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A Longitudinal Study of the Effect of Subcutaneous Estrogen Replacement on Bone in Young Women With Turner's Syndrome

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ABSTRACT

It is desirable that young women with primary ovarian failure achieve normal peak bone mass to reduce the subsequent risk of osteoporosis, and that there are management strategies to replace bone that is already lost. While estrogen (E_2) is generally considered to prevent bone loss by suppressing bone resorption, it is now recognized that estrogen also exerts an anabolic effect on the human skeleton. In this study, we tested whether estrogen could increase bone mass in women with primary ovarian failure. We studied the mechanism underlying this by analyzing biochemical markers of bone turnover and iliac crest biopsy specimens obtained before and 3 years after E_2 replacement. Twenty-one women with Turner's syndrome, aged 20–40 years, were studied. The T scores of bone mineral density at lumbar spine and proximal femur at baseline were −1.4 and –1.1, respectively. Hormone replacement was given as subcutaneous E_2 implants (50 mg every 6 months) with oral medroxy progesterone. Serum E_2 levels increased incrementally from 87.5 pM at baseline to 323, 506, 647, and 713 pM after 6 months and 1, 2, and 3 years of hormone replacement therapy (HRT), respectively. The bone mineral density at the lumbar spine and proximal femur increased after 3 years to T scores of −0.2 and −0.4, respectively. The cancellous bone volume increased significantly from 13.4% to 18.8%. There was a decrease in activation frequency, but the active formation period was increased by HRT. There was a significant increase in the wall thickness from 33.4 μm at baseline to 40.9 μm after 3 years of HRT, reflecting an increase in bone formed at individual remodeling units. Although there was an early increase in biochemical markers of bone formation, these declined thereafter. Our results show that estrogen is capable of exerting an anabolic effect in the skeleton of young women with Turner's syndrome and low bone mass. (J Bone Miner Res 2003;18:925–932)

Key words: estrogen, Turner's syndrome, bone histomorphometry

INTRODUCTION

Turner's syndrome (TS) is a common chromosomal abnormality with an approximate incidence of 1:2500 female live births. Low bone mineral density (BMD) and osteoporosis are the most common complications in women with primary ovarian failure caused by TS, affecting up to 45% of individuals, often two to three decades earlier than that noted in postmenopausal osteoporosis.(1–6) The low bone mass persists into adulthood and middle age despite patients being on long-term estrogen (E_2) replacement.(2,5–7)

It remains unclear whether the osteopenia is intrinsic to the chromosomal abnormality or results from delayed and inadequate bone formation accompanying estrogen deficiency during development and in adulthood.(2,3) The near-universal prevalence of low bone mass regardless of karyotype, and also in women with ovarian dysgenesis not attributed to TS, suggests that chromosomal abnormality per se is not a major contributor to the osteopenia and that chronic estrogen deficiency is the more likely etiological factor.(1–3,5–7) Furthermore, low growth hormone concentrations have been reported in adolescents with TS, and this may contribute to the delayed bone formation during development.(8,9) It also remains unknown whether the normal increase in bone density, as is expected in the third decade of life, can be achieved by correcting the estrogen deficiency. Attainment of optimum peak bone mass provides protection against developing osteoporosis in later life.

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While estrogen is generally thought to prevent bone loss by suppressing bone resorption, estrogen is increasingly recognized to also exert an anabolic effect in bone. Estrogen has been shown to stimulate osteoblast differentiation and activity in vitro and to increase bone formation and bone mass in animals.[10–12] In humans, an anabolic role for estrogen before skeletal maturation has been implicated by the low peak bone mass in E_2-deficient adolescent girls and in men with rare genetic syndromes of E_2 deficiency.[13,14] More recently, estrogen replacement has been shown to stimulate bone formation and increase bone mass in postmenopausal women with osteoporosis.[15,16]

