Physiological Estrogen Replacement Therapy for Puberty Induction in Girls: A Clinical Observational Study

Carina Ankarberg-Lindgren^a Berit Kriström^b Ensio Norjavaara^a

^aGöteborg Pediatric Growth Research Center, Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Göteborg, and ^bInstitute of Clinical Science/Pediatrics, Umeå University, Umeå, Sweden

Key Words

Estrogen deficiency · Hormone replacement therapy, transdermal · Ovarian failure · Turner syndrome · Growth

Abstract

Background/Aim: The goal of estrogen replacement therapy (ERT) in girls with hypogonadism is to achieve the endocrine milieu similar to natural puberty, where transdermal administration is the most physiological route. The aim of the study was to evaluate guidelines for the induction of puberty with transdermal estradiol (E₂) patches in a large outpatient setting. **Methods:** In a retrospective study, serum E₂ levels from 18 clinics were analyzed at the Göteborg Pediatric Growth Research Center laboratory, as part of the initiation of ERT in girls with hypogonadism. Exclusion criteria were pubertas tarda and pubertal arrest. Eighty-eight observations (50 with Turner syndrome, TS) were included. Serum E₂ levels were determined by extraction + radioimmunoassay (detection limit 4 pmol/l) and analyzed in relation to the dose of Evorel® (25 µg/24 h, containing 1.60 mg estradiol hemihydrate; Janssen-Cilag Pharmaceutica N.V., Beerse, Belgium). **Results:** There was a linear relationship between serum E₂ and the weight-based dose, with $r = 0.56$, $p < 0.0001$ for all observations and $r = 0.59$, $p < 0.0001$ for the TS study group. Linear

regression analysis for doses of 0.05–0.07 µg/kg resulted in serum levels of 17–23 pmol/l (TS 17–24 pmol/l) and doses of 0.08–0.12 µg/kg in 26–39 pmol/l (TS 27–39 pmol/l). **Conclusions:** For the initiation of ERT with nocturnally administered E₂ patches, we recommend *reduced* starting doses of 0.05–0.07 µg/kg, with the goal of mimicking E₂ levels during gonadarche. In older girls, when breast development is of high priority, the starting dose can still be 0.08–0.12 µg/kg.

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Introduction

The overall goal of estrogen replacement therapy (ERT) in girls with hypogonadism is to establish an age-appropriate endocrine milieu resulting in normal growth, bone mass accrual, uterine growth and maturation, development of secondary sexual characteristics and cognitive functions, at a tempo consistent with the peer group [1, 2]. The very early part of the pubertal growth spurt and the development of secondary sexual characteristics are regulated by increasing serum levels of nocturnal estrogens, where low serum estrogen levels are growth promoting and higher levels are needed for complete breast and body development [3, 4].

Study Material

The retrospective study consisted of registered serum E₂ levels from samples sent for analysis to the Göteborg Pediatric Growth Research Center laboratory, at the Queen Silvia Children's Hospital, Göteborg, Sweden. All clinical information on the referrals was recorded, together with the assay result. The samples were submitted from 18 clinics in Sweden. Included were those girls who broadly followed the guidelines by Ankarberg-Lindgren et al. [5] for puberty induction with ERT. With this retrospective design, the study lacks confirmatory clinical and laboratory data on the diagnosis. The analysis has to rely on the diagnosis recorded. Furthermore, no information of compliance was found.

E₂ Determination

Serum E₂ concentrations were determined in duplicate by an assay which involves a diethyl ether extraction step prior to radioimmunoassay (Spectria® Estradiol RIA; Orion Diagnostica, Espoo, Finland) [1, 6]. The extraction E₂ radioimmunoassay has been validated especially for pediatric use [6] and is accredited by the SWEDAC quality control agency in Sweden, SS-EN ISO 15189 (No. 1899). The lower limit of detection was 4 pmol/l. The cross-reactivity of the E₂ antiserum was below 1% for 30 E₂-related steroids. The intra-assay coefficient of variation was 8–17% for concentrations in the 4–100 pmol/l range, while the interassay coefficient of variation was 19% at 6 pmol/l, below 12% for 10–40 pmol/l, and below 10% for 70 pmol/l and above.

