Metabolic Effects of Oral Versus Transdermal 17β-Estradiol (E$_2$): A Randomized Clinical Trial in Girls With Turner Syndrome

L. Torres-Santiago, V. Mericq, M. Taboada, N. Unanue, K. O. Klein, R. Singh, J. Hossain, R. J. Santen, J. L. Ross, and N. Mauras

Nemours Children's Clinic (L.T.-S., M.T., N.M.), Jacksonville, Florida 32207; University of Florida (L.T.-S.), Jacksonville, Florida 32209; Institute of Maternal and Child Research (V.M.), University of Chile, Santiago 1058, Chile; Nemours Children's Clinic (M.T.), Orlando, Florida 32806; University of California, San Diego (K.O.K.), San Diego, California 92093; Mayo Clinic (R.S.), Rochester, Minnesota 55905; DuPont Hospital for Children (J.H.), Wilmington, Delaware 19803; University of Virginia Medical Center (R.J.S.), Charlottesville, Virginia 22903; and Jefferson Medical Center (J.L.R.), Philadelphia, Pennsylvania 19107

Context: The long-term effects of pure 17β-estradiol (E$_2$) depending on route of administration have not been well characterized.

Objective: Our objective was to assess metabolic effects of oral vs transdermal (TD) 17β-E$_2$ replacement using estrogen concentration-based dosing in girls with Turner syndrome (TS).

Patients: Forty girls with TS, mean age 16.7 ± 1.7 years, were recruited.

Design: Subjects were randomized to 17β-E$_2$ orally or TD. Doses were titrated using mean E$_2$ concentrations of normally menstruating girls as therapeutic target. E$_2$, estrone (E$_1$), and E$_1$ sulfate (E$_1$S) were measured by liquid chromatography tandem mass spectrometry and a recombinant cell bioassay; metabolites were measured, and dual-energy x-ray absorptiometry scan and indirect calorimetry were performed.

Main Outcome: Changes in body composition and lipid oxidation were evaluated.

Results: E$_2$ concentrations were titrated to normal range in both groups; mean oral dose was 2 mg, and TD dose was 0.1 mg. After 6 and 12 months, fat-free mass and percent fat mass, bone mineral density accrual, lipid oxidation, and resting energy expenditure rates were similar between groups. IGF-1 concentrations were lower on oral 17β-E$_2$, but suppression of gonadotropins was comparable with no significant changes in lipids, glucose, osteocalcin, or highly sensitive C-reactive protein between groups. However, E$_1$, E$_1$S, SHBG, and bioestrogen concentrations were significantly higher in the oral group.

Conclusions: When E$_2$ concentrations are titrated to the normal range, the route of delivery of 17β-E$_2$ does not affect differentially body composition, lipid oxidation, and lipid concentrations in hypogonadal girls with TS. However, total estrogen exposure (E$_1$, E$_1$S, and total bioestrogen) is significantly higher after oral 17β-E$_2$. TD 17β-E$_2$ results in a more physiological estrogen milieu than oral 17β-E$_2$ administration in girls with TS. (*J Clin Endocrinol Metab* 98: 2716–2724, 2013)

Estrogen is indispensable for normal physiological function in females including breast development and feminization, the growth and differentiation of primary

sex organs, skeletal maturation, consolidation, and growth. Although a variety of types of estrogen can achieve full feminization in hypogonadal females, it re-

Abbreviations: BMD, bone mineral density; BMI, body mass index; CEE, conjugated equine estrogen; CV, coefficient of variation; E$_1$, estrone; E$_2$, estradiol; E$_1$S, E$_1$ sulfate; FFM, fat-free mass; HDL, high-density lipoprotein; hsCRP, highly sensitive C-reactive protein; REE, resting energy expenditure; TD, transdermal.

mains unclear what is the most physiological route and dose to use in hypogonadal girls. Micronized 17β-estradiol (E_2), identical to the product of the ovary, is available for administration via different routes and is the most physiological form of estrogen available.

In the postmenopausal literature, a number of studies have previously suggested that estrogen given orally has deleterious effects on body composition and lipid oxidation as compared with transdermal (TD) administration (1, 2) and that it could serve as a functional GH antagonist by suppressing IGF-1 (3). However, this has not been consistently confirmed (4, 5). Many of these studies used not only different routes of delivery, but also different forms of estrogen, including conjugated equine estrogen (CEE), which has been the most widely used form of estrogen for the induction of puberty in girls with Turner Syndrome in the United States (6); yet this formulation has more than 100 individual estrogenic compounds of different biological potency not detected in conventional assays.

A recent pharmacokinetic and pharmacodynamic study in girls with Turner syndrome using the same type of 17β-E_2 orally and TD showed that high-dose TD (0.075 mg) 17β-E_2 resulted in a pharmacokinetic profile of E_2 and estrone (E_1) concentrations closer to that of normally menstruating adolescents when compared with low-dose oral and TD and high-dose oral. With a recombinant cell bioestrogen assay, high-dose TD dosing also achieved bioestrogen concentrations closest to normal. None of the formulations significantly affected IGF-1 concentrations, and all affected cholesterol minimally and highly sensitive C-reactive protein (hsCRP) concentrations similarly. We concluded that, among identical forms of 17β-E_2, high-dose TD (0.075 mg) was the most physiological, at least using short-term metabolic markers (7).

