Long-term hormone replacement therapy preserves bone mineral density in Turner syndrome

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Abstract

Context: Reduced bone mineral density (BMD) and increased risk of fractures are present in many women with Turner syndrome (TS).

Objective: Examine longitudinal changes in BMD in TS and relate changes to biochemical parameters.

Design: Prospective, pragmatic, and observational study. Examinations at baseline and follow-up (5.9 ± 0.7 years).

Setting: Tertiary hospital.

Participants: Fifty-four women with TS (43.0 ± 9.95 years).

Interventions: Hormone replacement therapy (HRT) and calcium and vitamin D supplementation.

Main outcome measures: BMD (g/cm²) measured at lumbar spine, hip, and the non-dominant forearm. Bone formation and resorption markers, sex hormones, IGF1, and maximal oxygen uptake.

Results: At follow-up, forearm BMD, radius ultradistal BMD, and hip BMD remained unchanged, radius 1/3 BMD declined (0.601 ± 0.059 vs 0.592 ± 0.059, P = 0.03), while spine BMD increased (0.972 ± 0.139 vs 1.010 ± 0.144, P < 0.0005). Bone formation markers did not change over time in TS. Bone resorption markers decreased over time in TS. Testosterone, IGF1, and maximal oxygen uptake was significantly reduced in TS.

Conclusion: Longitudinal changes in BMD in TS were slight. BMD can be maintained at most sites in well-informed women with TS, being encouraged to maintain a healthy lifestyle, including HRT and intake of calcium and vitamin D.

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women with TS. Participants were after the first DEXA scan advised to exercise regularly with weight-bearing activities, adhere to a healthy diet, take calcium and vitamin D supplementation if vitamin D levels were low, and keep taking HRT. Baseline data from this study have previously been presented (4).

**Materials and methods**

**Subjects**

The final study group consisted of 54 patients with TS aged 22–65 years (mean 37 years) at baseline. Originally, 59 women participated in the study, but three had died (two from aortic dissection), and two declined further participation. They were diagnosed by chromosome analysis. Karyotypes were distributed as follows: 45 X (n=24); 45, X/46, XX (n=5); karyotypes with isochromosomes (Xq or Xp) or deletions (n=15); karyotypes with Y chromosome material (n=5); karyotypes with a marker or ring chromosome (n=5). All patients were recruited through the National Society of Turner Contact Groups in Denmark.

Exclusion criteria were untreated hypothyroidism or hyperthyroidism, present or past malignant diseases, clinical liver disease, or treatment with drugs known to interfere with bone metabolism (e.g. glucocorticoids). Originally, six of the patients had menstruated spontaneously but all had experienced premature ovarian failure at inclusion. At inclusion, 52 received conventional HRT consisting of 17β-estradiol (E2; 2 mg) for the entire cycle and norethisterone (1 mg), medroxyprogesterone (10 mg), or levonorgestrel (0.25 mg) for 10 days every cycle. Two participants had chosen not to receive HRT. All were interviewed concerning the age at menarche (if present), age at the start of induction of puberty (by exogenous estrogen), age at premature menopause (if present), duration of HRT, and age at cessation with HRT (when relevant), enabling summation of total estrogen exposure (in years) and years of estrogen insufficiency, estimated as the number of years between the age of 13 and 53 years, during which participants neither were taking HRT, nor had spontaneous menstrual bleedings. These variables were used in subsequent statistical computations. The intake of two tablets a day of calcium (400 mg/tablet) and vitamin D3 (10 µg/tablet) was recommended to TS participants with low levels of 25-hydroxy-vitamin D (25-OHD) at baseline. We do not have information concerning the compliance to vitamin D intake. Two participants with TS had received GH during adolescence for <2 years, while all other participants had never received GH. All subjects received oral and written information concerning the study prior to giving written informed consent. The protocol was approved by the Aarhus County Ethical Scientific Committee as an extension of a previous protocol (# 1994/2929).

