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Estrogen Replacement in Turner Syndrome: Literature Review and Practical Considerations

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Narrative statement

The literature and recommendations for estrogen replacement have changed and expanded significantly over the past ten years, particularly regarding risks and benefits of estrogen replacement options. No consolidated review provides systematic practical guidelines for treatment in girls with Turner syndrome in the literature. This review and recommendations will be valuable to the clinician and will highlight areas where further research is needed. The comprehensive Tables will provide practical help to clinicians.

Search Strategies

We conducted a systematic search of PubMed for all research articles related to TS and puberty, including key words: estrogen, estradiol, growth, puberty, pubertal onset, as well as each of the variables under risks and benefits.

Context. Most girls with Turner Syndrome have hypergonadotropic hypogonadism and need hormonal replacement – for induction of puberty and later maintaining secondary sex characteristics, attaining peak bone mass, and uterine growth. The optimal estrogen replacement regimen is still being studied.

Evidence Acquisition. We conducted a systematic search of PubMed for research related to TS and puberty, including key words: estrogen, growth, puberty.

Evidence Synthesis. The goals of replacement are to mimic normal timing and progression of physical and social development while minimizing risks. Treatment should begin at 11 – 12 years old, with dose increases over 2 – 3 years. Initiation with low doses of estradiol is crucial to preserve growth potential. Delaying estrogen replacement may be deleterious to bone and uterine health. For adults who have undergone pubertal development, we suggest transdermal estrogen and oral progestin and discuss other approaches. We also discuss linear growth, lipids, liver function, blood pressure, neurocognition, socialization, bone and uterine health as related to hormonal replacement.

Conclusions. Evidence supports the effectiveness of starting pubertal estrogen replacement with low-dose transdermal estradiol (E_2). When transdermal E_2 is not available or the patient prefers, evidence supports use of an oral micronized E_2 or intramuscular preparation. Only when these are unavailable, should ethinyl estradiol be prescribed. We recommend against the use of conjugated estrogens. Once progestin is added many women prefer the ease of use of a pill

containing both an estrogen and progestin. The risks and benefits of different types of preparations, with examples, are discussed.

We reviewed the literature on estrogen replacement to induce puberty and minimize risks. Evidence supports starting with low-dose transdermal E2 and mimic average pubertal progression.

Introduction

The 2017 updated guidelines from the International Turner Syndrome Consensus Group have just been published in the European Journal of Endocrinology (1) and endorsed by the European Society of Endocrinology, the Endocrine Society, the Pediatric Endocrine Society, the European Society for Pediatric Endocrinology, the European Society of Human Reproduction and Embryology, the American Academy of Pediatrics and the Society for Endocrinology (UK). The American Heart Association and European Society of Cardiology also had official delegates at the meeting. The present paper expands on those guidelines specifically relating to puberty and estrogen replacement.

Turner syndrome (TS) defines phenotypic females who have one X chromosome and complete or partial absence of the second X chromosome. TS is characterized by physical features including a classic facial appearance, neck webbing, short stature, and lymphedema, as well as ovarian insufficiency, sensorineural hearing loss, congenital cardiovascular disease, renal anomalies, some neurodevelopmental disorders, and increased risk of thyroid and celiac disease. TS affects 25 – 50 per 100,000 females, and there is a very broad clinical spectrum of presentation. Some individuals have all the features mentioned above and others have minimal features, with or without short stature and ovarian insufficiency. The karyotype in TS ranges from complete 45,X to forms of mosaicism in which there is a normal (46,XX or 46,XY) cell line, and an abnormal second (or third) cell line (2).

Turner syndrome is usually accompanied by hypergonadotropic hypogonadism due to gonadal dysgenesis and ensuing primary or secondary amenorrhea. Most TS patients will therefore need hormonal replacement therapy – first for induction of puberty and then for maintaining secondary sex characteristics, attaining peak bone mass, and normalizing uterine growth for possible pregnancy later. This review focuses primarily on estrogen hormone replacement in the care of girls with TS.

The optimal estrogen replacement therapy regimen to induce pubertal development and maintain beneficial effects in adults is still being studied. A significant body of literature to date supports the effectiveness and theoretical benefits of starting pubertal estrogen replacement with low-dose transdermal estrogen (E_2) although there is no study to date of transdermal use from initiation of puberty until adulthood. Theoretical benefits of transdermal use include: the more physiologic route of delivery, avoiding first-pass effects in the liver that include the accumulation of un-physiologic estrogens observed after the oral route (3) and that are associated with a pro-coagulation state (4) and increased risk of stroke (5).

Estrogen forms, timing of replacement, dosing, route of administration, duration of treatment and monitoring treatment are here presented. We also review evidence relevant to optimizing the outcome and minimizing the risk of estrogen replacement in puberty as regards growth, lipids, liver health, bone health, uterine health, and thrombosis risk, as well as socialization and neurocognitive benefits.

Spontaneous puberty in girls with Turner Syndrome

Approximately one-third of girls with Turner syndrome have spontaneous breast development that may progress to menarche, occurring most often in girls with mosaicism (6,7). Regular menstrual cycles occur in ~6% (8) of these young women.

Laboratory markers of ovarian function

Elevated gonadotropins, luteinizing hormone (LH) and particularly follicle stimulating hormone (FSH) indicate ovarian failure (9,10). FSH concentrations are higher in girls with 45,X karyotype compared to those with mosaic karyotype. LH and FSH in girls with TS are elevated after birth, then decline to levels similar to girls with normal ovarian function during mid-childhood, and rise again in the peripubertal years (9,11) or at the time of loss of previous ovarian function. Low anti-Müllerian hormone levels and undetectable inhibin B levels have been reported to predict ovarian failure in TS (9,12). Seventy girls with TS and 2406 girls without TS had LH, FSH, and inhibin B measured prior to estrogen treatment (9). Ovarian function was related to whether girls had 45X or a mosaic karyotype. According to these data, undetectable inhibin B may predict the absence of spontaneous puberty, but the specificity was low. Anti-Müllerian hormone in 120 girls with TS predicted no ovarian function when < 4 pmol/L (0.56 pg/mL) and predicted ovarian function when > 19 pmol/L (12).

Treatment options for induction of puberty and maintenance of feminization

Estrogen forms available for replacement

Estradiol (E_2) is the natural form of estrogen that is secreted and binds to the estrogen receptor in humans (13). Ethinyl estradiol (EE) is a very potent synthetic E_2 analogue that is not metabolized to E_2. It binds to both estrogen receptors α and β. EE has an ethinyl group covalently attached at the 17α-position. EE is taken up in unmodified form and retained by estrogen target tissues for a longer time than is E_2. The E_2 precursor estrone acts after being metabolized to E_2. Equine estrogens, the major components of the widely used conjugated equine estrogens (CEE), consist of over 100 forms of estrogens of different receptor affinity and potency. Estrogens are metabolized in the liver by microsomal cytochrome P-450 with aromatic hydroxylation at either C2 or C4 position as the major route. Other pathways include formation of glucuronide conjugates and sulfation (14-16).

Table 1 lists commonly available, lower-dose estrogen treatments for pubertal induction, and considerations for their use. **Table 2** lists some common progestin and estrogen/progestin combination replacement options after pubertal induction is complete. The reader should be aware that availability and trade names differ among countries. The list is not all-inclusive. We present data from various routes and preparations, but list other preparations for reference, with the caution that studies have not been done in TS with each preparation listed. **Table 3** summarizes published low-dose estrogen treatments for puberty induction in TS.

Timing and dose

The goals of replacement are to mimic the normal progression of puberty in girls while maximizing growth potential and minimizing risks. Delaying estrogen replacement may be deleterious to bone, uterine, and psychosocial health parameters (17). To mimic normal physical and social development, initiation of treatment should begin at 11 – 12 years of age if gonadotropins are elevated, or AMH is low. LH and FSH may be measured yearly starting at age 11, based on average age of pubertal onset. If gonadotropins are normal for age, observation for spontaneous puberty is appropriate, with future replacement therapy if gonadal failure occurs.

Incremental dose increases at approximately 6 month intervals can mimic the normal pubertal tempo until adult dosing is reached over a 2 - 3 year period. This theoretically translates into 25-100% increase in dose every 6 months for 4 – 6 dose changes between the initiation and adult doses portrayed in the **Table 1**. However, no studies to date have rigorously studied outcomes in relation to the rate of dose increase for the different preparations.

In general, the studies summarized in Table 3, report onset of breast buds within 6 months in most girls (18-22). Each of these regimens results in pubertal stage 4 breasts in an average of 2.25 years, which is similar to that in TS girls with spontaneous puberty (1.9 years), as well as in the general population (21).