In follow-up to these studies, we aimed to investigate the differential, long-term effects on body composition and energy metabolism of oral vs TD 17β-E_2 replacement, specifically the effects on fat metabolism in girls with Turner syndrome treated for 12 months. We also sought to determine the feasibility of estrogen concentration-based dosing in puberty.

Subjects and Methods

This study was approved by the Institutional Review Boards of the Nemours Children's Clinics in Jacksonville, Florida, and Jefferson, Philadelphia, PA, and by the Institute of Maternal and Child Research, University of Chile in Santiago. Written informed consent was obtained from parents/guardians and subjects (older than 18 years of age), and children's assent was obtained. This study was registered in ClinicalTrials.gov (NCT00837616).

Study subjects

Forty girls with Turner syndrome (45X and related karyotypes) between 13 and 20 years of age were recruited using Institutional Review Board-approved advertising and followed among the 3 participating centers at the Nemours Children's Clinic, Jacksonville (coordinating center), Nemours Jefferson, and Clínica las Condes/University of Chile, Santiago, Chile. Subjects had completed or nearly completed their linear growth, and any previous GH therapy was discontinued at least 6 months before study participation. Estrogen replacement therapy was discontinued for at least 6 weeks before baseline studies. All girls were hypogonadal, defined as lack of or inadequate ovarian function leading to retardation in sexual development and evidenced by castrate levels of gonadotropins. Subjects with significant obesity (body mass index [BMI] > 36 kg/m²) or history of systemic illness were excluded.

Study design

A physical examination was performed and a fasting blood sample withdrawn at baseline. Body composition analysis was performed using dual-energy x-ray absorptiometry. Substrate oxidation and resting energy expenditure (REE) rates were measured fasting by indirect calorimetry using a mouthpiece.

Subjects were randomized into 2 groups, assigned to take oral vs TD 17β-E_2 starting at doses of either 0.5 mg orally daily (Estrace; Bristol Myers Squibb, New York, New York) or 0.0375 mg twice/wk TD (Vivelle TD system; Novartis Pharmaceuticals, East Hanover, New Jersey). Lower initial doses were chosen to introduce estrogens in incremental concentrations to account for potential variations in absorption, possibly resulting in different concentrations. Subjects took estrogen for 3 weeks, progesterone (Provera 10 mg) was added from days 14 to 21 monthly for cyclical withdrawal bleeding, and the hormonal cycle was repeated after the 28th day. Visits occurred 1 and 2 months from randomization, and a blood sample for 17β-E_2 and E_1 was repeated. E_2 doses were titrated between visits in the first 3 months to achieve E_2 levels within the normal range determined by the average of E_2 concentrations in the follicular and luteal phases of the menstrual cycle of a group of 20 normally menstruating healthy adolescent girls measured in the same assay reported by us previously (7). Doses were titrated to 1, 1.5, or 2 mg of oral or 0.05, 0.075, or 0.1 mg of TD 17β-E_2 as needed. Routine clinic visits occurred at 3-month intervals, and study parameters were repeated at 6 and 12 months. Doses continued to be adjusted subsequently as needed during office visits.

Assays

Blood samples for measurement of plasma lipids, 17β-E_2, E_1, E_1 sulfate (E_1S), bioestrogen, LH, FSH, IGF-1, osteocalcin, hsCRP, SHBG, insulin, and glucose concentrations were obtained. E_2 and E_1 concentrations were measured in serum at the mass spectrometry laboratory at Mayo Clinic (Rochester, Minnesota) using liquid chromatography and tandem mass spectrometry as previously described (8) with a quantitation limit for E_2 of 2.5 pg/mL and an intra-assay coefficient of variation (CV) of 20%. This assay has proven superior in accuracy and specificity over commercial RIAs and other assays (9–11). E_1S was also measured by liquid chromatography and tandem mass spectrometry at Mayo with an intra- and interassay CV of 3.8% and 6.0%, respectively. In addition, total bioestrogens were measured in plasma by a recombinant cell bioassay, using a *Saccha-*

romyces cerevisiae yeast transformed with plasmids encoding the human estrogen receptor, with a sensitivity of 0.2 pg/mL (0.73 pmol/L) as previously described (12). LH, FSH, IGF-1, lipids, hsCRP, osteocalcin, SHBG, insulin, and glucose were measured by standard assays (Luminex, Beckman DXC 800, GM7 analyzer, RIA, Human IGF-I Quantikine ELISA Kit from R&D Systems, Minneapolis, Minnesota) at the study's core laboratory at Nemours Children's Clinic-Jacksonville and at Baptist Medical Center, Jacksonville, Florida. The intra-assay CVs were as follows: IGF-1, 4.3%; LH and FSH, 6.3% and 7.2%, respectively; total cholesterol, 2.2%; high-density lipoprotein (HDL), 4.9%, triglycerides, 3.5%; hsCRP, 4.7%; osteocalcin, 5%; SHBG, 4.3%; insulin, 10%; and glucose, 1.4%.