Previous studies investigating the role of estrogen replacement in women with TS have found that the degree of bone loss was related to duration of amenorrhea. However, except for one study in which estrogen maintained bone mass in adolescents with TS,[17] E_2 treatment generally did not restore bone mass to normal.[2,3,5,6,18] While conventional estrogen replacement merely prevents bone loss in women with postmenopausal osteoporosis, we have shown that estrogen given as subcutaneous implants stimulates bone formation and increases cancellous bone volume.[16] These implants cause a higher serum level of estrogen than conventional oral hormone replacement therapy (HRT). In this study, we tested whether estrogen, given as subcutaneous implants, could increase the bone mass in young women with primary ovarian failure caused by TS, a high-risk group for osteoporosis. We studied the mechanism underlying this by analyzing biochemical markers of bone turnover and iliac crest biopsy specimens obtained before and 3 years after E_2 replacement.

MATERIALS AND METHODS

Patient selection and follow-up

Adult women with TS, aged 20–40 years, were invited to participate in the study. We selected 25 women, the majority of whom were members of the UK Turner's syndrome support group, and the rest were recruited from the gynecological endocrinology and infertility clinics. The diagnosis of TS was confirmed from karyotype results in their hospital records. Any relevant medical history, other investigation results, and drug history were also noted. Those with any high-risk factor for osteoporosis other than ovarian failure, who suffered from medical disorders, or used any drugs other than HRT that are known to affect calcium or bone metabolism were excluded from the study. Demographic features including age, height, weight, and body mass index (BMI) were recorded. Patients were asked about any personal history of fractures or family history of osteoporosis. The type of previous HRT used, age at commencement, and duration of use were noted. None of the patients had been treated with growth hormone or had thyroid disease.

The study was approved by the hospital ethics committee, and informed consent was obtained from the participants before each bone biopsy. It was considered unethical to have a placebo control group of TS women in a long-term study because the need for HRT in these patients is well established. We also did not obtain ethical approval to perform bone biopsy in a control group of women with normal ovarian function.

After recruitment, each woman was advised to discontinue previous estrogen therapy. This washout period lasted for 6 months. At the beginning of the study, we performed the following investigations: (1) serum levels of estradiol, follicle stimulating hormone (FSH), and biochemical markers of bone formation and resorption; (2) bone density at the lumbar spine and proximal femur by DXA scan; and (3) bone histomorphometry on transcortical iliac crest biopsy. After the baseline investigations, all participants received a 50 mg estradiol implant (Organon Laboratories Ltd., Cambridge, UK), inserted subcutaneously in the anterior abdominal wall and replaced at 6-month intervals. They were also given oral medroxy progesterone acetate (MPA) 5 mg daily (Upjohn Ltd., Crawley, UK) for 10 days each calendar month to protect against endometrial hyperplasia.

The study participants were advised to continue the HRT regimen and avoid any other treatment that alters bone metabolism, including calcium supplementation. Three withdrew from the study between the second and third year because of heavy or irregular periods. At the end of 3 years, the remaining 22 participants agreed to have another bone biopsy, which was successful in 21 women. Hormone and bone marker assays were repeated at 6, 12, 24, and 36 months after starting the therapy, and DXA scans were performed annually.

Bone biopsy and histomorphometry

Before each bone biopsy, the participants were given two courses of tetracycline spaced 12 days apart, and the biopsy was performed 4 days after the second course. Transcortical iliac crest biopsy was performed under local anesthesia using a 7.5-mm trephine at a standard site about 2 cm posterior to the anterior-superior iliac spine and 2 cm inferior to the iliac crest summit. Pre-therapy samples were taken from the right side and post-therapy samples from the left. Bone biopsy cores, which included both cortices and an intact intervening cancellous area, were considered suitable for analysis.

The specimens were fixed in 70% alcohol, dehydrated through graded alcohol, and embedded undecalcified in resin (London Resin Co. Ltd, Basingstoke, UK). Sections were cut from two levels separated by 200 μm, and nonconsecutive sections were selected for study. Seven-micrometer sections were stained with Goldner's trichrome and toluidine blue, and 12-μm sections were prepared unstained for fluorescence microscopy to identify the tetracycline labeling. For each sample, two sections were examined with bright field illumination and two sections under ultraviolet light, and bone histomorphometric measurements were performed using a semiautomated computer-assisted image analyzer (Osteomeasure; Osteometrics Inc., Atlanta, GA, USA).

