REVIEW

Pathophysiology of Growth Hormone Secretion Disorders and Their Impact on Bone Microstructure as Measured by Trabecular Bone Score

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Summary

This article is focused on endocrine-mediated osteoporosis caused by growth hormone (GH) disorders; adult GH deficiency and acromegaly. GH and insulin like growth factor-1 (IGF-1) stimulate linear bone growth through complex hormonal interactions and activates epiphyseal prechondrocytes. GH, *via* receptor activator of nuclear factor-kappaB (RANK), its ligand (RANK-L), and the osteoprotegerin system, stimulates production of osteoprotegerin and its accumulation in bone matrix. Malfunction of this mechanism, could lead to specific bone impairment. However, the primary problem of bone disease in GH secretion disorders is the primary prevention of osteoporotic fractures, so it is important to determine bone quality that better reflects the patient's actual predisposition to fracture. A method estimating bone quality from lumbar spine dual X-ray absorptiometry (DXA) scans is trabecular bone score (TBS). TBS in addition to bone mineral density (BMD) is a promising predictor of the osteoporotic fracture risk in women with postmenopausal osteopenia. In acromegaly TBS better defines risk of fracture because BMD is normal or even increased. TBS helps to monitor the effect of growth hormone therapy. Despite these findings, TBS should not be used alone, but a comprehensive consideration of all fracture risk factors, BMD and bone turnover markers is necessary.

Key words

Growth hormone • Insulin like growth factor-1 • Bone • Bone microstructure • Trabecular bone score

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Secondary endocrine-mediated osteoporosis

Osteoporosis is characterized by low bone mass and microarchitectural deterioration of the skeleton leading to bone fragility and a predisposition to fractures. Secondary osteoporosis is defined as low bone mass with microarchitectural alterations in bone leading to fragility fractures in the presence of an underlying disease or medication. Among osteoporosis population, 30 % of females and up to 80 % of males have secondary cause of osteoporosis contributing to osteoporosis. It is important to exclude secondary osteoporosis in all patients, because the management of the disease may differ (Mirza and Canalis 2015). The most common endocrine diseases associated with osteoporosis are: glucocorticoid-induced osteoporosis, hyperthyroidism, hypogonadism, hyperparathyroidism, diabetes mellitus, growth hormone deficiency and acromegaly. In this review, last two

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conditions and their impact on bone, especially bone microarchitecture, are presented.

Trabecular bone score

Trabecular bone score (TBS) is non-invasive method designed to obtain insight into trabecular micro-architecture based upon pixel gray-level variations in the dual X-ray absorptiometry (DXA) images of lumbar spine. While TBS is indirect measurement, it correlates with micro-computed tomography (μCT) measures in *ex vivo* studies. In humans, TBS is associated with trabecular microarchitecture and bone strength by high resolution peripheral quantitative computed tomography (HRpQCT). TBS is the methodology applied to DXA images that quantify local variations in grey level and differentiate between 3-dimensional (3D) micro-architectures that exhibit the same BMD but different trabecular characteristics. TBS is not a direct physical measurement of trabecular microarchitecture, but rather an overall descriptor of bone quality. A low TBS value is associated with fewer, less well-connected and more widely distributed trabeculae, while high TBS values are correlated with better trabecular structure. To summarize, it is well documented that low TBS is associated with a higher risk of fracture independent of BMD (Hans *et al.* 2011, Boutroy *et al.* 2013, Briot *et al.* 2013, McCloskey *et al.* 2016).

