Clinical Endocrinology

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Journal:	*Clinical Endocrinology*
Manuscript ID:	CEN-2008-000353.R2
Manuscript Type/Office:	2 Original Article - Europe, excluding UK
Date Submitted by the Author:	n/a
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Key Words:	Turner Syndrome, Puberty, Estrogen treatment, uterine dimensions, breast development

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

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This study was financially supported by Novo Nordisk A/S Bagsvard, Denmark

Short title: Pubertal development in TS

Keywords: estrogen treatment, Turner syndrome, puberty, uterine dimensions, breast development,

Word count: 4168
Number of figures: 4
Number of Tables: 2

Abstract

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

Objective: To describe the pubertal development and uterine dimensions achieved by low-dose 17β-estradiol 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 E1, starting estrogen treatment with a low dose 17β-estradiol (5 μg/kg/day) during GH-treatment at age 12.7(0.7). Hormone levels were measured at start, 3 months after start and after increasing 17β-estradiol dosage. Uterine dimensions were measured in 39 TS women at age 19.9 (2.2).

Results: Although breast and pubic-hair development were similar to that in normal Dutch girls up to B5 and P5, breast development was 2 years later. Before estrogen therapy, E2-levels were comparable to those in prepubertal girls. With a 17β-estradiol dose of 5 μg/kg/d, these levels increased significantly, becoming comparable to normal late pubertal or adult concentrations, whereas SHBG-levels were unchanged. At adult 17β-estradiol dose, SHBG had increased significantly. Uterus shape was juvenile in 4(10.2%), cylindrical in 4, 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 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 were subnormal. It

remains unclear whether this was related to E2-dosage, timing or duration, or factors related to TS.

Introduction

Besides short stature, gonadal dysgenesis is one of the main characteristics in Turner Syndrome (TS). It leads to lack of estrogens, 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's 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 (app. 2-5%)[1-3]. In most of the girls with TS, puberty has to be induced with exogenous estrogens.

Uterine dimensions in untreated girls and young women with TS are small for age, and are described as pre-pubertal[4, 5]. While some studies have reported that estrogen therapy in early to mid-adolescence leads to normal uterine development[6, 7], others have reported that development of the uterus after estrogen therapy is suboptimal[8-10]. However, the differences between these studies – which included different age ranges, routes of estrogen treatment, and forms of estrogen 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 estrogen therapy on adult height, and on whether estrogen therapy should be started during GH treatment or afterwards[11-14]. Recently, various 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 estrogens in a low dose did not negatively influence height velocity or adult height.

Little is known about breast development in TS girls with estrogen treatment started at an appropriate age in a low dose. The present study therefore describes the breast development and uterine dimensions that followed after puberty induction, started at an appropriate age, using low doses of oral 17β-estradiol. We also discuss whether measurements of serum levels of estradiol (E2), estrone (E1), gonadotropins, and sex-hormone binding globulin (SHBG) are useful in evaluating induced pubertal development.

Patient and Methods

Study design

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

GH trial: As previously described by Van Pareren et al[15], 68 previously untreated girls with TS started in an open-randomized multi-center growth hormone (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 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 SDS of the TS girls was -**

2.8(0.9) SDS compared to normal Dutch girls[20] and 0.2(1.0) SDS compared to Turner references[21]. Every three months during the GH trial, their height and weight were measured, and their pubertal stage according to Tanner[19] was assessed. 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 SD-score using the references for healthy Dutch girls[20] or the references for North European untreated girls with TS[21].

In order to induce puberty, a daily oral dose of micronized 17β-estradiol was given to girls aged 12 and over who had already undergone at least four years of GH treatment. In the first two years, a dose of 5 μg/kg body weight/day (equivalent to 0.05 μg ethinyl estradiol/kg/d) was given; in the third year the dose was raised to 7.5 μg/kg/d, and thereafter it was 10 μg/kg/d (tablets containing 0.1 mg micronized 17β-estradiol). After two years of estrogen therapy, cyclic progestagen therapy 5 mg/d 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 estrogen therapy was postponed for one year. If, one year later, Tanner breast stage was still B2, estrogen therapy was started according to the schedule as described above. If Tanner breast stage was ≥ B3, no estrogens were given.

Post GH trial: Six months after GH therapy ended, one more visit took place as part of the GH trial. Thereafter, the regular check-ups were performed by the girls' pediatric endocrinologist. A follow-up study took place mean(SD) 4.8 (2.0) 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, estrogen 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/d.