We measured both static and dynamic histomorphometric parameters as defined by the American Society of Bone and Mineral Research[19]: (1) cancellous bone volume (%), volume of mineralized and nonmineralized bone (osteoid) to total bone tissue volume; (2) trabecular thickness (μm), mean trabecular plate thickness; (3) trabecular separation

(μm), mean distance between trabeculae; (4) trabecular number (no./mm^2), number of trabeculae in a defined area; (5) wall thickness (μm), distance from the cement line to the quiescent trabecular surface of completed bone packets; (6) osteoid thickness (μm), mean osteoid thickness; (7) osteoid surface (%). osteoid-covered surface to total cancellous bone surface; (8) eroded surface (%), extent of resorption lacunae to cancellous bone surface; (9) single-labeled surface (sLS; %), the extent of single-labeled surface to cancellous bone surface; (10) doubled-labeled surface (dLS; %), extent of double-labeled surface to cancellous bone surface; (11) mineralizing surface (MS/BS; %), the extent of labeled (dL + 1/2sL) surface to cancellous bone surface; (12) mineral apposition rate (μm/day), mean distance between double-labeled lines divided by the labeling interval of 14 days; (13) adjusted appositional rate (AjAR = MAR $\times$ MS/OS; μm/day), amount of new bone mineralized per day per unit of osteoid-covered surface; (14) bone formation rate (BFR/BS = [MS/BS $\times$ MAR]/100; μm^3/μm^2/day $\times 10^{-2}$), amount of new bone mineralized per day per unit of cancellous bone surface; (15) activation frequency (AcFrq = BFR/W.Th; year^{-1}), frequency by which new remodeling cycles are initiated at a random location on the cancellous bone surface; (16) formation period (FP = WTh/AjAR; day), time required for an individual remodeling site to complete bone formation; (17) active formation period (AcFP = WTh/MAR; days), osteoblast life span; and (18) total period (TP; days), time required to complete a remodeling cycle.

Assessments were confined to the center of the cancellous bone, avoiding the transitional zone. Length measurements were made at 100$\times$ and width measurements at 400$\times$. Osteoid was measured when it exceeded 3 μm in thickness. Four equidistant width measurements were taken for osteoid thickness and wall thickness. Measurements were corrected for obliquity of sections and presented in three-dimensional terms. To avoid the inter-observer variation in the result, all samples were analyzed independently by one histomorphometrist (SF) who was blinded to the patient's identification, their BMD results, and time of biopsy with treatment.

Hormone assays

Serum estradiol and FSH were measured by an automated ELISA using the ES700 kits (Roche Diagnostics Ltd., Lewes, East Sussex, UK). The interassay precision for estradiol was 14.9%, 6.5%, and 8.0% at serum levels of 148, 856, and 2135 pM, respectively. The interassay precision for FSH was 2.9%, 2.7%, and 3.0% at serum levels of 7.6, 16.7, and 46.3 U/liter, respectively.

Biochemical markers of bone turnover

Blood samples were collected at a fixed time (10:00 a.m. to 11:00 a.m.) on each visit to avoid diurnal variation in levels of biochemical markers of bone turnover. Serum samples were separated immediately, divided in several aliquots, and stored at -20°C until analyzed in a single batch. Osteocalcin and carboxy terminal pro-peptide of type I pro-collagen (PICP) were measured as markers of bone formation, and deoxypyridinoline (DPD) and cross-linked carboxyterminal telopeptide of type I collagen (ICTP) as markers of bone resorption. Serum PICP was measured by a radioimmunoassay (RIA), which has an intra-assay CV of 2.1–3.7% and interassay CV of 3.6–6.6% (Orion Diagnostica, Espoo, Finland). Serum osteocalcin was estimated by an immunoassay with an intra-assay CV of 1.4–3.3% and an interassay CV of 1.8–3.8% (Roche Diagnostic GmbH, Mannheim, Germany). Serum ICTP was measured by a RIA with an intra-assay CV of 4.1–7.9% and an interassay CV of 2.8–6.2% (Orion Diagnostica). Serum DPD assay is a RIA with a precision of an intra-assay CV of 3.7–5.1% and an interassay CV of 5.5–8.8% (Nichols Institute Diagnostics BV, Wijchen, Netherlands).

