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NOVEL APPROACHES TO EVALUATE BONE QUALITY IN PARATHYROID GLAND DISEASES

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A Nonna Nanda e
a tutte le persone che affrontano
la vita con spontanea curiosità

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STATEMENT OF ORIGINALITY

Unless otherwise stated, the work described in this thesis was carried out at Campus Bio-Medico University of Rome, Italy.

The author designed the studies that are reported in this thesis and/or analyse and described the results.

I hereby state that this thesis entitled “Novel techniques to evaluate bone quality in parathyroid gland diseases” has not been submitted for a degree or any other qualification at any other university.

Gaia Tabacco, March 2023

STATEMENT OF ATTRIBUTION

1) Author's contributions for the following trial: **"*Dxa-based bone strain index: a new tool to evaluate bone quality in primary hyperparathyroidism"***

Conceived and designed the trial: Gaia Tabacco, Anda Mihaela Naciu and Andrea Palermo. *Performed the trial*: Gaia Tabacco, Anda Mihaela Naciu, Stefania Falcone and Andrea Palermo. *Analyzed and interpretated the data*: Gaia Tabacco, Anda Mihaela Naciu, Carmelo Messina, Luca Rinaudo, Roberto Cesareo, Stefania Falcone, Gianfranco Sanson, Nicola Napoli, John P Bilezikian, Fabio M Ulivieri and Andrea Palermo. *Wrote the manuscript:* Gaia Tabacco, Anda Mihaela Naciu, Carmelo Messina, John P Bilezikian, Fabio M Ulivieri and Andrea Palermo.

2) Author's contributions for the following trial: **"*DXA-based bone strain index in normocalcemic primaryhyperparathyroidism"***

Conceived and designed the trial: Gaia Tabacco, Anda Mihaela Naciu and Andrea Palermo. *Performed the trial*: Gaia Tabacco, Anda Mihaela Naciu, and Andrea Palermo. *Analyzed and interpretated the data*: Gaia Tabacco, Anda Mihaela Naciu, Carmelo Messina, Luca Rinaudo, Roberto Cesareo, Gianfranco Sanson, Nicola Napoli, Fabio M Ulivieri and Andrea Palermo. *Wrote the manuscript:* Gaia Tabacco, Anda Mihaela Naciu, Carmelo Messina, Fabio M Ulivieri and Andrea Palermo.

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ABBREVIATIONS

BMI: body mass index

BMD: bone mineral density

BMSi Bone Material Strength index

BSI: bone strain index

CaSR: Ca^{2+}sensing receptor

CKD-EPI: Chronic Kidney Disease–Epidemiology Collaboration

CTX: C-terminal crosslinking telopeptides of type I collagen

ECF: extracellular calcium

eGFR: estimated glomerular filtration rate

FEM: finite element method

FGF23: fibroblast growth factor 23

FHH: familial hypocalciuric hypercalcemia

FN: femoral neck

hypoPT: hypoparathyroidism

HRpQCT: high-resolution peripheral quantitative computed tomography

IDI Indentation Distance Increase (

LS: lumbar spine

MCS: mental component summary

MEN: multiple endocrine neoplasia

microCT: micro-computed tomography

NPT2a: sodium-coupled transporters a

NPT2c: sodium-coupled transporters c

NHPT: normocalcemic hyperparathyroidism

OC: osteocalcin

OPG: osteoprotegerin

P1NP: N-terminal crosslinking propeptides of type I procollagen

PHPT: primary hyperparathyroidism

PKA: protein kinase A

PKC: protein kinase C

PLC: phospholipase C

PTH: parathyroid hormone

PTHR1: PTH receptor

PTHrP: PTH-related peptide

PTX: parathyroidectomy

QOL: quality of life

RANK: receptor activator of nuclear factor kappa B

RANKL: receptor activator of nuclear factor kappa B ligand

RPI: reference Point Indentation

SF-36: 36-Item Short Form Health Survey

SHPT: secondary hyperparathyroidism

TBS: trabecular bone score

TH: total hip

TRAP-5b: tartrate-resistant acid phosphatase 5b

TRPV6: transient receptor potential vanilloid type 6

TSH: thyroid-stimulating hormone

vBMD: volumetric BMD

VFA: vertebral fracture assessment

VFx: vertebral fractures

25(OH)D3: 25-hydroxyvitamin D3

1,25(OH)2D3: 1,25-dihydroxyvitamin D3

CHAPTER 1

GENERAL BACKGROUND

1.1 Bone quality

The term bone quality describes the bone structure that contribute to bone strength independently of BMD. The bone strength is composed by *structural properties* such as geometry, bone size and shape, by *microarchitecture properties* such as trabecular architecture and cortical thickness/porosity and by *material properties* such as mineral-to-matrix ratio, crystal size, collage cross-links(1). All these parts are largely interdependent in a way that an abnormality in one on them will often drive changes in others. The recent attention in bone quality derives from the observations that the traditional gold standard measure of bone strength, the BMD, does not always correctly estimated the fracture risk. The development of tool that can estimate other components other than BMD and bone turnover have increased the interest in this field(2).

The size of bone has an effect on overall fragility. Studies have indeed showed that smaller bone are more fragile. This is true at the vertebral level but also at the hip where femoral strength is partly related to the hip axis length, which is a marker for the ability of the femur to reduce the impact of a fall (1). The microarchitecture plays an important role on overall bone fragility. Trabecular and cortical bone display different microarchitecture features. The trabecular architecture include the orientation, thickness, and spacing of the trabeculae and the connection between trabeculae. The trabecular failure has a central role in bone strength and it can be described as a reduction in trabeculae that are perpendicular to the direction of the load and an overall reduction of trabecular interconnection. The cortical architecture includes cortical thickness and integrity and the cortical failure include the reduction of the thickness and an increase in cortical porosity(1). There are different

techniques that can estimate different components of bone quality (table 1)

Table 1: Assessment of bone quality (2)

Variable	Technique
Bone turnover	Biochemical markers, histomorphometry
Bone microarchitecture	Histomorphometry, µCT, SR-µCT, HR-MRI, pQCT
Bone mineralization	Microradiography, qBSEI, SAXS, spectroscopy
Microdamage	Histology, confocal microscopy
Matrix/mineral composite	FTIR, TEM, SAXS, raman spectroscopy biochemestry

µCT- micro computed tomography, SR synchroton radiation , HR-MRI magnetic resonance imaging, pQCT peripheral computed tomography, qBSEI quantitative backscattered electron imaging, FTIR Fourier Trasform Infrared, TEM transmission electron microscopy, SAXS- small angle X-Ray scattering

1.2 Assessment of bone strength

1.2.1 Dual-energy X-ray absorptiometry

Dual-energy X-ray absorptiometry (DEXA) is a technique able to measure BMD using spectral imaging. DEXA is the most widely used tool to estimate bone strength and is the gold standard to evaluate osteoporosis. In adults, osteoporosis is diagnosed based on a T-score equal to or below -2.5 SD. However, lot of individuals sustain a fragility fractures with a T- score above this cutoff. This can be explained by the fact that DEXA scanners create two-dimensional images of complex three-dimensional structures, and assess the bone density as the quotient of the bone mineral content divided by the bone area. A clear pitfall of this technique is that a larger bone will carry superior strength, but could have actually the same bone density as a smaller bone(3). Furthermore, DEXA does not differentiate between the cortical and trabecular bone compartment and cannot provide additional information on bone microarchitecture(4). To overcome the DEXA

limitations, other tools have been developed to better estimate the bone strength and they can be divided in two main group: Dexa-derived tool namely trabecular bone score (TBS) and Bone strain index (BSI) and non dexa-derived tool such as high-resolution peripheral quantitative computed tomography (HRpQCT).

1.2.2 Trabecular Bone Score

TBS has emerged as a novel grey-level texture measurement that, using variograms of 2D projection images, estimates the variation in grey-level texture from one pixel to the adjacent pixels. TBS is not an indirect measurement of bone microarchitecture reflecting the trabecular number, the trabecular separation and the connectivity density(4)**.** TBS is calculated by re-analysis of lumbar spine DXA images and can be also calculated retrospectively. In recent years, there has been growing interest in the use of TBS, as a surrogate of bone microarchitecture for the stratification of fracture risk. Studies demonstrated that low TBS is associated with both a history of fracture and the incidence of new one, independently by aBMD. That means that TBS has sufficient power to enhance risk fracture stratification of Dexa. The prospective data are still not conclusive and further studies are needed to clarify if change in TBS can predict fracture risk reduction. Other studies demonstrated that TBS may play a role in secondary osteoporosis such as glucocorticoid excess, hyperparathyroidism and type-2 diabetes(4). The TBS meets the need for a noninvasive method for evaluate bone microarchitecture, however it does not take into account all necessary information to evaluate the resistance of bone to loads, is an indirect measure and it is limited only to lumbar site(5).

1.2.3 Bone Strain Index

The Bone strain index is a brand new DXA-derived index, calculated with Finite Element Analysis (FEA) on a greyscale of the distribution of density measured on both spine and femoral scans(6). Comparing to BMD and TBS, BSI calculation includes information on density distribution, bone geometry and resistance to loadings on local areas. In addition, BSI also includes data on geometry and

specific-conditions load applied to the bone according to patient's weight. BSI uses the finite element method (FEM). FEM consists of dividing an object into simpler parts and apply to them the laws of classical mechanics. Forces and constraints applied to specific regions of bone, determinate internal stresses and strains, that are depend by on the magnitude and the type of the force, the bone geometry and the stiffness of each simple part in which the bone has been divided. In the BSI, FEM analysis is automatically calculated by placing forces and constraints on a triangular mesh resulting from bone segmentation on DXA images. At the lumbar spine, each vertebra is loaded on the upper surface and constrained to the lower, according to Colombo et al. (7). At the femur, the BSI algorithm is based on a premise of lateral fall, with constraints placed both on the head and the lower part of the shaft and with a subject-specific impact force related to the weight of the person that is applied to the greater trochanter(6).

The software provides a graphic representation of the deformation index with a color map follows a ramp from blue (low strain) to green (intermediate strain), yellow, and red (high strain) and also a numeric value indicating an increase of the risk fracture proportionated to the increase of the strain (8). So far, clinical studies showed promising results of the ability of BSI to correctly stratify subject at high risk of fracture in primary and secondary osteoporosis(6).

1.2.4 HRpQCT

High-resolution peripheral quantitative computed tomography (HR-pQCT) has arisen as a noninvasive imaging technique with an isotropic voxel size of 82 μm (I generation) or 61 μm (II generation), that consents the assessment of volumetric bone density and microarchitecture of cortical and trabecular bone compartments. The principal information given by HRpQCT are summarized in table 2.

Table 2: principal parameters measured by HRpQCT adapted from (4)

Parameter (abbreviation)	Description	Units
Volumetric bone mineral density (vBMD) measures		
1. Total (Tt.vBMD)	Total volumetric density	mg HA/cm^3
2. Cortical (Ct.vBMD)	Cortical volumetric density	mg HA/cm^3
3. Trabecular (Tb.vBMD)	Trabecular volumetric density	mg HA/cm^3
Cortical (Ct.) measures		
4. Area (Ct.Ar)	Mean area occupied by cortical bone	mm^2
5. Thickness (Ct.Th)	Mean cortical thickness, calculated directly or indirectly as ratio of cortical bone volume to outer bone surface	mm
6. Porosity (Ct.Po)	Cortical porosity, calculated using void-voxel or density-based method	%
Trabecular (Tb.) measures		
7. Thickness (Tb.Th)	Mean thickness of trabeculae	mm
8. Number (Tb.N)	Mean number of trabeculae per unit length	mm^{-1}
9. Separation (Tb.Sp)	Mean distance between trabeculae	mm
Finite-element analysis (FEA) measures		
10. Stiffness	Whole bone stiffness	N/mm
11. Failure load	Estimated maximum load	N

To date, HR-pQCT represents the gold standard for the non-invasive evaluation of bone quality and studies showed its ability for fracture-risk prediction(4). Despite the promising results this tool is still used only for researcher purpose due to the high costs, and there are some concerns if the sites evaluated by the machine (radius and tibia) are informative about clinically important sites such as spine or hip.(4)

1.3 Calcium

Calcium in one of the most common elements in the body. Almost the totality of calcium (99%) is deposited in the skeleton as hydroxyapatite crystal [Ca10(PO4)6(OH)2], allowing the mechanical weight-bearing properties of bones. Soft tissues, blood and extracellular fluid contain about 1% of the remaining "non-bone" calcium. The 50% of this "non-bone" calcium is free in circulation as ionized calcium (the biological active form), while 40% is linked to proteins (mainly albumin and also globulins) and 10% is complexed to ions (i.e. bicarbonate, calcium phosphate, lactate, and calcium citrate) (9,10).While the bone represents a source of calcium to carry on some biological process and to maintain blood ionized calcium levels, non-bone calcium is crucial for vascular and muscle functions, intracellular signaling, glycogen metabolism, nerve transmission and hormone secretion(11). The body maintains the extracellular calcium level (ECF) within a rather narrow range to guarantee the numerous cellular mechanisms that calcium regulates (12).

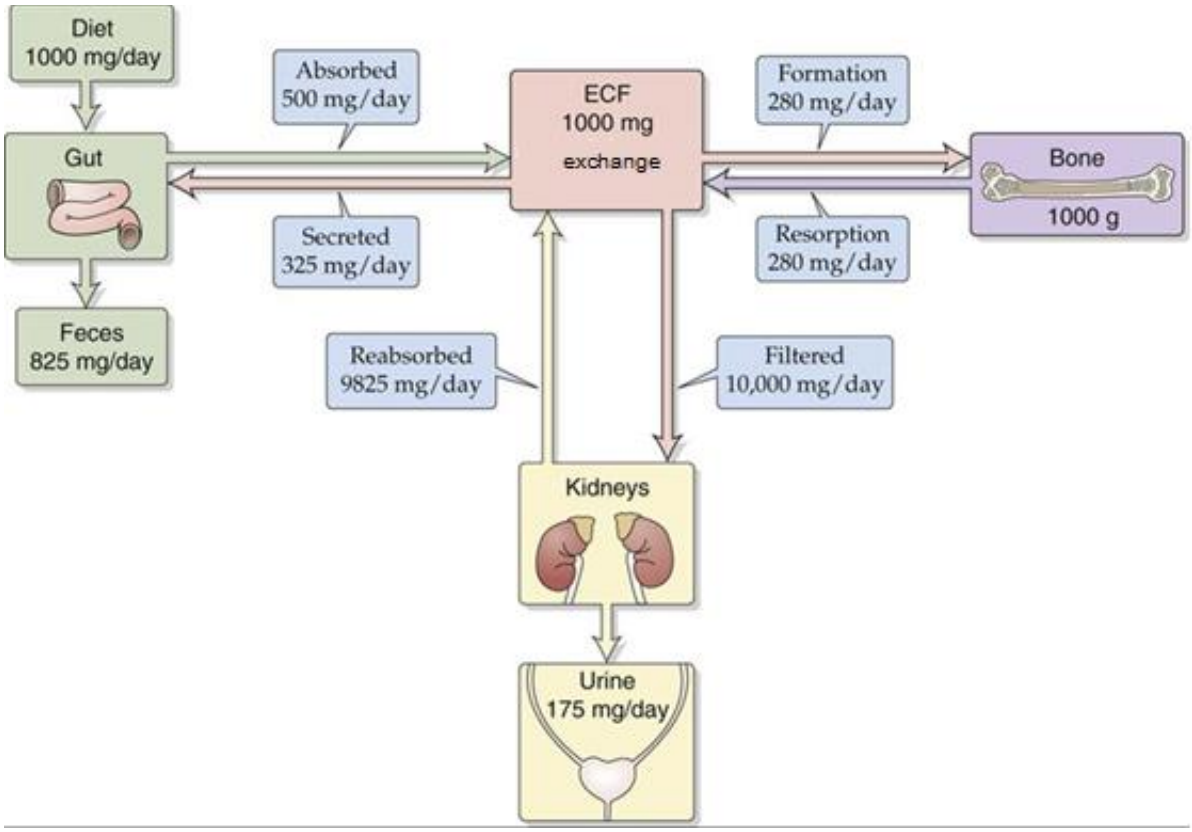
Even if only the ionized calcium is the biologically active calcium, in routine clinical practice, the total serum calcium is normally measured. The normal range of total serum calcium in healthy subjects is between 8.4 and 10.2 mg/dL (2.12 to 2.62 mmol/L). The normal levels of ionized calcium are 4.65–5.25 mg/dL (1.16–1.31 mmol/L). All the condition that affects albumin may also affect the estimation of serum calcium concentration. Indeed, in this case is recommend to measure directly the ionized calcium that remain quite stable. Otherwise it is possible to "correct" the total calcium level to the current albumin levels with some formulas. One of the most common used is the following formula: corrected calcium (mg/ dL) = measured total calcium (mg/dL) + 0.8 (4.0 – serum albumin [g/dL])(13).

