Letrozole versus testosterone for promotion of endogenous puberty in boys with constitutional delay of growth and puberty: a randomised controlled phase 3 trial

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Summary

Background The treatment of constitutional delay of growth and puberty (CDGP) is an underinvestigated area in adolescent medicine. We tested the hypothesis that peroral aromatase inhibition with letrozole is more efficacious than intramuscular injection of low-dose testosterone in inducing puberty in boys with CDGP.

Methods We did a randomised, controlled, open-label trial at four paediatric centres in Finland. Boys aged at least 14 years with CDGP who wanted medical intervention and exhibited the first signs of puberty were randomly assigned in blocks of ten to receive either six intramuscular injections of low-dose testosterone (about 1 mg/kg bodyweight) every 4 weeks for 6 months or peroral letrozole 2·5 mg once daily for 6 months. All boys were followed up for 6 months after the end of treatment. The primary outcomes were changes in testicular volume and hormonal markers of puberty at 6 months after treatment initiation, which were assessed in all participants who received the assigned treatment. All patients were included in the safety analysis. This study is registered with ClinicalTrials.gov, number NCT01797718.

Findings Between Aug 1, 2013, and Jan 30, 2017, 30 boys were randomly assigned to receive testosterone (n=15) or letrozole (n=15). One boy in the testosterone group was excluded from the primary analyses because of a protocol deviation. During treatment, boys in the letrozole group had higher serum concentrations of luteinising hormone, follicle-stimulating hormone, testosterone, and inhibin B than did boys in the testosterone group. Testicular growth from baseline to 6 months was greater in the letrozole group than in the testosterone group (7·2 mL [95% CI 5·2–9·3] *vs* 2·2 mL [1·4–2·9]; between-group difference per month 0·9 mL [95% CI 0·6–1·2], p<0·0001). Most adverse events were mild. One boy in the testosterone group had aggressive behaviour for 1 week after each injection, and one boy in the letrozole group had increased irritability at 6 months.

Interpretation Letrozole might be a feasible alternative treatment to low-dose testosterone for boys with CDGP who opt for medical intervention. However, the risks and benefits of manipulating the reproductive axis during early puberty should be weighed carefully.

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Introduction

About 2% of adolescents have delayed sexual maturation due to constitutional delay of growth and puberty (CDGP).[1] CDGP is diagnosed by exclusion, and its treatment, if requested by the patient, should involve an evidence-based pharmacological intervention to promote skeletal growth while minimising epiphyseal maturation and gonadal injury.[2,3] Although androgenic drugs, such as oxandrolone and fluoxymesterone, have been used to treat boys with CDGP,[4,5] the standard of care is low-dose testosterone administered via various routes, doses, and periods of time.[2,3,5–7]

Although treatment with low-dose testosterone promotes androgenic signs of puberty, it might initially suppress, rather than activate, the hypothalamic–pituitary–gonadal (HPG) axis,[7,8] and its puberty-promoting effects on gonadotropin secretion and testicular growth emerge later during treatment or after treatment cessation.[6,7,9] Only a few randomised trials have investigated the effects of testosterone on the progression of puberty,[3,6,10] rendering the optimal treatment of CDGP an under-investigated area in adolescent medicine.

Testosterone increases serum IGF-1 concentrations and growth velocity and promotes skeletal maturation in boys with CDGP.[6,9–11] Although treatment with testosterone is not thought to affect adult height,[12] no prospective studies have investigated this effect. An ideal drug for boys with CDGP would promote androgenic signs of puberty and testicular growth, increase height and lean mass gain, and minimise epiphyseal maturation.[3] We anticipated that these requirements would be met by the peroral aromatase inhibitor letrozole, which inhibits the conversion of androstenedione to estrone and testosterone to estradiol.[13] Letrozole thus lowers oestrogen concentrations, which

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Research in context

Evidence before this study

We searched PubMed and MEDLINE up to March 31, 2018, without language restrictions, using the terms "delayed puberty" AND "male" AND "therapeutics", for randomised controlled trials. The search identified 222 papers, from which we selected ten that included sufficient evidence on the management of constitutional delay of growth and puberty (CDGP) in boys and were written in English. Only seven studies had addressed the effects of intramuscular low-dose testosterone injections on pubertal progression, and none had compared aromatase inhibitor monotherapy with testosterone for the management of CDGP. Furthermore, the studies included few patients, varying doses and treatment periods, and participants who had already started puberty.

Added value of this study

To our knowledge, this is the first randomised controlled trial to compare aromatase inhibitor monotherapy with testosterone therapy for boys with CDGP who have early signs of puberty. Our data suggest that letrozole is a feasible alternative to low-dose testosterone for CDGP in boys who present with the first biochemical or clinical signs of puberty, select medical intervention, and expect comprehensive physiological changes of puberty, including testis growth. Both treatments accelerated linear growth (testosterone slightly more than letrozole), and so low-dose testosterone might be the primary option for boys who prioritise rapid height gain during treatment. Both treatments were well tolerated and had no detrimental effects on bone mineralisation.

Implications of all the available evidence

Our findings provide a basis for the design of future studies of aromatase inhibitors in boys with CDGP aiming to optimise their medical management. The effect of aromatase inhibition and other puberty-promoting treatments on psychosocial wellbeing and long-term health outcomes should be addressed in future studies.

delays epiphyseal maturation—an effect previously investigated for its ability to increase adult height in prepubertal and early pubertal boys with idiopathic short stature and in midpubertal boys with CDGP.[8,14,15] These landmark studies also showed that letrozole activates gonadotropin and testosterone secretion from very early puberty (ie, when repression of HPG axis activity during childhood is lessened).[8,14] Thus, letrozole seems to lead to immediate rather than delayed activation of the HPG axis in adolescent boys.

The efficacy of aromatase inhibitor monotherapy in augmenting puberty has not yet been compared with that of conventional low-dose testosterone, and knowledge gaps exist in the effects of low-dose testosterone on HPG axis function. Moreover, the effects of these two treatment regimens on the progression of growth and puberty should be investigated further. We aimed to test the hypothesis that letrozole is more efficacious than low-dose testosterone in expediting puberty in boys with CDGP.

