Association between L-thyroxine treatment, GH deficiency, and radiological vertebral fractures in patients with adult-onset hypopituitarism

G Mazziotti, M Mormando¹, A Cristiano, A Bianchi¹, T Porcelli, A Giampietro¹, F Maffezzoni, V Serra, L De Marinis¹ and A Giustina

Chair of Endocrinology, University of Brescia, A.O. Spedali Civili di Brescia, 25123 Brescia, Italy and ¹Pituitary Unit, Department of Endocrinology, Catholic University of Rome, 00168 Rome, Italy

Correspondence should be addressed to A Giustina
Email a.giustina@libero.it

Abstract

Objective: In this study, we aimed at evaluating the association between radiological vertebral fractures and levo-thyroxine (L-T4) replacement doses in adult patients with hypopituitarism.

Design: Cross-sectional study.

Methods: We studied 74 adult hypopituitary patients (males, 43; females, 31; mean age, 57 years; and range, 23–79) with central hypothyroidism treated with L-T4 (median daily dose: 1.1 mg/kg). All patients also had severe GH deficiency (GHD) and 38 of them were replaced with recombinant GH. Vertebral fractures were assessed by a quantitative morphometric analysis performed on thoracic and lumbar spine lateral X-ray.

Results: Radiological vertebral fractures were found in 23 patients (31.1%) in association with untreated GHD ($P = 0.02$), higher serum free $T_4$ levels ($P = 0.03$), a higher daily dose of L-T4 ($P = 0.005$), and a longer duration of hypopituitarism ($P = 0.05$). When GHD was treated, the prevalence of vertebral fractures was more frequent ($P = 0.03$) in patients receiving high L-T4 doses (third tertile: $> 1.35$ μg/kg per day) as compared with patients who were treated with lower drug doses (first tertile: $< 0.93$ μg/kg per day). Such a difference was not observed in patients with untreated GHD who showed a higher prevalence of vertebral fractures regardless of L-T4 daily doses. Multivariate analysis showed that untreated GHD (odds ratio: 4.27, 95% CI 1.27–14.33; $P = 0.01$) and the daily dose of L-T4 (odds ratio: 4.01, 95% CI 1.16–14.39; $P = 0.03$) maintained a significant and independent association with vertebral fractures in patients with central hypothyroidism.

Conclusions: Our data suggest for the first time that a relative overtreatment with L-T4 may influence the fracture risk in some patients with hypopituitarism.

Introduction

Thyroid hormones play an important role in the control of longitudinal growth and have physiological stimulatory effects on bone remodeling and bone mass (1). However, when thyroid hormones increase, bone remodeling is excessively stimulated with consequent bone loss, decrease in bone mineral density (BMD), and increase in fracture risk (2). Interestingly, an increased risk of fragility fractures has been reported even in patients exposed to mild excess of thyroid hormones, as it occurs during over-replacement of hypothyroidism (3, 4, 5, 6).

Skeletal fractures occur in a relevant percentage of adult patients with hypopituitarism (7, 8, 9) and growth hormone deficiency (GHD) is generally considered as the most important factor determining skeletal fragility in this clinical context (10). However, most adult GHD patients have other pituitary deficiencies that may influence the
skeletal health in patients with hypopituitarism. We had previously reported a high prevalence of radiological vertebral fractures in hypopituitarism in relationship with untreated GHD (9, 11) and overtreatment of secondary hypoadrenalism (12). However, it is still unclear whether levo-thyroxine (L-T4) treatment may also influence the fracture risk in this clinical setting. As a matter of fact, an overtreatment of secondary hypothyroidism may easily occur in patients with hypopituitarism, as the monitoring of replacement therapy is made difficult by the lack of a good biochemical biomarker of thyroid hormone action. In fact, thyrotropin (TSH) is not useful to determine the adequacy of L-T4 replacement therapy in central hypothyroidism, which is the opposite to what happens in monitoring treatment of primary hypothyroidism (13).

Based on the results of our previous studies (9, 11, 12), we designed a post hoc analysis with the aim to evaluate the association between radiological vertebral fractures and L-T4 dose in patients with central hypothyroidism and coexistent treated and untreated GHD. To avoid potential biases, we restricted the analysis to patients without hypoadrenalism or to those who were treated with glucocorticoid doses lower than those already reported to be associated with a high prevalence of vertebral fractures (12).