Girls with TS are very short and have a very short adult height potential, typically 20 cm less than the average female population in all countries studied. Growth hormone (GH) treatment is an FDA-approved therapy to promote growth in these girls, and the earlier it is started, the better the growth promotion. However, the expectations of intervention are modest, in general GH therapy results in a net gain of 1cm/yr of treatment (23,24). In girls in whom GH treatment has been delayed, consideration of initiation of GH prior to low dose estrogen is particularly important to optimize growth. There are no data to support the specifics of timing in such cases, but rather an individualized judgement, balancing the desire for taller height versus the desire for more rapid feminization. When height is a greater concern, often GH treatment can be initiated prior to low dose E_2, however, we recommend that E_2 not be delayed past 14 years of age. When feminization is a greater concern, GH and E_2 can be started simultaneously.

Initiation with low doses of E_2 is crucial to preserve growth potential whether or not growth hormone treatment has already been initiated. Very low dose EE and E_2 do not interfere with growth response to growth hormone therapy when started $\leq$ 12 years of age (25,26).

Progestins

TS patients have a normal uterus anatomy, so progestin must be added once breakthrough bleeding occurs, or after 2 years of E_2 treatment, to minimize the risks of endometrial hyperplasia, namely, irregular bleeding and endometrial cancer associated with prolonged unopposed estrogen (27,28).

Progestins are divided into several classes (**Table 4**) and individual agents can bind to the progesterone receptor as well as the androgen, glucocorticoid and mineralocorticoid receptors (29). Each progestin exerts differential effects on these various receptors and accordingly, unique, non-class action effects. In addition to the progestational effects, the 19-nor-derivatives are associated with androgenic, medroxyprogesterone-acetate with glucocorticoid agonistic, and drospirenone with anti-androgenic and anti-mineralocorticoid actions, whereas progesterone is more specific to progestational effects. The combined oral contraceptives (OCs) containing progestins are divided into first, second, third, and fourth generation OCs. First generation OCs contain 50 mcg of the estrogen mestranol and the progestogen norethynodrel (eg Enovid^R). Most later generation pills utilize 20-35 mcg of EE as the estrogen. Second generation progestogens include norethindrone, and its acetate, ethynodiol diacetate, and levonorgestrel. Third generation progestogens include desogestrel, norgestimate and gestodene. Fourth generation pills include drospirenone. All OCs increase the risk of venothrombotic episodes (VTE). A recent guideline (30) concludes that combinations of EE with the third generation (desogestryl, norgestimate, gestodene) or fourth generation (drosperinone) progestogens have a slightly higher risk of VTE than those containing first and second generation. Micronized progesterone is associated with a lesser risk (31).

Regimens of estrogen plus a progestin can be either combined-sequential with an estrogen for 21-25 days and the progestin for only 10-14 days, or combined-continuous with both sex steroids continuously. The estrogen is given for up to 21 -25 days to cause the endometrium to become proliferative and the progestin in combination with the estrogen induces the luteal phase of the endometrium. Ten days of a progestin each month protects against estrogen induced endometrial hyperplasia, and three months of combined continuous estrogen plus a progestin is also protective (32). The combined-sequential regimens are associated with menstruation and are preferred in younger women, whereas the combined-continuous prevent uterine bleeding, an attractive factor in older women. Intrauterine devices containing a progestin block endometrial hyperplasia and unwanted bleeding and can be used along with an estrogen, and can be especially attractive for women with bleeding problems on either transdermal or oral combined formulations. Availability of products varies by country (see **Table 2**).

Route: oral vs. transdermal (TD) comparisons

E_2 is normally secreted into the systemic circulation, the liver receives the same dose as other somatic tissues, and a systemic route of estrogen delivery is physiologic (13). In contrast, estrogen given orally reaches the systemic circulation only after absorption into the portal venous system and metabolism by the liver, thus exposing the liver to a greater dose of estrogen than the rest of the body.

Transdermal E_2 is the most widely used of these physiologic E_2 options, but the commercially available forms (patches and gels) are designed for the adult female market and thus the lowest dose forms are 4-10-fold greater than are appropriate to deliver early pubertal E_2 blood levels. The main strategies that have been advocated to fractionate transdermal E_2 in a manner that is appropriate for early puberty are based on different perspectives on normal pubertal E_2 physiology.

Currently, the lowest dose patch commercially available delivers 14 µg/day of E_2, and the most widely used low-dose patches deliver 25 µg/day. One method to deliver lower doses is to cut the patch in smaller pieces. Patches with a matrix design can be easily cut, while patches with a reservoir technology should not be cut. The disadvantages of cutting patches are that handling the smaller pieces may be difficult and that this is not recommended by the products' labels. However, there is clinical experience with this, especially in Scandinavia. There a group showed that a fractionated patch dose (one-quarter patch of 25 µg dose = approximately 6.2 µg or even less) applied overnight mimicked the normal early morning serum E_2 peak and fell back to baseline within a few hours of patch removal (22). If one does not want to cut the patches, it has also been proposed that cyclic administration of patches, commencing with the application of a 14-25 µg patch for 1 week monthly, may achieve similar results, although we have no data at this time with this method (25,33). This proposal comes from an expert committee of the Pediatric Endocrine Society, which recommended initiating cyclic therapy with 25 µg/d of transdermal E_2 for 1 wk and then gradually increasing the duration of patch application to 3 weeks per month before increasing the patch size. Support for this recommendation includes not only considerations of convenience and manufacturer recommendations against patch fractionation, but evidence of efficacy of cyclic administration of depot systemically delivered E_2 (25). Evidence also exists that estrogenization of the vaginal mucosa lags behind changes in serum E_2 by about 1 week (3,34) suggesting that the pituitary-ovarian axis activity normally commences with attenuated cyclicity (35). Expert discussion of this method, however, suggests that 1 week with and 3 weeks without E_2 would cause such variable changes in plasma E_2 concentrations

during these 4 weeks that may not mimic physiology. Further data are needed before conclusions can be made regarding the optimum mode of patch application recommendation.

Two studies in the Mauras' group have directly compared the transdermal and oral routes of E_2 administration in teenagers (3,36). The pharmacokinetics and pharmacodynamics of different doses of E_2 given orally vs. transdermally were examined in a group of girls with TS. Transdermal E_2 results in E_2, E_1, and bio-estrogen concentrations closer to normal and achieves greater suppression of LH/FSH in lower doses compared with normally menstruating girls without TS (37). The metabolic effects of oral vs. transdermal E_2 were further compared in 40 late-teen girls with TS followed for 1 year (3). The study found no differences in body composition, bone mineralization or plasma lipids when the plasma E_2 levels were titrated to those of normally menstruating adolescents. Although no metabolic differences were observed, oral estrogen was associated with a marked increase in conjugated estrogen precursors such as estrone sulfate and increased serum estrogenic bioactivity. This is concerning in the context of the increased thromboembolic risk observed with oral estrogen in epidemiological studies, although there are no data to suggest that such problems are present in TS (See Cardiovascular Risk section). Some European countries use an E_2 gel (**Table 1**) but it is very difficult to give a small enough dose for pubertal induction, and there is only one study in girls with TS (38).

Depot route

A randomized controlled trial showed that early, very low-dose, depot E_2 monthly injections stimulated normal pubertal growth and development in conjunction with growth hormone treatment (25). This remains a viable alternative in the USA, although less attractive due to the pain of injection.

Practical considerations

Estrogen treatment is crucial for girls with TS, first to induce puberty and then to maintain healthy levels for all the reasons described here. Individualizing treatment to optimize compliance is important, and helping girls understand how easy it is to help them have breast development consistent with their peers should be encouraged. Based on literature and theoretical principles presented here, we suggest the following practical approach to feminize girls with TS: initiate puberty with low dose TD E_2, when available, starting with half of a 14 µg patch applied weekly, or a whole 14 or 25 µg patch for one week per month at 11-12 years of age (**Table 1**) and increase every 6 – 12 months based on response and growth potential. When not available, or for physician or patient alternative preference, consider approaches discussed above as well as in **Tables 1 and 2**.

For the adult patient with TS, no long-term studies have assessed the optimal dose, route, or duration of E_2 treatment. Our recommendations are based on available data in both women with TS as well as other hypogonadal patients. The effects of hormone treatment in TS may be different from what is observed in other patient populations, and caution is needed when extrapolating data from postmenopausal studies (37). With those cautions, the type and route must be negotiated with the patient taking into account the preference of the patient, the size of the uterus (for possible oocyte donation), bone and body composition assessed by DEXA, blood pressure and quality of life, as well as other considerations (see Outcomes and Risks sections below). Adult transdermal replacement doses of 50 – 150 µg/d or oral replacement doses of 2-4 mg of E_2 will often be sufficient. Oral progestin for 10 days per month (combined sequential approach) or continuous progestin regimens are suggested (analogous to the

combined/continuous methodology commonly used for menopausal hormone therapy (39). If bleeding irregularities occur or if the patient prefers, an intra-uterine progestin coated device can be used together with either continuous oral or transdermal E_2. This will reduce bleeding irregularities and often abolish bleedings and the need for systemic progestin use. Close collaboration with a gynecologist with knowledge of TS is very useful.