Body composition, bone mineral density, and substrate oxidation rates

Body composition was measured using either a Hologic Discovery A (S/N 45903 Software) (Hologic, Waltham, Massachusetts) or a Lunar DPX-L (4.7 Software) (Lunar, Madison, Wisconsin). Each subject used the same scanner for the duration of the entire study. Daily calibration using company-provided phantoms was performed. Indirect calorimetry was measured using the same instrument for all the visits, either a CPX-MAX or Deltatrac II indirect calorimeter (using a mouthpiece), after a 12-hour fast, and calculations were made using gas exchange equations (13). Data are presented as kilocalories per kilogram fat-free mass (FFM) per day.

Free E_2 calculations

We calculated free E_2 using total E_2, E_1, and SHBG and its binding affinity constants, using the equations of Södergård et al (14), using a fixed concentration of albumin of 4.3 mg/L as suggested by Vermeulen et al (15)

Statistical analysis

A sample size of 36 (18 in each group) was determined based on the principal study outcome, ie, differential changes in body composition, by using a 2-sided *t* statistic allowing us to detect a minimal mean difference of 0.7 of the SD of the corresponding measurement with a power of 80% at a 5% level of significance. Data were summarized by treatment group and assessment time point. A mixed-model repeated-measures ANOVA model, with an appropriate covariance structure, was used to compare the mean changes in each of the study variables between treatments over time. Change from baseline was used as the response variable and treatment group, assessment time points, interaction of treatment group and time points, and baseline values of the corresponding variables were used as explanatory variables. Model assumptions were checked before analyses, and an appropriate transformation or a nonparametric method was used when there was a violation of model assumption. All tests were 2-tailed at the 5% level of significance. The statistical packages SPSS (version 18.0) and SAS (version 9.2) were used for data analyses. Data are presented as mean ± SE or median with confidence interval for metrics not normally distributed.

Results

Forty-one girls with Turner syndrome (45X or related karyotypes) who met inclusion criteria were recruited, and 1 patient dropped out early due to logistical difficulties in follow-up. Forty subjects completed the study, 20 in each group (oral vs TD). Their clinical characteristics are summarized in Table 1. Subjects were well matched for age, BMI, height, and previous treatment with GH. Compliance was assessed by frequent phone calling and pill and patch counting.

E_2 and E1

Mean baseline E_2 concentrations for the oral and TD groups were 5.9 ± 2.4 and 4.9 ± 1.5 pg/mL, respectively (Figure 1, A and B). Baseline E_2 concentrations were elevated in 4 subjects with spontaneous puberty despite elevated gonadotropins (3 in the oral and 1 in the TD group) and their data deleted from the estrogen concentration analysis. E_2 levels increased comparably between groups achieving concentrations similar to the average of normally menstruating adolescents (96 ± 11 pg/mL) by 3 months in >85% of the girls in both groups. Concentrations at 6 months were 83 ± 14 pg/mL on oral E_2 and 68 ± 13 pg/mL on TD, and at 12 months concentrations were 124 ± 19 pg/mL for the oral group and 74 ± 17 pg/mL for the TD group (*P* = .15 overall). The average daily dose of 17β-E_2 administered to achieve these concentrations was 2 mg orally and 0.1 mg TD.

We calculated a comparable increase in free E_2 concentrations throughout the 12 months of the study at all time points in the oral group (0 months, 0.07 ± 0.13 pg/mL; 6 months, 0.23 ± 0.14 pg/mL; 12 months, 0.42 ± 0.39 pg/mL) and TD group (0 months, 0.07 ± 0.17 pg/mL; 6 months, 0.33 ± 0.26 pg/mL; 12 months, 0.33 ± 0.25 pg/mL), reaching similar levels to those calculated in the normal controls (0.39 ± 0.18 pg/mL, *P* = .645).

Mean baseline E_1 concentrations were 12.9 ± 2.9 and 11.6 ± 1.3 pg/mL in the oral and TD groups, respectively; however, E_1 concentrations increased significantly more in the oral than TD group, both at 6 months (oral, 356 ±