Statistical Analyses

Relationships between E₂ dose and obtained serum E₂ were estimated with Pearson's correlation analyses. Linear regression analysis and correlation analysis were performed with Origin 9.0, OriginLab Corp., Northampton, Mass., USA. For girls in whom more than one observation was made for a given dose, the median was used in the statistical analyses.

Ethics Statement

Informed consent was obtained, in accordance with the Swedish Biobank Act (Biobankslagen SFS 2002:297), in the form of parental agreement to the inclusion of their child's samples and referrals in a biobank.

Results

All Kinds of Hypogonadism

For the 29 girls who started ERT at 0.05–0.07 µg/kg, all had serum E₂ levels above the reference interval for prepuberty (B1) [7]. Sixteen of them had levels in the peripubertal range (7–24 pmol/l), Tanner breast stage 1–2 (B1–2), while 12 were within the reference interval for Tanner breast stage 2 (B2; 24–47 pmol/l), and 1 was just above the B2 range (fig. 2a).

Of the 42 girls receiving the dose 0.08–0.12 µg/kg, 20 still had low levels corresponding to B1–2; 18 had serum E₂ within the reference interval for B2, while 4 had levels above the B2 interval (fig. 2a).

Fig. 2. Serum E₂ concentrations in relation to E₂ dose per kilogram body weight from a matrix patch (Evorel; 25 µg/24 h, containing 1.60 mg E₂ hemihydrate; Janssen-Cilag Pharmaceutica) in girls with hypogonadism. The morning serum samples were drawn with the patch still in situ. The gray area depicts the reference interval for girls in early puberty divided into three areas: peripubertal levels, i.e. levels seen during assumed gonadarche in girls with Tanner breast stage 1–2 (B1–2), breast stage 2 (B2) and the transition stage between breast stages 2 and 3 (B2–3), respectively. **a** Eighty-eight samples from 54 girls with any kind of hypogonadism. Linear regression analysis showed that serum E₂ (pmol/l) = 307.2 × E₂ dose (µg/kg) + 1.7 ($r = 0.56$, $p < 0.0001$). **b** Fifty E₂ samples from a subgroup of 31 girls with TS. Linear regression analysis showed that serum E₂ (pmol/l) = 314.3 × E₂ dose (µg/kg) + 1.6 ($r = 0.59$, $p < 0.0001$).

Among the 17 girls who were treated with 0.13–0.15 µg/kg, only 9 had E₂ levels within the B2 interval, while 5 had E₂ levels in the B2–3 interval (46–77 pmol/l) and 3 in the Tanner breast stage 3 (B3) interval. The relationship between E₂ dose per kilogram body weight and the E₂ serum concentration for the total group is shown in figure 2a. Linear regression analysis for doses of 0.05–0.07 µg/kg resulted in serum levels of 17–23 pmol/l and doses of 0.08–0.12 µg/kg resulted in 26–39 pmol/l.

Table 1. Doses of pubertal ERT converted to the corresponding individual patch doses

Dose, µg/kg	Weight, kg	Proportion of Evorel (25 µg/24 h) patch	Proportion of Evorel (50 µg/24 h) patch	Evorel dose, µg	Goal
0.05–0.07	≤40	1/14–1/12	1/24	2	Mimicking peripubertal E ₂ levels (7–24 pmol/l), which is seen in girls at gonadarche, with or without breast development
	41–60	1/8	1/16	3	
	60–85	1/7–1/6	1/14–1/12	4	
	>85	1/4	1/8	6	
0.08–0.12	<40	1/8	1/16	3	Breast development; serum E ₂ level 24–46 pmol/l
	35–50	1/7–1/6	1/14–1/12	4	
	50–80	1/4	1/8	6	
	>80	2/7	1/7	7	

Turner Syndrome

A subgroup analysis was performed on 50 blood samples from the 31 girls with TS. A linear relationship was found between E₂ dose and obtained serum levels (fig. 2b). Linear regression analysis for doses of 0.05–0.07 µg/kg resulted in serum levels of 17–24 pmol/l and doses of 0.08–0.12 µg/kg resulted in 27–39 pmol/l. When the two functions (TS vs. all kinds of hypogonadism) were compared, there was no significant difference.