Methods

Study design and participants

This randomised, controlled, phase 3, open-label trial was done at the paediatric endocrinology outpatient clinics of Helsinki University Hospital (Helsinki), Turku University Hospital (Turku), Kuopio University Hospital (Kuopio), and Kymenlaakso Central Hospital (Kotka) in Finland. Eligible boys were aged 14 years or older; had presented with delayed puberty, with the first signs of HPG axis activation; and had a mean testicular volume of at least 2·5 mL but less than 4 mL and serum testosterone concentration of less than 5 nmol/L (or serum testosterone concentration ≥1 nmol/L even if the mean testicular volume was <2·5 mL). In July, 2015, eligibility criteria were expanded to include boys who were at Tanner genital stage 2 and had serum testosterone concentrations of less than 3 nmol/L.

Study exclusion criteria were chronic diseases, primary or hypogonadotropic hypogonadism, known chromosomal anomalies, and chronic medication use that could potentially adversely affect bone mineralisation (excluding inhaled corticosteroids). Patients had been thoroughly investigated for family history of micropenis, and history of cryptorchidism, coloboma, anosmia or hyposmia, and hearing loss (ie, additional phenotypic features suggestive of congenital hypogonadotropic hypogonadism). Patients and guardians were also questioned about history of head trauma, headaches, and visual disturbances. During clinical examinations, patients were assessed for pigmentation defects, split hand and foot malformation, cleft lip and palate, missing teeth, external ear anomalies, and synkinesia (mirror movements of the upper limbs). In biochemical analyses, attention was paid to inhibin B concentrations because low concentrations have been associated with congenital hypogonadotropic hypogonadism,[16] especially when coexisting with small testis size.[17] In the diagnostic work-up, we checked the results of gonadotropin-releasing hormone (GnRH) stimulation tests given that the absence of a gonadotropin response might confirm the presence of severe congenital hypogonadotropic hypogonadism.[18]

Eligible boys were informed about the likely self-limiting nature of their condition and could choose either to be in the watchful waiting group or to receive treatment. Patient data were stored in the hospitals' patient information systems, and the data used for analyses were anonymised with a two-digit code. The Finnish National Committee on Medical Research Ethics and the Finnish Medicines Agency approved the study protocol, and the principles of Good Clinical Practice and

the Declaration of Helsinki were followed. Written informed consent was obtained from all participants and their guardians.

Randomisation and masking

Participants who opted to receive treatment (instead of watchful waiting) were randomly assigned in blocks of ten to receive either letrozole or testosterone for 6 months. The randomisation sequence was generated with a computer in the hospital pharmacy of Helsinki University Hospital. The allocation of each participant was kept in a sealed, opaque envelope, which was opened at the first study visit, at which time participants became unmasked to treatment assignment. Clinicians were not masked to treatment allocation.

Procedures

Both medications were provided by the hospital pharmacy of Helsinki University Hospital. Peroral letrozole 2·5 mg was administered once daily for 6 months, whereas low-dose testosterone (Sustanon 250; Aspen Nordic, Ballerup, Denmark) was injected intramuscularly every 4 weeks for 6 months (six injections in total). The dose of testosterone given to a patient was dependent on their weight (roughly 1 mg/kg), with the closest weight-based dose being chosen for each patient: 0·15 mL (37·5 mg) for boys weighing 37·5–43·7 kg, 0·2 mL (50·0 mg) for boys weighing 43·8–56·2 kg, 0·25 mL (62·5 mg) for boys weighing 56·3–68·7 kg, and 0·3 mL (75 mg) for boys weighing more than 68·8 kg. Participants and their guardians were informed about the correct dose, and testosterone was injected by a nurse at the participant's school or at a health-care centre. The dates and injected doses were written in a form that was reviewed at treatment completion. For boys treated with letrozole, medication adherence was assessed at 3-month and 6-month visits.

Study visits were at 0, 3, 6, and 12 months. For the testosterone group, the 3-month visit was scheduled for 1 week after the fourth injection of testosterone, and the 6-month visit occurred 4 weeks after the last injection. Thus, blood samples from 3 months and 6 months represented peak and trough concentrations of exogenous testosterone, respectively,[19] thereby enabling detection of the treatment effect (augmentation of gonadotropin secretion) at two different timepoints in relation to the preceding testosterone injection. Such augmentation of gonadotropin secretion has been reported to occur in some boys with CDGP even after 0·3 years of peroral testosterone undecanoate.[7] For the letrozole group, samples at 3 and 6 months were obtained during treatment.

At all visits, boys were physically examined (including measurement of height, weight, puberty stage, and testicular size) by an investigator (usually TV but also HH, RV, or JT). Height was measured with a wall-mounted Harpenden Stadiometer (Holtain, Crosswell, UK), with 0·1 cm precision, and weight was measured with a stabilised and calibrated scale. Pubertal progression was recorded as Tanner stage,[20] and testicular dimensions were measured with a ruler.[21] Testicular volume (in mL) was calculated with the formula: length (cm)×width² (cm)×0·52.[22]

At baseline and 12 months, an indwelling intravenous cannula was inserted for blood sampling and GnRH stimulation tests were done by intravenous injection of a GnRH analogue (Relefact LH-RH 0·1 mg; Aventis Pharma, Frankfurt, Germany; 3·5 µg/kg, all boys received a dose of 100 µg) as a single bolus, followed by measurement of serum gonadotropin concentrations for up to 90 min (0, 20, 30, and 60 min for luteinising hormone [LH] and 0, 30, 60, and 90 min for follicle-stimulating hormone [FSH]). Blood and urine samples were taken between 0800 h and 0900 h, and sera and urine were stored at –80°C for subsequent analyses. At all visits, blood samples were taken for measurement of blood count and concentrations of LH, FSH, inhibin B, estradiol, and testosterone; concentrations of lipids, IGF-1, insulin, and glucose were measured by the hospital laboratory at 0, 6, and 12 months.

Serum testosterone concentrations were measured with the API3000 liquid chromatography–tandem mass spectrometer (LC-MS/MS; AB Sciex, Concord, ON, Canada) or, for the three boys treated in Kuopio University Hospital, with the API3200 LC-MS/MS (AB Sciex, Concord, ON, Canada).[23] Circulating estradiol was measured with a TQ-5500 LC-MS/MS (AB Sciex, Concord, ON, Canada). Gonadotropins were quantified with an immunoelectrochemiluminometric assay on an automated immunoanalyser (Modular Analytics E170; Roche Diagnostics, Mannheim, Germany), while circulating inhibin B was measured with an ELISA (A81304; Beckman Coulter, Brea, CA, USA). Circulating IGF-1 was quantified with an immunochemiluminometric assay on the IMMULITE 2000 XPi analyser (Siemens, Llanberis, UK); with an immunoenzymometric assay (Mediagnost, Reutlingen, Germany); or, in the three boys treated in Kuopio University Hospital, with an immuno-chemiluminometric assay on the Liaison XL analyser (Diasorin, Saluggia, Italy). Urine LH was quantitated with a time-resolved immunofluorometric assay (AutoDELFIA LH Spec; PerkinElmer, Turku, Finland).