Table 1. Clinical Characteristics of Study Subjects

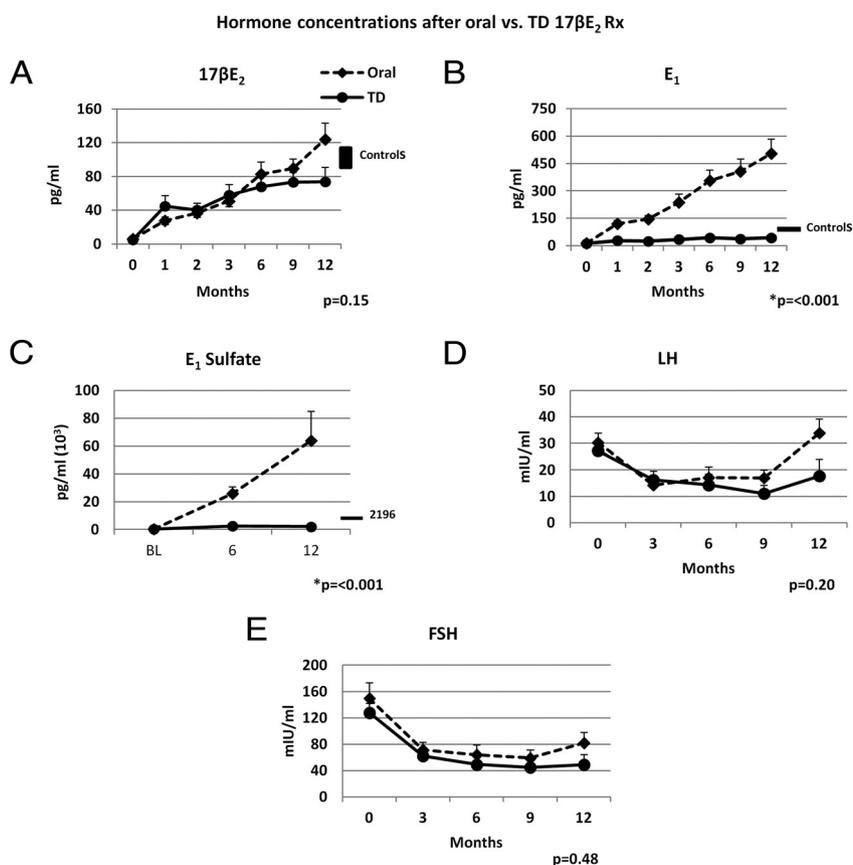|	Oral 17β-E_2	TD 17β-E_2
n	20	20
Age, y	16.7 ± 1.7	16.7 ± 1.8
Height SD score	−2.9 ± 1.1	−2.5 ± 1.7
BMI, kg/m^2	25.7 ± 5.9	24.5 ± 4.3
Subjects on prior GH	13 (65%)	14 (70%)
Years off GH	2.0 ± 1.7	1.5 ± 1.0
Karyotypes		
45,X	10	11
45,X/isochromosomes	6	3
45,X/46,X (rX)	0	2
45,X/46,Xr(X) (p11.3q21)	1	0
46,X del(X) p11.2	0	1
45,X/46,XX	2	1
45,X/46,XY	1	2

Figure 1. Mean $\pm$ SE plasma concentrations of E_2 (A), E_1 (B), E_1S (C), LH (D), and FSH (E) after oral or TD estradiol treatment (Rx) over 12 months. E_2 levels achieved were comparable between the groups ($P = .15$) and similar to healthy age-matched controls averaged from follicular and luteal phase values (bar on the right). E_1 and E_1S concentrations were much higher after oral E_2 than TD ($P < .001$). Gonadotropins decreased comparably over 12 months between groups ($P > .20$ for both), although there was an increase in both oral and TD LH at 12 months. Black bars represent controls. Analysis was done by mixed-model repeated-measures ANOVA. To convert to SI units, multiply by 3.67 for E_2, 3.699 for E_1, and 2.73 for E_1S.

59 pg/mL; TD, 43 ± 6 pg/mL) and at 12 months (oral, 504 ± 80 pg/mL; TD, 43 ± 7 pg/mL, $P < .001$, overall) and as compared with normal girls (70 ± 7 pg/mL).

Estrone sulfate (E_1S)

E_1S concentrations were similar at baseline in the 2 groups (oral, 311 ± 55 pg/mL; TD, 336 ± 77 pg/mL, $P = .66$) and significantly lower than those of normally menstruating adolescents (2196 ± 357 pg/mL) (Figure 1C). Concentrations increased significantly more in the oral group than in the TD group after 6 months of 17β-E_2 administration (oral, $+25\,595 \pm 4934$ pg/mL; TD, $+2308 \pm 382$, $P < .001$) and continued to increase by 12 months in the oral while it remained stable in the TD group (oral, $+63\,638 \pm 21\,088$ pg/mL; TD, 1875 ± 414, $P < .001$). Concentrations achieved after TD E_2 replacement were comparable to those of normally menstruating adolescents ($P = .95$), whereas they were significantly higher in the oral group ($P < .001$). P values are based on log-transformed data.

Recombinant cell bioestrogen

Bioestrogen concentrations were comparable at baseline in the 2 groups (oral, 15 ± 5 pg/mL; TD, 26 ± 9 pg/mL, $P = .75$) and substantially lower than those of normally menstruating girls previously reported (231 ± 12 pg/mL) (7). Concentrations increased after 6 months of E_2 replacement in the oral group (183 ± 23 pg/mL) vs TD group (141 ± 21 pg/mL). By 12 months, concentrations in the oral group were significantly higher than the TD group (257 ± 19 vs 131 ± 25 pg/mL, $P < .001$). Sixty-five percent of bioestrogen values in the oral group were above the upper limits of detection of the assay.