Duration

Once adult replacement doses are reached, treatment should continue until the time of usual menopause around age 51-53 years, when the risks versus benefits of continuing should be assessed, individualized, and reassessed annually (32,39). Combined estrogen and progestin treatment duration is limited by increased risk of breast cancer (40), however, there are no clinical or epidemiological data among TS to suggest that breast cancer is a problem. Actually, breast cancer seems to occur less frequently among women with TS (1), although diminished overall estrogen exposure may be a factor. Estrogen therapy alone after menopausal age has a more favorable risk/benefit ratio allowing more flexibility in duration but is only indicated in women having undergone hysterectomy (41). There will often be a continued need for education of the TS patient in order to explain the beneficial effects of hormonal replacement therapy on multiple organ systems, in order to maintain adherence to therapy.

Monitoring treatment

Routine monitoring of serum LH or FSH is not recommended during estrogen treatment as levels remain elevated in agonadal women until higher levels of estrogen are given (42). The suppression of gonadotropins was comparable after oral and TD E_2 when doses were titrated to similar serum E_2 levels (3). Estradiol measurement using a sensitive assay (e.g., liquid or gas chromatography with tandem mass spectrometry) allows titrating dosage if desired, though E_2 levels for optimal linear growth remain to be determined. Clinical assessment, patient satisfaction, patient age, and, often, residual growth potential are the primary determinants for dose increase. If potential for taller stature is still possible, girls may remain on lower estrogen doses longer. If girls are already older at initiation, the duration of time until adult dosing may be shortened.

Adult replacement transdermal doses of 50 – 200 µg/d typically allow women to reach normal adult plasma E_2 concentrations. The normal range of estradiol in cycling women is very wide with early follicular phase levels as low as 20-40 pg/mL (75 – 150 pmol/L) and midcycle peak of 200-600 pg/mL (730 – 2200 pmol/L), and some experts replace to these levels (3). When oral estrogen is used, adult replacement doses of 2 – 4 mg of E_2 will result in normal circulating E_2 levels (i.e. approximately 100 – 155 pg/mL (367 - 568 pmol/L))(42) and may lead to normal levels of FSH and LH in some women (42,43). However, women with TS lack inhibin (44), so normalizing LH and FSH is not the goal per se (45,46). Optimizing all the health benefits and minimizing the risks is the goal and it is important to remember that this must be individualized.

Optimize outcomes/minimize risks: growth, lipids, liver, bone health, uterine health, and thrombosis risk

Estrogens and linear growth

Low-dose estrogen regimens do not appear to interfere with growth response to growth hormone therapy when begun at 11-12 yr of age at low doses (25,26,47,48). Ultra-low dose oral EE (starting at 25 ng/kg/d, ages 5-12 y) in childhood TS has been reported but is not currently

recommended based on an increased risk of earlier thelarche and no proven benefit to growth or pubertal outcome (49).

A consistent effect of physiologic E_2 replacement on IGF-1 concentration has not been established (3). IGF-1 concentrations tended to be lower on oral than TD E_2 (-16 ± 12 vs. 28 ± 12 ng/mL at 12 months (p=0.059), while an earlier study from the same group showed no change in IGF1 concentration after oral or TD therapy (50). TD application caused a decrease in IGF binding protein-3 and GH binding protein compared with an increase of IGFBP3 and unchanged level of GHBP after oral administration (51). In contrast, contraceptive doses of oral EE are known to suppress IGF-1 (52,53). In a small study (13 girls), bone age advanced less on TD E_2 than oral E_2 (Δchronological age/Δbone age 2.2 vs. 0.58, p=0.005). At the same time, growth velocity was greater on TD E_2 than oral E_2 at 1 y (4.35 vs. 3.8 cm/y, p=0.022), suggesting overall better growth (22).

Estrogens and Metabolism

Lipids

Although there are theoretical reasons to be concerned about the relative systemic and hepatic hyperestrogenism of low-dose oral estrogens vs low-dose transdermal E_2, evidence thus far does not indicate that the hepatic effects on lipids or binding proteins causes an appreciable clinical difference between the two forms of treatment (**Table 5**)(3,36,54). With the exception of one study reporting significantly higher HDL-cholesterol after oral estradiol (36), there were no significant differences in lipids between groups with different routes of estrogen administration (3,36).

Estrogen deficiency in TS is associated with elevated intrahepatocellular lipids (55). Notably, while liver enzymes are elevated in untreated TS (43,51,56,57) exogenous estrogen-progestin administered orally or transdermally reduces these levels (43,52,58). However, withdrawal of estrogen substitution did not influence liver enzymes (59,60). There was no evidence of liver toxicity from estrogen replacement therapy (54).

Glucose, insulin

The risk of both type 1 and type 2 diabetes mellitus is increased in patients with TS across all ages (61). However, there were no significant differences in glucose (3), insulin tolerance (62,63) fasting insulin concentration, protein turnover and lipolysis (64), osteocalcin or highly sensitive C-reactive protein (3), BMI, or waist to hip ratio (63,65) between groups with transdermal versus oral estrogen treatment (**Table 5**). Glucagon and insulin (during oral glucose tolerance testing) as well as insulin resistance tended to be lower following evening oral E_2 administration (0.3 -0.5 mg/day)(66). Hyperinsulinemia was suppressed to normal by both EE and CEE. A recent study in 104 girls with TS followed up to 7 years after GH therapy showed no negative influence of GH treatment on β cell function, which is also reassuring as most of these girls continued on estrogen therapy (67).

Estrogens and Bone Density

Maintenance of bone health is crucial for women with TS. Delaying estrogen replacement is deleterious to bone health. Initiating and maintaining estrogen therapy as outlined above during puberty and adulthood is important for bone density accrual and prevention of fractures. In girls with TS, transdermal E_2 administration (25-37.5 µg/d) has been reported as better than CEE (0.3-0.45 mg/d) for spine bone mineral density in one study (0.12 ± 0.01 vs. 0.06 ± 0.01 g/cm^2, p=0.004)(19). A recent study suggests that a higher than usual oral dose (4 vs 2 mg) during early

adulthood improves body composition (increased muscle mass) and increases bone formation markers, which, although bone mineral density (BMD) was not increased during the study period, in the long run could improve overall bone health (68).

Some adult women with Turner syndrome prefer combined estrogen and progestin pill options for convenience sake (69). Few studies have directly compared transdermal estrogen regimens with oral regimens in women with premature ovarian insufficiency including patients with Turner syndrome. The better powered studies indicated improved lumbar spine density on a physiologic sex steroid replacement regimen (100-150 µg E_2 daily + 400 mg vaginal progesterone 2 wk/mo)(46).

On the basis of these studies, the guidelines written by the European Society of Human Reproduction and Embryology favored transdermal estradiol in women with premature ovarian failure and commented that oral contraceptive pills may be appropriate for some women but effects on BMD are less favorable (70-72). More comprehensive long-term studies will be necessary to confirm these results and to examine fracture rates.

Estrogens and Uterine Growth

Data concerning the influence of different routes of estrogen therapy on uterine volume is still inconclusive because route, dose, age at onset of treatment, and duration of treatment all influence uterine growth (19,21, 73-77). However, it is clear the longer the duration of treatment and the higher the dose of estrogen, the better the chances of normalizing uterine size, which is important only if pregnancy options are pursued. One study in 12 girls with TS reported uterine length significantly greater with TD E_2 (25-37.5 µg/d) compared to CEE (0.3-0.45 mg/d) (4.13 ± 0.39 vs. 1.98 ± 0.39 cm, p=0.003) and uterine volume greater (22.2 ± 4.4 vs. 4.0 ± 4.4 mL, p=0.02)(15). Higher than usual doses are often necessary before oocyte donation, where oral doses up to 8 mg have been used for up to 2 years in order to achieve satisfactory uterine growth (78).

Estrogens/Progestin therapy and Cardiovascular Risk

Although there have been no studies in children, or women with TS, we recommend against CEE use in view of thromboembolic and cardio-vascular disease risks reported in postmenopausal women, especially in the first year of treatment using oral estrogen, and in women with existing risk factors like obesity (4,5,79-81).

E_2 replacement therapy, oral or transdermal, lowers blood pressure (79-81), although E_2 causes salt and water retention (82). This contrasts with EE-containing contraceptives, which raise blood pressure significantly unless containing an anti-mineralocorticoid progestin (83).

Recent publications showed no increased risk of stroke with progesterone, pregnane derivatives, or nortestosterone derivatives (5,84). However, norpregnane derivatives were found to increase risk (5). Studies have not been done in TS comparing various progestin options.

Several studies examining both oral E_2 and oral conjugated estrogens vs transdermal E_2 replacement in the postmenopausal setting have shown increased thromboembolic risk, especially in the first year of treatment in the oral group, more pronounced in women with existing risk factors such as obesity (5,42,85). Studies directly comparing thromboembolic risk in women with Turner Syndrome have not been done.

Screening for thromboembolic risk, through measurement of Factor V Leiden and prothrombinase levels, should be done in girls with a personal or family history of VTE, however, routine screening is not recommended, and screening is done only to educate the family on risks, not to postpone estrogen therapy (86). Transdermal estrogen is the preferred treatment in these girls.