**Subjects and methods**

**Subjects**

We retrospectively screened 119 patients with adult-onset hypopituitarism attending our outpatient clinics in the period between 2005 and 2013. Out of these patients, 81 (68.1%) had been already evaluated in previous studies (9, 11, 12). The inclusion criteria were: i) severe GHD, as defined by a peak GH response to a stimulation test $<3$ µg/l (14) and ii) chronic L-T4 treatment (>1 year) for overt hypothyroidism, as defined by serum free T4 (FT4) values below reference ranges regardless of serum TSH values. The daily dose of L-T4 was calculated as the mean of doses over 2 years preceding the study and was expressed per kilogram of body weight measured at the time of study entry. Exclusion criteria were: i) previous diagnosis of Cushing’s disease, acromegaly, and hyperprolactinemia; ii) treatment with daily doses of hydrocortisone or cortisol acetate higher than 28 or 35 mg respectively, which were shown to be associated with a higher prevalence of vertebral fractures in hypopituitary patients (12); iii) treatment with anti-osteoporotic drugs except for calcium and vitamin D; iv) treatment with drugs causing osteoporosis (15) with the exception of those used to replace hypopituitarism; v) prolonged immobilization; vi) trauma; and vii) previous surgical intervention on the spine.

For this study, 74 patients (males, 43; females, 31; median age, 57 years; range, 23–79) meeting the inclusion and exclusion criteria were enrolled. Of them, 69 patients developed hypopituitarism after neurosurgery for non-functioning macroadenoma, whereas five patients were affected by empty sella. At enrollment, 38 patients were on replacement therapy with recombinant human GH, whereas the remaining 36 patients never received this treatment. It was noted that 48 patients had central hypoadrenalism and all of them were on treatment with cortisol acetate or hydrocortisone at the enrollment. Moreover, 49 patients had also hypogonadism and 19 of them received replacement therapy with androgens or estrogens. The remaining 30 patients (males, 12; females, 18; median age, 61; and range, 30–79) had untreated hypogonadism.

The study protocol was approved by the ethics committees and the patients gave informed consent to the study.

**Methods**

BMD of the lumbar spine was measured by dual-energy X-ray absorptiometry (DXA; Lunar Prodigy 8743, GE Medical Systems, Madison, WY, USA). BMD was expressed as g/cm². Fractured vertebrae were excluded from the lumbar BMD analysis.

Vertebral fractures were assessed by a quantitative morphometric approach. In brief, using a translucent digitizer and a cursor, six points were marked on each vertebral body to describe vertebral shape. Anterior (Ha), middle (Hm), and posterior (Hp) vertebral heights were measured and height ratios (Ha/Hp, Hm/Hp, Hp/Hp of the above vertebrae, Hp/Hp of the below vertebrae) were calculated for each vertebra from T4 to L4; the fractures were defined mild, moderate, and severe based on a height ratio decrease of 20–25%, 25–40%, and more than 40% respectively (16).

**Biochemical measurement**

Serum insulin-like growth factor 1 (IGF1) levels were measured by Immulite 2000 (DPC, Los Angeles, CA, USA). Serum FT4 concentrations were measured by the double-antibody RIA (Technogenetics, Milan, Italy); serum TSH was assayed by an immunoradiometric
method (DIA-Sorin, Saluggia, Italy). Normal ranges were 0.3–3.5 mU/l and 7–18 pg/ml for TSH and FT4 respectively. Urinary cortisol was measured using the RIA (Spectria, Orion Diagnostica, Espoo, Finland); in our laboratory, the reference range was 36–137 mg/24 h.

**Statistical analysis**

All data were expressed as the median and range. Unpaired data were compared using the Mann–Whitney U test. Frequencies were compared using the $\chi^2$-test with the Fisher correction, when appropriate. A logistic regression model was used in the statistical analysis of risk factors for the occurrence of vertebral fractures. Statistical significance was assumed when $P$ values were $\leq 0.05$.

**Results**

Radiological vertebral fractures were found in 23 patients (31.1%). Of them, 14 patients had a single fracture, whereas nine patients had two or more vertebral fractures. The fractures were mild in 11 patients, while the remaining 12 patients had moderate or severe fractures.

Fractured patients had more frequently untreated GHD, higher serum FT4 values, longer disease duration and were treated with higher daily doses of $\beta$-T4 as compared with patients who did not have fracture, without significant differences in age, sex, lumbar BMD, prevalence of treated hypoadrenalism and untreated hypogonadism, urinary cortisol values, and serum TSH and IGF1 values (Table 1).

