, in view of potential adverse effects such as virilization due to hyperandrogenism and the development of ovarian cysts.

Safety concerns

Estrogen receptors and aromatase activity are ubiquitously present throughout the body, illustrating that estrogen signaling is crucial. Potential safety concerns of inhibition of estrogen biosynthesis can be deduced from findings in patients with aromatase deficiency,[4,5,7,108–112] patients with estrogen deficiency or hypertestosteronemia, and from animal studies.[18,44,45,48,113] However, lifelong estrogen deficiency, such as in patients with aromatase deficiency, as well as postnatal exposure to aromatase inhibitors in animal models—equivalent to treating from infancy through puberty—do not translate to the same level of exposure achieved in humans thus far. In this section, we shall only discuss safety concerns in men treated with aromatase inhibitors.

Hypothalamic–pituitary–gonadal axis

In early and midpubertal boys, estrogen is the primary sex steroid responsible for the central negative feedback effect on gonadotropin secretion.[9,114] After the onset of central puberty, blockade of estrogen biosynthesis by letrozole increases the circulating levels of LH and FSH, nocturnal LH pulse amplitude, and GnRH-induced LH response, but does not influence nocturnal LH pulse frequency.[9,114]

As a result of increased secretion of LH, gonadal testosterone production is stimulated. Mean serum testosterone concentration at Tanner stage IV (genital stage) reached supraphysiological levels of 30.9 nmol/l and slightly over 50.0 nmol/l during letrozole treatment in boys with ISS and delayed puberty, respectively,[8,9] a value approximately three times higher than that in controls. This increase may enhance the progression of physical signs of puberty.[9] Treatment with anastrozole results in less stimulated androgen secretion,[10] presumably owing to the lower potency of this agent compared with letrozole.[115]

Whether aromatase inhibitor therapy influences the timing and progression of puberty is unclear. In a study in young prepubertal boys, aromatase inhibitor treatment did not influence circulating sex steroid concentrations or basal gonadotropin levels, nor the onset of puberty.[9] In boys with constitutional delay of growth and puberty, a greater proportion of letrozole-treated individuals entered puberty compared with placebo-treated boys.[11]

No reports on severe androgenic adverse effects, such as facial acne, have been published.[11] Theoretically, aromatase inhibitors might have an effect on prostatic

hypertrophy and cancer,[15] but this finding has not been reported. Hypertestosteronemia might cause erythrocytosis,[15,116] which has indeed been observed in pubertal boys treated with aromatase inhibitors[18] and in men with aromatase deficiency.[111] In boys treated with letrozole and testosterone, increases in hemoglobin levels correlated with testosterone levels.[117] Erythrocytosis can increase the risk of thrombotic events.[18] To our knowledge, the influence of hypertestosteronemia on behavior has not been systematically studied. Another theoretical risk of hypertestosteronemia is a decline in adiponectin. However, serum adiponectin levels declined similarly in letrozole-treated boys with ISS and untreated controls, although a trend towards lower values was observed in letrozole-treated boys.[118] Treatment of adolescent boys with letrozole,[8,9,11] but not with anastrozole,[10] enhanced testicular growth, most probably due to elevated gonadotropin levels. Circulating inhibin B increased in letrozole-treated pubertal boys in a similar manner[9] or even more[8] than in placebo-treated boys.

Findings in knockout animal models have suggested that some level of estrogen receptor α activation and aromatase activity is required for normal spermatogenesis,[13,119–121] and letrozole treatment of monkeys resulted in reduced sperm count and quality.[122] In humans, estrogens produced locally in sperm can influence its fertilizing capacity.[123] However, no differences in sperm parameters were found between GH-deficient boys who received anastrozole several years earlier and controls.[124] In fact, aromatase inhibitors have been employed to increase FSH secretion in the treatment of male infertility.[53,125]

Bone health

Sex hormones have an important role in bone development and remodeling, particularly in puberty, with a distinct sexual dimorphism.[126] As indicated by the bone phenotype of men with aromatase deficiency or estrogen resistance, long-term deprivation of estrogen action results in low bone mass.[2,4,5,7] Furthermore, aromatase inhibitors in women have been shown to increase bone turnover and fracture risk.[127] This finding has raised concerns that aromatase inhibitor therapy during childhood or adolescence could adversely influence the accrual of peak bone mass and increase the risk of osteoporosis.

BMD

BMD has been monitored in four controlled trials using aromatase inhibitors in adolescent boys.[8–11] During treatment with letrozole for 1–2 years in boys with delayed puberty[8,11] or ISS,[9,128] areal BMD of the lumbar spine and femoral neck increased in a similar fashion to placebo, as evaluated by dual-energy X-ray absorptiometry (DXA). Similarly, in a study of GH-deficient boys, 3 years of anastrozole treatment in combination with GH had no adverse influence on areal BMD.[10] Letrozole treatment also had no adverse effect on bone mineral apparent density, a measure of volumetric BMD.[8,9,11] Furthermore, short-term treatment of adolescent males with anastrozole did not influence kinetically measured rates of bone calcium turnover.[34]

These observations suggest that aromatase inhibitor treatment for 1–3 years in male adolescents has no adverse influence on bone mass accrual. This neutral effect on BMD is potentially explained by bone-protective effects of stimulated androgen secretion, which stimulates periosteal bone formation and bone expansion.[128–130] However, DXA is a 2D measurement technique and gives no data on bone quality in different bone compartments or on bone geometrical properties.

Considering that peripubertal aromatase inhibitor treatment of male rats impairs bone geometry and trabecular bone quality,[44,45] more data are needed on the effect of aromatase inhibitors on trabecular bone density and structure in adolescent boys. In addition, the relationship between development of muscle mass and bone mass during aromatase inhibitor therapy has not yet been characterized.

Bone turnover

Longitudinal follow-up data of serum and urine markers of bone turnover have been reported in two studies on letrozole treatment in adolescent boys.[128,131] In the placebo groups, the levels of bone turnover markers increased during follow-up and, as expected, correlated positively with growth velocity. In letrozole-receiving boys, the levels of some markers of bone resorption and formation remained at the pretreatment level, suggesting lower bone turnover rate. In contrast with the placebo group, the changes in markers of bone turnover correlated poorly with changes in circulating estradiol, testosterone or IGF-1, or with growth velocity.[128] As boys with delayed puberty or ISS had elevated levels of circulating testosterone after the onset of puberty, the lower rate of bone turnover might reflect androgen-induced inhibition of bone resorption.[132,133] The effect of this finding on bone strength is unclear.

