NEW DIAGNOSTIC AND THERAPEUTIC ASPECTS OF SUBCLINICAL AND CLINICAL FORMS OF PARATHYROID GLAND DISEASES

Andrea Palermo, MD

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University Campus Bio-Medico, Rome, Italy

In collaboration with

Academic Unit of Bone Metabolism,

University of Sheffield, Sheffield, UK

…To my family and to the people who have been protecting me from the heaven

STATEMENT OF ORIGINALITY

Unless otherwise stated, the work described in this thesis was carried out at the University Campus Bio-Medico, Rome, Italy and at Academic Unit of Bone Metabolism, University of Sheffield, UK.

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 "**New Diagnostic and therapeutic aspects of subclinical and clinical forms of parathyroid gland diseases**" has not been submitted for a degree or any other qualification at any other university.

Andrea Palermo, September 2016

1. RESEARCH QUESTION

Can alendronate and vitamin D improve the bone mineral density in patients with normocalcemic primary hyperparathyroidism?

ABSTRACT

Introduction: Normocalcemic primary hyperparathyroidism (NPHPT) is defined by normal serum calcium and consistently elevated PTH levels after ruling out the causes of secondary hyperparathyroidism. It is likely that subjects with NPHPT may develop kidney and bone disease. As no data on the pharmacological treatment of NPHPT are available, we aimed to investigate the effects of alendronate and cholecalciferol on both BMD and bone biochemical markers in postmenopausal women with NPHPT. Safety of vitamin D was evaluated as secondary endpoint.

Methods and design: The study was a prospective open label randomized trial comparing 15 postmenopausal women with NPHPT (PMW-NPHPT), treated with oral alendronate plus cholecalciferol (treated group) and 15 PMW-NPHPT treated only with cholecalciferol (control group). Blood samples were obtained at baseline and after 3, 6, and 12 months. Bone turnover markers (BTM) were measured at baseline, 3, and 6 months, respectively. BMD was assessed at baseline and after 12 months.

Results: After 1 year of treatment, BMD increased significantly at the lumbar, femoral neck, and hip level in the treated group, but not in the control group ($p = 0.001$). No differences were found between or within groups in serum calcium, PTH, and urinary calcium levels. BTM significantly decreased in the treated group but not in the control group, at 3 and 6 months ($p<0.001$), respectively. No cases of hypercalcemia or hypercalciuria were detected during the study.

Conclusion: The results of this study indicate that alendronate/cholecalciferol may increase BMD in postmenopausal women with NPHPT. Alendronate/cholecalciferol or vitamin D alone does not affect serum or urinary calcium.

2. RESEARCH QUESTION

What is the prevalence and the metabolic bone profile of the normocalcaemic hypoparathyroidism?

ABSTRACT

Introduction: There are no consistent data on the prevalence and bone status of normocalcaemic hypoparathyroidism (NHYPO) as defined by normal adjusted calcium and low PTH level. Our aim was to determine the prevalence and the metabolic bone profile of NHYPO in older women, assessing its evolution over time. The second objective was to evaluate the prevalence of other calcium metabolic disorders.

Methods and design: The Osteoporosis and Ultrasound Study (OPUS) is a 6-yr prospective study of fracture-related factors. A total of 2419 older women (age 55-79 yrs) and 258 younger women (age 30-40 yrs) participated. Complete follow-up data were available in 1416 subjects. After calculating the adjusted calcium according to James' formula, we identified 'abnormal' calcium and PTH using Mahalanobis distances and allocated older women into different pathological categories using reference intervals from the healthy young women.

Results: We identified 57 subjects with NHYPO (2·4%). These women had lower than expected bone turnover as assessed by bone alkaline phosphatase (-14·5%, 95% CI: -26·2 to -3·0, P = 0·007), CTX (-66·3%, 95% CI: -74·0 to -56·4, P < 0·001) and osteocalcin (-36·8%, 95% CI: -45·6 to -26·6, P < 0·001). After 6 years, of the 35 NHYPO subjects with follow-up data, none developed overt hypoparathyroidism and only 15 (0·6%) subjects had persistent evidence of NHYPO. We also identified 86 subjects (3·6%) affected by hyperparathyroid hypercalcaemia.

Conclusion: This is the first large population-based study to investigate NHYPO in older women. NHYPO is fairly common, not always persistent and is characterized by low bone turnover.

3. RESEARCH QUESTION

Can PTH(1-34) treatment restore the calcium and phosphate balance and improve the quality of life in adult subjects with post-operative hypoparathyroidism?

ABSTRACT

Introduction: Conventional therapy for hypoparathyroidism consists of calcium and calcitriol, but sometimes normal serum calcium cannot be maintained, and/or this approach might lead to nephrocalcinosis, nephrolithiasis, or renal insufficiency. The objective of the study was to investigate the effects of 6 months of PTH(1-34) treatment in adult subjects with postoperative hypoparathyroidism and to evaluate quality-of-life changes.

Methods and design: This is an Italian multicentric 2-year prospective, open-label study that has included 42 subjects with surgical hypoparathyroidism (90% females, age range 34-77 y). The intervention included a twice-daily PTH(1-34) 20 μg sc injection. At baseline and after 6 months of PTH(1-34) treatment, calcium and vitamin D supplementation requirements, serum calcium, phosphate, creatinine, alkaline phosphatase, uric acid, and 24-hour urinary calcium excretion were evaluated. Quality of life was evaluated by the Rand 36-Item Short Form Health Survey covering eight domains of physical and mental health.

Results: The mean serum calcium levels significantly increased from baseline to 15 days (7.6 ± 0.6 vs 9.1 ± 0.9 mg/dL, $P < .001$) and remained stable until the end of the observational period, despite a significant reduction in calcium and vitamin D supplementation. Phosphate levels gradually decreased from baseline to the sixth month ($P = .005$ for the trend), whereas the alkaline phosphatase increased ($P < .001$). Data from the Rand 36-Item Short Form Health Survey showed a significant improvement in the mean scores of all eight domains ($P < .001$).

Conclusion: This is the largest study that demonstrates the effectiveness of PTH(1-34) in the treatment of adult patients with postsurgical hypoparathyroidism, and it shows that PTH(1-34) may improve the mental and physical health in hypoparathyroid subjects.

4. RESEARCH QUESTION

Can PTH(1-34) prevent the onset of hypocalcemia and shorten the duration of hospitalization in subjects with high risk of post-surgical after thyroidectomy?

ABSTRACT

Introduction: Subjects undergoing thyroidectomy may experience severe hypocalcemia often requiring extended hospitalization with increased healthcare costs. Up to now, there are no studies evaluating the use of teriparatide for prevention of postoperative hypocalcemia. The objectives of this study are to evaluate whether teriparatide can prevent post-surgical hypocalcemia and shorten the hospitalization in subjects at high risk of hypocalcemia following thyroid surgery.

Methods and design: this is a prospective Phase II Randomized Open Label Trial conducted in the Surgical ward of the University Campus Bio-Medico. 26 subjects (6 males, 20 females, mean age 53.4, SD 17.0) with iPTH<10 pg/ml 4 hours after thyroidectomy have been enrolled. Subjects have been randomized (1:1) to receive subcutaneous administration of 20 mcg of teriparatide every 12 hours until the discharge (treatment group) or to follow the standard clinical care (control group).

The Main Outcome Measure were Adjusted serum calcium, duration of hospitalization, calcium/calcitriol supplementation.

Results: Treated patients had a lower risk of hypocalcemia than controls [RR 0.26 (95% CI:0.09- 0.723)]. The median duration of hospitalization was 3 days (IQR:1) in control subjects and 2 days (IQR:0) in treated subjects (P=0.012). One month after discharge, 10 out of 13 subjects in the treatment group had stopped calcium carbonate supplements, while only 5/13 in the control group had discontinued **calcium. The**

ANOVA for repeated measures showed a significant difference in calcium supplements between groups at one month visit (P=0.04) as well as a significant difference between discharge and one month visit in the treatment group (P for interaction time group =0.04)

Conclusions: Our findings suggest that Teriparatide may prevent post-surgical hypocalcemia, shorten the duration of hospitalization and reduce the need for calcium and vitamin D supplementation after discharge in high risk subjects after thyroid surgery.

STATEMENT OF ATTRIBUTION

1)Author's contributions for the following trial: **"*Effects of alendronate and vitamin D in patients with normocalcemic primary hyperparathyroidism"***.

Conceived and designed the trial: Andrea Palermo, Fabio Vescini, Roberto Cesareo. *Performed the trial*: Andrea Palermo, Fabio Vescini, Roberto Cesareo, Giuseppe Campagna, Roberto Cianni, Silvia Manfrini, Valreio Pasqualini, Francesca Romitelli. *Analyzed the data*: Andrea Palermo, Enrico Di Stasio, Grimaldi Franco. *Wrote the manuscript:* Andrea Palermo, Fabio Vescini, Roberto Cesareo.

2)Author's contributions for the following trial: **"*Normocalcemic Hypoparathyroidism: Prevalence and Effect on Bone Status in Older Women. The OPUS Study"***.

Conceived and designed the trial: Richard Eastell, David M. Reid, Christian Roux, Dieter Felsenberg, Claus-C Glüer. *Performed the trial*: Fatma Gossiel, David M. Reid, Christian Roux, Dieter Felsenberg, Claus-C Glüer, Andrea Palermo and Richard Eastell. *Analyzed the data*: Richard Jacques, Richard Eastell, Andrea Palermo. *Wrote the manuscript:* Andrea Palermo, Richard Eastell.

3)Author's contributions for the following trial: **"*PTH(1–34) for the primary prevention of postthyroidectomy hypocalcemia: the THYPOS trial"***.

Conceived and designed the trial: Andrea Palermo, Paolo Pozzilli, Silvia Manfrini. *Performed the trial*: Andrea Palermo, Giuseppe Mangiameli, Gaia Tabacco, Filippo Longo, Silvia Irina Briganti, Silvia Manfrini, Daria Maggi, Anda Naciu, Angelo Lauria Pantano, Nicola Napoli, Silvia Angeletti, Paolo Pozzilli, Pierfilippo Crucitti. *Analyzed the data*: Andrea Palermo, Claudio Pedone, Fabio Vescini. *Wrote the manuscript:* Andrea Palermo, Fabio Vescini.

4)Author's contributions for the following trial: **"*PTH(1–34) for Surgical*

Hypoparathyroidism: A Prospective, Open-Label Investigation of Efficacy and Quality of Life".

Conceived and designed the trial: Andrea Palermo, Assunta Santonati, Silvia Manfrini, Fabio Vescini. *Performed the trial*: Assunta Santonati, Andrea Palermo, Ernesto Maddaloni, Daniela Bosco, Antonio Spada, Franco Grimaldi, Bruno Raggiunti, Raffaele Volpe, Silvia Manfrini, Fabio Vescini. *Analyzed the data*: Andrea Palermo, Ernesto Maddaloni. *Wrote the manuscript:* Andrea Palermo, Fabio Vescini

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PUBLICATIONS AND PAPER DERIVED FROM THIS WORK

Cesare R, Di Stasio E, Vescini F, Campagna G, Cianni R, Pasqualini V, Romitelli F, Grimaldi F, Manfrini S & **Palermo A.** ***Effects of alendronate and vitamin D in patients with normocalcemic primary hyperparathyroidism***. *Osteoporosis Internationa 2015;26(4):1295-1302.*

Palermo A, Jacques R, Gossiel F, Reid MD, Roux C, Felsenberg D, Glueer CC, Eastell R. ***Normocalcemic hypoparathyroidism: prevalence and effect on bone status in older women. The Opus study***. *Clin End 2015; 82:861-823*

Santonati A*, **Palermo A***, Maddaloni E, Bosco D, Spada A, Grimaldi F, Raggiunti B, Volpe R, Manfrini S, Vescini F and the Hypoparathyroidism AME group. ***PTH(1–34) for Surgical Hypoparathyroidism:A Prospective, Open-Label Investigation of Efficacy and Quality of Life***. *JCEM 2015, 100(9):3590–3597.* *They contributed equally to this work

Palermo A, Mangiameli G, Tabacco G, Longo F, Pedone C, Briganti SI, Maggi D, Vescini F, Naciu A, Lauria Pantano A, Napoli N, Angeletti S, Pozzilli P, Crucitti P, Manfrini S. ***PTH(1–34) for the primary prevention of postthyroidectomy hypocalcemia: the THYPOS trial.*** *JCEM 2016* (Accepted August 9, 2016.)

ABBREVIATIONS

ADIS: Agonist-Driven Insertional Signaling

ALP: total alkaline phosphatase

BALP: Bone-specific alkaline

BMC: Bone Mineral Content

BMD: Bone Mineral Density

CaSR: Ca^{2+}sensing receptor

CTX: C-terminal crosslinking telopeptides of type I collagen

DPD : deoxypyridinoline

ECD: extracellular domain

ECF: extracellular fluid

FGF23: fibroblast growth factor 23

GlucR: glucagon receptor

GPCR: G protein-coupled receptor

MafB : v-maf musculoaponeurotic fibrosarcoma oncogene homolog B

MAPK mitogen-activated protein kinase

NHPT: Normocalcemic hyperparathyroidism

NPHPT: Normocalcaemic PHPT

NTX: N-terminal crosslinking telopeptides of type I collagen

nVDRE: negative vitamin D-responsive element

OC: Osteocalcin

OHP: hydroxyproline

P1CP: C-terminal crosslinking propeptides of type I procollagen

P1NP: N-terminal crosslinking propeptides of type I procollagen

PAS: Parathyroid Assessment of Symptoms Score

PHPT: primary hyperparathyroidism

PKA: protein kinase A

PTH: Parathyroid hormone

PTHR1: PTH receptor

PTHrP: PTH-related peptide

PTX : parathyroidectomy

PYD: pyridinoline

REMSA: RNA electrophoretic mobility shift assays

RXR: retinoic acid X receptor

TMD: transmembrane domain

TMs: transmembrane helices

VDIR: VDR interacting repressor

VDR: vitamin D receptor

VDR: vitamin D receptor

VRE: vitamin D response element

CHAPTER 1

GENERAL BACKGROUND ON CALCIUM METABOLISM AND PTH

1.1 Calcium

In adults, the body contains about 1,000g of calcium, of which 99% is located in the mineral phase of bone as the hydroxyapatite crystal [Ca10(PO4)6(OH)2]. The crystal plays a key role in the mechanical weight-bearing proper- ties of bone and serves as a ready source of calcium to support a number of calcium-dependent biological systems and to maintain blood ionized calcium within the normal range. The remaining 1% of total body calcium is located in the blood, extracellular fluid, and soft tissues. Of the total calcium, the ionized fraction (50%) is the biologically functional portion of total calcium and can be measured clinically; 40% of the total is bound to albumin in a pH-dependent manner; and the remaining 10% exists as a complex of either citrate or PO4 ions [1].

About cellular system, cytosol calcium is 10−6 M, which creates a 1,000- fold gradient across the plasmatic membrane [extracellular fluid (ECF) calcium is 10−3 M] that favors calcium entry in the cell. There is an electrical charge across the plasmatic membrane of about 50mV with the cell interior negative. Thus, the chemical and electrical gradients across the plasmatic membrane favor calcium entry, which the cell must defend against to preserve cell viability [1]. Calcium-induced cell death is largely prevented by several mechanisms including extrusion of calcium from the cell by adenosine triphosphate (ATP)-dependent energy driven calcium pumps and calcium channels; Na– Ca exchangers; and the binding of intracellular calcium by proteins located in the cytosol, endoplasmic reticulum (ER), and mitochondria. Calcium binding to ER and mitochondrial sites buffer intracellular calcium and can be mobilized to maintain cytosol calcium levels and to create pulsatile peaks of calcium to mediate membrane receptor signaling that regulate a variety of biologic systems.

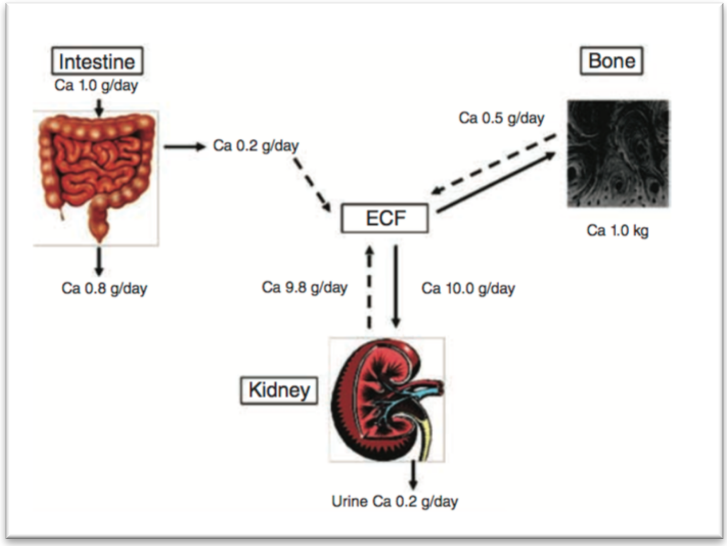
Blood Calcium is generally transported in part bound to proteins (about 45%), primarily albumin; another part (about 10%) bound to small anions such as citrate; another part (about 45%) in the free or ionized condition [2]. It has been demonstrated that only the ionized calcium can move into cells and activate several cellular activities but most clinical laboratories measure total serum calcium concentrations. Total serum calcium concentration in normal helathy subjects usually range between 8.5 and 10.5 mg/ dL (2.12 to 2.62mM). The normal range of ionized calcium is 4.65–5.25mg/dL (1.16–1.31mM). When protein concentrations (in particular albumin) vary, total calcium levels may change, instead the ionized calcium should remain quite stable. Events such as dehydration/hemoconcentration may elevate serum albumin and falsely elevate total serum calcium. This is the reason why is important to "correct" the total calcium level by subtracting 0.8mg/dL from the total calcium for every 1.0 g/dL by which the serum albumin concentration is greater than 4 g/dL. Instead, if albumin levels are low, total calcium should be corrected by adding 0.8mg/dL for every 1.0g/dL by which the albumin is less than 4g/dL. Moreover, changes in blood pH can alter the equilibrium constant of the albumin–Ca2+ complex, with acidosis reducing the binding and alkalosis enhancing it. In conclusion, changes in serum protein or pH need measurement of the ionized calcium level to determine the physiologic serum calcium level. The ECF calcium concentration must be maintained within a rather narrow range because calcium regulates several cellular functions such as protein secretion, muscle contraction, neuronal excitability, coagulation etc.

The gut, the skeleton and the kidney carry out a major role in assuring calcium homeostasis. In general, in a normal healthy subject, if 1,000 mg of calcium are ingested in the diet per day:

- about 200 mg will be absorbed.
- about 10g of calcium will be filtered daily through the kidney and most will be reabsorbed, with about 200mg being excreted in the urine.
- The normal 24-hour excretion of calcium might vary between 100-300 mg per day .

The skeleton rapresents the major storage site for calcium in the body (1kg of calcium). Furthermore, a normal bone turnover provides about 500mg of calcium to blood from the bone, instead the same amount of calcium is captured by bone from the blood (Figure. 1).

Figure 1. Calcium Balance[3]



Strict regulation of the ECF calcium concentration is guaranteed through the action of calcium-sensitive cells that regulate the hormone productions [4]. Indeed, they act on specific cells (in the bone, kidney and bowel) which can respond by altering fluxes of calcium to maintain ECF calcium. In particular a reduction in ECF calcium stimulates release of PTH that can act to enhance bone resorption and liberate both calcium and phosphate from the skeleton. PTH can also stimulates calcium reabsorption in the kidney and, at the same time, inhibits phosphate reabsorption increasing phosphaturia. The phenomenon of Hypocalcemia and PTH elevation are both able to enhance 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] [4], which in turn will stimulate intestinal calcium absorption, and to a lesser extent renal phosphate reabsorption. The the mobilization of calcium from bone, the increased absorption of calcium from the gut, and the increased reabsorption of filtered calcium along the

nephron lead to the balance restoration of the ECF calcium and consequently to inhibit further production of PTH and 1,25(OH)2D3. Conversely, if calcium raised above the normal range, reduction of PTH and calcitriol secretion would occur. The effect of suppressing the release of PTH and 1,25(OH)2D3 and stimulating CaSR diminishes skeletal calcium release, decreases calcium absorption from the bowel and renal calcium reabsorption, and restores the elevated ECF calcium to normal.

1.1.1 Intestinal calcium transport

Net intestinal calcium absorption can be determined by the external balance technique in which a diet of known composition with a known amount of calcium is ingested, and urine calcium excretion and fecal calcium loss are measured. Negative absorption occurs when net absorption declines to about 200 mg calcium per day (5.0 mmol). The portion of dietary calcium absorbed varies with age and amount of calcium ingested and may vary from 20% to 60%. Rates of net calcium absorption are high in growing children, during growth spurts in adolescence, and during pregnancy and lactation. The efficiency of calcium absorption increases during prolonged dietary calcium restriction to absorb the greatest portion of that ingested. Net absorption declines with age in men and women, and so increased calcium intake is required to compensate for the lower absorption rate. Fecal calcium losses vary between 100 and 200mg per day (2.5 to 5.0mmol). Fecal calcium is composed of unabsorbed dietary calcium and calcium contained in intestinal, pancreatic, and biliary secretions. Secreted calcium is not regulated by hormones or serum calcium. About 90% of absorbed calcium occurs in the large surface area of the duodenum and jejunum. Increased calcium requirements stimulate expression of the epithe- lial calcium active transport system in the duodenum, ileum, and throughout the colon sufficient to increase fractional calcium absorption from 20% to 45% in older men and women to 55% to 70% in children and young adults.1,25(OH)2D3 increases the efficiency of the small intestine and colon to absorb alimentary calcium. Active calcium absorption can obtain about 10–15% of a dietary load [7]. Reductions in dietary calcium intake can increase PTH secretion and 1,25(OH)2D3 production.

Increased 1,25(OH)2D3 can then increase expression of TRPV6, resulting in enhanced fractional calcium absorption and compensation for the dietary reduction [5].

Intestinal epithelial calcium transport includes both an energy-dependent, cell-mediated saturable active process that is largely regulated by 1,25(OH)2D3, and a passive, diffusional paracellular path of absorption that is driven by transepithelial electrochemical gradients. The cell-mediated pathway involving the TRPV6 calcium channel is saturable with a Kt (1/2 maximal transport) of 1.0 mM. Passive diffusion increases linearly with luminal calcium concentration. In adults fed a diet low in calcium, enhanced 1,25(OH)2D3 production increases the effi- ciency of absorption through an increase in saturable calcium transport. During high dietary calcium intake absorption 1,25(OH)2D3 is suppressed, and passive para- cellular transport accounts for most all absorption.

