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.

Introduction

Turner syndrome (TS) occurs in about 50 per 100 000 live-born girls (1, 2). Cardinal stigmata in TS include reduced final height, cardiovascular malformations, and premature ovarian failure associated with infertility (3). Premature ovarian failure, if untreated, leaves patients deficient of female sex hormones from early childhood and onwards. Several studies have shown decreased bone mineral density (BMD) in girls as well as in younger and middle-aged women with TS (4–8). This finding may largely be due to the two-dimensional nature of dual energy x-ray absorptiometry (DEXA) scans (9), which fails to account for the reduced height in TS leading to lower BMD a priori. Studies of volumetric BMD, taking actual body height into account, have shown largely similar volumetric BMD in TS and controls (4, 10). Regardless of BMD, end-point studies have documented increased fracture frequency in girls and women with TS (11–13). It is not entirely clear whether a primary bone defect exists in TS (4, 14), perhaps due to skeletal dysmorphogenesis (15) caused by haploinsufficiency of the short stature homeobox-containing gene (SHOX) (16, 17) or rather endocrine or metabolic defects exists (18), resulting in estrogen deficiency and other endocrine deficiencies (4).

Premature ovarian failure in TS can lead to osteoporosis, if untreated, but should not be considered analogous to postmenopausal osteoporosis or surgical menopause, both manifestations of a state of estrogen withdrawal, but be viewed as a prolonged phase of suboptimal bone accretion in the non-estrogenically treated adolescent with TS. The optimal time of pubertal induction in TS and the optimal dose of hormonal replacement therapy (HRT) during adolescence, early, and late adulthood are uncertain, as is the age at which to stop therapy (19).

Bone metabolism is a key parameter to monitor during HRT in premature ovarian failure. In order to study the effect of HRT on longitudinal bone changes in TS, we conducted a longitudinal, observational, pragmatic study of BMD, and biochemical indices in adult
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 patients 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 either were not 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 (VO₂ max) (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 CV of 8%. P-C-telopeptide fragments of type I collagen (ICTP) (25) and P-procollagen III amino-terminal propeptide (PIIIINP )
(26) were analyzed by RIAs from Orion Diagnostica (Oulunsalo, Finland). Intra- and interassay CV’s were <6 and <8% respectively. P-ostecalcin (N-MID-osteocalcin), P-intact parathyroid hormone (PTH), and P-procollagen I amino-terminal propeptide (PINP) (27) were analyzed by electrochemiluminescence immunoassays using an automated instrument (Elecsys 2010, Roche). The osteocalcin (OC) method detects intact OC as well as the large N-mid terminal fragment (28). Intra- and interassay CV’s were 2–4 and 4–7% respectively for all assays. P-25-OH-vitamin D2 and D3 were analyzed by isotope dilution liquid chromatography–tandem mass spectrometry on a API300 TM mass spectrometer (Applied Biosystems, Inc., Carlsbad, CA, USA) by a method adapted from Maunsell et al. (29). The interassay CV for P-25OHD2 and P-25OHD3 were 8–9%. 25OHD is presented as the sum of 25OHD2 and P-25OHD3. We measured vitamin D-binding protein (DBP) by an immunonephelometric assay using a Behring Nephelometer II (Dade Behring, Liederbach, Germany) with an interassay CV of 5% (30), and serum total ALP and urinary creatinine by standard laboratory methods. As a measure of ‘free vitamin D’, we calculated a 25-OHD/DBP ratio.

We measured serum insulin-like growth factor-1 (IGF1) by non-competitive TR-IFMA, and IGF binding protein (IGFBP)-3 by IRMA (Diagnostics Systems Laboratories, Inc., Webster, TX, USA). We measured serum E2 and testosterone by a commercial time-resolved fluoroimmunoassay (DELFIA), Wallac, Inc., Turku, Finland).

### 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).

### 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 (years)</td>
<td>37.2 ± 9.7</td>
<td>42.8 ± 9.9</td>
<td>&lt;0.0005b</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>146.2 ± 6.2</td>
<td>145.7 ± 6.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.5 ± 13.7</td>
<td>58.7 ± 13.0</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>26.8 ± 5.3</td>
<td>27.4 ± 5.4</td>
<td>0.057</td>
</tr>
<tr>
<td>VO2max (ml O2/kg min)</td>
<td>39.0 ± 9.9</td>
<td>36.6 ± 10.3</td>
<td></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>&lt;0.0005c</td>
<td></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.
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 osteopena</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 osteopena</td>
<td>19</td>
<td>22</td>
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</tbody>
</table>

*Paired $t$-test.

Figure 1 Longitudinal changes in site-specific BMD during the 5.9-year follow-up period among women with TS. Error bars indicate s.d.s. Significant changes in BMD are noted in the figure. Radius 1/3, BMD of proximal part of distal third of the forearm; radius UD, BMD of the ultradistal part of the forearm.
markers of bone resorption

Hormones

With TS receive appropriate HRT late in life and for a
nutrition, physical activity, local growth factors, and a
depends on several factors, such as genetic background,
observed reduction of BMD in TS. Peak bone mass
might not be the only explanatory factors for the
Still, differences in body size and bone scan technique
and spine between TS and age-matched control women.
only minimal differences in volumetric BMD at the hip
consider the smaller size of the TS subjects as shown
sional nature of DEXA scanning, which does not
decreased in TS in line with previous studies (2–6).

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-dimen-
sional 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
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
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 therefore 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 duplicates 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 measurements 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 pronounced 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 comparison 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 testosterone (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/creatinine 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 mineralization 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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References

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