Cortical bone

Letrozole treatment in boys with ISS resulted in enhanced cortical bone growth, as evaluated by the metacarpal index.[128] The change in metacarpal index correlated positively with mean testosterone:estradiol ratio during treatment, supporting the view that androgens increase and estrogens decrease periosteal bone formation.[129,130] Cortical bone size is an important predictor of bone strength,[134] and the metacarpal index associates negatively with the risk of wrist and forearm fractures in children, even after adjusting for DXA-measured BMD.[135]

Findings in a 17-year-old boy with aromatase deficiency and sufficient testosterone level suggest a more complex regulation of cortical bone growth by sex steroids. In this boy, estrogen treatment increased bone cross-sectional area and cortical thickness, but not volumetric BMD.[136] Indeed, a certain level of signaling through the estrogen receptor α in bone tissue might be required for the androgen-induced cortical bone anabolic response to occur.[137]

Vertebral deformities

Estrogen deficiency and aromatase inhibitor treatment have been associated with impaired trabecular bone

development in male mice,[38,43,44] and low bone mass with severe kyphoscoliosis was evident in a 27-year-old man with aromatase deficiency.[7] These findings raise the concern that aromatase inhibitor treatment during adolescence might impair the strength of vertebral bodies rich in trabecular bone. Post-treatment vertebral body morphology has been evaluated by MRI in two cohorts of men who had received letrozole.[104] In men with ISS, letrozole treatment for 2 years during prepuberty or early puberty was associated with a significantly increased risk of vertebral body deformities compared with placebo, with five of 11 letrozole-treated men showing mild anterior wedging or compression deformities. These mild deformities probably reflect disturbed vertebral body growth rather than true compression fractures. By contrast, in men with delayed puberty treated for 1 year during early or midpuberty, letrozole had no effect on vertebral morphology.[104]

Body proportions and composition

From observations in aromatase-deficient men, one would expect therapy with aromatase inhibitors to decrease trunk length and increase leg length. In a follow-up study on boys with delayed puberty and ISS, this phenomenon was indeed observed, although the differences between groups were not statistically significant.[104]

Therapy with aromatase inhibitors has no effect on BMI.[10,118] In a study of boys with GH deficiency, no difference in fat free mass or percentage fat mass (as evaluated by skin fold measurements) between anastrozole and placebo groups was found during 3 years of treatment.[10] By contrast, letrozole decreased the percentage of fat mass after the start of puberty in boys with ISS.[118] The difference between letrozole and anastrozole in fat mass response might be explained by GH co-treatment in the anastrozole study and/or by the higher androgen levels induced by letrozole than by anastrozole, because the change in testosterone correlated negatively with the change of percentage fat mass in letrozole-treated boys.[118]

Metabolic parameters

Letrozole treatment effectively blocked the pubertal increase in both circulating estradiol and IGF-1 in two studies,[8,9] but this finding was not confirmed in the Iranian study.[11] This relatively low IGF-1 level probably reflects a lack of pubertal stimulation of GH secretion, which is known to be estrogen-mediated.[138] This suppression of the GH–IGF-1 axis was also observed in healthy male adolescents treated with anastrozole,[34] but not in boys with GH deficiency,[10] presumably owing to the co-administration of GH. LDL cholesterol, apolipoprotein B and triglyceride levels were not affected by aromatase inhibitor treatment in male adolescents.[10,11,118,139] By contrast, during progression of puberty, letrozole reduced HDL cholesterol levels (particularly the HDL2 cholesterol subclass) by approximately 0.5 mmol/l,[118,139] due to increased HDL cholesterol catabolism.[140,141] A similar decrease was noted in apolipoprotein A-I levels. The change in HDL cholesterol concentration was negatively associated with the change in testosterone levels and positively associated with the change in adiponectin levels.[118] After discontinuation of treatment, HDL cholesterol levels were similar in the letrozole-treated and control groups. This reduction in HDL cholesterol levels has not been observed in pubertal boys treated with anastrozole, which is potentially explained by GH co-treatment and/or lower testosterone levels in these individuals.[8–10,34] Serum lipoprotein(a) decreased in pubertal boys treated with letrozole in an inverse relationship to serum testosterone.[118,139]

Aromatase inhibition during male puberty results in a hormonal milieu characterized by normal to high testosterone concentration, low estradiol concentration and low IGF-1 concentration. The net effect of these changes on insulin sensitivity appears to be neutral[10,34,118] or beneficial,[139] as evaluated by levels of fasting serum insulin, glucose or homeostasis model assessment (HOMA) index. Fasting insulin concentrations of adolescent boys decreased during treatment with letrozole and increased in boys receiving placebo; the changes in fasting serum insulin and IGF-1 levels correlated positively, suggesting that aromatase inhibitor treatment attenuates the development of insulin resistance of puberty by preventing the activation of the GH–IGF-1 axis.[139,142] Adiponectin levels declined similarly in letrozole-treated and placebo-treated pubertal boys.[118]

Cognitive function

Several lines of evidence suggest that estrogen has a role in the regulation of cognitive functions.[143] Temporal lobe neurons are capable of synthesizing estrogen locally through aromatization of androgenic precursors, and estrogen receptor β is expressed abundantly in the hippocampus of the human brain,[144] an area known to be important for memory function. Estrogen might have a role in the regulation of verbal memory performance, also in adult men, whereas direct androgen effects are possibly more important for spatial memory.[145]

Given that aromatase inhibitors cross the blood–brain barrier,[146] aromatase inhibitor treatment might theoretically result in disturbance of cognitive function. However, this observation was not documented in studies including adult women and men.[147,148] Also, in adolescents, no effect of letrozole on cognitive performance was found, as evaluated by a selection of cognitive tests focusing on verbal and visuospatial performance and memory.[149] However, the number of patients was small and only a few boys reached late stages of puberty in that study, making further investigation of this issue necessary.

Safety parameters during follow-up

If a clinician decides to prescribe aromatase inhibitors off-label to pediatric patients, we propose to follow up several efficacy and safety parameters (Box 1). The primary concern is still the treatment's influence on bone health, so we recommend performing BMD measurements and evaluation of vertebral morphology every year. Suggestions to assess vitamin D status have been made, and at least standard doses of calcium and vitamin D supplementation are recommended in patients treated with aromatase inhibitors.[18] At present, we do not recommend

using aromatase inhibitors in children or adolescents with a known primary or secondary disease that affects bone health. Hemoglobin and hematocrit are to be checked, in view of the risk of erythrocytosis.