At the end of GH therapy, pubertal development was re-evaluated in girls who had had spontaneous start of puberty and no estrogen treatment during GH therapy. If progression of breast development was insufficient in these girls, estrogen 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 estrogen 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 6 girls were included in the evaluation of breast development and evaluation of serum hormone levels (see below). Two girls dropped out of the study three and six months after start of estrogen therapy, and could not thus be included in the evaluation of the pubertal development and hormone levels. This left n=56 for analysis (Figure 1), 50 of whom had karytotype 45,X, and six a variant karyotype.

Thirty-nine girls participated in the follow-up study, a mean of 4.8 (2.0) years after the end of GH treatment. Twenty-five did not participate in the follow-up study, due either to 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 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 (Figure 1). Five of the six girls with spontaneous start of puberty had started estrogen treatment at GH discontinuation. Thirty-one girls out of the 39 had karyotype 45,X, and eight had a variant karyotype.

The GH trial protocol was approved by the Medical Ethics Committees of each participating center, and the follow-up study protocol was approved by the Medical Ethics Committee at Erasmus University Medical Center. 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 three months using the criteria and definitions described by Tanner[19]. After the GH trial, data was collected from the hospital information systems of the different hospitals, up to a mean age of 16.7 (1.2), 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, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and SHBG were measured at start of estrogen therapy, three to six months after start of estrogen treatment, and after each increase in estrogen dosage. Estrogens 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 hours after estrogen administration[23]. Concentrations of serum E2 and E1 were measured using radioimmuno-assay 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 estrogen sulfates and glucuronides, were below 0.3% with exception of E1 (10%), estriol (0.32%), E1 glucuronide (1.8%) and E1 sulfate (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 were 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 variation coefficients were 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 described 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). Mean duration of estrogen 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

designed 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 *x* maximum width *x* maximum length *x* 0.523[25]. Girls who had entered puberty spontaneously were included in the analysis of the pelvic ultrasound.

Statistical methods

Unless indicated otherwise, results were expressed as mean (SD). Repeated measurement models were used to compare serum hormone levels with the different oral estradiol dosages over time. As oral estrogen dose was increased more rapidly to an adult dose after discontinuation of GH, not all girls had an estrogen dose of 7.5 µg/kg/d (n=38) and 10 µg/kg/d (n=21). Hormone levels at the adult estradiol 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 estradiol 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/d) and with baseline measurement. Geometric mean and 95% confidence interval of the estimates are given. Spearman's correlations were used to assess the relation between breast stage and serum E2 levels; between duration from breast stage B2-B4 and age at start of estrogen therapy; and between serum levels of E2 and serum levels of LH, FSH, SHBG, and E1. Student's t-tests were performed to analyze 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 11.8–15.0 yr. The mean GH duration before start of estrogen therapy was 6.2 (1.9) years. The mean GH duration after start of estrogen therapy was 2.6 (0.9) years. Six girls entered puberty spontaneously, one of whom had regular menstrual cycles. The remaining five started estrogen 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 curve of the breast and pubic hair development respectively in our treated TS population (top panel) and in the normal Dutch population (bottom panel), as presented previously by Mul et al.[22], adapted with permission. The dotted line presents the crude data. The P_{50} **chronological** age can be read from the figures. These figures also show the interval between the Tanner stages.

The different breast stages advanced gradually, and were comparable to development in the normal Dutch female population up to B5[22], albeit with a two-year

delay (Figure 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 estrogen treatment one year later according to the protocol.

The development of pubic hair in the Turner girls was similar to that in the normal Dutch female population (Figure 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 bleedings 1.5 (1.2) years after start of dydrogesterone 5 mg/d, and 30 started to have withdrawal bleedings 0.5 (0.6) years after start of a dydrogesterone dose of 10 mg/d.

Serum hormone levels

Figure 4 shows the geometric mean and the 95% confidence interval of serum E2, E1 LH, FSH, and SHBG concentrations at the different oral 17β-estradiol dosages. At 24.7 pmol/l (95%CI 22-28), the mean serum E2 concentration at start of estrogen therapy (mean age 12.7(0.7) years) was comparable to that in prepubertal girls (mean age 9.1 yr) with serum estradiol 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-estradiol dose of 5 μg/kg/d, 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/d (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-estradiol dosage.