1.2 **PTH and mineral homeostasis**

PTH has an important role in calcium homeostasis. In particular, Ca^{2+} is maintained within a very narrow range of about ±1–2% of its mean value in any given individual, although the normal range in the population as a whole encompasses a normal range of ≈ 8.5–10.5 mg/dl, i.e., ± 10% [6]. Serum phosphorus varies over a somewhat wider range, 2.5–4.5 mg/dl. There can be severe clinical consequences when the extracellular concentrations of these two mineral ions deviate above or below their normal limits, so the availability of homeostatic systems ensuring maintenance of their circulating levels within the respective normal ranges is critical.

A central element within this system is the parathyroid cell's capacity to detect small (≈ 1–2%) deviations in Ca^{2+} from its normal level and to respond with appropriate alterations in the secretion of parathyroid hormone (PTH) so as to restore normocalcemia. The remarkable sensitivity of the parathyroid glands to changes in Ca^{2+} is a consequence of the steep inverse sigmoidal relationship between PTH release to Ca^{2+} (figure 2), which ensures large changes in PTH in response to small perturbations in Ca^{2+} from its normal level (figure 3).

Figure 2 **Steep inverse sigmoidal relationship between Ca^{2+} and PTH in vivo** [3]

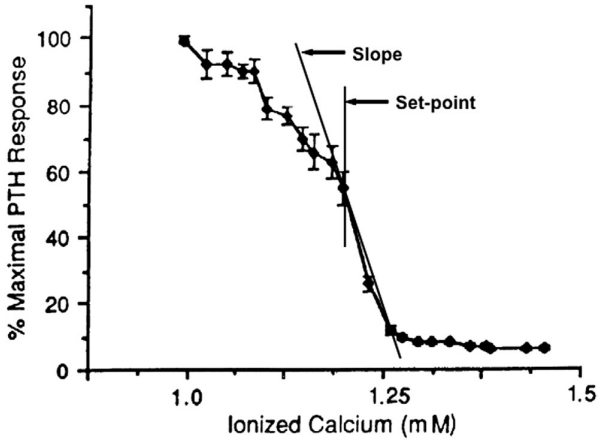
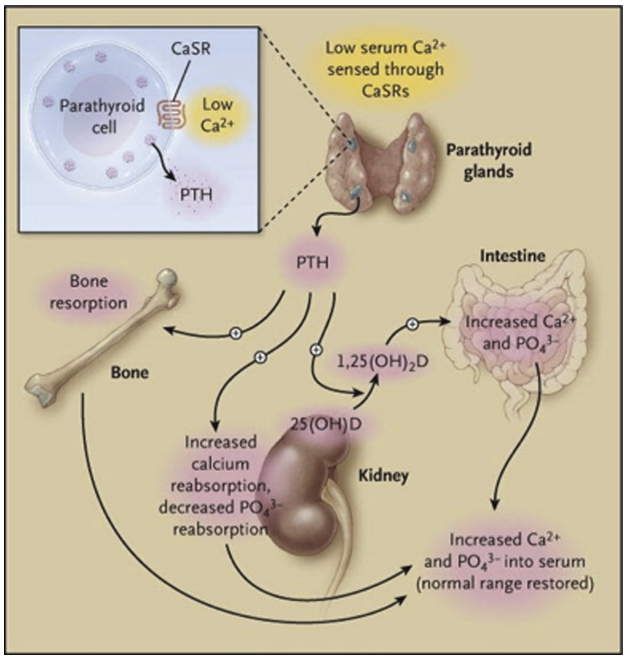


Figure 3. **The principal hormones and tissues that participate in extracellular Ca^{2+} homeostasis**. [3]



PTH's primary role is to serve as a key Ca2 + -elevating hormone, a role it shares with 1,25(OH)2 D3 . Four important actions of PTH function to restore normocalcemia in this setting [6,7]:

1) it enhances renal reabsorption of Ca^{2+} in both the cortical thick ascending limb and distal convoluted tubule of the kidney;

2) it increases renal synthesis of 1,25(OH)2 D3 from 25(OH)D3 in the proximal

tubule, which then increases intestinal absorption of Ca^{2+} and phosphate through distinct transport systems;

3) it directly stimulates net release of Ca^{2+} and phosphate from bone. The skeletal actions of PTH participating in Ca^{2+} homeostasis may involve acute changes in bone turnover and/or mobilization of a pool of soluble mineral ions at the bone surface [8,9].

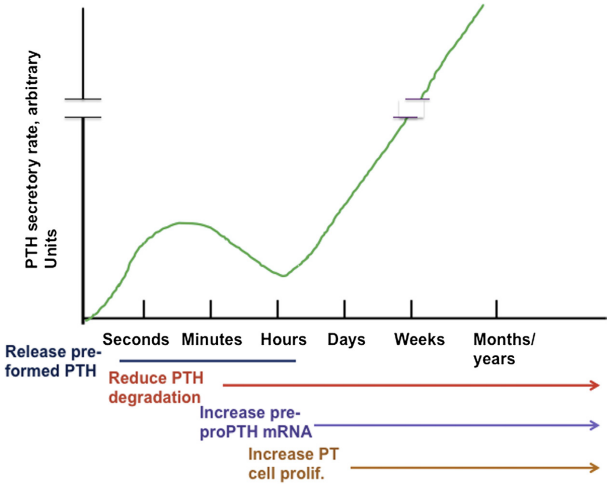
4) Finally, PTH promotes renal phosphate excretion, an action that can contribute to excretion of any excess phosphate arising from increased GI absorption and/or net release from bone. The hypo- and hyperphosphatemia that can be observed in subjects with hyper- or hypoparathyroidism [10], respectively, speak to the physiologically relevant role of PTH in contributing to the maintenance of phosphate homeostasis.

The parathyroid glands exhibit several adaptive responses to hypocalcemia that enhance their secretory capacity beyond simply the rapid release of preformed PTH, which lasts 1–2 hours [11,12] should restoration of normocalcemia not be achieved by the sequence of events just outlined. The variables modulating the overall secretory rate of normal parathyroid glands are:

- Changes in the minute-to-minute secretion of preformed PTH
- Changes in the rate of PTH degradation
- Changes in the level of PTH gene expression
- Changes in parathyroid cellular proliferation
- Changes in parathyroid cellular apoptosis
- Changes in proportion of active and inactive secretory cells in the parathyroid gland

In particular, in addition to the initial secretion of preformed PTH, stored PTH takes place within seconds to minutes: decreased intracellular degradation of PTH (beginning as early as 20 minutes after exposure of the cells to high Ca^{2+}) [13,14], thereby augmenting the availability of intact PTH(1–84), increased levels of PTH mRNA (within an hour or less in some cases and lasting weeks or more) [15] and augmented parathyroid cellular proliferation (within 2 days and lasting essentially indefinitely should hypocalcemia persist that long) [16–18] (figure 4).

Figure 4. **Time course of PTH changes after hypocalcemia** [3]



Normal parathyroid glands have the capacity to increase their mass by 10–100-fold or more in the setting of chronic hypocalcemia, so-called secondary hyperparathyroidism (HPT), especially in patients with chronic renal insufficiency. The time course of these various adaptive changes, extending from seconds to indefinite duration, ensure that there is an immediate response, but also components of the response that persist for as long as is needed, without any "windows" where an enhanced secretory response is not present. For instance, exhaustion of preformed hormone occurs at a time when PTH degradation has decreased ensuring the availability of more PTH(1–84) until increased expression of the preproPTH gene and, later, activation of parathyroid cellular proliferation begin.

Over the past decade, however, it has become apparent that there is another homeostatic system critical for maintaining phosphate homeostasis and, to a lesser extent, Ca^{2+} homeostasis. Central to this mechanism is the phosphaturic hormone, fibroblast growth factor 23 (FGF23), which is secreted by osteocytes, osteoblasts that have become encased within bone during bone formation [19] (Figure 5.1 and 5.2).

Figure 5.1 **Interaction between FGF 23, PTH, 1,25 OH vit D and PO_4** [3]

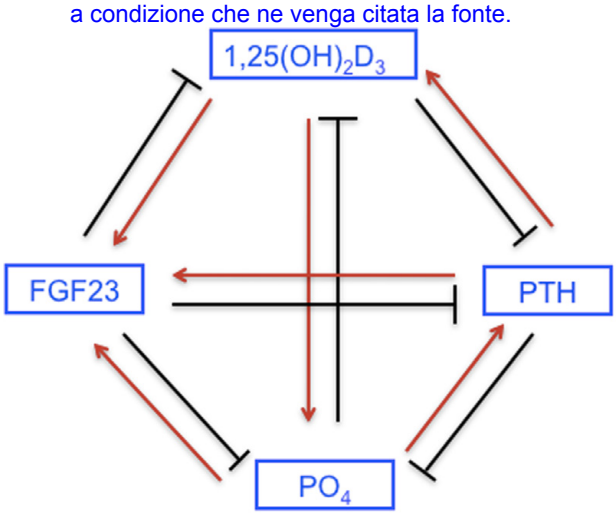
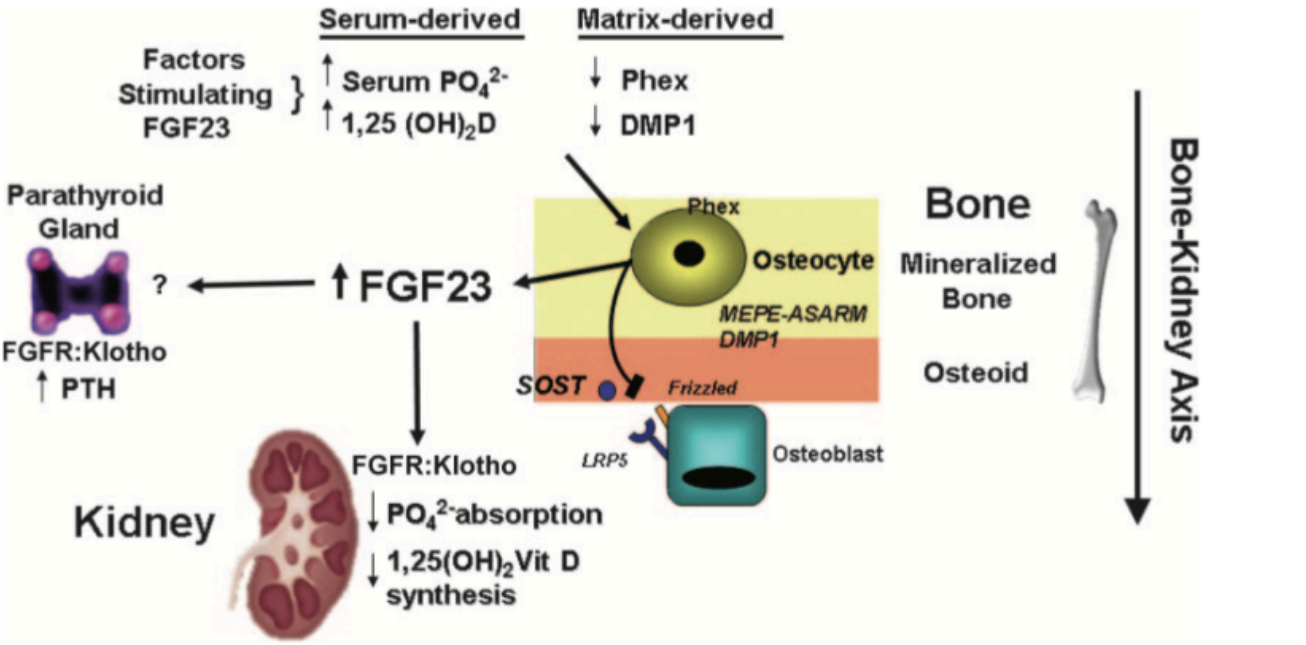


Figure 5.2 **Role of FGF 23** [20]



FGF23 also engages in cross-talk with the hormones that participate in Ca^{2+} homeostasis (figure 5.1). Note the sequence of homeostatic events that occur in

response to a phosphate load, which stimulates FGF23 production. FGF23 then promotes a normalization of serum phosphate, principally through its potent phosphaturic action on the kidney [21]. FGF23 also inhibits the renal production of 1,25(OH)2 D3 , thereby reducing absorption and release of phosphate from intestine and bone, respectively. Since 1,25(OH)2 D3 stimulates FGF23 production, the interactions of 1,25(OH)2 D3 with FGF23 constitute a negative feedback loop whereby high FGF23 levels inhibit 1,25(OH)2 D3 production, which will tend to inhibit further FGF23 production. Since both hyperphosphatemia and a decrease in 1,25(OH)2 D3 stimulate parathyroid function ,18 the increase in PTH, in conjunction with an elevation in the level of FGF23, further augment the renal phosphate wasting. Hyperphosphatemia also stimulates the production of both FGF23 and PTH [22] both laviand the resultant decrease in phosphate will reduce production of both FGF23 and PTH. It has become apparent over the past decade that there are also important direct and indirect interactions between FGF23 and PTH [23].

1.3 PTH action on the skeleton

1.3.1 Anabolic effect

Studies of mice with genetically targeted deletion of PTH (PTH "knock-out" mice) have demonstrated that the lack of PTH leads to a decrease in metaphyseal steoblasts and a reduction in trabecular bone in fetal and neonatal mice. These observations are consistent with an important physiologic role for PTH in promoting development of the fetal and newborn skeleton, and therefore in bone anabolism [24]. This anabolic role in early development may be recapitulated later in life as demonstrated by the fact that endogenous PTH also appears necessary for optimal fracture healing in mice [25]. Exogenous PTH has also been reported to increase fracture repair in animals [26] and its effect may be tempered by endogenous levels of the hormone [25]. Although there is robust evidence from animal experimentsthat PTH can improve normal fracture healing, this has still not been demonstrated beyond a reasonable doubt in humans. Two controlled clinical trials with PTH(1–34) or PTH(1–84), one of radial wrist

fractures [27] and one of pelvic rami fractures [28], have reported encouraging results and appear to show acceleration of normal fracture healing, but both have methodological shortcomings that to date preclude definitive conclusions regarding the utility of PTH as a pharmacologic agent for treatment of fracture repair. In patients with hypoparathyroidism, variable effects on trabecular bone have been reported as measured by bone densitometry [29]. In keeping with the importance of PTH for maintenance of trabecular bone, an increased prevalence of subclinical vertebral fractures indicative of vertebral fragility has been reported in patients with hypoparathyroidism [30]. Fracture healing studies have not, however, been reported in patients with hypoparathyroidism. In patients with primary hyperparathyroidism (PHPT), bone densitometry shows that trabecular bone in sites such as the vertebrae is preserved despite elevated concentrations of circulating PTH. These findings are confirmed by analysis of bone biopsy specimens by micro-computed tomography (microCT). The overall findings were of greater than average values for trabecular bone volume, and preservation of trabecular bone microarchitecture in most patients with PHPT as noted from assessment of trabecular number, connectivity, and separation [31]. Clinical studies in which exogenous PTH(1–34) [32] or PTH(1–84) [33] were given to patients with osteoporosis have shown that both molecules improve bone mineral density (BMD) and bone microarchitecture. BMD increases occur predominantly in areas rich in trabecular bone, such as veterbrae. Improvement in trabecular bone volume and connectivity and in trabecular number, as well as increases in plate-like relative to rod like structures, have been documented by microCT analyses of iliac crest biopsies [34]. PTH(1–34) has been reported to increase bone formation rates in iliac crest biopsies, on the trabecular, endosteal, and periosteal surfaces. The increased bone formation on periosteal surfaces suggests that PTH may lead to an increase in bone diameter. The major effects on trabecular bone appear to account for the reduction in vertebral fractures reported with both PTH(1–84) and PTH(1–34). PTH(1–34) has also been shown to reduce extra-vertebral fractures [32], possibly as a result of its effect on increasing periosteal apposition,

leading to augmented periosteal width, and therefore improved long bone strength. Overall, both animal and human studies of endogenous PTH deficiency or excess, or of exogenous PTH administration, provide compelling evidence that PTH is particularly important for normal trabecular bone quantity and quality and that significant increases in periosteal apposition may occur, which may also be beneficial.

Parathyroid hormone-related protein (PTHrP) is closely related to PTH, and was initially discovered in the search for a PTH-like factor that causes hypercalcaemia of malignancy [35]. In contrast to the mild fetal phenotype in knock-out mice lacking PTH, targeted deletion of PTHrP produces severe dysmorphic features in the appendicular skeleton of the neonate because of the crucial role of PTHrP in normal development of the growth plate. Therefore, although PTHrP and PTH were both assumed to be osteolytic calcium-modulating hormones, these studies showed that both are also anabolic, with PTHrP being critical for normal growth plate development and PTH playing an essential role in bone anabolism. The discovery of such different skeletal phenotypes in PTH and PTHrP knock-out mice was surprising in view of the fact that PTH and PTHrP are both believed to elicit cell signaling via interaction of their homologous N-terminal domains with a common G-protein-coupled receptor (GPCR), the type 1 PTH/ PTHrP receptor (PTHR1) [36]. However, differences in the timing and location of expression of PTH and PTHrP during development, differences in the duration of binding of each hormone to its receptor, and additional intracrine signaling by PTHrP has been described, all of which may contribute to the variable actions of the two related hormones [37]. Osteoblastic PTHrP may also contribute to the maintenance of skeletal mass by transducing mechanical forces [38]. Finally, in humans, heterozygous loss-of-function mutations in the gene encoding PTHrP are associated with brachydactyly type E and short stature [39], polymorphisms in the PTHrP gene have been associated with attainment of peak bone mass in young men[40] and specific allelic variants have been associated with reductions in BMD [41], suggesting that skeletal anabolic activity is also of importance

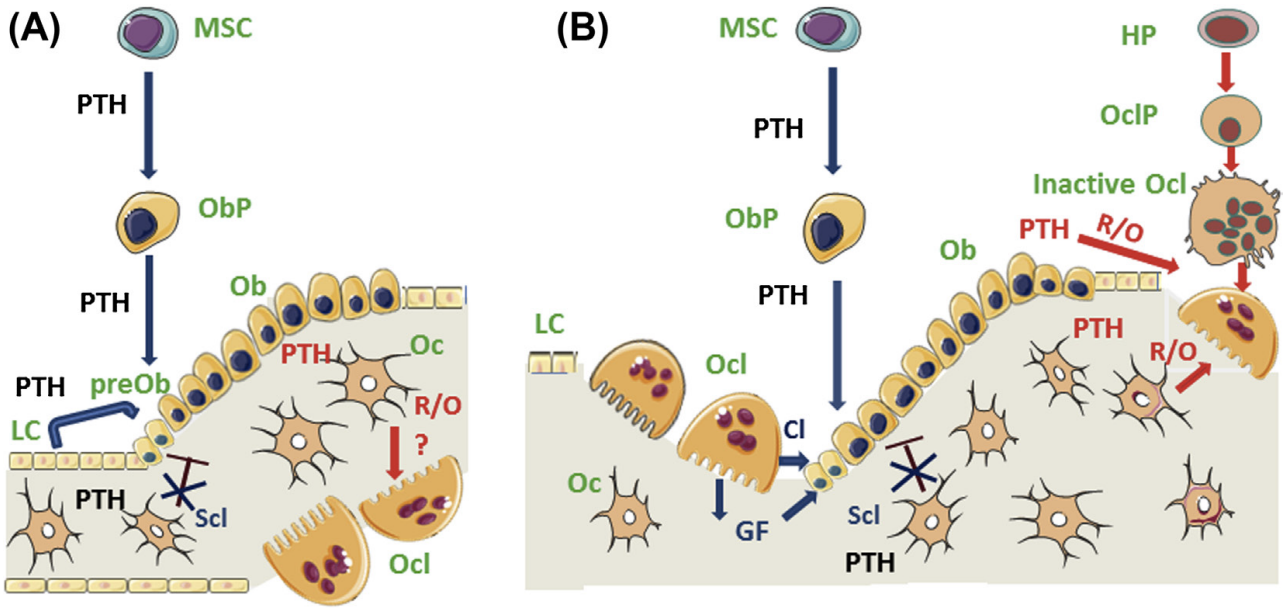
in adult humans. These and additional observations [42] indicate that circulating PTH essentially cooperates with PTHrP in carrying out physiologic anabolic bone activity. In osteoporosis clinical trials, PTHrP(1–36) has been shown to be a potent anabolic agent, raising the likelihood that NH2 - terminal forms of PTHrP will be efficacious once optimal analogs and doses have been defined.55 PTH use in skeletal anabolic therapy may be regarded therefore as an attempt to reproduce, in part, the local anabolic skeletal action of PTHrP [43].

In response to intermittent administration of PTH in osteoporosis in humans, analysis of the temporal patterns of circulating bone formation and bone resorption markers has indicated that there is an early phase of stimulation of bone formation without resorption, which is followed subsequently by stimulation of bone resorption [44]. Excess formation may proceed for a time after remodeling has been initiated in response to PTH so that a smaller amount of bone may still be accrued, but the increase in bone accrual is ultimately limited by enhanced osteoclastic resorption, and a plateau effect occurs. The period during which the effects of PTH on bone formation relative to resorption are maximal has been termed the "anabolic window" [45]. Histomorphometric analyses of iliac crest bone biopsies [46] have supported the concept that PTH stimulates bone formation without prior resorption, and it has been estimated that in early phases of treatment with intermittent PTH(1–34), such modeling-based bone formation may account for over 20% of the new formation in trabecular and endocortical bone; with prolonged treatment, as bone remodeling is stimulated, modeling may account for less than 10% of new bone formation. The PTH-induced bone formation can therefore occur not only on quiescent bone surfaces, but also in areas of remodeling in which there is overfilling of bone resorption cavities due to increased formation relative to resorption [47]. Thus, PTH-induced increases in the activation frequency of remodeling units may enhance its bone-forming effect. In animal studies, bone formation in trabecular bone and on the endocortical surface appears to result from a combination of Wnt-driven increased osteoblast number and resorption-dependent osteoblast activity [48].