Adult growth hormone deficiency

Growth hormone (GH) has pleiotropic effects. In GH deficiency, replacement therapy reduces cardiovascular morbidity and mortality, improves lipid profiles, changes body composition, and increases quality of life (Rosen *et al.* 1994, Arwert *et al.* 2005, Touskova *et al.* 2016). GH acts on target tissues directly, or *via* insulin-like growth factor 1 (IGF-1), which is produced mostly by hepatocytes. GH treatment of adults with GH deficiency (GHD) increases bone mineral density (BMD) and likely reduces fragility fracture risk (Rota *et al.* 2008, Conway *et al.* 2009, Elbornsson *et al.* 2012b, Appelman-Dijkstra *et al.* 2014, Krantz *et al.* 2015, Mo *et al.* 2015). However, a clear understanding of GHD- induced osteopenia, the effects of this deficiency on bone quality/architecture, and how GH treatment may affect BMD and bone architecture (Barake *et al.* 2014) is lacking. GH stimulates linear bone growth through complex hormonal interactions and activates epiphyseal prechondrocytes. GH, *via* receptor activator of nuclear factor-kappaB (RANK), its ligand (RANK-L), and the osteoprotegerin system, stimulates production of osteoprotegerin and its accumulation in bone matrix. IGF-1 and GH affect the regulation of bone remodeling across the lifespan. The anabolic effects of GH are important to attain peak bone mass, and to achieve appropriate trabecular bone micro-architecture during late adolescence and early adulthood which affects fracture risk later in life. Prior studies demonstrate a positive effect of GH replacement on BMD, bone turnover markers, and fracture risk (Amato *et al.* 1993, Degerblad *et al.* 1995, Biermasz *et al.* 2001, Clanget *et al.* 2001, Davidson *et al.* 2004, Arwert *et al.* 2005, Rota *et al.* 2008, Conway *et al.* 2009, Jorgensen *et al.* 2011, Elbornsson *et al.* 2012a, Elbornsson *et al.* 2012b). In our previous multi-centric prospective study (Kuzma *et al.* 2014) in 147 adults (mean age 35 yrs) with GHD during 24 month of recombinant human GH (rhGH) replacement treatment an effect of rhGH on bone turnover markers, BMD and TBS was assessed. After baseline visit, during the whole treatment, the IGF-1 levels were within the reference range.

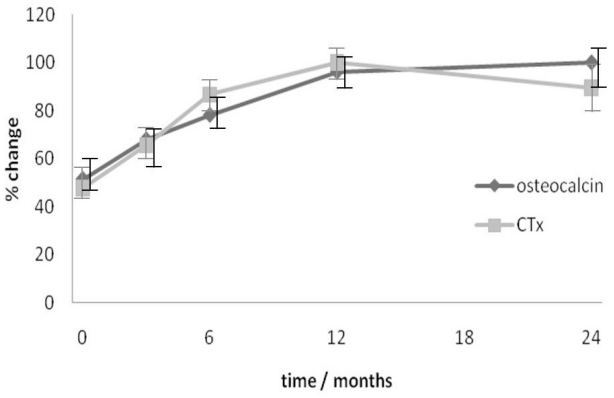

Fig. 1. Bone turnover markers during 24 month rhGH treatment.

The bone markers increased significantly after 2 years of treatment (OC levels (N=98) of 95.8 %; $p<0.001$, CTx (N=95) levels of 82.3 %; $p<0,0001$). During the first year of treatment, both bone markers were increasing. After the first year of rhGH treatment CTx was decreasing in contrast to OC. (Fig. 1). No significant difference in bone turnover markers was observed between genders and onset of GHD. A significant increase in the BMD lumbar spine (L-spine) was observed at month 24 of the treatment (0.88 g/cm^2 vs. 1.02 g/cm^2, increase of 14 %, $p<0.0001$) (Fig. 2). This