The main source of calcium is the diet and currently the dietary recommended calcium intake is between 800 and 1200 mg/day (14). The "fractional calcium absorption" is the rate of calcium absorbed by the intestine, and it is estimated to be around 20-30% of the total intake (15). In healthy young adults, 175 mg out of 1000

mg calcium that we should ingest, it will be absorbed and it will be part of the exchanging pool (plasma, bone, cells). The remain part of calcium is excreted in the faeces (75%) (16). The calcium balance changes during life: it is positive during skeletal growth in children, zero in adults, and negative in aging. The kidney regulates the balance under hormonal controls, and so in adults, it will excrete 175 mg of calcium in the urine per day, neutralizing the gut intake. The strict regulation of calcium absorption and excretion keeps the extracellular ionized calcium in the normal range thanks to exchange of calcium to and from essential stores (10).

The skeleton is principal storage site for calcium (1000 g of calcium) and with gut and kidney regulates calcium homeostasis. The bone regulates calcium homeostasis thanks to the bone turnover. Bone turnover delivers about 500 mg of calcium to blood from the bone, while the same amount of calcium is deposited by bone from the blood maintaining the neutral balance. (Figure 1).

Figure 1: Calcium homeostasis adapted from (17)



The regulation of the ECF calcium concentrations is under control of calcium-sensitive cells that control the hormone production and release (18). The decrease in ECF is sensed by the CaSR at the parathyroid gland level, resulting is a release of PTH. PTH acts on bone resulting in the release of both calcium and phosphate from the skeleton, and in the same time stimulates calcium reabsorption in the kidney and inhibits phosphate reabsorption increasing phosphaturia with the net results of an increase on calcium levels. Both hypocalcemia and PTH elevation determine the conversion of the 25-hydroxyvitamin D3 (25(OH)D3), to the active form of vitamin D 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) (18), that increases the intestinal absorption of calcium. This integrated hormonal mechanism restores serum calcium levels and closes the negative feedback loop (10,15).

Intestinal epithelial calcium transport consist of two mechanisms, an active and saturable process that is under control of 1,25(OH)2D3, and a passive, paracellular way of absorption that is not saturable and it is under control of luminal calcium concentration. The amount of calcium absorbed at this point is influenced by current calcium and active vitamin D levels, indeed. However, other factors such as age, gender, race, lactation, pregnancy and diseases can affect calcium absorption. All together these factors are responsible for a variation in intestine calcium absorption from 20 to 60%.

Normally, in the large surface area of the duodenum and jejunum about 90% of calcium is absorbed passively. Increased calcium demand stimulates the expression of the epithelial calcium active transport system in the duodenum, ileum, and throughout the colon able to improve fractional calcium absorption from 20 to 70%. On the other hand, reduced dietary calcium intake can increase PTH secretion and consequently 1,25(OH)2D3 production. 1,25(OH)2D3 stimulates the expression of the transient receptor potential vanilloid type 6 (TRPV6), an apical calcium channel that permits the entrance of calcium into the enterocyte. Once in the cell, it seems that the calcium-binding protein calbindin-D9k helps the diffusion of calcium in the

cytoplasm where it is released at the basolateral side through the intestinal plasma membrane pump PMCA1b (19). High dietary calcium intake stimulates 1,25(OH)2D3 suppression, and passive transport accounts for most all absorption (20).

1.4 PTH and mineral homeostasis

PTH is an 84-amino acid peptide and is the major regulator of blood calcium levels. Avoiding major fluctuation in serum calcium levels is essential for the body so an of homeostatic systems ensuring maintenance of calcium in a physiologic narrow way is vital (21,22). The CaSR in parathyroid cell detects small variations in Ca^{2+} (≈ 1–2%) with the effect to regulate accordingly the PTH secretion(23). PTH regulates serum calcium by different mechanisms: (i) stimulate the release of calcium and phosphorus from the bone thanks to the indirect activation of osteoclasts (24,25); (ii) stimulates the renal synthesis of 1,25(OH)2 D3 from 25(OH)D3 in the proximal tubule that increase intestine calcium absorption; (iii) decreases the renal calcium excretion (26); finally (iv) stimulates renal phosphate excretion. This last PTH-action explains the hypo- and hyperphosphatemia in subjects with hyper- or hypoparathyroidism (27), respectively.

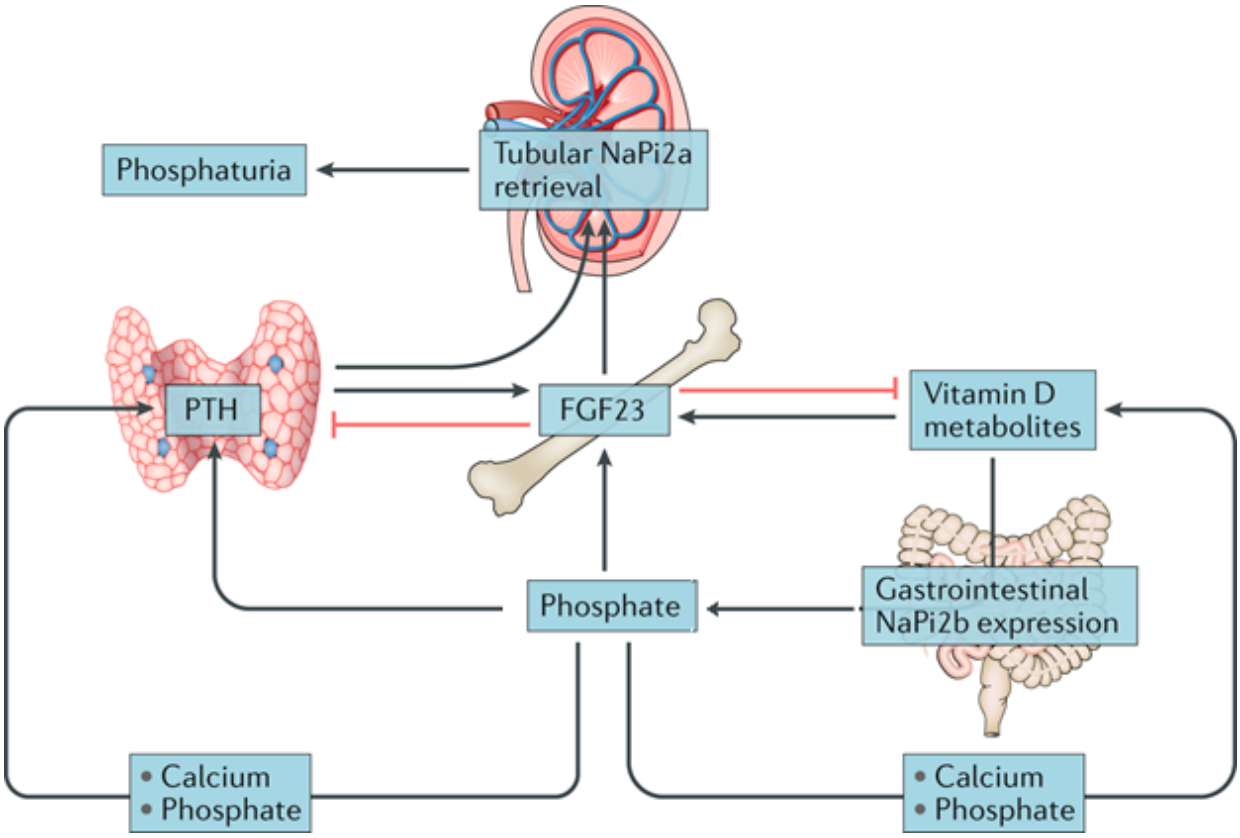
Briefly, if CaSR senses high calcium levels it will reduce PTH secretion thus increasing renal calcium excretion (28). On the other hand, if CaSR senses low calcium levels it will release PTH with increase in renal calcium excretion, and an increased release of calcium from bones (28). Other factors have an impact on PTH levels, such as epinephrine, calcitonin, vitamin D, magnesium, and phosphate (29).

During the day, the release of PTH follows the change of calcium, phosphate and calcitriol (30). About 20% to 30% of PTH secretion is pulsatile with a peak in the first hours of the day, a nadir in the late morning and a second, lower peak in the afternoon, (30). A chronic decreases in serum calcium will results in chronic secretion of PTH (31). The parathyroid glands, indeed, have the capacity to increase their mass by 10–100-fold or more under chronic stimulus as for example in patients with chronic renal insufficiency.

An opponent to the action of PTH and another regulator of phosphate and, to a little extend, also calcium metabolism is the hormone called fibroblast growth factor 23 (FGF23). FGF23 is secreted by osteocytes, and seems to have an inhibitory effect

on PTH production and secretion during normocalcemia. During hypocalcemia, on the opposite is the FGF23 that is inhibited (32). FGF23, as PTH, exerts phosphaturic effect on the kidney (inhibits NaPi-II cotransporters) but, contrary to PTH, inhibits the renal production of 1,25(OH)2D3(Figure 2)(33)

Figure 2 Calcium and phosphorus homeostasis adapted from(33)



1.5 PTH action on skeleton

The effect of PTH on bone starts with the interaction with a specific receptor: PTH type 1 receptor (PTHR1), a member of the G protein–coupled transmembrane receptor family B, mainly expressed in bone but also kidney and cartilage. PTHR1 is a member of the family G-protein-coupled receptor (GPCR)(34) and PTH/PTHR1 interaction stimulates four different intracellular signalling cascades: i) GαS-adenylyl cyclase-cAMP-protein kinase A (PKA); ii) Gαq-phospholipase C (PLC) β-inositol triphosphate-cytoplasmic Ca2+-protein kinase C,18; iii) Gα12/13-hospholipase D-transforming protein RhoA19 a; and iv) β-arrestin-extracellular signal-regulated kinase 1/2 (ERK1/2). Osteoblasts are the target cells of PTH action and the intermittent or chronic simulation of PTHR1 at this level accounts for the different response observed at skeletal level(35).

Specifically, in presence of a chronic increase of serum PTH levels, as for instance during PHPT, the stimulation of PTHR1 will determine a catabolic effect of PTH on bone. The OPG–RANKL–RANK pathway plays an important role in PTH-induced bone resorption. Specifically, the prolonged PTH/PTHR1 interaction determines the transcription of RANKL and on the other hands, the reduction of its decoy receptor, OPG, with a net catabolic effect(36). In vitro e in vivo studies confirmed that the activation of osteoclastogenesis indirectly occurs through the "hyperstimulation" of osteoblasts.

The anabolic effect on the skeleton by PTH is instead seen when PTH/PTHR1 interaction is intermittent. The anabolic effect of PTH is due to an increase in bone formation secondary to an increase in osteoblastogenesis, a reduction of osteoblast apoptosis, and activation of quiescent lining cells as shown by histological studies(37). Moreover, the intermitted PTH stimulates bone formation also through the canonical Wnt/β-catenin pathway by increasing β-catenin levels(38). Finally, PTH is a known inhibitor of sclerostin, a protein encoded by SOST gene, a Runx2 target gene. Sclerostin is a glycoprotein that inhibit bone formation (39). Thus, the

inhibition of sclerostin represents an essential PTH-mediated action enabling the increase of bone formation(40,41). In the last decade exogenous PTH(1–34) (42) and PTH(1–84) (43) have been used to treat subjects with osteoporosis. The intermittent daily administration of PTH peptides have demonstrated to improve BMD and bone microarchitecture (44). The effects on trabecular bone with the use of both PTH(1–84) and PTH(1–34) treatment are confirmed by the reduction of vertebral fractures (VFx). PTH(1–34) has also demonstrated to diminish extra-VFx (42). The intermittent administration of PTH at the beginning stimulates bone formation and subsequently also bone resorption (45). This phenomenon is called the “anabolic window” (46).

1.6 PTH action on kidney

PTH regulates calcium and phosphate transport, vitamin D biosynthesis and degradation at the kidney level. Thanks to Fuller Albright and his research team, it has been discovered that the principal effect of PTH at this level is to determine a rapid increase of phosphate excretion. PTH increases calcium and magnesium absorption, while inhibits phosphate, bicarbonate, sodium and potassium absorption.

The renal phosphate transport occurs mainly in the proximal tubules, where two sodium-coupled transporters NPT2a (SLC34A1)1 and NPT2c (SLC34A3) regulates uptake of phosphate from luminal fluid. PTH down-regulates NPT2a and, to a minor extent, NPT2c resulting in an inhibition of renal phosphate transport. The renal tubule reabsorbs approximately 97.5% of calcium. Interestingly, the majority of calcium absorption occurs in proximal tubules (47), however, PTH exerts its effect on calcium absorption at distal nephron sites. Calcium reabsorption in proximal tubules is passive, thanks to the osmotic driving force of active sodium transport that permits the paracellular calcium mobility by creating a concentration gradient for calcium diffusion. PTH regulates the active calcium absorption via a cellular transport mechanism (47,48). The effect are mediated by PTH1R, (49), abundantly expressed throughout the renal tubule (50). PTHR1 regulates TRVP5 (51) on the luminal membrane of the distal nephron, (52), and determines calcium influx indirectly thanks to the calcium-sensing protein calmodulin (53). Calcium influx inactivates channel transports avoiding excessive calcium absorption. In the kidney, PTH also regulates the production of active form of vitamin D: calcitriol. Calcitriol is produced after the sequential hydroxylation of cholecalciferol in the liver by the 25-vitamin D hydroxylase (CYP2R1) and then 1α -hydroxylation by 25-hydroxyvitamin D3 1α -hydroxylase (CYP27B1) in the kidney. This last step happens in proximal tubules and is highly stimulated by PTH. 1,25(OH)2D3 is degraded to 24,25(OH)2D3, an inactive metabolite, via side-chain oxidation regulated by 1,25(OH)2D3 -24-hydroxylase (CYP24A1), and this process is also

regulates by PTH action (54). Dietary calcium, phosphate, FGF3 and 1,25(OH)2D3 itself regulates the production of vitamin D by inhibit the 1α –hydroxylase (55), and by increases CYP24, and finally by reducing PTH transcription (56), in a complex and perfectly regulated negative feedback loop.

1.7 Hypoparathyroidism

Hypoparathyroidism (hypoPT) is characterized by low serum calcium and low or inappropriately low-normal serum PTH. HypoPT is a rare disease and can be divided in two main forms: acquired or inherited. The acquired form is more common (75% of cases) and derives from the accidental removal or damage of parathyroid glands during neck surgery for thyroid or parathyroid diseases and it is called, indeed, post-surgical. After that, the second cause in adults is the autoimmune disease, involving either the parathyroid glands, or multiple other endocrine glands. Different rare infiltrative disorders, metastatic disease, ionizing radiation exposure, or rare diseases explain the residual cases (57).

The prevalence of the disease is different from country to country. In the United States, it has been estimated to be 37/100,000 (58,59). In Italy, the prevalence ranges from 5.3 to 27 per 100,000 (60,61). In Denmark, it is around 22/100,000 (62,63) and it is less frequent in other countries(64). The prevalence is different if we divide the postsurgical and genetic forms. The incidence of postsurgical temporary hypoparathyroidism has been estimated to range 25.4–83% (65) and permanent hypoparathyroidism range from 0.12 to 4.6% of cases (66). Autoimmune isolated hypoparathyroidism is quite rare with an estimated prevalence of 3.8% (67). This autoimmune form can be part of APS-1 (67). The worldwide prevalence of APS-1 is estimated 1/1,000,000, but the prevalence is more frequent in: Finns (1:25,000), Sardinians 1:14,500 in, Iranian Jews 1:9000(68). However, even though the variability within and between countries, hypoparathyroidism is a rare disorder of calcium homeostasis.

1.7.1 Post-surgical hypoparathyroidism

Post-surgical hypoparathyroidism is characterized by an inadequate production of PTH after surgery. The most common neck surgeries that are associated with risk of post-surgical HypoPT are total or subtotal thyroidectomy and parathyroid surgery for the removal of a single or different parathyroid adenomas. The rate of thyroid

surgery is, of course, greater than parathyroid surgery. Whatever is the reason for surgery, hypoPT can start early after operation, between hours or 1-2 days in a transient form that recover within 6 months after surgery, or permanent or chronic if persists after 6-12 months. Thyroid surgery represents a risk for parathyroid glands for different reasons: parathyroid gland can be accidentally removed or damaged during electrocoagulation or indirectly damaged after ligation of thyroid arteries if also the parathyroid arteries are inadvertently closed. In order to avoid these problems during surgery the parathyroid glands must be identified at the time of thyroidectomy. To guarantee their blood supply, it is important to ligate thyroid vessels as far as possible from of parathyroid arteries(69). The type of surgery also influences the risk of post-surgical hypoPT. The need of radical neck lymph node dissections, basedow disease and repeated surgery increase the risk (70).

After surgery for parathyroid diseases, hypoPT is typically transient. Indeed, if PHPT is severe, after the removal of the hyperfunctionally gland the remaining gland may require a short period of time before recover the full functional activity. (71). Chronic HypoPT after parathyroid surgery is more common if more than one parathyroid glands are removed.

Clinical presentation. The hallmark of the disease is the hypocalcemia, defined by a low circulation ionized serum calcium level. The symptoms of hypocalcemia are related to the neuromuscular irritability and the degree of symptoms depends on the actual calcium levels and how quickly the calcium drops. If the decrease in serum calcium is mild, hypocalcemia may be asymptomatic. Typically, acute hypocalcemia is symptomatic and it starts with numbness and tingling in the fingertips and buccal region, followed by paresthesias in the extremities. Then symptoms can evolve in signs of tetany such as carpal or pedal spasm as for example the Trousseau sign. (72)

In order to clinical assess the hypocalcemia, it can be evaluated the Chvostek sign, that consists of a contraction of facial muscles after light percussion on the facial

nerve near its outlet close to the external auditory meatus. Severe acute hypocalcemia may evolve in broncho- and laryngospasm, cardiologic alterations i.e. prolonged QT interval on electrocardiogram and rarely arrhythmias or congestive heart failure (73).

