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ORIGINAL ARTICLE

Puberty induction in Turner syndrome: results of oestrogen treatment on development of secondary sexual characteristics, uterine dimensions and serum hormone levels

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Summary

Background Besides short stature, gonadal dysgenesis leading to a lack of oestrogen is one of the main characteristics of Turner syndrome (TS). In most TS girls, puberty is induced with exogenous oestrogens.

Objective To describe the pubertal development and uterine dimensions achieved by low-dose 17β-oestradiol (17β-E2) orally started at an appropriate age. Additionally, to determine whether serum hormone levels aid evaluation of pubertal progression.

Design In 56 TS girls, we prospectively studied pubertal stage, serum E2, LH, FSH, SHBG and oestrone (E1), starting oestrogen treatment with a low-dose 17β-E2 (5 μg/kg/day) during GH treatment at mean (SD) age 12·7 (0·7) years. Hormone levels were measured at start, 3 months after start and after increasing 17β-E2 dosage. Uterine dimensions were measured in 39 TS women at age 19·9 (2·2) years.

Results Although breast and pubic hair development were similar to that in normal Dutch girls up to Tanner stage B5 and P5, respectively, breast development was 2 years later. Before oestrogen therapy, E2 levels were comparable to those in prepubertal girls. With a 17β-E2 dose of 5 μg/kg/day, these levels increased significantly, becoming comparable to normal late pubertal or adult concentrations, whereas SHBG levels were unchanged. At the adult 17β-E2 dose, SHBG had increased significantly. Uterus shape was juvenile in four (10·2%), cylindrical in four and mature-adult shaped in 31 (79·5%) of TS patients.

Conclusions During GH treatment in TS girls, normal breast development up to B5 can be mimicked, with just a 2-year delay. In a clinical setting, serum hormone levels provide no additional information for evaluating pubertal progression. After age-appropriate pubertal induction, uterine dimensions in women aged nearly 20 years were subnormal. It remains unclear whether this was related to E2 dosage, timing or duration, or factors related to TS.

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Introduction

Besides short stature, gonadal dysgenesis is one of the main characteristics in Turner syndrome (TS). It leads to lack of oestrogens, which play an essential role in changes occurring during female puberty, such as the development of secondary sexual characteristics, the establishment of fertility, and the pubertal growth spurt.

Between 5% and 10% of women with TS start pubertal development spontaneously, more frequently in women with mosaic TS than in those with the 45,X karyotype (40% *vs.* 8%). However, very few of these women maintain ovarian function, and spontaneous pregnancies are rare (approximately 2–5%).[1–3] In most of the girls with TS, puberty has to be induced with exogenous oestrogens.

Uterine dimensions in untreated girls and young women with TS are small for age, and are described as prepubertal.[4,5] While some studies have reported that oestrogen therapy in early to mid-adolescence leads to normal uterine development,[6,7] others have reported that development of the uterus after oestrogen therapy is suboptimal.[8–10] However, the differences between these studies, which included different age ranges, routes of oestrogen treatment and forms of oestrogen therapy, indicate that their results are difficult to compare. Most of these studies did not have a standardized puberty-induction treatment protocol.

The debate on TS has often focused on the effect of oestrogen therapy on adult height, and on whether oestrogen therapy should be started during GH treatment or afterwards.[11–14] Some studies, including our own, have shown that it is not necessary to delay the induction of puberty when GH treatment has been optimized,[15–17] and that oestrogens in a low dose do not negatively influence height velocity or adult height.

Little is known about breast development in TS girls with oestrogen treatment started at an appropriate age in a low dose. The present study

therefore describes the breast development and uterine dimensions that followed puberty induction, started at an appropriate age, using low doses of oral 17β-oestradiol (17β-E2). We also discuss whether measurements of serum levels of E2, oestrone (E1), gonadotrophins and SHBG are useful in evaluating induced pubertal development.

Patients and methods

Study design

This study was performed in a population of patients with TS who participated both in a prospective GH trial and in a cross-sectional follow-up study.

GH trial. As described by van Pareren et al.,[15] 68 previously untreated girls with TS started in an open-randomized multicentre GH dose–response study that began in the Netherlands in November 1989. In this prospective study, the diagnosis of TS was based on both characteristic physical features and complete or partial absence of the second sex chromosome, with or without cell-line mosaicism. Subjects were included if their chronological age was between 2 and 11 years and their height below the 50th percentile according to normal Dutch references,[18] as we did not want to treat girls taller than the median Dutch girl. Pre-study height standard deviation score (SDS) of the TS girls was –2·8 (0·9) compared to normal Dutch girls[19] and 0·2 (1·0) compared to Turner references.[20] Every 3 months during the GH trial, the height and weight of the TS girls were measured, and their pubertal stage assessed according to Tanner[21]. The GH treatment was discontinued when final height had been attained; in the study protocol, this was defined as a height velocity of less than 1 cm over 6 months. Height and weight were expressed as SDS using the references for healthy Dutch girls[19] or the references for North European untreated girls with TS.[20]