At baseline and 12 months, an experienced paediatric radiologist (ST-S) reviewed thoracic x-rays of boys in the letrozole group for disc-space narrowing, wedging, compression, and irregularity of lumbar and thoracic vertebrae (T2 to L3), according to the classification for paediatric vertebral body morphology.[24] At these visits, bone age was ascertained from x-rays of the left hand and wrist in all participants with an automated bone age assessment (BoneXpert),[25] except for two boys whose bone ages were ascertained by a paediatric endocrinologist (MH) using the Greulich and Pyle method.[26] Predicted adult height was estimated with the BoneXpert adult

height predictor.[27] At 0, 6, and 12 months, areal bone mineral densities were measured from lumbar spine (L1 to L4), hip, and total body less head with dual-energy x-ray absorptiometry (Lunar Prodigy; GE Healthcare, Madison, WI, USA, or Hologic Discovery A; Hologic Waltham, MA, USA). Bone mineral apparent densities of lumbar spine (L1 to L4) were calculated as described previously.[28]

During the trial, treatment safety was monitored carefully. At all visits, patients were questioned about back pain, gastrointestinal symptoms, eye or vision problems, neurological or psychiatric symptoms, operations, fractures, and urinary problems. Dates of testosterone injections and missed doses of letrozole were recorded.

Outcomes

The primary outcomes were changes in testicular volume and hormonal markers of puberty (serum testosterone, LH, FSH, and inhibin B and urinary LH) 6 months after initiation of treatment. Secondary outcomes were changes in bone mineral density and body composition, including lean body mass and percentage body fat.

Statistical analysis

The sample size calculation was based on testicular volume only. We estimated that the minimum clinically significant mean difference between groups in change in testicular volume from baseline to 6 months would be 3 mL.[14] Assuming an SD of 2·5 mL,[14] we estimated that 22 boys (11 in each group) would provide 80% power to detect this difference, with an α value of 0·05.

All boys who received the assigned treatment were included in the primary analyses; the safety analysis included all study participants. We analysed testicular growth during treatment (0–6 months of the study) using a mixed model repeated measures analysis, with time and time by treatment as fixed effects. The model assumptions were assessed visually with diagnostic residual plots. We calculated p values for parameter estimates in the linear mixed models using Satterthwaite's approximation for degrees of freedom. We estimated the overall treatment effect (0–12 months of the study) by comparing mean changes in testicular volume between groups using an unpaired *t* test. We did a sensitivity analysis for testicular growth using the same linear mixed model but including only boys who were followed up in Helsinki University Hospital.

Linear mixed models could not be fitted satisfactorily to hormonal data, and so differences between groups in changes in hormone values (following log-transformation to normality) during treatment were analysed with mixed repeated measures ANOVA, with treatment as the between-participant factor and time (months) as the within-participant factor, followed by comparisons between group means with unpaired *t* tests. Generalised estimating equation models for hormone data (0–6 months) were fitted with first-order autoregressive correlation structure. To test whether possible differences between treatment groups persisted beyond treatment discontinuation, we compared changes in testicular volume and hormone values during the study period (ie, between baseline and 12 months) using unpaired *t* tests. To adjust for potential differences between groups at baseline, we repeated the analyses with inclusion of treatment group as a covariate in the linear mixed model and generalised estimating equation models.

We assessed between-group differences in measures obtained at baseline, 6 months, and 12 months (ie, only once during therapy) using unpaired *t* tests (for areal bone mineral densities, bone mineral apparent densities, and laboratory measures other than reproductive hormones). We compared growth velocity before treatment, during treatment, and for the entire study period using unpaired (for between-group comparisons) and paired (for within-group comparisons) *t* tests. Tanner stages were analysed with Fisher's exact test, and correlations between hormonal markers of puberty and dual-energy x-ray absorptiometry measures were analysed with Spearman's rank correlation. Within-group changes in reproductive hormone concentrations were analysed with one-way repeated measures ANOVA, and Bonferroni correction was used for within-group pairwise analyses. All p values are two-tailed, and a p value of less than 0·05 indicates a statistically significant difference.

To depict the natural course of delayed puberty, we included data from an untreated group of patients with CDGP, which included participants who fulfilled the inclusion criteria but selected watchful waiting instead of treatment and nine retrospectively identified boys who also fulfilled the study inclusion criteria.[17] Patients who selected watchful waiting were followed up for 12 months in Helsinki University Hospital.

Statistical analyses were done with SPSS for Windows, version 22.0, and R version 3.4.4.

This study is registered with ClinicalTrials.gov (number NCT01797718).

Role of the funding source

The funders had no role in the study design, study conduct, data analysis, or data interpretation. The corresponding author had access to all of the data in the study and had the final responsibility for the decision to submit for publication.

Results

Between Aug 1, 2013, and Jan 30, 2017, 142 boys were screened for eligibility, of whom 35 were enrolled (figure 1). Overall, 30 boys were randomly assigned to the letrozole group (n=15) or to the testosterone group (n=15) and five selected watchful waiting. None of the boys in the treatment groups dropped out of the study, although one participant in the testosterone group was excluded because of a protocol deviation (figure 1). Baseline characteristics were mostly similar between treatment groups (table 1), and all patients had detectable gonadotropin responses.

In the testosterone group, hormone concentrations at 3 months and 6 months reflected peak and trough concentrations of exogenous testosterone, respectively, whereas the values in the letrozole group represented the effects of ongoing letrozole treatment (figure 2; appendix). There was a significant interaction effect between time and treatment group on the concentrations of all reproductive hormones from baseline to 6 months (figure 2; appendix). Changes from baseline to 6 months in concentrations of estradiol were lower in the letrozole group than in the testosterone group, whereas changes over that time in concentrations of urinary LH and serum LH, FSH, testosterone, and inhibin B were greater in the letrozole group than in the testosterone group (all p<0·0001; figure 2). The overall changes in reproductive hormone concentrations between baseline and 12 months did not differ significantly between groups (figure 2). Sensitivity analyses adjusting for potential between-group differences at baseline did not change the results (appendix).