BMD

BMD was measured at the lumbar spine and proximal femur using a Hologic 1000 QDR DXA scanner (Hologic, Waltham, MA, USA). The CV for the densitometer calculated with daily use of a spinal phantom was 0.67% during the course of the study. The precision in vivo was assessed by serial scans in 10 healthy premenopausal volunteers. The CV was 0.98% at the lumbar spine and 1.21% at the proximal femur. BMD results were presented as absolute values (g/cm^2) and as SD and percentages above or below the mean result of young female adults (T score). The T score enabled assessment of the severity of osteoporosis and degree of improvement with therapy.

Statistical analysis

Bone histomorphometry and DXA scan variable results were not normally distributed and thus are presented as median with interquartile range. Similarly, the changes in these variables with therapy were measured as median difference with 95% CI, and the significance was assessed by Wilcoxon matched-pairs signed-ranks test. Spearman correlation coefficient was used to analyze the relation between variables because they were not normally distributed. Multiple regression analysis was performed for those histomorphometric variables that significantly changed with therapy. Age, BMI, duration of HRT, pre-therapy histomorphometry results, and post-therapy serum estradiol levels were used as covariates to assess their individual influence on the post-therapy histomorphometry results. Serum levels of biochemical markers of bone turnover were not normally distributed, and Friedman two-way ANOVA was performed to estimate the significance of changes with therapy.

RESULTS

The results of 21 women with TS who had satisfactory pre- and post-therapy transcortical iliac crest biopsy specimens were analyzed. This included 12 women with pure TS and 9 women with mosaic TS. Their mean age at the beginning of the study was 31.4 years (range, 20–40 years), and none of them had any previous pregnancies. The mean height, weight, and BMI of these women before therapy were 1.5 m (range, 1.4–1.8 m), 56.4 kg (range, 40–80 kg), and 23.6 kg/m^2 (range, 18.8–31.3 kg/m^2), which changed minimally after 3 years to 1.6 m (range, 1.4–1.8 m), 56.4 kg (range, 40–80 kg), and 23.5 kg/m^2 (range, 18.2–31.3 kg/

TABLE 1. BONE HISTOMORPHOMETRIC PARAMETERS IN YOUNG WOMEN WITH TURNER'S SYNDROME ON SUBCUTANEOUS ESTROGEN REPLACEMENT FOR 3 YEARS

Histomorphometry	*Pre-therapy**	*Post-therapy**	p *Value*
Structural parameters			
Cancellous bone volume (%)	13.37 (10.39–17.30)	18.83 (15.86–24.61)	0.0001
Trabecular thickness (μm)	104.23 (88.69–128.40)	142.51 (123.16–177.72)	0.0001
Trabecular separation (μm)	650.17 (621.87–725.28)	563.97 (461.69–678.17)	0.0071
Trabecular number (no./mm^2)	1.29 (1.21–1.37)	1.36 (1.26–1.66)	0.0173
Static parameters			
Wall thickness (μm)	33.38 (29.30–36.75)	40.91 (37.14–44.11)	0.0002
Osteoid thickness (μm)	5.99 (4.59–6.94)	10.03 (8.29–11.96)	0.0002
Osteoid surface (%)	13.94 (6.80–18.86)	3.64 (2.43–7.20)	0.0003
Eroded surface (%)	15.04 (11.81–19.81)	7.06 (4.39–9.52)	0.0001
Dynamic parameters			
Single-labeled surface (%)	2.57 (1.21–6.05)	0.71 (0.00–1.63)	0.0009
Doubled-labeled surface (%)	2.15 (0.62–5.58)	0.45 (0.00–1.69)	0.0442
Mineralizing surface (%)	4.37 (1.42–6.74)	1.10 (0.00–2.31)	0.0071
Mineral apposition rate (μm/d)	0.62 (0.42–0.76)	0.52 (0.24–0.69)	0.1842
Adjusted appositional rate (μm/d)	0.19 (0.08–0.31)	0.13 (0.00–0.32)	0.5430
Bone formation rate/BS (μm^3/μm^2/d)	10.27 (4.37–17.15)	2.61 (0.00–5.89)	0.0126
Activation frequency (year^{-1})	0.30 (0.13–0.59)	0.13 (0.05–0.34)	0.0590
Formation period (days)	182.41 (97.88–331.26)	179.13 (76.95–457.02)	0.2787
Activation formation period (days)	50.82 (42.08–74.61)	93.98 (54.20–152.93)	0.0019
Total period (days)	1313.77 (618.17–2810.48)	2821.59 (1084.51–7312.22)	0.0844