Socialization and Neurocognitive benefits

Estrogen replacement in TS girls may improve motor speed, verbal and nonverbal processing time compared to placebo-treated TS patients (87,88). In TS adolescents. oral estrogen therapy improved self-reported self-esteem and psychological well-being over time. At the same time their parents reported improvement in problem behaviors (89). Data concerning adults with TS have not been so optimistic. TS adults had relative difficulty with measures of spatial/perceptual skills, visual-motor integration, affect recognition, visual memory, attention, and executive function. These deficits were apparent in TS women despite evidently adequate estrogen treatment (90,91). Age of onset of puberty influenced sexual experience in one study (92) but not in another (93). A more recent follow up report suggests that women with TS face more challenges in areas of sexual confidence and self-esteem (94).

The young women with TS who reached normal height and had age-appropriate pubertal development reported normal health-related quality of life (HRQoL); satisfaction with breast development (and height) had a positive influence on several HRQoL scales (95). Puberty should be induced at a physiologically appropriate age in patients with Turner syndrome to optimize self-esteem, social adjustment, and timing of initiation of the patient's sex life. However, one study showed that neither estrogen use nor age of puberty influenced sexual function in TS patients (93).

Oxandrolone Effect on Puberty

Oxandrolone is a non-aromatizable weak androgen with direct growth promoting effects. Low-dose oxandrolone has been shown to act synergistically with GH to increase linear growth in several well-controlled studies (46,96-98). However, oxandrolone may also increase hirsutism and clitoral size slightly and modestly slow pubertal progression (by 1.3 y) and delay menarche in response to estrogen replacement (99). These effects are usually minor and/or transient. One study indicates that normal adult breast size is subsequently attained as oxandrolone is discontinued and adult estrogen replacement is instituted (100). Pubic hair stage was not affected. Therefore, a reasonable suggestion is that treatment with oxandrolone 0.03–0.05 mg/kg/day (maximum 2.5 mg/day) starting from the age of 10 years onwards be considered as adjunctive therapy only in very short TS girls (99,101).

Future Research

At the present time, limited data from random controlled trials are available in girls with TS regarding estrogen treatment options, as discussed above. There continues to be a paucity of data in girls and women with TS regarding the regimens discussed here and the long-term compliance. Questions for future research include: 1) What is the optimal protocol for pubertal induction, including dose, route, and rate of progression?; 2) What are the optimal circulating levels of estradiol during each phase of pubertal induction?; 3) What is the optimal dosing, preparation and timing of progestin-induced uterine bleeding?; 4) How long should estradiol treatment be continued in women with TS?; 5) What is the optimal method and timing for monitoring bone health in women with TS?; 6) What is the optimal regimen to promote uterine growth?; 7) If an OC is used for treatment, which are preferable in women with TS?; and 8) What is the effect of oral contraceptive pill placebo days on the hormonal milieu in women with TS?.

Summary and Conclusion

In summary, we suggest that estrogen replacement should mimic normal physical and social development for timing and progression of puberty, starting between 11-12 years of age and increasing over 2 – 3 years. This regimen improves socialization and growth, and optimizes uterine and bone health. Neurocognitive benefits are inconclusive.

When available, low-dose E_2 administered by a systemic route is preferred, and evidence supports its effectiveness and theoretical benefits. When transdermal E_2 is not available, or compliance is an issue, evidence supports use of oral micronized E_2 or depot E_2 preparations. Only when these forms of E_2 are unavailable, should other forms of estrogen be prescribed. Progestin should be added once vaginal bleeding occurs or after 2 years of estrogen treatment. At that time some women prefer the ease of use of an oral combination of estrogen and progestin. Some preparations are safer than others, and availability varies by country, but ordinarily the benefit of good compliance to a chosen regimen outweighs the risks. Treatment is monitored by patient satisfaction and growth and development measures.

None, Karen Klein

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The authors have nothing to disclose directly related to this manuscript.
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References

1. Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, Lin AE, Mauras N, Quigley CA, Rubin K, Sandberg DE, Sas TCJ, Silberbach M, Söderström-Anttila V, Stochholm K, van Alfen-van derVelden JA, Woelfle J, Backeljauw PF; International Turner Syndrome ConsensusGroup. Clinical practice guidelines for the care of girls and women with Turner syndrome: proceedings from the 2016 Cincinnati International Turner Syndrome Meeting, Eur J Endocrinol. 2017; Sep;177(3):G1-G70.
2. Cameron-Pimblett A, La Rosa C, King TFJ, Davies MC, Conway GS. The Turner syndrome life course project: Karyotype-phenotype analyses across the lifespan. Clin Endocrinol (Oxf). 2017;87:532-538
3. Torres-Santiago L, Mericq V, Taboada M, Unanue N, Klein K, Singh R, Hossain J, Santen R, Ross J, Mauras N. Metabolic effects of oral vs. transdermal 17 beta estradiol (E_2): a randomized clinical trial in girls with Turner Syndrome. J Clin Endocrinol Metab. 2013;98:2716-2724
4. Khaled Mohammed, Abd Moain Abu Dabrh, Khalid Benkhadra, Alaa Al Nofal, Barbara G. Carranza Leon, Larry J. Prokop, Victor M. Montori, Stephanie S. Faubion, and Mohammad Hassan Murad. Oral vs Transdermal Estrogen Therapy and Vascular Events: A Systematic Review and Meta-Analysis J Clin Endocrinol Metab, 2015;100(11):4012–4020
5. Marianne Canonico, Laure Carcaillon, Geneviève Plu-Bureau, Emmanuel Oger, Archana Singh-Manoux, Pascale Tubert-Bitter, Alexis Elbaz, Pierre-Yves Scarabin, Postmenopausal

Hormone Therapy and Risk of Stroke Impact of the Route of Estrogen Administration and Type of Progestogen, Stroke. 2016;47:00-00
6. Tanaka T, Igarashi Y, Ozono K, Ohyama K, Ogawa M, Osada H, Onigata K, Kanzaki S, Kohno H, Seino Y, Takahashi H, Tajima T, Tachibana K, Tanaka H, Nishi Y, Hasegawa T, Fujita K, Yorifuji T, Horikawa R, Yokoya S. Frequencies of spontaneous breast development and spontaneous menarche in Turner syndrome in Japan. Clin Pediatr Endocrinol. 2015;24(4):167-73.
7. Negreiros LP, Bolina ER, Guimarães MM. Pubertal development profile in patients with Turner syndrome. J Pediatr Endocrinol Metab. 2014;27(9-10):845-9.
8. Pasquino AM, Passeri F, Pucarelli I et al: Spontaneous pubertal development in Turner's syndrome. Italian Study Group for Turner's Syndrome. J Clin Endocrinol Metab. 1997;82: 1810-3
9. Hagen CP, Main KM, Kjaergaard S, Juul A. FSH, LH, inhibin B and estradiol levels in Turner syndrome depend on age and karyotype: longitudinal study of 70 Turner girls with or without spontaneous puberty. *Human Reproduction*. 2010;25(12):3134–3141.
10. Fechner PY, Davenport ML, Qualy RL, Ross JL, Gunther DF, Eugster EA, Huseman C, Zagar AJ, Quigley CA; Toddler Turner Study Group. Differences in follicle-stimulating hormone secretion between 45,X monosomy Turner syndrome and 45,X/46,XX mosaicism are evident at an early age. J Clin Endocrinol Metab. 2008;91(12):4896-902.
11. Conte FA, Grumbach MM, Kaplan SL.A diphasic pattern of gonadotropin secretion in patients with the syndrome of gonadal dysgenesis. J Clin Endocrinol Metab. 1975; Apr;40(4):670-4.
12. Lunding SA, Aksglaede L, Anderson RA, Main KM, Juul A, Hagen CP, Pedersen AT. AMH as Predictor of Premature Ovarian Insufficiency: A Longitudinal Study of 120 Turner Syndrome Patients. J Clin Endocrinol Metab. 2015; Jul;100(7):E1030-8.
13. Rosenfield RL, Perovic N, Devine N, Mauras N, Moshang T, Root AW, Sy JP. Optimizing estrogen replacement treatment in Turner syndrome. Pediatr 1998;102:486-488
14. Lee AJ, Cai MX, Thomas PE, Conney AH, Zhu BT Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. Endocrinology 2003; Aug;144(8):3382-98
15. Lepine J, Bernard O, Plante M, Tetu B, Pelletier G, Labrie F, Belanger A, Guillemette C Specificity and regioselectivity of the conjugation of estradiol, estrone, and their catecholestrogen and methoxyestrogen metabolites by human uridine diphospho-glucuronosyltransferases expressed in endometrium. The Journal of clinical endocrinology and metabolism 2004; Oct;89(10):5222-32
16. Levesque E, Turgeon D, Carrier JS, Montminy V, Beaulieu M, Belanger A Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP glucuronosyltransferase. Biochemistry 2001; Apr 3;40(13):3869-81
17. Misra M, Katzman D, Miller KK, Mendes N, Snelgrove D, Russell M, Goldstein MA, Ebrahimi S, Clauss L, Weigel T, Mickley D, Schoenfeld DA, Herzog DB, Klibanski A Physiologic estrogen replacement increases bone density in adolescent girls with anorexia nervosa. J Bone Miner Res 2011;26:2430-2438
18. Cakir ED, Saglam H, Eren E, Ozgur T, Tarim OF. Retrospective evaluation of pubertal development and linear growth of girls with Turner Syndrome treated with oral and transdermal estrogen. J Pediatr Endocrinol Metab. 2015;28:1219-1226