Increased osteoclastic activity, via both direct (clastokine-driven) and indirect (bone matrix-released growth factor-driven) mechanisms increase the osteoblast pool; conversely, a reduction in osteoclastic activity would be expected to reduce the osteoblast pool and diminish PTH-stimulated bone formation. From studies in mice with targeted deletion of RANK, where osteoclasts are deficient, the capacity of PTH to exert its anabolic effect was significantly diminuished even if it has not been suppressed. These results suggest that osteoclasts are not strictly required for PTH anabolism, which presumably still occurs via modeling-based bone formation. Nevertheless, the magnitude of the PTH anabolic response appears to depend on the extent of the remodeling space, as determined by the number and activity of osteoclasts on bone surfaces [49]. This concept is consistent with the observation that there is diminished capacity of PTH to exert its anabolic effect after treatment with bisphosphonates [50] or after targeted deletion of the c-fos proto-oncogene, which is known to be essential for osteoclastogenesis [51]. In studies in oophorectomized mice in which two mechanistically different anti-resorptive agents were employed, i.e., a bisphosphonate and OPG, each anti-resorptive increased femoral and lumbar spine BMD, presumably by inhibiting resorption more than formation. When mice were co-treated with PTH and either anti-resorptive, additive increases in BMD in the femur and supra-additive increases in the lumbar spine were observed [52]. This is in keeping with a previous study in rats in which the combination of PTH(1–34) and OPG increased bone mineral density (BMD) and bone strength more than either agent individually [53]. Nevertheless, anabolic effects of PTH were blunted (but not eliminated) when animals were first pretreated with either antiresorptive before receiving the hormone [54]. Thus, pretreatment of the animals with the anti-resorptives, by reducing osteoclastic cells, may also have depleted the pool of mesenchymal osteoblast precursors [55]. By contrast, in the co-treatment paradigm, the PTH-induced stimulation of osteoblasts would have been initiated prior to any reduction in the osteoblast pool resulting from a decrease in osteoclasts, and PTH-induced bone formation may have been prolonged prior to initiation of the counteracting resorptive

action of the osteoclasts. This may then have facilitated the extension of the anabolic window. Pretreatment of osteoporotic women with a bisphosphonate has, as in animal studies, also been reported to blunt the anabolic response to PTH [56]. However, studies of co-treatment with PTH and an anti-resorptive have been conflicting. Thus, co-treatment with PTH(1–34) and alendronate in osteoporotic men [57] and women [58] produced no additive or synergistic effect, whereas combined treatment using both PTH(1–34) and zoledronic acid provided the greatest and most rapid increments in BMD when both spine and hip sites were considered [59]. Furthermore, combined treatment with PTH(1–34) and a RANKL-inhibiting antibody (denosumab) increased BMD more than either agent alone [60]. Therefore, the timing of the introduction of PH relative to the anti-resorptive appeared to be important, with less capacity of PTH to exert its full anabolic effect the longer the anti-resorptive was in use prior to initiating PTH. Consequently, osteoclasts, when in abundance, may limit PTH-stimulated bone accrual by their resorptive action, but when deficient, may also limit the anabolic effects of PTH in part at least by decreasing the osteoblast pool.

Figure 6. **Anabolic action of PTH in the setting of (A) modeling and (B) remodeling of bone** [3]



PTH can increase mature, bone-forming osteoblasts by stimulating all stages of osteoblastogenesis, including commitment of mesenchymal stromal cells (MSC) to osteoblast progenitors (ObP), and proliferation and differentiation

of osteoblast progenitors to preosteoblasts (preOb) and then to mature osteoblasts (Ob). PTH may also stimulate lining cells (LC) to differentiate into osteoblasts and may increase the osteoblast pool by inhibiting apoptosis of osteoblasts. PTH can also act on osteocytes (Oc) to inhibit release of sclerostin (Scl) and therefore facilitate increased osteoblast numbers and activity. In the process of modeling (A), increased osteoblasts are activated by PTH on surfaces devoid of prior osteoclastic bone resorption, i.e., bone formation is not tightly coupled to bone resorption and increased bone may be accrued. Osteoblastic bone formation and osteoclastic bone resorption occur on different bone surfaces. In remodeling (B), osteoclastic bone resorption initiates the remodeling cycle followed by osteoblastic filling of the resorption cavities. The bone-forming osteoblast pool may be increased by clastokines (Cl) released by osteoclasts, and/or by growth factors (GF) released by osteclastic degradation of the bone matrix. PTH may then further enhance the active osteoblast pool (Ob), leading to overfilling of the resorption space and net bone formation. PTH stimulation of osteoblasts and osteocytes may lead to production of an increased ratio of RANKL to osteoprotegerin (R/O) resulting in stimulation of all steps in osteoclastogenesis including stimulation of hematopoietic progenitors (HP) and mononuclear osteoclast precursors (OclP), fusion to form inactive multinucleated osteoclasts (Inactive Ocl), and activation of these cells to produce active bone resorbing osteoclasts (Ocl). Osteoclastic resorption may therefore limit the quantity of PTH-stimulated bone that is accrued. Whether PTH-stimulated osteocytes produce increases in RANKL to osteoprotegerin (R/O) in modeling is uncertain. Blue arrows and lettering denote steps in stimulation of osteoblastic cells. Red arrows and lettering denote steps in stimulation of osteoclastic cells.

1.3.2 Catabolic effect

Although PTH exerts a physiologic action to maintain trabecular bone mass in the fetus and also in the neonate, by 4 months of age PTH-null mice on a normal diet exhibit increased rather than decreased bone mass, especially in cortical bone [61]. This is consistent with reduced bone resorption normally carried out by PTH to maintain normocalcemia. Consequently, in the postnatal environment of the older animals, where external calcium sources differ from those in the fetus and neonate and where regulation of calcium homeostasis differs, PTH appears to defend against a decrease in serum calcium by increasing bone resorption more than formation. In patients with hypoparathyroidism, increased cortical bone has also been observed as measured by bone densitometry [29]. In contrast, in PHPT, where excess PTH circulates, preferential reduction of cortical relative to trabecular bone has been noted by bone densitometry and analysis of bone biopsy specimens by microCT. Thus, in the vast majority of patients with PHPT cortical thickness is reduced and increased cortical porosity is observed. BMD loss may therefore occur predominantly in areas of increased cortical bone such as the distal third of the radius and to a lesser extent in the hip. Some

patients, generally with a more severe form of PHPT, may also have reductions in lumbar spine BMD and an abnormal trabecular bone compartment with reduced trabecular volumetric density, reduced trabecular number, and increased trabecular spacing [62]. In the most advanced cases, generalized bone loss as well as localized brown tumors (osteotitis fibrosa cystica), bone cysts, and sub-periosteal resorption of phalanges and the distal clavicle may occur, all compatible with a marked increase in bone remodeling. Fracture risk may be increased in the more severe forms of PHPT but whether this occurs in the milder forms is uncertain. Overall, therefore, these and other [63] animal and human studies provide abundant evidence that PTH has discrete effects on trabecular and cortical bone compartment, sparing trabecular bone and diminishing cortical bone, at least until very high concentrations of circulating PTH are attained when more generalized bone resorption may occur to release calcium from skeletal stores.

1.4 PTH and Kidney

The kidneys are a primary target of PTH action, where it regulates phosphate and calcium transport, vitamin D biosynthesis and degradation, and intermediary metabolism. So much of what we recognize as direct renal actions of PTH stem from the enduring and insightful observations of Fuller Albright and his investigative team at the Massachusetts General Hospital. With the capacity to measure calcium and phosphate in plasma and urine, they established that the primary effect of PTH was a rapid elevation of phosphate excretion; increases of plasma calcium followed more slowly, and persisted longer following cessation of parathyroid hormone extract.These elementary studies of mineral ion balance served as a foundation for the abundant work that followed, from which we now enjoy a greater understanding of the cell and molecular mechanisms underlying these phenomena.

1.4.1 PTH and Phosphate

PTH promotes the absorption of calcium and magnesium, while inhibiting absorption

of phosphate, bicarbonate, sodium, and potassium. The most prominent and first action of PTH to be identified in the kidney was the inhibition of phosphate absorption. As for much of our understanding of PTH actions, Fuller Albright made many of the seminal observations. These included infusing PTH extract into a patient with idiopathic hypoparathyroidism [64]. Albright noted that urinary phosphate excretion increased promptly and preceded the rise in serum calcium. The opposite effects were noted after removal of the parathyroid glands. Based on these observations, Albright concluded that PTH primarily affects phosphate rather than calcium excretion. Further, because there was no initial change of serum calcium, which increased only after a delay and then rose abruptly, Albright proposed that the increase of calcium was secondary to desaturation of serum calcium phosphate, leading to dissolution of bone and release of calcium. These findings and their remarkable interpretation set the stage for the considerable insights to the cell and molecular events that we now understand are responsible for these actions. Renal phosphate transport is essentially restricted to proximal tubules, where two sodium-coupled transporters NPT2a (SLC34A1)1 and NPT2c (SLC34A3) mediate uptake of phosphate from luminal fluid. PTH inhibits renal phosphate transport by the endocytic retrieval and metabolic down-regulation of brush border NPT2a and, to a lesser extent, NPT2c. Recent findings indicate that persistent exposure to PTH not only down-regulates Npt2a expression but also reduces Npt2a mRNA stability [65]. Brush border (apical) expression of Npt2a requires the last three amino acids (Thr, Arg, and Leu), while the dibasic Lys-Arg amino acid motif (KR) located within an intracellular loop is needed for PTH-induced internalization. NPT2c/Npt2c lacks the Thr-Arg-Leu motif, but nonetheless localizes to apical cell membranes. The structural determinants for apical membrane tethering of Npt2c have not been defined. Furthermore, despite the absence of an identified tethering element in Npt2c, its endocytic retrieval following challenge with PTH is delayed considerably longer than is observed with Npt2a [66]. Remarkably, the identity, mechanism, and possible regulation of basolateral phosphate efflux have not been defined. PTH promotes

endocytosis of Npt2a and Npt2c in polarized OK cells and in isolated tubule preparations. Notably, apical effects of PTH are preferentially mediated by a phospholipase C (PLC) and protein kinase C (PKC) signaling mechanism, whereas basolateral PTH effects seem primarily to proceed through the adenylyl cyclase and protein kinase A pathway (PKA). Consistent with this view, in the absence of apical NHERF1 in proximal tubules PTHR1 coupling to PLC is deficient and is attended by reduced PTH-induced Npt2a internalization. In contrast to the PTHR1, which signals both through Gs-coupled activation of adenylyl cyclase and Gq/11-coupled activation of PLC, the dopamine-1 receptor (DRD1/Drd1a) couples exclusively to Gs.

Blockade of PKA abolishes dopamine-inhibitable phosphate transport in mouse proximal tubules. 40 Thus, PKA activation would seem to be necessary and sufficient for the acute effects of PTH on phosphate transport [67]. Using mice harboring a signaling selective PTHR1 acting solely through the PKA pathway established that sustained hypophosphatemia requires PLC/PKC actions [68]. Despite the unequivocal requirement for PKA/PKC action to initiate Npt2a endocytosis, the phosphorylation state of the transporter itself does not seem to be affected by P[68]TH treatment [69]. PTH importantly promotes targeted phosphorylation of NHERF1, which then releases bound Npt2a to initiate internalization and thereby inhibit phosphate transport [70]. The phosphorylation status and effects of PTH on Npt2c have not been characterized. It remains to be determined how apical and baso-lateral signals emanating from PTHR1 activation are integrated to permit spatiotemporal coordination of phosphate transport. As alluded to earlier, apical brush border localization of Npt2a depends upon the PDZ protein NHERF1/ EBP50. In the absence of NHERF1, apical Npt2a expression is diminished with increased cytoplasmic protein accumulation, and is accompanied by a corresponding elevation of urinary phosphate excretion [71]. The mechanism by which NHERF1 regulates phosphate transport is more complex than previously appreciated. Recent studies show that NHERF1 assembles a ternary complex with Npt2a and ezri. Mutations, truncations, or other disruptions of this macromolecular structure impair or prevent PTH-dependent inhibition of Npt2a [72]

and result in constitutive urinary wasting of phosphate [73]. PTH inhibition of proximal tubule phosphate absorption depends on PKA action. This effect of PTH entails the biosynthetic formation of cAMP, which escapes from the cells, with much of it appearing in urine. Although vasopressin and many other Gs-coupled G protein-coupled receptors are expressed in the nephron, at physiologic concentrations they exert little, if any, effect on cAMP excretion. Thus, urinary, or nephrogenous, cAMP is a virtual reflection of PTHR1 action in proximal tubules; and nephrogenous cAMP is considered to be a reliable index of parathyroid function.

1.4.2 PTH and calcium

Calcium is absorbed by most nephron segments and, indeed, the majority of calcium recovery occurs in proximal tubules. However, the tight hormonal regulation of calcium absorption by PTH is restricted to distal nephron sites including cortical thick ascending limbs, distal convoluted and connecting tubules. Conflicting views attend the possibility of regulated calcium absorption by collecting ducts.

Calcium transport in proximal tubules is largely thermodynamically passive, proceeding through the lateral intercellular space, or so-called paracellular pathway. By creating an osmotic driving force for water absorption, active sodium transport drives paracellular calcium movement either by generating a concentration gradient for calcium diffusion or by convection/solvent drag. Experimentally eliminating the driving forces for passive calcium movement uncovered a small amount of transcellular calcium absorption. In contrast to the passive calcium transport by proximal tubules, calcium absorption in distal tubules is entirely transcellular and energetically active. Cortical thick ascending limbs represent a hybrid situation, where basal calcium transport proceeds passively through tight junctions and is driven by the electrochemical gradient established by active sodium transport that results in a lumen-positive voltage. PTH stimulates active calcium absorption through a cellular transport mechanism. Notably, in proximal tubules and thick ascending limbs calcium and sodium absorption occur in parallel, where increases of sodium

movement are accompanied by elevated calcium transport. Conversely, decreases of sodium transport, for instance, that attend the action of loop diuretics such as bumetanide or furosemide, are accompanied by diminished rates of calcium absorption. This is not the case in distal tubules, where sodium and calcium movement are inversely related. Here, decreased sodium absorption pursuant to administration of a thiazide diuretic or the presence of mutations in the NaCl cotransporter, SLC12A3, that occur in Gitelman syndrome, are associated with elevated calcium absorption. This is the situation with Gitelman syndrome, which is characterized in part by hypocalciuria. Thus, in cortical thick ascending limbs, early and late distal convoluted tubule active calcium transport follows a cellular pathway that is stimulated by PTH. Notably, in human kidneys, PTH receptor activity is concentrated in early distal convoluted tubules, with far lower action in latter tubule segments. In the mouse, PTH receptor activity is likewise primarily localized to cortical ascending limbs and early distal convoluted tubules, with virtually negligible activity in late distal convoluted tubules or cortical collecting tubules. Cellular calcium entry across apical membranes of late distal convoluted tubules and connecting tubules is mediated by the V5 transient receptor potential channel, TrpV5.55 TrpV5 knock-out in mice results in a multi-fold increase in calcium excretion [74]. Unexpectedly, proximal salt and water absorption were dramatically reduced, resulting in a substantial increase in the distal delivery of calcium and a marked diuresis. It is quite possible that distal tubule calcium transport, which is known to depend on the delivered load, is overwhelmed in this setting, resulting in some or even much of the calcium wasting attributed to the loss of TrpV5.

Despite the marked urinary calcium loss, serum calcium and PTH levels were well maintained, and serum levels of 1,25(OH)2 D are elevated. The compensatory support of serum calcium arises at the expense of skeletal calcium. The mechanism envisioned by which PTH regulates TrpV5-mediated calcium influx is markedly indirect, and involves the calcium-sensing protein calmodulin. Calcium influx through TrpV5 induces rapid channel inactivation, which prevents excessive

absorption of calcium. This inactivation is mediated by binding of the calcium-sensing protein calmodulin to the last ≈ 30 residues of the carboxy terminus of the channel. PTH activation of the cAMP–PKA pathway results in phosphorylation of threonine at position 709 in the carboxy terminus of TrpV5. This phosphorylation blocks binding of calmodulin, and hence increases channel-open probability with an attendant increase of calcium entry [75]. These findings suggest that PKA activation is necessary and sufficient to stimulate calcium channel entry, at least in late distal convoluted tubules. A somewhat different mechanism was advanced by Gesek et al., who showed that PTH stimulation of calcium entry in early distal convoluted tubule cells needs activation of both PKA/PKC and is due to hormone-induced hyperpolarization of apical membranes and stimulation of a heteromeric, voltage-sensitive calcium channel [76]. These disparities suggest that additional mechanisms of PTH-dependent apical calcium entry remain to be defined. Net calcium absorption requires that once calcium has entered the distal tubule it must cross basolateral cell membranes. The plasma membrane calcium ATPase (PMCA, ATP2B1) and the Na/Ca exchanger (NCX1, SLC8A1) have been implicated in this process. In our view, PMCA1b/4b are responsible for resting levels of calcium efflux, whereas NCX1 mediates the electrogenic extrusion of Ca2+ that is engaged upon PTH-induced cellular hyperpolarization [77]. A contrary view suggests that PTH increases the abundance of TrpV5 entry channels and NCX1-mediated extrusion [78]. How such a transcriptionally dependent system could respond rapidly enough to capture the nearly immediate action of PTH on renal calcium conservation is unclear.

1.4.3 PTH and Vitamin D

The biologically active form of human and other mammalian vitamin D is 1,25-dihydroxyvitamin D3 (1,25[OH]2 D3 ; calcitriol). Stated briefly, it is formed by 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 (1α -hydroxylase; CYP27B1) in the kidney. This latter step proceeds in

proximal tubules and is highly regulated by PTH. 1,25[OH]2 D3 is degraded to 24,25[OH]2 D3, an inactive metabolite, through side-chain oxidation mediated by 1,25[OH]2 D3 -24-hydroxylase (CYP24A1), and this too is regulated by PTH action, though in distal tubule cells as well as in proximal tubules [79]. 1,25[OH]2 D levels are controlled not only by PTH but also by FGF23, dietary calcium and phosphate, and by 1,25[OH]2 D3, which inhibits the 1α –hydroxylase [80], induces CYP24, and also suppresses PTH transcription [81], in a tightly regulated negative feedback circuit. Most studies implicate cAMP and PKA in mediating the stimulatory effects of PTH on 1α -hydroxylase. cAMP modulates 1α -hydroxylase gene expression by phosphorylation of the cAMP-response element-binding protein (CREB) and its binding to cAMP-response elements (CRE) [82]. As noted earlier, PTHR1 stimulation is capable of activating PKC, and this latter pathway also has been implicated in 1α -hydroxylase regulation. PKC, however, has been reported to decrease [83] but also to increase [84] 1α -hydroxylase activity. These apparently contradictory findings may result from complex phosphorylation schemes, including the possibility that CREB is phosphorylated by PKC, or by actions of PKC on the 24-OHase to reduce 1,25[OH]2 D3 , which in turn may affect PTH sensitivity. In addition to its stimulatory effect on the 1α -hydroxylase, PTH down-regulates vitamin D receptor (VDR) expression [85]. PTH-mediated down-regulation of renal VDR may block suppression of the 1α –hydroxylase by 1,25[OH]2 D3 and induction of the 24-hydroxylase, respectively, thereby increasing serum 1,25[OH]2 D3 levels.

RESEARCH PROJECT N°1

EFFECT OF ALENDRONATE AND VITAMIN D IN PATIENTS WITH NORMOCALCEMIC PRIMARY HYPERPARATHYROIDISM.

CHAPTER 2: **SPECIFIC BACKGROUND**

2.1 Primary Hyperparathyroidism

Primary hyperparathyroidism (PHPT) is characterized by hyperactivity of one or more parathyroid glands, disordered calcium homeostasis, and a consequent increase in serum calcium and elevated or inappropriately present circulating levels of parathyroid hormone (PTH) [86]. In the 1940's Fuller Albright described the classic manifestations of PHPT as a disorder of bones and stones. Indeed, historically, PHPT was characterized best by skeletal and kidney involvement. This clinical landscape changed rather dramatically in the 1970s with the advent of the multichannel autoanalyzer that provided a serum calcium concentration whenever the biochemical screening test was ordered. The diagnosis of PHPT was made much more commonly thereafter by mild hypercalcemia, lack of any specific symptomatology, or obvious renal or bone disease [87]. Currently, up to 80% of patients with PHPT in parts of the world where biochemical screening is routine have "asymptomatic" PHPT [86]. Most patients with PHPT have a single, benign adenoma. A smaller percentage, about 15% to 20%, have multigland disease, including multiple adenomas and hyperplasia. Multigland disease is more common in familial syndromes such as multiple endocrine neoplasia (MEN) 1 or 2 [88]. Parathyroid carcinoma is rare, occurring in fewer than 1% of patients with PHPT [86]. Gene mutations can be associated with the development of parathyroid tumors. These genes include MEN1, calcium-sensing receptor (CASR), HRPT2, RET (familial forms), and PRAD1/cyclin1 (sporadic tumors) [89].

2.1.1 Diagnosis

Excessive secretion of PTH from one or more parathyroid glands causes hypercalcemia, which constitutes the biochemical hallmark of PHPT. The repeated finding of hypercalcemia in routine biochemical tests is a clue to the diagnosis of PHPT, especially in individuals older than 50 years and in postmenopausal women [90]. About 45% of serum calcium is bound to albumin, thus total serum calcium level should be adjusted using the formula: corrected calcium = measured serum calcium in mg/dL + [0.8 (4-serum albumin in g/dL)]. The measurement of ionized calcium could be useful in selected cases, such as in patients with hyper- or hypoalbuminemia, thrombocytosis, Waldenström macroglobulinemia, and myeloma. In the latter two instances, hypercalcemia may be present but the ionized serum calcium is normal (artifactual hypercalcemia) [86]. A cohort study based on the population of Tayside used the following biochemical criteria for diagnosing PHPT:1 albumin-corrected serum calcium above 10.22 mg/dL (reference values: 8.4–10.22 mg/dL) at least on two occasions, with serum intact PTH, measured by different assays, above 28 pg/ml (reference values: 19–65 pg/mL; or 2) albumin-corrected serum calcium above 10.22 mg/dL on only one occasion plus serum intact PTH above 65 pg/mL [91]. Although not all patients selected by these criteria, namely, hypercalcemia and PTH levels within the reference range, have histological confirmation, it seems reasonable to consider inappropriately normal serum PTH levels, in the presence of sustained hypercalcemia, as indicative of the diagnosis of PHPT. Other causes of hyperparathyroidism such as the use of thiazide diuretics and lithium, vitamin D deficiency, bisphosphonates, and renal failure should be excluded. Tertiary hyperparathyroidism in renal failure, in addition to genetic causes such as familial hypocalciuric hypercalcemia also need to be excluded. The differential diagnosis with conditions that lead to hypercalcaemia should be considered. The finding of a normal corrected serum calcium associated with elevated serum PTH in the absence of other causes help to establish the diagnosis of normocalcemic PHPT [92]. Serum PTH levels may be elevated, in the absence of PHPT, in subjects with

glomerular filtration rates between 40 and 60 ml/min. Hypercalcemia with very low or undetectable plasma PTH levels is present in malignant diseases, where PTHrP is often responsible for serum calcium elevation [90].