* Median (interquartile range).

m^2), respectively. All had been on HRT for a mean duration of 10.4 years (range, 2–18 years). The type and dose of estrogen included oral contraceptive pill (30 mg ethinyl estradiol; n = 11), conjugated estrogen (0.625 mg; n = 6), estradiol valerate (2 mg; n = 3), and ethinyl estradiol (1 mg; n = 1). The women were all subjected to a 6-month washout period before commencing subcutaneous estrogen replacement therapy. Although two women had suffered from fractures of the distal radius, none of them had a family history of osteoporosis.

Table 1 summarizes the bone histomorphometric results before and after 3 years of subcutaneous estrogen replacement therapy. The cancellous bone volume showed a significant increase after HRT with a median (95% CI) change of 5.39% (3.86–8.98%; Table 1). This was accompanied by architectural changes in cancellous bone, which included an increase in trabecular thickness and trabecular number and a decrease in trabecular separation, showing a median (95% CI) change of 32.15 μm (25.79–53.88 μm), 0.07 no./mm^2 (0.01–0.21 no./mm^2), and 99.39 μm (14.11–152.71 μm), respectively. There was also a significant increase in wall thickness and osteoid thickness with a median (95% CI) rise of 7.80 μm (4.76–10.87 μm) and 3.41 μm (2.83–7.30 μm), respectively. However, there was a decrease in osteoid surface and eroded surface with a median (95% CI) decrease of 7.75% (4.84–11.54%) and 8.93% (6.27–12.37%), respectively.

Single-labeled, double-labeled, and mineralized surfaces were all reduced, showing a median (95% CI) change of 2.09% (1.364.36%), 0.83% (0.02–3.45%), and 1.55% (0.93–5.40%), respectively (Table 1). There was also an associated decrease in activation frequency and bone formation rate (BFR/BS) with a median (95% CI) change of 0.19 year^{-1} (0.03–0.63 year^{-1}) and of 6.61 μm^3/μm^2/day (1.90–13.67 μm^3/μm^2/day), respectively. However, mineral apposition rate and adjusted apposition rate were not significantly altered. Total period and formation period did not change significantly, but the active formation period showed a median (95% CI) increase of 27.3 days (11.8–55.2 days).

There was no difference in bone histomorphometric parameters between women with pure TS and mosaic TS. Neither age nor the duration of past HRT correlated with any histomorphometric parameters, either before or after therapy. Similarly height, weight, and BMI had no influence on pre- or post-therapy histomorphometric results. However, the change in wall thickness correlated inversely with its respective pre-therapy results, which indicates that the lower the baseline value, the greater the improvement with therapy (r = −0.61; p = 0.003). None of the other histomorphometric parameters showed such a relationship.

Serum estradiol levels progressively increased, whereas serum FSH levels progressively declined during course of implant therapy (Table 2). The mean serum estradiol over the whole period of study, which represented a cumulative effect of the therapy, was 543.3 pM (range, 345.5–931.5 pM). Post-therapy wall thickness correlated both with mean serum estradiol level during the whole study (r = 0.46; p = 0.035) and serum estradiol at the end of the study (r = 0.52; p = 0.016). Multiple linear regression analysis confirmed an independent influence of serum estradiol level on wall thickness at the end of the study (p = 0.035). None of the other histomorphometric parameters, either before or after therapy, had a significant correlation with serum estradiol levels.