19. Nabhan ZM, Dimeglio LA, Qi R, Perkins SM, Eugster EA. Conjugated oral versus transdermal estrogen replacement in girls with Turner syndrome: a pilot comparative study. J Clin Endocrinol Metab. 2009;94:2009-2014
20. van Pareren YK, de Muinck Keizer-Schrama SM, Stijnen T, Sas TC, Jansen M, Otten BJ, Hoorweg-Nijman JJ, Vulsma T, Stokvis-Brantsma WH, Rouwe CW, Reeser HM, Gerver WJ, Gosen JJ, Rongen-Westerlaken C, Drop SL. Final height in girls with turner syndrome after long-term growth hormone treatment in three dosages and low dose estrogens. J Clin Endocrinol Metab. 2003;88:1119-1125
21. Bannink EM, van Sassen C, van Buuren S, de Jong FH, Lequin M, Mulder PG, de Muinck Keizer-Schrama SM. Puberty induction in Turner syndrome: results of oestrogen treatment on development of secondary sexual characteristics, uterine dimensions and serum hormone levels. Clinical endocrinology 2009;70:265-273
22. Ankarberg-Lindgren C, Elfving M, Wikland KA, Norjavaara E. Nocturnal application of transdermal estradiol patches produces levels of estradiol that mimic those seen at the onset of spontaneous puberty in girls. J Clin Endocrinol Metab. 2001;86:3039-3044
23. Bondy CA. Care of girls and women with Turner syndrome: a guideline of the Turner syndrome study group. J Clin Endocrinol Metab 2007 92 10-25.
24. Soriano-Guillen L, Coste J, Ecosse E, Leger J, Tauber M, Cabrol S, Nicolino M, Brauner R, Chaussain JL, Carel JC. Adult height and pubertal growth in Turner syndrome after treatment with recombinant growth hormone. *J Clin Endocrinol Metab* 2005;90:5197-204
25. Rosenfield RL, Devine N, Hunold JJ, Mauras N, Moshang T, Jr., Root AW. Salutary effects of combining early very low-dose systemic estradiol with growth hormone therapy in girls with Turner syndrome. J Clin Endocrinol Metab. 2005;90:6424-6430
26. Ross JL, Quigley CA, Cao D, Feuillan P, Kowal K, Chipman JJ, Cutler GB, Jr. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. N Engl J Med. 2011;364:1230-1242
27. Shifren JL, Gass MLS The North American Menopause Society Recommendations for Clinical Care of Midlife Women. Menopause: The Journal of The North American Menopause Society 2014; 21(10), 1-25
28. Fournie A, Berrino F, Clavel-Chapelon. Unequal risks for breast cancer associated with different hormone replacement therapies: results from the E3N cohort study. [Erratum appears in Breast Cancer Res Treat. 2008; Jan;107(2):307-8] Breast Cancer Research & Treatment. 107(1):103-11, 2008
29. Stanczyk FZ, Hapgood JP, Winder S, Mishell DR Jr. Progestogens used in postmenopausal hormone therapy: differences in their pharmacological properties, intracellular actions, and clinical effects. Endocr Rev 2013;34(2):171-208
30. Combined hormonal contraception and the risk of venous thromboembolism: a guideline. Fertil and Steril 2017;107(1) 43-51
31. Devineni D, Skee D, Vaccaro N, Massarella J, Janssens L, LaGuardia KD, Leung AT, Pharmacokinetics and pharmacodynamics of a transdermal contraceptive patch and an oral contraceptive. J Clin Pharmacol. 2007;47(4):497.
32. Santen RJ, Allred DC, Ardoin SP, Archer DF, Boyd N, Braunstein GD, Burger HG, Colditz GA, Davis SR, Gambacciani M, Gower BA, Henderson VW, Jarjour WN, Karas RH, Kleerekoper M, Lobo RA, Manson JE, Marsden J, Martin KA, Martin L, Pinkerton JV, Rubinow DR, Teede H, Thiboutot DM, Utian WH. Executive Summary: Postmenopausal

Hormone Therapy: An Endocrine Society Scientific Statement. J Clin Endocrinol Metab, July 2010;95(Suppl 1):S1–S66
33. Rosenfield RL, DiMeglio LA, Mauras N, Ross J, Shaw ND, Greeley SA, Haymond M, Rubin K, Rhodes ET. Commentary: Launch of a quality improvement network for evidence-based management of uncommon pediatric endocrine disorders: Turner syndrome as a prototype. J Clin Endocrinol Metab. 2015;100:1234-1236
34. Rosenfield RL, Fang VS, Dupon C, Kim MH, Refetoff S. The effects of low doses of depot estradiol and testosterone in teenagers with ovarian failure and Turner's syndrome. The Journal of clinical endocrinology and metabolism. 1973;37:574-80
35. Rosenfield RL, Bordini B, Yu C. Comparison of detection of normal puberty in girls by a hormonal sleep test and a gonadotropin-releasing hormone agonist test. The Journal of clinical endocrinology and metabolism. 2013;98(4):1591-601
36. Taboada M, Santen R, Lima J, Hossain J, Singh R, Klein KO, Mauras N. Pharmacokinetics and pharmacodynamics of oral and transdermal 17β estradiol in girls with Turner syndrome. J Clin Endocrinol Metab. 2011; Nov;96(11):3502-10.
37. Trolle C, Hjerrild B, Cleemann L, Mortensen KH, Gravholt CH, Sex hormone replacement in Turner syndrome. Endocrine 2012; Apr;41(2):200-19.
38. Piippo S, Lenko H, Kainulainen P & Sipilä I. Use of percutaneous estrogen gel for induction of puberty in girls with Turner syndrome. *J Clin Endocrinol Metab* 2004;89 3241-7
39. Stuenkel CA, Davis SR, Gompel A, Lumsden MA, Murad MH, Pinkerton JV, Santen RJ. Treatment of Symptoms of the Menopause: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2015; Nov;100(11):3975-4011
40. Morch LS, Skovlund CW, Hannaford PC, Iversen L, Fielding S, Lidegaard O. Contemporary hormonal contraception and the risk of breast cancer. N Engl J Med 2017;377:2228-2239
41. The 2012 Hormone Therapy Position Statement of The North American Menopause Society. Menopause: The Journal of The North American Menopause Society 2012;19(3)257-271
42. Ostberg JE, Storry C, Donald AE, Attar MJN, Halcox JPJ, Conway GS. A dose response study of hormone replacement in young hypogonadal women: Effect on intima media thickness and metabolism. Clin Endocrinol 2007; 66:557-564
43. Koulouri O, Ostberg J, Conway GS.Liver dysfunction in Turner's syndrome: prevalence, natural history and effect of exogenous oestrogen. Clin Endocrinol (Oxf). 2008; Aug;69(2):306-10.
44. Gravholt CH, Naeraa RW, Andersson AM, Christiansen JS, Skakkebaek NE. Inhibin A and B in adolescents and young adults with Turner's syndrome and no sign of spontaneous puberty. Hum Reprod. 2002;Aug;17(8):2049-53.
45. Taylor AE, Adams JM, Mulder JE, Martin KA, Sluss PM, Crowley WF Jr. A randomized, controlled trial of estradiol replacement therapy in women with hypergonadotropic amenorrhea. J Clin Endocrinol Metab. 1996; Oct;81(10):3615-21.
46. Crofton PM, Evans N, Bath LE, Warner P, Whitehead TJ, Critchley HO et al. Physiological versus standard sex steroid replacement in young women with premature ovarian failure: effects on bone mass acquisition and turnover. Clinical Endocrinology 2010;73(6):707-714.