PTH assays have evolved significantly. The second generation assays (so-called "intact" PTH or "total" PTH assays) detect both the 1–84 primary amino acid sequence of PTH (considered the biologically active full-length moiety), and other large fragments with uncertain biological activity, for example, the truncated PTH(7–84). This fragment, besides being found in PHPT, can also be detected in normal persons and accumulates in patients with renal failure. A so-called third-generation or biointact assay that measures only the full-length PTH(1–84) molecule was developed to help solve this problem. However, there are few studies comparing the diagnostic sensitivity between the second- and third-generation assays, and although some show the superiority of the latter, others do not [93]. Laboratory evaluation should include renal function tests and serum measurement of 25OHD. The 24-hour urinary calcium and the serum creatinine level should also be measured. A calcium clearance/creatinine clearance ratio less than 0.01 suggests, but does not prove, familial hypocalciuric hypercalcemia. Serum phosphorus levels are usually found to be low in severe disease, and low–normal in milder forms [90]. Specific markers of bone formation (osteocalcin, bone-specific alkaline phosphatase) or bone resorption (deoxypyridinoline, N-telopeptide, and C-telopeptide) tend to be in the normal–high range or slightly above the reference values [94]. A renal ultrasound should be performed if history suggests nephrolithiasis, and can be considered even in the absence of these symptoms to rule out nephrocalcinosis or nephrolithiasis, findings that would argue for surgical intervention. BMD, measured by dual energy X-ray absorptiometry (DXA), should be evaluated at the lumbar spine, femur, and distal 1/3 radius in all patients with PHPT [95]. Although vitamin D levels may differ by latitude and skin pigmentation worldwide, there is growing evidence that even people living in areas with abundant sunlight may not attain adequate vitamin D stores. In our population, the prevalence of vitamin D deficiency is high particularly in

postmenopausal women and old men. Using several cutoff points for serum 25OHD, vitamin D deficiency was found in 8% of the postmenopausal women considering values below 15 ng/ml (37.5 nmol/L), in 24% of the patients considering values below 20 ng/ml (50 nmol/L), and in 43% considering values below 25 ng/ml (62.5 nmol/L). These data show a prevalence similar to that in the US, but greater than that found in Canada and Scandinavian countries, and reinforce the idea that the abundant presence of sunlight may not prevent vitamin D deficiency in postmenopausal women. Serum 25OHD is considerably lower in patients with severe PHPT than in those with asymptomatic disease [86]. Serum C-telopeptide (CTX) is highly correlated with serum 25OHD in severely affected patients, whose levels are also considerably higher than those of patients with milder forms of PHPT [96].

2.1.2 Epidemiology

PHPT was a less common disorder when the diagnosis was relatively dependent on symptoms related to more severe and longer-standing hyperparathyroidism. In the early twentieth century, it was assumed that PHPT was uncommon given the great rarity of osteitis fibrosa cystica. However, in 1934 Fuller Albright reported 17 cases of PHPT, three of whom did not have evidence of metabolic bone disease [64]. From these observations, it was concluded that PHPT may be more common than previously identified and could have variable clinical manifestations, with urinary tract involvement being observed more frequently than skeletal involvement [64]. This seminal publication was critical, and thus changed the perception of the epidemiology of this disease. PHPT became increasingly identified based on serum calcium measurement, particularly in patients with renal calculi. Early population-based screening studies measuring serum calcium were influential in leading to the next series of findings regarding the epidemiology of PHPT. The initial reports of routine serum calcium analysis identified apparently asymptomatic individuals with hypercalcemia with a prevalence of PHPT estimated at 100–200 cases per 100,000.5–7 Similarly, a health screening survey over 10.25 years in Sweden, which included

serum calcium measurement, suggested that asymptomatic hypercalcemia had an even higher prevalence, identifying 6% of the screened population of adults, excluding those taking thiazides, with hypercalcaemia verified on repeat measurement [97]. More recent prevalence estimates from Norway in 1994–1995 suggest PHPT has a prevalence of 3.6–13.9%, depending on the defining biochemical criteria in older women [98]. This study emphasized the importance of the biochemical criteria utilized in defining PHPT, as well as the population studied to determine its prevalence. Thus, lower serum calcium and PTH thresholds for diagnosis, female sex, and older age are associated with a higher prevalence of PHPT. Outside of laboratory biochemical screening, prevalence of PHPT has also been estimated utilizing parathyroid pathology. An autopsy study in 422 Swedish subjects without advanced renal disease (mean age 65 years) demonstrated histologic parathyroid hyperplasia in 7% and adenomas in 2.4% [99]. It was concluded that histologic parathyroid disease may be more common than biochemical hyperparathyroidism, and early forms of histologic PHPT may have only mild abnormalities in calcium biomarkers. The first US population-based assessment of PHPT from Rochester, MN, confirmed that routine measurement of serum calcium in automated chemistry panels led to a four-fold increase in its incidence [100]. The Rochester Epidemiology Project was utilized to assist with retrospective identification of subjects, and subsequently, medical records PHPT [101]. Automated serum chemistry panels measured 12 biochemical analytes, including serum calcium, and were used to screen patients at the Mayo Clinic for multiple medical disorders. The average annual incidence of PHPT before June 30, 1974 was 7.8 per 100,000, but the year after the introduction of routine serum calcium measurement with automated chemistry panels the incidence peaked at 51.1 per 100,000. Similarly, from the first 6 months to the second half of 1974, the incidence rate of PHPT increased from 24.0 per 100,000 person-years to 129.4 per 100,000 person-years, which represented the peak observed rate in Rochester, MN [102]. After the initial detection of previously prevalent cases, the incidence rate dropped to 27.7 per 100,000 during the last 1.5 years of the initial

study, which was thought to represent the true rate in an environment of automated measurement of serum calcium [100]. A similar sharp rise and fall in the incidence of diabetes mellitus was observed in Rochester after routine blood glucose measurement was started in 1959 [103]. The incidence of PHPT in Rochester after the initial detection of previously prevalent cases was consistent with estimates in Sweden and the United Kingdom at that time [104]. The higher incidence rate was attributed to more thorough case ascertainment of subjects with asymptomatic hypercalcaemia [105]. Throughout the 1980s the incidence of PHPT in Rochester, MN, steadily decreased, and by 1992 the incidence had declined to four cases per 100,000 person-years, suggesting a fundamental modification about the prevalence and incidence of the disease [106]. The overall age- and sex-adjusted incidence was 20.8 per 100,000 person-years from 1983 to 1992 [102]. Furthermore, the decline occurred despite improving case ascertainment through the inclusion of laboratory data in identifying hypercalcemia, in addition to the clinical diagnosis.

Although other studies also suggested lower incidence rates, these were based on surgical cases rather than population-based assessment [107]. The cause of the lower incidence rate during this time period was not clear. The decrease in incidence was not related to less frequent measurement of calcium, since 20% of Rochester residents were tested each year during this period. Changes in estrogen replacement therapy also did not explain the unexpected decrease in the incidence of PHPT, since estrogen-containing prescriptions did not decline during this time [108] and identical declining trends were observed in men [102]. Throughout most of the 1990s the lower incidence rate of PHPT persisted [106]. The persistently lower rate was seen despite thorough case ascertainment, this time identifying patients with elevated serum calcium or PTH measurements, in addition to the clinical diagnosis of PHPT. The overall age- and sex-adjusted incidence of 21.6 per 100,000 person-years in 1993–2001 was less than the annual rate of 29.1 per 100,000 person-years in 1982–1992, and 82.5 per 100,000 person-years from July 1974 to 1982. Despite the continued lower incidence of PHPT in Rochester, MN, the number of parathyroidectomies

remained relatively stable at 230–300 per year [106], suggesting that serum calcium was still measured on a relatively frequent basis. An important regulatory change eliminated the use of automated chemistry panels on June 13, 1996 in Rochester, MN, thereby necessitating individual orders for serum calcium measurements. Despite a 30% reduction in serum calcium measurements after this change, a difference in the patient profile was not observed, as most patients remained asymptomatic with mild hypercalcaemia [106].

Furthermore, a rise in PHPT incidence was noted in 1998, which suggested that another important change in the incidence rate might be occurring. Three more recent population-based studies, from Scotland, the United States, and Denmark, have demonstrated that the incidence of PHPT is higher than previously reported, suggesting that the incidence had increased from 2000 to 2010 [91]. The first European study reporting a higher incidence rate of PHPT emerged from Tayside, Scotland, where between 1997 and 2006 retrospective electronic database inquiry of serum calcium results and medical record diagnoses showed an incidence rate of 57.8 to 142.7 per 100,000 person-years in women and 22.8–79.5 in men (overall, 41.3–113.0 per qEuropean incidence rates of less than six per 100,000 person-years [109]. Prevalence of PHPT in 2006 was 672 per 100,000 in Tayside. Notably, the number of calcium tests increased by 10–15% per year over the study period, but did not show cyclicity in the annual incidence of PHPT [91]. Subsequently, a retrospective electronic record study identifying diagnostic codes for PHPT in Denmark from 1977 to 2010, but utilizing hospital admissions records only until 1990, demonstrated a progressive rise in incidence rates from 1990 to 2010 [110]. Furthermore, a threefold increase in incidence was observed from 1998 to 2010. Although the increase was seen in both men and women, the largest increase in incidence occurred among women more than 50 years of age. Despite the increasing incidence rate in Denmark, the rate of 16 per 100,000 in 2010 remained below other reports during the same period [111]. The most recent study on the incidence of PHPT was the second performed in North America, but importantly, in an ethnically diverse population.

Yeh and colleges evaluated the incidence and prevalence of PHPT within the Kaiser Permanente Southern California database, containing a racially mixed population (Asian 5%, Black 8.8%, Hispanic 26.5%, other 23%, White 36.7%) [111]. Retrospective electronic analysis of patients with elevated serum calcium measurements from 1995 to 2010 showed that the mean age-adjusted incidence of PHPT was 65.5 per 100,000 person-years in women and 24.7 per 100,000 person-years in men. These higher rates than previously described may in part be explained by the lack of individual medical record review to adjust for potential confounding clinical factors that are unrecognized in the electronic databases. Although the proportion of Kaiser Permanente enrollees with at least one serum calcium measure grew from 6.8 to 12.7% in women and 5.3 to 8.9% in men over the study period, this increase did not correlate with the incidence of PHPT. By 2010, the age-adjusted prevalence of PHPT was 232.7 per 100,000 in women and 85.5 per 100,000 in men.

2.1.3 Epidemiology in subjects with Head or Neck Radiation Exposure

Ionizing radiation is a recognized risk factor for the development of sporadic PHPT due to parathyroid adenomas [112]. Use of therapeutic head/neck radiation in the 1930s and 1940s in the United States for benign conditions such as acne has been associated with surgery for parathyroid adenomas three decades thereafter [113]. In a prospective study of 4297 patients who received radiation to the tonsils before 16 years of age, 32 patients developed clinical PHPT, with an incidence of 18.7 per 100,000 person-years below the age of 40 years and 171 per 100,000 among those 40–60 years [113]. This is an estimated 2.9- and 2.5-fold increased incidence rate of PHPT for each age group, respectively. As expected, these individuals also had a high proportion of thyroid tumors (84%) and thyroid cancers (31%). Survivors from the Hiroshima atomic bomb also had a four-fold increase in the risk of PHPT [114]. Although it is possible that fallout from nuclear testing from 1951 to 1962 could have led to an increased incidence of PHPT years later, to date there is no convincing evidence that this has occurred [106].

2.1.4 Racial differences

PHPT appears to be more common among blacks compared to other races [111]. From 1995 to 2010 the age-adjusted incidence of PHPT was 92 and 46 per 100,000 in black women and men, respectively, compared to 81 and 29 per 100,000 in white women and men, 52 and 28 per 100,000 in Asian women and men, and 49 and 17 per 100,000 in Hispanic women and men [111]. The racial differences in this study were more apparent with increased age. Indeed, the highest reported prevalence rate of PHPT was in black women, affecting 921.5 per 100,000 in the 70–79 year age range.

2.1.5 Sex and age distribution

PHPT affects women more commonly than men, but the difference is not seen until menopause. In fact, the incidence rate in men and women <45 years of age is essentially the same, whereas at ≥ 45 years, the ratio of incidence in women to men is 2:1. Increasing age is also associated with higher incidence rates in both men and women, but the effect is more pronounced in women. The highest incidence rate of PHPT is seen in women 65–74 years of age, with an annual incidence of 99 per 100,000 person-years compared to a rate of 17.2 per 100,000 person-years in men of the same age [106]. In comparison, before 45 years of age the incidence rates are 7.7 and 6.0 per 100,000 person-years in men and women, respectively [106]. One reason for the increased incidence rate of PHPT in women as they age may be related to relatively abrupt menopausal estrogen decline. Estrogen inhibits activation of bone remodeling, suppresses bone resorption, and maintains bone formation [115]. In addition, estrogen deficiency is likely the major cause of postmenopausal bone loss, primarily resulting in accelerated cortical bone loss [115], which is the skeletal site most affected in PHPT.95 Hence, menopause may unmask early PHPT with the efflux of calcium from the skeleton that occurs with estrogen deficiency [116].

2.1.6 Mortality

Conflicting information exists regarding the risk of death in patients with PHPT.

Accurate estimates of death due to PHPT from death certificates are not feasible since endocrine disorders are often not included. In 2005, only 83 deaths due to hyperparathyroidism and other disorders of the parathyroid gland from the International Classification of Diseases (ICD)-Tenth Revision code E21.3, most of which were not specified as primary versus secondary hyperparathyroidism, were reported in the US with an estimated crude death rate of 0.35 per million per year for hyperparathyroidism [65]. The only data regarding mortality in North America come from Rochester, MN. In this population-based cohort, overall survival was not adversely affected among unselected patients in the community, and, in fact, was better than expected (RR = 0.69; 95% CI = 0.57–0.83) [117]. Death due to cancer and cardiovascular disease was also lower than expected. However, more severe hypercalcemia was an independent predictor of mortality, with serum calcium levels of ≥ 11.2 mg/dL associated with significantly reduced survival ($p < 0.001$) [117]. Data from a Danish case-control study of patients who had parathyroidectomy also demonstrated that patients from 1979 to 1990 with more severe serum calcium elevations than reported in North America had an increased risk of mortality compared to patients operated on from 1991 to 1997 with less severe hypercalcaemia [118]. Evaluation of referral patients undergoing parathyroidectomy at the Mayo Clinic in Rochester, MN, from 1980 to 1984 also did not have reduced survival after parathyroidectomy [119]. As opposed to North American data on mortality, European data based largely on patients with surgical PHPT who had more severe disease showed an increased risk of death [120]. One of these studies suggested that the increased mortality risk normalized approximately 5 years after parathyroidectomy in patients with milder PHPT operated on in 1980 [113]. However, other reports have suggested a persistently elevated risk of death several years after parathyroidectomy [113]. Other European studies in patients with mild to moderate PHPT who were observed have also demonstrated an increased risk of death [121]. More recently, a retrospective population-based study in Tayside, Scotland, from 1997 to 2006 in untreated patients with mild PHPT (mean baselineserum calcium 10.32 mg/dL and

maximum serum calcium <11.6 mg/dL) demonstrated an increased risk of all-cause mortality (SMR 2.62; 95% CI 2.39–2.86), cancer death (SMR 2.95; 95% CI 2.48–3.49), and cardiovascular mortality (SMR 2.68; 95% CI 2.34–3.05). Baseline PTH rather than serum calcium was associated with increased mortality [122]. The same investigators also demonstrated an increased risk of mortality and cardiovascular death in the same cohort of patients with mild untreated PHPT, with each patient matched to five population-based age, gender, and calendar year of PHPT diagnosis controls [123].

2.1.7 Cost of primary hyperparathirodism

PHPT is diagnosed with the measurement of serum calcium with a concomitant serum PTH. Additionally, serum albumin, creatinine, and phosphorus are commonly measured in this setting. Hence, diagnosing PHPT is relatively inexpensive, estimated to cost US$110.40 per patient aged 45 years and older when diagnosed through routine serum calcium screening [124]. Fortunately, hospitalization for severe hypercalcemia is rare, as is parathyroid carcinoma and death related to PHPT [65]. The incidence of hospitalization for PHPT in 1999 was estimated to be eight per 100,000 per year for all PHPT ICD-9 code (252.0) at hospital discharge, and 1.8 per 100,000 per year for PHPT being the first-listed code clark [125]. Notably, the hospitalization rate due to PHPT steadily declined over time from 4.7 per 100,000 per year in 1977 to 2.9 per 100,000 per year in 1986. The decline may in part be due to the creation of a separate code ICD for secondary hyperparathyroidism with the transition from ICD-8 to ICD-9. Nonetheless, the majority of patients with PHPT have relatively mild disease without serious complications. Therefore, the majority of cost of this disease at present is likely to be related to the initial evaluation and subsequent treatment, which, in general,is annual observational surveillance or parathyroidectomy, rather than hospitalization or death. Once diagnosed, the decision to observe or proceed with parathyroidectomy determines the majority of the cost of PHPT. Based on the 2009 NIH Consensus Conference criteria for parathyroidectomy,

approximately 50–60% of patients with PHPT will meet surgical criteria initially. Approximately 25% of asymptomatic patients with PHPT will develop evidence of progressive disease over 10 years, and 37% over 15 years, and therefore require surgical consideration. In clinical practice, some patients with uncomplicated asymptomatic PHPT who do not meet surgical criteria will undergo surgery and others meeting criteria will not [126]. Indeed, opinion determining which patients with PHPT should undergo parathyroid surgery varies among endocrine surgeons as well as endocrinologists [127]. After diagnosis of PHPT, the clinical evaluation to assess if complications are present often includes three-site dual-energy X-ray absorptiometry bone mineral density of the spine, hip, and non-dominant wrist, and imaging for nephrolithiasis by computed tomography (CT) abdomen and pelvis without contrast, renal ultrasound, or kidney ureter bladder (KUB) plain film radiographs with tomography. Due to the high prevalence of vitamin D deficiency in PHPT [128], serum 25-hydroxyvitamin D is also commonly measured. Finally, to distinguish PHPT from familial hypocalciuric hypercalcemia, 24-hour urine calcium and creatinine are often assessed, especially if surgery is a consideration, there is a family history of hyperparathyroidism or hypercalcemia, the patient has persistent PHPT after initial surgery, and/or the serum calcium level has not been documented to be normal on prior measurements. Localization of the parathyroid pathology is usually undertaken once a decision is made to proceed with surgery. Several studies have reviewed the cost of preoperative localization [129], but, ultimately, the decision regarding which test to utilize is determined by the test that as the highest sensitivity and specificity at the institution performing the study. Earlier estimates from Sweden between 1965 and 1979 reported a parathyroidectomy rate of 5–10 per 100,000 per year. In 1999, parathyroidectomy (ICD-9 code 06.8) was performed on approximately 12,000 hospitalized patients in the US, with an estimated parathyroidectomy rate of 4.4 per 100,000 per year [65]. The cost of parathyroidectomies in the US was $282 million annually based on 1996 dollars. Several innovations in the surgical management of PHPT, combining preoperative localization with intraoperative rapid parathyroid

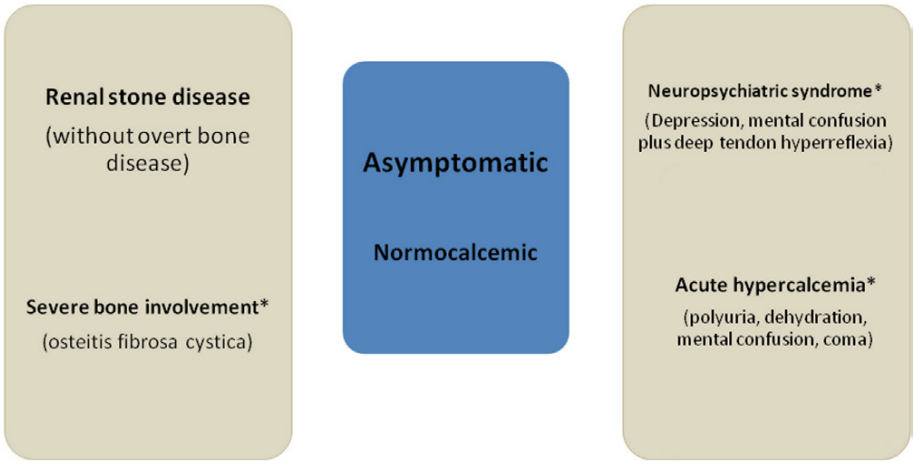
hormone assay [130] and radioguided parathyroidectomy [131] have occurred since these cost estimates. These advances have led to many patients requiring surgery having outpatient minimally invasive parathyroidectomies rather than traditional bilateral neck exploration, which is generally reserved for patients with prior neck surgery, recurrent or persistent PHPT, multi-gland disease, and disease that is not localized on preoperative imaging [132]. Although these innovations in general have not improved surgical outcomes, they have reduced the costs of surgery by reducing surgical time and length of stay in the hospital [133]. More recent cost estimates of parathyroid surgery based on 2005 dollars and using Medicare charge and reimbursement data (excluding lost productivity) suggest that the cost of single-gland parathyroidectomy is $ 3733, with multi-gland parathyroidectomy costing $ 4433, and reoperation being the most expensive procedure at $5711 [127]. More recent estimates based on Center for Medicare and Medicaid Service (CMS) clinical fee schedule amounts for parathyroidectomy indicate higher costs than this in 2013. The increased cost of reoperation emphasizes the importance of utilizing an experienced parathyroid surgeon with high cure rates. Given the increased risk of complications during reoperation for persistent PHPT, some have suggested that the costs of reoperation are more than twice the cost of initial surgery [134]. Fortunately, cure occurs in ≥ 95% with initial parathyroidectomy, and complications are seen in only 1–3% [135]. Variation in the care of patients with PHPT has important clinical and financial implications. Based upon the practice at the Mayo Clinic in 1977, it was estimated that the cost of observation would exceed the cost of early surgery after 5.5 years. Similarly, more recent cost-effectiveness analyses have suggested that surgery is preferable to observation or pharmacologic therapy in asymptomatic patients with a life expectancy of 5 years [136]. Current cost estimates do not include data regarding disability, due in part to the lack of evidence and clarity regarding outcomes of long-term observation versus surgery in mild asymptomatic PHPT.