To induce puberty, a daily oral dose of micronized 17β-E2 was given to girls aged 12 years and over who had already undergone at least 4 years of GH treatment. In the first 2 years, a dose of 5 μg/kg body weight/day (equivalent to 0·05 μg ethinyl oestradiol/kg/day) was given; in the third year the dose was raised to 7·5 μg/kg/day, and thereafter it was 10 μg/kg/day (tablets containing 0·1 mg micronized 17β-E2). After 2 years of oestrogen therapy, cyclic progestagen therapy 5 mg/day was added in the first 14 days of the month. If start of puberty had developed spontaneously into Tanner breast stage B2, the start of oestrogen therapy was postponed for 1 year. If, 1 year later, Tanner breast stage was still B2, oestrogen therapy was started according to the schedule as described above. If Tanner breast stage was ≥ B3, no oestrogens were given.

Post-GH trial. Six months after GH therapy ended, one more visit took place as part of the GH trial. Thereafter, regular check-ups were performed by the girls' paediatric endocrinologist. A follow-up study took place 4·8 (2·0) [mean (SD)] years after the end of GH therapy. As well as other assessments, this involved assessment of the pubertal stages and pelvic ultrasound of the internal genitalia.

After the end of GH therapy, oestrogen treatment was increased to an adult dose of 1 mg/day, and additionally to 2 mg/day. Cyclic progesterone dosage was increased to 10 mg/day.

At the end of GH therapy, pubertal development was re-evaluated in girls who had had spontaneous start of puberty and no oestrogen treatment during GH therapy. If progression of breast development was insufficient in these girls, oestrogen substitution therapy was initiated.

Study subjects

As described above, 68 girls with TS started in the GH trial. Four girls dropped out before the start of oestrogen therapy and could not be included in this evaluation. Fifty-six girls out of the 64 had karyotype 45,X, and eight had a variant karyotype.

Six girls entered puberty spontaneously, four with karyotype 45,X and two with a variant karyotype; none of these six girls were included in the evaluation of breast development and the evaluation of serum hormone levels (see below). Two girls dropped out of the study 3 and 6 months after start of oestrogen therapy and could not therefore be included in the evaluation of the pubertal development and hormone levels. This left $n = 56$ for analysis (Fig. 1), 50 of whom had karyotype 45,X and six a variant karyotype.

Thirty-nine girls participated in the follow-up study, a mean (SD) of 4·8 (2·0) years after the end of GH treatment. Twenty-five did not participate in the follow-up study due to either lack of motivation ($n = 18$) for reasons including psychological problems, practical considerations or lack of interest. One was lost to follow-up due to emigration, and six were not included due to mental retardation or autism. Of the remaining participants, six had undergone a spontaneous start of puberty, and in 33, puberty was induced according to the study protocol (Fig. 1). Five of the six girls with spontaneous start of puberty had started oestrogen treatment at GH discontinuation. Thirty-one girls out of the 39 had karyotype 45,X, and eight had a variant karyotype.

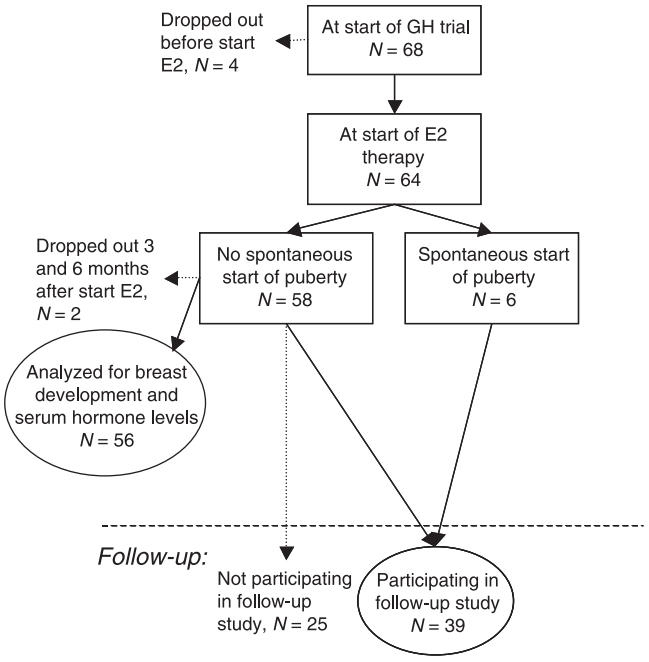

Fig. 1 Flow diagram of the patients in the initial GH trial and the follow-up study.

The GH trial protocol was approved by the Medical Ethics Committees of each participating centre, and the follow-up study protocol was approved by the Medical Ethics Committee at Erasmus University Medical Centre. Written informed consent was obtained from the girls and/or their parents.