47. Gault EJ, Perry RJ, Cole TJ, Casey S, Paterson WF, Hindmarsh PC, Betts P, Dunger DB, Donaldson MD. Effect of oxandrolone and timing of pubertal induction on final height in Turner's syndrome: randomised, double blind, placebo controlled trial. *BMJ* 2011; 342:d1980.
48. Perry RJ, Gault EJ, Paterson WF, Dunger DB, Donaldson MD. Effect of oxandrolone and timing of oral ethinylestradiol initiation on pubertal progression, height velocity and bone maturation in the UK Turner study. Horm Res Paediatr. 2014;81:298-308
49. Quigley CA, Wan X, Garg S, Kowal K, Cutler GB, Jr., Ross JL Effects of low-dose estrogen replacement during childhood on pubertal development and gonadotropin concentrations in patients with Turner syndrome: results of a randomized, double-blind, placebo-controlled clinical trial. J Clin Endocrinol Metab. 2014;99:E1754-1764
50. Mauras N, Shulman D, Hsiang HY, et al: Metabolic effects of oral versus transdermal estrogen in growth hormone-treated girls with turner syndrome. J Clin Endocrinol Metab. 2007;92:4154-60
51. Gravholt CH, Naeraa RW, Fisker S, Christiansen JS. Body composition and physical fitness are major determinants of the growth hormone-insulin-like growth factor axis aberrations in adult Turner's syndrome, with important modulations by treatment with 17 beta-estradiol. J Clin Endocrinol Metab. 1997; Aug;82(8):2570-7.
52. Kam GY, Leung KC, Baxter RC, Ho KK Estrogens exert route- and dose-dependent effects on insulin-like growth factor (IGF)-binding protein-3 and the acid-labile subunit of the IGF ternary complex. J Clin Endocrinol Metab. 2000;85:1918-1922.
53. Weissberger AJ, Ho KK, Lazarus L. Contrasting effects of oral and transdermal routes of estrogen replacement therapy on 24-hour growth hormone (GH) secretion, insulin-like growth factor I, and GH-binding protein in postmenopausal women. JCEM 1991;72:374-381
54. Roulot D, Degott C, Chazouillères O, Oberti F, Calès P, Carbonell N, Benferhat S, Bresson-Hadni S, Valla D. Vascular involvement of the liver in Turner's syndrome. Hepatology. 2004; Jan;39(1):239-47.
55. Ostberg JE, Thomas EL, Hamilton G, Attar MJ, Bell JD, Conway GS. Excess visceral and hepatic adipose tissue in Turner syndrome determined by magnetic resonance imaging: estrogen deficiency associated with hepatic adipose content. J Clin Endocrinol Metab. 2005; May;90(5):2631-5.
56. Gravholt CH, Poulsen HE, Ott P, Christiansen JS, Vilstrup H. Quantitative liver functions in Turner syndrome with and without hormone replacement therapy. Eur J Endocrinol. 2007;Jun;156(6):679-86.
57. Larizza D, Locatelli M, Vitali L, Viganò C, Calcaterra V, Tinelli C, Sommaruga MG, Bozzini A, Campani R, Severi F. Serum liver enzymes in Turner syndrome.Eur J Pediatr. 2000; Mar;159(3):143-8
58. Elsheikh M, Hodgson HJ, Wass JA, Conway GS. Hormone replacement therapy may improve hepatic function in women with Turner's syndrome. Clin Endocrinol (Oxf). 2001; Aug;55(2):227-31.
59. El-Mansoury M1, Berntorp K, Bryman I, Hanson C, Innala E, Karlsson A, Landin-Wilhelmsen K. Elevated liver enzymes in Turner syndrome during a 5-year follow-up study. Clin Endocrinol (Oxf). 2008; Mar;68(3):485-90.
60. Albareda MM, Gallego A, Enríquez J, Rodríguez JL, Webb SM. Biochemical liver abnormalities in Turner's syndrome. Eur J Gastroenterol Hepatol. 1999; Sep;11(9):1037-9.
61. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. J Clin Epidemiol 1998;51:147-158.

62. Guttmann H, Weiner Z, Nikolski E, Ish-Shalom S, Itskovitz-Eldor J, Aviram M, Reisner S, Hochberg Z. Choosing an oestrogen replacement therapy in young adult women with Turner syndrome. Clin Endocrinol (Oxf). 2001; Feb;54(2):159-64.
63. Alves ST, Gallichio CT, Guimarães MM: Insulin resistance and body composition in Turner syndrome: Effect of sequential change in the route of estrogen administration. Gynecol Endocrinol. 2006;22:590-4
64. Mauras N, Shulman D, Hsiang HY et al: Metabolic effects of oral versus transdermal estrogen in growth hormone-treated girls with turner syndrome. J Clin Endocrinol Metab. 2007;92:4154-60
65. Reinehr T, Lindberg A, Toschke C, Cara J, Chrysis D, Camacho-Hübner C. Weight gain in Turner Syndrome: Association to puberty induction? - Longitudinal analysis of KIGS data. Clin Endocrinol (Oxf). 2016;85(1):85-91
66. Naeraa RW, Gravholt CH, Kastrup KW, Svenstrup B, Christiansen JS. Morning versus evening administration of estradiol to girls with turner syndrome receiving growth hormone: impact on growth hormone and metabolism. A randomized placebo-controlled crossover study. Acta Paediatr. 2001; May;90(5):526-31.
67. Baronio F, Mazzanti L, Girtler Y, Tamburrino F, Lupi F, Longhi S, Fanolla A, Radetti G. The Influence of GH Treatment on Glucose Homeostasis in Girls With Turner Syndrome: A 7-Year Study, J Clin Endocrinol Metab. 2017; Mar 1;102(3):878-883. 3179.
68. Cleemann L, Holm K, Kobbernagel H, Kristensen B, Skouby SO, Jensen AK, Gravholt CH. Dosage of estradiol, bone and body composition in Turner syndrome: a 5-year randomized controlled clinical trial, Eur J Endocrinol. 2017; Feb;176(2):233-242.
69. Cartwright B, Robinson J, Seed PT, Fogelman I, Rymer J. Hormone Replacement Therapy Versus the Combined Oral Contraceptive Pill in Premature Ovarian Failure: A Randomized Controlled Trial of the Effects on Bone Mineral Density. J Clin Endocrinol Metab 2016;101(9):3497-3505.
70. European Society for Human Reproduction and Embryology (ESHRE) Guideline Group on POI, Webber L, Davies M, Anderson R, Bartlett J, Braat D et al. ESHRE Guideline: management of women with premature ovarian insufficiency. Human Reproduction 2016;31(5):926-937.
71. Herrmann M, Seibel MJ. The effects of hormonal contraceptives on bone turnover markers and bone health. Clinical Endocrinology 2010;72(5):571-583.
72. Lopez LM, Grimes DA, Schulz KF, Curtis KM, Chen M. Steroidal contraceptives: effect on bone fractures in women. Cochrane Database of Systematic Reviews 2014 Jun 24 2014;(6):CD006033.
73. Paterson WF, Hollman AS, Donaldson MD. Poor uterine development in Turner syndrome with oral oestrogen therapy. Clin Endocrinol (Oxf). 2002; Mar;56(3):359-65
74. Bakalov VK, Shawker T, Ceniceros I, Bondy CA. Uterine development in Turner syndrome. J Pediatr. 2007; Nov;151(5):528-31, 531.e1.
75. Rodrigues EB, Braga J, Gama M, Guimarães MM. Turner syndrome patients' ultrasound profile. GynecolEndocrinol 2013; Jul;29(7):704-6.
76. Elsedfy HH1, Hamza RT, Farghaly MH, Ghazy MS. Uterine development in patients with Turner syndrome: relation to hormone replacement therapy and karyotype. J PediatrEndocrinolMetab. 2012;25(5-6):441-5.
77. Cleemann L, Holm K, Fallentin E, Skouby SO, Smedegaard H, Møller N, Borch-Christensen H, Jeppesen EM, Wieslander SB, Andersson AM, Cohen A, Højbjerg Gravholt C,