After neck surgery it is recommend to check the albumin-corrected serum calcium or ionized calcium represent to assess postsurgical hypocalcemia. Intact PTH and phosphate measurement can further establish parathyroid gland function after surgery (74). The onset of surgical hypoparathyroidism is responsible for late discharge after neck surgery. Accurate surgical techniques, as for instance, minimally invasive surgery (75) and the use of local or loco-regional anesthesia (76), represent the best way to prevent hypocalcemia. However, it cannot be sufficient. Measuring PTH serum levels during surgery or immediately thereafter represent a reliable way to predict the onset of surgical hypoparathyroidism (72).

1.7.2 Complications

The principal complications associated with hypoPT are reported in table 3

Table 3: Principal complications of Hypoparathyroisim adapted from (77).

Complication/symptom	Number of patients/controls	Crude OR (95% CI)	Adjusted HR/OR (95% CI)
Nephrocalcinosis/nephrolithiasis	**9414/45,463**	**2.63 (2.29–3.01)**	**1.88 (1.68–2.12)**
Renal insufficiency	**9264/45,253**	**6.22 (5.74–6.74)**	**3.67 (2.44–5.52)**
Cataract	**1466/6074**	**2.08 (1.66–2.61)**	**2.13 (1.65–2.75)**
Seizures	**1500/6406**	**2.83 (2.26–3.53)**	**3.22 (2.51–4.11)**
Arrhythmia	**1078/4679**	**1.62 (1.23–2.12)**	**1.37(1.05–1.79)**
Ischemic heart disease	**1078/4679**	**1.55 (1.24–1.94)**	**1.26 (1.02–1.56)**
Depression	**1140/4749**	**2.21 (1.69–2.89)**	**1.89 (1.37–2.61)**
Infection	**9245/44,390**	**1.96 (1.82–2.11)**	**2.30 (1.75–3.02)**
All-cause mortality	**1358/5980**	**1.47 (1.25–1.74)**	**1.80 (1.49–2.17)**
Anxiety	930/2674	2.64 (1.46–4.78)	1.42 (0.26–7.76)
Any fracture	1545/6118	1.20 (1.01–1.42)	1.05 (0.72–1.53)
Vertebra fracture	1248/4800	1.95 (1.35–2.82)	1.25 (0.43–3.61)
Stroke	1078/4679	1.49 (1.09–2.02)	1.31 (0.97–1.76)
Myocardial infarct	1078/4679	1.18 (0.77–1.81)	0.98 (0.64–1.51)
Upper extremities fracture	1078/4679	1.28 (0.95–1.74)	–
Lower extremities fracture	1078/4679	1.35 (0.92–1.98)	–
Humerus or wrist fracture	1078/4679	0.91 (0.58–1.41)	–
Intracranial calcification	391/2515	5.92 (3.62–9.67)	–
Neuropsychiatric disease	918/2644	1.69 (1.37–2.08)	–

Kidney is particularly affected during hypoPT for two main principal reasons. First,

the lack of PTH action in the distal renal tubule determines an increase in the glomerular filtered calcium leading to hypercalciuria (65). Second, the treatment with high dose of calcium increases the load of calcium filtered by kidney. Hypercalciuria may be followed by nephrolithiasis, nephrocalcinosis, and renal insufficiency (78). The evidences on ability of replacement therapy to reduce urinary calcium excretion are not conclusive to date, yet PTH 1-84 demonstrated the ability to prevent renal insufficiency over time(77).

Cataracts is a well known complication of both postsurgical (55%) (79)and idiopathic hypoparathyroidism (41–51%)(80). Typically it is posterior and begin in the periphery of the lens.

Another typical complication of hypoPT is the basal ganglia calcification and it is associated with longer duration and higher serum phosphorus levels (81). Usually this manifestation is asymptomatic but rarely can be associated with cognitive impairment (82).

Another typical findings in subject with HypoPT is the reduced quality of life (QOL) independently from serum calcium levels (83,84). The QoL has been evaluated with the generic 36-Item Short Form Health Survey (SF-36), (57,83) that showed a reduction in both physical and mental domains of QoL. The replacement treatment with PTH analogues has been associated with amelioration of QoL score in some but not all studies(85–87).

1.7.3 Skeletal involvement

Chronic hypoPT is associated with low bone turnover state. Serum markers of bone formation such as procollagen type 1 amino-terminal propeptide (P1NP), osteocalcin (OC), or bone-specific alkaline phosphatase (BAP) and markers of bone resorption such as tartrate-resistant acid phosphatase 5b (TRAP-5b) and serum C-telopeptide (CTX) are typically low or in the lower half part of normal range(88–90). In addition, Rubin et al. reported also low numbers of circulating osteogenic

precursor cells (91) and an increase in sclerostin levels (92).

This low bone turnover state reflects the BMD above average compared to age- and sex-matched controls (93). Also the postmenopausal bone loss due to estrogen deficiency is attenuated compared to woman without hypoparathyroidism (94). Trabecular volumetric BMD (vBMD), cortical vBMD and cortical thickness measured by peripheral quantitative computed tomography (pQCT) are all superior in hypoparathyroidism than controls (95). Higher cortical vBMD and lower cortical porosity is also highlighted by high-resolution peripheral quantitative computed tomography (HRpQCT) (96). Comprehensive information has been also provided by histomorphometric analyses of the percutaneous iliac crest bone biopsies. Cancellous, endocortical, and intracortical bone sites are impaired (97) with higher trabecular bone volume and trabeculae thickness(98). The three dimensional (3D) analytical capability of microcomputed tomography (μCT) showed also the impairment of cortical bone that is thicker and the cortical porosity is lower (99).

The increased BMD does not reflect a reduction in fracture risk. Rather, concern exists for an increased risk because of the low remodeling activity. However, data on fracture risk in hypoparathyroidism are sparse. Actually, finite element analysis showed a normal mechanical strength and not an increase or decrease in fracture risk (100).

The treatment with calcium and active vitamin D supplementation is not able to restore the altered microarchitecture in hypoPT. PTH therapy can address some of these abnormalities helping to restore bone turnover (98).

1.7.4 Evaluation and management

The management of HypoPT can be divided in two different phases: acute and chronic

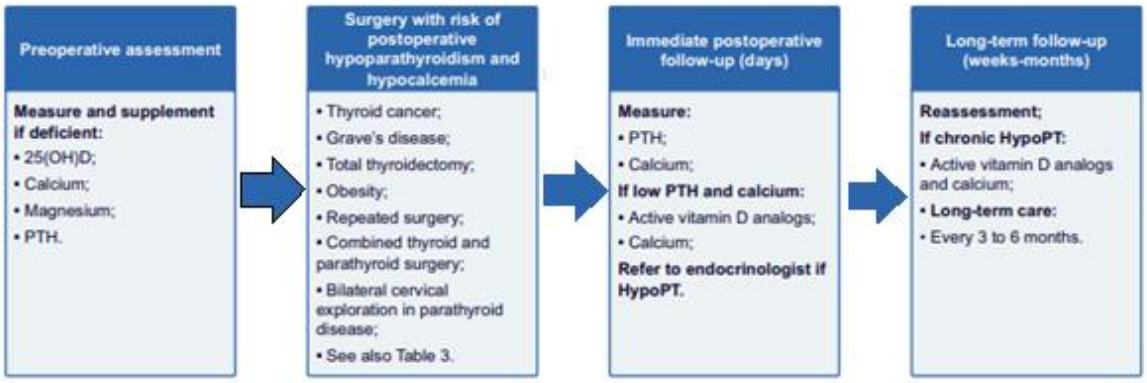
Acute management: the latest guideline suggests to start i.v. calcium supplementation in acute severe hypocalcemic symptoms, such as carpal or pedal

spasm or even seizures and laryngospasm(101). Acute hypocalcemia can also occur in subject on chronic management if other factors perturb the equilibrium such as gastrointestinal illness.

The therapy suggested in case of symptoms is: i) intravenous infusion of 1 to 2 ampules of 10% calcium gluconate (93 mg of elemental calcium/10 mL) in 50 mL of 5% dextrose over 15 to 30 minutes: ii) a slower and prolonged infusion of calcium gluconate, 0.5–1.5 mg/ kg body weight/hour, over an 8- to 10-hour period. During both phases, continuous electrocardiogram monitoring is recommended, and calcium levels should be monitored every 4 to 6 hours (102).

The perioperative management to avoid acute hypocalcemia in post-surgical subjects is illustrated in Figure 3.

Figure 3 Perioperative management of patients at risk of hypoparahyroidismadapted from (101)



Chronic management. The chronic management of HypoPT conventionally consists of active vitamin D analogues and calcium supplements. As PTH is responsible for 1α-hydroxylation of vitamin D in the proximal tubule, the treatment consists of 1α-hydroxylated analogues of vitamin D (101). About calcium supplements, calcium carbonate is typically preferred for is high bioavailability

(contains 40% of elemental calcium). However, calcium carbonate can be associated with bloating and constipation and alternatively, calcium citrate can be used. Calcium citrate does not require gastric acid to be absorbed and does not release carbon dioxide after ingestion so it is not associated with gastrointestinal discomfort (103). Patients can require 1 to 2 g of supplemental calcium per day at it is recommended to take calcium in 500 mg per meal. The major goals for chronic management are to avoid symptomatic hypocalcemia for patients and at the same time ameliorate/prevent the complications of the disease (102). Here the latest recommendations for the management of cronich hypoPT(101):

1.1. Treat with calcium and active vitamin D analogue therapy, with a goal to raise serum calcium (albumin adjusted or ionized) into the target range; ie, the lower half of the normal reference range or just below the normal reference range. At this time, it is not clear how to best balance the doses of calcium and active vitamin D analogue therapy.

1.2. Alleviate symptomatic hypocalcemia while avoiding hypercalcemia.

1.3. Avoid hypercalciuria when titrating calcium and active vitamin D analogue therapy, aiming for low normal plasma calcium levels. The panel proposes achieving a 24-hour urinary calcium level <6.25/7.5 mmol/24 hours (250/300 mg/24 hours) for adult women and men, respectively.

1.4. Avoid hyperphosphatemia. Panel members prescribe calcium supplements with meals to serve as phosphate binders, implement a low phosphate diet in adults and judiciously use active vitamin D analogue therapy. No data are available on the use of other types of phosphate binders in HypoPT.

1.5. Treat to normalize plasma magnesium levels. Magnesium supplements can be used as tolerated by the patient.

1.6. Aim to achieve a 25-hydroxyvitamin D level in the normal reference range.

1.7. Consider treating hypercalciuria with thiazide diuretics in conjunction with a

low sodium diet with careful monitoring of serum magnesium, potassium, and renal function.

Conventional therapy with calcium and vitamin D generally is not able to achieve these goals neither to control the rate of complications. Actually, the use of high dosage of calcium and vitamin D sometimes may increase some complications i.e nephrolithiasis and ectopical calcification.

Some authors tried to overcome this issue and to improve the management of hypoparathyroidism by restoring the PTH levels with the use of PTH analogues.

Up to now, the only molecule approved for the treatment of hypoPT is the rhPTH(1-84) but its use is limited to subjects that are not adequately controlled with conventional treatment(101). The pivotal study with rhPTH(1-84) was the REPLACE that showed that PTH was able to maintain normal serum calcium with 50% or greater reduction in oral calcium and active vitamin D doses (104). This study was followed by REPEAT, that confirmed and repeated these good results over the time(105). Other evidences further demonstrated that long term therapy with rhPTH(1-84) determined a reduction in urinary calcium excretion (106), a stabilization of renal function and an improvement in quality of life (87), but not a reduction of nephrolithiasis(101). Some authors also showed the efficacy of PTH(1-34) twice a day to improve the serum calcium levels (107,108). There are new therapies on the horizon that potentially can improve the management of hypoPT, the majority target the parathyroid hormone receptor(PTH1R), and one target the calcium-sensing receptor (CaSR)(101)

1.8 Primary hyperparathyroidism

Primary hyperparathyroidism is characterized by high serum calcium levels and high or inappropriately normal PTH levels due to the hyperactivity of one or more parathyroid glands (109). Until 1970, PHPT was the disease of bone, groans and stones for the important skeletal and kidney involvement. The inclusion of serum calcium levels in the biochemical screening tests after 1970 definitely changed this clinical phenotype thanks to the early diagnosis of PHPT. Nowadays the diseases in often mild and asymptomatic (110). In around 80% of cases, PHPT is due to a single, benign adenoma, alternately about 15% to 20% it can be related to multigland disorder, as multiple adenomas or hyperplasia. Multigland disease are typical in syndromes such as multiple endocrine neoplasia (MEN) 1 or 2 (111). Parathyroid carcinoma is quite rare disorder, occurring in less than 1% of subjects with PHPT (109) and may be associated with gene mutations such as include MEN1, CaSR, HRPT2, RET (familial forms), and PRAD1/cyclin1 (sporadic tumors) (112).

PHPT is far more common in postmenopausal women, with a female/male ratio of 3 to 4:1. However, before the age of 45 the ratio in the same between sexes. The incidence is different in race with the higher prevalence among blacks, followed by whites and the lower in Asians and Hispanics(113). This prevalence reflect also the high prevalence in the United States and Western Europe, around 0.86% (114), while in other countries such as Asia and Latin America the incidence and prevalence are increasing probably for the inclusion in the routine evaluation of serum calcium (115).

The diagnosis of PHPT has been made by the presence of hypercalcemia in addition to PTH levels that are inappropriately high for the hypercalcemic state. Usually, the PTH levels are frankly elevated, but they can also be within the upper normal range matching the definition of inappropriate high PTH levels. The main differential diagnosis of PTH-dependent hypercalcemia include the therapy with thiazide diuretics or lithium (116,117). Other conditions that can increase PTH levels are

vitamin D deficiency, bisphosphonates and denosumab, and renal failure including secondary hyperparathyroidism (118). A peculiar but rare disease that need to be excluded is the familial hypocalciuric hypercalcemia (FHH). This condition is an asymptomatic genetic disorder characterized by lifelong moderate hypercalcemia along with normo- or hypocalciuria and elevated plasma parathyroid hormone (PTH).

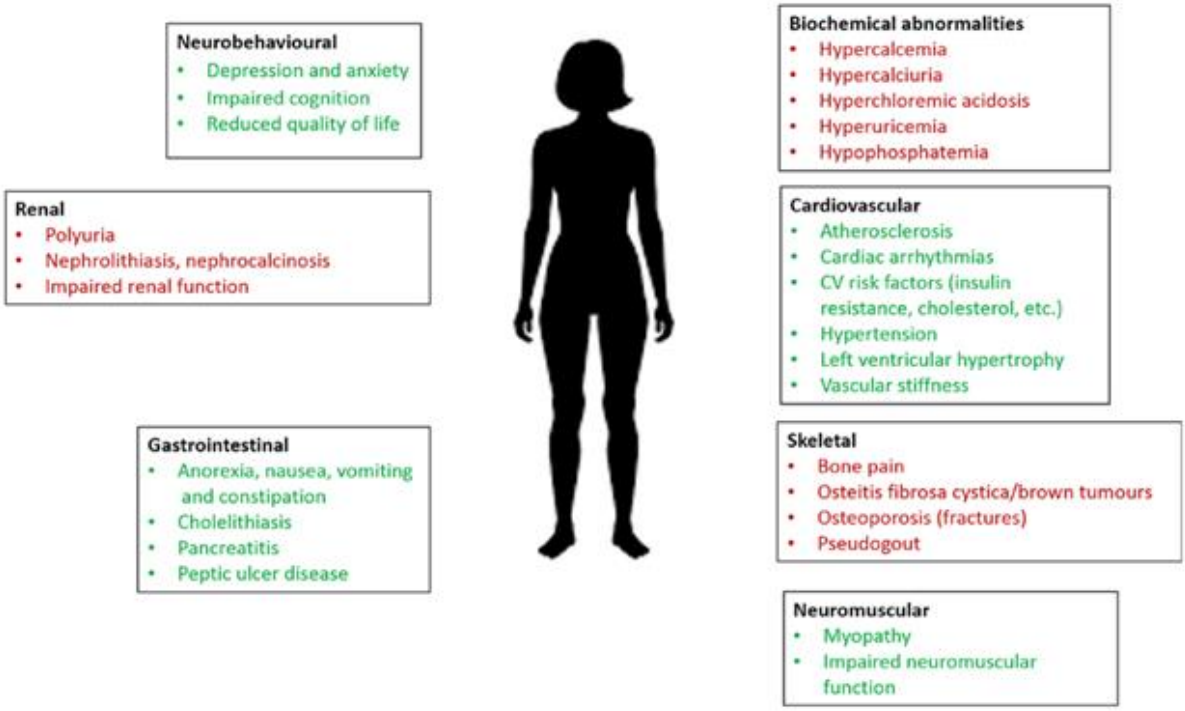
Hypercalcemia associated with very low or undetectable PTH levels is typical of malignant diseases, where the production of PTHrP determines elevation of serum calcium (119).

In case of uncertain diagnosis is important also to look at the PTH assays used. Indeed, the assays have evolved significantly during the years and different generations of PTH assays are used. The older ones, called “intact” PTH or second-generation PTH assays can give false high PTH levels because it cross-reacts with an N-terminal truncated PTH fragment called (7–84) PTH or non-(1–84) PTH (120). The more recent assay, third generation, overcome this issue (121).