In girls with McCune–Albright syndrome, whether or not aromatase inhibitors are used, it is necessary to follow up basal and stimulated gonadotropin levels to recognize the onset of central puberty. When pubertal onset occurs, a GnRH analogue should be added, to avoid excessive stimulation of the ovaries.[69] Furthermore, regular sonography of the uterus and ovaries is mandatory.

Design of future studies

The use of aromatase inhibitors in childhood and adolescence is off-label and, therefore, by definition experimental. For clarification of its potential role and benefit–risk ratio in increasing adult stature, studies are needed to directly compare letrozole, anastrozole, GH, GH plus an aromatase inhibitor, and placebo in boys with short stature in an early stage of puberty. Outcome parameters should include predicted and achieved adult height and extensive safety parameters, including thorough assessment of vertebral abnormalities. Although, admittedly, no study comparing various ages at treatment start and durations of treatment exists, we believe that, in future studies, aromatase inhibitors could be administered to boys in an early stage of puberty (Tanner stage 2–3) for a duration of 2–3 years.

Conclusions

For the practicing clinician the answers to two questions are important: first, which are the medical conditions in which off-label use of aromatase inhibitors can be considered in pediatrics, given current data on efficacy and safety; and second, which aromatase inhibitor should be used preferentially? The answers to the first question are summarized in Table 3. We believe that sufficient arguments are in favor of the use of aromatase inhibitors in the treatment of aromatase excess syndrome and for an aromatase inhibitor plus antiandrogen to treat patients with testotoxicosis, in spite of the low number of cases reported. Aromatase inhibitors can be considered in the management of patients with Peutz–Jeghers syndrome or McCune–Albright syndrome, but the results are suboptimal and variable. Data to support the use of aromatase inhibitors in congenital adrenal hyperplasia and girls with functional follicular ovarian cysts are insufficient. Aromatase inhibitors appear ineffective in pubertal gynecomastia.

For the treatment of boys with short stature and/or delayed puberty, considerable evidence indicates that aromatase inhibitors effectively delay bone maturation and thereby increase PAH.[8–11,97] When compared with GnRH analogues, advantages of aromatase inhibitors include that pubertal development and growth spurt are less affected, which is expected to have positive effects on psychosocial wellbeing. Furthermore, bone mass accrual as well as body composition appear to be less affected by aromatase inhibitors than by GnRH analogues. However, near-adult height was only reported in one study, and studies differed widely in trial design, diagnosis, age, pubertal stage, height, preparations, duration and co-treatment with other compounds (GH or testosterone). Certainly, safety concerns remain, particularly an increased risk of vertebral deformities in

Box 1 | Follow-up of aromatase inhibitor therapy

Medical history and physical examination

- Signs of androgen excess (for example, acne)
- Height, weight, sitting height
- Pubertal staging, testicular growth
- Musculoskeletal symptoms (joint and back pain)

Laboratory investigations

- LH, FSH, testosterone, estradiol, inhibin B
- Alanine transaminase, aspartate transaminase
- Total, HDL and LDL cholesterol
- Hemoglobin, hematocrit
- Blood leukocytes
- Vitamin D

Radiological assessment

- Bone age
- BMD and bone mineral apparent density of the lumbar spine and femur
- Peripheral quantitative CT (pqCT)
- Vertebral morphology by radiography (lateral projection)

Suggested co-treatment

- Calcium and vitamin D supplementation

Table 3 | Off-label use of aromatase inhibitors

Conditions according to efficacy of off-label aromatase inhibitors use	*n**	Quality of evidence‡
Probably effective		
Aromatase excess syndrome	3	IIIB
Testotoxicosis (if combined with bicalutamide)	20	IIB
Probably effective, but safety concerns		
Short stature and/or delayed puberty	83	IB
Probably partially effective		
Peutz–Jeghers syndrome	3	IIIB
McCune–Albright syndrome	37	IIB
Probably ineffective		
Gynecomastia	82	ID
Insufficient data		
Congenital adrenal hyperplasia	16	IIIC
Functional follicular ovarian cysts	1	IIIC

*Reported number of patients treated with third-generation aromatase inhibitors. ‡The qualities of evidence are: I (data from at least one properly randomized controlled trial), II (data from other clinical studies) and III (data from opinions of respected authorities based on clinical experience, descriptive studies or reports of expert committees). The strengths of recommendation are: A (good evidence to support use), B (moderate evidence to support use), C (poor evidence to support recommendation), D (moderate evidence against use) and E (strong evidence against use).[14]

boys treated at a young age. We, therefore, believe that aromatase inhibitors must be considered experimental in all growth disorders, and their use should ideally be limited to well-controlled studies. If a clinician still considers aromatase inhibitor treatment in an individual case, thorough monitoring of possible adverse effects, particularly vertebral deformities, is mandatory.

With respect to the comparison between letrozole and anastrozole, the difference in potency would suggest that in boys with testotoxicosis and in girls with McCune–Albright syndrome, letrozole might be more effective than anastrozole. In boys with short stature, a less severe blockade of aromatase may have advantages, for example, less elevated serum testosterone levels and possibly a milder effect on vertebral bone health.

Review criteria

The following databases were searched: PubMed (1946 to March 2011), EMBASE (OVID-version, 1980 to March 2011), Web of Science (1945 to March 2011) and COCHRANE Library (1898 to March 2011). For the three concepts, all relevant keyword variations were used, not only in the controlled vocabularies of the various databases, but the free text word variations of these concepts as well. This search strategy was optimized for all consulted databases, taking into account the differences of the various controlled vocabularies as well as the differences of database-specific technical variations (for example, the use of quotation marks). In total, 660 references were identified. For more details see supplementary information online.