Measurements

Secondary sexual characteristics. During the GH trial, pubertal stage was determined every 3 months using the criteria and definitions described by Tanner.[21] After the GH trial, data were collected from the hospital information systems of the different hospitals, up to a mean age of 16·7 (1·2) years, range 14·5–19·8 years. Breast development was compared to that in healthy Dutch girls ($n = 3562$).[22]

Serum hormone levels. The serum concentrations of E2, E1, LH, FSH and SHBG were measured at start of oestrogen therapy, 3–6 months after start of oestrogen treatment, and after each increase in oestrogen dosage. Oestrogens were administered on the morning of the hospital visit. To measure the serum E2 levels at the highest expected serum concentration, blood samples were taken 4–6 h after oestrogen administration.[23] Concentrations of serum E2 and E1 were measured using radioimmunoassay kits provided by Diagnostic Products Corporation (Los Angeles, CA) and Diagnostic Systems Laboratories (Webster, TX). In the E2 assay, cross-reactivities of all other steroids tested, including oestrogen sulphates and glucuronides, were below 0·3%, with the exception of E1 (10%), oestriol (0·32%), E1 glucuronide (1·8%) and E1 sulphate (0·58%). Similarly, in the E1 assay the only cross-reactions exceeding 0·3% were those for E2 (1·25%) and 16α-hydroxy-E1 (0·46%). Intra- and interassay coefficients of variation (CVs) were, respectively, below 10·2% and 8·8% for E2 and below 9·4% and 11·1% for E1. Sensitivities of the assays were 10·0 and 4·4 pmol/l for E2 and E1, respectively. LH, FSH and SHBG were estimated using luminescence-based immunometric assays (Immulite 2000, Diagnostic Products Corporation). Intra- and interassay CVs were, respectively, below 3·5% and 7·1% for LH, below 3·0% and 5·8% for FSH and below 4·8% and 6·0% for SHBG. Assay sensitivities were 0·1 IU/l for the gonadotrophins and 0·2 nmol/l for SHBG.

Serum E2 levels were compared to normal levels as described by Sehested *et al.*,[24] who studied a normal female population ($n = 403$) without oral contraceptives during pubertal development. Serum E2, LH and FSH were reported according to breast stage, median chronological age and range.

Pelvic ultrasound. Pelvic ultrasound was part of a follow-up study performed 4·8 (2·0) years after the end of GH therapy, when the girls were aged 19·9 (2·2) years. Mean duration of oestrogen therapy at ultrasound measurement was 7·1 (2·2) years. The ultrasonographic examination was performed transabdominally according to the conventional full-bladder technique. The ovaries were measured when visible, and their volume was calculated. The fundo-cervical (FC) ratio was calculated as: [anterior-posterior (AP) diameter of the fundus]/(AP diameter of the cervix). Uterine shape was described as juvenile (cervix larger than fundus), cylindrical (mid-childhood with cervix and fundus approximately the same), and mature-adult shape (fundus larger than cervix).[25] Uterine volume was calculated according to the formula for a prolate ellipsoid: maximal depth × maximum width × maximum length × 0·523.[25] Girls who had entered puberty spontaneously were included in the analysis of the pelvic ultrasound.

Statistical methods

Unless indicated otherwise, the results are expressed as mean (SD). Repeated measurement models were used to compare serum hormone levels with the different oral E2 dosages over time. As oral oestrogen dose was increased more rapidly to an adult dose after discontinuation of GH, not all girls had an oestrogen dose of 7·5 μg/kg/day ($n = 38$) and 10 μg/kg/day ($n = 21$). Hormone levels at the adult E2 dosage of 1 and 2 mg/day were taken together for analyses. As serum could not be collected from all patients at the adult oral E2 dose, the repeated measurement was performed separately in the subgroup concerned ($n = 19$) for purposes of comparison with the last measurement available (5, 7·5 or 10 μg/kg/day) and with the baseline measurement. Geometric means and 95% confidence intervals (CIs) of the estimates are given. Spearman's correlations were used to assess the relationship between breast stage and serum E2 levels; between duration from breast stage B2–B4 and age at start of oestrogen therapy; and between serum levels of E2 and serum levels of LH, FSH, SHBG and E1. Student's *t*-tests were performed to analyse the differences in uterine length, shape and volume between women having karyotype 45,X and those with a variant karyotype, and between woman who had spontaneous pubertal onset and those who had not. *P*-values < 0·05 were considered to be statistically significant. All calculations were performed with SPSS 11·5.

Results

Study subjects

In the 56 girls without spontaneous start of pubertal development, the mean age at start of puberty induction was 12·7 (0·7) years, with a range of 11·8–15·0 years. The mean GH duration before start of oestrogen therapy was 6·2 (1·9) years. The mean GH duration after start of oestrogen therapy was 2·6 (0·9) years. Six girls entered puberty spontaneously, one of whom had regular menstrual cycles. The remaining five started oestrogen therapy at GH discontinuation, at a mean age of 15·1 (1·1) years.