Gonadotropins

As expected, gonadotropins were elevated in all subjects at baseline (Figure 1, D and E). Mean LH levels were 30 ± 4 and 27 ± 4 mIU/mL in the oral and TD groups respectively, and FSH was 150 ± 24 and 128 ± 14 mIU/mL. There was a significant and comparable decrease in FSH concentrations at 6 and 12 months in the oral and TD groups (6 months: oral, -75 ± 12 mIU/mL, vs TD, -84 ± 12 mIU/mL; 12 months: oral, -59 ± 12 mIU/mL, vs TD, -84 ± 12 mIU/mL, $P = .48$). Overall, changes in LH were similar in both groups at 6 months (oral, -11.3 ± 4 mIU/mL; TD, -14 ± 4 mIU/mL) and at 12 months (oral, $+4 \pm 4$ mIU/mL; TD, -10 ± 4 mIU/mL) ($P = .20$).

Body composition

The principal study outcome was the difference between groups in changes in body composition after 12 months of treatment (Figure 2, A–E). Girls gained a comparable amount of weight at 6 months (oral, $+0.9 \pm 0.6$ kg; TD $+0.6 \pm 0.6$ kg, $P = .68$) and at 12 months (oral, $+1.1 \pm 0.6$ kg; TD, $+1.9 \pm 0.6$ kg, $P = .37$) as well as in BMI throughout the study (6 months: oral, $+0.26 \pm 0.28$ kg/m^2; TD, $+0.22 \pm 0.28$ kg/m^2, $P = .97$; 12 months: oral, $+0.075 \pm 0.38$ kg/m^2; TD, $+0.65 \pm 0.38$ kg/m^2, $P = .2$). Both groups had a modest but comparable decrease of percent fat mass at 6 and 12 months (6 months oral, $-0.54\% \pm 0.47\%$; TD, $-1.63\% \pm 0.47\%$, $P = .14$; 12
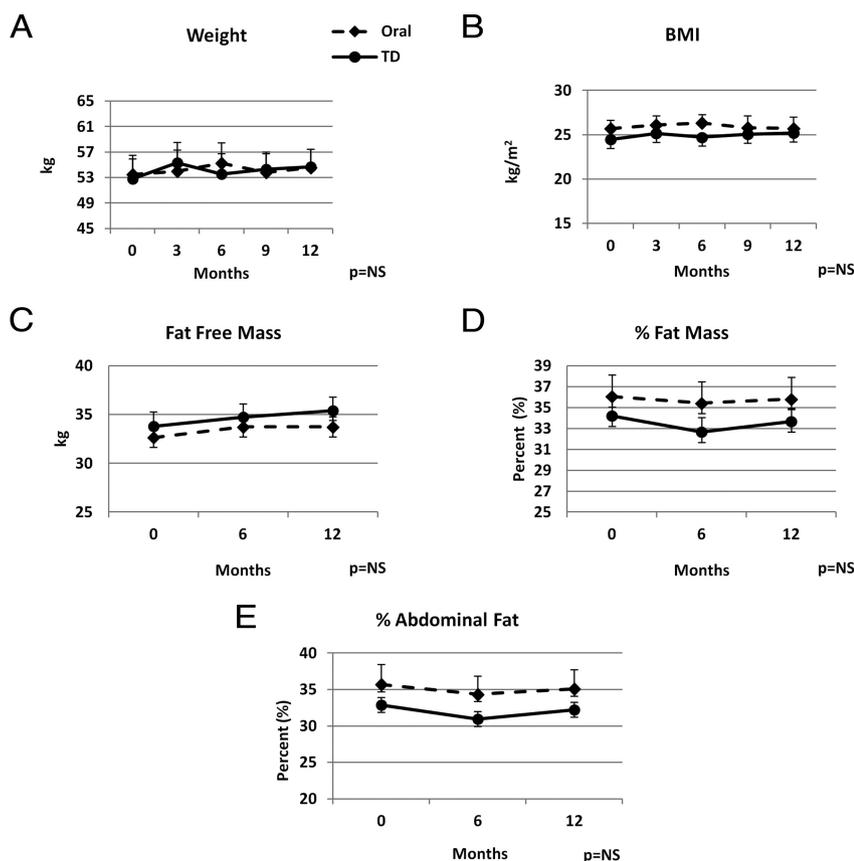
Figure 2. Mean ± SE measures of body composition during 12 months of oral and TD 17β-E_2 treatment in girls with Turner syndrome for weight (A), BMI (B), FFM (C), percent fat mass (D), and percent abdominal fat (E). Overall, there were no significant differences between groups in any parameter at 12 months ($P > .22$ for all).

Lipid concentrations, including HDL, low-density lipoprotein, triglycerides, total cholesterol, fasting plasma glucose, insulin, osteocalcin, and hsCRP remained stable over 12 months with no significant differences between groups. SHBG concentrations were comparable at baseline and similar to those of normal adolescents (491 ± 37 nmol/L, $P = .24$). However, estradiol administration resulted in an increase in SHBG in the oral group and no change in the TD group (Table 2).