**Methods**

We examined all participants in the morning. We had instructed all women not to eat or drink anything other than bottled mineral water and to met fasting in the laboratory. After blood was drawn, serum was separated and stored at −20 °C in multiple vials for later analysis. We measured body weight to the nearest 0.1 kg on an electronic scale and body height was measured to the nearest 0.5 cm, with the subjects in underwear and barefooted. BMI was calculated as weight (kg) divided by height (m) squared.

BMD (g/cm²) was measured at the lumbar spine (L2–L4), the hip (femoral neck and trochanteric region), and the non-dominant forearm (total, ultradistal (radius UD), and proximal part of distal third (radius 1/3) by DEXA on Hologic 1000/w or 2000/w osteodensitometers (Hologic, Inc., Waltham, MA, USA). Cross-calibration was ensured through the use of double measurements of a phantom. Precision of BMD was 1.5% for the lumbar spine, 2.1% for the femoral neck, and 1.9% for the UD forearm. These quantities included cross-over calibration, change in hardware, change in technicians, and long-term stability (<0.2%/years). T-scores were calculated, and results were categorized as ‘osteoporosis’ if the T-score ≤−2.5 or ‘osteopenia’ when the T-score is between −1.0 and −2.5 according to the WHO (20).

A 6-min submaximal exercise test with continuous monitoring of the heart rate was performed on a bicycle ergometer (Monark Ergometric 829 E, Monark exercise AB, Varberg, Sweden) using a workload of 300–1200 kpm/min, depending on age and reported physical activity by the subject. The mean heart rate during the last 2 min of work (>120 beats/min) was used for calculation of the maximal aerobic capacity (VO2max) (21). This indirect measure of maximal aerobic capacity has been shown to correlate well with a direct measure of maximal aerobic capacity, with a coefficient of variation (CV) of <10% (22), which in our laboratory has a day-to-day intra-individual CV of 9% (unpublished observations).

**Assays**

We measured urine N-terminal cross-linking telopeptide of type I collagen (NTX) by an immunometric assay using a Vitros ECI analyser (Ortho Clinical Diagnostics, Amersham), and data are presented as a ratio (NTX/creatinine ratio). This assay employs monoclonal antibodies against human NTX (23). We measured plasma bone-specific alkaline phosphatase (bone ALP) using an enzyme immunoassay based on monoclonal anti bone ALP antibodies (Metra BAP method, Quidel Corporation, San Diego, CA, USA) (24). This assay performed with a CVtotal of 8%. P-C-telopeptide fragments of type I collagen (ICTP) (25) and P-procollagen III amino-terminal propeptide (PIIINP)
Table 1 Anthropometric data in the Turner syndrome (TS) group at baseline and at follow-up (mean and S.D. or median (range)).

<table>
<thead>
<tr>
<th></th>
<th>TS baseline</th>
<th>TS follow-up</th>
<th>P* (TS baseline versus TS follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>54</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>37.2 ± 9.7</td>
<td>42.8 ± 9.9</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>146.2 ± 6.2</td>
<td>145.7 ± 6.1</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.5 ± 13.7</td>
<td>58.7 ± 13.0</td>
<td>0.1</td>
</tr>
<tr>
<td>BMI (kg/cm²)</td>
<td>26.8 ± 5.3</td>
<td>27.4 ± 5.4</td>
<td>0.02</td>
</tr>
<tr>
<td>VO₂max (ml O₂/kg min)</td>
<td>39.0 ± 9.9</td>
<td>36.6 ± 10.3</td>
<td>0.057</td>
</tr>
<tr>
<td>Estrogen exposure (years)</td>
<td>15.0 (0–37)</td>
<td>20.0 (0–44)</td>
<td>&lt;0.0005b</td>
</tr>
<tr>
<td>Estrogen insufficiency (years)</td>
<td>6.0 (0–40)</td>
<td></td>
<td>&lt;0.0005c</td>
</tr>
</tbody>
</table>

*Paired t-test, unless otherwise indicated.

bWilcoxon signed-ranks test.

cOne sided t-test, expecting the estrogen insufficiency period to be 0 years.