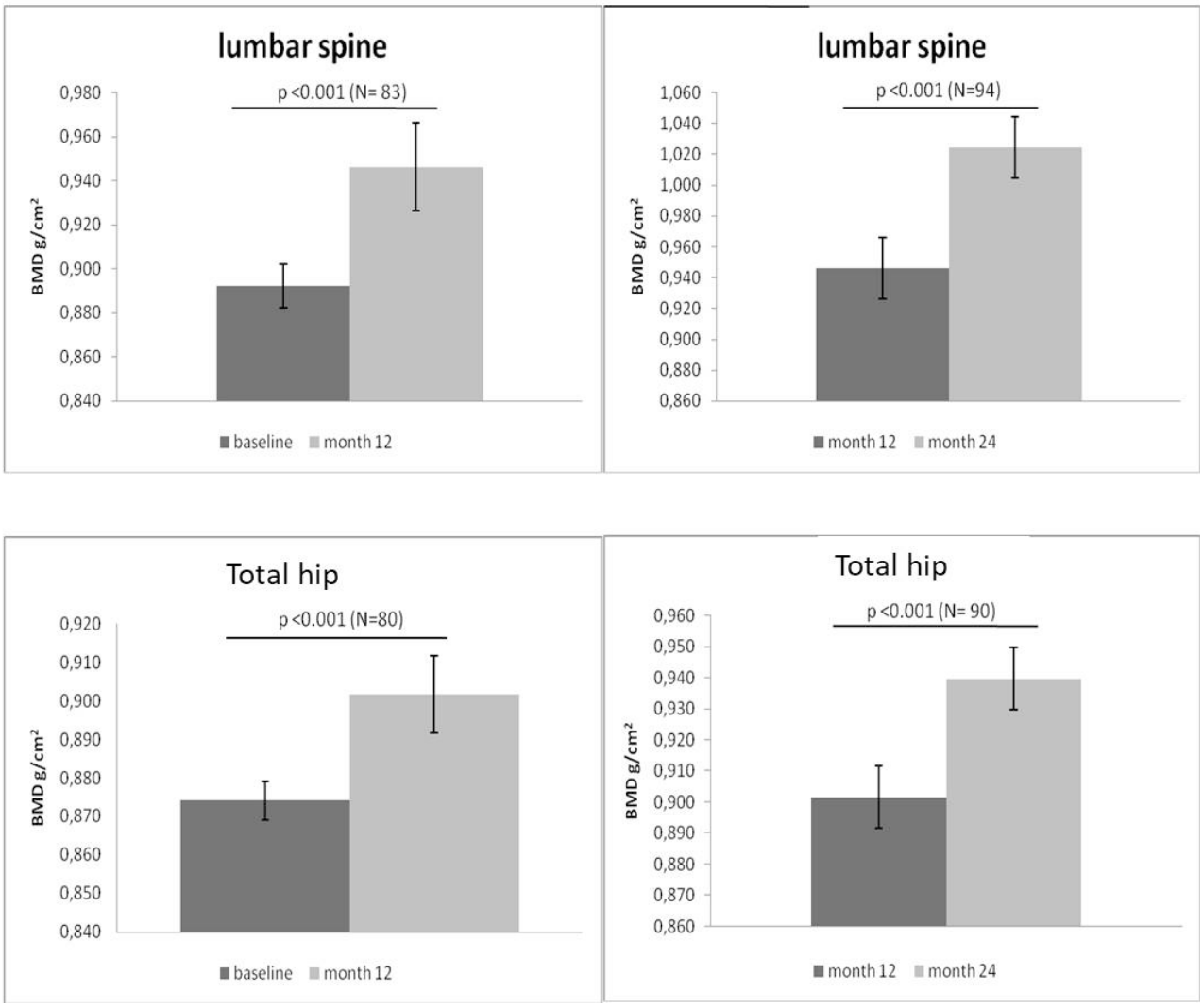

Fig. 2. BMD increase during 24 months of rhGH treatment

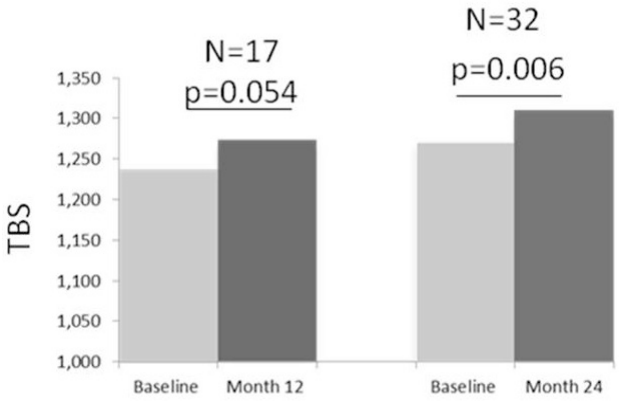

Fig. 3. Increase in TBS in the subset of GHD patients during 24-months of treatment.

increase was significantly higher in male patients compared to females (+15 % in males vs. 10 % in females, p=0.037). In male patients, an initial decrease of BMD was noted. Total hip (TH) BMD after 2 years has increased of 6.7 % (0.86 g/cm^2 at vs. 0.93, p=0.05) (Fig. 1). BMD scans of lumbar spine were analysed in a subgroup (N=32, 19 males, 15 AO-GHD) of patients to obtain TBS. An increase of 4 % (p=0.02) after two years of treatment with rhGH was observed (Fig. 3).