Conflicting data are available on the risk of death in subjects affected by PHPT. It seems that PHPT is associated with increase cardiovascular mortality especially if untreated (122–124). Parathyroidectomy seem to be associated with a reduction of mortality after 5 years of surgery in some studies (125).

The clinical presentation changed over the years. The common presentation in countries that uses routine calcium testing is the asymptomatic disease, with mild hypercalcemia as main symptom and also different degree of osteoporosis, silent nephrolithiasis, nephrocalcinosis, or asymptomatic vertebral fractures(126). Hypercalcemia can have different effect in different system as showed in figure 4 and the degree of hypercalcemia can cause different symptoms as polyuria, polydipsia, dehydration, acute kidney injury, gastrointestinal symptoms as nausea, vomiting, constipation, also headache and altered mental status. PTH can also have a direct effect on some systems such as bone and kidney.

Figure 4 Symptoms and organ involvement in patients with primary hyperparathyroidism adapted from(126)



In the recent years the measurement of PTH in normocalcemic subjects for the evaluation for example of osteoporosis changed again the clinical presentation of PHPT with the discovery of the new form: the normocalcemic hyperparathyroidism (NHPT) (118).

1.8.1 Bone involvement

The bone involvement is a PHPT hallmark, even in its mild forms. Bone remodeling is increased, as demonstrated by elevated bone turnover markers (126). The classic and more severe bone involvement of PHPT is the osteitis fibrosa cystica. This condition is due to the increased bone resorption that determines periostal bone resorption causing bone pain, deformities, and pathologic fractures. This is typically visible at the distal phalanges with characteristic radiologic signs. Radiologic signs may also be present in the skull, acknowledged as “salt-and pepper” pattern. Local destructive lesions, bone cysts, and “brown tumors” typically in the long bones and

pelvis represent other skeletal manifestations.

Another manifestation is the reduction in BMD at all sites. The typical reduction of BMD according to DXA is at the distal one-third radius levels, a site enriched of cortical bone, with a possible preservation in site rich in cancellous bone such as the lumbar spine (127). However, latest non-invasive skeletal imaging technologies, confirmed the involvement at all sites and explaining the increased vertebral fractures in PTPH(128,129). One of this technique is the TBS, that showed in different studies the trabecular impairment independently of preserved lumbar BMD in PHPT (100,130,131). Data from HRpQCT studies confirmed trabecular impairment at the radius and tibia (130,132,133) with reduction of volumetric BMDs (total, cortical, and trabecular), cortical thickness, and trabecular number(126). These alterations have been confirmed also in study using Peripheral QCT (pQCT). The bone involvement determines an increased risk of fractures that has been confirmed in a recent systematic review and meta-analysis of studies showing two-fold increase in risk of all fractures in PHPT compared to controls and particularly at forearm and hip(126). Parathyroidectomy seems to reduce fracture risk. Antiresorptive therapy have been demonstrated to increase BMD, but there are no data available on fracture risk in PHPT (134).

1.8.2 Renal involvement

Hypercalcemia and hypophosphatemia in PTH are related to the actions of PTH on the kidney tubule. At the kidney level PHPT determines hypercalciuria and nephrolithiasis consisting of calcium oxalate or calcium phosphate calculi (135). Different studies demonstrated a rate of kidney stones about 15–20% of PHPT, with a higher frequency if ultrasound screening is performed until 55%(136,137). Besides nephrolithiasis, the kidneys may be affected in other ways, such as nephrocalcinosis and reduction of creatinine clearance (138).

1.8.3 Other clinical manifestations

Besides the skeleton and the kidneys, PHPT can involve other systems. Weakness and fatigue due to atrophy of type II muscle fibers (139) can be present. Depression, anxiety, irritability, other mood disorder until cognitive impairment has been described with a reduction of QOL (140,141). Parathyroidectomy can improve o restore these symptoms (142–144). There are different and emerging evidence that PHPT is associated with higher cardiovascular morbidity and mortality but the pathogenic mechanisms are still not clear. PHPT has been associated with hypertension, atherosclerosis, increased vascular stiffness, valve calcification, left ventricular hypertrophy, and arrhythmias (145,146), but the reversibility after parathyroidectomy remains variable (147,148).

1.8.4 Treatment

Parathyroid surgery. Parathyroidectomy still remains the only way to treat and cure PHPT (149). Surgery is recommended in all patients with classical symptoms of PHPT and the latest guidelines updated the indication for parathyroidectomy in asymptomatic forms of the disease have been addressed by the last guidelines (Table 5)(150)

Table 4. Indications for surgery in primary hyperparathyroidism adapted from (150)**.**

Parameter	1990	2002	2008	2013	2022
Serum Calcium (>upper limit of normal)	1–1.6 mg/dL (0.25–0.4 mmol/L)	1.0 mg/dL (0.25 mmol/L)	1.0 mg/dL (0.25 mmol/L)	1.0 mg/dL (0.25 mmol/L)	1.0 mg/dL (0.25 mmol/L)
Skeletal	BMD by DXA: *Z*-score < −2.0	BMD by DXA: *T*-score < −2.5 at any site	BMD by DXA: *T*-score < −2.5 at any site Previous fragility fracture	a. BMD by DXA: *T*-score < −2.5 at lumbar spine, total hip, femoral neck or distal 1/3 radius b. Vertebral fracture by X-ray, CT, MRI, or VFA	a. BMD by DXA: *T*-score < −2.5 at lumbar spine, total hip, femoral neck or distal 1/3 radius b.Vertebral fracture by X-ray, CT, MRI or VFA
Renal	a. eGFR reduced by >30% from expected. b. 24-Hour urine for calcium >400 mg/day (>10 mmol/day)	a. eGFR reduced by >30% from expected b. 24-Hour urine for calcium >400 mg/day (>10 mmol/day)	a. eGFR <60 cc/min b. 24-Hour urine for calcium not recommended	a. eGFR <60 cc/min b. 24-hour urine for calcium >400 mg/day (>10 mmol/day) and increased stone risk by biochemical stone risk analysis c. Presence of nephrolithiasis or nephrocalcinosis by X-ray, ultrasound, or CT	a. eGFR <60 cc/min b. Complete 24-hour urine for calcium >250 mg/day in women (>6.25 mmol/day) or > 300 mg/day in men (>7.5 mmol/day) c. Presence of nephrolithiasis or nephrocalcinosis by X-ray, ultrasound, or CT
Age	<50 years	<50 years	<50 years	<50 years	<50 years

Preoperative localization is recommend in subjects candidates for minimally invasive parathyroidectomy, in recurrent or persistent disease after surgery (151–153). The first-line localization techniques are technetium-99m-sestamibi [with or without single photon emission computed tomography (SPECT)] or neck ultrasound. Often, these techniques are inconclusive and other second line like ^{11}C-Methionine/ ^{1}C-choline-PET/CT or 4D- CT showed a good performance in localizing affected glands in subjects with non-conclusive first line imaging (154–156). Negative results not preclude surgical option (157).

Focused minimally invasive parathyroidectomy is the treatment of choice in subjects single gland disease at the preoperative evaluation (153). This procedure can be strengthened by the intraoperative dosage of PTH. The half-life of PTH is 3–5 minutes, so after removal of the adenoma the circulating PTH rapidly drops. A reduction of PTH of 50% at 10 or 20 minutes after resection is indicative of successful surgery (158). Potential complications of surgery include damage of the recurrent laryngeal nerve resulting in hoarseness and decreased voice volume, and permanent hypoparathyroidism.

After surgery, there is an increased risk of transient hypocalcemia that can be prevented or managed with calcium supplementation during the first postoperative week. A peculiar post operative complication is the so called “hungry bone syndrome” that is a prolonged hypocalcemia and hypophosphatemia as a consequence of rapid deposition of calcium and phosphate into bone for the rapid reduction of bone turnover. After successful surgery, serum calcium and PTH concentrations normalize, bone density improves after at least one year and continue to improve over time (159).

Non-surgical management Subjects who do not meet surgery criteria need to be

monitored (160). There are, however, subject that cannot undergo surgery. Long-term observational studies report a stability of biochemical parameters for several years (161), however BMD appears to abruptly decline at cortical sites after 10 years of follow-up and almost 40% of subjects met one or more indications for parathyroidectomy over 15 years (159).

In subjects undergo medical management, the latest guidelines suggest to control serum calcium concentrations once to twice yearly together with annual control of serum creatinine and urinary calcium excretion. It is also recommended annual or biannual bone densitometry at the spine, hip, and also at distal one-third site of the forearm(150).

To controls serum calcium levels, thiazide diuretics and lithium should be not used if possible. Cincalcalcet is approved to control serum calcium levels in PHPT, SHPT and parathyroid carcinoma. Cinacalcet is a calcimimetic agents that act on CaSR function with a subsequent decrease in PTH synthesis and secretion, and so a reduction in the serum calcium level. Studies showed the ability of Cinacalcet in normalize and maintain the reduction across a different range of disease severity (162). Neither BMD nor urinary calcium excretion improved after cinacalcet treatment. Moreover, there are no information of potential reduction of nephrolithiasis risk reduction.

The medical management also includes the normalization of vitamin D level, with the aim to have levels of 21–30 ng/ml with conservative doses of cholecalciferol (600–1000 IU daily) to avoid further increase of PTH (163). Active vitamin D metabolites should not be used to adjust vitamin D deficiency in PHPT.

Bisphosphonates have been evaluated as a possible medical strategy to controls the loss of BMD and also to contain the serum calcium levels. The major part of the studies assessing bisphosphonate used alendronate and showed the ability to successfully reduce bone turnover and increase BMD in subjects with PHPT. Impact on serum calcium concentrations has been erratic with a null effect on serum PTH

concentrations There are very few data on men (164). To date there are not conclusive data on the ability of bisphosphonate in reducing fracture risk in PHPT(150)

Denosumab showed to successfully improve BMD and reduce bone turnover in subjects with PHPT (165). Denosumab appeared as effective as parathyroidectomy in increasing BMD but also improved TBS (166).

1.9 Normocalcemic hyperparathyroidism

Normocalcemic primary hyperparathyroidism (NHPT) is a new form of PHPT described for the first time in 2008. NHPT is defined by normal adjusted total calcium

and normal ionized calcium levels along with elevated intact PTH (utilizing either a second or third generation assay) on at least two occasions over 3–6 months after all other causes for secondary hyperparathyroidism have been excluded(150). Despite more than 10 years from its first definition the natural history, management and real prevalence are still matter of debate.

2.7.1 Epidemiology

The prevalence of NHPT is quite different in the cohorts. In the nonreferral populations, i.e. The Osteoporotic Fractures in Men (MrOS) and Dallas Heart Study (DHS) study, the prevalence reported was 0.4 and 0.6%, respectively (167). Similarly, in Italy, the prevalence of NHPT was 0.44% (168). The prevalence of the disease in referral centers rates from 0.1% and 8.9%, however many studies overestimate the prevalence due to a not correct exclude all causes of secondary hyperparathyroidism(126).

1.9.1 Pathophysiology and diagnosis

At the beginning, one of the common hypotheses was that NHPT is as an early, ‘subclinical’ phase of PHPT (169). Other suggested that NHPT could be due to a target resistance to the actions of PTH that failed to suppress PTH in response to calcium levels (170). The most recent hypothesis contemplate: i) an increase in the calcium set point for PTH release; ii) mild initial PHPT, iii) or FHH, iv) calcium-sensing receptor

polymorphisms), v) age, as PTH increases with age. Some, but not all reports have shown progression to hypercalcemia or intermittent hypercalcemia, during time. (150)

For correct NHPT diagnosis, serum calcium should be measured as either total albumin-adjusted and ionized calcium. It is important to measure calcium over time at at least twice within a 6-month period in order to exclude transient hypercalcemia (160). Then, it is mandatory to rule out secondary causes of hyperparathyroidism. The most frequent cause is the low vitamin D levels. Usually, high PTH levels came back to normal range after 3 months of vitamin D supplementation and with vitamin D levels above 30 ng/mL (171). Renal insufficiency is probably the second most common cause of elevation of PTH. The Kidney Disease Outcomes Quality Initiative (KDOQI) and Kidney Disease Improving Global Outcomes (KDIGO) guidelines stare that serum PTH start to rise when eGFR is below 60 mL/min/1.73 m2 (172).

Other than that, to be confident in correct diagnosis of NHPT, some additional conditions need to be excluded such as: daily calcium intake below 700 mg/day, gut malabsorption, idiopathic hypercalciuria, drugs as loop diuretics, lithium, bisphosphonates, denosumab and antiepileptic medications (173,174).

1.9.2 Clinical aspects

Often the diagnosis of NHPT follow the screening for secondary causes of osteoporosis, that one of the reason why in patients with NHPT there is an high prevalence of osteoporosis (46-55%) (175–179). However, the prevalence of VFx is lower when compared with PHPT (175). Despite the know catabolic effect of PTH at the cortical levels the BMD at distal radius seems to be preserved in NHPT compared PHPT (175,176,179). Moreover by HRpQCT, NHPT subjects showed only an impairment in some cortical assets with normal trabecular parameters (180). Finally NHPT bone turnovers are not elevated(167,175,181).

Despite the normal calcium excretion, nephrolithiasis is quite common in NHPT and is similar to PHPT (13–18%) (175,176,178,179,181). NHPT could be associated with an increase cardiovascular risk maybe related to the impact of PTH and/or calcium on the cardiac and endothelial cells and cardiac conduction system (182).

1.9.3 Management

To date there is not consensus on the correct management of NHPT. The Fifth International Workshop Guidelines for the Management of Primary Hyperparathyroidism do not recommend surgery(150) others can suggest surgery only after “experienced endocrine review”(101). It is recommended to measure serum calcium, phosphate, alkaline phosphatase, 25(OH)D3, creatinine, and PTH levels every year, and to perform a DXA scan every 1-2 years.

Few studies have evaluated the benefit of alendronate showing the ability in improving BMD (183). The impact of medical therapies on cardiovascular and neurocognitive symptoms in NHPT are unknown.

CHAPTER 2: **RESEARCH PROJECT N°1**

DXA-Based Bone Strain Index: A New Tool to Evaluate Bone Quality in Primary Hyperparathyroidism.*

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2.1 Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disorder. It is characterised classically by hypercalcemia and high or inappropriately normal PTH levels. Major complications of PHPT include nephrolithiasis, osteoporosis, and fragility fracture(184), even if there is a high prevalence of asymptomatic disease in Western countries (185,186).Although increased fractures risk at both vertebral and non-vertebral sites are well established clinically, bone mineral density (BMD) is typically reduced at the distal 1/3 forearm, a primarily cortical bone site, with relative preservation of the lumbar spine, a predominantly trabecular site(187–189). High-resolution peripheral quantitative computed tomography (HRpQCT) has resolved these discrepant findings by demonstrating at the microarchitectural level that trabecular bone is also affected adversely in PHPT(132,190). However, as primarily a research tool, HRpQCT is not widely available. Other more readily available indices are needed to evaluate bone quality in the clinical setting. Recently, a pilot study applied 3D-DXA software to evaluate the hip. It confirmed the deterioration of cortical bone but not trabecular bone (191).

Trabecular bone score (TBS) is a useful non-invasive index of bone quality in PHPT. Studies on postmenopausal women with PHPT showed a reduction in TBS values (131,192), in contrast to lumbar spine DXA in which values are closer to normative controls. Questions remains whether vertebral fractures (VFs) can be readily detected in this clinical setting(193,194).

Bone Strain Index (BSI) is a new DXA-derived skeletal parameter of deformation that is based on Finite Element Method (FEM)(195). It can be applied both to lumbar and femoral DXA scans(8). Recent clinical studies showed the usefulness of BSI as a complementary tool, together with other clinical risk factors, to identify patients at risk of fracture(196,197)and to characterize better young patients affected by secondary osteoporosis(198,199). Patients treated with teriparatide experienced an improvement of BSI over time (200).

The aim of this study was to compare the BSI measured at lumbar spine and hip in subjects with PHPT versus controls. We also explored a possible association of BSI with morphometric vertebral fractures in PHPT.

2.2 MATERIALS AND METHODS

2.2.1Study design and population

We performed a case–control study including 150 subjects. Cases (n=50) included subjects with PHPT who were consecutively enrolled from September 2017 to December 2019, at Campus Bio-Medico University of Rome. PHPT was defined as elevated or normal PTH concentrations and persistently elevated total, albumin-

corrected, or ionized serum calcium levels (at least 2 different determinations, at least 3 months apart). Exclusion criteria were the following: any other condition that can affect bone and calcium metabolism; use of drugs affecting bone and calcium metabolism (i.e. use of glucocorticoids); early menopause; a history of possible high-energy VFs; metabolic bone diseases such as Paget disease and osteogenesis imperfecta.

For each case, two controls were identified from electronic medical records of the outpatient clinic of endocrinology at Campus Bio Medico University of Rome, where they were referred for unrelated diseases (eg, thyroid nodules with euthyroidism). Controls were matched by age (±2 years), gender and date of consultation at the outpatient clinic and were recruited using the aforementioned exclusion criteria. We included control subjects who had a BMD test, thoracolumbar radiograph and blood testing to evaluate calcium metabolism for the first time. In all control subjects with osteoporosis and/or fragility fractures, secondary causes of osteoporosis were ruled out.