2.2 Clinical Aspects of Primary hyperparathirodism (figure 7)

The presentation of PHPT has changed around the world. This phenomenon was first observed in the US and Europe, where most patients do not suffer from overt skeletal or renal complications of PHPT. However, in some regions the disease still presents with its traditional manifestations, that is, severe bone involvement. Thus, the presentation and some complications of PHPT are not necessarily the same in different countries. Rarely, neuropsychiatric symptoms or acute hypercalcemia may be the predominant presentation.

Figure 7 Clinical presentation of primary hyperparathyroidism [3].

* Uncommon forms



After the classical descriptions of PHPT by Fuller Albright in the first half of the twentieth century reports emerged about 40 years ago confirming a rise in the incidence of the disease,2 which was accompanied by a major shift in its pattern of clinical presentation toward a less symptomatic form [100]. The increase in the recognition of PHPT occurred in two phases. First, in the 1930s, PHPT was discovered to be a cause of nephrolithiasis, and second, in the early 1970s with the introduction of routine biochemical screening. As would be expected, the inclusion of the calcium measurement in the biochemical screening led to a shift in the presentation of PHPT toward a disease diagnosed as a result of evaluation for asymptomatic hypercalcaemia. Subsequently, a decline in the incidence was reported

from Rochester, Minesota [102], which could be best explained by the expected decrease in incidence that follows a period of increased case finding after the introduction of biochemical screening. Most PHPT patients in the past were middle-aged and had severe skeletal symptoms due to generalized osteopenia and osteitis fibrosa cystica. Fragility fractures, bone pain, and neurologic complications were a clinical reality, as were skeletal deformation and an altered body stature.1 Most patients had fairly pronounced hypercalcaemia and it was soon recognized that recurrent renal stones also accompanied PHPT.

2.2.1 Clinical Aspects in United States

Asymptomatic disease has been the dominant clinical phenotype of PHPT in the United States for the past 40 years. Overt skeletal manifestations are most uncommon and the incidence of nephrolithiasis has declined to 17–20% [137]. Over the past decade, yet another phenotype of PHPT has emerged due to an increasingly proactive approach to the evaluation of subjects with low bone density. In these subjects, the PTH level is typically measured as part of an extensive evaluation for low bone mass. Subjects are being discovered with elevated PTH levels despite consistently normal serum total and ionized calcium concentrations [138]. These individuals do not have any known secondary cause for an elevated PTH level. The clinical phenotype of normocalcaemic PHPT (NPHPT) has been considered the most important change in the presentation of PHPT in the USA over the past 10 years [87]. The incidence of PHPT peaks in the seventh decade, a phenomenon that is also seen today in countries such as China and Brazil where asymptomatic cases are becoming more frequent than in the past [139]. Most cases occur in women (74%), but the incidence is similar in men and women younger than 45 years of age [106]. Recently published data from an epidemiological study showed substantial differences in the incidence and prevalence of PHPT within a racially mixed population. The study population included 13,327 enrollees within the Kaiser Permanente healthcare system in Southern California between 1995 and 2010. Subjects had PHPT as defined by at least one elevated serum calcium level (>10.5 mg/dL, 2.6 mmol/L) and elevated or inappropriately

normal parathyroid hormone levels, after exclusion of secondary or renal and tertiary hyperparathyroidism cases. The incidence of PHPT fluctuated from 34 to 120 per 100,000 person-years (mean 66) among women, and from 13 to 36 (mean 25) among men. With advancing age, the incidence increased and sex differences became pronounced (incidence 12–24 per 100,000 for both sexes younger than 50 years; 80 and 36 per 100,000 for women and men aged 50–59 years, respectively; and 196 and 95 for women and men aged 70–79 years, respectively). The incidence of PHPT was highest among blacks (92 women; 46 men, $P < 0.0001$), followed by whites (81 women; 29 men), with rates for Asians (52 women, 28 men), Hispanics (49 women, 17 men), and other races (25 women, 6 men) being lower than that for whites ($P < 0.0001$). The prevalence of PHPT tripled during the study period, increasing from 76 to 233 per 100,000 women and from 30 to 85 per 100,000 men. Racial differences in prevalence mirrored those found in incidence [111]. PHPT may be part of genetic syndromes, the most common being MEN1, with an estimated prevalence of 2–3 per 100,000. The prevalence for MEN2A or other rare genetic forms of PHPT have not been established.

2.2.2 Clinical Aspects in Europe

In Europe, asymptomatic PHPT has become the predominant form of presentation as well, although some differences may exist in comparison to the United States. A study in 16,000 unselected subjects in Sweden showed persistent hypercalcemia during 2 successive years in 1.07% of individuals over 25 years of age. Hypercalcemic cases were followed for decades, but the diagnosis of PHPT, likely responsible for most cases of hypercalcemia, was unequivocally established only in 16 parathyroidectomized individuals [140]. In another population-based screening in Sweden, PHPT was considered to be the cause of hypercalcemia in 0.3% of adult men and 1.6% of corresponding women [141]. A study performed between 1997 and 2006 in Tayside, Scotland, UK, identified 2709 patients (70.8% female) diagnosed with PHPT by the end of 2006. The mean age of women (68 years) was older than

that of men (64 years) at baseline. The prevalence of diagnosed PHPT in Tayside increased from 1.82 per 1000 population in 1997 to 6.72 per 1000 population in 2006 (P< 0.001). The prevalence of PHPT was higher in females, and the female preponderance increased with age. The annual prevalence ratio between women and men was stable at around 2.5 each year and there was a 3–4-year cyclical incidence rate varying from 4.13 to 11.30 per 10,000 person-years [91].A retrospective, cross-sectional, observational study compared the biochemical and skeletal manifestations of male and female PHPT patients in two Caucasian populations (US and Italian) matched for age and body mass index (BMI). Although the mean serum calcium level were significantly higher in Italian men compared to women and in the Italians as a group compared to the US patients, the mean serum ionized calcium levels were similar. Moreover, mean serum PTH levels did not differ either between the genders or between the countries. After adjusting for BMI, the mean bone mineral density (BMD) at the proximal hip in female US patients was significantly higher than in the Italian women. Thus, it appears that despite similar levels of circulating PTH, Italian patients have more pronounced effects of the disease as assessed by serum total calcium, and more significant reductions in cortical bone in women as assessed by BMD. These data may support the idea that PHPT in urope may present somewhat differently than in the US. In another study designed to address how PHPT impacts the Italian hospital healthcare system, the authors found a decreased trend in hospitalization from 2006 to 2011, most likely because of economic issues. They also report a concomitant increase in the age of the patients, and, most notably, a progressive increase in the frequency of parathyroidectomy among patients admitted for PHPT.

2.2.3 Clinical Aspects in Latin American

In this country, only a few data are available on PHPT and they mainly come from case reports or small case series with the majority of patients presenting with a symptomatic phenotype. During the last decade, large series along with

epidemiological studies have been reported especially from Brazil [142]. In our institution, routine serum calcium measurements have been used as part of medical examination for the last 30 years. In the first large report of 124 patients with PHPT, 47% presented with no symptoms related to the disease, while 25% presented with severe skeletal involvement and osteitis fibrosa cystica, 25% with renal stone disease without overt bone involvement, and 2% with the typical neuropsychiatric syndrome. This same pattern was also seen in the city of Sao Paulo [142]. BMD was extremely low in severely affected patients but showed remarkable recovery following surgical cure. Serum PTH and bone markers were considerably higher in these patients, who also had a high rate of vitamin D deficiency, and the localization of the parathyroid lesion was easier than in asymptomatic patients. Regarding etiology, 87% had histological confirmation of a single adenoma. In a study from Argentina, 38 of 87 (44%) PHPT patients (78 females) had kidney stones. Most (83%) of the patients sent to surgery had a single parathyroid adenoma. It has been performed an epidemiological study to determine the prevalence of PHPT in individuals aged 18 years or more attending public and private endocrine centers. The diagnostic criteria for PHPT were as follows: elevated serum calcium on two occasions plus serum PTH above the 75th percentile for the reference population (57 pg/ml). The prevalence of PHPT among 4207 patients was 0.78% (95% CI 0.52–1.04); 81.8% of them were asymptomatic. The female/male ratio was 7.2:1, and 89.7% of these women were postmenopausal. Mean age was 61.1 ± 15.7 years, serum calcium 10.63 ± 1.33 mg/dL, and serum PTH 182.48 ± 326.51 pg/mL. Osteitis fibrosa cystica was present in 6.1%; nephrolithiasis in 18.2%, and neuropsychiatric syndrome in 3%; 51.5% had fatigue and 39.3% muscle weakness; 63.6% hypertension; 33% type 2 diabetes mellitus; 18.2% depression; 6.1% peptic ulcer and MEN1.16. It has been also evaluated the differences between asymptomatic mild hypercalcemic and normocalcemic PHPT, which is increasingly recognized in our country due to the widespread inclusion of serum PTH as part of the osteoporosis workup. In a series of 70 patients with PHPT (33 normocalcemic and 37 with mild hypercalcemia), it has been found no

statistically significant difference in the frequency of nephrolithiasis [18.2% in normocalcemic and 18.9% in the hypercalcemic patients (P = 0.937)] and the history of previous fractures [15% in normocalcemic patients and 10.8% of hypercalcemic patients (P = 0.726)]. [143].

2.2.4 Clinical aspects in Asia

In Beijing, China, over a long period, 1958–1993, patients with PHPT demonstrated a much higher serum calcium level (12.4 ± 1.1 vs. 10.7 ± 10.7 mg/dL), and a remarkably higher PTH concentration (21.4- vs. 1.86-fold the upper limit of normal) than their American counterparts. Strikingly, 97% of the Beijing PHPT patients suffered from skeletal lesions (osteitis fibrosa cystica, osteoporosis, and pathological fractures), kidney stones, and other features of classical PHPT. Recently, from 2000 to 2010, it has been reported a change in clinical patterns of PHPT in Chinese patients [144]. In this decade, a total of 249 consecutive PHPT patients coming from 17 out of 31 provinces in China were recognized and treated in a single clinical center in Shanghai. As compared to patients from Western countries [145] Chinese patients were younger and less likely to be women (F:M 2.07:1 vs. approximately 3:1 in Western countries). Their serum calcium (11.7 ± 1.4 mmol/L), PTH 402 pg/mL [(range 103–2700), normal range 15–65 pg/mL], and creatinine levels were all significantly higher, while serum albumin and mean 25-hydroxyvitamin D (25OHD) concentrations (13 ng/mL; range 5.2–29.9) were much lower. Sixty percent of PHPT patients manifested classical symptoms related to PHPT, such as polydipsia, polyuria, urolithiasis, bone pain, fatigue, etc. Nearly 6% of PHPT cases in Chinese patients had parathyroid carcinoma. NPHPT was not identified [144]. Despite symptomatic PHPT still being common, there was a dramatic increase of asymptomatic PHPT cases in the Shanghai cohort from less than 20% before 2006 to approximately 50% in the 2007–2010 period. This change was mainly driven by more frequent routine serum calcium testing and the incidental discovery of parathyroid nodules on neck ultrasonography, performed for the evaluation of thyroid disease [146]. Similar to the

experience of Western countries, a survey in Hong Kong demonstrated a steady seven-fold increase in the diagnosis of PHPT cases over a period of 30 years, from 1973 to 2002,30 with the observation of a shift from a disease with significant metabolic complications to a milder form of PHPT, often with asymptomatic presentation. In Asian countries, like India, Iran, Saudi Arabia, and Thailand, there is still a predominance of symptomatic disease, with overt skeletal and renal manifestations. Asymptomatic PHPT in these other Asian countries is rarely seen (0–2.2%).

2.2.5 Bone disease

The classic bone disease of PHPT is osteitis fibrosa cystica, a disorder which causes bone pain, deformities, and pathologic fractures. Excessive bone resorption due to the elevated concentrations of PTH is associated with several typical radiologic signs. Sub-periosteal bone resorption of the distal phalanges is the most sensitive radiologic feature. It is appreciated best on the radial side of the middle phalanges. Radiologic changes may be present in the skull, namely the so-called "salt-and pepper" pattern. Local destructive lesions, bone cysts, and "brown tumors" in the long bones and pelvis constitute other skeletal manifestations of the disease. Both the non-specific demineralization and the specific radiologic manifestations outlined above reflect the catabolic skeletal actions of PTH. All these abnormalities are associated with marked decreases in BMD at all sites. Non-specific generalized skeletal demineralization is sometimes evident in the absence of the above features of hyperparathyroid bone disease, and this is particularly true in asymptomatic patients in whom low BMD is not associated with the clinical or radiographic manifestations seen in overt bone disease In asymptomatic PHPT, the predominant catabolic effect of PTH is seen at the distal 1/3 radius, a cortical site. This, together with the fact that the lumbar spine, a trabecular site, is less affected, has given rise to the notion that the trabecular skeleton may be relatively preserved in patients with asymptomatic PHPT [147]. Recent data obtained by high resolution QCT imaging as well as other imaging modalities

have raised questions about the selectivity of PTH to erode cortical bone in mild PHPT [148]. After parathyroidectomy, BMD increases even in those with minimal evidence of demineralization. In addition, BMD is almost completely restored after successful parathyroidectomy, in patients with osteitis fibrosa cystica associated with extreme bone loss [149].

Generalized osteopenia and fractures were a regular feature of the form of PHPT seen many years ago. Data on the disease as it is seen today in the United States, however, are both limited and less clear. Interpretation and generalization from available reports is limited by design issues, including retrospective study design, small size, selection of patients and controls, and variable definitions of vertebral fractures.

Most cohort studies report an increased risk of any fracture [150]. In terms of site specificity, data from bone density and histomorphometry might lead one to expect that, in PHPT, fracture incidence would not be increased at the spine, whereas fractures of the distal radius or other cortical sites might be increased. Consistent with this notion, the risk of forearm fractures has been reported to be increased [150]. On the other hand, the result with respect to vertebral fractures is discordant with boe density and histomorphometry data, given the relative preservation of bone density at the spine, and sparing of cancellous bone on biopsy analysis. Although vertebral fracture risk was not found to be increased in several studies [151], most studies report an increased risk, [152] even in patients with asymptomatic disease [152]. Initially, this discrepant finding was thought to be due to surveillance bias, as the skeleton of patients who carry the diagnosis of PHPT is monitored far more carefully than most, and vertebral fractures might be diagnosed with increased frequency in this closely observed population, in whom back pain is more likely to lead to an X-ray. It would also seem logical to anticipate an increased incidence of hip fractures associated with cortical bone loss in this disease, but PHPT is not a dominant feature in any series of hip fracture patients, and hip fractures are not a dominant feature in any series of elderly patients with PHPT. Thus, the increased incidence of forearm fractures, at a site containing more cortical bone, is consistent with these expectations. Until a

multicenter trial with sufficient statistical power is performed, the question of fracture incidence in PHPT in the United States will not be resolved. After parathyroidectomy, fracture risk seems to decline. In patients followed without surgery, antiresorptive drugs have been shown to increase BMD in placebo-controlled trials, but there are no data available on whether these agents reduce fracture risk in PHPT [153].

2.2.6 Nephrolithiasis

The frequency of a renal stone disorder varies considerably among PHPT cohorts and has decreased during recent decades. This traditional sign is clinically easy to recognize and is an undisputed indication for parathyroidectomy [154]. Although the incidence of nephrolithiasis in PHPT has diminished along with the incidence of bone disease, renal stones are still seen [155]. Most series now place the rate of kidney stones at 15–20% of all patients with PHPT, with a lower (7%) frequency evaluated by ultrasound in patients with asymptomatic PHPT [155]. Besides nephrolithiasis, the kidneys may be affected in other ways. Deposition of calcium phosphate crystals throughout the renal parenchyma, a process known as nephrocalcinosis, may occur. Nephrocalcinosis may or may not be associated with frank stones and/or a reduction in creatinine clearance [155].

2.2.7 Other clinical manifestations

PHPT has the potential to involve organ systems besides the skeleton and the kidneys. The common complaints of weakness and fatigue were associated with a particular neuromuscular syndrome, characterized histologically by atrophy of type II muscle fibers [156].Current experience suggests that the weakness and easy fatigability reported by hyperparathyroid patients are no longer associated with overt neurologic findings, and the neuromuscular exam is usually normal in fatigued patients. However, subclinical abnormalities in detailed electroneurographical studies of peripheral nerves have been reported in some but not all cohorts with asymptomatic

PHPT [156]. Studies reporting psychiatric manifestations have found the prevalence to be significantly higher than in seemingly appropriate control groups. Affective and neurasthenic symptoms are the most common of these disturbances. In particular, the patients have increased fatigue, weakness, and anxiety, as well as mood swings, irritability, and apathy [157]. More recent studies substantiate significant decreases in the quality of life with influences on physical, emotional, and social functions, and body pain and vitality [158]. Self-rating scales nevertheless have supported the existence of a psychological morbidity even in asymptomatic females with normocalcaemic or mildly hypercalcemic PHPT [158]. Peptic ulcer disease, formerly regarded as a frequent complication, is now predominantly seen in patients with MEN1 syndrome, in whom PHPT and peptic ulcer disease may coexist. Aside from this specific association, there is continuing debate over a pathophysiologic link between these two relatively common disorders. Similarly, the association between PHPT and acute pancreatitis, apart from that related to hypercalcemia per se , remains to be established [159]. Despite the increasing evidence that PHPT may contribute to increased cardiovascular morbidity and mortality, the pathogenic mechanisms of this association are still not completely understood. Various abnormalities have been found in conjunction with PHPT, including hypertension, premature atherosclerosis, valve calcification, left ventricular hypertrophy, and arrhythmias. Walker et al. found no evidence of increased left ventricular mass, or diastolic dysfunction, in patients with biochemically mild PHPT. However, the finding of increased serum calcium and PTH levels in patients with diastolic dysfunction suggests that the disease severity may determine the presence of cardiac manifestations in PHPT [160]. In another study, Iwata et al. identified that mild PHPT was associated with subclinical aortic valve calcification and this was predicted by serum PTH concentrations. Serum PTH, but not serum calcium, was a more important predictor of aortic valve calcification than well-accepted cardiovascular risk factors [161].

2.3 Quality of life

At the time of the last International Workshop on Asymptomatic PHPT in 2008, three RCTs of PTX versus observation upon QOL and psychological functioning in asymptomatic PHPT patients with mild hypercalcemia (calcium 10.2–10.8 mg/dl) had been published. Despite using the same tool [the Short Form-36 general health survey (SF-36), which measures functional health and well-being], findings varied between studies. For the first time, Rao and colleagues have revieled no difference in baseline SF-36 scores between PHPT patients and normal values[162]. Anyway, surgery was associated with a significant benefit in social functioning and emotional role function on the SF-36. In the second RCT, Bollerslev and colleagues [163] have investigated the effect of PTX versus medical observation upon QOL in a large, multinational trial in Scandinavia. They have shown that PHPT scored lower in all psychological domains and the mental component summary of the SF-36 compared to a large, age- and sex-matched reference population at baseline. At 2 years, physical function worsened in the observation group, although this parameter did not improve in those who underwent PTX. Similarly, surgery provided no consistent improvement in the psychological domains of functioning.

Th authors have concluded that PHPT is characterized by impaired QOL but surgery did not provide any significant benefits.

Recently, Ambrogini et al. have studied the effect of surgery vs oberservation on QOL in 50 PHPT subjects (n=50), none of whom met the Asymptomatic PHPT Workshop guidelines for surgery [164]. It has been recorded that the SF-36 evaluation was similar between PHPT and normal control subjects.At 1 year, PTX group had a higher emotional role function score instead emotional role function did not improve after surgery as it did in the Rao study. In conclusion, the authors have recorded a beneficial effect of PTX on bodily pain, general health, vitality, and mental health but no worsening in the observation group was noted. While the balance of data in these three RCTs does support a marginally beneficial effect of PTX on QOL, the findings across studies were inconsistent. The last International Workshop, therefore, did not

add impaired QOL to the criteria for PTX but identified the need for more data in this area [165]. Since that time, QOL has been evaluated by a number of observational studies with a focus on the long-term benefits of PTX. Pasieka et al. reported 10-year data on QOL after PTX [166]. QOL was assessed using the Parathyroid Assessment of Symptoms Score (PAS), which has been shown to correlate with SF-36 scores [167]. Data were available on 78 of Pasieka's original 122 symptomatic and asymptomatic PHPT patients (mean calcium 11 mg/dl) and 39 of 58 thyroidectomy study participants who were followed for a mean of 10 and 11 years, respectively. At baseline, PHPT had worse QOL compared to controls. PTX resulted in a reduction in PAS that was sustained for 10 years versus controls in whom there was no change over time. Amstrup et al. [168] also reported on long-term effects of PTX on QOL. This study utilized the SF-36 and included a cross-sectional analysis of 51 PHPT patients (symptoms and calcium not reported) who had been successfully treated with PTX at least 5 years earlier versus 51 population-based, age-matched controls. Their results indicated that reduced QOL persisted in former PHPT patients who had been cured by surgery. The authors concluded that a change in surgical guidelines was not warranted though they acknowledged that the reduced QOL in their participants was difficult to attribute solely to the history of PHPT. In contrast, Leong et al. studied QOL using the same tool (SF-36) in 24 PHPT patients (median calcium 11.2 mg/dl) pre- and 6 months postoperatively. Preoperative scores were lower than national averages in all eight domains. Post-PTX, there were improvements in six of eight domains: physical and social functioning, physical and emotional role limitations, energy, and mental health. Median physical component summary score and the mental component summary scores were improved such that the mental component summary was comparable to the national average. Other recent data come from small studies with methodological flaws. Results from an uncontrolled study in Japan are difficult to interpret given the lack of a validated questionnaire, but post-PTX there were no changes in asymptomatic PHPT (mean calcium 11.0 mg/dl) patients' subjective assessment of neuropsychological symptoms including tiring easily,

forgetfulness, decreased concentration, depression, irritability, uneasiness, or sleeplessness [169]. In a small (n = 22) Brazilian study, the authors noted that those with long-standing PHPT (13.7 years on average) had lower QOL as assessed by the SF-36 (functional capacity, physical limitations, general state of health, and vitality) compared to those with a more recent diagnosis. There were, however, no differences in mental health and the authors did not adjust for age, which was on average 6 years older in those with long-standing PHPT [158]. In summary, both RCT and observational studies tend to suggest impaired QOL in patients with PHPT. However, specific areas of impairment and results regarding improvement after PTX both from observational studies and larger RCTs are conflicting. It is not currently possible to predict any specific change in quality of life with PTX and current data do not support sending a patient to surgery for this purpose.