For the 21 TS girls who started E₂ treatment on 0.05–0.07 µg/kg, 12 had serum E₂ levels within the B1–2 interval, while 8 observations were within the B2 interval and 1 was just above the B2 range. Furthermore, in 18 girls receiving the dose 0.08–0.12 µg/kg, 12 had E₂ levels in the B1–2 interval, 4 were within the B2 interval, and 2 were in the interval seen both in breast stages 2 and 3 (B2–3). However, in the 11 girls who were treated with a higher dose (0.13–0.15 µg/kg), 5 had E₂ levels within the B2 interval while 4 had E₂ levels in the B2–3 interval and 2 in the B3 interval (fig. 2).

In table 1, the obtained doses of E₂ (µg/kg) are converted to the corresponding individual patch doses. This study was made with 25-µg patches; however, if the 25-µg patches are not available, the surface of a 50-µg patch is double the size of a 25-µg patch, and our results may be converted accordingly.

Discussion

We have previously published a recommendation that transdermal E₂ matrix patches should be cut to administer low doses; with nocturnal application, the serum E₂ levels and diurnal rhythm in normal girls undergoing puberty can be closely mimicked [5]. Our regimen has become a worldwide guideline [8–10]. However, the pub-

lished guidelines were based on a limited group of hospitalized patients [5]; in the present study, we evaluated how this regimen works in an outpatient setting, using an even lower starting dose, testing a larger group of hypogonadal girls and assessing the reported dose in relation to the achieved morning level of serum E₂.

The results from this study suggest that the ERT starting dose should be 0.05–0.07 µg/kg, which is lower than in our previous study [5]. With this reduced regimen, the risk of too high a serum E₂ concentration, compared to the reference interval for Tanner stage B2, will be reduced. This is also supported by recent results from girls in spontaneous puberty, demonstrating an association of serum E₂ levels of 13–31 pmol/l with increased height velocity or onset of the pubertal growth spurt [3].

For clinics *with* access to ultrasensitive methods for determination of childhood E₂, we recommend starting with a dose of 0.05–0.07 µg/kg and following the morning serum E₂ level within 1–2 weeks, when the patient is comfortable with the regime. This makes it possible to rapidly adjust the dose if the individual target serum level is not reached.

For clinics *without* access to an ultrasensitive E₂ assay, we recommend starting with the same dose of 0.05–0.07 µg/kg and following the clinical signs of breast development or increment in uterine size by ultrasound. If no effect is found on breast development after 9–15 months, the dose can be increased. If breast development starts within 3 months, consider reducing the dose if the aim is low E₂ levels in the beginning of puberty, or refraining from dose increment for at least 12 months. This consideration could be applied to other effects of E₂, such as development of the uterus and advancement of bone age, but we have limited data on how E₂ levels relate to uterine development, and we cannot give any recommendations on these measures.

For those clinical situations in which breast development is a higher priority than a continued favorable growth – for example in older girls or in girls with TS and pubertal arrest – we would recommend starting with higher doses, such as 0.08–0.12 µg/kg; these girls may already have been exposed to pubertal levels of E₂.

Our recommendation to give 4 µg (1/7 to 1/6 of a 25-µg patch, or 1/14 to 1/12 of a 50-µg patch) for puberty induction in girls weighing approximately 63 kg is in line with obtained serum E₂ concentrations in postmenopausal women using the 50-µg Evorel patch. The average serum E₂ concentration of 184 pmol/l in an average 63-kg woman using the 50-µg patch [11] is about 12 times higher than our target level for serum E₂ for puberty induction; thus, our recommended dose of 4 µg is 1/12 of the adult dose.

Physiological age is preferable as a guide to deciding when to start the treatment, as it supports normal bone mineral accrual [12], and there is also the psychological advantage for the girl to reach puberty at the same age as her peers [13]. Earlier ERT may also improve cognitive function, as demonstrated in girls with TS [14]. For these reasons, there is international consensus to induce puberty in girls with TS around the age of 12 years [8], which is approximately 1 year later than the average pubertal onset in normal girls.

In the present study, some of the girls started ERT earlier (girls aged 9–11 years) than the recommendation from guidelines [8]. The reason for an earlier start of pubertal ERT was to mimic the peripubertal levels (gonadarche) of E₂ seen 1–2 years before the start of breast development [15]. And indeed, the present study shows that it is possible. Whether it is possible to mimic the prepubertal levels by cutting the patch to even smaller pieces needs to be studied in the future.