Statistical analysis

All statistical calculations were performed with SPSS for Windows version 11.0 (SPSS, Inc., Chicago, IL, USA). Data were examined by two-tailed paired or the Mann–Whitney two-tailed test as appropriate. Results are expressed as mean ± s.d. (range). However, when not parametrically distributed, data are presented as median and range. Significance levels < 5% were considered significant. Pearson or Spearman correlation was used where appropriate, to examine relationships between DEXA scan-derived BMD, bone markers and estrogen exposure, age, and body size (height, weight, and BMI).

Multiple backward stepwise linear regression models were constructed to examine the principal determinants of changes in BMD, where independent variables were omitted from the model when P > 0.1. As biochemical determinants, all bone markers, vitamin D-related markers, and hormones were studied.

Results

During the 5.9-year study period, there was a slight increase in weight among TS and a decrease in height, which resulted in a significant increase in BMI. The median estrogen insufficiency period was 6 years with a wide variation (Table 1).

BMD: longitudinal changes in TS

T-scores (and Z-scores, not shown) were decreased at the spine and the hip, both at baseline and at follow-up. The repeated DEXA scannings after 5.9 years showed a decline in 1/3 radius BMD. Total forearm BMD, UD forearm BMD, and hip BMD remained unchanged, while spine BMD increased (Fig. 1). Accordingly, T-score at the spine increased and decreased at the hip. At follow-up, 4 (7%) at the spine and 9 (17%) at the hip respectively were categorized as having osteoporosis based on BMD measurements according to the WHO definition (Table 2).

Individual changes in UD forearm BMD and hip BMD showed a positive correlation with changes in weight (r = 0.334, P = 0.018 and r = 0.408, P < 0.05 respectively). No correlation was found between changes in weight and changes in spine BMD, total forearm, or 1/3 radius BMD. No correlations were found between changes in BMD at any of the regional sites and changes in estrogen exposure, except spine BMD, or changes in age or body size (height and weight).
The changes in BMD during the observation period at any site were correlated to the changes seen at all other sites (all \( P < 0.05 \), individual results not shown).

Bone markers and hormones: longitudinal changes in TS

Comparing the longitudinal changes in bone markers in TS, we found a significant reduction in the levels of urine NTX/creatinine ratio and ICTP and an increase in the level of PTH. All bone formation markers were unchanged (Table 3).

The decreased NTX/creatinine ratio was negatively correlated to changes in total forearm BMD, UD BMD, and spine BMD (\( r = -0.286 \) to \(-0.551\), all \( P < 0.05 \)). Change in NTX/creatinine ratio was positively correlated to changes in other bone markers like ICTP, total ALP, and PINP (\( r = 0.313–0.379\), all \( P < 0.05 \)), but negatively correlated to changes in 25-OHD (\( r = -0.323\), \( P < 0.01 \)).

The decrease in ICTP was negatively correlated to changes in total forearm BMD and spine BMD (\( r = -0.328 \) to \(-0.413\), all \( P < 0.05 \)). Change in PTH was negatively correlated with changes in ICTP (\( r = -0.291\), \( P < 0.05 \)), but not to changes in vitamin D. Both at baseline and during follow-up, there were no significant correlations between PTH and vitamin D-related variables (25-OHD, DBP and 25-OHD/DBP ratio).

The recorded small changes in bone ALP and 25-OHD were not found to be significantly correlated with changes in BMD at any of the bone sites, nor with the other biochemical bone markers.

When studying changes in formation (PINP and total OC, but not bone ALP) and resorption markers (ICTP and NTX/creatinine ratio), there was evidence for continuing tight coupling between bone resorption and bone formation as seen from significant positive correlations between the changes in these markers (all \( P < 0.05 \)).

We found a significant decrease in serum testosterone (42%) and IGF1 (21%) with time, while serum E\(_2\) (10%) increased slightly, and IGFBP-3 (22%) increased significantly.