Uterus and ovaries in girls and young women with Turner syndrome evaluated by ultrasound and magnetic resonance imaging. Clin Endocrinol (Oxf). 2011; Jun;74(6):756-61.
78. Foudila T, Söderström-Anttila V, Hovatta O. Turner's syndrome and pregnancies after oocyte donation Hum Reprod. 1999; Feb;14(2):532-5.
79. Gravholt CH, Naeraa RW, Nyholm B, Gerdes LU, Christiansen E, Schmitz O, Christiansen JS. Glucose Metabolism, Lipid Metabolism, and Cardiovascular Risk Factors in Adult Turner's Syndrome: The impact of sex hormone replacement. *Diabetes Care*, 1998:21:1062-1070
80. Langrish JP, Mills NL, Bath LE, Warner P, Webb DJ, Kelnar CJ, Critchley HOD, Newby DE, Wallace WHB. Cardiovascular Effects of Physiological and Standard Sex Steroid Replacement Regimens in Premature Ovarian Failure *Hypertension* 2009, 53:805-811
81. Mortensen KH, Anderson AH, Gravholt CH. Cardiovascular Phenotype in Turner Syndrome—Integrating Cardiology, Genetics, and Endocrinology *Endocrine Reviews* 2012; 33:677-714
82. Stachenfeld NS, DiPietro L, Palter SF, Nadel ER. Estrogen influences osmotic secretion of AVP and body water balance in postmenopausal women. Am J Physiol. 1998;274:R187-95
83. Oelkers W, Foidart JM, Dombrovicz N, Welter A, Heithecker R. Effects of a new oral contraceptive containing an antimineralocorticoid progestogen, drospirenone, on the renin-aldosterone system, body weight, blood pressure, glucose tolerance, and lipid metabolism. J Clin Endocrinol Metab. 1995;80:1816-21
84. Renoux C, Dell'aniello S, Garbe E, Suissa S.Transdermal and oral hormone replacement therapy and the risk of stroke: a nested case-control study. BMJ. 2010; Jun 3;340:c2519
85. Sweetland S, Beral V, Balkwill A, Liu B, Benson VS, Canonico M, Green J, Reeves GK; Million Women Study Collaborators. Venous thromboembolism risk in relation to use of different types of postmenopausal hormone therapy in a large prospective study. J Thromb Haemost. 2012;Nov;10(11):2277-86
86. Baber RJ, Panay N, Fenton A The IMS Writing Group. 2016 IMS Recommendations on women's midlife health and menopause hormone therapy, Climacteric, 2016;19:2, 109-150
87. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler GB Jr.Effects of estrogen on nonverbal processing speed and motor function in girls with Turner's syndrome. J ClinEndocrinolMetab. 1998; Sep;83(9):3198-204.
88. Ross JL, Roeltgen D, Feuillan P, Kushner H, Cutler GB Jr.Use of estrogen in young girls with Turner syndrome: effects on memory. Neurology. 2000; Jan 11;54(1):164-70.
89. Ross JL, McCauley E, Roeltgen D, Long L, Kushner H, Feuillan P, Cutler GB Jr.Self-concept and behavior in adolescent girls with Turner syndrome: potential estrogen effects. J ClinEndocrinolMetab. 1996; Mar;81(3):926-31
90. Ross JL, Stefanatos GA, Kushner H, Zinn A, Bondy C, Roeltgen D.Persistent cognitive deficits in adult women with Turner syndrome. Neurology. 2002; Jan 22;58(2):218-25.
91. Ross JL, Stefanatos GA, Kushner H, Bondy C, Nelson L, Zinn A, Roeltgen D.The effect of genetic differences and ovarian failure: intact cognitive function in adult women with premature ovarian failure versus turner syndrome. J Clin Endocrinol Metab. 2004; Apr;89(4):1817-22.
92. Carel JC[1], Elie C, Ecosse E, Tauber M, Léger J, Cabrol S, Nicolino M, Brauner R, Chaussain JL, Coste J. Self-esteem and social adjustment in young women with Turner syndrome--influence of pubertal management and sexuality: population-based cohort study. J Clin Endocrinol Metab. 2006; Aug;91(8):2972-9.

93. Sheaffer AT, Lange E, Bondy CA. Sexual function in women with Turner syndrome. J Womens Health 2008; Jan-Feb;17(1):27-33.
94. Fjermestad KW, Naess EE, Bahr D, Gravholt CH. A 6-year follow-up survey of health status in middle-aged women with Turner Syndrome, Clin Endocrinol 2016, 85:423-429.
95. Bannink EM, Raat H, Mulder PG, de Muinck Keizer-Schrama SM. Quality of life after growth hormone therapy and induced puberty in women with Turner syndrome. J Pediatr. 2006; Jan;148(1):95-101.
96. Nilsson KO, Albertsson Wikland K, Alm J, Aronson S, Gustafsson J, Hagenas L, Hager A, Ivarsson SA, Karlberg J, Kristrom B, Marcus C, Moell C, Ritzen M, Tuvemo T, Wattsgard C, Westgren U, Westphal O, Aman J. Improved final height in girls with Turner's syndrome treated with growth hormone and oxandrolone. *J Clin Endocrinol Metab* 1996; 81 635-640.
97. Menke LA, Sas TC, de Muinck Keizer-Schrama SM, Zandwijken GR, de Ridder MA, Odink RJ, Jansen M, Delemarre-van de Waal HA, Stokvis-Brantsma WH, Waelkens JJ, Westerlaken C, Reeser HM, van Trotsenburg AS, Gevers EF, van BS, Dejonckere PH, Hokken-Koelega AC, Otten BJ, Wit JM. Efficacy and safety of oxandrolone in growth hormone-treated girls with turner syndrome. *J Clin Endocrinol Metab* 2010; 95:1151-1160.
98. Zeger MP, Shah K, Kowal K, Cutler GB, Jr., Kushner H, Ross JL. Prospective study confirms oxandrolone-associated improvement in height in growth hormone-treated adolescent girls with Turner syndrome. *Horm Res Paediatr* 2011; 75:38-46.
99. Sas TC, Gault EJ, Bardsley MZ, Menke LA, Freriks K, Perry RJ, Otten BJ, de Muinck Keizer-Schrama SM, Timmers H, Wit JM, Ross JL, Donaldson MD. Safety and efficacy of oxandrolone in growth hormone-treated girls with Turner syndrome: evidence from recent studies and recommendations for use. Horm Res Paediatr. 2014;81:289-297.
100. Freriks K, Sas TC, Traas MA, Netea-Maier RT, den Heijer M, Hermus AR, Wit JM, van Alfen-van der Velden JA, Otten BJ, de Muinck Keizer-Schrama SM, Gotthardt M, Dejonckere PH, Zandwijken GR, Menke LA, Timmers HJ. Long-term effects of previous oxandrolone treatment in adult women with Turner syndrome. Europ J Endocrinol. 2013;168:91-99
101. Sheanon NM, Backeljauw PF 2015 Effect of oxandrolone therapy on adult height in Turner syndrome patients treated with growth hormone: a meta-analysis. Int J Pediatr Endocrinol 2015;18

Table 1. Some common low-dose estrogen treatment options for pubertal induction in TS, and considerations for use.

Preparation *	**Doses available, frequency, route**	**Starting dose of puberty**	**Increase approximately every 6 m to adult dosing**	**Considerations for use**
Transdermal options (some brands)		3-7 µg/day	25-100 µg/day	See text on applying patches
Menostar (matrix)	14 µg weekly TD	½ patch weekly	Only used for low dosing, not full replacement	Easiest way to give low dose; once a week dosing
Vivelle Dot (matrix)	25, 37.5, 50, 75, 100 µg twice weekly	¼ patch weekly, or 1 patch per month (no patch other 3 weeks)	25-100 µg twice weekly	Designed for twice weekly, but can give once per week to increase dose slower
Vivelle Mini (matrix)	25, 37.5, 50, 75, 100 µg twice weekly	Too small to consistently cut	25-100 µg twice weekly	Smaller size patch, but not smaller dosing
Generic (different brands in different countries; ex: Oesclim Estradot, Evorel, Systen)	25, 37.5, 50, 75, 100 µg twice weekly	¼ patch weekly, or 1 patch per month (no patch other 3 weeks)	25-100 µg twice weekly	Once a week dosing can be used
Estraderm (matrix)	50, 100 µg twice weekly	Not small enough to	50-100 µg twice	Can't use to intiate puberty

		initiate puberty	weekly	
E_2 gel		0.25 mg/pump	1 pump daily	Only available in some countries at the low dose
Estragel 0.06%	0.75 mg E_2/pump			
Divigel (0.1%)	0.25, 0.5, 0.1 mg E_2/pump			
Oral options				
17β-estradiol (E_2) (ex: Estrace, Cetura)	0.5, 1, 2, 4 mg daily	½ pill daily	1-4 mg/day	Cheapest option, brands vary by country
Ethinyl Estradiol (EE)		2 µg/day	10-20 µg/day	Not available in many countries
Premarin (CEE)	0.3, 0.625, 0.9, 1.25 mg daily	½ pill daily	0.625-1.25 mg/day	Not available in many countries, not recommended based on safety
Depot options				
Depot E_2 (cypionate)	5 mg/ml	0.2 mg/m	2 mg/m	Not available in Europe

* The reader should be aware that availability and trade names differ among countries. The list is not all-inclusive.

Table 2. Some common progestin and estrogen/progestin combination replacement options after pubertal induction is complete.

Adding Progestin options	**Doses available, frequency and route**	**Not needed to initiate puberty**	**Add once bleeding occurs or after 2 years**	**Notes**
Medroxyprogesterone acetate	10 mg daily for 10 days		Give with TD E_2, or alone for 10 days	
Micronized progesterone (Prometrium)	100 mg daily		Give continuously with TD E_2	Less breast cancer risk long term
Combined E_2/Progestin sequential patch - some brand options		Do not use to initiate puberty		
Climara Pro	E_2 0.045 mg/levonorgestrel 0.015 mg/24 h		1 patch weekly	
Combipatch	E_2 0.045 mg/norethidrone 0.14 or 0.25 mg/24 h		1 patch weekly	
Evo-Sequi	E_2 50 µg /norethisterone acetate 170 µg/24 h		2 patches weekly	
Combined E_2/Progestin sequential pills		Do not use to initiate puberty		
Trisequens	E_2 2 mg /norethisterone acetate 1 mg		1 pill/day	
Divina plus	Estradiolvalerate 2 mg/Medroxyprogesterone acetate 10 mg		1 pill/day	
Femoston	E_2 and dydrogesterone 1/10 or 2/10 mg		1 pill/day	
Oral contraceptive pills		Do not use to initiate puberty		

* There are multiple types of oral contraceptive pills, which differ in estrogen dose, sequential vs continuous, and type and dose of progestin. The reader is referred to the text to outline general principles.