Blocking the formation of estradiol by letrozole resulted in acute elevation of circulating concentrations of LH and testosterone, which had largely subsided by 12 months (figure 2; appendix). Circulating FSH concentrations followed a similar pattern to circulating LH concentrations, whereas inhibin B concentrations increased steadily during treatment in the letrozole group (figure 2; appendix).

In the testosterone group, serum concentrations of both LH and inhibin B concentrations were decreased at 3 months compared with baseline (figure 2; appendix). In one boy treated with testosterone, inhibin B remained suppressed (57 ng/L) 6 months after cessation of treatment. No differences between groups were observed in GnRH-stimulated LH and FSH concentrations at baseline or at 12 months. Concentrations of haemoglobin, haematocrit, insulin, glucose, and cholesterol during the study period are shown in the appendix.

During the 6 months of treatment, the mean testicular volume increased by 7·2 mL (95% CI 5·2–9·3) in the letrozole group and by 2·2 mL (1·4–2·9) in the testosterone group (between-group difference per month 0·9 mL, 95% CI 0·6–1·2; p<0·0001; figure 3A). Between baseline and 12 months, boys treated with letrozole had a larger increase in testicular volume than did boys treated with testosterone (9·8 mL [95% CI 7·5–12·0] *vs* 6·1 mL [4·9–7·3]; p=0·0059; figure 3A). In sensitivity analyses including only the 23 boys who were followed up in Helsinki University Hospital, testicular growth during treatment was faster in boys treated with letrozole than in boys treated with testosterone (between-group difference per month 1·0 mL [95% CI 0·7–1·3]; p<0·0001). For the testicular volume primary endpoint, we did an exploratory intention-to-treat analysis that included the boy who had erroneously received intramuscular testosterone 250 mg repeatedly for 3 months; the results of this analysis were not different from those of the primary analysis (appendix).

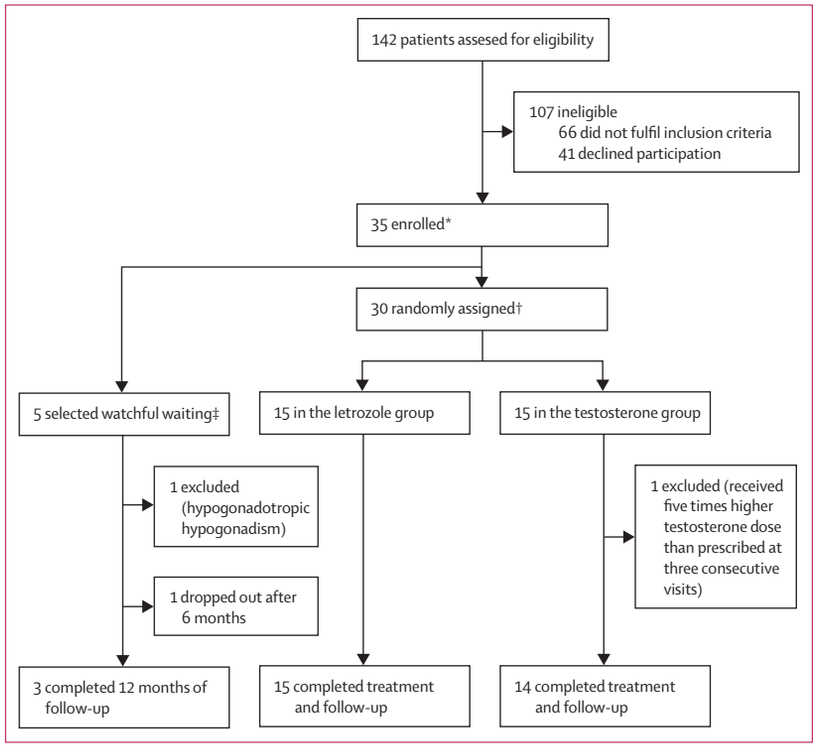

Figure 1: **Trial profile**
*Including 29 boys in Helsinki University Hospital, three in Kuopio University Hospital, two in Kymenlaakso Central Hospital, and one in Turku University Hospital. †Including 24 boys in Helsinki University Hospital, three in Kuopio University Hospital, two in Kymenlaakso Central Hospital, and one in Turku University Hospital. ‡All were in Helsinki University Hospital.

	Letrozole (n=15)	Testosterone (n=14)
Age (years)	14·8 (14·2 to 15·1)	14·9 (14·5 to 15·1)
Testicular volume (mL)	2·9 (2·4 to 3·5)	3·4 (2·9 to 3·8)
Height (cm)	153·7 (149·6 to 157·9)	158·1 (154·5 to 161·7)
Height SDS	–2·3 (–2·8 to –1·8)	–1·7 (–2·2 to –1·2)
BMI (kg/m^2)	20·3 (17·8 to 22·8)	20·2 (17·9 to 23·2)
Growth velocity (cm per year)	3·9 (3·4 to 4·4)	4·5 (3·6 to 5·4)
Bone age (years)	12·3 (11·6 to 12·9)	12·5 (11·9 to 13·1)
Predicted adult height (cm)	174·9 (170·9 to 178·3)	178·4 (176·5 to 181·6)
LH (IU/L)	2·1 (1·5 to 2·7)	2·2 (1·3 to 3·1)
FSH (IU/L)	2·4 (1·7 to 3·0)	2·6 (1·9 to 3·2)
Testosterone (nmol/L)	1·9 (1·3 to 2·4)	2·3 (1·5 to 3·0)
Estradiol (pmol/L)	9·7 (7·0 to 12·3)	14·0 (6·5 to 21·5)
Inhibin B (ng/L)	174 (143 to 206)	201 (150 to 253)
IGF-1 (nmol/L)	31 (26 to 37)	36 (30 to 42)

Data are mean (95% CI). BMI=body-mass index. FSH=follicle-stimulating hormone. LH=luteinising hormone. SDS=standard deviation score.