Conclusively, a positive effect of rhGH on bone mass based on the increase of BMD and bone turnover markers was observed. Additionally, TBS increased after 2 years of treatment concordant with positive effect on bone microarchitecture.

There is a potential relationship of vitamin D deficiency with GHD. It is known that IGF-1 stimulates renal 1 α-hydroxylase and may affect bone metabolism by this mechanism (Joseph *et al.* 2008). This fact is supported by analysis of children with rickets, in which serum IGF-1 significantly increased after vitamin D treatment (Soliman *et al.* 2008, Bereket *et al.* 2010). Associations between 25(OH)D and IGF-1 levels have

been found in healthy subjects (Gomez *et al.* 2004, Bogazzi *et al.* 2011) and patients with active acromegaly (Halupczok-Zyla *et al.* 2015). In one retrospective analysis (Ameri *et al.* 2013) was found that GHD subjects with 25(OH)D levels below 15 ng/ml have significantly lower IGF-1 levels than those whose 25(OH)D was above 15 ng/ml. therefore, it is likely that vitamin D affects the bone response to rhGH replacement. Our next study (Kuzma *et al.* 2016) of 57 AO-GHD patients (29 males/28 females, mean age 34.4 years) treated with rhGH during 24 month showed potential beneficial effects of sufficient 25(OH)D3 levels on bone microarchitecture, as measured by TBS. All patients received rhGH in standard IGF-1-normalizing rhGH replacement regimen and were divided according 25(OH)D levels in two groups with cutoff defined as the 50th percentile at each time point of follow up. 25(OH)D3 were measured at baseline and at months 12 and 24 of treatment. Serum 25(OH)D3 was assessed by chromatographic determination on a simple isocratic HPLC system with UV detection. To prevent overt vitamin D deficiency and adverse effect of secondary hyperparathyroidism, patients with vitamin D inadequacy at baseline, defined as a circulating 25(OH)D level <50 nmol/l, received cholecalciferol 800 IU and calcium 1000 mg daily as recommended by International Osteoporosis Foundation (IOF).

In the entire study population after 24 months of GH replacement, LS BMD increased by 7.6 % and TH BMD increased by 4.5 % (both $p<0.05$), no significant effect on TBS in the entire study cohort was observed. There was no difference between TH and LS BMD change after 24 months according to 25(OH)D percentile analysis. However, a significant difference of TBS change from baseline based on vitamin D status was observed. Subjects above 50th percentile of 25(OH)D had TBS increase of +1.39±3.6 % (mean TBS 1.37±0.13) in comparison to TBS decrease of -1.36±5.6 % (mean TBS 1.29±0.15) in subjects whose 25(OH)D was below the 50th percentile ($p< 0.05$) (Fig. 4). A limitation of this study is that a so-called "treat to target" strategy for vitamin D supplementation was not utilized. It is likely that despite provision of a commonly recommended daily cholecalciferol dose, many of our vitamin D insufficient participants likely did not achieve an optimal 25(OH)D status. It is possible that the low 25(OH)D group remained vitamin D deficient despite supplementation thus leading to a decline in TBS despite daily supplementation. Future studies evaluating the relationship of GH treatment with vitamin D status should assure that vitamin D supplement doses do achieve optimal 25(OH)D levels.