Mean LH concentrations at an oral 17β-estradiol dose of 5 μg/kg/d were lower than before start of 17β-estradiol (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-estradiol dosages of 7.5 and 10 μg/kg/d were higher relative to the levels at the 5 μg/kg/d, and also significantly higher than before start. FSH concentrations showed a significant decrease after the start of estrogen therapy. SHBG concentrations did not change after starting the lowest dose of 5 μg/kg/d, and decreased significantly with a dose of 7.5 and 10 μg/kg/d. The serum SHBG levels at the adult oral dose of 1 or 2 mg/d (mean 48 nmol/l 95%CI 43-55 nmol/l) were significantly higher than the serum SHBG levels before start (37nmol/l 95%CI 33-42 nmol/l) and than during the preceding estrogen 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 more, 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 estrogen 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 estrogen therapy in girls with Turner Syndrome (TS) is to induce puberty and feminization as physiologically as possible, without 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 the mean

population (Fig. 2). Induction was started with a very low dose of 17β-estradiol for two years before the estrogen dose was slowly increased. 17β-Estradiol is a natural estrogen, which has less pronounced effects on the coagulation factors, lipid profiles and blood pressure than synthetic estrogens[26]. The low dose did not affect height velocity[15].

Although it has previously been reported 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 estrogen administration differ between studies. Chernausek et al[11] used conjugated equine estradiol in a daily dose of 0.3 mg (~0.48 mg 17β-estradiol) orally over six months, following this with 0.625 mg daily – a higher estrogen 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 an lower dose of estrogens from age 12 years onwards enhanced height velocity while preserving adult height. Age at start of puberty induction with percutaneous estrogen in the study of Piippo et al[7] had a large range (10.7 to 17.7 years).

Our study shows that low dose of oral estrogen results in normal breast development in the majority of Turner women up to B5, just two years later than in their peers. As adult height is not affected by this low dose, it is possible to start this estrogen 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 estrogen dose regimen. Although after stop of GH treatment estrogens 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 having TS.

Our results also showed that the older the TS girls at start of estrogen therapy, the faster the progression through puberty. This can probably be explained by the faster increase in estrogen 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 estrogen 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 estrogen dose were comparable to normal late pubertal or adult serum E2 concentrations[24]. The lowest E2 (5 µg/kg/d) dose suppressed LH and FSH. However, during treatment with the double E2 dose (10 µg/kg/d), 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 estrogen substitution in the

Turner girls, and the relatively high gonadotropin levels, both indicate that the overall serum E2 effect at the low estradiol dose was small.

In postmenopausal women receiving 2 mg estradiol per day, gonadotropins decreased significantly [32, 33], resulting in LH and FSH levels which are comparable to[33] or lower[32] than the levels during treatment with the adult dose reported in our study. Whereas the lower 17β-estradiol 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-estradiol, in whom SHBG levels also increased significantly[33].

As expected, ovarian volumes were small. Remarkably, 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]. Furthermore, the 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[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) was suboptimal compared to that in women of the same age. However,

there is no normative data in literature on uterine length and shape in women aged 15-25. Although Paterson et al reported similar uterine length in an estrogen-treated Turner population of a similar age range, uterine shape was less mature than in our population[8]. One explanation for the differences between their study and ours may be the age at initiation of the estrogen replacement and the period of estrogen therapy before ultrasound measurement, which were not reported in their paper. Furthermore, the form of estrogen treatment may have been of influence, as they used ethinyl estradiol, of which the pharmaco-kinetics are different from those of 17β-estradiol.

TS women participating in an ovum-donation IVF program were reported to have a lower successful pregnancy rate per embryo-transfer[39, 40]. It has already been proposed that, for optimum endometrial response and improved outcome, higher constant estrogen replacement was 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 program. It has been suggested that lacking small amounts of estrogens during childhood or starting estrogen replacement later than at the physiological age of the serum estradiol 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, a higher daily dose of estradiol 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 estrogens and/or a higher estrogen dose may result in normal uterine dimensions at an adult age. Alternatively, other factors related to

TS may underlie uterine dimensions that remain subnormal. But as puberty induction in our study started two years after the onset of physiological puberty, it is also possible that uterine development has been subject to delay.

In conclusion, our study shows that when a low dose of oral 17β-estradiol is started at an appropriate age to induce puberty in girls with TS, the breast development is comparable to normal up to B5, with just a two-years 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 estrogen and/or a higher estrogen 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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Figure 1: Flow diagram of the patients in the initial GH-trial and the follow-up study.

Figure 2: Breast stages according to Tanner in girls with Turner Syndrome (*top panel*) who started 17β-estradiol at an appropriate age compared to a normal Dutch reference population (*bottom panel*) (Mul, D., et al., Pediatr Res, 2001. **50**(4): p. 479-86.). Adapted from Mul et al (Mul, D., et al., Pediatr Res, 2001. **50**(4): p. 479-86.) with permission.

Figure 3: Pubic-hair stages according to Tanner in girls with Turner Syndrome (*top panel*) who started 17β-estradiol at an appropriate age compared to normal Dutch reference population (*lower panel*) (Mul, D., et al., Pediatr Res, 2001. **50**(4): p. 479-86.). Adapted from Mul et al (Mul, D., et al., Pediatr Res, 2001. **50**(4): p. 479-86.) with permission.