1. Santen, R. J., Brodie, H., Simpson, E. R., Siiteri, P. K. & Brodie, A. History of aromatase: Saga of an important biological mediator and therapeutic target. *Endocr. Rev.* **30**, 343–375 (2009).
2. Smith, E. P. *et al.* Estrogen resistance caused by a mutation in the estrogen-receptor gene in a man. *N. Engl. J. Med.* **331**, 1056–1061 (1994).
3. Conte, F. A., Grumbach, M. M., Ito, Y., Fisher, C. R. & Simpson, E. R. A syndrome of female pseudohermaphrodism, hypergonadotropic hypogonadism, and multicystic ovaries associated with missense mutations in the gene encoding aromatase (P450arom). *J. Clin. Endocrinol. Metab.* **78**, 1287–1292 (1994).
4. Morishima, A., Grumbach, M. M., Simpson, E. R., Fisher, C. & Qin, K. Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J. Clin. Endocrinol. Metab.* **80**, 3689–3698 (1995).
5. Carani, C. *et al.* Effect of testosterone and estradiol in a man with aromatase deficiency. *N. Engl. J. Med.* **337**, 91–95 (1997).
6. Bilezikian, J. P., Morishima, A., Bell, J. & Grumbach, M. M. Increased bone mass as a result of estrogen therapy in a man with aromatase deficiency. *N. Engl. J. Med.* **339**, 599–603 (1998).
7. Herrmann, B. L. *et al.* Impact of estrogen replacement therapy in a male with congenital aromatase deficiency caused by a novel mutation in the *CYP19* gene. *J. Clin. Endocrinol. Metab.* **87**, 5476–5484 (2002).
8. Wickman, S., Sipilä, I., Ankarberg-Lindgren, C., Norjavaara, E. & Dunkel, L. A specific aromatase inhibitor and potential increase in adult height in boys with delayed puberty: a randomised controlled trial. *Lancet* **357**, 1743–1748 (2001).
9. Hero, M., Norjavaara, E. & Dunkel, L. Inhibition of estrogen biosynthesis with a potent aromatase inhibitor increases predicted adult height in boys with idiopathic short stature: a randomized controlled trial. *J. Clin. Endocrinol. Metab.* **90**, 6396–6402 (2005).
10. Mauras, N. *et al.* Anastrozole increases predicted adult height of short adolescent males treated with growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years. *J. Clin. Endocrinol. Metab.* **93**, 823–831 (2008).
11. Salehpour, S. *et al.* A double-blind, placebo-controlled comparison of letrozole to oxandrolone effects upon growth and puberty of children with constitutional delay of puberty and idiopathic short stature. *Horm. Res. Paediatr.* **74**, 428–435 (2010).
12. Cernich, J., Jacobson, J. D., Moore, W. V. & Popovic, J. Use of aromatase inhibitors in children with short stature. *Pediatr. Endocrinol. Rev.* **2**, 2–7 (2004).
13. Dunkel, L. Use of aromatase inhibitors to increase final height. *Mol. Cell Endocrinol.* **254–255**, 207–216 (2006).
14. Shulman, D. I., Francis, G. L., Palmert, M. R. & Eugster, E. A. Use of aromatase inhibitors in children and adolescents with disorders of growth and adolescent development. *Pediatrics* **121**, e975–e983 (2008).
15. Geffner, M. E. For debate: Aromatase inhibitors to augment height: have we lost our inhibitions? *Pediatr. Endocrinol. Rev.* **5**, 756–759 (2008).
16. Dunkel, L. Update on the role of aromatase inhibitors in growth disorders. *Horm. Res.* **71** (Suppl. 1), 57–63 (2009).
17. Geffner, M. E. Aromatase inhibitors to augment height: continued caution and study required. *J. Clin. Res. Pediatr. Endocrinol.* **1**, 256–261 (2009).
18. Diaz-Thomas, A. & Shulman, D. Use of aromatase inhibitors in children and adolescents: what's new? *Curr. Opin. Pediatr.* **22**, 501–507 (2010).
19. Kish, M. A. Guide to development of practice guidelines. *Clin. Infect. Dis.* **32**, 851–854 (2001).
20. AGREE Collaboration. Development and validation of an international appraisal instrument for assessing the quality of clinical practice guidelines: the AGREE project. *Qual. Saf. Health Care* **12**, 18–23 (2003).
21. Cavalli, A. *et al.* Enantioselective nonsteroidal aromatase inhibitors identified through a multidisciplinary medicinal chemistry approach. *J. Med. Chem.* **48**, 7282–7289 (2005).
22. Sebastian, S., Takayama, K., Shozu, M. & Bulun, S. E. Cloning and characterization of a novel endothelial promoter of the human *CYP19* (aromatase P450) gene that is upregulated in breast cancer tissue. *Mol. Endocrinol.* **16**, 2243–2254 (2002).
23. Bulun, S. E. *et al.* The human *CYP19* (aromatase P450) gene: update on physiologic roles and genomic organization of promoters. *J. Steroid Biochem. Mol. Biol.* **86**, 219–224 (2003).
24. Labrie, F., Bélanger, A., Cusan, L. & Candas, B. Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. *J. Clin. Endocrinol. Metab.* **82**, 2403–2409 (1997).
25. Corbin, C. J. *et al.* Isolation of a full-length cDNA insert encoding human aromatase system cytochrome P-450 and its expression in nonsteroidogenic cells. *Proc. Natl Acad. Sci. USA* **85**, 8948–8952 (1988).
26. Grumbach, M. M. & Auchus, R. J. Estrogen: consequences and implications of human mutations in synthesis and action. *J. Clin. Endocrinol. Metab.* **84**, 4677–4694 (1999).
27. Drop, S. L., De Waal, W. J. & De Muinck Keizer-Schrama, S. M. Sex steroid treatment of constitutionally tall stature. *Endocr. Rev.* **19**, 540–558 (1998).
28. Juul, A. *et al.* Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. *J. Clin. Endocrinol. Metab.* **78**, 744–752 (1994).
29. Mauras, N. *et al.* Pharmacokinetics and dose finding of a potent aromatase inhibitor, aromasin (exemestane), in young males. *J. Clin. Endocrinol. Metab.* **88**, 5951–5956 (2003).
30. Bisagni, G. *et al.* Letrozole, a new oral non-steroidal aromastase inhibitor in treating postmenopausal patients with advanced breast cancer. A pilot study. *Ann. Oncol.* **7**, 99–102 (1996).
31. Bajetta, E. *et al.* Double-blind, randomised, multicentre endocrine trial comparing two letrozole doses, in postmenopausal breast cancer patients. *Eur. J. Cancer* **35**, 208–213 (1999).
32. Karmazin, A., Moore, W. V., Popovic, J. & Jacobson, J. D. The effect of letrozole on bone age progression, predicted adult height, and adrenal gland function. *J. Pediatr. Endocrinol. Metab.* **18**, 285–293 (2005).
33. Mauras, N. *et al.* Pharmacokinetics and pharmacodynamics of anastrozole in pubertal boys with recent-onset gynecomastia. *J. Clin. Endocrinol. Metab.* **94**, 2975–2978 (2009).
34. Mauras, N., O'Brien, K. O., Klein, K. O. & Hayes, V. Estrogen suppression in males: metabolic effects. *J. Clin. Endocrinol. Metab.* **85**, 2370–2377 (2000).
35. Geisler, J., Haynes, B., Anker, G., Dowsett, M. & Lønning, P. E. Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *J. Clin. Oncol.* **20**, 751–757 (2002).
36. Chagin, A. S. & Sävendahl, L. Oestrogen receptors and linear bone growth. *Acta Pædiatr.* **96**, 1275–1279 (2007).
37. Fisher, C. R., Graves, K. H., Parlow, A. F. & Simpson, E. R. Characterization of mice deficient in aromatase (ArKO) because of targeted