In our previous study, we found that low doses of ≤0.08 µg/kg may not necessarily induce breast development [5]. However, after the first administration of patches in doses of 0.08–0.12 µg/kg, breast development occurred within 3–6 months for the majority. It was also found that girls with E₂ levels above the B2 reference interval on the initial dose developed to stage B3 within 10 months of the start of ERT [5]. Nevertheless, some girls need higher doses, possibly due to individual breast sensitivity to E₂, all drawing attention to the need for close monitoring of serum E₂ levels in relation to clinical development.

There are both benefits and risks associated with pubertal ERT. The most serious risk is that no product is authorized for this indication; thus, treatment is per-

formed off label with formulas intended for adults, which may result in adult E₂ levels and too fast a pubertal development [16–18], or in attempts to manipulate adult doses to mimic E₂ levels seen during spontaneous puberty [5, 19].

Nonetheless, transdermally administered ERT can achieve serum E₂ levels, as well as a diurnal rhythm, similar to spontaneous puberty and the associated risks are low. Transdermal ERT has minimal, if any, effect on hepatic clotting factor production and other protein changes associated with the liver first-pass effect. Whereas oral ERT in postmenopausal women is associated with an increased risk of deep vein thrombosis and pulmonary thromboembolism [20–22], breast cancer [23], reduced insulin-like growth factor-I levels, increased fat mass and reduced lean body mass [24], none of these effects are evident with transdermal ERT. Furthermore, studies on physiological sex steroid replacement therapy (transdermal E₂ and progesterone) in postmenopausal women and women with premature ovarian failure resulted in lower blood pressure and improved renal function compared to oral regimens [22, 25]. Transdermal ERT in girls with TS has shown a faster bone accrual in the spine, increased uterine growth, improved overall body composition and lower luteinizing hormone/follicle-stimulating hormone concentrations compared to oral ERT [17, 26, 27]. Moreover, ethinyl E₂ – a potent synthetic estrogen used in oral contraception – has different binding properties compared to natural estrogen (17β-E₂ in transdermal preparations), in that ethinyl E₂ has a fourfold selectivity for estrogen receptor α compared to 17β-E₂, which has similar selectivity for both α- and β-forms [28].

This is not a randomized, placebo-controlled clinical trial and has all the weaknesses that an observational study has. In addition, hospitalized patients are more closely controlled regarding the dose, compared to patients in outpatient settings. Nevertheless, even if there was a relatively high interindividual variance in the present study, we found a linear relationship between ERT dose and serum E₂ levels, in the TS group as well as in the group with hypogonadism of various etiologies. Although E₂ levels of 24–30 pmol/l were seen over the dose range from 0.05 to 0.15 µg/kg, a doubling of the dose resulted in an approximate doubling of the serum E₂ level. This indicates that the results may be applied to any kind of hypogonadism.

We conclude that for the initiation of ERT in order to induce pubertal development with the nocturnally administered patch, the recommended starting dose is

0.05–0.07 µg/kg, equivalent to 2–6 µg from an E₂ patch. In older girls, when breast development is a high priority, the starting dose can be 0.08–0.12 µg/kg, corresponding to 3–7 µg from an E₂ patch.

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Disclosure Statement

C.A.-L., B.K. and E.N. declare no conflicts of interest.