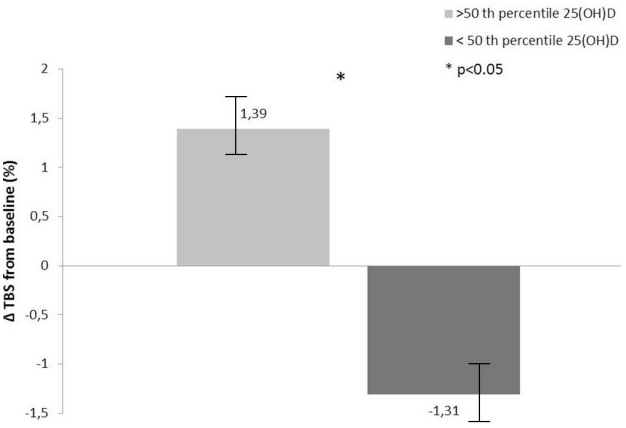

Fig. 4. Change in TBS according to vitamin D levels in patients with treated with rhGH. Patients with 25OHD levels above 50th percentil had significant increase in TBS in comparison to decrease of TBS in those vitamin D levels under 50th percentil.

This study is showing positive effect of GH treatment on BMD with no effect of vitamin D status. However, a differential effect of GH on TBS was observed with a TBS increase only in the cohort with 25(OH)D above the 50th percentile. As such, it is possible that an optimal effect of GH treatment to improve bone quality, represented by TBS, in GHD adults is achieved only in those with sufficient 25(OH)D levels. Further studies are needed to solve this issue.

Acromegaly

Opposite to GHD, it is not surprising that some authors suggested positive effects of GH hypersecretion on bone (Riggs *et al.* 1972, Kayath and Vieira 1997, Vestergaard and Mosekilde 2004). However, several studies find increased prevalence of asymptomatic vertebral fractures in patients with acromegaly, regardless of bone mineral density (BMD) or disease activity (Bonadonna *et al.* 2005, Claessen *et al.* 2013, Mazziotti *et al.* 2013). Patients with acromegaly have elevated bone turnover markers (Stepan *et al.* 1979, Scillitani *et al.* 1997, Ueland *et al.* 2001) but BMD as measured by DXA may not adequately estimate fracture risk (Mazziotti *et al.* 2015). Several quantitative computed tomography studies in acromegaly patients found poorer trabecular bone parameters, such as higher trabecular separation and decreased trabecular number and bone volume to tissue volume, in comparison to healthy controls (Maffezzoni