disruption of the *Cyp19* gene. *Proc. Natl Acad. Sci. USA* **95**, 6965–6970 (1998).
38. Oz, O. K. *et al.* Bone has a sexually dimorphic response to aromatase deficiency. *J. Bone Miner. Res.* **15**, 507–514 (2000).
39. Oz, O. K. *et al.* Bone phenotype of the aromatase deficient mouse. *J. Steroid Biochem. Mol. Biol.* **79**, 49–59 (2001).
40. Britt, K. L. *et al.* The ovarian phenotype of the aromatase knockout (ArKO) mouse. *J. Steroid Biochem. Mol. Biol.* **79**, 181–185 (2001).
41. Jones, M. E., McInnes, K. J., Boon, W. C. & Simpson, E. R. Estrogen and adiposity—utilizing models of aromatase deficiency to explore the relationship. *J. Steroid Biochem. Mol. Biol.* **106**, 3–7 (2007).
42. Gevers, E. F., Wit, J. M. & Robinson, I. C. Effects of long-term gonadotrophin-releasing hormone analog treatment on growth, growth hormone (GH) secretion, GH receptors, and GH-binding protein in the rat. *Pediatr. Res.* **43**, 111–120 (1998).
43. Vanderschueren, D. *et al.* Aromatase inhibition impairs skeletal modeling and decreases bone mineral density in growing male rats. *Endocrinology* **138**, 2301–2307 (1997).
44. van Gool, S. A. *et al.* Impaired body weight and tail length gain and altered bone quality after treatment with the aromatase inhibitor exemestane in male rats. *Horm. Res. Paediatr.* **73**, 376–385 (2010).
45. Bajpai, A. *et al.* Peripubertal aromatase inhibition in male rats has adverse long-term effects on bone strength and growth and induces prostatic hyperplasia. *J. Endocrinol.* **207**, 27–34 (2010).
46. Eshet, R. *et al.* The aromatase inhibitor letrozole increases epiphyseal growth plate height and tibial length in peripubertal male mice. *J. Endocrinol.* **182**, 165–172 (2004).
47. Turner, K. J., Morley, M., Atanassova, N., Swanston, I. D. & Sharpe, R. M. Effect of chronic administration of an aromatase inhibitor to adult male rats on pituitary and testicular function and fertility. *J. Endocrinol.* **164**, 225–238 (2000).
48. van Gool, S. A. *et al.* Marginal growth increase, altered bone quality and polycystic ovaries in female prepubertal rats after treatment with the aromatase inhibitor exemestane. *Horm. Res. Paediatr.* **73**, 49–60 (2010).
49. Mouridsen, H. *et al.* Letrozole therapy alone or in sequence with tamoxifen in women with breast cancer. *N. Engl. J. Med.* **361**, 766–776 (2009).
50. Gibson, L., Lawrence, D., Dawson, C. & Bliss, J. Aromatase inhibitors for treatment of advanced breast cancer in postmenopausal women. Cochrane Database of Systematic Reviews, Issue 4. Art. No.: CD003370 doi:10.1002/14651858.CD003370.pub3 (2009).
51. Casper, R. F. & Mitwally, M. F. Review: aromatase inhibitors for ovulation induction. *J. Clin. Endocrinol. Metab.* **91**, 760–771 (2006).
52. Bohlmann, M. K. *et al.* Off-label use of aromatase inhibitors: an alternative in reproductive medicine and in other nonmammary diseases? [German]. *Gynäkologische Endokrinologie* **6**, 221–228 (2008).
53. Raman, J. D. & Schlegel, P. N. Aromatase inhibitors for male infertility. *J. Urol.* **167**, 624–629 (2002).
54. Shozu, M. *et al.* Estrogen excess associated with novel gain-of-function mutations affecting the aromatase gene. *N. Engl. J. Med.* **348**, 1855–1865 (2003).
55. Demura, M. *et al.* Regional rearrangements in chromosome 15q21 cause formation of cryptic promoters for the *CYP19* (aromatase) gene. *Hum. Mol. Genet.* **16**, 2529–2541 (2007).
56. Stratakis, C. A. *et al.* The aromatase excess syndrome is associated with feminization of both sexes and autosomal dominant transmission of aberrant P450 aromatase gene transcription. *J. Clin. Endocrinol. Metab.* **83**, 1348–1357 (1998).
57. Martin, R. M. *et al.* Familial hyperestrogenism in both sexes: clinical, hormonal, and molecular studies of two siblings. *J. Clin. Endocrinol. Metab.* **88**, 3027–3034 (2003).
58. Binder, G. *et al.* Dominant transmission of prepubertal gynecomastia 58. Binder, G. *et al.* Dominant transmission of prepubertal gynecomastia due to serum estrone excess: hormonal, biochemical, and genetic analysis in a large kindred. *J. Clin. Endocrinol. Metab.* **90**, 484–492 (2005).
59. Kara, C., Kutlu, A. O., Tosun, M. S., Apaydin, S. & Senel, F. Sertoli cell tumor causing prepubertal gynecomastia in a boy with Peutz–Jeghers syndrome: the outcome of 1-year treatment with the aromatase inhibitor testolactone. *Horm. Res.* **63**, 252–256 (2005).
60. Lefevre, H. *et al.* Prepubertal gynecomastia in Peutz–Jeghers syndrome: incomplete penetrance in a familial case and management with an aromatase inhibitor. *Eur. J. Endocrinol.* **154**, 221–227 (2006).
61. Grandone, A. *et al.* Prepubertal gynecomastia in two monozygotic twins with Peutz–Jeghers syndrome: two years' treatment with anastrozole and genetic study. *Horm. Res. Paediatr.* **75**, 374–379 (2011).
62. Weinstein, L. S. *et al.* Activating mutations of the stimulatory G protein in the McCune–Albright syndrome. *N. Engl. J. Med.* **325**, 1688–1695 (1991).