Bone mineral density (BMD)

Whole-body BMD Z-scores were low at baseline in most subjects (SD score, $-2.2 ± 0.3$ for the oral group and $-2.0 ± 0.2$ for the TD group, $P = .99$) as was the lumbar BMD Z-score (oral, $-1.8 ± 0.3$; TD, $-1.5 ± 0.3$, $P = .8$) (Figure 4). There was significant but similar improvement in whole-body and lumbar BMD Z-scores over 12 months between groups (whole body: oral, $+0.22 ± 0.09$; TD, $+0.29 ± 0.09$, $P = .23$; lumbar: oral, $0.19 ± 0.07$; TD, $0.20 ± 0.07$, $P = .91$).

months oral, $-0.14\% ± 0.56\%$; TD, $-0.64\% ± 0.56$, $P = .5$). A significant but comparable increase in FFM was observed within each group over time between TD and oral (6 months oral, $+1.03 ± 0.37$ kg; TD, $+1 ± 0.37$ kg, $P = .962$; 12 months oral, $+1.03 ± 0.37$ kg; TD, $+1.67 ± 0.4$ kg; $P = .54$). Abdominal fat decreased comparably over 12 months in both groups ($P = .39$) at 6 months ($-1.1\% ± 0.63\%$ vs $-2.2\% ± 0.65\%$ for oral and TD, respectively) and at 12 months (oral, $-0.35\% ± 0.8\%$; TD, $-0.9\% ± 0.8\%$).

IGF-1, lipids, and SHBG

Baseline IGF-1 concentrations were similar between groups: median 197 (range 80–390) ng/mL for the oral group and 170 (95–288) ng/mL for the TD group ($P = .3$). Mean changes in IGF-1 concentrations in the oral and TD groups, respectively, were $+2 ± 12$ and $+24 ± 12$ ng/mL at 6 months and $-16 ± 12$ and $+28 ± 12$ ng/mL at 12 months ($P = .059$ overall changes between groups). However, IGF-1 concentrations remained within physiological range for age at all time points regardless of treatment route (Figure 3).

Lipid oxidation rates and REE

Lipid oxidation and REE rates were unaffected by 17β-E_2 treatment regardless of route of delivery at 6 and 12 months (Table 2).

Discussion

To our knowledge, this is the first randomized clinical trial assessing the metabolic effects and body composition changes in girls with Turner syndrome using the same form of 17β-E_2 given orally or TD for a year while titrating doses to raise E_2 concentrations similar to those of normally menstruating adolescents. We observed comparable effects on body composition, bone mineral content accrual, lipoprotein profiles, markers of inflammation, blood glucose and insulin concentrations, and substrate oxidation rates in both groups treated for 12 months. This was despite a tendency for lower IGF-1 in the group receiving oral therapy. However, oral 17β-E_2 administration was associated with significantly higher E_1, E_1S, and estrogen bioassay concentrations, resulting in a less phys-

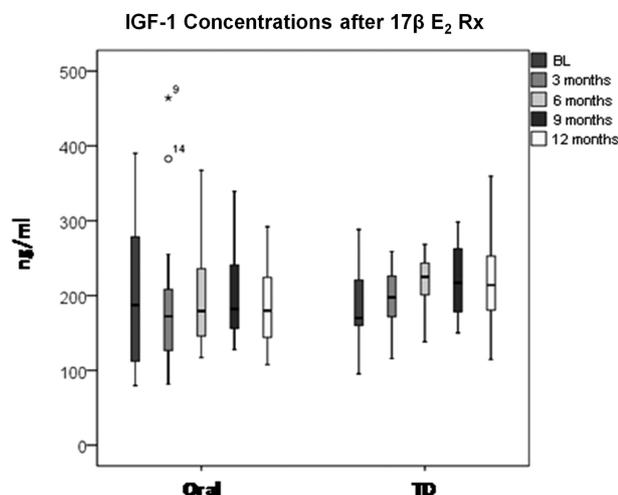**IGF-1 Concentrations after 17β E$_2$ Rx**

Figure 3. Box plots of median IGF-1 concentrations over 12 months after treatment (Rx) with 17β-E$_2$. Overall, IGF-1 concentrations decreased in the oral group and increased in the TD group at 6 and 12 months (P = .059 between groups) (normal range: prepubertal girls, 118–664 ng/mL; pubertal, 208–1060 ng/mL, data from subjects in Ref. 35).

iological estrogen milieu. These results represent the most detailed analysis, to date, on the effect of the route of estrogen delivery on metabolism and concentration of estrogens in hypogonadal girls.

Girls with Turner syndrome had baseline E$_2$ and gonadotropin concentrations comparable to menopausal women, offering a unique model to study the effects of estrogen administration, minimizing confounding effects of other products of the intact gonad. A previous study in girls with Turner syndrome (16) aimed to compare oral and TD routes of administration using 2 different types of estrogen, 17β-E$_2$ TD and CEE orally, the latter containing a large number of estrogenic compounds of different potency that cannot be readily measured and that could have variable effects depending on the target tissue.