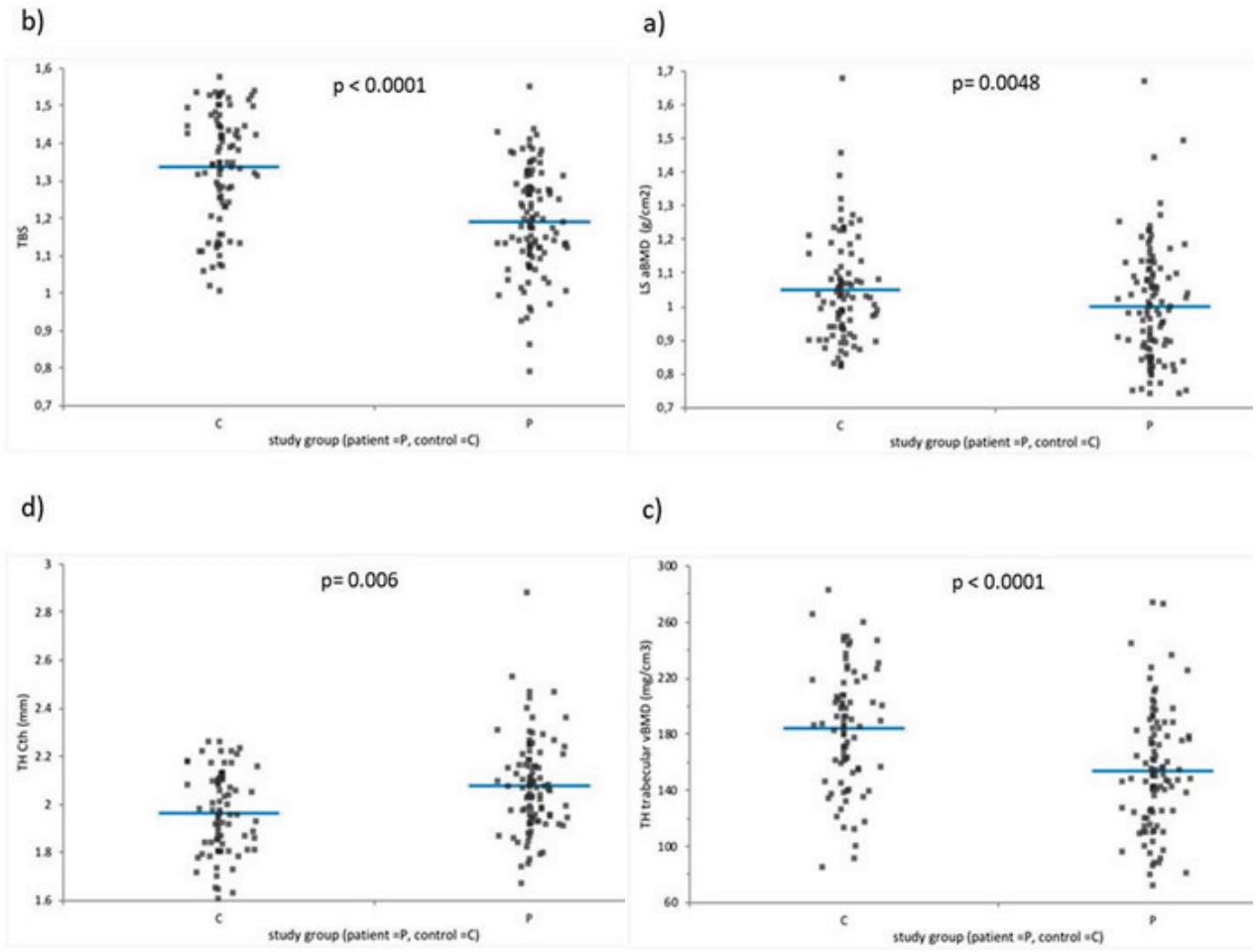

Fig. 5. Comparison of LS aBMD, TBS, TH trabecular vBMD and TH Cth in patients with acromegaly and healthy controls. A lower LS aBMD, TBS and trabecular vBMD at TH and higher Cth was observed in patients with acromegaly in comparison to healthy controls.

et al. 2016). These conclusions are supported by studies with bone microidentation (Malgo *et al.* 2017) and TBS (Godang *et al.* 2016, Hong *et al.* 2016). Finally, bone histomorphometry in acromegaly patients with VFs, showed impaired trabecular parameters (decreased trabecular thickness and increased trabecular separation), in comparison to healthy sex and age-matched adults and also increased cortical thickness and porosity among acromegaly subjects (Carbonare *et al.* 2018).

In our recent cross-sectional study, DXA-derived bone parameters areal BMD (aBMD), trabecular bone score (TBS) and 3D-SHAPER in patients with acromegaly were compared (Kuzma *et al.* 2019). 106 patients with acromegaly (mean age 56.6 years, BMI 30.2 kg/m^2) and 104 control subjects (mean age 54.06 years, BMI 28.4 kg/m^2) were included. Acromegaly patients were heavier, had higher IGF-1 and P1NP levels but lower LH, FSH, TSH, ACTH levels (all $p<0.05$). Considering areal DXA bone parameters, acromegaly patients had significantly lower ($p<0.05$) lumbar spine aBMD and TBS when compared to controls while no differences were observed for aBMD at the femoral neck and total hip. Using the 3D modelling approach, acromegaly patients had thicker cortical thickness (Cth) at both the femoral neck ($p<0.05$) and TH ($p\leq0.001$), but significantly lower Trabecular vBMD at both sites [neck ($p<0.05$) and TH ($p<0.001$)]. After adjustment for weight, LS aBMD, TBS and TH Trabecular vBMD remained significantly lower ($p=0.0048$, <0.0001 and <0.0001 respectively) in acromegaly patients compared with controls. In contrast, Cth (at TH and Neck) remained significantly thicker ($p=0.006$) in acromegaly patients than in controls (Fig. 5).

Considering bone parameters only (2D and 3D), the best multivariate model (Model 1) discriminating patients with and without acromegaly included TBS, TH Trabecular vBMD and TH Cth parameters while aBMD LS and weight were excluded from the model (all $p>0.1$). Model 1 (Fig. 6) was associated with the presence of acromegaly as reported by an OR per SD decrease of 4.85 [3.09-7.62] and an AUC of 0.845 [0.787-0.903].