63. Shenker, A. *et al.* Severe endocrine and nonendocrine manifestations of the McCune–Albright syndrome associated with activating mutations of stimulatory G protein GS. *J. Pediatr.* **123**, 509–518 (1993).
64. Foster, C. M. *et al.* Ovarian function in girls with McCune–Albright syndrome. *Pediatr. Res.* **20**, 859–863 (1986).
65. Feuillan, P. P. McCune–Albright syndrome. *Curr. Ther. Endocrinol. Metab.* **6**, 235–239 (1997).
66. Feuillan, P. P. *et al.* Treatment of precocious puberty in the McCune–Albright syndrome with the aromatase inhibitor testolactone. *N. Engl. J. Med.* **315**, 1115–1119 (1986).
67. Feuillan, P. P., Jones, J. & Cutler, G. B. Jr. Long-term testolactone therapy for precocious puberty in girls with the McCune–Albright syndrome. *J. Clin. Endocrinol. Metab.* **77**, 647–651 (1993).
68. Nunez, S. B., Calis, K., Cutler, G. B. Jr, Jones, J. & Feuillan, P. P. Lack of efficacy of fadrozole in treating precocious puberty in girls with the McCune–Albright syndrome. *J. Clin. Endocrinol. Metab.* **88**, 5730–5733 (2003).
69. Feuillan, P. *et al.* Letrozole treatment of precocious puberty in girls with the McCune–Albright syndrome: a pilot study. *J. Clin. Endocrinol. Metab.* **92**, 2100–2106 (2007).
70. Mieszczak, J., Lowe, E. S., Plourde, P. & Eugster, E. A. The aromatase inhibitor anastrozole is ineffective in the treatment of precocious puberty in girls with McCune–Albright syndrome. *J. Clin. Endocrinol. Metab.* **93**, 2751–2754 (2008).
71. Engiz, O., Berberoglu, M., Siklar, Z., Bilir, P. & Ocal, G. Treatment of autonomous ovarian follicular cyst with long-term anastrozole therapy. *Indian J. Pediatr.* **76**, 950–951 (2009).
72. Eugster, E. A. Peripheral precocious puberty: causes and current management. *Horm. Res.* **71**, 64–67 (2009).
73. Laue, L. *et al.* Treatment of familial male precocious puberty with spironolactone and testolactone. *N. Engl. J. Med.* **320**, 496–502 (1989).
74. Laue, L., Jones, J., Barnes, K. M. & Cutler, G. B. Jr. Treatment of familial male precocious puberty with spironolactone, testolactone, and deslorelin. *J. Clin. Endocrinol. Metab.* **76**, 151–155 (1993).
75. Leschek, E. W., Jones, J., Barnes, K. M., Hill, S. C. & Cutler, G. B. Jr. Six-year results of spironolactone and testolactone treatment of familial male-limited precocious puberty with addition of deslorelin after central puberty onset. *J. Clin. Endocrinol. Metab.* **84**, 175–178 (1999).
76. Kreher, N. C., Pescovitz, O. H., Delameter, P., Tiulpakov, A. & Hochberg, Z. Treatment of familial male-limited precocious puberty with bicalutamide and anastrozole. *J. Pediatr.* **149**, 416–420 (2006).
77. Lenz, A. M. *et al.* Bicalutamide and third-generation aromatase inhibitors in testotoxicosis. *Pediatrics* **126**, e728–e733 (2010).
78. Eyssette-Guerreau, S. *et al.* Effectiveness of anastrozole and cyproterone acetate in two brothers with familial male precocious puberty. *J. Pediatr. Endocrinol. Metab.* **21**, 995–1002 (2008).
79. Reiter, E. O. *et al.* Bicalutamide plus anastrozole for the treatment of gonadotropin-independent precocious puberty in boys with testotoxicosis: a phase II, open-label pilot study (BATT). *J. Pediatr. Endocrinol. Metab.* **23**, 999–1009 (2010).
80. Eugster, E. A. *et al.* Height outcome in congenital adrenal hyperplasia caused by 21-hydroxylase deficiency: a meta-analysis. *J. Pediatr.* **138**, 26–32 (2001).
81. Van der Kamp, H. J. *et al.* Longitudinal analysis of growth and puberty in 21-hydroxylase deficiency patients. *Arch. Dis. Child.* **87**, 139–144 (2002).
82. Bonfig, W., Bechtold, S., Schmidt, H., Knorr, D. & Schwarz, H. P. Reduced final height outcome in congenital adrenal hyperplasia under prednisone treatment: deceleration of growth velocity during puberty. *J. Clin. Endocrinol. Metab.* **92**, 1635–1639 (2007).
83. Merke, D. P. *et al.* Flutamide, testolactone, and reduced hydrocortisone dose maintain normal growth velocity and bone maturation despite elevated androgen levels in children with congenital adrenal hyperplasia. *J. Clin. Endocrinol. Metab.* **85**, 1114–1120 (2000).
84. Warne, G. L., Grover, S. & Zajac, J. D. Hormonal therapies for individuals with intersex conditions: protocol for use. *Treat. Endocrinology* **4**, 19–29 (2005).
85. Braunstein, G. D. Gynecomastia. *N. Engl. J. Med.* **328**, 490–495 (1993).
86. Ma, N. S. & Geffner, M. E. Gynecomastia in prepubertal and pubertal men. *Curr. Opin. Pediatr.* **20**, 465–470 (2008).
87. Nydick, M., Bustos, J., Dale, J. H. Jr & Rawson, R. W. Gynecomastia in adolescent boys. *JAMA* **178**, 449–454 (1961).
88. Plourde, P. V. *et al.* Safety and efficacy of anastrozole for the treatment of pubertal gynecomastia: a randomized, double-blind, placebo-controlled trial. *J. Clin. Endocrinol. Metab.* **89**, 4428–4433 (2004).
89. Lawrence, S. E., Faught, K. A., Vethamuthu, J. & Lawson, M. L. Beneficial effects of raloxifene and tamoxifen in the treatment of pubertal gynecomastia. *J. Pediatr.* **145**, 71–76 (2004).
90. Grumbach, M. M. Estrogen, bone, growth and sex: a sea change in conventional wisdom. *J. Pediatr. Endocrinol. Metab.* **13** (Suppl. 6), 1439–1455 (2000).