<table>
<thead>
<tr>
<th>No fractures</th>
<th>Fractures</th>
<th>$P$ values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>51</td>
<td>23</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57 (23–79)</td>
<td>59 (25–78)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>21/30</td>
<td>10/13</td>
</tr>
<tr>
<td>Body weight</td>
<td>80 (50–120)</td>
<td>82 (51–126)</td>
</tr>
<tr>
<td>Duration of disease (years)</td>
<td>5.0 (1.0–16.5)</td>
<td>9.7 (1.5–13.0)</td>
</tr>
<tr>
<td>Lumbar BMD (g/cm²)</td>
<td>1.1 (0.60–1.59)</td>
<td>1.0 (0.74–1.45)</td>
</tr>
<tr>
<td>Untreated hypoadrenalism (%)</td>
<td>36 (72.0%)</td>
<td>12 (52.2%)</td>
</tr>
<tr>
<td>Untreated GHD (%)</td>
<td>21 (45.7%)</td>
<td>9 (39.1%)</td>
</tr>
<tr>
<td>Serum TSH (mU/l)</td>
<td>0.34 (&lt;0.005–3.0)</td>
<td>0.23 (&lt;0.005–1.8)</td>
</tr>
<tr>
<td>Serum FT4 (pg/ml)</td>
<td>10.3 (6.7–14.7)</td>
<td>11.6 (9.0–15.0)</td>
</tr>
<tr>
<td>Serum IGF1 (ng/ml)</td>
<td>109 (15–285)</td>
<td>120 (25–270)</td>
</tr>
<tr>
<td>Urinary cortisol values (mg/24 h)</td>
<td>65 (24–110)</td>
<td>70 (19–113)</td>
</tr>
<tr>
<td>Daily dose of $\beta$-T4 (µg/kg per day)</td>
<td>0.96 (0.3–1.9)</td>
<td>1.2 (0.50–2.15)</td>
</tr>
</tbody>
</table>

F, females; M, males; BMD, bone mineral density; GHD, growth hormone deficiency; TSH, thyrotropin; FT4, free thyroxine; IGF1, insulin-like growth factor 1; $\beta$-T4, levo-thyroxyne.

Stratifying the patients for tertiles of serum FT4 values, we found a significantly ($P=0.02$) higher prevalence of vertebral fractures in patients with higher serum FT4 values (third tertile: >12.2 pg/ml) as compared with patients with the lowest values (first tertile: <9.0 pg/ml) (44.4 vs 13.6%; $P=0.02$).

Patients were also stratified for treatment of GHD and tertiles of daily doses of $\beta$-T4 (Fig. 1). As a result of GHD treatment, the prevalence of vertebral fractures was found to be greater in patients receiving high $\beta$-T4 doses (third tertile: >1.35 µg/kg per day) as compared with patients given lower doses (first tertile: <0.93 µg/kg per day). Such a difference was not observed in patients with untreated GHD who showed a higher prevalence of vertebral fractures, all treated vs untreated GHD.

**Figure 1**

Prevalence of radiological vertebral fractures in patients with hypopituitarism, stratified for treatment of growth hormone deficiency (GHD) and tertiles of $\beta$-thyroxine ($\beta$-T4) daily doses. *$P<0.05$ third tertile vs first and second tertiles of $\beta$-T4 doses; **untreated vs treated GHD.
Table 2  Determinants of vertebral fractures in hypopituitary patients with central hypothryroidism. Results of logistic multivariate analysis.

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Odds ratios</th>
<th>95% CI</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum FT4</td>
<td>1.17</td>
<td>0.87–1.69</td>
<td>0.25</td>
</tr>
<tr>
<td>Daily dose of L-T4</td>
<td>4.01</td>
<td>1.16–14.39</td>
<td>0.03</td>
</tr>
<tr>
<td>Untreated GHD</td>
<td>4.27</td>
<td>1.27–14.33</td>
<td>0.01</td>
</tr>
<tr>
<td>Duration of hypopituitarism</td>
<td>1.01</td>
<td>0.99–1.02</td>
<td>0.10</td>
</tr>
<tr>
<td>Age</td>
<td>1.02</td>
<td>0.98–1.06</td>
<td>0.27</td>
</tr>
</tbody>
</table>

GHD, growth hormone deficiency; FT4, free thyroxine; L-T4, levo-thyroxine.

fractures, as compared with treated GHD, regardless of L-T4 daily doses (Fig. 1).