Table 1: **Baseline characteristics**

During the study period, puberty progression did not differ between groups (p=0·54 for Tanner genital stage and p=0·24 for Tanner pubic hair stage; figure 3B and C). Both treatments increased growth velocity from

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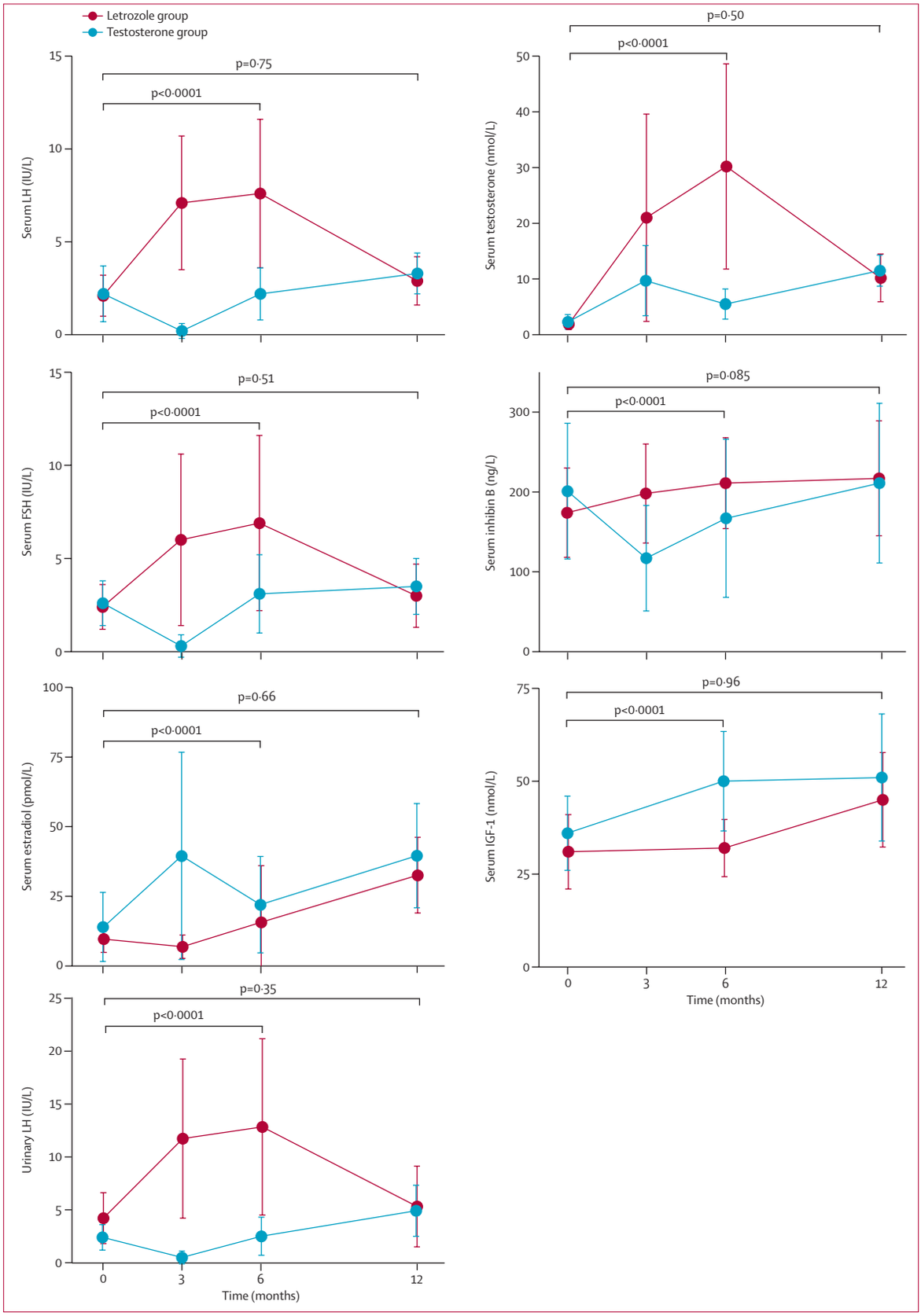

Figure 2: **Change over time in hormonal markers of puberty**
Circles represent means and whiskers indicate SDs. p values are for between-group differences in changes from baseline to 6 months (calculated with repeated measures ANOVA) or 12 months (calculated with unpaired *t* test). At 3 months and 6 months, the samples were obtained 1 and 4 weeks after the preceding testosterone injections, respectively, and while on medication in the letrozole group. In the testosterone group, the hormone concentrations at 3 and 6 months thus reflect peak and residual effects of exogenous testosterone, respectively, whereas the values in the letrozole group represent the effect of ongoing treatment. LH=luteinising hormone. FSH=follicle-stimulating hormone.