Table 3. Summary of published low-dose estrogen treatment for puberty induction in TS

Author, Journal, Year	**Subjects**	**Estrogen Treatment, Route and Dose**	**Outcomes**	**Height**
Ankarberg-Lindgren, JCEM, 2001	8 girls with TS (12 - 16 y) and 7 with other hypogonadism	TD E_2 6 µg - 18 µg given just 12 h overnight	B2 in 3 - 6 m in 75% of girls on low dose, and B3 in 2 y on higher dose; TD dose correlated with serum E_2 ($p<0.001$)	No height data
Van Pareren, JCEM, 2003	60 girls with TS, ± spontaneous puberty	Oral E_2 5 µg/kg X 2y → 7.5 µg/kg X 1y → 10 µg/kg after 4 y GH	B2 onset in 0.2 y avg	No neg effect on height or growth velocity vs spontaneous puberty
Piippo, JCEM 2004	23 girls with TS	E_2 gel 0.1 mg X1y→ 0.2 mg X1 y → 0.5 mg X1y → 1 mg X1y → 1.5 mg X1 y	Pubertal advance about 1 stage per year treatment with 50% ≥ B2 at 6 m	Adult height 153.1 ± 4.8 cm
Soriano-Guillen, JCEM 2005	704 girls with TS ± spontaneous puberty	Oral EE 1-5 µg/d; oral E_2 0.5 mg/d; TD ¼ of a 25	No data on rate of pubertal progression	TD E_2 taller than oral by 2.1 cm avg; but shorter than

		µg/d patch		spontaneous
Rosenfield, JCEM, 2005	14 girls with TS, compared with NCGS registry, 12 - 15 yo	Depot E_2 0.2 mg/m with increase of 0.2 mg every 0.5 y; GH also given 0.05 mg/kg/d	Half of girls B1 --> B2 in 0.5 y; and increased one stage per 0.5 y with each 0.2 mg increase in dose; On 1 mg - 100% B3-B5 by 2 y and menarche in 62.5% by 2.5 y	Lowest dose had greatest GV; FH > PAH at start of Rx; FH > GH alone, growth not as good as in TS with spontaneous puberty
Nabhan, JCEM, 2009	12 girls with TS, 11.3 - 17 y	Oral CEE (0.3 -0.45 mg/d) vs. TD (25 µg/d for 6 m --> 37.5 µg/d for 6 m)	B3-4 by 1 y in 83%; no change in 17%; TD group had greatest increase in spine density, BMD, uterine length and volume	No height data
Bannick, Clin Endocrinol, 2009	56 girls with TS, 11 - 18 yo	Oral E_2 (5 µg/kg/d) X 2 yrs; with progression to 7.5 and 10 µg/kg/d	Breast stage progressed in same timing as average Dutch population: B1 --> B2 in 0.2 y; B1 --> B4 in 2.1 y	No height data
Torres-Santiago, JCEM, 2013	40 girls with TS, 13-20 y	Oral E_2 (avg 2 mg) vs. TD E_2 (avg 0.1 mg) - dose titrated to plasma E_2	No difference in body composition, BMD, or lipids between groups	No height data
Ross, NEJM, 2011; and Quigley, JCEM, 2014	144 girls with TS analyzed for growth; 123 girls with TS analyzed for puberty, 5 - 12.5 yo	Oral EE: 25 ng/kg/d, 5–8 y; 50 ng/kg/d, >8–12 y; >12 y, escalating from 100 ng/kg/d; ± GH	EE dose decreased for breast before 12 yo or vaginal bleeding before 14 y; Age of menarche similar to general population; Earlier breasts for girls who received the early low dose	GH + EE group height SDS increase of 0.58 compared to increase of 0.26 in GH alone
Perry, Hormone Res in Pediatr, 2014	92 girls with TS, 7 - 13 y	Oral EE: 2 µg/d Y 1; 4 2 µg/d Y 2; 6/8/10 2 µg/d increases every 4 m in Y 3	B1-->B2 in 0.65 y and to B4 in 2.25 y	Growth not as good as with depot E_2 reported
Cakir, J Pediatr Endocrinol Metab, 2015	13 girls with TS, 11 - 17 y	Oral E_2 (0.5 mg/d) vs. TD (4.5 µg/d)	B1-->B3-4 in 1 y	BA advance less with TD (ΔCA/ΔBA 2.2 vs. 0.58, p=0.005); GV greater on TD at 1 y (4.35 vs. 3.8, p=0.022)

Legend: B2 (3,4) – breast stage 2 (3,4); BA – bone age; CA – chronologic age; GV – growth velocity; FH – final height; PAH – predicted adult height; GH – growth hormone

Table 4. Classification of Progestins

Classification	**Progestin**
Natural	Progesterone
Synthetic	
Pregnane derivatives	
Acetylated	Medroxyprogesterone acetate
	Megestrol acetate
	Cyproterone acetate
Nonacetylated	Chlormadinone acetate
	Dydrogesterone
	Medrogestone
19-Norpregnane derivatives	
Acetylated	Nomegestrol acetate
	Nesterone
Nonacetylated	Demegestone
	Promegestone
	Trimegestone
Nor-testosterone	
Ethinylated Estranes	Norethindrone (norethisterone)
	Norethindrone acetate
	Ethynodiol diacetate
	Norethynodrel
	Lynestrenol
	Tibolone
13-Ethylgonanes	Levonorgestrel
	Desogestrel
	Norgestimate
	Gestodene
Nonethinylated	Dienogest
	Drospirenone

Table 5. Estrogen treatment and Metabolic Outcome Data

Author, journal, year	(n)	Treatment	Main metabolic measure outcome
Jospe, J Pediatr Endocrinol Metab, 1995	8	Oral E_2: 100 ng/kg/d *vs.* TD E_2: 0.0125 mg/kg/d	Oral, but not TD, increased serum HDL
Gravholt, Diabetes Care, 1998, Atherosclerosis, 2000, J Clin Endocrinol Metab, 1997	15 (oral) 8 (TD)	Oral: 2 mg/day E_2 days 1–22, + 1 mg/d norethisterone acetate days 13–22, and 1mg/d E_2 days 23–28 vs. TD E_2 50 mg/day for 28 days + oral 1 mg norethisterone days 13–22	No difference between oral and TD in insulin sensitivity, body composition changes, 24h ambulatory blood pressure, IGF-I, liver function tests and lipids
Gussinyé, Horm Res, 2000	12	TD E_2: 100 µg/d	BMD and BMD z-score values significantly increased; no significant differences in BMI, calcium intake and physical activity habits
Guttmann, Clin Endocrinol (Oxf) 2001	17	CEE: 0.625 mg/d *vs.* EE: 30 µg/d	Hyperinsulinemia was suppressed to normal by both EE and CEE Lipid profiles were normal on both regimens. PTH and 1,25-dihydroxyvitamin D levels increased on HRT (EE > CEE), and phosphorus decreased Alkaline phosphatase, osteocalcin and urinary deoxypyridinoline cross-links (DPD) were high off therapy; the former two suppressed to high-normal levels on the EE regimen, but not on CEE.
Naeraa, Acta Paediatr, 2001	9	morning Oral E_2 6-11 µg/kg/d *vs.* evening Oral E_2 6-11 µg/kg/d	During OGTT in the morning, glucagon and insulin were lower following evening E_2administration as well as insulin resistance tended to be lower
Alves, Gyncecol Endocrinol, 2006	9	CEE: 0.625 mg/d *vs.* TD E_2 (gel): 1.5 mg/d	No difference in BMI, WHR, or insuline tolerance between CEE and TD E_2 During TD: tendency to increased total lean mass
Mauras, JCEM, 2007	11	Low dose Oral E_2: 0.5 mg/d	LDL/HDL cholesterol responses were variable among groups
Taboada, JCEM, 2011	10	Low dose TD E_2: 0.0375 mg/d High dose Oral E_2: 2.0 mg/d High dose TD E_2: 0.075 mg Oral E_2 0.5, 1, 2 mg/d for 2 weeks each *vs.* TD E_2 0.025, 0.0375, 0.05 mg/d for 2 weeks each	Neither Oral nor TD E_2adversely affected rates of protein turnover, lipolysis, and lipid oxidation rates or plasma lipids, fibrinogen, or fasting insulin concentrations
Torres-Santiago, JCEM, 2013	40	Oral E_2: 2 mg *vs.* TD E_2: 0.1 mg	Similar fat-free mass, %fat mass, bone mineral density accrual, lipid oxidation, resting energy expenditure rates No significant changes in lipids, glucose, osteocalcin, hs-CRP
Reinehr, Clin Endocrinol (Oxf), 2016	490	Oral vs. TD (no details availabe)	The duration and dose of estrogens, its route of administration did not correlate significantly to changes of BMI-SDS

Legend: E_2 - estradiol, TD - transdermal; CE- conjugated estrogen; EE- ethinyl estradiol; OGTT- oral glucose tolerance test; BMI- body mass index; WHR- waist to hip ratio; BMD- bone mineral density; SDS- standard deviation score