2.4 Normocalcemic primary hyperparathyroidism

Normocalcemic Hyperparathyroidism (NPHPT) is a subclinical pathological condition characterized by normal total and ionized serum calcium concentrations and consistently elevated PTH levels[170] after ruling out the causes of secondary hyperparathyroidism such as vitamin D deficiency and kidney failure. In particular:

Vitamin D deficiency. There is an inverse relationship between PTH and 25-hydroxyvitamin D. At some reduced level of 25-hydroxyvitamin D, the parathyroidglands are signaled to increase PTH secretion. Exactly what the threshold value for 25-hydroxyvitamin that leads to an increase in PTH is controversial. The Institute of Medicine report states that there is no conclusive evidence that levels of 25-hydroxyvitamin D $\geq$ 20 ng/ml are regularly associated with increases in PTH levelsin population sampling. However, these studies are confounded by the lack of any prospective data that would track an individual's PTH level as the 25-hydroxyvitamin D levels is increased from 20 ng/mL to 30 ng/mL. Anyway, to be confident in the diagnosis of normocalcemic primary hyperparathyroidism, it would seem advisable to ensure that the 25-hydroxyvitamin D level is greater than 30

ng/mL. There is another reason for requiring the 25-hydroxyvitamin D level to be truly sufficient. Occasionally normocalcemic patients who demonstrate high PTH levels will become hypercalcemic when 25-hydroxyvitamin D levels are raised to over 30 ng/mL. In these situations, the correct diagnosis is traditional hypercalcemic primary hyperparathyroidism that was masked by the vitamin D deficiency.

Reduction of kidney function. It has been well demonstrated that PTH strats to rise when GRF fall down <60ml/min[171].

Medications. It has been described that Hydrochlorothiazide [172] and lithium [173] can be associated with elevation of PTH.

Hypercalciuria. This phenomenon may be associated with a rise in PTH levels.

Gastrointestinal disorders associated with calcium malabsorption [174].

In order to make a correct diagnosid it's mandatory to measure ionized calcium as sometimes occasional normal total calcium value is seen in hypercalcemic individuals [175]. In conclusion, *the diagnostic criteria for normocalcemic primary hyperparathyroidism, therefore, should include consistently normal albumin-adjusted total serum calcium and normal ionized calcium.*

It has been formally described in 2008 during the Third International Workshop on the Management of Asyntomatic Primary Hyperparathyroidism[176]. Rao and colleagues supported the hypothesis that NPHPT belongs to a first phase of a biphasic chronology related to its clinical development [177] (figure 8). The second phase rapresents the "hypercalcemic state" of the disease. Unfortunately, no available data are able to predict if and when the normocalcaemic subjects fall in the second phase category or remain stable in the first category. Other author[170] have proposed that this condition could be related to a mild target organ resistance to the actions of PTH because normocalcemic subjects have demonstrated inadequate suppression of PTH in response to an oral calcium load compared to hypercalcemic subjects. Moreover the ability of PTH to reduce tubular phosphate reabsorption and to stimulate 1,25-dihydroxyvitamin D synthesis was reduced in NPHPT[170].

Most of the reports of NPHPT have largely come from referral centers in which subjects were evaluated for a metabolic bone disease therefore the observation that they have skeletal involvement is not surprising. In table 1 is summarized the prevalences of NPHPT in various referral centers according to different diagnostic criteria used by the different invetigtors.

Table 1. Prevalence of Normocalcemic PHPT in Various Populations [3]

Study	Population	Prevalence	Comments
Cusano et al.[47]	Men ≥65 years, US (MrOS)	0.4%	Excluding renal failure (GFR <60 cc/min), vitamin D deficiency (25-hydroxyvitamin D <20 ng/dL), thiazide diuretic use
Lundgren et al.[48]	Women 55–75 years, Sweden	0.5%	Secondary etiologies of hyperparathyroidism not excluded, although pathologic evidence of parathyroid disease noted in some subjects
Misra et al.[49]	Men and women >45 years, US (NHANES)	1.0%	Excluding renal failure (GFR <60 cc/min) and vitamin D deficiency (25-hydroxyvitamin D <30 ng/dL)
Cusano et al.[47]	Men and women 18–65 years, US (DHS)	3.1% (Baseline) 0.6% (Follow-up)	Excluding renal failure (GFR <60 cc/min), vitamin D deficiency (25-hydroxyvitamin D <20 ng/dL), thiazide diuretic and lithium use
Garcia-Martin et al.[50]	Postmenopausal women, Spain	6%	Excluding renal disease, vitamin D deficiency (25-hydroxyvitamin D <30 ng/dL), and malnutrition
Berger et al.[51]	Men and women 19–97 years, Canada (CaMos)	16.7%	Excluding vitamin D deficiency (25-hydroxyvitamin D <20 ng/dL)

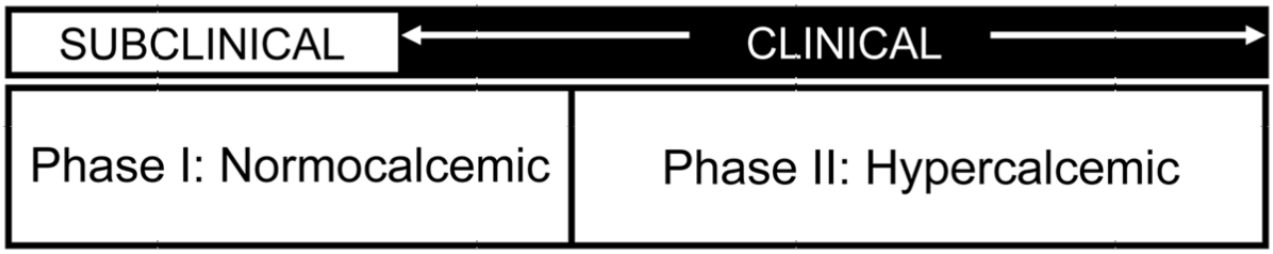
National Health and Nutrition Examination Survey, NHANES; The Osteoporotic Fractures in Men Study, MrOS; Dallas Heart Study, DHS; Canadian Multicentre Osteoporosis Study, CaMos.

These patients had been identified among symptomatic individuals referred for further evaluation or treatment of hyperparathyroidism. As a result, the phenotype was not seen incidentally but in a referral population[178].

However, Charopoulos and colleagues[178] tried to investigate the bone impairment in NPHPT in comparison to "hypercalcemic" subjects. The authors have analysed the cortical and trabecular component assessed by DXA and they have found that PTH catabolic effects were noted in both groups but more pronounced in hypercalcemic subjects than in normocalcemic subjects. Although cortical geometric properties were also adversely affected in subjects with NPHPT, trabecular properties were preserved.

Figure 8 **The two-phase hypothesis of the evolution of primary hyperparathyroidism, from**

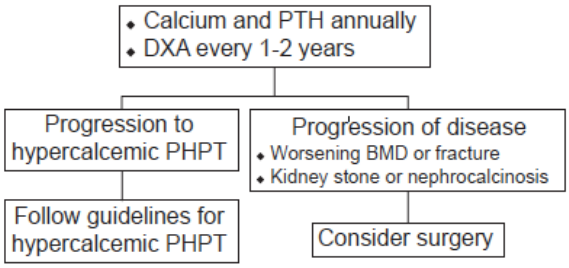
asymptomatic normocalcemic to symptomatic hypercalcemic disease [87]



We do not have significant and enough data to understand the natural history of this condition. The available data seem to support the concept that worsening of biochemical features of the NPHPT is slow. Indeed, Tordjman [179] and Garcia-Martin et al. [180] did not record any significant change in serum calcium, PTH or development of hypercalcaemia or nephrolithiasis, or fracture over the time.
Conversely, in the Columbia cohort [138], a symptomatic population at diagnosis, 40% of NPHPT subjects developed further signs of primary hyperparathyroidism during the mean follow-up period (max 8 years). Hypercalcemia developed in 19% of these individuals. The subjects who became hypercalcemic tended to be older, had higher baseline serum calcium levels, and higher baseline urinary calcium excretion.

The new guidelines for the management of NPHPT [181] suggest to monitor these subjects in the same way we monitor those with asymptomatic hypercalcemic primary hyperparathyroidism: annual serum calcium, PTH and bone mineral density assessment. If the disease evolves into the hypercalcemic form, then the published guidelines from the Fourth International Workshop would be reasonable to follow. Progression of the disease in other ways, such as worsening bone density, a fracture, or a kidney stone would signal a more proactive surgical approach to the disease, even if patients continue to be normocalcaemic (figure 9).

Figure 9. Management guidelines for normocalcemic PHPT proposed by the Fourth International Workshop on the Management of Asymptomatic PHPT [3]



2.5 Treatment

2.5.1 Surgery

Surgery provides the only option for cure of PHPT. While surgery is indicated in all patients with classical symptoms of primary hyperparathyroidism (overt bone disease or kidney stones, or if they have survived an episode of acute primary hyperparathyroidism with life-threatening hypercalcemia), there is considerable controversy concerning the need for intervention in patients who have no clear signs or symptoms of their disease. To date, four national and international conferences (1990, 2002, 2008, 2014) have updated guidelines for surgical intervention [181] (table 2).

Table 2. Guidelines for Surgery in Asymptomatic PHPT: A Comparison of Current Recommendations With Previous Ones [181]

	1990	2002	2008	2013
Measurement[b]				
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)
Skeletal	BMD by DXA: Z-score <−2.0 (site unspecified)	BMD by DXA: T-score <−2.5 at any site[b]	BMD by DXA: T-score <−2.5 at any site[b]	A. BMD by DXA: T-score < −2.5 at lumbar spine, total hip, femoral neck, or distal 1/3 radius[b]
			Previous fragility fracture[c]	B. Vertebral fracture by x-ray, CT, MRI, or VFA
Renal	A. eGFR reduced by >30% from expected B. 24-h urine for calcium >400 mg/d (>10 mmol/d)	A. eGFR reduced by >30% from expected B. 24-h urine for calcium >400 mg/d (>10 mmol/d)	A. eGFR < 60 cc/min B. 24-h urine for calcium not recommended	A. Creatinine clearance < 60 cc/min B. 24-h urine for calcium >400 mg/d (>10 mmol/d) and increased stone risk by biochemical stone risk analysis[d] C. Presence of nephrolithiasis or nephrocalcinosis by x-ray, ultrasound, or CT
Age, y	<50	<50	<50	<50

Abbreviations: eGFR, estimated glomerular filtration rate; MRI, magnetic resonance imaging. Patients need to meet only one of these criteria to be advised to have parathyroid surgery. They do not have to meet more than one.
[a] Surgery is also indicated in patients for whom medical surveillance is neither desired nor possible and in patients opting for surgery, in the absence of meeting any guidelines, as long as there are no medical contraindications.
[b] Consistent with the position established by the ISCD, the use of Z-scores instead of T-scores is recommended in evaluating BMD in premenopausal women and men younger than 50 y (11).
[c] The history of a fragility fracture at any site would define someone as having a complication of PHPT, and thus the individual would be automatically considered to be a surgical candidate.
[d] Most clinicians will first obtain a 24-hour urine for calcium excretion. If marked hypercalciuria is present (_400 mg/d [_10 mmol/d]), further evidence of calcium-containing stone risk should be sought by a urinary biochemical stone risk profile, available through most commercial laboratories. In the presence of abnormal findings indicating increased calcium-containing stone risk and marked hypercalciuria, a guideline for surgery is met.

In particular, asymptomatic patients are now advised to have surgery if they have [181]

1) Serum adjusted calcium greater than 1 mg/dl above the upper limit of normal; **2) Significantly reduced creatinine clearance (less than 60 cc/min).** Moreover, renal stone evaluation is now recommended by renal imaging with x-ray, ultrasound, or CT. If stones or nephrocalcinosis is present, surgery is recommended. Furthermore, twenty-four-hour urine for calcium will help in the differential diagnosis of FHH. If marked hypercalciuria is present (> 400 mg/d), a more complete urinary biochemical stone profile should be considered. In presence of abnormal findings indicating increased calcium-containing stone risk and marked hypercalciuria, a guideline for surgery is met

3) Bone density more than 2.5 SD below young normal control subjects at any site (T-score: –2.5 or below) or fragility fracture; reductions in bone density

continue to be a cause for concern in PHPT, either as patients present or as they are monitored. Surgery is recommended for peri- or postmenopausal women and men age 50 and older who have a T-score of - 2.5 or less at the lumbar spine, femoral neck, total hip, or distal 1/3 radius. In premenopausal women and in men under 50, the Z score of - 2.5 is recommended as the cut-point below which surgery is advised. The use of Z-scores instead of T-scores is consistent with the International Society of Clinical Densitometry (ISCD) official position in evaluating BMD in this population. This recommendation recognizes, however, that in PHPT, other effects of PTH on bone size and structure could influence fracture proclivity. Other approaches to skeletal evaluation, such as vertebral x-ray, VFA, TBS, or HRpQCT may provide information to help in the decision to recommend surgery. Substantial trabecular disease by TBS or HRpQCT could support a recommendation for surgery, recognizing that these modalities are not routinely available and thus cannot be widely used. About fracture, If a vertebral fracture is present by x-ray or VFA, surgery is recommended, even if there is no prior documentation.

4) **Subjects younger than 50 years of age.**

Because surgery is an acceptable approach even in patients who do not meet surgical guidelines, some physicians will recommend surgery for all patients with primary hyperparathyroidism; others will not recommend surgery unless clear-cut complications of primary hyperparathyroidism are present. Similarly, some patients do not want to live with a curable disease while others may be unwilling to face the risks of surgery, although surgical indications are present. Finally, advances in surgical techniques may also shift the balance in favor of intervention in the eyes of some patients and their physicians.

Preoperative localization was initially used to identify the location of an ectopic parathyroid gland. Today it is used to identify candidates for minimally invasive parathyroidectomy (MIP) as well as in disease that is recurrent or persistent after surgery [182–184]. These techniques should not be used to make the diagnosis; rather,

they should guide the surgeon once a diagnosis is made. The most widely used localization modalities are technetium-99m-sestamibi [with or without single photon emission computed tomography (SPECT)] or ultrasound. The former is excellent in single gland disease but is often inaccurate in multigland disease. Other localization techniques include four-dimensional (4D) CT scanning, magnetic resonance imaging (MRI), and the invasive modalities of selective venous sampling or arteriography. Radioisotopic imaging and ultrasound are best for parathyroid tissue that is located in proximity to the thyroid, whereas CT and MRI approaches are better for ectopically located parathyroid tissue. Arteriography and selective venous studies are reserved for those individuals in whom the noninvasive studies have not been successful. In patients who have undergone prior unsuccessful surgery, localization by two different modalities is suggested.

Surgery. Even without localization, an experienced parathyroid surgeon will find the abnormal parathyroid gland(s) 95% of the time in the patient who has not had previous neck surgery. The glands are notoriously variable in location, requiring the surgeon' s knowledge of typical ectopic sites such as intrathyroidal, retroesophageal, the lateral neck, and the mediastinum. Four-gland exploration was long considered the gold standard surgical approach. It remains the procedure of choice in patients with no suggestive localization studies and those with hereditary disease or lithium-induced disease, in whom multigland involvement is common. Today, focused MIP is rapidly becoming the procedure of choice in patients in whom preoperative localization has localized single gland disease [184]. MIP or unilateral exploration require the capability to measure intraoperative PTH levels. Taking advantage of the short half-life of PTH (3–5 minutes), an intraoperative PTH level is drawn shortly after resection [185]. If the PTH level falls by 50% and is within the normal range, the adenoma that has been removed is considered to be the only source of abnormal glandular activity, and the operation is terminated. There is some concern about the 50% decline rule, because if the PTH level falls by more than 50% but remains frankly elevated, other glandular sources of PTH may remain. In the case of

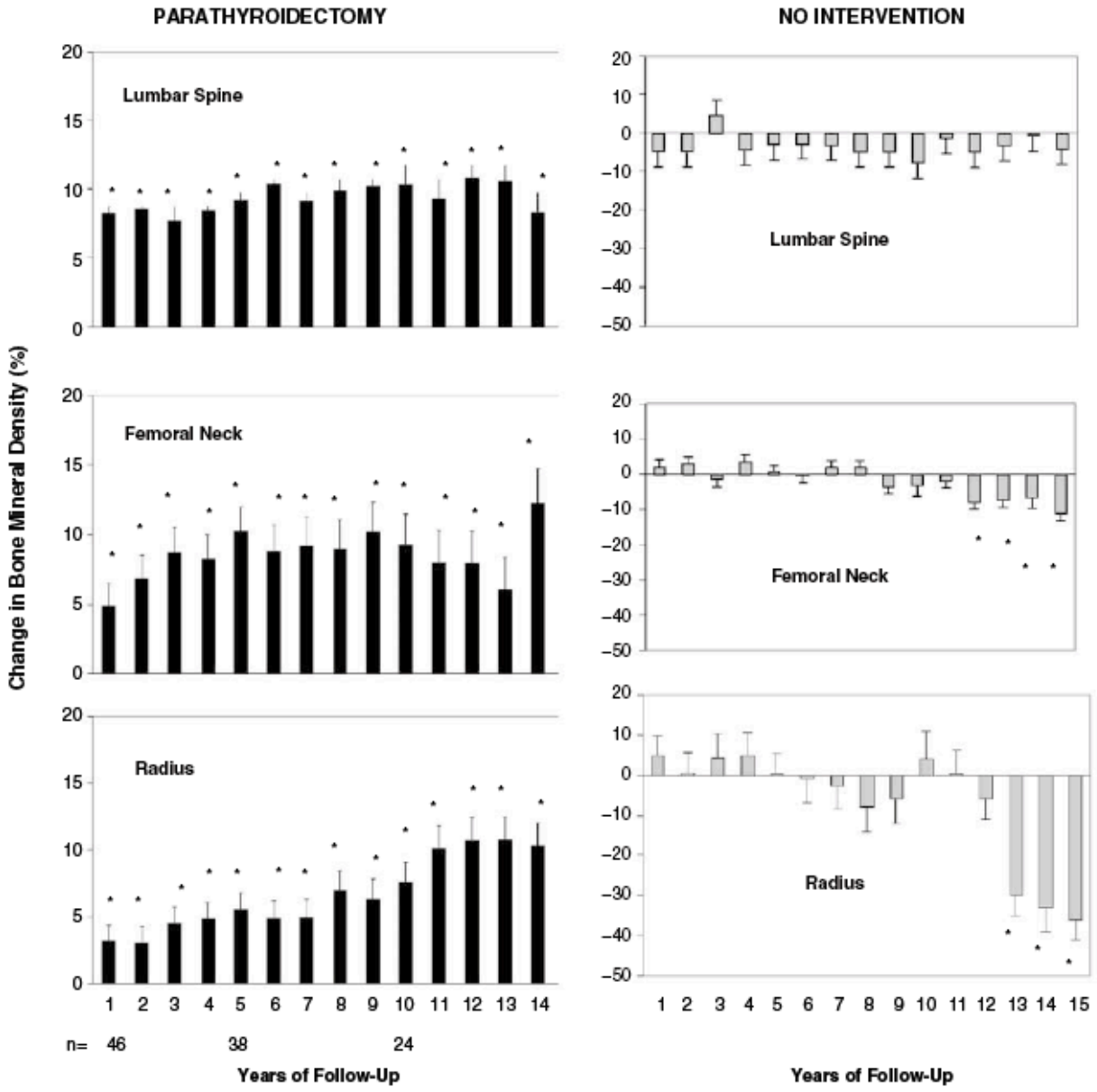
multiglandular disease, the approach is to remove all tissue except for a fragment of parathyroid tissue that is left in situ or autotransplanted into the nondominant forearm. Potential complications of surgery include damage to the recurrent laryngeal nerve, which can lead to hoarseness and reduced voice volume, and permanent hypoparathyroidism in those who have had previous neck surgery or who undergo subtotal parathyroidectomy (for multiglandular disease). Postoperatively, the patient may experience a brief period of transient hypocalcemia, during which time the normal but suppressed parathyroid glands regain their sensitivity to calcium. This happens within the first few days after surgery but can be prevented in most cases by providing patients with several grams of calcium on a daily basis during the first postoperative week. Prolonged postoperative symptomatic hypocalcemia as a result of rapid deposition of calcium and phosphate into bone ("hungry bone syndrome") is rare today. Such patients may require parenteral calcium for symptomatic hypocalcemia. After successful surgery, the patient is cured. Serum biochemistries and PTH levels normalize. Long-term observational data confirm short-term randomized trial fi ndings showing that bone density improves in the first several years after surgery [186]. The cumulative increase in bone mass at the lumbar spine and femoral neck can be as high as 12%, an increase that is sustained for years after parathyroidectomy (Figure 10). It is noteworthy that substantial improvement is seen at the lumbar spine, a site where PTH seems to protect from age-related and estrogen-deficiency bone loss. Because patients who have vertebral osteopenia or osteoporosis sustain an even more impressive improvement in spine BMD after cure, they should be routinely referred for surgery, regardless of the severity of their hypercalcemia.

2.5.2 Non surgical approach

Most patients who are not surgical candidates for parathyroidectomy do well when they are managed conservatively. In most such patients, biochemical indices (serum calcium, PTH, 1,25-dihydroxyvitamin D, and urinary calcium excretion) and BMD remain stable over the first decade of observation [187]. However, those patients

followed for longer than that period begin to show evidence of bone loss, particularly at the more cortical sites (hip and radius) [186].

Figure 10. Long term effect of surgery vs non intervention in hyperparathyroidism



In a 15-year observational cohort, 37% of patients with asymptomatic primary hyperparathyroidism had biochemical or bone densitometric evidence of disease progression. Those under the age of 50 years have a far higher incidence of progressive disease than do older patients (65% vs 23%), supporting the notion that younger patients should be referred for parathyroidectomy [188]. Finally, today, as in

the day of classical primary hyperparathyroidism, patients with symptomatic disease do poorly when observed without surgery. Thus, the data support the safety of observation without surgery only in selected patients with asymptomatic primary hyperparathyroidism, and even in those, indefinite observation is not clearly desirable.