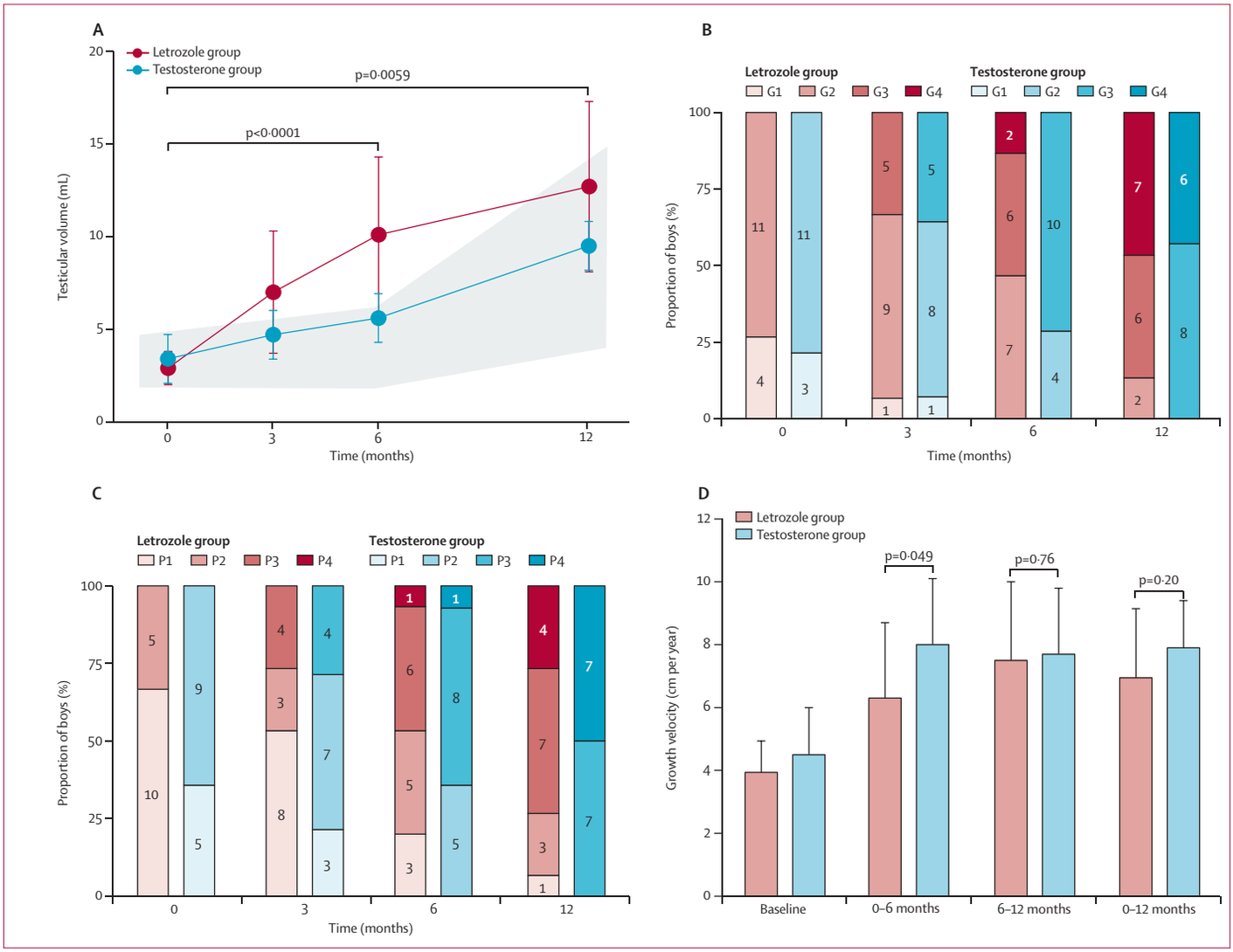

Figure 3: **Change over time in testicular volume, Tanner stages, and growth velocity**

(A) Change over time in testicular volume; circles represent means and whiskers indicate SDs; the shaded area depicts testicular growth (10–90th percentile range) in boys with untreated constitutional delay of growth and puberty (data were available for 13 boys at baseline, eight at 6 months, and 12 at 12 months); p values are for between-group differences in changes from baseline to 6 months (calculated with linear mixed model) or 12 months (calculated with unpaired *t* test). (B) Change over time in Tanner genital stages; numbers in columns indicate numbers of patients. (C) Change over time in Tanner pubic hair stages; numbers in columns indicate numbers of patients. (D) Change over time in growth velocity; p values are for difference in growth velocity between groups at each timepoint.

baseline to 6 months (p=0·0026 for letrozole and p=0·00026 for testosterone; figure 3D; appendix). During this period, boys receiving testosterone grew slightly faster (p=0·049; figure 3D) and had higher IGF-1 concentrations (p<0·0001; figure 2). Growth velocities were similar between the groups during follow-up (p=0·76) and over the entire study period (p=0·20; figure 3D; appendix).

During the 12 months of the study, the progression in bone age did not differ between boys treated with letrozole (0·7 years per year [95% CI 0·5–1·0]) and those treated with testosterone (1·0 years per year [0·7–1·5]; p=0·13). The changes in predicted adult height were similar between treatment groups (1·7 cm [95% CI 0·4–3·0] in the letrozole group *vs* 1·5 cm [0·1–2·9] in the testosterone group; p=0·78).

Between baseline and 6 months, boys treated with testosterone had a larger increase in lumbar spine bone mineral density than did boys treated with letrozole (0·03 g/cm² [95% CI 0·01 to 0·05] *vs* 0·01 g/cm² [–0·01 to 0·02]). Between baseline and 12 months, left hip and lumbar spine bone mineral densities increased more in the testosterone group than in the letrozole group (0·07 g/cm² [95% CI 0·04 to 0·11] *vs* 0·03 g/cm² [0·01 to 0·06] for left hip and 0·08 g/cm² [95% CI 0·05 to 0·11] *vs* 0·04 g/cm² [0·01 to 0·07] for lumbar spine; figure 4; appendix). However, no between-group differences were observed in lumbar spine bone mineral