TABLE 2. SERUM HORMONE LEVELS IN YOUNG WOMEN WITH TURNER'S SYNDROME ON SUBCUTANEOUS ESTROGEN REPLACEMENT FOR 3 YEARS

		*Post-therapy**			
Hormones	*Pre-therapy**	*6 months*	*12 months*	*24 months*	*36 months*
Estradiol (pMol/liter)	87.54 (62.70–112.38)	323.52 (280.12–366.92)	506.42 (425.84–587.01)	644.85 (541.49–748.22)	713.57 (634.56–792.57)
FSH (IU/liter)	40.16 (25.35–54.97)	11.68 (3.12–20.23)	3.73 (2.13–5.32)	2.17 (1.39–2.94)	1.98 (1.47–2.48)

* Mean (95% CI).

TABLE 3. BONE MINERAL DENSITY (BMD) AND T SCORE AT THE LUMBAR SPINE AND PROXIMAL FEMUR IN YOUNG WOMEN WITH TURNER'S SYNDROME ON SUBCUTANEOUS ESTROGEN REPLACEMENT FOR 3 YEARS

		*Post-therapy**			
	*Pre-therapy**	*12 months*	*24 months*	*36 months*	p *Value*
Lumbar spine					
BMD (gm/cm^2)	0.893 (0.839–0.964)	0.964 (0.901–1.024)	0.983 (0.937–1.054)	1.021 (0.968–1.073)	<0.0001
T score (SD)	−1.40 (−1.88–−0.75)	−0.76 (−1.32–−0.20)	−0.58 (−1.00–0.07)	−0.23 (−0.72–0.24)	<0.0001
Proximal femur					
BMD (gm/cm^2)	0.840 (0.788–0.894)	0.867 (0.801–0.906)	0.893 (0.836–0.944)	0.907 (0.873–0.954)	<0.0001
T score (SD)	−1.13 (−1.55–−0.67)	−0.90 (−1.45–−0.57)	−0.64 (−1.16–−0.25)	−0.44 (−0.91–−0.14)	<0.0001

* Median (interquartile range).

TABLE 4. CHANGES IN BONE MARKERS IN YOUNG WOMEN WITH TURNER'S SYNDROME ON SUBCUTANEOUS ESTROGEN REPLACEMENT FOR 3 YEARS

		*Post-therapy**				
Bone markers	*Pre-therapy**	*6 months*	*12 months*	*24 months*	*36 months*	p *Value*
PICP (μg/liter)	100.00 (93.00–138.00)	137.00 (98.50–171.00)	131.00 (104.00–158.50)	121.00 (91.00–137.50)	98.00 (85.50–130.00)	<0.0001
Osteocalcin (ng/ml)	11.90 (8.55–16.00)	16.40 (12.00–22.55)	17.10 (12.80–22.50)	14.90 (10.55–18.05)	11.00 (9.45–13.50)	<0.0001
ICTP (μg/liter)	3.70 (3.20–4.63)	4.45 (3.75–4.94)	4.10 (3.10–4.63)	3.60 (3.15–4.70)	3.40 (2.90–4.10)	<0.0001
DPD (nMol/liter)	1.96 (1.54–2.61)	1.90 (1.72–2.79)	2.30 (1.51–2.59)	2.10 (1.25–2.42)	1.60 (1.23–1.88)	<0.0001

* Median (interquartile range).

The BMD results showed significant improvement in each patient, both at the lumbar spine and proximal femur. The median percentage rise (95% CI) at the lumbar spine was 12.70 (10.22–15.18) and at the proximal femur was 8.39 (6.93–9.85). T score at both sites improved from osteopenic levels before therapy to normal levels after 3 years of E_2 implant (Table 3). However, the increase in BMD levels at both sites did not correlate with either the absolute value or changes in any histomorphometric parameters.

The circulating levels of biochemical markers of bone formation, and to a lesser extent, bone resorption showed an initial rapid rise lasting 6–12 months followed by a slow decline reaching below baseline values by 36 months (Table 4). There was no correlation between these biochemical markers and corresponding parameters assessed by bone histomorphometry.