A set of general medical guidelines is recommended for patients who do not undergo surgery [181]. Serum calcium levels should be measured once to twice yearly with annual assessment of serum creatinine and annual or biannual bone densitometry at the spine, hip, and distal one-third site of the forearm. Adequate hydration and ambulation are always encouraged. Thiazide diuretics and lithium should be avoided if possible, because they may worsen hypercalcemia. There is no good evidence that patients with primary hyperparathyroidism show significant fluctuations of their serum calcium as a function of dietary calcium intake. **Dietary calcium** intake should therefore be moderate, as low calcium diets could theoretically lead to further stimulation of PTH secretion. High calcium intake (more than 1 g/day) should be avoided in patients whose 1,25- dihydroxyvitamin D levels are elevated. We still lack an effective and safe therapeutic agent approved for the medical management of primary hyperparathyroidism in most patients. **Oral phosphate** will lower the serum calcium in patients with primary hyperparathyroidism by 0.5–1 mg/dl. Phosphate seems to act by three mechanisms: (1) interference with absorption of dietary calcium, (2) inhibition of bone resorption, and (3) inhibition of renal production of 1,25-dihydroxyvitamin D. Phosphate, however, is not recommended as an approach to management, because of concerns related to ectopic calcification in soft tissues as a result of increasing the calcium–phosphate product. Moreover, oral phosphate may lead to an undesirable further elevation of PTH levels. Gastrointestinal intolerance is another limiting feature of this approach. In postmenopausal women, **estrogen therapy** remains an option in those women desiring hormone replacement for treatment of symptoms of menopause [189]. The rationale for estrogen use in primary hyperparathyroidism is based on the known antagonism by estrogen of PTH-

mediated bone resorption. Although the serum calcium concentration does tend to decline after estrogen administration (by 0.5 mg/dl), PTH levels and the serum phosphorous concentration do not change. Estrogen replacement may improve BMD in these patients as well. A few data suggest that the **selective estrogen receptor modulator**, raloxifene, may have a similar effect on serum calcium levels in postmenopausal women with primary hyperparathyroidism [190].

The prevalence of **vitamin D** deficiency is more frequent in patients with PHPT than in geographically matched populations, independently of the cut-off values used for defining vitamin D status [191]. A recent Italian study reported a lower prevalence of vitamin D deficiency using a cut-off of serum 25OHD below 20 ng/mL [192]. Several mechanisms may contribute to the development of vitamin D deficiency in patients with PHPT [163]. The accelerated catabolism of 25OHD in response to the increased serum levels of 1,25(OH)2 D, [193] and the increased body mass index seen in patients with PHPT [194] are the more likely explanations. In PHPT, the vitamin D status may influence the clinical expression of the disease. Severe, symptomatic PHPT is frequently diagnosed in regions were vitamin deficiency is endemic and osteitis fibrosa cystica is still rather common [195]. Moreover, an inverse relationship between serum 25OHD levels and adenoma weight has been observed in countries where vitamin D deficiency is widespread [195]. Finally, in patients with asymptomatic PHPT, low serum 25OHD values have been associated with a more severe expression of the disease [196], including higher bone remodeling and lower BMD [197] and cardiovascular abnormalities in some [198] but not all studies [199]. Coexisting vitamin D deficiency/insufficiency in patients with mild PHPT may counteract the effect of PTH to increase serum and urinary calcium levels. Serum and urinary calcium may be in the normal range, leading to diagnostic uncertainty. Vitamin D repletion will allow the full-blown biochemical expression of PHPT and the proper stratification for management recommendation. Successful parathyroidectomy is followed by a spontaneous increase of serum 25OHD [164]. The Third International Workshop on Asymptomatic PHPT recommended that serum 25OHD be measured in all patients

with PHPT, and that D-deficient subjects be repleted to achieve a serum level of 25OHD greater than 20 ng/mL before any treatment decision [181]. The Endocrine Society also recommends correcting vitamin D deficiency, suggesting to reach serum values of 25OHD >30 ng/mL. This strategy should be extended to all individuals with suspected normocalcemic PHPT, where the diagnosis can only be confirmed once optimal levels of 25OHD have been reached. A few studies have addressed the effects of vitamin D supplementation in patients with PHPT. Gray et al. treated 21 patients with mild PHPT (serum calcium <12 mg/mL) and 25OHD <20 ng/mL with cholecalciferol for 12 months [200]. The mean $\pm$ SD (unless otherwise stated all data are expressed as mean $\pm$ SD) serum 25OHD at 6 and 12 months' observation were 30 $\pm$7 and 31$\pm$ 6 ng/mL, respectively. Serum levels of 1,25(OH)2 D did not change significantly. Mean serum calcium was unchanged following vitamin D supplementation, but one patient showed a rise of serum calcium from 10.5 to 11.9 mg/dL, accompanied by symptoms of hypercalcemia. Plasma PTH levels declined by about 25% and an inverse relationship was found at 6 months between the change in serum 25OHD and PTH. Mean 24-h urinary calcium did not significantly change, even though three patients had 24-h urinary calcium excretion >400 mg/dL after 6 months of vitamin D repletion. No patient developed symptomatic nephrolithiasis. The decrease of serumPTH was associated with a significant fall of serum total alkaline phosphatase and a tendency to decline in urine N-telopeptide (NTX). No change in BMD at the lumbar spine and femoral hip was observed. Tucci et al. prospectively followed 56 patients with PHPT for up to 34 weeks. Their serum calcium and 25OHD levels ranged from 10.6 to 12 mg/dL and from 7 to 24 ng/mL, respectively [201]. Treatment consisted of weekly ergocalciferol for 8 weeks, followed by individualized dosing of cholecalciferol or ergocalciferol in order to maintain serum 25OHD >30 ng/mL. After 10 weeks of therapy the mean serum 25OHD increased from 15 to 35 ng/mL ($P < 0.0001$). There was no change in mean serum calcium, PTH, and 24-h urinary calcium to creatinine ratio or urinary NTX. Serum calcium and 25OHD remained stable until the last observation after 34 weeks.

Velayoudom-Cephise et al. prospectively followed 22 patients with PHPT and serum 25OHD <30 ng/mL for 6 months. They were treated with either ergocalciferol or cholecalciferol [202]. Measurements at 6 months showed a modest, but statistically significant (P = 0.006), increase in mean serum 25OHD concentration and a statistically significant decline in mean serum calcium, PTH, osteocalcin (from 58 ±16 to 53 ±15 ng/mL, P = 0.011) and cross laps (from 5711 ± 4124 to 4247 ± 2191 pmol/L, P = 0.009). Serum phosphate significantly increased from 2.4 ± 0.5 to 2.8 ± 0.6 mg/dL (P = 0.018). Twenty-four hour urinary calcium excretion did not significantly change. Femoral neck T-score significantly increased from −2.3 ± 0.49 to −1.75 ± 0.86 (P =0.001). Rao et al. reported on 28 consecutive postmenopausal women with PHPT treated with cholecalciferol for a mean of 17 months [203]. Serum 25OHD significantly increased and serum PTH decreased. Serum calcium remained stable and renal function tests were unchanged. No patient developed nephrolithiasis. Wagner et al. retrospectively reviewed 35 patients with PHPT and vitamin D deficiency treated with different regimens of vitamin D supplementation for 5 months [204]. Serum 25OHD significantly increased in both groups. Serum calcium remained stable in the high-dose group and slightly decreased in the low-dose group. Serum PTH and creatinine did not change. No cases of nephrolithiasis occurred. Isidro and Ruano prospectively evaluated 27 patients with mild PHPT (mean serum calcium 10.8 ± 0.5 mg/ dL) and vitamin D deficiency (mean serum 25OHD 11.5 Å} 3.2 ng/mL) treated for 1 year with calcifediol [205]. Basal serum PTH levels (mean 188 ± 135 pg/mL) were inversely correlated with serum 25OHD. The starting dose of calcifediol ranged between 480 and 940 units daily and the dose was adjusted up to a maximum dose of 960 units daily. Mean serum 25OHD significantly increased during follow-up (28.6 ±13.0 at 12 months). Mean serum PTH significantly declined compared with baseline at 3 and 6 months, whereas no difference was observed at 12 months. Serum calcium, phosphate and alkaline phosphatase did not change. Two patients had an increase of serum calcium of 0.8 mg/dL after 3 months, without any calcium-related complaints. Mean 24-h urinary calcium significantly increased at 3

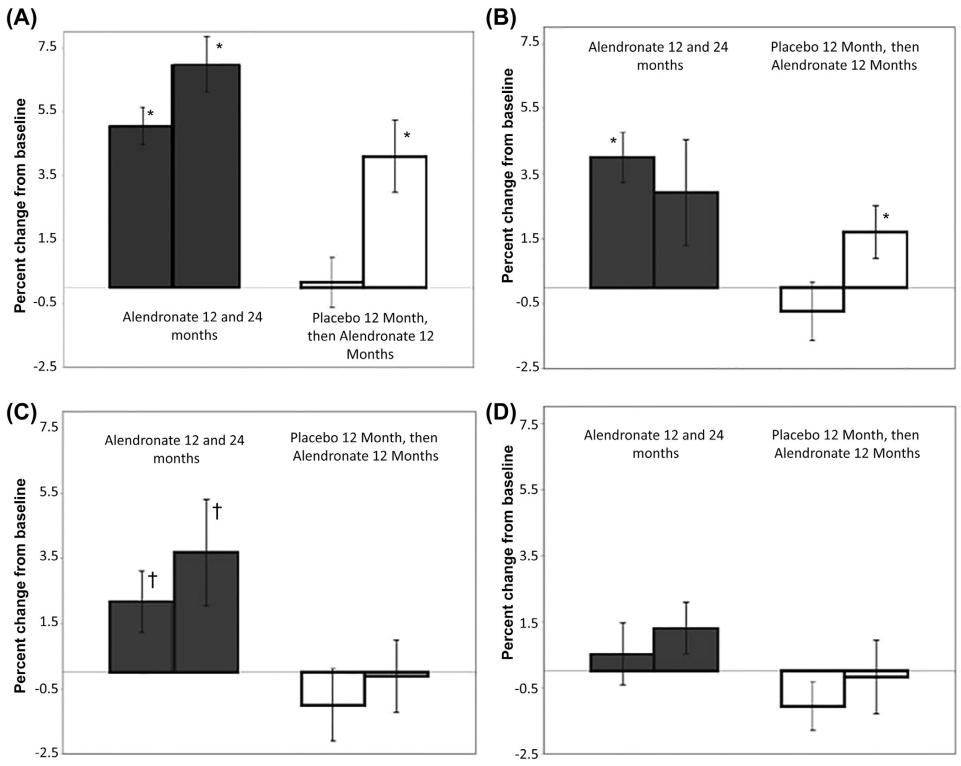
and 12 months. Three patients developed hypercalciuria while on the minimum dose of calcifediol and treatment was withdrawn; six patients developed hypercalciuria while taking 720 or 960 units daily and excretion returned to normal values upon reduction of the calcifediol dose. No patient experienced complaints related to hypercalciuria. In summary, the available data in patients with mild PHPT and coexistent vitamin D deficiency/insufficiency suggest that vitamin D supplementation is safe and potentially beneficial. Indeed, only one case of symptomatic hypercalcaemia 41 and six cases of hypercalciuria were observed in 162 patients receiving vitamin D supplementation [206]. Serum PTH declined in most studies suggesting that patients with PHPT and vitamin D deficiency/ insufficiency have a further increase of serum PTH driven by the D-deficient status, which might be deleterious particularly for the skeleton. On the other hand, the use of calcifediol appears to be associated with a greater risk of hypercalciuria. Nonetheless, no patient manifested complaints related to the increased urinary calcium excretion. Based on these findings, patients with PHPT and low serum 25OHD levels should be treated with daily doses of vitamin D not exceeding 1000 units, with close monitoring of serum and urinary calcium during the first few months and less frequently afterwards. Active vitamin D metabolites should not be used to correct vitamin D deficiency in PHPT. Vitamin D deficiency should also be corrected in patients planning to undergo surgery, because of the greater risk of postoperative hypocalcemia and secondary hyperparathyroidism. This association was clearly shown by Stewart et al. in a series of 190 patients with PHPT who underwent minimally invasive parathyroidectomy for a single adenoma [207].

Bisphosphonates have been investigated as a possible medical approach to primary hyperparathyroidism. Pamidronate, risedronate, and alendronate are all nitrogen-containing bisphosphonates that have been evaluated in PHPT. In small studies, administration of both pamidronate [208] and risedronate [209] led to transient decreases in serum calcium. These decreases in serum calcium were associated with rises in serum PTH [210]. The majority of the studies evaluating bisphosphonate therapy in PHPT have

been conducted using alendronate and this bisphosphonate has been found to effectively decrease bone turnover and increase BMD in patients with PHPT. Effects on serum calcium levels have been inconsistent. Serum PTH levels have been stable with no statistically significant change. Rossini et al. randomly assigned 26 elderly women with PHPT to treatment with oral alendronate (10 mg on alternate days) or no treatment for 2 years.55 Markers of bone turnover declined and remained suppressed in the active treatment group. BMD increased at the lumbar spine and hip (6.6 ± 3.5 and 1.2 ± 1.4% compared with baseline, respectively). The BMD changes in women given alendronate were similar to those observed in a group of women who underwent parathyroidectomy. A transient decline of serum calcium and urinary calcium excretion and a statistically significant increase in serum PTH were also noted. In an open label study by Parker et al., alendronate 10 mg/day was given to 32 patients with PHPT for 2 years. This treatment resulted in a significant increase in BMD at the lumbar spine by 7.3 ± 1.7%.56 There were non-significant transient changes in serum calcium and PTH. These levels returned to baseline by 3 months. In a randomized placebo-controlled study, 10 mg of alendronate was given to 40 postmenopausal women daily for 48 weeks [211]. Following the treatment period there was a 24-week treatment withdrawal phase. BMD at the femoral neck, distal 1/3 radius, and lumbar spine was monitored. Significant increases were observed at the lumbar spine and femoral neck with alendronate therapy with stable BMD at the distal 1/3 radius. There were no significant changes in serum PTH. A significant decrease in serum calcium from 11.3 to 11.0 mg/dl P = 0.018) was observed with alendronate therapy at 48 weeks. There was no significant change in serum PTH or urinary calcium excretion. An international double-blind placebo-controlled trial evaluated the effect alendronate on BMD, bone turnover, serum calcium, and PTH in individuals with PHPT [212]. Forty-four patients were enrolled in the study with the active treatment group receiving 10 mg/day of alendronate for 2 years. The placebo group received placebo in the first year and was then switched to active therapy with alendronate in the second year. BMD was monitored every 6 months, and bone

turnover markers, serum calcium, and PTH were monitored every 3 months. After 2 years, the alendronate-treated group demonstrated a significant increase in the lumbar spine BMD in comparison to baseline by 6.8 ± 0.94% ($P < 0.001$) (Figure 11). The placebo group showed no significant change from baseline in the first year. A 4.1% increase in the lumbar spine BMD was observed in the second year of the study when the placebo group was switched to alendronate treatment. Total hip BMD increased significantly in the active treatment group (4.01%) after 1 year.

Figure 11. Effect of treatment with alendronate or placebo on lumbar spine (A), total hip (B), femoral neck (C), and distal 1/3 radius (D) BMD. *, significantly higher ($P < 0.001$) compared with baseline; †, significantly higher ($P < 0.05$) compared with baseline (560).



There was no further increase during the second year of treatment. The placebo group showed no significant change in the first year. In the second year the placebo group was crossed over to alendronate and had a significant 1.7% increase in total hip BMD (Figure 11). There were no significant BMD changes at the distal 1/3 radius. Alendronate did result in a significant reduction in the markers of bone turnover.

Urinary NTX levels decreased by 66% (P< 0.001) at 3 months and remained suppressed throughout the treatment period. In the placebo group, no changes were seen until the crossover to alendronate and this was associated with a similar decrease in urinary NTX. No significant changes were observed in serum calcium or PTH levels.

There are very limited data evaluating bisphosphonate therapy in men with PHPT. Many of the studies mentioned above included men in their sample, but did not isolate them for a comparison in effectiveness with women. In the international study evaluating alendronate in PHPT, the male patients were evaluated separately and compared with the postmenopausal women [213]. Of the 44 patients who were studied there were 28 postmenopausal females and nine males. All participants met the inclusion criteria of confirmed hypercalcemia and elevated serum PTH levels by immunoradiometric assay. In addition, they also displayed reduced bone density in at least one skeletal site (lumbar, hip, spine, or distal 1/3 radius). The male and female groups had similar ratios in terms of ethnicity and similar mean age. The participants did not differ in any clinically meaningful way other than the distal 1/3 radius BMD being lower among postmenopausal women. The nine men receiving alendronate therapy showed a similar response to treatment, with increases in BMD at lumbar spine and total hip, and decreases in the bone turnover markers, as seen in postmenopausal women. The placebo group exhibited no difference in BMD or biochemical markers of bone turnover. The BMD increases at the lumbar spine and total hip were not significantly different between men and women. At the distal 1/3 radius, BMD increased more in men. Thus, men demonstrated a similar response to alendronate as seen in postmenopausal women with respect to impact on bone turnover markers and lumbar spine and total hip, but not distal 1/3 radius BMD. In summary, the RCT data published to date with alendronate have consistently demonstrated improvements in bone density and reductions in bone turnover. Serum calcium and PTH levels appear to be stable. There are no fracture data. Bisphosphonate therapy requires further study with respect to its impact on bone

strength and fracture risk.

Calcimimetic agents alter the function of the extracellular calcium-sensing receptor, increasing the affinity of the parathyroid cell calcium receptor for extracellular calcium, leading to increased intracellular calcium, a subsequent reduction in PTH synthesis and secretion, and ultimately a fall in the serum calcium. Indeed, cinacalcet have shown normalization of serum calcium for up to 5.5 years and sustained decreases in serum calcium levels across a wide range of disease severity [214]. However, this agent does not provide the equivalent of a "medical parathyroidectomy" because it does not improve bone density. There are no data on the effect of calcimimetics on constitutional or neuropsychological symptoms or fracture. Cinacalcet is approved for use in parathyroid cancer and was FDA approved for the treatment of severe hypercalcemia in patients with primary hyperparathyroidism who are unable to undergo parathyroidectomy. However, it is not currently recommended for general use in patients who are candidates for parathyroidectomy.

2.6 Bone Turnover Markers and PHPT

Characteristically in PHPT, increased bone metabolism is reflected in elevated levels of BTMs [215]. Moreover, subjects with more active disease will present higher levels of BTMs than those with less active disease. In particular, Silverberg and colleagues[216] have demonstrated that BTMs are not always very elevated when cohorts are averaged and compared with normal reference ranges. In a group of 66 asymptomatic subjects with PHPT, baseline mean values of the bone formation and resorption markers such as ALP and PYD, DPD, and OHP were all at the upper limit of the normal reference range. These levels did not show any tendency to change over a 6-year period of observation, [217] although it was previously shown that BTMs tend to be increased further with duration of disease [218].

PTH levels fall within minutes after the surgical removal of the pathological parathyroid gland(s), followed soon thereafter by normalization of serum calcium

levels. A rapid reductions of BTM have been shown immediately after PTX, followed by a more gradual reduction in bone formation markers. Reductions in BTMs are associated with BMD increments [186]. In a longitudinal 2 years study [219] and in a shorter one[220] (6 months), significant reductions in both formation and resorption markers were found after curative surgery.

Patients were then followed in a 36-month extension study [221]. By the end of the extension period, all BTMs were within the normal range.While a previous study [222] failed to show an association between BTMs levels before PTX and changes in BMD, this study have found that the levels of BTM before PTX were positively correlated with eventual changes in lumbar spine BMD [223]. These findings have been confirmed by another 1 year prospective study conducted in 53 subject with PHPT.

These studies show that BTMs normalize post-PTX and that probably patients with the highest turnover will experience the greatest gains in BMD.

In a small cohort of older PTHP subjects who underwent PTX, Tamura [224] have found that PTX had induced a BTM level decrease from baseline values at 1 year post-PTX. BTMs maintained lower compared to baseline values during the subsequent 5 years [225]. Overall, the data from these studies show that after PTX, BTMs routinely decline and if elevated prior to surgery fall into the normal range, with the greatest reductions occurring within the first postoperative year. Bone resorption markers respond more rapidly to PTX followed thereafter by reductions in bone formation markers. After surgery, a possible anabolic window is created, as early bone formation predominates due to the initial and preferential reduction in bone resorption.

Moreover, Boudou and colleagues have investigated the BTM trend after PTX in subjects with PHPT[128] who were divided into three groups according to the levels of BMT: a) high bone turnover (both elevation of CTX and P1NP); b) high bone resorption; c) normal bone turnover.

After surgery, significant reductions in CTX levels were observed in all three

subgroups, with the high turnover group showing the most prominent decline [128]. Conversely, P1NP significantly increased in the high turnover and high resorption groups. Interleukin-6 (IL-6) levels have been shown to be elevated in PHPT patients [226]. Moreover, Grey et al.21 showed that not only IL-6 levels are elevated in PHPT, but also IL-6 soluble receptor (IL-6sR), and that both IL-6 and IL-6sR levels decreased after PTX. Changes in IL-6 after PTX were tracked in a 12-day longitudinal study [227]. Similar to previous reports, the study showed that IL-6 baseline levels were higher in PHPT patients compared to controls. After PTX, IL-6 levels increased as early as 1–2 hours after surgery, peaking by day 1 and declining thereafter. IL-6 also increased postoperatively in the surgical control group, which did not undergo PTX but who underwent cholecystectomy, sigmoid colectomy or hemicolectomy, peaking by day 2 followed by a decline.

However, data are contrasting because there are studies that have reported also elevation in IL-6 after PTX probabily due to the inflammatory response to the surgery[228].

Recently, sclerostin has been studied in PHPT. Previous studies have demonstrated that PTH is a regulator of sclerostin [229]. It would be expected that sclerostin levels would be reduced in patients with PHPT and higher in patients with hypoparathyroidism, and such expectations have been confirmed. Compared to euparathyroid controls, sclerostin levels are higher in hypoparathyrodism [230]. In PHPT, sclerostin levels are only minimally lower than control subjects [231,232]. In a small cohort of PHPT patients who underwent PTX, sclerostin levels increased as early as 2 days postoperatively returning to the age reference range within 10 days and remaining stable up to a year throughout the study visits [233].