Secondary sexual characteristics

The average age (50th percentile) (P10–P90) for attaining the different stages of breast and pubic hair development are shown in Table 1. Figures 2 and 3 present the reference curves of the breast and pubic hair development, respectively, in our treated TS population and in the normal Dutch population, as presented previously by Mul *et al.*,[22] adapted with permission. The broken lines in these figures present the crude data. The P_{50} chronological age can be read from the figures. These figures also show the interval between the Tanner stages.

Table 1. Mean (range) chronological age at which the different stages of secondary sexual characteristics were reached

	Turner population P50 (P10–P90)		Normal Dutch population* $N = 3562$ P50 (P10–P90)	
Stage	Breast stage	Pubic hair stage	Breast stage	Pubic hair stage
2	$N = 56$	$N = 56$		
	12·67 (12·06–13·53)	10·32 (8·28–12·36)	10·72 (9·0–12·2)	11·01 (9·4–12·5)
3	$N = 54$	$N = 54$		
	13·76 (12·79–15·76)	11·87 (10·07–13·67)	11·90 (10·5–13·1)	11·89 (10·6–13·2)
4	$N = 49$	$N = 55$		
	15·13 (13·61–18·12)	13·15 (11·57–14·72)	12·84 (11·5–14·5)	12·68 (11·4–14·3)
5	$N = 23$	$N = 46$		
	19·23 (15·19–NA)	15·13 (13·25–NA)	14·34 (12·5–19·5)	13·76 (12·1–17·7)

*Mul *et al.*[22]

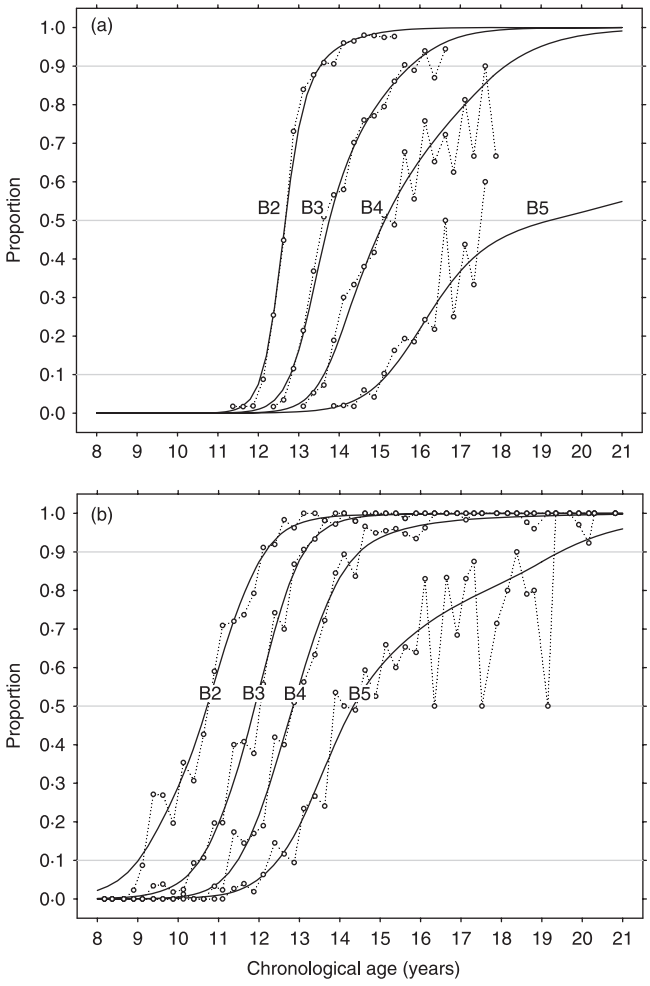

Fig. 2 Breast stages according to Tanner in girls with Turner syndrome (a) who started 17β-E2 at an appropriate age compared to (b) a normal Dutch reference population. Adapted from Mul *et al.*[22] with permission.