Figure 4: Geometric mean and 95% confidence interval at different oral 17β-estradiol dosages of serum estradiol (pmol/l)(5a), LH (IU/L)(5b), FSH (IU/L)(5c), SHBG (nmol/l)(5d), and estrone (pmol/l)(5e). 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 ug/kg/d compared to preceeding dosage of 5 respectively 7.5 ug/kg/d, ***c***: adult dose of 1 or 2 mg/day compared to the levels at the last oral dosage taken.

Figure 1

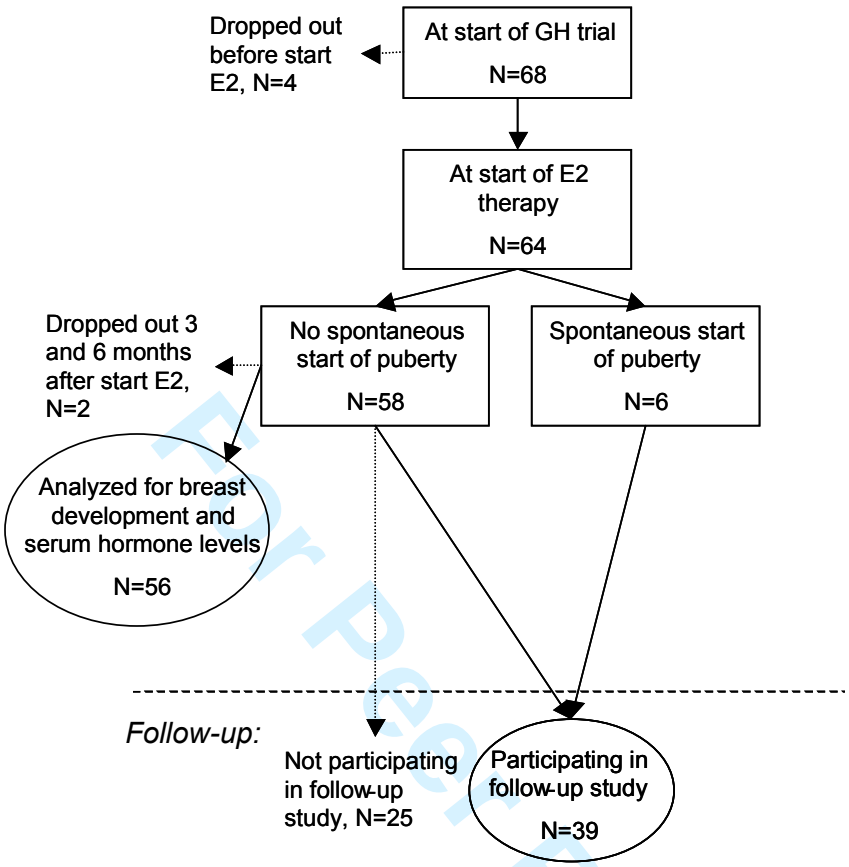


Figure 2

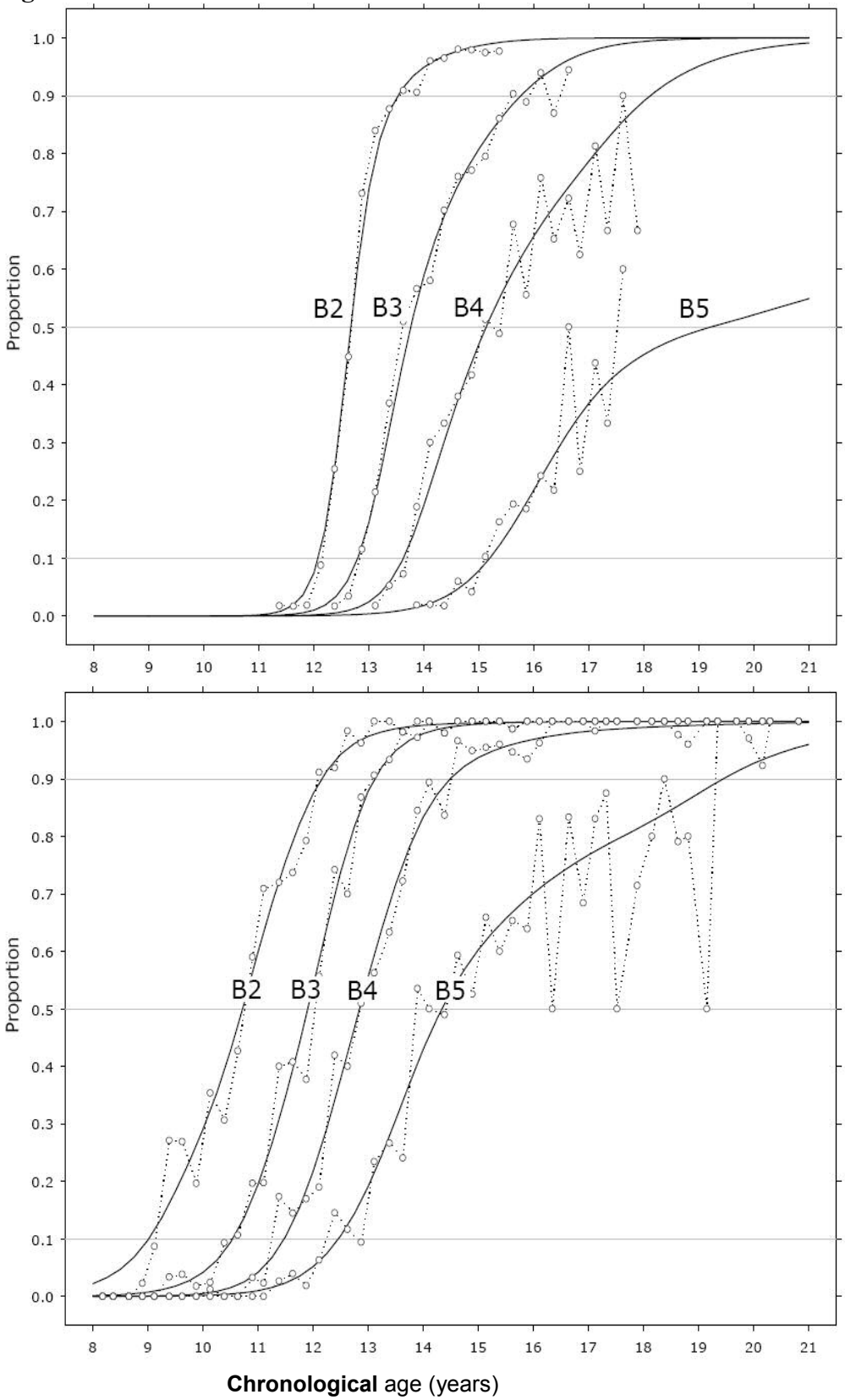

Proportion
B2
B3
B4
B5
Proportion
B2
B3
B4
B5
Chronological age (years)