Multiple regression models

Baseline variables predicting change in spine BMD

The principal determinants of longitudinal change in spine BMD were evaluated by backward multiple linear regression with measures of anthropometry, maximal oxygen uptake, and biochemical markers at baseline as independent variables. Significant variables were chosen from the Spearman’s correlation analyses (results not shown).

In a multivariate model (\( r = 0.572\), \( P = 0.001 \)) with change in spine BMD in TS as the dependent variable, body height (\( P = 0.01 \)), estrogen exposure time (\( P = 0.03 \)), and IGF1 (\( P = 0.06 \)) were all explanatory variables.

Table 2 Mean and s.d. (range) of bone mineral density (g/cm\(^2\)) among Turner syndrome (TS). Data are also presented as T-scores, and categorized according to the WHO criteria for osteoporosis and osteopenia.

<table>
<thead>
<tr>
<th></th>
<th>TS baseline</th>
<th>TS follow-up</th>
<th>( P^a ) (TS baseline versus TS follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm (total), (g/cm(^2))</td>
<td>0.49±0.05 (0.37–0.59)</td>
<td>0.50±0.06 (0.38–0.61)</td>
<td>0.2</td>
</tr>
<tr>
<td>Radius (1/3), (g/cm(^2))</td>
<td>0.60±0.06 (0.47–0.72)</td>
<td>0.59±0.06 (0.48–0.72)</td>
<td>0.03</td>
</tr>
<tr>
<td>Radius UD, (g/cm(^2))</td>
<td>0.40±0.06 (0.27–0.54)</td>
<td>0.41±0.06 (0.28–0.55)</td>
<td>0.1</td>
</tr>
<tr>
<td>Spine, (g/cm(^2))</td>
<td>0.97±0.14 (0.59–1.24)</td>
<td>1.01±0.14 (0.69–1.28)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Spine T-score</td>
<td>-0.76±1.22 (−4.11–1.46)</td>
<td>-0.62±1.30 (−3.52–1.24)</td>
<td>0.02</td>
</tr>
<tr>
<td>WHO osteoporosis</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>WHO osteopenia</td>
<td>14</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Hip, (g/cm(^2))</td>
<td>0.85±0.13 (0.60–1.14)</td>
<td>0.85±0.14 (0.59–1.18)</td>
<td>0.4</td>
</tr>
<tr>
<td>Hip T-score</td>
<td>-1.04±1.32 (−3.10–2.13)</td>
<td>-1.27±1.37 (−3.52–2.05)</td>
<td>0.04</td>
</tr>
<tr>
<td>WHO osteoporosis</td>
<td>9</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>WHO osteopenia</td>
<td>19</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \)Paired \( t \)-test.
Markers of bone resorption
- Urine NTX ratio
- Urine N-terminal cross-linking telopeptide of type 1 collagen:
  - TS baseline: 61.2 (9–389)
  - TS follow-up: 47.2 (18–322)
  - P<0.0005

Table 3 Bone markers, vitamin D-related markers and hormones (mean ± s.d. (range) or median and range).

<table>
<thead>
<tr>
<th>Markers of bone formation</th>
<th>TS baseline</th>
<th>TS follow-up</th>
<th>P (TS baseline versus follow-up)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaline phosphatase (U/l)</td>
<td>63.1 ± 28.6 (23–162)</td>
<td>66.0 ± 27.6 (22–148)</td>
<td>0.3</td>
</tr>
<tr>
<td>Bone specific alkaline phosphatase (U/l)</td>
<td>15.9 ± 4.75 (8–32)</td>
<td>16.1 ± 4.47 (8–30)</td>
<td>0.4</td>
</tr>
<tr>
<td>Osteocalcin (μg/l)</td>
<td>20.9 (10–67)</td>
<td>20.7 (11–93)</td>
<td>0.8b</td>
</tr>
<tr>
<td>PINP (μg/l)</td>
<td>51.6 ± 28.3 (13–166)</td>
<td>49.7 ± 25.1 (19–150)</td>
<td>0.5</td>
</tr>
<tr>
<td>PIIINP (μg/l)</td>
<td>4.23 ± 1.40 (2.2–8.1)</td>
<td>4.24 ± 1.53 (2.1–8.8)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Markers of bone resorption
- ICTP (μg/l)                               | 4.35 ± 1.39 (2.2–8.7)       | 3.93 ± 1.21 (2.0–7.7)         | 0.03                             |