DISCUSSION

TS is a common chromosomal abnormality with an approximate incidence of 1:2500 female live births. More than one-half of patients with TS have a mosaic chromosomal component. Low bone density, and the consequent higher incidence of fracture, is a well-recognized risk in women with primary ovarian failure caused by TS. Although it has been suspected to be genetically determined, there is no evidence to support this, and bone density has been found to be uniformly low, irrespective of the diagnosis of ovarian dysgenesis (46XX), TS (45X), or Turner's mosaics.[1–3,5,6,18,20]

Maturation of bones may be restricted in women with TS, resulting in a delay or failure to attain peak bone mass. The delayed skeletal growth results in a 1- to 3-year lag in bone age relative to chronological age and may lead to an underestimation of bone mineralization.[3,18] Although these adolescent girls have low BMD for chronological age and bone age, when adjusted for height age, the lumbar bone density in pre-pubertal TS patients lies within the normal range. Despite this, most adult TS cases have a low bone mineral content (BMC). The absence of pubertal bone growth and failure of continued bone formation in reaching normal peak bone mass are the most likely explanations for the low bone density. This is of significance because attainment of optimum peak bone mass confers protection against subsequent risk of osteoporosis.

Despite the lack of evidence that the low bone mass in TS represents an intrinsic feature of the chromosomal alteration, there is also insufficient data to suggest that it results from ovarian hormone deficiency. Although low bone mass is a well-recognized feature of amenorrhea, the current belief is that estrogen deficiency per se is not the primary cause of osteoporosis in TS. This is because the BMC remains low despite estrogen replacement therapy, even when this is commenced during puberty.[18,20] In addition, abnormal growth hormone secretion is also thought to play a role in the delayed bone development.[8,9]

Conventional doses of oral estrogen replacement are usually sufficient for development of secondary sexual characteristics, symptom relief, and induction of regular periods in young women with ovarian failure. However, doubts about its efficacy in protecting bones have been raised because there is still a higher incidence of osteoporotic fractures in these women despite long-term estrogen replacement. Osteoporosis is one of the most common complications of TS. It has been shown that osteopenia and osteoporotic fractures occur more frequently in TS with relative risks of 10.1 (2.1–30.9) and 2.7 (1.4–4.6), respectively.[21] An alternative explanation is that the physiological levels of serum estradiol that may be required to optimize bone formation are not achieved with standard doses of HRT.

Physiological and supraphysiological levels of estrogen have been shown to stimulate osteoblastic recruitment and activity, leading to increased bone volume in animals.[12,22,23] There is also in vitro evidence that estradiol may stimulate osteoblastic differentiation and function.[10,11] The standard doses of HRT commonly used result in relatively low serum estradiol levels (<200 pM), only reaching that of the early to mid-follicular and late luteal range of the normal menstrual cycle. These basal levels of estradiol may be sufficient to suppress bone resorption but are inadequate to stimulate bone formation.[24,25] This merely serves to prevent bone loss but is inadequate in the management of low bone mass resulting from deficient bone formation. Increased bone resorption has been found in previous studies of women with TS, and this is suppressed by estrogen treatment.[20,26] The subcutaneous route used in our study ensures complete compliance. This is much less of a problem in a group of young women who are accustomed to the notion of regular menstruation and are motivated to not only prevent future osteoporosis but wish to maintain an optimum hormonal milieu for assisted conception. The estrogen implants also enable much higher estradiol levels to be achieved, similar to that observed in the late follicular and mid-luteal phase, and a more physiological estradiol to estrone ratio by avoiding the hepatic first-pass effect. One year after commencement of estrogen implant therapy, nearly all the women in our study had estradiol levels in the mid-luteal range (450 pM), and the mean estradiol level over the treatment period was also within this range, but still was below that observed during the ovulatory surge (>740 pM).