Figure 3

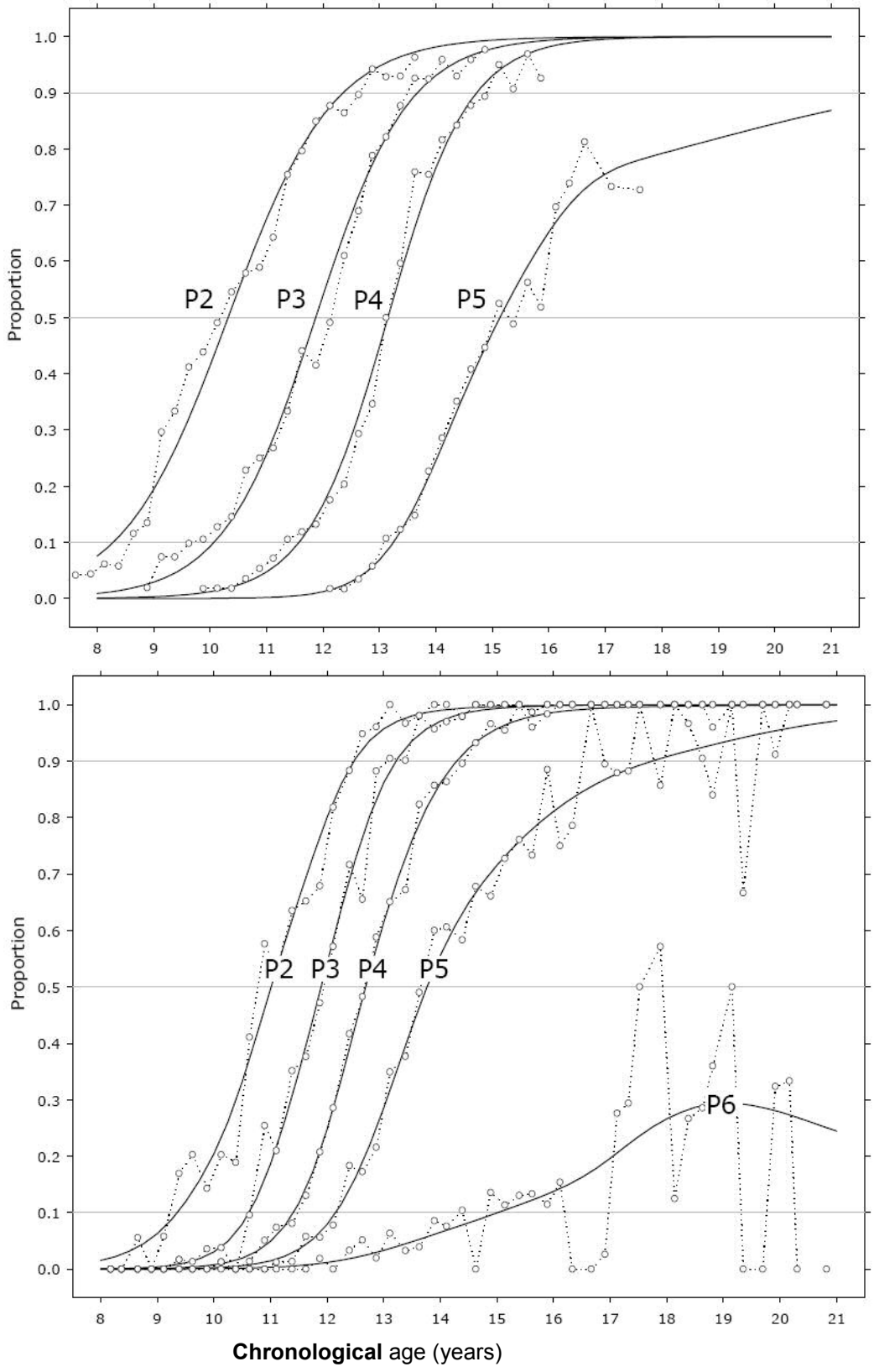
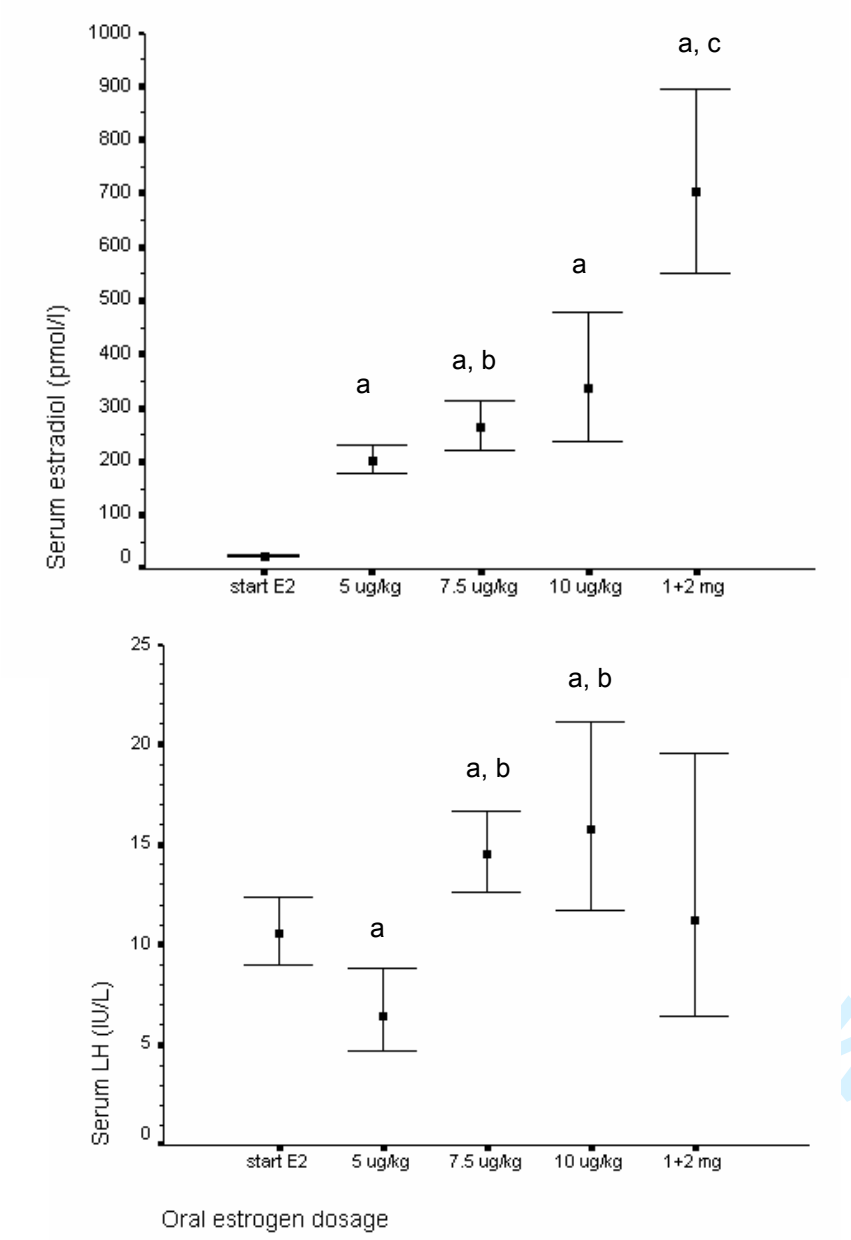