Vitamin D related markers
- 25-OH-vitamin D (nmol/l)                  | 58.8 ± 26.3 (36–137)        | 62.3 ± 21.4 (30–115)          | 0.3                              |
- PTH (pmol/l)                              | 3.2 (1.1–15.9)              | 3.8 (1.1–20.8)                | 0.001b                           |
- Vitamin D binding protein (mg/l)          | 248.9 ± 39.4 (162–379)      | 249.4 ± 52.1 (196–335)        | 0.9                              |
- 25-OH-vitamin D/D vitamin DBP ratio       | 0.24 ± 0.08 (0.09–0.44)     | 0.25 ± 0.08 (0.12–0.47)       | 0.5                              |

Hormones
- Estradiol (nmol/l)                        | 0.21 (0.02–0.63)            | 0.23 (0.06–0.89)              | 0.03b                            |
- Testosterone (nmol/l)                     | 1.10 (0.08–3.54)            | 0.77 (0.04–5.42)              | 0.02b                            |
- IGFl (μg/l)                               | 154.2 ± 54.9 (52–317)       | 127.3 ± 42.5 (67–244)         | <0.0005                          |
- IGFBP-3 (μg/l)                            | 3477 ± 600 (2249–5331)      | 4231 ± 795 (2459–6025)        | <0.0005                          |

Discussion

This is the first study to demonstrate that adult TS women on proper HRT can maintain BMD. The principal results of the study show equivocal changes in BMD at different sites in TS, in whom the great majority (96%) received appropriate HRT. Overall, there were only small changes in BMD at the sites examined. Although we found a significant increase in BMD at the spine, the change was small but nevertheless possibly clinically relevant. Likewise, the significant reduction in BMD at the radius was only small, but could be of clinical significance. Thus, we conclude that in a pragmatic observational set-up, BMD can be maintained at most sites in well-informed young to middle-aged individuals with TS, by encouraging them to maintain a healthy lifestyle including regular HRT and a high intake of calcium and vitamin D.

BMD at all sites (forearm, spine, and hip) was decreased in TS in line with previous studies (2–6). These results are likely influenced by the two-dimensional nature of DEXA scanning, which does not consider the smaller size of the TS subjects as shown in previous studies (4), where we showed that there are only minimal differences in volumetric BMD at the hip and spine between TS and age-matched control women. Still, differences in body size and bone scan technique might not be the only explanatory factors for the observed reduction of BMD in TS. Peak bone mass depends on several factors, such as genetic background, nutrition, physical activity, local growth factors, and a number of hormones (31, 32). Evidently, many women with TS receive appropriate HRT late in life and for a shorter duration than is recommended (5, 33, 34). According to the WHO definition, more women with TS than controls were categorized as having osteoporosis and osteopenia.

Adolescent girls with TS with spontaneous puberty have BMD in the normal range, while in TS girls who underwent induced puberty, BMD was in the osteopenic range in all and in the osteoporotic range in 30% (8), and young TS does not attain peak BMD despite proper HRT started in adolescence (35). This suggests that estrogen plays a role in obtaining and maintaining maximal BMD. Since the general agreement among physicians is to continue HRT until the age of normal menopause (around the age of 55 years), the age at debut of pubertal induction ultimately influences the total exposure of the bones to estrogen in years. The optimal estrogen dosage regimen to induce and maintain pubertal development in order to mimic the physiological levels of E2 in normal adolescents is not known. Other reports also suggest that normal gonadal function during puberty and late adolescence is required for adequate skeletal mineralization, as well as attaining and maintaining normal bone mass (8). However, girls with TS are often introduced late to estrogens to avoid stunting of growth conferred by the compounds. Recent evidence shows that regimens initiating E2 treatment at the age of 12 years permit a normal pace of puberty without interfering with the positive effect that GH has on final height.