We defined asymptomatic subjects as those with primary hyperparathyroidism and no overt signs of the disease namely: osteitis fibrosa cystica, vertebral fractures, nephrocalcinosis, reduced renal function, muscle weakness, peptic ulcer disease, pancreatitis, neurocognitive symptoms (201).

Biochemical analysis

Fasting blood samples were collected for the following: serum total calcium (normal, 8.4-10.2 mg/ dL) albumin (normal 3.2-4.6 g/dl), serum phosphate (normal,

2.3-4.7 mg/ dL) creatinine (normal 0.55-1.2 mg/dl) and 25 OH vitamin D (normal, 30-100 ng/mL), that were measured by automated methods. Calcium values were corrected for albumin concentration. Ionized serum calcium (normal, 1.13- 1.32 mmol/L) was measured by a potentiometric method on GEM PREMIER 4000 analysers (Werfen, Le Pré-Saint-Gervais, France). Intact PTH (normal, 14-72pg/mL) was measured by an immunochemiluminometric assay using a Modular E170 automatic analyser (Roche Diagnostics, Indianapolis, IN, USA). Serum levels of beta CrossLaps (CTX) were assayed by the Cobas β-CrossLaps (ECLIA; β-CrossLaps/Serum, Roche Diagnostics, Basel, Switzerland), which uses two monoclonal antibodies against β-cross-linked CTX according to the manufacturer's protocol. Serum levels of P1NP were analyzed by Cobas Total P1NP (ECLIA; Roche Diagnostics) automated analyser.

Dual-energy X-ray absorptiometry

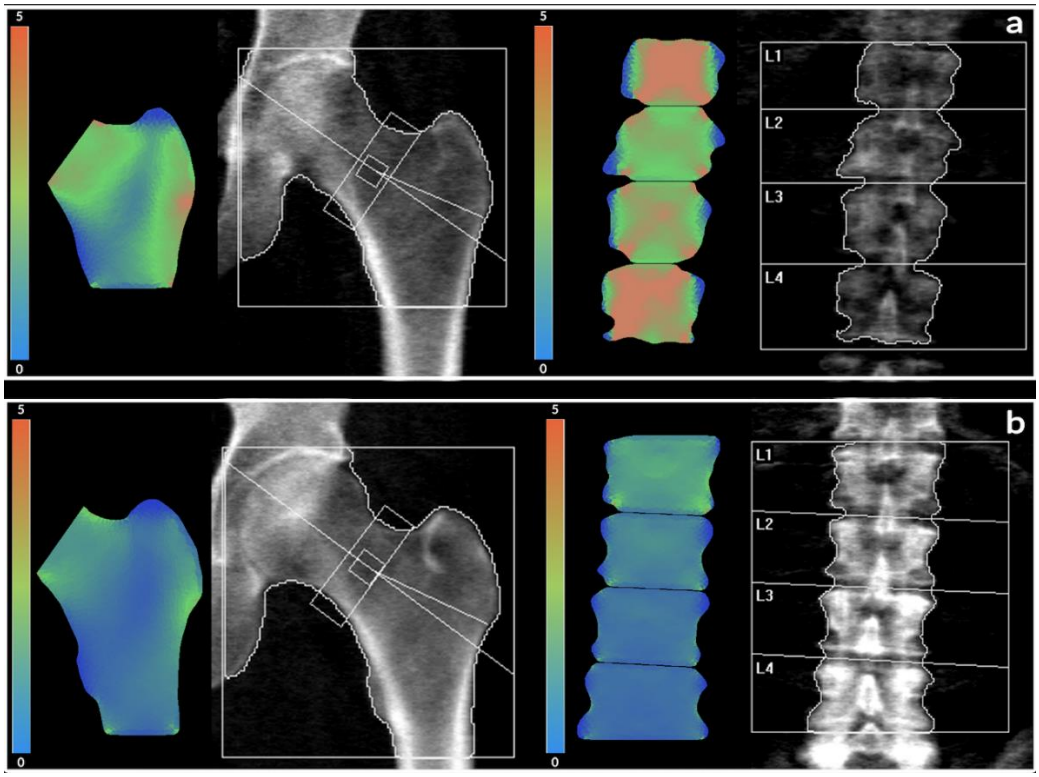
We measured BMD by DXA at the lumbar spine (L1-L4), total hip, femoral neck, and non-dominant forearm (one-third distal radius) (Hologic Discovery QDR Instrument, MA, USA, version 13.3:3; coefficients of variation LS: 0.97% FN:1.49% TH: 1.36%). Data were reported for absolute BMD, T-scores values (SD difference from mean values of sex-matched young, healthy individuals) and Z-score (SD difference form mean values of age and sex-matched healthy individuals).All scans were performed according to the International Society for Clinical Densitometry (ISCD) guidelines (202). Fractured vertebrae and vertebrae with structural changes were excluded from the analysis (T-score difference with the

adjacent vertebra > 1.0). TBS was automatically derived from the same lumbar spine DXA region by dedicated software (Insight TBS, Medimaps Groupe, Geneve, Switzerland; version: 3.0.2.0).

Bone Strain Index

For BSI analysis, raw data from the DXA image were sent to a separate workstation in which the BSI software (Tecnologie Avanzate s.r.l., Torino, Italy) is installed. For the lumbar spine, BSI computation was determined by dividing each vertebra of the lumbar scan into several triangles following the contour provided by DXA software mapping (203). The DXA lumbar image was analyzed by a pattern drawn with the load applied to the upper plate and the constraints to each vertebra's lower plate(204). At the femoral site, BSI was calculated on the premise of a lateral fall, with force applied to the greater trochanter and the constraints applied to the femoral head and shaft (205). In both spine and femur, stiffness of the elements was defined by the empirical relations described by Morgan et al.(206), at each anatomic site. The resulting amount of strain presented graphically in different colors, led to the identification of areas with the higher strain concentration. The BSI value represents the average equivalent strain in the regions defined by DXA analysis, assuming a higher strain level (high BSI) indicates higher fracture risk (Figure 5). FEA computation was automatically performed on the computer by dedicated software both for lumbar spine and hip, using the same region of interest that is utilized for BMD calculations (8,207). Each BSI examination takes about 5 seconds. The BSI analysis was conducted as a blinded analysis.

Figure 5 Femoral and Lumbar Spine BSI images in a subject with hyperparathyroidism (a) and in a control subject (b). The corresponding values of BSI were:
a. Femoral neck BSI 2.33; Total Hip BSI 1.94; Lumbar spine BSI 3.02
b. Femoral neck BSI 1.13; Total Hip BSI 1.17; Lumbar spine BSI 0.93.



Vertebral fracture assessment (VFA)

VFA utilized dedicated vertebral fracture assessment software. Conventional spinal radiographs (T4-L4) in the lateral and the anteroposterior projections were also performed to confirm VFs among those with scoliosis and disk space osteoarthritis (208). A single experienced investigator read all images and scored VFs using the Genant semi quantitative method (grade 1, mild; grade 2, moderate; grade 3, severe) (209).

Ethics

The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Principles of Good Clinical Practice. Local ethics committees approved the research protocol and all participants gave informed consent allowing their anonymised information to be used for data analysis.

2.2.2 Statistical analysis

According to our sample size, there will be a 80% probability to declare as statistically significant at $\alpha = .05$ a standardised difference of 0.5 between groups ("medium-sized" difference according to Cohen)(210). Data distribution was evaluated using the Kolmogorov–Smirnov test. The difference between the means was analysed using the unpaired Student t-test, after determining whether equal variance could be attributed to the subgroups according to Levene's test. The nominal variables were described as a number and percentage and analysed with contingency tables and the χtest.

The associations between FN-BSI, TH-BSI, LS-BSI, LS-BMD, TH-BMD, FN-BMD, calcium, PTH, phosphate, 25 OH vitamin D, CTX, P1NP and TBS were tested by either Pearson's product-moment correlation or Spearman's correlation as appropriate.

The performance of the FN-BSI, TH-BSI and LS-BSI in detecting patients with prevalent VFs was tested by calculating the area under the receiver operating characteristics curve (AUC), whose results were interpreted as follows: 0.50 to 0.59: poor; 0.60 to 0.69: moderate; 0.70 to 0.79: good; 0.80 to 0.89: very good; and ≥ 0.90:

excellent discrimination(211). For the only variable showing at least moderate accuracy in discriminating VFs (i.e., LS-BSI) the optimal cut-off value was determined based on the maximum Youden index. Based on the identified optimal cut-off value, the sensitivity, specificity and accuracy of LS-BSI were calculated.

Several logistic regression models were run to test the predictive power of the LS-BSI to predict VFs, adjusted for a different combination of relevant predictors such as age, sex, BMI, CTX, P1NP, and TBS. LS-BSI was converted into a dummy variable according to the identified threshold. The possible multicollinearity between continuous variable was tested by computing the variable inflation factor (VIF); no more than moderate correlation (VIF ranging from 1.2 to 2.2) was detected. Since age, BMI, CTX, P1NP, and TBS were non normally distributed, logarithmic or reciprocal transformations were performed, as appropriate, to achieve a more normal data distribution. For both age and TBS, the transformed data showed a worst distribution; consequently, untransformed data were used. Results were presented as odds ratio (OR) and 95% confidence intervals (CI). The coefficient of the statistical models' determination was calculated based on the Nagelkerke R^2, and the overall performance of the logistic models in predicting VFs was quantified with respect to discrimination as described by the AUC.

We also divided the population into subgroups in osteoporosis, osteopenia and normal, based on the T-score according to WHO criteria (212) and then, we evaluated the BSI in these subgroups.

A p-value of <0.05 was considered significant. Statistical analysis was performed by SPSS version 26.0 statistical package (SPSS, Inc.).

2.3 RESULTS

Clinical, biochemical and radiologic characteristics

Table 5 describes the population's baseline characteristics, with corresponding differences between the two groups.

There were no differences between groups in terms of age, sex, menopausal age, years after menopause, but PHPT patients had a significantly higher BMI. The albumin-adjusted total serum calcium and PTH levels were significantly higher in the PHPT group, as expected ($p<0.001$); while phosphorus levels were significantly lower in PHPT compared to controls ($p<0.001$) (Table 5).

A between-groups statistically significant difference was showed for FN BMD (PHPT 0.633 ± 0.112 vs controls 0.666 ± 0.081 p= 0.042) and 1/3 distal radius BMD (PHPT 0.566 ± 0.07 vs controls 0.625 ± 0.06 $p<0.001$). There were no differences for TH- and LS-BMD. A between-groups statistically significant difference was showed for FN T-score (PHPT -1.95 ± 1.0 vs controls -1.66 ± 0.72 p= 0.043) and 1/3 distal radius T-score (PHPT -2.19 ± 1.17 vs controls -1.24± 0.81 $p<0.001$). Osteoporosis was evident in 17% of controls and in 34% of subjects with PHPT (p=0.01). Osteopenic T-scores were evident in 79% of controls and in 58% of subjects with PHPT (p=0.003). The majority of individuals with PHPT (62%) were asymptomatic.

The TBS was significant lower in PHPT compared to controls (1.24 ± 0.09 vs 1.30 ± 0.10 p=0.001).

BSI was significantly worse (higher) between PHPT and control subjects at the FN (1.72 ± 0.41 vs 1.49 ± 0.35 p=0.001) at the TH (1.51 ± 0.33 vs 1.36 ± 0.25, p=0.002) and at the LS (2.28 ± 0.59 vs 2.02 ± 0.43 p=0.009). Figure6 describes the differences in BSI scores between PHPT and control patients.

In subjects with osteoporosis, there was a significant difference between PHPT and controls in BSI at the FN (2.04±0.29 vs 1.79±0.28 p=0.01) and the TH (1.76±0.28 vs 1.59±0.21 p=0.04). In the group with osteopenic T-scores, PHPT subjects showed a higher BSI at the LS (2.26±0.56 vs 2.00±0.42 p=0.01).A statistically significant higher prevalence of morphometric VFs was showed in the PHPT group (p<0.001).

The bivariate correlation analysis showed that TBS was significantly associated with LS-BSI (r=-0.59 p=0.001).

In controls there is a significant difference in BSI between osteoporotic (n=17) and non- osteoporotic subjects (i.e. osteopenic T-scores n=79 and normal T-scores n=4) at the LS (2.25±0.38 vs 1.98±0.43 P=0.017), TH (1.59±0.21 vs 1.31±0.24, P<0.001) and FN (1.44±0.34 vs 1.79±0.28, P<0.001)

Table 5. Baseline characteristics of the study population.

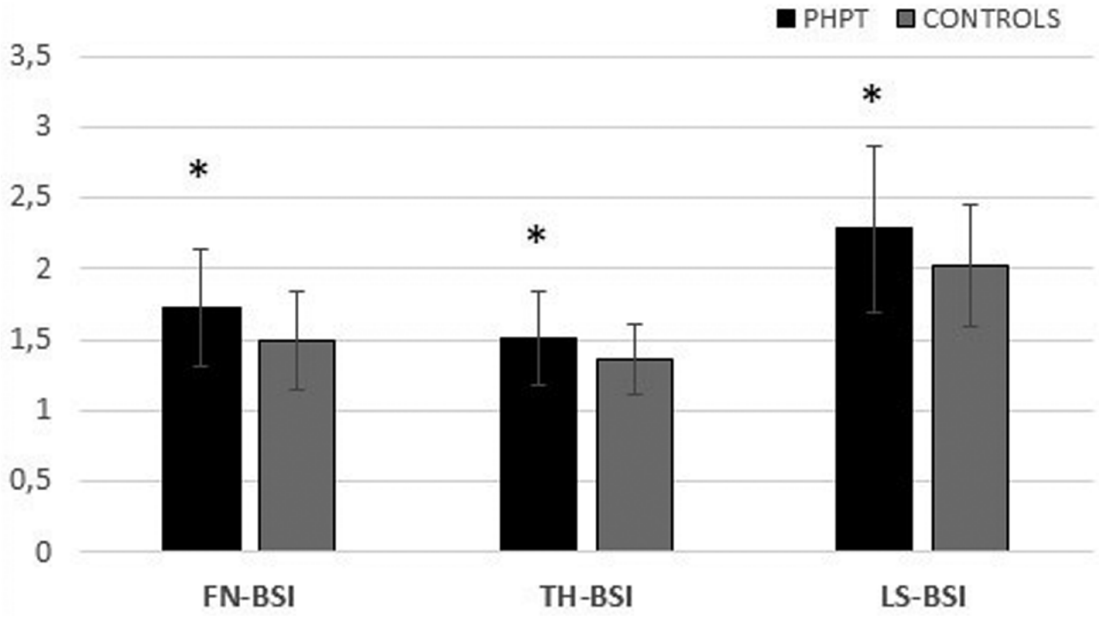
Variable	n	PHPT(n=50)	Control(n=100)	p-value
Demographic and clinical features				
Age, years	150	65.2 ± 11.6	65.2 ± 8.2	0.996
Sex, female	150	47 (94%)	95 (95%)	0.535
BMI, kg/m^2	150	27.9 ± 6.0	25.5 ± 4.4	**0.011**
Menopause age, years	142	50.2 ± 3.9	49.8 ± 3.1	0.517
Length of menopause, years	142	15.1 ± 9.6	15.7 ± 8.2	0.731
Biochemical analysis				
Albumin-adjusted serum total calcium, mg/dL	150	10.8 ± 0.4	9.4 ± 0.4	**<0.001**
Serum phosphorus, mg/dL	150	2.8 ± 0.5	3.6 ± 0.5	**<0.001**
PTH, pg/dL	150	143.6 ± 72.4	52.6 ± 13.7	**<0.001**
25-OH vitamin D, ng/mL	150	32.0 ± 7.8	31.6 ± 12.0	0.849
Serum creatinine, mg/dL	150	0.7 ± 0.2	0.7 ± 0.1	0.886
GFR, ml/min/m^2	150	88.0 ± 24.6	82.4 ± 15.5	0.146
CTX, ng/mL	50	0.5 ± 0.3	--	--
P1NP, ng/mL	50	71.9 ± 41.8	--	--
Bone measures				
Vertebral fractures	150	18 (36.7%)	10 (10%)	**<0.001**
1/3 DR-BMD, g/cm^2	89	0.566 ± 0.07	0.625 ± 0.06	**<0.001**
1/3 DR Z-score	89	-0.40 ± 1.02	0.42 ± 0.83	**<0.001**
FN-BMD, g/cm^2	150	0.633 ± 0.112	0.666 ± 0.081	**0.042**
FN Z-score	150	-0.43 ± 0.99	-0.15 ± 0.81	0.070
FN-BSI	150	1.72± 0.41	1.49 ± 0.35	**0.001**
TH-BMD, g/cm^2	150	0.793 ± 0.136	0.824 ± 0.102	0.130
TH Z-score	150	-0.01 ± 1.15	0.20 ± 0.90	0.207
TH-BSI	150	1.51 ± 0.33	1.36 ± 0.25	**0.002**
LS-BMD, g/cm^2	150	0.869 ± 0.197	0.884 ± 0.129	0.632
LS Z-score	150	0.13 ± 1.97	0.19 ± 1.31	0.867
LS-BSI	150	2.28 ± 0.59	2.02 ± 0.43	**0.009**
TBS	150	1.24 ± 0.09	1.30 ± 0.10	**0.001**
LS T-score adjusted for TBS	150	-2.40 ± 1.05	-1.82 ± 1.02	**0.002**
LS Z-score adjusted for TBS	150	-0.41 ± 1.04	0.27 ± 0.99	**<0.001**
Osteoporosis	150	17 (34%)	17 (17%)	**0.01**
Osteopenia	150	29 (58%)	79 (79%)	**0.003**

PHPT hyperparathyroidism; BMI: body mass index; CTX: beta CrossLaps; P1NP: procollagen amino-terminal peptide; BMD: bone mineral density; BSI: Bone Strain Index; DR: distal radius; FN: femoral neck; LS: Lumbar Spine; TH: total hip, TBS: Trabecular Bone Score.