We achieved normal plasma concentrations of E$_2$ in both groups. Free estradiol concentrations, as calculated by the equations in Södergård et al (14), were also comparable between the groups and similar to those of our healthy controls. Estrone and E$_1$S concentrations, however, were much higher in the oral group at all time points after treatment, likely related to hepatic conversion of E$_2$ to E$_1$ by 17β-hydroxysteroid dehydrogenase type 1 and to E$_1$S through sulfotransferase. These precursors serve as an estrogen reservoir that can be converted back to estradiol in peripheral tissue (17).

Bioestrogen levels were also much higher in the oral group, suggesting that E$_1$ and potentially E$_1$S may have inherent estrogenic activity as detected by some contribution to activation of the estrogen receptor in the bioassay. Interestingly, bioestrogen concentrations in the oral group were comparable to those previously reported by us in

controls; however, at 12 months, ~65% of the values in the oral group were above the detection limit of the assay (>270 pg/mL), suggesting that the bioestrogen concentrations were indeed higher in the oral group than in controls. These differences cannot be elucidated with these data. The bioassay used was specifically engineered with extreme sensitivity to low estrogen levels, but at higher estrogen concentrations, it likely causes estrogen receptor saturation, making distinctions less useful (12, 18–20). The significantly higher levels of SHBG observed in the oral group also evidence a bigger hepatic effect of oral estrogens. Both E$_1$ and E$_1$S exert biological activity in a variety of systems, including mammary tumor growth in vivo (21), and these precursors likely contribute to the higher bioestrogen levels observed in the oral group. Collectively, our data suggest using the oral route causes a larger total estrogen exposure and is less physiological, despite normal measured E$_2$ and calculated free E$_2$ concentrations in both groups.

Both routes of 17β-E$_2$ replacement had similar effects in suppressing gonadotropin concentrations. The FSH decrease was not as robust as LH's likely due to diminished inhibin production in Turner syndrome. Interestingly, at 12 months, both groups had a relative and similar increase in LH concentrations despite normal E$_2$ concentrations. The mechanisms of these observations are not fully understood, but this time-dependent escape from this restraint by LH may represent withdrawal of the paracrine effects of endogenous GnRH and/or increased dopaminergic tone induced by estrogen as previously suggested in girls with Turner syndrome (22). The comparable effect of both forms of estrogen on LH and FSH suppression but a greater effect of oral estrogen on SHBG levels provides direct evidence that oral estrogens exert differential effects on tissue but similar effects at the pituitary level.

In our previous pharmacokinetic study, IGF-1 concentrations were not different between oral and TD administration; however, those interventions were very short-term (2 weeks) (7). In the present studies, IGF-1 concentrations decreased over time after oral whereas they increased mildly after TD 17β-E$_2$, with the difference between the groups approaching significance (P = .059). These results are similar to previous studies using oral and TD estrogen preparations (1, 3, 23) and the hepatic first-pass effect of oral estrogen has been postulated as a principal mechanism possibly due to up-regulation of suppressors of cytokine signaling (SOCS)-2 and -3, which inhibit GH signaling (24). However, this mildly lower IGF-1 in the oral group was not associated with differences in body composition after 12 months.

FFM increased significantly but similarly in both groups, accompanied by a parallel weight increase, sug-

Table 2.   Metabolites, Hormones, and Substrate Oxidation Rates[a]

	Oral			TD		
	Baseline	**6 mo**	**12 mo**	**Baseline**	**6 mo**	**12 mo**
Cholesterol, mg/dL						
Total	165 ± 7	167 ± 6	168 ± 7	158 ± 5	155 ± 5	153 ± 6
LDL, mg/dL	95 ± 4	97 ± 5	93 ± 4	96 ± 4	91 ± 4	88 ± 5
HDL, mg/dL	51 ± 4	52 ± 3	56 ± 3	47 ± 2	47 ± 2	50 ± 2
TG, mg/dL	95 ± 16	89 ± 9	95 ± 19	75 ± 8	83 ± 8	70 ± 9
Glucose, mg/dL	77 ± 2	76 ± 3	78 ± 4	78 ± 3	75 ± 3	72 ± 4
Insulin, μU/mL	14 ± 2	16 ± 3	19 ± 3	13 ± 2	13 ± 2	15 ± 2
SHBG,[b] nmol/L	465 ± 63	684 ± 83	615 ± 113	384 ± 43	384 ± 33	396 ± 62
Osteocalcin, ng/mL	26 ± 3	24 ± 3	27 ± 3	24 ± 11	27 ± 4	28 ± 5
hsCRP,[c] mg/L	1.7 (0.2–5.8)	1.4 (0.2–9.7)	1.3 (0.2–6.7)	1.0 (0.2–3.9)	0.9 (0.2–5.7)	1.1 (0.2–5.2)
Lipid Ox, kcal/FFM/d	9.2 ± 2.6	9.4 ± 1.9	10.0 ± 2.8	7.1 ± 2.9	9.3 ± 1.2	7.3 ± 1.6
REE, kcal/FFM/d	35 ± 1	35 ± 1	36 ± 1	35 ± 1	34 ± 1	34 ± 1

Abbreviations: LDL, low-density lipoprotein; Ox, oxidation; TG, triglyceride.