AUC of model 1 was significantly greater than AUCs of each of the three co-factors included in this model (all $p<0.01$). This study shows that acromegaly patients after multivariate analysis have lower TBS, trabecular vBMD and higher Cth, as measured by a novel method 3D-Shaper, in comparison to healthy controls. TBS showed the best sensitivity to distinguish between acromegaly and non-acromegaly subjects, proving suitability of TBS as the surrogate of bone microarchitecture in this form of secondary osteoporosis.

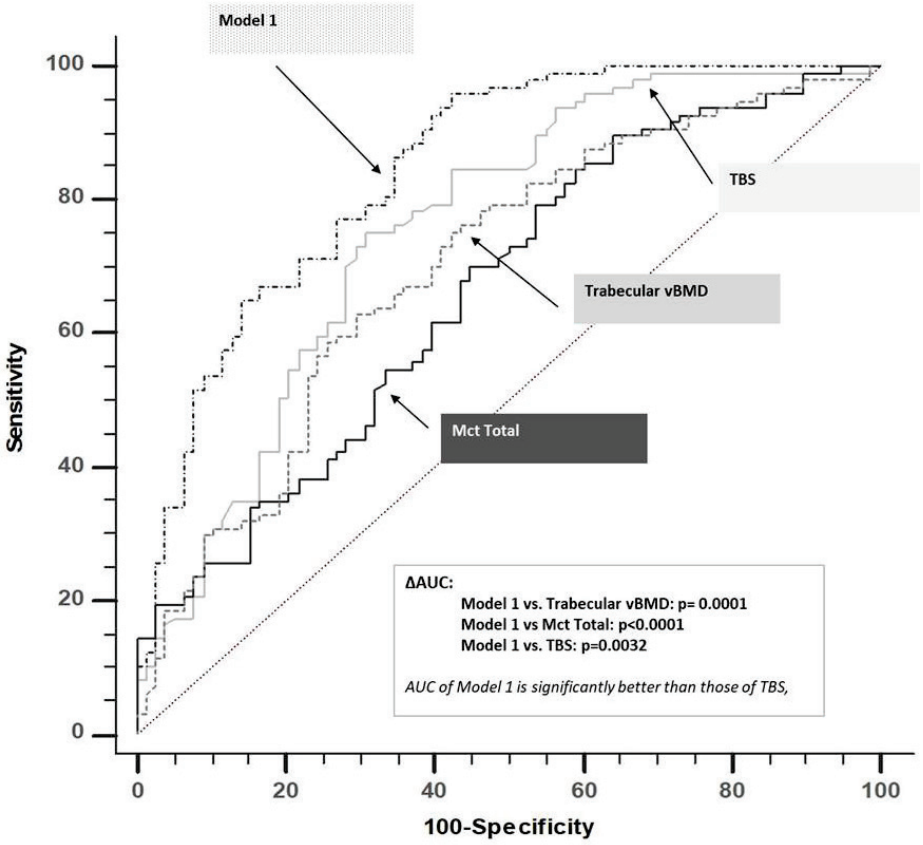

Fig. 6. Considering bone parameters only (2D and 3D), the best multivariate model (Model 1) discriminating patients with and without acromegaly included TBS, TH Trabecular vBMD and TH Cth. However, all three parameters together (Model 1) showed the highest sensitivity and specificity.

Conclusion

Treatment of GHD with GH increases BMD at all sites over time. RhGH has a biphasic effect on bone; represented by initial phase associated with an increase in bone resorption and a decrease in BMD and a second phase characterized by an increase in bone formation and in BMD, usually after 6–12 months of treatment. In our studies, positive effect on BMD and bone microstructure, as assessed by TBS, was observed. Another study shows that positive GH effect on TBS is maintained only when sufficient vitamin D levels are reached. Acromegaly is associated with increased bone remodeling, and significantly higher prevalence of vertebral fractures. Our study shows possibility to use TBS to distinguish acromegaly patients who are at risk of vertebral fractures. According to results of these studies, TBS seems as the suitable surrogate of bone microstructure and thus fracture risk in patients with GH secretion disorders.

Conflict of Interest

There is no conflict of interest.

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