Data reported as mean ± SD, except for Sex, Asymptomatic PHPT, Vertebral fractures, osteoporosis and osteopenia (number and percentage).

The difference between the means was analysed using the unpaired Student t-test. The nominal variables were analysed with contingency tables and the χ test.

Figure 6. Evaluation of BSI in PHPT and control group. Data are presented as mean (bars) and standard deviation (Whiskers). *p < 0.01



Association between BSI parameters and VFs in PHPT subjects

Using ROC analysis, we evaluated the accuracy of FN-, TH- and LS-BSI, LS-, FN-, TH- and 1/3 distal radius- BMD, TBS and TBS-adjusted LS T-score in detecting patients with prevalent VFs. LS-BSI was the only parameter showing a trend toward a statistically significant difference in subjects with morphometric VFs (no VFs: 2.14 ± 0.62; VFs: 2.48 ± 0.48; p=0.051). No difference was detected either for FN-BSI (no VFs: 1.72 ± 0.47; VFs: 1.73 ± 0.33 p=0.95) or for TH-BSI (No VFs: 1.52 ± 0.40; VFs: 1.51 ± 0.21; p=0.94). Similarly, LS-BSI showed a moderate accuracy for discriminating VFs (AUC 0.667; 95% CI 0.513-0.820; p=0.054), while FN- (AUC 0.532; 95% CI 0.369-0.696; p=0.709) and TH-BSI (AUC 0.529; 95% CI 0.368-0.689; p=0.740) showed poor accuracy. For LS-BSI, the optimal cut-off value was

set at 2.2; based on this threshold, sensitivity, specificity, and accuracy were 77.8%, 61.3% and 67.4%, respectively.

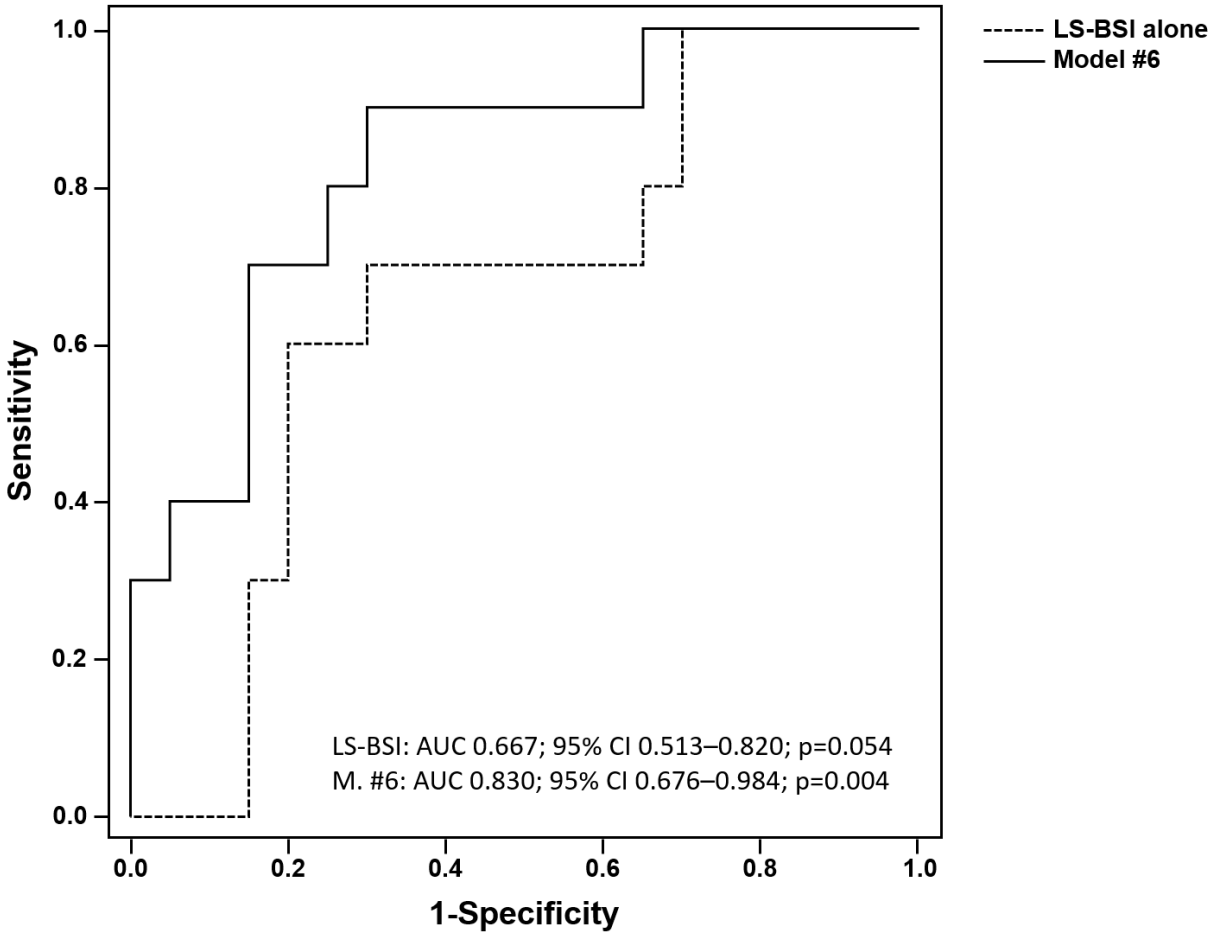
Table 6 shows the results of the multivariate analysis. In all explored regression models, adjusted LS-BSI ≥ 2.2 was a statistically significant independent predictor of morphometric VFs risk, with an adjusted OR ranging from 5.7 to 15.1. None of the considered adjustment covariates (i.e., sex, age, BMI, CTX, P1NP, and TBS) showed a statistically significant association with VFs in any of the explored regression models. LS-BSI showed a progressively greater discrimination power in identifying patients with VFs risk when adjusted for other relevant covariates (AUC ranging from 0.74 to 0.83) (Table 6 and Figure 7).

Table 6. Performance of LS-BSI to predict vertebral fractures in logistic regression models adjusted for different covariates

Model	R^2	LS-BSI ≥ 2.2 adjusted OR; 95% CI (*p*-value)	Adjustments	AUC (95% CI); *p*-value
# 1	0.243	6.887 (1.628–29.138); *p*=0.009	Sex, Age	0.768 (0.597–0.938); *p*=0.019
# 2	0.270	7.709 (1.752–33.924); *p*=0.007	Sex, Age, BMI	0.795 (0.618–0.972); *p*=0.009
# 3	0.376	9.602 (1.251–73.696); *p*=0.030	Sex, Age, BMI, CTX, P1NP	0.825 (0.666–0.984); *p*=0.004
# 4	0.189	5.739 (1.244–26.481); *p*=0.025	TBS	0.735 (0.535–0.935); *p*=0.039
# 5	0.251	7.152 (1.355–37.755); *p*=0.020	TBS, Sex, Age, BMI	0.795 (0.616–0.974); *p*=0.009
# 6	0.388	15.120 (1.059–215.786); *p*=0.045	TBS, Sex, Age, BMI, CTX, P1NP	0.830 (0.676–0.984); *p*=0.004

LS-BSI: lumbar spine Bone Strain Index. CI: confidence interval. AUC: area under the receiver operating characteristics curve; BMI: Body Mass Index (kg/m^2). CTX: Collagen Telepeptide (ng/mL). P1NP: Aminoterminal Propeptide (ng/mL). TBS: Trabecular Bone Score.

Figure 7 ROC curve of LS-BSI alone or adjusted for confounders for detecting PHPT patients with prevalent vertebral fractures. Covariates in Model #6: LS-BSI, TBS, Sex, Age, BMI, CTX, P1NP.



2.4 Discussion

DXA is a key aspect of skeletal evaluation in bone loss syndromes such as PHPT, (213–217), but it does not provide insight into skeletal microstructure. Thus, other imaging modalities are needed. The BSI is a recently developed option in this regard. BSI is based on FEM algorithms that evaluate the stress/strain conditions of the bone when a force is applied, simulating structural deformation. Higher BSI values indicate lower bone strength. This methodology has shown promise in predicting the risk of vertebral fracture in subjects with osteoporosis (197). This is the first study to evaluate BSI in subjects with PHPT. Compared to age-matched controls, patients with PHPT have higher BSI values at the lumbar spine, femoral neck, and total hip.

In agreement with previous studies (218), our cohort showed no differences between groups in total hip BMD and lumbar spine BMD. In contrast, BSI was significantly higher (i.e. worse) at all skeletal sites in the PHPT group. The increased BSI at the lumbar spine, a site enriched in trabecular bone, supports the hypothesis that trabecular bone, in addition to cortical bone, is adversely affected in PHPT. To date, HRpQCT has been the only technique able to quantitatively detect trabecular involvement in PHPT (132), at relevant sites, resolving the inconsistency between increased fracture risk at both vertebral and non-vertebral sites and relatively well preserved trabecular bone as determined by BMD (218,219). The advantage of BSI rests in its availability, through the DXA image of lumbar spine and hip regions, areas that HRpQCT cannot directly measure.

Another DXA-derived parameter that can assess bone quality in subjects with PHPT is TBS (130,131,192,194,220). However, the predictive ability of TBS to identify VFs in PHPT is still a matter of debate (130,131,192–194,220,221). Our study confirmed a reduction of TBS in PHPT, but, in our cohort, TBS could not detect vertebral fractures. LS-BSI was the only parameter able to predict vertebral fractures. In PHPT, an LS-BSI over the threshold of 2.2, showed an approximately 6-fold increased risk of vertebral fractures. Of interest is a correlation between LS-BSI and TBS. This is not surprising because both methods address the bone quality of the trabecular compartment of bone. However, BSI can quantitate bone strength more precisely than TBS.

The evaluation of BSI in control subjects with osteoporosis showed a higher BSI at lumbar spine, femoral neck and total hip than control subjects without osteoporosis. When we compared the controls with osteoporosis versus patients with PHPT and osteoporosis, there is a significant increase in femoral neck BSI in PHPT. This could suggest that in PHPT there are additional factors that impair bone quality than those found typically in postmenopausal women with osteoporosis. Of perhaps greater importance is the evaluation of BSI in subjects with T-scores in the osteopenic range. In these subjects, LS-BSI was higher as compared to controls. This particularly interesting observation indicates early involvement of the trabecular compartment of bone. It provides, thus, even more information vis a vis fracture risk than DXA in this disease. Detecting deterioration of bone quality in osteopenic subjects is particularly relevant since skeletal impairment, either by DXA or by the fracture itself, is currently a major criterion for decision-making in PHPT(160,222,223). Further research might offer more insights into how this new method might aid overall skeletal assessment in PHPT.

A recent multicenter study performed BSI analysis of lumbar spine images showing its ability to predict re-fracture in patients with severe osteoporosis(197), and this finding was also confirmed by an Artificial Intelligence-based study(224). The BSI was a significant independent predictor of a subsequent re-fracture. Another study investigated the effect of teriparatide on different DXA-based parameters, including BSI (200). That study showed a positive effect of teriparatide on TBS and BSI, suggesting that the increase in BMD was accompanied by increased bone

strength(200). Finally, a study on a cohort of patients with mastocytosis showed a good correlation between BSI and biochemical index of disease's activity and also the BSI's ability to discriminate whose patients with a higher risk of vertebral fracture (225). This methodology, thus, has the potential to lend insight into secondary causes of osteoporosis.

If further prospective studies confirm these findings, BSI may well become a useful tool to identify subjects at higher risk of vertebral and non-vertebral fractures.

The study has some limitations. First, the cross-sectional design of the study needs to be followed by prospective investigations. Second, as a single site study, we await further single and multi-site studies to add insights into our observations. We expect that larger prospective studies may well demonstrate the utility of BSI to evaluate bone quality in PHPT.

CHAPTER 3: **RESEARCH PROJECT N°2**

DXA-BASED BONE STRAIN INDEX IN NORMOCALCEMIC PRIMARY HYPERPARATHYROIDISM*

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3.1 Introduction

Normocalcemic hyperparathyroidism (NHPT) was described for the first time in 2008. It is defined by normal levels of albumin-adjusted total serum calcium and ionized calcium with consistently elevated PTH values after ruling out secondary causes of hyperparathyroidism(226). This relative young condition's natural history and clinical consequences are not fully clarified yet (227). Heterogeneous studies using different definitions of NHPT do not draw definitive conclusions (227). Our group previously showed that a preserved bone phenotype with normal bone turnover, no significant BMD impairment, and no increased prevalence of vertebral fractures (VFs) characterise NHPT compared to subjects with primary hyperparathyroidism (PHPT) (175). Additionally, the trabecular bone score (TBS) in NHPT also seems to be preserved (228). Recently a new DXA-derived parameter of bone quality has been developed and it is called the Bone Strain Index (BSI) (8). This is a deformation index based on the Finite Element Method (FEM and can be applied both to lumbar and femoral DXA scans (195). The BSI has shown its usefulness in characterizing secondary osteoporosis (198,199) and stratifying fractures risk (196,197). We recently applied this index in a cohort of subjects with primary hyperparathyroidism showing that BSI was significantly impaired at all

skeletal sites and was able to identify PHPT subjects at high risk of fractures (229). In this study, we aim to assess BSI in subjects with NHPT.

3.2 Materials and methods:

This case–control study includes 170 subjects: 40 subjects with NHPT, 50 subjects with PHPT and 80 controls. We included in this analysis 40 out of 47 NHPT subjects already enrolled in a previous study(175) as in 7 NHPT subjects of the original cohort the DXA scans were not available at the time of BSI evaluation. We consecutively enrolled subjects with NHPT at bone outpatient clinics of three different referral centers of Lazio, Italy, with the following proportion: S. M. Goretti" Hospital, Latina: 60%, Campus Biomedico: 35% and San Giovanni Hospital, Rome: 5%. The biochemical and radiological procedures were centralized in a single center. PHPT and NHPT were defined according to International Guidelines (226).

NHPT was defined as a persistently normal total, albumin-corrected, and ionized serum calcium concentrations and persistently elevated PTH levels (at least 2 different determinations, at least 3 months of difference between the determinations), after ruling out secondary causes of hyperparathyroidism such as renal disease (glomerular filtration rate <60 mL/min), hypovitaminosis D (25–OH Vitamin D <30 ng/mL), hypercalciuria. All NHPT patients were taking oral cholecalciferol in order to maintain adequate 25-OH Vitamin D levels.

PHPT was defined as elevated or normal PTH concentrations and persistently elevated total, albumin-corrected, or ionized serum calcium levels (at least 2 different determinations, at least 3 months of difference between the determinations).

We excluded subjects with any other condition that can affect bone and calcium metabolism, such as familial hypocalciuric hypercalcemia; calcium intake below 700 mg/day, malabsorption diseases, other diseases known to affect bone metabolism (thyrotoxicosis, bowel diseases, chronic hepatic disease, depression, history of Cushing's, alcoholism, smokers, diabetes, obesity, eating disorders and rheumatological or hematological diseases); administration of drugs affecting bone and calcium metabolism (diuretics, lithium, bisphosphonates, denosumab, significant use of glucocorticoids within the past 2 years or any treatment that could affect calcium metabolism), history of possible high-energy vertebral fractures (VF), metabolic bone diseases such as Paget disease and osteogenesis imperfecta.

For each case (NHPT), 2 **controls** were identified from electronic medical records of the outpatient clinic of endocrinology at Campus Bio-Medico University of Rome, where they were referred for unrelated diseases (eg, thyroid nodules with euthyroidism) and participated in prevention program for early detection of osteoporosis. Controls were matched by age (±2 years), gender, and date of consultation at the outpatient clinic and were recruited using the aforementioned exclusion criteria. We included control subjects who had a BMD test, thoracolumbar

radiograph, and blood testing to evaluate calcium metabolism for the first time. In all control subjects with osteoporosis and/or fragility fractures, secondary causes of osteoporosis were ruled out.

The BSI analysis was conducted in a separate workstation (Tecnologie Avanzate s.r.l., Torino, Italy) starting from the raw data of the DXA images. The BSI value represents the average equivalent strain in the regions defined by DXA analysis, where a higher strain level (high BSI) indicates higher fracture risk(229). The BSI analysis and Vertebral Fracture Assessment were conducted as a blinded analysis.