Although estrogen has been reported to increase BMD in TS,[17] this is the first longitudinal study showing increase in BMD corroborated by increased cancellous bone volume by estrogen treatment in women with TS. This is remarkable given the short period of treatment. The increase in bone volume was caused at least in part by increase in wall thickness. Increase in wall thickness reflects increased bone formation at a bone remodeling unit level. This may be because of increased numbers of osteoblasts recruited to individual bone remodeling units, increased activity of individual osteoblasts, and/or increased active life-span of osteoblasts. We found an increase in active formation period, and because the formation period is essentially unchanged, this suggests that the active life-span of osteoblasts is proportionately increased and that this is a mechanism by which the increased bone formation has occurred. This may be caused by reduced apoptosis of osteoblasts by estradiol.[27] The large early rise in serum osteocalcin and PICP suggests that osteoblast numbers are also increased. This phenomenon has also been observed in previous studies using transdermal estrogen.[28] The increase in wall thickness was related to serum estradiol levels. This, in turn, suggests that the increased numbers and active lifespan of osteoblasts may be related to estrogen levels. We did not find an increase in the mineral apposition rate or adjusted mineral apposition rate to suggest increased activity of individual osteoblasts. The decrease in labeled bone surface and bone formation rate, as with the decrease in osteoid and eroded surfaces and activation frequency, reflects suppression of bone turnover, a well-recognized effect of estrogen treatment, and therefore does not negate the stimulatory effect that estrogen may also exert on osteoblasts. We were, however, surprised to find that biochemical markers of bone resorption were transiently increased in the first year, although to a lesser extent than those of bone formation. This is contrary to the expected action of estrogen in suppressing bone resorption. The exact reason for the transient increased bone resorption is unclear, but we cannot rule out a biphasic action of estrogen on bone resorption.[29] Thus, by the end of 3 years, the effect of estrogen seems to be increased wall thickness and osteoid thickness at the level of the bone remodeling unit and decreased activation frequency at the level of local bone tissue, but the circulating biochemical markers reflecting whole body bone turnover remained relatively unchanged from baseline. Our results suggest that the increase in cancellous bone volume and BMD caused by estrogen treatment is due to suppression of bone resorption and also to increase in bone formation. A weakness of the study, however, is the lack of an appropriate age-, height-, and weight-matched healthy control group.

Because our cohort is comprised of adults, the increase in bone mass as assessed by bone densitometry and by bone histomorphometry suggests that the increase is not due to bone growth, but represents an increase in the amount of pre-existing bone. Normalization of the T score in these women suggests that peak bone mass can be optimized in patients with Turner's syndrome, thereby conferring some protection against future osteoporosis.

Our results show that larger doses of estrogen given as implants, which achieve E_2 levels at the higher end of the physiological range, are capable of exerting an anabolic effect in the skeleton of young women with TS and low bone mass. High estrogen levels, as are found in pregnancy, are also associated with an increase in bone mass.[30] This may serve as additional storage for calcium to be mobilized during lactation. Recent evidence from in vitro reporter gene assays and estrogen receptor knock-out animals suggests that low and high levels of estrogen may cause differential activation of estrogen receptors (ER) α and β, and in so doing, exert differential effects on bone resorption and bone formation.[31,32] At low estrogen levels, ER β is predominantly activated and reduce overall cellular sensitivity to estradiol. This may be only sufficient to suppress bone resorption. At higher levels of estrogen, both ER α and β are activated, and bone formation is stimulated.[31] Our current findings in young women and those of earlier studies showing an anabolic effect of estrogen in the bones of elderly postmenopausal women treated with estrogen implants[15,16] suggest that these findings may extend to the human skeleton. It is likely that under normal circumstances, bone resorption and turnover are suppressed by basal levels of estrogen, but the higher levels, observed in the late follicular and mid-luteal phase, enhance bone formation that is already started. There are analogous circumstances elsewhere in biology. For example, while at very low concentrations, macrophage-colony-stimulating factor (M-CSF) inhibits apoptosis, at intermediate levels, it stimulates proliferation, and at the highest levels, it induces differentiation. This has important implications for the understanding of the action of estrogen on the skeleton and for the development of estrogen and estrogen-like compounds for the management of osteoporosis.

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