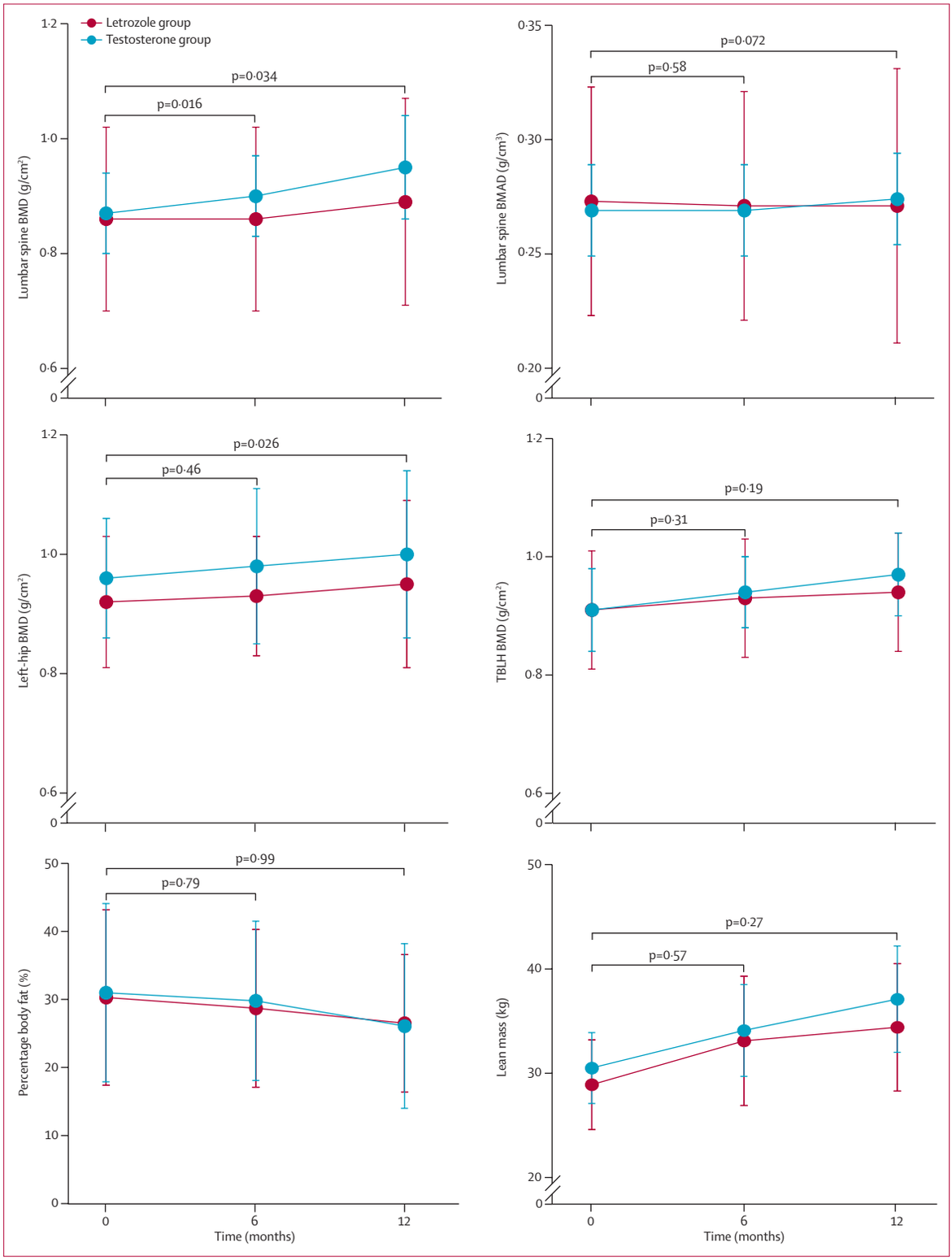

Figure 4: **Change over time in BMD, BMAD, percentage body fat, and lean mass**
Circles represent means and whiskers indicate SDs. p values are for between-group differences in changes between baseline and 6 months or 12 months. BMD=bone mineral density. BMAD=bone mineral apparent density. TBLH=total body less head.

apparent density or total body less head bone mineral density at any timepoint (figure 4).

Increments in lean mass were similar in the letrozole and testosterone groups both during treatment (4·2 kg [95% CI 2·5–5·8] and 3·5 kg [2·1–5·0], respectively) and over the entire study period (5·5 kg [3·9–7·0] and 6·6 kg [5·0–8·3], respectively; figure 4; appendix). To assess the independent effect of androgens on muscle mass, we examined the association between lean body mass and testosterone concentration in the letrozole group. The change in lean mass during treatment showed a strong positive correlation with the treatment-related change in serum testosterone (r=0·74, p=0·0083). None of the letrozole-treated boys had deformities in thoracic or lumbar spine x-rays at baseline or 12 months.

Puberty progressed in all treated boys, and none were readmitted to the outpatient clinic for re-evaluation of delayed puberty. Five (33%) patients in the letrozole group and six (40%) in the testosterone group reported adverse events during the treatment period (ie, at the 3 month or 6 month visit). These were mostly mild, and included back pain (n=1), dry eyes (n=1), and enuresis (n=1) in the letrozole group, and back pain (n=1), nausea after injection (n=1), migraine (n=1), and testicular pain (n=1) in the testosterone group (table 2). A moderate-to-severe adverse event was recorded in one boy treated with low-dose testosterone; he had aggressive behaviour for 1 week after each injection. Apart from increased irritability in one participant at 6 months, none of the boys in the letrozole group reported any treatment-related adverse event, despite some boys exhibiting significantly elevated serum testosterone concentrations (up to 56·6 nmol/L). No patient had severe or long-term musculoskeletal symptoms.

Two boys in the letrozole group had short treatment pauses: one because of elevated concentrations of aspartate aminotransferase and alkaline phosphatase (treatment was stopped for 2 weeks) and the other because of myocarditis caused by parainfluenza virus infection (treatment was paused for 8 days). In four boys treated with letrozole, serum testosterone concentration exceeded 30 nmol/L at the 3-month visit, and their letrozole dose was reduced to 2·5 mg once every other day for the last 3 months of treatment.

	Letrozole group (n=15)	Testosterone group (n=15)
Any adverse event	9 (60%)	8 (53%)
Mild adverse events		
Musculoskeletal symptoms (transient back pain, joint pain)	3 (20%)	3 (20%)
Gastrointestinal symptoms (abdominal pain, diarrhoea, nausea)	2 (13%)	1 (7%)
Neurological and vision symptoms (dry eyes, migraine)	2 (13%)	1 (7%)
Genitourinary symptoms (frequent urination, pain in testicle)	1 (7%)	1 (7%)
Moderate-to-severe adverse events		
Fracture (hand or wrist fracture)	1 (7%)	1 (7%)
Infections (myocarditis)	1 (7%)	0
Elevated liver enzymes	1 (7%)	0
Mood and behavioural symptoms (irritability, aggressive behaviour)	1 (7%)	1 (7%)

Data are number of patients (%). Patients could have more than one adverse event. Adverse events include those before, during, and after treatment.

Table 2: **Adverse events**

Discussion

The results of this randomised, controlled, multicentre study suggest that, in boys with CDGP, both short-term letrozole and testosterone treatments stimulate growth, induce similar changes in body composition, and are well tolerated, but that low-dose testosterone is less efficacious than letrozole in promoting testicular growth. This finding is probably due to the opposite effects of the two drugs on HPG axis activity: whereas low-dose testosterone suppresses gonadotropin secretion, letrozole (an aromatase inhibitor) activates it through depletion of oestrogen. Boys treated with letrozole had larger testes than boys treated with testosterone 6 months after cessation of treatment (ie, at the time when testosterone-treated boys are expected to show HPG axis activation[6,7]). This persisting difference between groups was more likely due to rapid testicular growth during letrozole therapy than to androgen-mediated differences in HPG axis maturation given that the sex steroid and gonadotropin concentrations, as well as LH responses to GnRH at the end of the study, were similar between groups. Longer-term follow-up is required to confirm this hypothesis.

Reassurance and psychosocial counselling (if necessary) are the first and usually sufficient means of managing boys with self-limited delayed puberty if they present with the initial clinical signs of puberty. The boys enrolled in this study, however, chose medical intervention after receiving information about the likely benign nature of their condition. Based on their stage of puberty, the boys in our study represent the target group for medical intervention (appendix). Indeed, using testicular volume of 4 mL as a conservative clinical indicator of puberty onset,[29] 87% of boys in this study would have been classified as prepubertal at the start of treatment. This study was designed a priori to compare hormonal and physiological changes of puberty induced by low-dose testosterone or letrozole, rather than to compare boys who received either treatment with untreated boys.