91. Mauras, N. Strategies for maximizing growth in puberty in children with short stature. *Endocrinol. Metab. Clin. North Am.* **38**, 613–624 (2009).
92. Carel, J. C. *et al.* Consensus statement on the use of gonadotropin-releasing hormone analogs in children. *Pediatrics* **123**, e752–e762 (2009).
93. Carel, J. C. Management of short stature with GnRH agonist and co-treatment with growth hormone: a controversial issue. *Mol. Cell Endocrinol.* **254–255**, 226–233 (2006).
94. van Gool, S. A. *et al.* Final height outcome after three years of growth hormone and gonadotropin-releasing hormone agonist treatment in short adolescents with relatively early puberty. *J. Clin. Endocrinol. Metab.* **92**, 1402–1408 (2007).
95. Yanovski, J. A. *et al.* Treatment with a luteinizing hormone-releasing hormone agonist in adolescents with short stature. *N. Engl. J. Med.* **348**, 908–917 (2003).
96. Kreher, N. C., Eugster, E. A. & Shankar, R. R. The use of tamoxifen to improve height potential in short pubertal boys. *Pediatrics* **116**, 1513–1515 (2005).
97. Hero, M., Wickman, S. & Dunkel, L. Treatment with the aromatase inhibitor letrozole during adolescence increases near-final height in boys with constitutional delay of puberty. *Clin. Endocrinol. (Oxf.)* **64**, 510–513 (2006).
98. Martin, M. M., Martin, A. L. A. & Mossman, K. L. Testosterone treatment of constitutional delay in growth and development: effect of dose on predicted versus definitive height. *Acta Endocrinol. Suppl. (Copenh.)* **279**, 147–152 (1986).
99. Crowne, E. C., Shalet, S. M., Wallace, W. H., Eminson, D. M. & Price, D. A. Final height in boys with untreated constitutional delay in growth and puberty. *Arch. Dis. Child.* **65**, 1109–1112 (1990).
100. LaFranchi, S., Hanna, C. E. & Mandel, S. H. Constitutional delay of growth: expected versus final adult height. *Pediatrics* **87**, 82–87 (1991).
101. Albanese, A. & Stanhope, R. Does constitutional delayed puberty cause segmental disproportion and short stature. *Eur. J. Pediatr.* **152**, 293–296 (1993).
102. Albanese, A. & Stanhope, R. Predictive factors in the determination of final height in boys with constitutional delay of growth and puberty. *J. Pediatr.* **126**, 545–550 (1995).
103. Rekers-Mombarg, L. T. *et al.* Spontaneous growth in idiopathic short stature. European Study Group. *Arch. Dis. Child.* **75**, 175–180 (1996).
104. Hero, M., Toiviainen-Salo, S., Wickman, S., Mäkitie, O. & Dunkel, L. Vertebral morphology in aromatase inhibitor-treated males with idiopathic short stature or constitutional delay of puberty. *J. Bone Miner. Res.* **25**, 1536–1543 (2010).
105. Mauras, N., Welch, S., Rini, A. & Klein, K. O. An open label 12-month pilot trial on the effects of the aromatase inhibitor anastrozole in growth hormone (GH)-treated GH deficient adolescent boys. *J. Pediatr. Endocrinol. Metab.* **17**, 1597–1606 (2004).
106. Hagenas, L. Growth rate can be manipulated. Estrogen production in pubertal boys can be blocked by an aromatase inhibitor [Swedish]. *Lakartidningen*. **99**, 165–168 (2002).
107. Faglia, G., Arosio, M. & Porretti, S. Delayed closure of epiphyseal cartilages induced by the aromatase inhibitor anastrozole. Would it help short children grow up? *J. Endocrinol. Invest.* **23**, 721–723 (2000).
108. Balestrieri, A., Faustini-Fustini, M., Rochira, V. & Carani, C. Clinical implications and management of oestrogen deficiency in the male. *Clin. Endocrinol. (Oxf.)* **54**, 431–432 (2001).
109. Maffei, L. *et al.* Dysmetabolic syndrome in a man with a novel mutation of the aromatase gene: effects of testosterone, alendronate, and estradiol treatment. *J. Clin. Endocrinol. Metab.* **89**, 61–70 (2004).
110. Jones, M. E., Boon, W. C., Proietto, J. & Simpson, E. R. Of mice and men: the evolving phenotype of aromatase deficiency. *Trends Endocrinol. Metab.* **17**, 55–64 (2006).
111. Rochira, V., Zirilli, L., Madeo, B., Maffei, L. & Carani, C. Testosterone action on erythropoiesis does not require its aromatization to estrogen: Insights from the testosterone and estrogen treatment of two aromatase-deficient men. *J. Steroid Biochem. Mol. Biol.* **113**, 189–194 (2009).
112. Rochira, V. *et al.* Tall stature without growth hormone: four male patients with aromatase deficiency. *J. Clin. Endocrinol. Metab.* **95**, 1626–1633 (2010).
113. Gerardin, D. C. & Pereira, O. C. Reproductive changes in male rats treated perinatally with an aromatase inhibitor. *Pharmacol. Biochem. Behav.* **71**, 301–305 (2002).
114. Wickman, S. & Dunkel, L. Inhibition of P450 aromatase enhances gonadotropin secretion in early and midpubertal boys: evidence for a pituitary site of action of endogenous E. *J. Clin. Endocrinol. Metab.* **86**, 4887–4894 (2001).
115. Haynes, B. P., Dowsett, M., Miller, W. R., Dixon, J. M. & Bhatnagar, A. S. The pharmacology of letrozole. *J. Steroid Biochem. Mol. Biol.* **87**, 35–45 (2003).
116. Seftel, A. Testosterone replacement therapy for male hypogonadism: part III. Pharmacologic and clinical profiles, monitoring, safety issues, and potential future agents. *Int. J. Impot. Res.* **19**, 2–24 (2007).
117. Hero, M., Wickman, S., Hanhijärvi, R., Siimes, M. A. & Dunkel, L. Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. *J. Pediatr.* **146**, 245–252 (2005).
118. Hero, M., Ankarberg-Lindgren, C., Taskinen, M. R. & Dunkel, L. Blockade of oestrogen biosynthesis in peripubertal boys: effects on lipid metabolism, insulin sensitivity, and body composition. *Eur. J. Endocrinol.* **155**, 453–460 (2006).
119. Couse, J. F. & Korach, K. S. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr. Rev.* **20**, 358–417 (1999).
120. Robertson, K. M. *et al.* Impairment of spermatogenesis in mice lacking a functional aromatase (*Cyp19*) gene. *Proc. Natl Acad. Sci. USA* **96**, 7986–7991 (1999).
121. Pentikainen, V., Erkkilä, K., Suomalainen, L., Parvinen, M. & Dunkel, L. Estradiol acts as a germ cell survival factor in the human testis *in vitro*. *J. Clin. Endocrinol. Metab.* **85**, 2057–2067 (2000).
122. Shetty, G., Krishnamurthy, H., Krishnamurthy, H. N., Bhatnagar, S. & Moudgal, R. N. Effect of estrogen deprivation on the reproductive physiology of male and female primates. *J. Steroid Biochem. Mol. Biol.* **61**, 157–166 (1997).
123. Aquila, S. *et al.* Towards a physiological role for cytochrome P450 aromatase in ejaculated human sperm. *Hum. Reprod.* **18**, 1650–1659 (2003).
124. Mauras, N., Bell, J., Snow, B. G. & Winslow, K. L. Sperm analysis in growth hormone-deficient adolescents previously treated with an aromatase inhibitor: comparison with normal controls. *Fertil. Steril.* **84**, 239–242 (2005).
125. Ramasamy, R. *et al.* Successful fertility treatment for Klinefelter's syndrome. *J. Urol.* **182**, 1108–1113 (2009).
126. Bonjour, J. P. & Chevalley, T. Pubertal timing, peak bone mass and fragility fracture risk. *Bonekey Osteovision* **4**, 30–48 (2007).
127. McCloskey, E. V. Aromatase inhibitors and bone health. *Bonekey Osteovision* **3**, 5–13 (2006).
128. Hero, M. *et al.* Impact of aromatase inhibitor therapy on bone turnover, cortical bone growth and vertebral morphology in pre- and peripubertal boys with idiopathic short stature. *Horm. Res.* **71**, 290–297 (2009).
129. Lorentzon, M., Swanson, C., Andersson, N., Mellström, D. & Ohlsson, C. Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. *J.* Bone *Miner. Res.* **20**, 1334–1341 (2005).
130. Seeman, E. Clinical review 137: Sexual dimorphism in skeletal size, density, and strength. *J. Clin. Endocrinol. Metab.* **86**, 4576–4584 (2001).
131. Wickman, S., Kajantie, E. & Dunkel, L. Effects of suppression of estrogen action by the p450 aromatase inhibitor letrozole on bone mineral density and bone turnover in pubertal boys. *J. Clin. Endocrinol. Metab.* **88**, 3785–3793 (2003).
132. Leder, B. Z., LeBlanc, K. M., Schoenfeld, D. A., Eastell, R. & Finkelstein, J. S. Differential effects of androgens and estrogens on bone turnover in normal men. *J. Clin. Endocrinol. Metab.* **88**, 204–210 (2003).
133. Michael, H., Härkönen, P. L., Väänänen, H. K. & Hentunen, T. A. Estrogen and testosterone use different cellular pathways to inhibit osteoclastogenesis and bone resorption. *J. Bone Miner. Res.* **20**, 2224–2232 (2005).
134. Seeman, E. & Delmas, P. D. Bone quality—the material and structural basis of bone strength and fragility. *N. Engl. J. Med.* **354**, 2250–2261 (2006).
135. Ma, D. & Jones, G. The association between bone mineral density, metacarpal morphometry, and upper limb fractures in children: a population-based case-control study. *J. Clin. Endocrinol. Metab.* **88**, 1486–1491 (2003).
136. Bouillon, R., Bex, M., Vanderschueren, D. & Boonen, S. Estrogens are essential for male pubertal periosteal bone expansion. *J. Clin. Endocrinol. Metab.* **89**, 6025–6029 (2004).
137. Lee, K., Jessop, H., Suswillo, R., Zaman, G. & Lanyon, L. Endocrinology: bone adaptation requires oestrogen receptor α. *Nature* **424**, 389 (2003).
138. Metzger, D. L. & Kerrigan, J. R. Estrogen receptor blockade with tamoxifen diminishes growth hormone secretion in boys: evidence for a stimulatory role of endogenous estrogens during male adolescence. *J. Clin. Endocrinol. Metab.* **79**, 513–518 (1994).
139. Wickman, S., Saukkonen, T. & Dunkel, L. The role of sex steroids in the regulation of insulin sensitivity and serum lipid concentrations during male puberty: a prospective study with a P450-aromatase inhibitor. *Eur. J. Endocrinol.* **146**, 339–346 (2002).
140. Tikkanen, M. J. & Nikkilä, E. A. Regulation of hepatic lipase and serum lipoproteins by sex steroids. *Am. Heart J.* **113**, 562–567 (1987).
141. Sorva, R., Kuusi, T., Dunkel, L. & Taskinen, M. R. Effects of endogenous sex steroids on serum lipoproteins and postheparin plasma lipolytic enzymes. *J. Clin. Endocrinol. Metab.* **66**, 408–413 (1988).