Statistics Statistical analysis was performed using the IBM SPSS Statistics for Windows, version 22.0 (IBM Corp, Armonk, New York). Nominal variables were displayed as numbers and percentages; comparisons between the groups were analysed via a χ test. Continuous variables with normal distribution were presented as mean ± standard deviation (SD) and non-normally distributed variables as median and interquartile range (IQR). One-way analysis of variance (ANOVA) or nonparametric Kruskal-Wallis test were applied for all comparisons between the subgroups, as appropriate. In the cases of statistically significant intergroup differences, multiple pairwise comparisons were tested via Tukey post-hoc analysis (ANOVA) or Bonferroni correction (Kruskal-Wallis). For all tests, a level of $p=0.05$ was set for statistical significance. Correlation between serum PTH and BSI parameters was tested with Spearman's coefficient (r). Correlation strengths were defined as follows: 0-0.30, negligible; 0.30-0.50, low; 0.50-0.70, moderate; 0.70-0.90, high and 0.90-1, very high.

Ethics The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Principles of Good Clinical Practice. Local ethics committees approved the research protocol and all participants gave informed consent allowing their anonymized information to be used for data analysis

3.3 Results

Table 7 shows the baseline characteristics of the study population. The enrolled patients were mostly female (n=159; 93%). The range of total calcium, ionized calcium and PTH were 8.5–11.8 pg/mL, 1.1–1.7 mg/dL and 15.0–502.0 mg/dL, respectively. There were no differences in age, sex and menopause age between groups. 12 subjects with NHPT (30%) have ionized calcium in the higher quartile of normal range.

Table 8 shows the comparisons of BSI, BMD and TBS parameters between study groups. FN-BSI was lower in NHPT compared to PHPT (1.52 ± 0.31 vs 1.72 ± 0.42 p=0.031), while there were no differences between NHPT and controls. TH-BSI was lower in NHPT compared to PHPT (1.36 ± 0.23 vs 1.52 ± 0.34, p=0.030), while there were no differences between NHPT and controls. LS-BSI was not different between NHPT and both PHPT and controls. LS-BMD, FN-BMD, TH-BMD and TBS was similar between NHPT and both PHPT and controls. A statistically significant but negligible positive correlation was documented between serum PTH and BSI parameters (FN-BSI: r=0.170, p=0.020; TH-BSI: r=0.145, p=0.048; LS-BSI: r=0.170, p=0.020).

Table 7. Baseline characteristics and between-groups differences of enrolled population

Variable	Whole population (n=170)	PHPT (n=50)	NHPT (n=40)	Control (n=80)	*p*-value
Sex (male)	11 (6.5)%	3 (6.0)%	3 (7.5)%	5 (6.3)%	0.834
Age (years)	64.9 ± 9.3	65.2 ± 11.6	63.4 ± 9.0	65.4 ± 7.8	0.532
Menopause age (years)	50.2 ± 3.6	50.2 ± 3.9	50.9 ± 4.5	49.8 ± 3.0	0.383
Menopause length (years)	14.8 ± 8.6	15.2 ± 9.7	13.1 ± 9.0	15.3 ± 7.7	0.425
Body mass index	26.8 ± 5.1	27.9 ± 6.0[§]	27.4 ± 4.5	25.8 ± 4.4[§]	0.041
Overall vertebral fractures	36 (21.3%)	18 (36.7%)[§]	8 (20.0%)	10 (12.5%)[§]	0.005
Moderate-severe vertebral fractures	16 (9.5%)	10 (20.4%)[§†]	2 (5.0%)[†]	4 (5.1%)[§]	0.037
25(OH) vitamin D (ng/mL)	33.0; 27.0-38.0	32.7; 27.0-36.4[§]	35.3; 32.0-37.9[§†]	31.0; 23.5-40.0[†]	0.013
Albumin-adjusted serum calcium (mg/dL)	9. 8 ± 0.7	10.8 ± 0.4[§†]	9.4 ± 0.5[†]	9.4 ± 0.4[§]	<0.001
Ionized serum calcium (mmol/L)	1.3; 1.2-1.3	1.3; 1.3-1.4[§†]	1.2; 1.2-1.2[§]	1.2; 1.2-1.3[†]	<0.001
Parathyroid hormone (pg/mL)	81.6; 56.0-120.0	116.9; 104.0-153.2[§]	120.0; 109.3-141.0[†]	55.0; 44.5-64.0[§†]	<0.001
Phosphate (mg/dL)	3.2 ± 0.6	2.8 ± 0.5[§†‡]	3.1 ± 0.5[§†‡]	3.6 ± 0.5[§†‡]	<0.001

Creatinine (mg/dL)	0.7 ± 0.1	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.597
GFR (ml/min/1.73 m^2)	80.9; 68.0-99.5	87.4; 68.0-102.3	80.2; 64.8-103.8	78.3; 72.5-93.7	0.362

Data are presented as: "means ± standard deviations", "medians; interquartile range" or "number (percentage)". GFR: glomerular filtration rate.

§, †, ‡: statistically significant difference in pairwise comparisons (identical symbols identify pairs of values whose differences were statistically significant, i.e. p <0.05). Normal values: Total calcium 8.4-10.2 mg/dL, ionized calcium 1.13-1.32 mmol/L, iPTH 14-72 pg/mL

Table 8. Differences of BSI, BMD and TBS parameters between study groups

Method	Group	Parameter	*p*-value	Pairwise comparisons
Bone strain index (BSI)				
Femoral neck	PHPT	1.72 ± 0.42	0.001	PHPT vs NHPT: *p*=0.031
	NHPT	1.52 ± 0.31		PHPT vs Control: *p*=0.001
	Control	1.47 ± 0.35		NHPT vs Control: *p*=n.s.
Total hip	PHPT	1.52 ± 0.34	0.001	PHPT vs NHPT: *p*=0.030
	NHPT	1.36 ± 0.23		PHPT vs Control: *p*=0.001
	Control	1.34 ± 0.26		NHPT vs Control: *p*=n.s.
Lumbar spine	PHPT	2.28 ± 0.60	0.023	PHPT vs NHPT: *p*=n.s.
	NHPT	2.11 ± 0.65		PHPT vs Control: *p*=0.017
	Control	2.01 ± 0.44		NHPT vs Control: *p*=n.s.

Bone mineral density (BMD) g/cm^2				
Femoral neck	PHPT	0.63 ± 0.11	0.101	/
	NHPT	0.67 ± 0.11		/
	Control	0.67 ± 0.08		/
Total hip	PHPT	0.79 ± 0.14	0.100	/
	NHPT	0.84 ± 0.12		/
	Control	0.84 ± 0.10		/
Lumbar spine	PHPT	0.82 ± 0.19	0.327	/
	NHPT	0.88 ± 0.18		/
	Control	0.87 ± 0.14		/
Trabecular bone score (TBS)				
	PHPT	1.24 ± 0.10	0.010	PHPT vs NHPT: p=n.s.
	NHPT	1.29 ± 0.14		PHPT vs Control: p=0.009
	Control	1.30 ± 0.07		NHPT vs Control: p=n.s.

Data are presented as: "means ± standard deviations" or "medians; interquartile range". PHPT: primary hyperparathyroidism. NHPT: normocalcemic hyperparathyroidism. n.s.: non statistically significant

3.4 Discussion

This is the first study evaluating the BSI in subjects with NHPT. Since 2009 different studies have evaluated the prevalence and bone involvement in NHPT, but the strict criteria to define this condition were not followed in many studies (230), therefore the interpretation of the findings is tricky. In this study, we showed that in NHPT both trabecular and cortical compartment assessed by BSI seems not to be

significantly affected by the chronic elevation of PTH. So, differently from a subject with PHPT who showed a cortical and trabecular impairment with higher BSI value than controls (229), in NHPT the catabolic effect of PTH seems to be mitigated. Indeed, the BSI values of the hip, both femoral neck and total hip BSI, are similar to controls and significant better than hypercalcaemic hyperparathyroidism. These results align with our previous evidence of substantially normal bone phenotype in NHPT, assessed by DXA and TBS, while the biochemical profile is intermediate between PHPT and controls (175). As primary hyperparathyroidism is a secondary endocrine related osteoporosis where BMD evaluation does not fully help clinicians to stratify the risk of fractures (231), the lack impairment of BMD in NHPT could be related to the inability of BMD to catch the bone involvement in this condition. BSI showed to be an adequate tool to study the bone impairment in PTH induced bone fragility (229). Indeed, BSI includes information on density distribution, bone geometry and loadings differently from BMD and TBS, which are based on the quantification of bone mass and distribution averaged over the DXA scan (232). Thus, the results of this study might be a reliable confirmation of the poor involvement of bone structure during NHPT, and still casts doubts on the real existence of this pathological condition. These findings are also confirmed by the lower number of moderate-severe VFs in NHPT than PHPT.

Indeed, the population-based studies aimed to investigate the natural history of NHPT suggested that only about one-third of NHPT subjects confirmed their biochemical alterations over time (167,227).

Otherwise, NHPT might be an initial form of PHPT or a condition characterized by a resistance to the PTH action (170,233)

Prospective studies are needed to investigate if there is an evolution of bone involvement over time or, as shown in preliminary NHPT studies.

Our study has limitations: the cross-sectional study design and the lack of third-generation PTH assays. Further prospective studies are needed to confirm these findings and give an insight into the natural history of NHPT to improve knowledge and management of this condition.

CHAPTER 4: **RESEARCH PROJECT N°3**

BONE QUALITY, AS MEASURED BY TRABECULAR BONE SCORE IN NORMOCALCAEMIC PRIMARY HYPERPARATHYROIDISM *

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4.1 Introduction

Over the past several decades, phenotypic variability in the clinical presentations of primary hyperparathyroidism (PHPT) has become evident (185), due to the widespread use of PTH measurements in the evaluation of those with or suspect to have a metabolic bone disease.

Normocalcemic hyperparathyroidism (NHPT) was first described in 2008, as a condition characterized by levels of albumin-adjusted total serum calcium and ionized calcium consistently within normal limits associated with constantly elevated PTH values after ruling out secondary causes of high PTH (233,234).

Up to now, its true prevalence, natural history and clinical consequences have not been fully clarified. Nevertheless, although few previous studies reported bone impairment in patients with NHPT (176,178,235), the largest recent cross sectional evaluation showed no significant reduction of BMD nor increased number of fragility VFx compared to controls (175).

In both PHPT and NHPT, data on microstructural features by HRpQCT are available. While in PHPT patients, both cortical and trabecular microarchitectural abnormalities have been reported (132), in NHPT subjects HRpQCT showed an impairment in cortical geometric properties with preserved trabecular parameters (180).

However, HRpQCT remains a research tool and other more widely available techniques are required to evaluate in clinical settings bone quality in NPHPT.

TBS is a textural analysis of images acquired by DXA of the lumbar spine (LS) (5). Applied to assess bone microarchitecture, TBS correlates well with standard 3-dimensional (3D) parameters of bone microarchitecture, and appears to be independent of BMD (236,237).

In PHPT, TBS has not consistently stratified fracture risk (130,131,192–194,220); in NPHPT, there is essentially only one study (238).

In this report, we present TBS data from a cohort of NHPT patients compared with PHPT patients and control subjects (175).

4.2 Material and methods

4.2.1 Study design and population

Detailed inclusion and exclusion criteria have already been reported elsewhere (175). Briefly, in this study, we consecutively enrolled subjects with PHPT and NHPT at bone outpatient clinics of the following centers: Unit of Endocrinology and Diabetes, Campus Bio-Medico University of Rome; Department of Internal Medicine, "S. M. Goretti" Hospital, Latina; Department of Endocrinology, San Giovanni Addolorata Hospital, Rome and Department of Endocrinology, CTO “A. Alesini” Hospital, Rome.

NHPT was defined as a persistently normal total, albumin-corrected, and ionized serum calcium levels and persistently elevated PTH concentrations (at least 2 different evaluations), after ruling out the secondary causes of hyperparathyroidism such as renal function impairment (eGFR <60 mL/min), hypovitaminosis D (25(OH)D3 <30 ng/mL), daily calcium intake below 700 mg/day and hypercalciuria. All NHPT subjects received supplementation with oral cholecalciferol in order to maintain adequate 25(OH)D3 levels. PHPT was described as elevated or normal PTH levels and persistently elevated total, albumin-corrected, or ionized serum calcium levels (at least 2 different evaluations).

We excluded subjects with any other condition that can affect bone and calcium metabolism, malabsorption diseases, administration of drugs affecting bone and calcium metabolism, history of possible traumatic vertebral fractures, metabolic bone diseases such as Paget disease and osteogenesis imperfecta.

Control subjects without any abnormalities of calcium, phosphate and PTH levels were consecutively recruited based on the above-mentioned exclusion criteria from the outpatient clinic of endocrinology at Campus Bio Medico University of Rome, where they were referred for unrelated diseases (thyroid nodules with euthyroidism). Before enrollment, all controls had never performed a BMD evaluation. In all control subjects with low BMD, we ruled out secondary causes of osteoporosis in accordance with the current edition of Italian guidelines (239).

Clinical and biochemical evaluation. We performed a physical examination and reviewed the previous medical records in order to evaluate the clinical profile of the whole study population.

In the morning (from 8:00 to 8:30 AM) fasting blood samples were obtained. Serum total calcium (normal, 8.4–10.2 mg/dL), albumin (normal 3.2-4.6 g/dl), serum phosphate (normal, 2.3-4.7 mg/dL), creatinine (normal 0.55-1.2 mg/dl) and 25(OH) D (normal, 30-100 ng/mL) were measured using automated methods. Calcium values were corrected for albumin concentration using the formula: corrected calcium (mg/ dL) = measured total calcium (mg/dL) + 0.8 (4.0 – serum albumin [g/dL]). Ionized serum calcium (normal, 1.13–1.32 mmol/L) was measured by potentiometric method on the GEM PREMIER 4000 analyzers (Werfen, Le Pré-Saint-Gervais, France). Intact PTH (normal, 14-72 pg/ml) was measured by an immunochemiluminometric assay using the automatic analyzer Modular E170 (Roche Diagnostics, Indianapolis, Ind, USA) in the Laboratory at Campus Bio-Medico University of Rome. 24-h urinary calcium was assessed by calorimetry. eGFR was calculated using the MDRD equation. Serum levels of CTX were assayed by the β-CrossLaps (ECLIA; β-CrossLaps/Serum, Roche Diagnostics, Basel,

Switzerland), which uses two monoclonal antibodies against β-cross-linked CTX according to the manufacturer's protocol. Serum levels of P1NP were analysed by sensitive electrochemiluminescent detection technology and was formatted for the Cobas Total P1NP (Roche Diagnostics) automated analyser.

Dual-energy X-ray absorptiometry. BMD by DXA was measured at the LS (L1–L4), total hip (TH), femoral neck (FN), and non-dominant forearm (one-third distal radius-Radial) using a dual-energy X-ray absorptiometer (Hologic Discovery QDR Instrument, MA, USA). Data was reported as absolute bone density, Z-score (SD values from the mean for gender, ethnicity and age-matched healthy population) and T-scores (SD values from the mean for a sex- and young reference population). All scans were performed according to the International Society for Clinical Densitometry (ISCD) guidelines (202). Fractured vertebrae and vertebrae with structural changes were excluded from the analysis (T-score difference with the adjacent vertebra > 1.0).

Vertebral fracture assessment (VFA). VFx were evaluated by DXA scanning of the spine for VFA by quantitative morphometry (QM). In order to confirm these findings in presence of scoliosis and disc space osteoarthritis (208), conventional spinal radiographs (T4–L4) in lateral and antero-posterior projection was performed. A single experienced investigator read all images and scored VFx using the Genant semiquantitative method (grade 1 – mild, grade 2 - moderate, grade 3 - severe) (209).

Trabecular bone score. TBS was automatically derived from the same region of LS DXA by a dedicated software (Insight TBS, Medimaps Groupe, Geneve, Switzerland). TBS was calculated as the mean value of the individual determinations for LS vertebrae L1–L4. Vertebrae excluded for BMD measurement were also excluded for TBS evaluation at LS. Fracture-resistant microarchitecture is reflected by higher TBS instead lower values may reflect frail microarchitecture (236). We defined low TBS as TBS <1.200(240).

Ethics. The study was conducted in compliance with the Declaration of Helsinki and the International Conference on Harmonization Principles of Good Clinical Practice. The research protocol was approved by Local Ethics Committees and all participants gave informed consent allowing their anonymized information to be used for a data analysis.

4.2.2 Statistical analysis

The characteristics of the study participants were reported using mean and standard deviation for continuous variables and proportions for categorical variables, by groups defined according to the diagnosis of NHPT, PHPT or control group. Differences between groups were analyzed using analysis of variance for continuous variables and $\chi 2$ test for proportions, and a Tukey test for the post-hoc comparisons between groups was performed.

Receiver operating characteristic (ROC) curves analysis were created to estimate the best cut-off values of TBS, LS Z-score, LS Z-score adjusted for TBS (LS Z-score*TBS), LS T-score adjusted for TBS (LS T-score*TBS), Radial Z-score and FN Z-score for detecting patients with overall VFx or moderate-severe VFx. On the basis of these cut-offs, sensitivity (SN), specificity (SP) and overall accuracy of these parameters were calculated. A further analysis was performed to compare the diagnostic accuracy of FN Z-score alone with that of the combination FN Z-score plus TBS using the c statistic.

4.3 Results

Baseline characteristics

The study population consisted of 127 adults (47 subjects with NHPT, 41 with PHPT and 39 controls). Table 9 summarizes key patient demographics and baseline characteristics. The mean age of the study population was 64.1 years (SD 9.6); 91% female (postmenopausal women).