The risks and benefits of manipulating the reproductive axis during early puberty should be weighed carefully. For example, letrozole-induced gonadotropin secretion and ensuing high concentrations of intratesticular testosterone might affect development of seminiferous epithelium.[30] However, circulating concentrations of inhibin B remained stable during letrozole treatment in boys with CDGP in our study, as well as in midpubertal boys with CDGP and boys with idiopathic short stature after entering puberty in previous studies.[14,31] By contrast, boys with CDGP who

were treated with low-dose testosterone in this study exhibited a transient decline in serum gonadotropin and inhibin B concentrations. This decline was unexpected given that low-dose testosterone was previously shown to suppress FSH and LH, but not inhibin B, in midpubertal boys with CDGP.[31] During early puberty, proliferating immature Sertoli cells secrete inhibin B in an FSH-dependent manner,[32–34] and their number gradually becomes fixed, probably via androgen receptor-mediated differentiation.[30,35,36] The testosterone-mediated decline in inhibin B might thus reflect altered Sertoli cell development in early puberty, and future studies should investigate the clinical significance of this finding.

Optimal medical management of CDGP should promote growth, and this requirement was fulfilled by both treatment regimens. During treatment, boys treated with testosterone had higher IGF-1 concentrations and gained on average 0·8 cm more in height than did boys treated with letrozole. This difference was expected because oestrogen is known to stimulate the growth hormone–IGF-1 axis,[37,38] and we and others have shown that oestrogen depletion with aromatase inhibition prevents the pubertal increase in IGF-1 concentrations and delays the progression of bone age.[14,39] In this study, the rate of bone age progression did not differ significantly between groups, although this study was not powered to detect differences in epiphyseal maturation. To this end, letrozole might improve adult height by suppressing the rate of bone maturation in boys with CDGP, although available data are inconclusive.[40,41] We did not measure adult height, but the change in predicted adult height was similar in the two treatment groups. Long-term follow-up is needed before the effects on adult height, if any, are detectable.

In boys, percentage of fat mass decreases and lean mass and bone mass increase during puberty.[42] The changes in percentage body fat and lean mass were similar in the treatment groups, whereas bone mineral density measures increased less in boys treated with letrozole than in those treated with testosterone. However, there was no difference between groups when lumbar spine bone mineral density was adjusted for the size of the vertebrae by calculation of bone mineral apparent densities. Taken together, these findings suggest that aromatisable androgen (ie, testosterone) expedites bone mineral accrual more efficiently than does treatment with a compound with exclusive androgenic effects (ie, an aromatase inhibitor), but this difference might result from strong stimulation of bone growth or expansion by testosterone rather than impairment of true volumetric bone mineral density by letrozole. The effects of aromatase inhibition on bone mineralisation in pubertal boys are of clinical interest given that some degree of oestrogen activity is required for normal accrual of peak bone mass in men.[43] However, previous studies[14,31,43,44] of letrozole versus placebo in adolescent boys did not find any significant adverse effects for short-term letrozole treatment on bone mineral density, even after 2 years of treatment. We did not observe any vertebral body changes in boys treated with letrozole in this study, which is consistent with the concept that short-term aromatase inhibition has no detrimental effect on spine health when used after the onset of puberty.[45] Furthermore, no adverse effects were reported in previous studies[39,46] that administered the aromatase inhibitor anastrozole for a considerably longer period of time than letrozole was administered in this study.

This study has several limitations. We did not include a placebo group because we did not consider it to be ethically acceptable, and we used an open-label design, which was inevitable because peroral testosterone preparations are not used for children in Finland. We considered masking the investigator who collected data on the primary outcomes to treatment allocation, but this was not possible for practical reasons. Despite taking careful measures to exclude boys with congenital hypogonadotropic hypogonadism, some cases might not have been detected because no reliable method exists to differentiate between CDGP and congenital hypogonadotropic hypogonadism. In adolescence, diagnosis of hypogonadotropic hypogonadism is challenging, especially if gonadotropin deficiency is not complete.[18] Despite careful phenotyping and biochemical evaluation according to recent guidelines,[16] one boy who selected careful watchful waiting in our study was diagnosed with congenital hypogonadotropic hypogonadism soon after completion of the study. This diagnosis was based on the lack of spontaneous testicular growth during the study; low testosterone, gonadotropin, and inhibin B concentrations; and detection of a heterozygous missense mutation in *FGFR1* (c.1009G>C, p.[Gly337Arg]). Another limitation is that the long-term effects of puberty-promoting medications are not well known, and we speculate that the groups in our study will eventually catch up in terms of somatic changes. It should be noted that the difference in testicular growth between the study groups might be partly attributable to differences in serum testosterone concentration, and future studies could be designed to assess the relative roles of sex-steroid balance and gonadotropins in the treatment outcomes. Other potential sources of imprecision are the exclusion of one boy in the testosterone group because of a dosing error and the limited ability to ensure compliance to letrozole. Additionally, true dose-finding studies of letrozole in children have not yet been done, and so we used the fixed letrozole dose on the basis of previous studies.[14,30] Finally, the effect of aromatase inhibitor treatment on social function, body image, self-esteem, psychosocial wellbeing, bone architecture, and adult height are unclear and need to be addressed in future studies.

In conclusion, we found that letrozole was more efficacious than testosterone in promoting testicular growth, and both treatments significantly increased growth velocity (testosterone slightly more than letrozole), had no

detrimental effect on bone mineralisation, induced pubertal changes in body composition, and were well tolerated. Taken together, our data suggest that letrozole might be a feasible alternative to low-dose testosterone for boys with CDGP who present with the first biochemical or clinical signs of puberty, select medical intervention, decline injections, and expect comprehensive physiological changes of puberty, including testicular growth. By contrast, low-dose testosterone might be the primary option for boys who prioritise rapid height gain during treatment.

Contributors

MH, TR, PJM, HH, RV, JT, M-AP, and ST contributed to participant enrolment. TV, MH, TR, EH, and PJM designed the study. TV, HH, JT, M-AP, EH, ST, and RV acquired the data. ML designed the data analyses. TV, MH, TR, ML, LK, AT, KV, and ST-S analysed and interpreted the data. All authors contributed to drafting, reviewing, and editing the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Anonymised individual-participant data that underlie the primary endpoints of this trial will be made available 9 months after publication to investigators of methodologically reliable meta-analyses. Proposals may be submitted up to 24 months after publication of the Article and should be directed to the senior author (taneli.raivio@helsinki.fi). Data will be shared according to the EU General Data Protection Regulation and national and hospital data protection regulations.

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