The multivariate analysis showed that untreated GHD and daily dose of L-T4 maintained a significant and independent association with vertebral fractures in patients with central hypothyroidism (Table 2).

Discussion

This cross-sectional study showed an association between high replacement doses of L-T4 and vertebral fractures in patients with central hypothyroidism and coexistent GHD.

Chronic exposure to thyroid hormone excess may increase the risk of fractures (2). Bone resorption and formation are both accelerated in thyrotoxicosis and the remodeling cycle is shortened leading to high-bone-turnover osteoporosis (1). It is noteworthy that the detrimental skeletal effects of thyroid hormones may occur even in patients exposed to mild hormone excess particularly in post-menopausal women and older people, who are already at a higher risk of osteoporosis and fractures (3, 4, 5, 6). In fact, a high incidence of fractures was shown in older patients receiving high doses of L-T4 for treatment of primary hypothyroidism (5). These clinical observations prompted us to investigate the effects of L-T4 treatment on the skeleton in adult patients with hypopituitarism who can be considered as a population particularly predisposed to bone loss and fragility fractures (7, 8, 9, 10). However, the impact of T4 treatment on the skeleton in this specific clinical context is still largely unknown. Moreover, overtreatment of hypopituitarism may occur in some patients because replacement therapies do not completely mirror the endogenous hormonal production and their monitoring is also made difficult by the lack of good biomarkers of their action (12, 17). Specifically, patients with central hypothyroidism may be easily exposed to thyroid hormone excess as TSH is deficient and there are no other reliable markers of peripheral T4 action (13). As a matter of fact, patients with central hypothyroidism may develop complications of thyroid hormone overtreatment, such as osteoporosis and fractures.

Vertebral fractures are the hallmark of osteoporosis. In fact, they are the most common osteoporotic fractures with prevalence estimates of 35–50% among women older than 50 years (18). Only about one-third of vertebral fractures are clinically recognized (19) and the radiological and morphometric approach has emerged as the method of choice for evaluating the true prevalence and incidence of fractures in population studies (20). Using this approach, we already demonstrated a high prevalence of vertebral fractures in patients with hypopituitarism, in close relationship with untreated GHD and overtreatment of central hypoadrenalism (9, 11, 12). This study provides a first evidence for the existence of association between vertebral fractures and L-T4 doses in patients with central hypothyroidism not exposed to overtreatment with glucocorticoids. Indeed, we observed a significant increase in prevalence of vertebral fractures in patients receiving more than 1.3 µg/kg per day of L-T4 as compared with patients treated at lower doses. This cutoff, statistically calculated by subdividing the patients in tertiles, was slightly lower than that already proposed for defined L-T4 overtreatment in patients evaluated for extra-skeletal metabolic outcomes (21, 22, 23). On the other hand, the cutoff found in our patients was comparable with the maximum L-T4 dose recommended for the elderly patients with central hypothyroidism (21), based on the general concept that the detrimental effects of thyroid hormones occur more frequently in the elderly as compared with the young adults (24). However, in our patients, the negative effects of L-T4 excess on the skeleton seemed to be age independent, in agreement with previous observations that both young and aged subjects with hypopituitarism were potentially affected by skeletal fragility (10).

In our study, the association between L-T4 doses and vertebral fractures was observed only in patients with treated GHD, whereas when GHD was not treated the prevalence of vertebral fractures was high regardless of L-T4 doses. We did not investigate the specific mechanisms underlying the variable effects of T4 over-replacement on vertebral fractures in treated vs untreated GHD, but it could be hypothesized that in the latter patients the skeleton was relatively protected by the negative effects of T4 excess due to a blunted peripheral thyroid hormone activation (25, 26). As a matter of fact, GH status is a major determinant of T4 biological effects by stimulating the
deiodination of T4 in active triiodothyronine (T3) (25, 26). This physiological effect could explain why mainly patients with treated GHD were exposed to the negative effects of T4 over-replacement (25). Moreover, l-T4 overtreatment in patients with normal GH status may also be favored by the frequent need for higher doses of l-T4 to normalize serum FT4 in this clinical context (27). It is worthy to be mentioned that serum FT4 levels usually decrease during GH replacement and this trend may prompt the clinician to progressively increase l-T4 dose (28). However, this empirical approach does not consider that GH replacement increases the peripheral activation of T4 to T3 potentially exposing the patients to thyrotoxicosis at tissue level (28). This hypothesis was not proven in our patients, as we did not provide information on serum FT3 values. However, the final results of our study, i.e. the significant association between l-T4 doses and vertebral fractures in treated GHD, seem to further support the notion that target serum T4 levels should be different in GHD treated vs untreated patients (29).