References

- Norjavaara E, Ankarberg C, Albertsson-Wikland K: Diurnal rhythm of 17 beta-estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay. *J Clin Endocrinol Metab* 1996;81:4095–4102.
- Ankarberg-Lindgren C, Norjavaara E: Estradiol in pediatric endocrinology. *Am J Clin Pathol* 2009;132:978–980.
- Albin AK, Niklasson A, Westgren U, Norjavaara E: Estradiol and pubertal growth in girls. *Horm Res Paediatr* 2012;78:218–225.
- Janner M, Fluck CE, Mullis PE: Impact of estrogen replacement throughout childhood on growth, pituitary-gonadal axis and bone in a 46,XX patient with CYP19A1 deficiency. *Horm Res Paediatr* 2012;78:261–268.
- 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.
- Ankarberg-Lindgren C, Norjavaara E: A purification step prior to commercial sensitive immunoassay is necessary to achieve clinical usefulness when quantifying serum 17beta-estradiol in prepubertal children. *Eur J Endocrinol* 2008;158:117–124.
- Ankarberg-Lindgren C, Mahler TB, Norjavaara E: Biological reference intervals for serum estradiol and testosterone in children. *Horm Res Paediatr* 2013;80(suppl 1):179.
- Bondy CA, Turner Syndrome Study G: care of girls and women with Turner syndrome: a guideline of the Turner Syndrome Study Group. *J Clin Endocrinol Metab* 2007;92:10–25.
- Davenport ML: Approach to the patient with Turner syndrome. *J Clin Endocrinol Metab* 2010;95:1487–1495.
- Palmert MR, Dunkel L: Clinical practice. Delayed puberty. *N Engl J Med* 2012;366:443–453.
- Reginster JY, Albert A, Deroisy R, Colette J, Vrijens B, Blacker C, Brion N, Caulin F, Mayolle C, Regnard A, Scholler R, Franchimont P: Plasma estradiol concentrations and pharmacokinetics following transdermal application of Menorest 50 or System (Evorel) 50. *Maturitas* 1997;27:179–186, erratum in *Maturitas* 1997;28:193–195.
- Mora S, Weber G, Guarneri MP, Nizzoli G, Pasolini D, Chiumello G: Effect of estrogen replacement therapy on bone mineral content in girls with Turner syndrome. *Obstet Gynecol* 1992;79:747–751.
- Carel JC, Elie C, Ecosse E, Tauber M, Leger 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;91:2972–2979.
- 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 Clin Endocrinol Metab* 1998;83:3198–3204.
- Norjavaara E, Albertsson-Wikland K, Ankarberg-Lindgren C: A sensitive estradiol assay can predict the onset of puberty before thelarche. *Horm Res* 2005;64(suppl 1):23.
- Mauras N, Shulman D, Hsiang HY, Balagopal P, Welch S: Metabolic effects of oral versus transdermal estrogen in growth hormone-treated girls with Turner syndrome. *J Clin Endocrinol Metab* 2007;92:4154–4160.
- 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.
- 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. *Clin Endocrinol (Oxf)* 2009;70:265–273.
- Piippo S, Lenko H, Kainulainen P, Sipila I: Use of percutaneous estrogen gel for induction of puberty in girls with Turner syndrome. *J Clin Endocrinol Metab* 2004;89:3241–3247.
- Scarabin PY, Oger E, Plu-Bureau G: Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. *Lancet* 2003;362:428–432.
- Canonica M, Oger E, Plu-Bureau G, Conard J, Meyer G, Levesque H, Trillot N, Barrellier MT, Wahl D, Emmerich J, Scarabin PY, Estrogen Thromboembolism Risk Study G: Hormone therapy and venous thromboembolism among postmenopausal women: impact of the route of estrogen administration and progestogens: the Esther Study. *Circulation* 2007;115:840–845.
- L'Hermite M, Simoncini T, Fuller S, Genazzani AR: Could transdermal estradiol + progesterone be a safer postmenopausal HRT? A review. *Maturitas* 2008;60:185–201.
- Opatrny L, Dell'Aniello S, Assouline S, Suissa S: Hormone replacement therapy use and variations in the risk of breast cancer. *Br J Obstet Gynaecol* 2008;115:169–175; discussion 175.
- O'Sullivan AJ, Ho KK: Route-dependent endocrine and metabolic effects of estrogen replacement therapy. *J Pediatr Endocrinol Metab* 2000;13(suppl 6):1457–1466.
- Langrish JP, Mills NL, Bath LE, Warner P, Webb DJ, Kelnar CJ, Critchley HO, Newby DE, Wallace WH: Cardiovascular effects of physiological and standard sex steroid replacement regimens in premature ovarian failure. *Hypertension* 2009;53:805–811.
- Alves ST, Gallichio CT, Guimaraes MM: Insulin resistance and body composition in Turner syndrome: effect of sequential change in the route of estrogen administration. *Gynecol Endocrinol* 2006;22:590–594.
- Taboada M, Santen R, Lima J, Hossain J, Singh R, Klein KO, Mauras N: Pharmacokinetics and pharmacodynamics of oral and transdermal 17beta estradiol in girls with Turner syndrome. *J Clin Endocrinol Metab* 2011;96:3502–3510.
- Harris HA, Bapat AR, Gonder DS, Frail DE: The ligand binding profiles of estrogen receptors alpha and beta are species dependent. *Steroids* 2002;67:379–384.