142. Moran, A. *et al.* Association between the insulin resistance of puberty and the insulin-like growth factor-I/growth hormone axis. *J. Clin. Endocrinol. Metab.* **87**, 4817–4820 (2002).
143. Phillips, K. A., Ribi, K. & Fisher, R. Do aromatase inhibitors have adverse effects on cognitive function? *Breast Cancer Res.* **13**, 203 (2011).
144. Osterlund, M. K., Gustafsson, J. A., Keller, E. & Hurd, Y. L. Estrogen receptor β (ERβ) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERα mRNA. *J. Clin. Endocrinol. Metab.* **85**, 3840–3846 (2000).
145. Cherrier, M. M. *et al.* The role of aromatization in testosterone supplementation: effects on cognition in older men. *Neurology* **64**, 290–296 (2005).
146. Kil, K. E. *et al.* Synthesis and PET studies of [^{11}C-cyano]letrozole (Femara), an aromatase inhibitor drug. *Nucl. Med. Biol.* **36**, 215–223 (2009).
147. Jenkins, V. A. *et al.* Effects of anastrozole on cognitive performance in postmenopausal women: a randomised, double-blind chemoprevention trial (IBIS II). *Lancet Oncol.* **9**, 953–961 (2008).
148. Young, L. A., Neiss, M. B., Samuels, M. H., Roselli, C. E. & Janowsky, J. S. Cognition is not modified by large but temporary changes in sex hormones in men. *J. Clin. Endocrinol. Metab.* **95**, 280–288 (2010).
149. Hero, M., Maury, S., Luotoniemi, E., Service, E. & Dunkel, L. Cognitive effects of aromatase inhibitor therapy in peripubertal boys. *Eur. J. Endocrinol.* **163**, 149–155 (2010).
150. Dixon, J. M. *et al.* Letrozole suppresses plasma estradiol and estrone sulphate more completely than anastrozole in postmenopausal women with breast cancer. *J. Clin. Oncol.* **26**, 1671–1676 (2008).
151. de Jong, P. C. *et al.* Inhibition of breast cancer tissue aromatase activity and estrogen concentrations by the third-generation aromatase inhibitor vorozole. *Cancer Res.* **57**, 2109–2111 (1997).

Author contributions
All authors contributed equally to all aspects of the manuscript.

Supplementary information
Supplementary information is linked to the online version of the paper at www.nature.com/nrendo