Over the recent years, several studies provided evidence for a direct inhibitory effect of TSH on bone resorption (30). In animal models, the lack of TSH signal was shown to increase bone resorption leading to osteoporosis regardless of the effects of thyroid hormones (31). Also in humans, TSH was clearly shown to exert direct effects on bone remodeling (32) and low TSH levels were found to be associated with a high fracture risk in postmenopausal women with osteopenia or osteoporosis (33). Our study did not allow to clarify whether TSH deficiency may influence the fracture risk in hypopituitarism, as most of our patients had low TSH values and we did not include in the analysis patients without TSH insufficiency.

This study confirms that BMD is a poor predictor of fractures in patients with hypopituitarism (34). The relationship between BMD and risk of fracture is complex. Fracture risk is related to bone strength that is dependent on two main physical/structural factors: quantity and quality. BMD reflects bone quantity but not bone quality that consists of structural and material properties (35).

Over the last few years, there has been growing evidence that both GHD and GH excess (i.e. acromegaly) may affect the quality more than the quantity of the bone, leading to a poor correlation between BMD and fractures (9, 36).

Some limitations of our study merit mention. The cross-sectional design of this post hoc analysis did not allow to investigate the temporal and causal relationship between T4 (over)treatment and fracture risk in patients with hypopituitarism. The results of univariate analysis showing a correlation between duration of disease and vertebral fractures would be suggestive for a time-dependent effect of l-T4 overtreatment of fracture risk in hypopituitary patients, although future prospective studies are needed to clarify this aspect. Moreover, we did not measure biochemical markers of bone turnover, which could have provided reliable information on the change in the bone remodeling process in relation to l-T4 doses in patients with hypopituitarism. Although we excluded from this study patients treated with high doses of hydrocortisone already shown to be associated with a high prevalence of vertebral fractures, we cannot rule out possible overtreatment for central hypoadrenalism in all enrolled patients. In fact, the upper limit of daily hydrocortisone doses (i.e. 28 mg/day) allowed in our patients was chosen based on our previous study that suggested this as threshold dose for predicting fracture risk in hypopituitary patients (12). However, this dose is higher than that generally considered physiological (i.e. ~20 mg/day) (37). Besides this issue, glucocorticoid overtreatment may also be dependent on the degree of peripheral activation of cortisone in cortisol that is greatly influenced by GH (38, 39). Therefore, normal urinary cortisol values in all of our patients did not exclude a relative hydrocortisone overtreatment in some of them, taking into account that, in most cases, the determination of the dose is often empirical and based on clinical judgment (37). Clinical and experimental data suggest that sex steroid deficiency leads to an increase in bone resorption with high bone turnover and osteoporosis (40). These effects may amplify the negative effects of T4 excess on bone remodeling (41), but the small size of study group did not allow to investigate reliably the association between untreated hypogonadism and vertebral fractures in our patients undergoing treatment with l-T4. However, the role of treated and untreated hypogonadism on fracture risk of patients with hypopituitarism is still uncertain, as we observed a high prevalence of vertebral fractures in untreated GHD patients regardless of treatment of hypogonadism in a previous paper (11).

In conclusion, we found an additional risk factor for morphometric vertebral fractures in hypopituitarism, i.e. subtle thyrotoxicosis due to l-T4 overtreatment. Interestingly, this finding was independent of the use of inappropriately high doses of cortisol replacement, as patients were selected in order to avoid the interference of this risk factor known already (12). Therefore, caution should be used in exceeding 1.35 μg/kg per day of l-T4 in the replacement of hypopituitary patients due to potential bone detrimental effects, and particularly in GH-treated subjects.
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.

Funding
This study was partially supported by the Center for Research in Osteoporosis and Bone Metabolism (CROMO), the Glucocorticoid-Induced Osteoporosis Skeletal Endocrinology Group (GIOSEG), the University of Brescia, Italy, and the Italian Ministry for University and Research (MIUR).

References
13 Shimon I, Cohen O, Lubetsky A & Olchovsky D. Thyrotropin suppression by thyroid hormone replacement is correlated with thyroxine level normalization in central hypothyroidism. Thyroid 2002 12 823–827. (doi:10.1089/107072502760339406)


Received 4 February 2014
Revised version received 28 March 2014
Accepted 1 April 2014