[a] Results are shown as mean± SE, estimated from a mixed model containing the treatment group, time, and time interaction. To convert to SI units, multiply by 0.0259 for cholesterol, 0.0113 for TG, 0.0555 for glucose, and 7.175 for insulin.

[b] $P <$ .001 (oral vs TD at 6 and 12 months).

[c] hsCRP presented as median and range.

gesting that the latter was associated with increases in lean body mass because fat mass remained stable. There were no differential changes in BMI or percent abdominal fat during the study. This differs from previous studies where lean body mass improved only with TD E_2 (25) and total body fat mass increased using oral estrogen (1, 25). However, both these studies used CEE in contrast to 17β-E_2, making the current study comparisons more robust. Ul-

Figure 4. Whole-body (A) and lumbar (B) BMD over time after treatment with 17β-E_2. There was significant ($P <$ .05) but similar improvement in whole-body and lumbar BMD over 12 months between groups.

timately, IGF-1 levels remained within the low physiological range in both groups; hence, these mild differences in IGF-1 may be clinically inconsequential in girls who have completed growth.

Rates of lipid oxidation and REE in the fasted state were similar between groups. These findings are congruent with our previous observations in girls with Turner syndrome (26) and in fasting postmenopausal women (1), but differ from results of lower lipid oxidation during oral CEE replacement in postmenopausal women studied postprandially (25).

Girls with Turner syndrome showed no differences in fasting plasma lipids, insulin, or glucose concentrations with either form of estrogen treatment, similar to previous reports (5, 16, 26), although they differ from other studies in girls with Turner syndrome where ethinyl estradiol was found to increase HDL levels (27).

Previously, others have found similar concentrations of osteocalcin in adults with Turner syndrome as compared with controls (28), similar to the present study. Rates of BMD accrual were also comparable between groups, suggesting similar bone mineralization activity irrespective of route of 17β-E_2 delivery.

Despite similar changes in body composition, lipids, and lipid metabolism, can these differences in bioestrogen exposure matter in the long term? Recently, several studies looking at oral conjugated estrogens vs 17β-E_2 TD replacement 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 (29, 30), as well as an increased risk of stroke (31). Whether this apparent increased thromboembolic
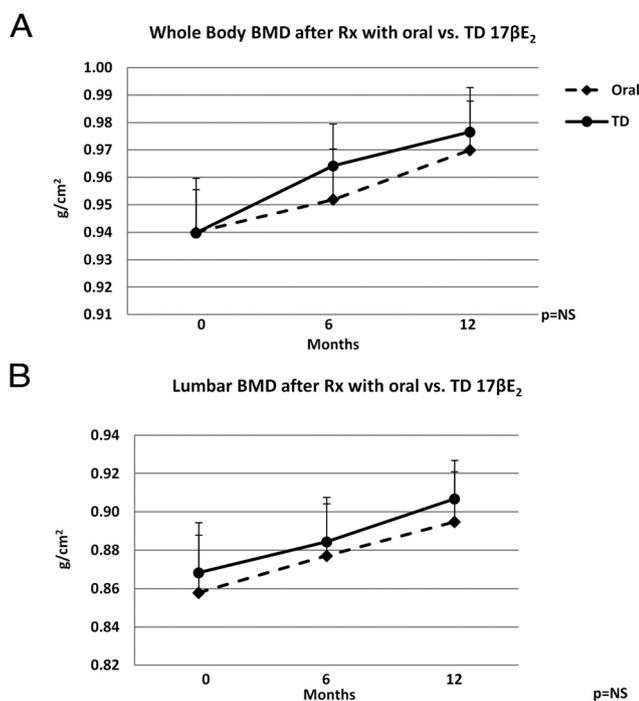
risk translates to oral 17β-E$_2$ as well is unknown; however, given the increased overall risk for cardiovascular disease in Turner syndrome (32–34), the long-term use of TD estradiol appears to be more physiological.

In conclusion, we demonstrated that when 17β-E$_2$ is given orally vs TD while titrating doses close to normal E$_2$ concentrations, the route of delivery does not differentially affect body composition, REE, substrate oxidation rates, metabolic parameters, gonadotropins suppression, or BMD accrual over 12 months of therapy in girls with Turner syndrome. This is despite relatively lower (albeit normal) IGF-1 concentrations in the oral group. However, despite similar E$_2$ concentrations, bioestrogen concentrations and total E$_1$ and E$_1$S concentrations were much higher after oral 17β-E$_2$, suggesting that TD 17β-E$_2$ results in a more physiological estrogen milieu than oral 17β-E$_2$ administration in girls with TS.

Acknowledgments

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