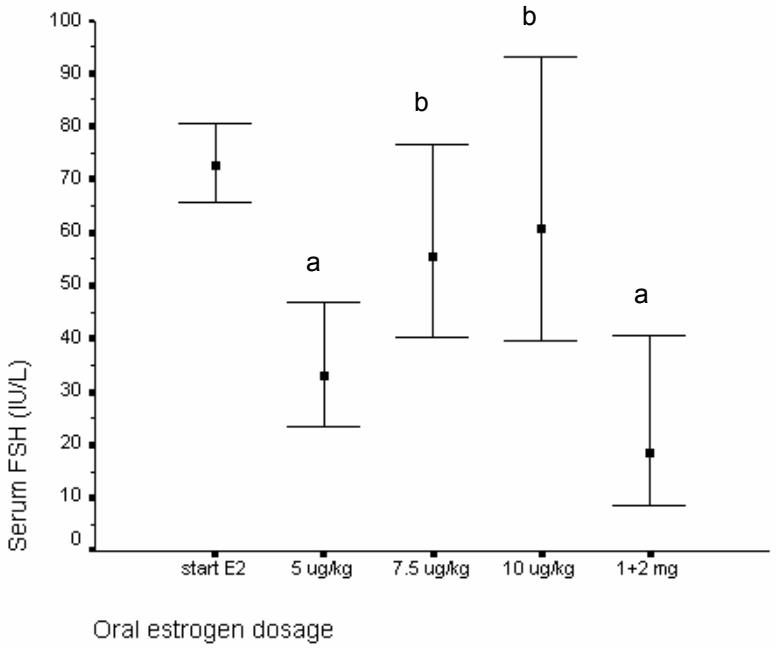
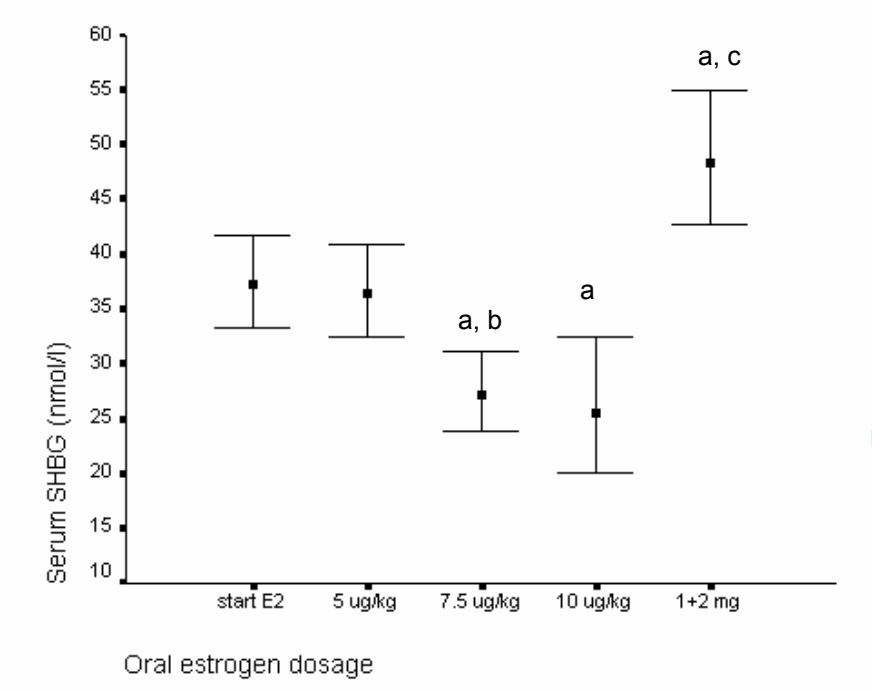