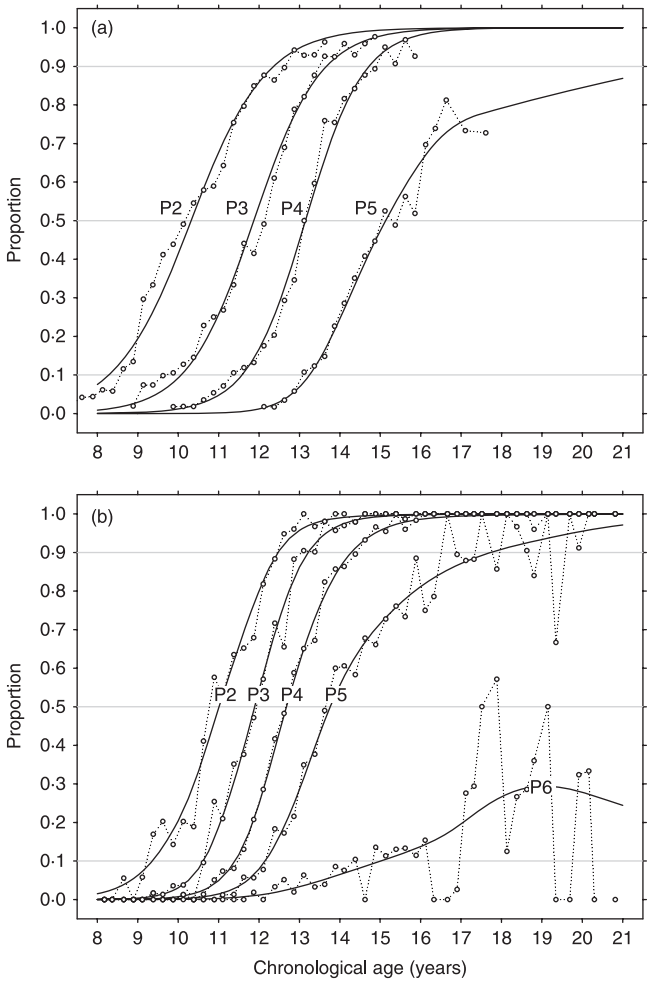

Fig. 3 Pubic hair stages according to Tanner in girls with Turner syndrome (a) who started 17β-E2 at an appropriate age compared to (b) a normal Dutch reference population. Adapted from Mul *et al.*[22] with permission.

Table 2. Mean ± SD (range) of uterine dimensions during follow-up study 7·1 (2·2) years after start of oestrogen therapy and 4·8 (2·0) years after discontinuation of GH

		Karyotype	
	Total group ($N = 39$)	45X ($N = 31$)	'Variant' ($N = 8$)
Chronological age (years)	19·9 ± 2·2 (15·0–23·4)	19·7 ± 2·1 (15·0–23·2)	20·7 ± 2·4 (16·6–23·4)
Uterus length (mm)	60·1 ± 15·9 (25·0–87·0)	58·9 ± 16·7 (25·0–87·0)	66·9 ± 11·4 (49·0–83·0)
Uterus volume (ml)	24·8 ± 15·0 (4·4–57·9)	22·3 ± 13·0* (4·4–57·5)	34·5 ± 19·0 (7·9–57·9)
Fundo-cervical ratio	1·5 ± 0·5 (0·8–2·8)	1·4 ± 0·5 (0·8–2·8)	1·5 ± 0·3 (1·1–2·0)

*Significant difference between patients with karyotype 45,X and 'variant' with $P < 0·05$.

The different breast stages advanced gradually, and were comparable to development in the normal Dutch female population up to B5,[22] albeit with a 2-year delay (Fig. 2, Table 1). At the age of 19 years, 50% of the TS women reached B5 compared to 90% of the normal Dutch women. Six of the 56 girls had some breast development (B2) without further spontaneous progression, and started oestrogen treatment 1 year later according to the protocol.

The development of pubic hair in the TS girls was similar to that in the normal Dutch female population (Fig. 3, Table 1). However, P5 was reached at 15·13 years in the TS girls compared to 13·76 years in the normal Dutch population, and P6 was not scored in the TS girls. Seventeen of the TS girls started to have withdrawal bleeding 1·5 (1·2) years after start of dydrogesterone 5 mg/day, and 30 started to have withdrawal bleeding 0·5 (0·6) years after start of a dydrogesterone dose of 10 mg/day.

Serum hormone levels

Figure 4 shows the geometric mean and the 95% CI of serum E2, E1, LH, FSH and SHBG concentrations at the different oral 17β-E2 dosages. At 24·7 pmol/l (95% CI 22–28), the mean serum E2 concentration at start of oestrogen therapy [mean age 12·7 (0·7) years] was comparable to that in prepubertal girls (mean age 9·1 years), with serum E2 levels of 22 pmol/l (95% CI < 18–52).[24] Measured at the expected peak concentration, the serum E2 concentration increased significantly to 202 (95% CI 176–231) pmol/l at a 17β-E2 dose of 5 μg/kg/day, which is comparable to normal late pubertal (breast stage 4/5) or adult serum E2 concentrations (B4: 162 pmol/l, 95% CI < 18–1094 pmol/l, B5: 182 pmol/l, 95% CI 27–1108 pmol/l, or adults: 289 pmol/l, 95% CI 74–1075 pmol/l, respectively).[24] The serum E2 levels at the adult oral dose of 1 or 2 mg/day (mean 703 pmol/l, 95% CI 552–895 pmol/l) were significantly higher than the serum E2 levels at the preceding dose. Serum E1 concentrations rose significantly along with the increase in 17β-E2 dosage.