In particular, patients with NHPT presented higher Radial BMD compared with PHPT (0.605, SD 0.080 vs 0.563, SD 0.078, P = .031). The prevalence of VFs was 28% in NHPT group, 23% in controls and 60% in PHPT group.

Table 9. General characteristics of the population.

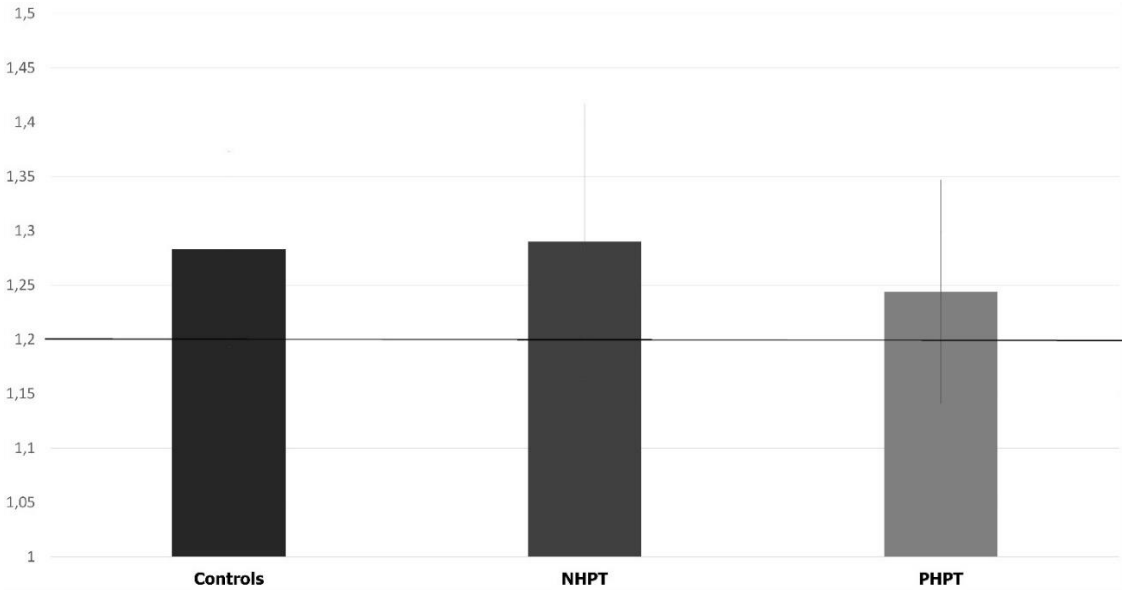
	Controls N: 39	NHPT N: 47	PHPT N: 41
Age (years)	64.7 (7)	63.8 (9.3)	63.9 (12)
Female (%)	90	91	93
BMI (kg/m^2)	26.2 (4.7)	26.7 (4.9)	27.4 (5.3)
Time from menopause (years)	14 (7.6)	13.7 (9.8)	14 (9.6)
eGFR (ml/min/1.73 m^2)	86.86 (15.65)	83.44 (23.68)	92.11 (24.46)
Serum calcium concentration (mg/dl)	9.5 (0.4)	9.4 (0.4)[c]	10.8 (0.4)[a]
Serum calcium ion concentration (mmol/l)	1.22 (0.05)	1.19 (0.05)[b, c]	1.35 (0.05)[a]
24-h urine Calcium (mg/24h)	192.3 (76)	196.1 (49.2)[c]	293.5 (146.3)[a]
Serum phosphate concentration (mg/dl)	3.8 (0.4)	3.2 (0.5)[a, c]	2.8 (0.5)[a]
Serum PTH concentration (pg/ml)	52.4 (15.4)	126.8 (29.5)[a]	139.1 (49.7)[a]
25-OH Vitamin D concentration (ng/ml)	28.6 (12.8)	36.7 (6.6)[a]	31.1 (7.8)[b]
Calcium*Phosphorus	35.66 (3.84)	29.55 (4.42)[a]	29.78 (5.26)[a]
Serum CTX concentration (ng/ml)	0.33 (0.21)	0.37 (0.18)	0.49 (0.27)*
Serum P1NP concentration (ng/ml)	50.12 (24.14)	61.33 (25.41)	73.09 (42.09)*
TBS	1.283 (0.09)	1.290 (0.127)	1.244 (0.103)
TBS<1.2 (%)	15.4	23.4	26.8
T-score*TBS	-1.95 (1.00)	-1.93 (1.30)	-2.37 (1.05)
Z-score*TBS	0.07 (0.93)	-0.04 (1.12)	-0.48 (1.06)[b]
LS BMD (g/cm^2)	0.904 (0.149)	0.893 (0.186)	0.880 (0.184)
LS T-score	-1.3 (1.3)	-1.4 (1.7)	-1.5 (1.6)
LS Z-score	0.3 (1.4)	0.2 (1.7)	0.1 (1.8)
FN BMD (g/cm^2)	0.671 (0.075)	0.659 (0.108)	0.633 (0.107)
FN T-score	-1.6 (0.7)	-1.8 (0.9)	-2 (1)
FN Z-score	-0.1 (0.6)	-0.2 (0.9)	-0.4 (0.8)
TH BMD (g/cm^2)	0.872 (0.097)	0.819 (0.125)	0.795 (0.126)[b]
TH T-score	-0.6 (0.7)	-1.1 (0.9)	-1.2 (1)[b]
TH Z-score	0.5 (0.7)	0.0 (0.9)	-0.0 (1.0)[b]
Radial BMD (g/cm^2)	0.620 (0.065)	0.605 (0.080)[d]	0.563 (0.078)[b]
Radial T-score	-1.3 (0.8)	-1.6 (1.2)[d]	-2.3 (1.3)[a]
Radial Z-score	0.3 (0.8)	0.0 (0.9)[d]	-0.5 (1.1)[a]
Overall vertebral fractures (%)	23	28	60[b]
Renal lithiasis (%)	3	13	10

Abbreviations: BMI = body mass index; BMD = bone mineral density; CTX = beta CrossLaps; eGFR = estimated glomerular filtration rate; FN = femoral neck; LS = lumbar spine; NHPT = normocalcemic hyperparathyroidism; PHPT = primary hyperparathyroidism; PTH = parathyroid hormone; TH = total hip; TBS = trabecular bone score.
Radial: 1/3 distal radius
Data reported as mean (SD) or n (%).
[a] $P < .001$ vs control group;
[b] $P < .05$ vs control group;
[c] $P < .001$ vs PHPT;
[d] $P < .05$ vs PHPT.

Trabecular bone score

We did not find any significant difference in terms of TBS and LS T-score*TBS between NHPT participants, PHPT group and controls (Figure 10; Table 9). The same results were observed considering LS T-score*TBS. We found a lower LS Z-score*TBS in PHPT participants when compared with controls (-0.48 ± 1.06 vs 0.07 ± 0.93, p: 0.017) (Table 9). Moreover, the prevalence of low TBS was 23.4% in NHPT, 26.8% in PHPT and 15.4% in controls, without statistically differences between groups. Furthermore, we found that subjects with low TBS had higher BMI compared with those with TBS>1.200 in both NHPT (30.42 kg/m2 ± 5.73 vs 25.75 kg/m2 ± 4.19, p: 0.034) and PHPT (32.28 kg/m2 ± 4.59 vs 25.82 kg/m2 ± 4.49, p<0.001) groups.

Figure 10. TBS in controls, NHPT and PHPT participants



Accuracy of trabecular bone score in detecting subjects with vertebral fractures

The ROC analyses have been performed to assess the best threshold values of TBS, LS Z-score, LS Z-score*TBS, LS T-score*TBS, Radial Z-score, FN Z-score for detecting all VFx in NHPT group. The results showed that all these parameters are not able to detect overall VFx in NHPT group (Table 10).

Table 10. Diagnostic performance of DXA-derived parameters and TBS in detecting overall VFx in NHPT participants

	AUC (95% CI)	Threshold	Specificity	Sensitivity	Accuracy
TBS	0.515 (0.327-0.703)	1.254	0.676	0.538	0.638
LS Z-score*TBS	0.45 (0.253-0.648)	-0.15	0.559	0.538	0.553
LS T-score*TBS	0.509 (0.321-0.698)	-2.45	0.706	0.462	0.638
LS Z-score	0.457 (0.269-0.645)	0.25	0.485	0.615	0.522
Radial Z-score	0.489 (0.289-0.688)	0.2	0.606	0.5	0.578
FN Z-score	0.678 (0.499-0.858)	-0.05	0.758	0.692	0.739
TH Z-score	0.569 (0.388-0.749)	-0.0	0.455	0.538	0.456

Abbreviations: DXA = dual-energy x-ray absorptiometry; FN = femoral neck; LS = lumbar spine; NHPT = normocalcemic hyperparathyroidism; TBS = trabecular bone score; TH = total hip; VF = vertebral fracture.
Radial: 1/3 distal radius.

Furthermore, LS T-score*TBS demonstrated a moderate accuracy for identifying moderate-severe VFx in subjects with NHPT (Table 11).

Table 11. Diagnostic performance of DXA-derived parameters and TBS in detecting moderate-severe VFx in NHPT participants

	AUC (95% CI)	Threshold	Specificity	Sensitivity	Accuracy
TBS	0.714 (0.461-0.967)	1.304	0.667	0.8	0.681
LS Z-score*TBS	0.81 (0.62-0.996)	0.55	0.833	0.8	0.83
LS T-score*TBS	0.719 (0.464-0.952)	-0.85	0.857	0.6	0.83
LS Z-score	0.705 (0.497-0.913)	-0.05	0.463	1	0.522
Radial Z-score	0.71 (0.54-0.88)	0.2	0.634	1	0.667
FN Z-score	0.624 (0.364-0.885)	-0.95	0.293	1	0.37
TH Z-score	0.544 (0.291-0.797)	-0.5	0.317	1	0.630

Abbreviations: DXA = dual-energy x-ray absorptiometry; FN = femoral neck; LS = lumbar spine; NHPT = normocalcemic hyperparathyroidism; TBS = trabecular bone score; TH = total hip; VF = vertebral fracture.
Radial: 1/3 distal radius.

Regarding PHPT group, TBS was not able to detect overall VFx (Threshold 1.292, AUC 0.52 95%CI 0.336-0.718) and the same findings have been confirmed also for the identification of moderate-severe VFx (data not shown).

The combination of FN Z-score with TBS does not improve the ability of overall VFs detection compared to FN Z-score alone in both NHPT (c statistic 0.683 vs 0.678, P=0.715) and PHPT (c statistic 0.569 vs 0.548, P=0,830) population.

4.4 Discussion

This is the first study that systematically evaluated the TBS, a surrogate marker of bone quality, in a large cohort of well-defined subjects with NHPT. We showed that compared to controls and PHPT, NHPT population did not have statistically different TBS values. Moreover, TBS tool may increase the accuracy of DXA in individuating only moderate-severe VFx, while it seems not to be able to capture patients with mild VFx.

It has been well demonstrated that bone strength and fracture risk are influenced also by other factors than bone mass (241), and this is one of the reasons why BMD assessed by DXA is not able to adequately predict the increased risk of fractures in patients with PHPT (128,129).

While some studies investigated bone quality by HRpQCT in PHPT subjects (132,190), only one investigation used this tool in NHPT patients. In that study, an impairment of cortical bone microstructure was reported, but the inclusion of subjects with 25(OH)D3 levels <30 ng/ml might have influenced that finding (180).

However, while HRpQCT still remains a research tool, TBS is a DXA-derived index of bone microarchitecture that is simple and easy to perform in clinical practice (237).

Indeed, TBS has been demonstrated to be an accurate tool in predicting fracture risk in many clinical settings of secondary osteoporosis (242,243). In particular, in subjects with diabetes, TBS captured fracture risk and skeletal deterioration more

accurately than BMD (244,245). Furthermore, TBS has greater discriminative power than BMD for bone quality deterioration and fracture risk assessment in subjects with glucocorticoid-induced osteoporosis (246), remarkably also in those with only a mild cortisol hypersecretion (247).

This latter finding has rendered the TBS determination in NHPT, which is considered by some authors a possible mild form of PHPT (110,248,249) as an interesting area of investigation.

However, the utility of TBS in predicting fragility fractures in patients with PHPT is still uncertain (130,131,192–194,220). In particular, some authors (131,192,194,220) showed a significant reduction of TBS in PHPT subjects that seems to be associated with VFx after adjustment for confounding factors. Likewise, Silva et al. (130) reported significant positive correlations between TBS and measurements of trabecular microarchitecture assessed by HRpQCT, suggesting that low TBS may also indicate impaired bone strength. Conversely, in the largest study published to date, Grigorie et al. (193) showed that even if PHPT patients had mean TBS values in the partially degraded range, TBS was not independently associated with fractures. In our cohort, PHPT showed a lower LS Z-score*TBS compared to controls even if it was not able to detect VFx in this clinical setting.

Moreover, Tay et al. did not reveal any significantly impaired values of TBS in nonobese PHPT subjects (221). We founded that both NHPT and PHPT with fully degraded skeletal microarchitecture, as measured by TBS, had higher BMI than those with TBS>1.200. These findings suggest the detrimental effect of obesity on bone quality, as previously reported in PHPT (221).

Longitudinal studies using TBS in PHPT are limited, and data regarding TBS changes after parathyroidectomy are largely contradictory (131,221).

To date, the only study investigating TBS in NHPT subjects (238) was not able to demonstrate differences in TBS between NHPT and asymptomatic PHPT patients.

The small sample size, the inability to rule out all causes of secondary hyperparathyroidism and the lack of fractures evaluation rendered the results of this study incomplete.

In accordance with that previous study, we did not find significant differences in terms of TBS or Z-score*TBS between NHPT and the other groups. Furthermore, this tool did not provide an additional advantage in detecting overall VFx compared to BMD. The ability to predict overall VFx remains poor even when combining the TBS with FN-Z-score, in both in the NHPT and PHPT groups. LS Z-score*TBS has an acceptable accuracy (83%) in detecting moderate-severe VFx in NHPT group. This may be in line with the fact that TBS seems to reflect moderate-severe deteriorations of bone quality while it is not able to detect small reductions in bone quality (250). Therefore, it is not surprising that LS Z-score*TBS could be usefully used for detecting patients with moderate-severe VFx, that represent a moderate-severe reduction of bone quality, rather that mild VFx, that account for a slight bone quality reduction (251).

We already showed that in NHPT bone density is not remarkably reduced and is similar to that the bone phenotype of healthy control subjects (175). This further data of unimpaired bone quality assessed by TBS in NHPT group support the hypothesis that this condition may represent a very early stage of classic PHPT (110,248,249). However, the finding that in NHPT the TBS determination could increase the accuracy in detecting moderate-severe VFx encourages further studies along these lines.

Our evaluation has particular strengths: we have studied one of the largest cohorts of NHPT and biochemical and radiological assessments were conducted in a single center. Our study is limited by its cross-sectional design and the lack of use of a third generation PTH assay.

Notwithstanding the afore-mentioned limitations, the results of the present study suggest that NHPT patients do not show significant impairment of bone

microarchitecture as assessed by TBS. In NHPT, TBS does not appear to identify patients with mild VFx. Rather, it might offer advantages in identifying those with moderate-severe VFx.

IMPACT OF MY RESEARCH PROJECT AND FUTURE PERSPECTIVES

Dxa-based bone strain index: a new tool to evaluate bone quality in primary hyperparathyroidism.

The stratification of fracture risk in subject with PHPT by DEXA did not fully explain the increased fractures risk at both vertebral and non-vertebral sites. Until recently, TBS was the only clinical tool to evaluate bone quality in PHPT with contrasting data on the ability to detect subject at high risk of fracture. This is the first study to evaluate the BSI in subjects with PHPT. BSI has shown promising results in predicting the risk of vertebral fracture in other secondary osteoporosis. Our results are in agreement with hrPQCT findings showing an impairment of both cortical and trabecular site. Further prospective studies are needed to confirm these results and BSI could become a suitable tool to identify subjects at higher risk of vertebral and non-vertebral fractures in this clinical setting.

Future perspectives

- A prospective trial aimed to evaluate the ability of BSI in predicting fractures.

-A prospective trial to evaluate the change in BSI before and after parathyroidectomy.

Dxa-Based bone strain index in normocalcemic primary hyperparathyroidism

Since 2009 different studies have evaluated the bone involvement in NHPT, but the results are still not clear. This is the first study evaluating the BSI in subjects with NHPT. In this study, we showed that in NHPT both trabecular and cortical compartment assessed by BSI seems not to be significantly affected by the chronic elevation of PTH. The results of this study might be a reliable confirmation of the poor involvement of bone structure in NHPT, and still casts doubts on the real

existence of this pathological condition. Prospective studies are needed to investigate if there is an evolution of bone involvement over time, as shown in preliminary NHPT studies.

Future perspectives

- A prospective trial aimed to evaluate change of BSI over time.

Bone quality, as measured by trabecular bone score in normocalcaemic primary hyperparathyroidism.

This is the first study that systematically evaluated TBS, in a large cohort of well-defined subjects with NHPT. TBS values were similar in NHPT and PHPT and controls. However, TBS tool can improve the accuracy of DXA in individuating moderate-severe VFx.

Future perspectives

-We designed a new trial aimed to evaluate the bone material properties assessed by microindentation technique in NHPT compared to PHPT and healthy subjects.

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