Figure 4


Serum estradiol (pmol/l)
1000
900
800
700
600
500
400
300
200
100
0
a
a, b
a
a, c
start E2
5 ug/kg
7.5 ug/kg
10 ug/kg
1+2 mg
Serum LH (IU/L)
25
20
15
10
5
0
a
a, b
a, b
start E2
5 ug/kg
7.5 ug/kg
10 ug/kg
1+2 mg
Oral estrogen dosage


Serum FSH (IU/L)
100
90
80
70
60
50
40
30
20
10
0
a
b
b
a
start E2
5 ug/kg
7.5 ug/kg
10 ug/kg
1+2 mg
Oral estrogen dosage


Serum SHBG (nmol/l)
60
55
50
45
40
35
30
25
20
15
10
a, b
a
a, c
start E2
5 ug/kg
7.5 ug/kg
10 ug/kg
1+2 mg
Oral estrogen dosage

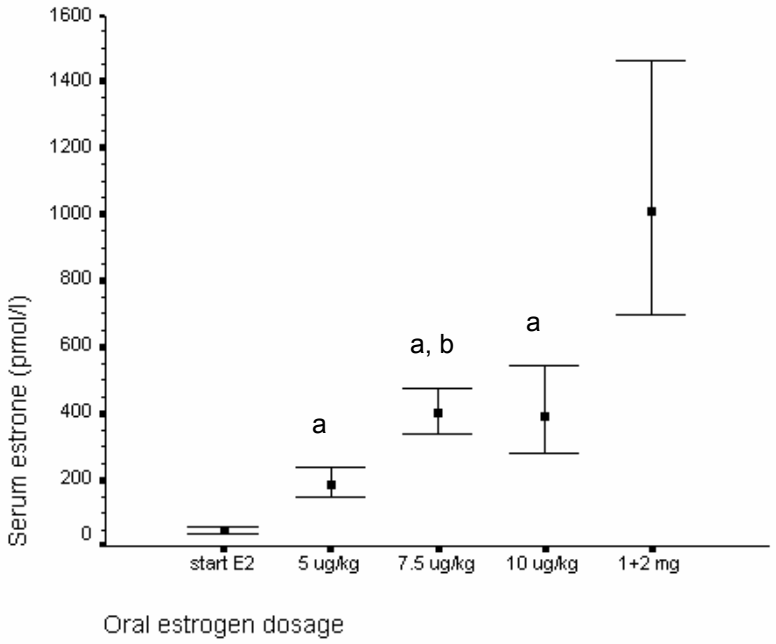

1600
1400
1200
1000
800
600
400
200
0
Serum estrone (pmol/l)
a
a, b
a
start E2
5 ug/kg
7.5 ug/kg
10 ug/kg
1+2 mg
Oral estrogen dosage

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

[a]: Mul, D., et al., Pediatr Res, 2001. **50**(4): p. 479-86.

Table 2: Mean ± SD (range) of uterine dimensions during follow-up study 7.1 (2.2) years after start of estrogen therapy, and 4.8 (2.0) years after discontinuation of GH.

*: Significant difference between patients with karyotype 45,X and 'variant' with $P<0.05$

Table 1:

	Turner population P50 (P10-P90)		**Normal Dutch population[a] N=3562 P50 (P10 – P90**	
Stage:	**Breast stage**	**Pubic hair stage**	**Breast stage**	**Pubic hair stage**
2	N=56 12.67 (12.06 – 13.53)	N=56 10.32 (8.28 – 12.36)	10.72 (9.0 – 12.2)	11.01 (9.4 – 12.5)
3	N=54 13.76 (12.79 – 15.76)	N=54 11.87 (10.07 – 13.67)	11.90 (10.5 – 13.1)	11.89 (10.6 – 13.2)
4	N=49 15.13 (13.61 – 18.12)	N=55 13.15 (11.57 - 14.72)	12.84 (11.5 – 14.5)	12.68 (11.4 – 14.3)
5	N=23 19.23 (15.19 – NA)	N=46 15.13 (13.25 – NA)	14.34 (12.5 – 19.5)	13.76 (12.1 – 17.7)

Table 2

	Total group **N=39**	**Karyotype**	
		45,X **N=31**	**'variant'** **N=8**
Chronological age	19.9 ± 2.2	19.7 ± 2.1	20.7 ± 2.4
(yrs)	15.0 – 23.4	15.0 – 23.2	16.6 – 23.4
Uterus length (mm)	60.1 ± 15.9	58.9 ± 16.7	66.9 ± 11.4
	25.0 – 87.0	25.0 – 87.0	49.0 – 83.0
Uterus volume (ml)	24.8 ± 15.0	22.3 ± 13.0*	34.5 ± 19.0
	4.4 – 57.9	4.4 – 57.5	7.9 – 57.9
Fundo-cervical ratio	1.5 ± 0.5	1.4 ± 0.5	1.5 ± 0.3
	0.8 – 2.8	0.8 – 2.8	1.1 – 2.0