Mean LH concentrations at an oral 17β-E2 dose of 5 μg/kg/day were lower than before the start of 17β-E2 (6·4 IU/l, 95% CI 4·7–8·8 IU/l and 10·6 IU/l, 95% CI 9·0–12·4 IU/l, respectively). The LH levels at oral 17β-E2 dosages of 7·5 and 10 μg/kg/day were higher relative to the levels at the 5 μg/kg/day, and also significantly higher than before the start. FSH concentrations showed a significant decrease after the start of oestrogen therapy. SHBG concentrations did not change after starting the lowest dose of 5 μg/kg/day, and decreased significantly with a dose of 7·5 and 10 μg/kg/day. The serum SHBG levels at the adult oral dose of 1 or 2 mg/day (mean 48 nmol/l, 95%CI 43–55 nmol/l) were significantly higher than the serum SHBG levels before start (37 nmol/l, 95%CI 33–42 nmol/l) and than during the preceding oestrogen dose.

Pelvic ultrasound

Uterus. Uterine dimensions are shown in Table 2. Uterine volume in patients with karyotype 45,X was significantly smaller than in patients with variant karyotype. There were no significant differences between patients with spontaneous start of puberty and those without. Fundo-cervial ratio (FCR) was juvenile (FCR < 1) in four patients (10·2%), cylindrical (FCR ≈ 1) in four others, and mature-adult shaped (FCR > 1) in 31 (79·5%). None of the patients with variant karyotype had a juvenile-shaped uterus, and one out of the eight had a cylindrically shaped uterus.

Ovaries. Streaks or no ovaries were detected in 17 girls out of the 39. In four girls (one with spontaneous puberty) one ovary was detectable, while in 18 girls (two with spontaneous puberty) two ovaries were visualized. The mean ovarian volume of all ovaries measured (n = 40) was 2·5 (2·2) ml. The volumes of the ovaries detected in the girls with start of spontaneous puberty were not significantly larger than those measured in the girls without spontaneous start of puberty. The girl with regular menstrual cycles had ovarian volumes of 1·3 and 1·5 ml.

Correlations

Age at start of oestrogen therapy was negatively correlated with the duration from B2 to B4 ($r = –0·30$, $P < 0·05$). Serum E2 concentration was positively correlated with breast stage ($r = 0·71$, $P < 0·001$). Serum E2 concentration was negatively correlated with serum FSH ($r = –0·31$, $P < 0·001$), but not with serum LH ($r = 0·01$, $P = 0·87$), nor with serum SHBG ($r = 0·04$, $P = 0·625$). It was also strongly correlated with serum E1 concentration ($r = 0·81$, $P < 0·001$).

Discussion

The main purpose of oestrogen therapy in girls with TS is to induce puberty and feminization as physiologically as possible, without