Out of the 54 TS women in the present study, only two, by choice, did not receive estrogen treatment at the time of examination, but the calculated median estrogen insufficiency in years at follow-up was still 6...

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years with a wide scatter. Inadequate availability of
estrogens to bone tissue from infancy to adulthood,
caused by early pre-pubertal ovarian dysfunction,
delayed puberty, lack of therapeutic estrogen/gestagen
regimens, and treatment non-compliance, should there-fore be considered as major factors responsible for the
observed low BMD values in some studies in patients
with TS (8). Despite recommendations regarding an
appropriate intake of calcium and vitamin D, we
observed a slight, but significant, increase in PTH but
also a slight insignificant increase in vitamin D, showing
mild secondary hyperparathyroidism. This result dupli-
cates earlier findings of low vitamin D levels in many
individuals with TS (5, 33), and reinforces the need to
stimulate an adequate intake of vitamin D and calcium
in TS, and exposure to sunlight, although we did not
find significant correlations between BMD measure-
ments and vitamin D in the present study. We believe
this may be due to the size of the study sample, since
studies in other populations have found vitamin D levels
to be a determinant of BMD. Here, we also document
lower levels of vitamin D-binding globulin, being
produced in the liver, which may be a consequence of
the frequent liver involvement in TS (36).

All markers of bone formation were unchanged
during follow-up, while we observed a rather pro-
nounced decrease in the resorption marker, NTX/
creatinine ratio, and ICTP, which correlated closely
and negatively to the observed changes in BMD,
indicating that the patients with the most pronounced
slowing of bone resorption had the greatest increase in
BMD during the 5.9-year period of observation. We also
observed a tight coupling between changes in markers
of bone formation and resorption, contrary to what we
observed in the initial baseline study from the present
cohort (4), where we found bone formation markers to
be comparable or only marginally elevated in compari-
son with controls, while bone resorption markers were
35–70% elevated (4). In the present population, it is
difficult to determine whether inadequate sex HRT or
the relative secondary hyperparathyroidism is the cause
of the enhanced resorption, or if it is a consequence of
other metabolic changes, such as low serum testoster-
one (18, 37) and low bioavailable IGF1 (38, 39), or the
genetic defect per se. The observed positive correlation
between plasma PTH and total OC and renal NTX/crea-
tinine corroborates a biological effect of PTH on bone in
Turner patients. It is, however, of great interest that the
constructed multiple linear regression model reveals
varying independent variables at the level of the spine.
We found a number of baseline independent
determinants of change in spine BMD, i.e. IGF1, body
height, and estrogen exposure time. Overall, the results
from the multiple regression analyses point towards
several hormonal systems, estrogen exposure time,
and anthropometric variables as being important
contributors to bone health in TS. The reported levels
of testosterone and IGF1 are lower than what we see in
controls (4), and we also observed quite pronounced
reductions over the course of the study. On the other
hand, HRT maintains serum E2 levels close to what is
seen in controls (4). In addition, we replicate findings of
low maximal oxygen uptake in women with TS in
comparison with controls. These findings suggest that
recommendations of a healthy lifestyle should include
advice on appropriate exercise to maintain bone health.

In conclusion, bone health can be maintained in most
women with TS when appropriate information is given,
and proper HRT as well as vitamin D and calcium
supplementation is recommended. Future studies
should determine the optimal estrogen dosage regimen
to induce and maintain pubertal development as well as
attain maximal bone mass acquisition and mineral-
ization in TS.

Declaration of interest
The authors declare that there is no conflict of interest that could be
perceived as prejudicing the impartiality of the research reported.

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