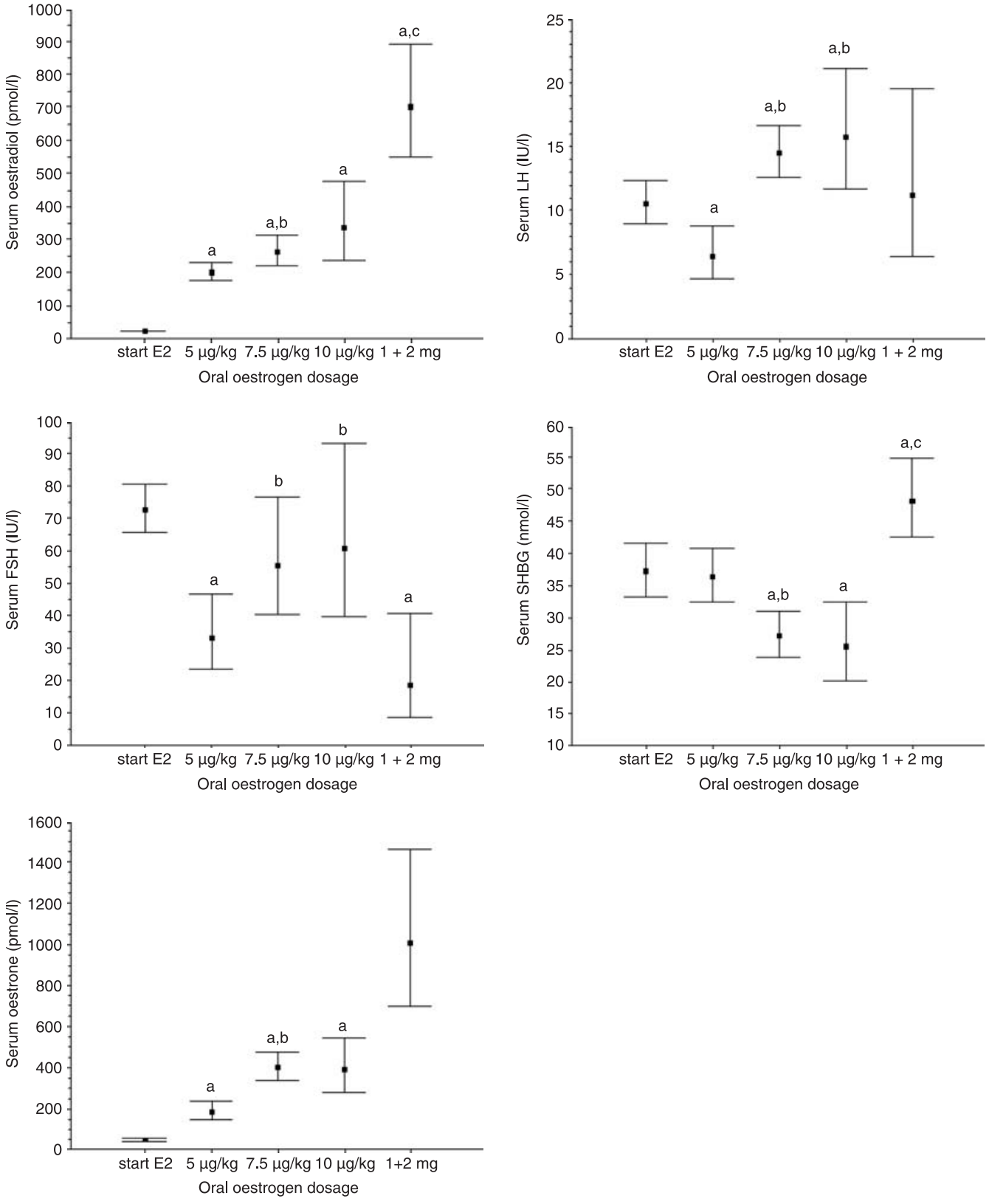

Fig. 4 Geometric mean and 95% confidence interval for (a) serum E2 (pmol/l), (b) LH (IU/l), (c) FSH (IU/l), (d) SHBG (nmol/l) and (e) E1 (pmol/l) at different oral 17β-E2 dosages. Analysis of repeated measurements produced the following significant differences (P-value < 0·05): A, compared to start E2; B, for the oral dosage of 7·5 and 10 µg/kg/day compared to preceding dosage of 5 and 7·5 µg/kg/day, respectively; C, adult dose of 1 or 2 mg/day compared to the levels at the last oral dosage taken.

sacrificing adult height. The present study shows the results of puberty induction during GH treatment in girls with TS, starting at an age of 12·7 (0·7) years. The age of 12 years was chosen to start puberty induction as it is near the normal range, just 2 years later than that of the mean population (Fig. 2). Induction was started with a very low dose of 17β-E2 for 2 years before the oestrogen dose was slowly increased. 17β-E2 is a natural oestrogen that has less pronounced effects on the coagulation factors, lipid profiles and blood pressure than synthetic oestrogens.[26] The low dose did not affect height velocity.[15]

Although it has been reported previously that normal progression through the breast stages is possible in girls with TS,[7,11] the results are difficult to compare, as the form, dosage and route of the oestrogen administration differ between studies. Chernausek *et al.*[11] used conjugated equine E2 in a daily dose of 0·3 mg (~0·48 mg 17β-E2) orally over 6 months, following this with 0·625 mg daily, that is a higher oestrogen dose than used in our study.[27] As their dosage was thus relatively high with a faster dosage increase, it was not beneficial for adult height.[11] Rosenfield *et al.*[17] showed that GH treatment with a lower dose of oestrogens from age 12 years onwards enhanced height velocity while preserving adult height. Age at start of puberty induction with percutaneous oestrogen in the study of Piippo *et al.*[7] had a large range (10·7–17·7 years).

Our study shows that a low dose of oral oestrogen results in normal breast development in the majority of Turner women up to B5, just 2 years later than in their peers. As adult height is not affected by this low dose, it is possible to start this oestrogen treatment at an appropriate age.

Breast development up to B5 was comparable to normal. At the age of 19 years, 50% of the TS women reached B5 compared to 90% of the normal Dutch women. The stunted breast development in late adolescence may be due to the oestrogen dose regimen. Although after cessation of GH treatment oestrogens were increased to an adult dose, this dose might not be sufficient to reach B5 in all Turner girls. Furthermore, the suboptimal breast development may be due to their having TS.

Our results also showed that the older the TS girls at start of oestrogen therapy, the faster the progression through puberty. This can probably be explained by the faster increase in oestrogen dose, which was increased to an adult dose after final height had been attained. This phenomenon mimics the natural situation in normal girls, where an earlier onset of puberty corresponds with a longer duration.[28]

Pubic hair developed similar to normal, although in our Turner girls P5 was reached later than in normal girls, and P6 was not observed at all. Pubic hair development had started in most girls before oestrogen treatment was initiated. This indicates that, although ovarian androgen production is lacking, start of adrenarche in girls with TS proceeds normally, which is in agreement with earlier reports.[29] The differences between the treated TS girls and the normal girls regarding P5 and P6 may be due to the reduced androgen levels in TS compared to normal, which is more pronounced later in adolescence.[30,31]

The peak levels of serum E2 at the lowest oral oestrogen dose were comparable to normal late pubertal or adult serum E2 concentrations.[24] The lowest E2 (5 μg/kg/day) dose suppressed LH and FSH. However, during treatment with the double E2 dose (10 μg/kg/day), also providing double serum E2 levels, LH and FSH levels increased. This effect may be a result of changed sensitivity of the E2 feedback, possibly due to an increasing age or to a higher tolerance. Furthermore, the E2 and E1 levels were measured at the expected highest serum concentration, which means that the *mean* serum value is lower. The lack of an increase in serum SHBG after starting oestrogen substitution in the Turner girls and the relatively high gonadotrophin levels both indicate that the overall serum E2 effect at the low E2 dose was small.

In postmenopausal women receiving 2 mg E2 per day, gonadotrophins decreased significantly,[32,33] resulting in LH and FSH levels that are comparable to[33] or lower[32] than the levels during treatment with the adult dose reported in our study. Whereas the lower 17β-E2 dosage during GH therapy did not significantly increase SHBG levels, these levels increased significantly after the adult dose. This was comparable to the situation in postmenopausal women taking 2 mg of 17β-E2, in whom SHBG levels also increased significantly.[33]

As expected, ovarian volumes were small. Of note, one girl with small ovaries had regular menstrual cycles, indicating up till now preserved hormonal function.

In our Turner population, uterine volume was smaller (mean 24·9 ml, range 4·4–57·9 ml) than that reported in normal female students of the same age who had never been pregnant (mean 61 ml, range 37–130 ml).[34] The same authors reported that the uterus had continued to grow several years after menarche, resulting in a larger uterine volume at young adulthood than in fully matured girls at the age of approximately 15 years. Most reports on uterine dimensions provide normative data up to the age of 15–16 years.[35–38] The uterine volume, length and shape in our Turner population were comparable to uterine dimensions in normal girls who have reached breast stage B5 and/or those aged 14–16 years.[35–37] As normal uterine volume increases after breast stage B5 has been reached, or after the age of 15, uterine length and/or shape will increase simultaneously. This may indicate that uterus development in our Turner population at age 19·9 (2·2) years was suboptimal compared to that in women of the same age. However, there are no normative data in the literature on uterine length and shape in women aged 15–25. Although Paterson *et al.* reported similar uterine length in an oestrogen-treated Turner population of a similar age range, uterine shape was less mature than in our population.[8] An explanation for differences between their study and ours may lie in the age at initiation of the oestrogen replacement and the period of oestrogen therapy before ultrasound measurement, as these were not reported in their paper. Furthermore, the form of oestrogen treatment may have been of influence, as they used ethinyl E2, of which the pharmacokinetics are different from those of 17β-E2.

TS women participating in an ovum donation IVF programme were reported to have a lower successful pregnancy rate per embryo transfer.[39,40] It has been proposed that, for optimum endometrial response and improved outcome, higher constant oestrogen replacement is needed before embryo transfer.[39] In our population we did not measure endometrial thickness, as we did not standardize the timing of the ultrasound before the timed withdrawal bleeding. Furthermore, if TS women attain normal adult uterine dimensions, this may also reduce the number of pregnancy-related problems

they face in an ovum donation IVF programme. It has been suggested that lacking small amounts of oestrogen during childhood or starting oestrogen replacement later than at the physiological age of the serum E2 increase, resulting in a hypoplastic uterus, may have irreversible effects.[8,9] However, Snajderova *et al.*[10] reported that, in adult TS women with a median age of 21·4 years, a higher daily dose of E2 was associated with a greater uterine length, and that uterine length was positively associated with uterine shape. These data suggest that possibly an earlier start of oestrogens and/or a higher oestrogen dose may result in normal uterine dimensions at an adult age. Alternatively, other factors related to TS may underlie uterine dimensions that remain subnormal. However, as puberty induction in our study started 2 years after the onset of physiological puberty, it is also possible that uterine development was subject to delay.

In conclusion, our study shows that when a low dose of oral 17β-E2 is started at an appropriate age to induce puberty in girls with TS, breast development is comparable to normal up to B5, with just a 2-year delay, and at the age of nearly 20 years uterine dimensions were subnormal. Pubic hair developed normally up to P5, whereas P5 was delayed and P6 was not observed at all, possibly due to decreased androgen levels as observed in TS. Serum hormone levels do not provide additional information for evaluating the progression through puberty in a clinical setting. Future studies are needed to explore whether an even earlier start with low-dose oestrogen and/or a higher oestrogen dose after attaining final height, possibly in combination with androgens, will result in normal pubertal and uterine development without compromising height potential.

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