In conclusion, BTMs are elevated or in the high normal range in PHPT, depending on the severity of the disease. It is possible that some of the discordance seen in the pattern of how bone markers respond to treatment could be explained by the different severity of PHPT in cohorts enrolled in different studies.

After PTX, bone resorption markers fall markedly and quickly, followed gradually

by a slower fall in bone formation markers. Nonsurgical, pharmacological approaches to PHPT with anti-resorptive agents, but not with cinacalcet, are also associated with reductions in BTMs that persist for as long as the drug is used.

CHAPTER 3

RESEARCH PROJECT N°1

EFFECT OF ALENDRONATE AND VITAMIN D IN PATIENTS WITH NORMOCALCEMIC PRIMARY HYPERPARATHYROIDISM *.

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3.1 Introduction

Primary hyperparathyroidism (PHPT) is a common endocrine disease characterized by an inappropriate elevation of the circulating parathyroid hormone (PTH) that provides stable hypercalcaemia. Monoclonal parathyroid adenoma and multi-glandular hyperplasia represent the main causes of this endocrine condition. The disease has been reported to affect bone, kidney and cardiovascular system health, but in recent years its clinical scenario has completely changed. The widespread use of multi-channel biochemistry and the increasing attention paid to parathyroid pathologies are the reasons why it is increasingly frequent to find sub-clinical or asymptomatic forms of PHPT by accident during the biochemical screening of primarily middle-aged or elderly patient [181]. This subclinical form of hyperparathyroidism has been recently described as asymptomatic primary hyperparathyroidism. The Fourth International workshop for the management of asymptomatic primary hyperparathyroidism (PHPT) stated that it is characterized by the lack of specific sign and/or symptoms that are traditionally associated with PTH excess or hypercalcaemia [181]. A new part of the spectrum of the asymptomatic primary hyperparathyroidism is represented by normocalcaemic primary hyperparathyroidism (NPHPT). NPHPT is defined by normal total and ionized serum calcium concentrations and consistently elevated PTH levels after ruling out known causes for secondary elevations of PTH, such as renal disease or vitamin D deficiency [137,170,234]. It was first formally recognized at the time of the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism in 2008 [165] but, due to the lack of evidence, the expert panel

was not able to provide a clear guideline for the its management. Although there is contrasting evidence about the metabolic consequences of NPHPT [179,180], it is likely that subjects with NPHPT can develop kidney and bone involvement [138]. Charopoulos et al demonstrated an impairment of cortical and cancellous skeleton sites in both normocalcemic and primary hyperparathyroidism even though the effect was accentuated in hypercalcemic patients [178]. In the last published guidelines for the management of PHPT, parathyroidectomy (PTX) is recommended for subjects with a BMD T-score at any of three sites (lumbar spine, proximal femur, or forearm) of less than -2.5 SD [235]. Moreover, in subjects with mild primary hyperparathyroidism, PTX is followed by a decrease in bone turnover with an increase in bone mass, although the cortical compartment doesn't show any improvement in a short term [236].

No conclusive data are available about the treatment with vitamin D in subjects with PHPT. Although treatment with vitamin D_3 [200] and vitamin D_2 [201] slightly decreases the level of PTH in patients with vitamin D deficiency, its effect on bone mineral density (BMD) and bone turnover markers (BMT) has not been well established. Only a randomized placebo controlled trial [237] together with an uncontrolled trial [238] have demonstrated a significant reduction of bone resorption markers with a parallel increase of lumbar spine BMD. Some concerns were raised on the safety of this treatment as other investigators have reported increased plasma and urine calcium levels after treatment with vitamin D [239]. A few randomized controlled trials have investigated the role of anti-resorptive agents on the management of PHPT. In particular oestrogen therapy [189] and bisphosphonates [153] were shown to increase femoral neck and lumbar spine BMD in subjects with mild primary hyperparathyroidism compared to untreated patients.

To date, no data on the pharmacological treatment of NPHPT are available. Due to this lack of data, our study aimed to investigate the effects of oral treatment with alendronate and vitamin D on BMD and on the biochemical markers of both bone metabolism and turnover in post-menopausal women presenting with NPHPT. As a secondary endpoint we aimed to assess the safety and effects of vitamin D

supplements in NPHPT patients.

3.2 Materials and methods

3.2.1 Study design and population

The study was a prospective investigator-initiated, randomized, open parallel-group trial comparing weekly oral alendronate (70mg) plus cholecalciferol (2800 IU) (Fosavance® 70mg/28000 IU, tablets) with only weekly cholecalciferol (Dibase® 10,000 IU: 2800 IU = 11 drops). Thirty postmenopausal women (mean age 57±4 years) with NPHPT, as defined by the Consensus Development Conference on the management of primary hyperparathyroidism, were enrolled (from January 2009 to March 2013). At baseline, blood samples and information on medical history, socioeconomic factors, and dietary habits (intake of milk and milk products, fish and fruit) as well as the use of vitamin supplements were collected. In particular dietary calcium intake was assessed by a questionnaire [240] that was completed after the first visit. Inclusion criteria were a menopausal state of at least five years, the presence of osteoporosis (defined by a BMD T-score at one or more skeletal sites lower than - 2.5 SD), elevated serum PTH, normal values of calcium after adjustment for serum albumin and normal vitamin serum levels (> 30 ng/ml). Exclusion criteria were secondary hyperparathyroidism, concurrent systematic illness, thyroid disease, hepatic or renal dysfunction and other disorders known to influence BMD. No patients had received estrogens, bisphosphonates, supplements of calcium and/or vitamin D or other drugs that could interfere with bone or mineral metabolism during the last 12 months. The subjects reported no personal or familial history of recurrent kidney stone disease.

Normocalcemia was defined as an adjusted plasma calcium level below the upper limit of reference range (in our lab this is set at 10.5 mg/dl). A state of hyperparathyroidism was defined as PTH levels in the upper third of the reference interval or above (> 65 pg/ml). Randomization was performed by the investigators using a computer-generated randomization code. The patients were randomized to

treatment with Fosavance® (treated group; n=15) or with cholecalciferol (control group; n=15). To limit the potential difference in terms of bioavailability, all the subjects were instructed to take either Fosavance® or cholecalciferol at the same time (in the morning 30 minutes before breakfast) in a fasting state. Overall dietary calcium intake was not adequate (treated group: 685 mg ± 89 mg; control group 703 mg ± 12 mg, p=ns).

All patients were maintained on a controlled diet with a calcium intake of about 1000 mg/day. The study was performed in accordance with the Declaration of Helsinki II and guidelines on Good Clinical Practice. Informed consent was obtained from all the patients and the study was approved by the local ethics committee.

Fasting blood samples for determination of serum PTH, calcium, phosphates and 25-OH vitamin D were obtained at baseline and after 3, 6 and 12 months. Osteocalcin and urine samples for the measurement of CTX and 24-h calcium excretion were obtained at baseline and after 3 and 6 months. Serum PTH (2.1% of intra-assay and 2.3% of inter-assay variability) and osteocalcin (2.4% of intra-assay and 2.2% of inter-assay variability) were measured using chemiluminescence immunoassays (Roche Diagnostics, Mannheim, Germany) on a Modular E170. Measurements of serum calcium (1.8% of intra-assay variability and 2% of inter-assay variability), phosphate (2.8% of intra-assay and 2.6% of inter-assay variability) and 24-h urinary calcium were determined using an Olympus AU 2700 automated multichannel analyser (Olympus, Tokyo, Japan). Calcium was corrected for serum albumin concentration according the following formula: [0.8*(4.0 – patient's albumin) + serum calcium]. Determination of 25-OH vitamin D (4.6% of intra-assay variability and 5.1% of inter-assay variability) was performed using a DiaSorin kit on a Liaison automated immunoassay analyzer (DiaSorin, Saluggia, Italy). Urinary concentrations of the amino-terminal cross-linked telopeptides of collagen (CTX) were measured with a commercial enzyme-linked immunoassay kit (Cross Laps ELISA, Osteometer, Denmark) (3.6% of intra-assay and 4.3% of inter-assay variability). BMD was measured using dual-energy X ray absorptiometry (DXA) (Hologic Discovery QDR

instrument, MA, USA). DXA was performed at L1–L4 lumbar spine (postero-anterior projection), total hip and femoral neck at baseline and after 12 months.

3.2.2 Statistical analysis

Data were analyzed using SPSS version 13.0 for Windows. Variables were expressed as mean ± SD. BMD was expressed as g/cm^2 and changes in BMD were shown either as percentage change or as absolute difference between values at 12^{th} month and baseline. Differences of data between treated and control patients at baseline were analyzed using unpaired Student's *t*-test, as appropriate. Changes over time for each patient were evaluated by repeated measures ANOVA for PTH, calcium, phosphate, 25-OH Vitamin D, osteocalcin, urinary calcium and CTX. Wilcoxon test was used to determine BMD variations. Differences were considered significant when $p<0.05$.

3.2.3 Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

3.3 Results

Baseline biochemical and BMD characteristics of the patients are displayed in table 3;

Table 3. Biochemical and BMD characteristics of treated and control patients at baseline. Data are expressed as mean ± SD.

		Treated group (n=15) mean±SD	Control group (n=15) mean±SD	p
	Age (years)	59 ± 5	57 ± 5	0.343
Biochemical data	Adjusted Calcium (mg/dl)	9.9 ± 0.5	9.7 ± 0.4	0.219
	Phosphate (mg/dl)	3.8 ± 0.2	3.9 ± 0.3	0.432
	PTH (pg/ml)	112 ± 21	106 ± 13	0.310
	25-OH-Vitamin D (ng/ml)	32 ± 5	34 ± 7	0.500
	CTX (ng/ml)	0.6 ± 0.1	0.7 ± 0.1	0.328
	Osteocalcin (ng/ml)	29 ± 5	27 ± 2	0.402
	24-h urinary calcium (mg/l)	185 ± 57	197 ± 48	0.518
BMD data	Lumbar L1-L4 (g/cm^2) (T score)	0.781 ± 0.071 (-2.3 ± 0.2)	0.772 ± 0.074 (-2.3 ± 0.2)	0.698
	Femoral neck (g/cm^2) (T score)	0.623 ± 0.095 (-2.0 ± 0.3)	0.643 ± 0.081 (-1.8 ± 0.2)	0.516
	Total femur (g/cm^2) (T score)	0.701 ± 0.112 (-2.0 ± 0.3)	0.710 ± 0.080 (-1.9 ± 0.2)	0.782

No significant differences were found between treated and control patients for PTH, calcium, phosphates, 25-OH vitamin D and osteocalcin concentrations, as well as for 24-h urinary calcium excretion and CTX. BMD at spine, total hip and femoral neck was similar in both groups of patients without significant differences (table 3). Table 4 shows biochemical data of treated and control patients at different time points. Over all time points, both groups did not show any significant differences in serum and urinary calcium levels as well as in serum, PTH and urinary calcium concentrations compared to baseline values (table 4).

A small, but statistically significant, positive trend was detected for 25-OH vitamin D

levels from baseline to the end of the study in the treatment group (table 4). A similar slightly significant trend was detected for phosphate. No difference was detected between groups, at each time point, for each variable.

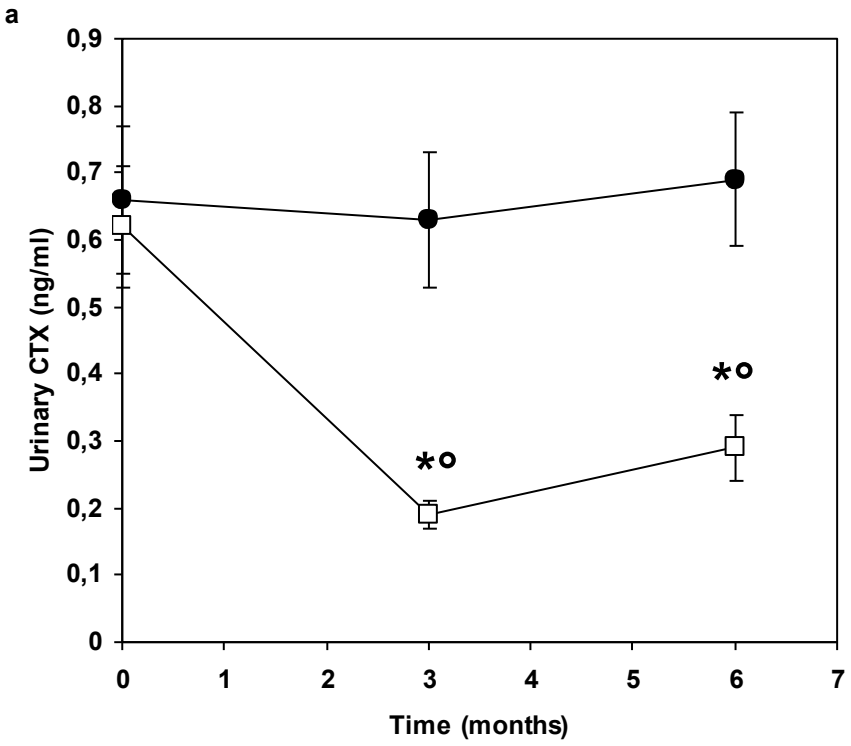
Table 4. Biochemical parameters of the two patient groups at different time points of the study period. Data are expressed as mean ± SD

	Biochemical data	baseline	3rd month	6th month	12th month	p
Treated Group (n=15)	Adjusted Calcium (mg/dl)	9.9 ± 0.5	9.5 ± 0.3	9.7 ± 0.4	9.6 ± 0.4	0.100
	Phosphate (mg/dl)	3.8 ± 0.2	3.9 ± 0.3	3.7 ± 0.4	3.8 ± 0.3	**0.002**
	PTH (pg/ml)	112 ± 21	110 ± 13	112 ± 15	110 ± 13	0.865
	25-OH-Vitamin D (ng/ml)	32 ± 5	36 ± 5	35 ± 4	36 ± 5	**0.021**
	CTX (ng/ml)	0.62 ± 0.09	0.19 ± 0.02	0.29 ± 0.05	-	**<0.001**
	Osteocalcin (ng/ml)	28 ± 5	15 ± 1	17 ± 3	-	**<0.001**
	24h-urinary calcium (mg/l)	185 ± 57	184 ± 61	194± 52	182 ± 62	0.769
Control group (n=15)	Adjusted Calcium (mg/dl)	9.7 ± 0.4	9.5 ± 03	9.6 ± 0.3	9.5± 0.3	0.287
	Phosphate (mg/dl)	3.9 ± 0.3	3.9 ± 0.4	3.9 ± 0.3	3.7 ± 0.4	0.156
	PTH (pg/ml)	106 ± 13	111 ± 11	105 ± 12	110 ± 14	0.173
	25-OH-Vitamin D (ng/ml)	34 ± 7	36 ± 4	34 ± 4	35 ± 4	0.392
	CTX (ng/ml)	0.66 ± 0.11	0.63 ± 0.10	0.69 ± 0.10	-	0.344
	Osteocalcin (ng/ml)	27 ± 2	25 ± 3	27 ± 2	-	0.595
	24h-urinary calcium (mg/l)	197± 48	191 ± 38	211 ± 53	183 ± 63	0.738

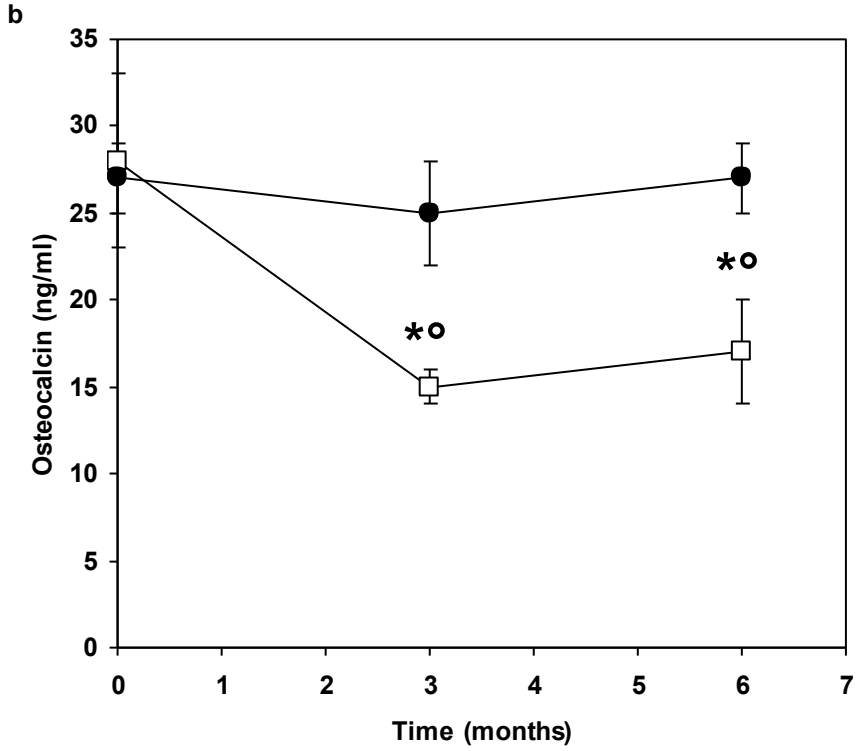
After three months, the treated group showed significantly decreased serum osteocalcin and urinary CTX concentrations (- 46.4% and -69.4% respectively; $p<0.001$ vs. baseline) and these values remained reduced after six months (-39.3% and -53.2% respectively, $p<0.001$ vs. baseline). The control group did not demonstrate any significant change in osteocalcin and CTX concentration at any time points. A significant difference in CTX and osteocalcin levels was detected between control and treated group both at 3 and 6 months ($p<0.001$ for each comparison)

(figure 12). BMD values at baseline and after 12 months are reported for both groups of patients in table 3. After a year of treatment, BMD in the treated group increased significantly at lumbar, femoral neck and total femur (p=0.001).

Figure 12. **Panel A.** Urinary CTX concentration at different timing in treated (☐) and control (●) groups. **Panel B**. Serum Osteocalcin concentration at different timing in treated (☐) and control (●) groups.



A significant decrement compared to the baseline value **(*)** is observed in the group under treatment at 3rd and 6th month ($p<0.001$). A significant decrement of urinary CTX concentration is observed in the group under treatment compared to the control group **(°)** at 3rd and 6th month ($p<0.001$).



A significant decrement compared to the baseline value **(*)** is observed in the group under treatment at 3rd and 6th month ($p<0.001$). A significant decrement of serum Osteocalcin is observed in the group under treatment compared to the control group **(°)** at 3rd and 6th month ($p<0.001$).

In particular, the greatest increment was at lumbar spine with a mean BMD increase of 4.7% (ranging from 3.0% to 8.0%). In the control group a significant BMD reduction was recorded at all measured sites compared with baseline (p=0.001). After 12 months, the alendronate plus vitamin D treatment group showed a higher significant increase of BMD (lumbar spine, femoral neck and total femur) than the control group treated only with cholecalciferol (p=0.001) (table 5).

Table 5. BMD measurements in treated and control groups of patients at baseline and after 12 months of observation. Differences between values at 12th month and baseline are expressed both as absolute difference (abs) and as percentage change (%).

BMD	Treated group (n=15) mean ± SD [abs;(%)]			Control group (n=15) mean ± SD [abs;(%)]		
	baseline	12 months	p	baseline	12 months	p
Lumbar L1-L4 (g/cm^2)	0.781 ± 0.071	0.819 ± 0.074 [0.038; (4.7)]	0.001	0.772 ± 0.074	0.759 ± 0.072 [-0.013; (-1.6)]	0.001
T score	-2.3 ± 0.2	-1.9 ± 0.2		-2.3 ± 0.2	-2.5 ± 0.2	
Femoral neck (g/cm^2)	0.623 ± 0.095	0.640 ± 0.092 [0.017; (2.6)]	0.001	0.643 ± 0.081	0.632 ± 0.083 [-0.011; (-1.7)]	0.001
T score	-2.0 ± 0.3	-1.8 ± 0.2		-1.8 ± 0.2	-2.0 ± 0.3	
Total femur (g/cm^2)	0.701 ± 0.112	0.732 ± 0.111 [0.031; (4.0)]	0.001	0.710 ± 0.083	0.700 ± 0.080 [-0.010; (-1.4)]	0.001
T score	-2.0 ± 0.3	-1.7 ± 0.2		-1.9 ± 0.2	-2.0 ± 0.2	

3.4 Discussion

This is the first study evaluating the effect of alendronate and vitamin D on patients affected by NPHPT. The underlying causes of NPHPT are still unknown but Rao et al hypothesized that NPHPT and primary hyperparathyroidism are two phases of the same disease: the first would represent the subclinical condition and the second would be the overt disease with elevation of calcium levels [177]. Moreover, Murani et al have shown that the resistance of kidney and bone to the actions of PTH could play an important role in the development of this subclinical condition [170].

Although the Fourth International workshop for the management of asymptomatic PHPT tries to clarify its diagnostic criteria and suggests the way to manage subjects with NPHPT, no prospective randomized controlled trials have evaluated the impact of the anti-resorptive therapy in this kind of population. Few randomized controlled trials have investigated the role of bisphosphonates in the management of PHPT. However, Rossini et al have demonstrated that 2 years of treatment with alendronate induced significant increases in BMD at lumbar spine, total hip and total body compared to control patients which showed a significant BMD reduction [153]. In another placebo controlled trial, alendronate caused an increase in lumbar spine and

total hip BMD as well as a decrease in BMT without any changes of ionised calcium, phosphorus and PTH [241]. Chow et al have obtained similar results with a significant improvement of spine and femoral neck BMD in subjects affected by PHPT after treatment with alendronate [242].

Our data have demonstrated the ability of alendronate to increase BMD in osteoporotic patients with NPHPT. As far as osteoporosis representing an indication for PTX, these findings might raise some doubts about the real need of parathyroidectomy in this group of patients. Moreover, our data seem to confirm the results of the meta-analysis by Sankaran et al on skeletal effects of interventions in mild primary hyperparathyroidism. The authors have shown that surgical treatment and anti-resorptive therapies increase BMD in mild PHPT to a similar degree and that medical treatment could represent a reasonable option in a patient with mild hyperparathyroidism and low BMD [243]. According to other observations in primary hyperparathyroidism [244], our results confirmed the effects of alendronate in decreasing both bone resorption and formation markers also in patients with NPHPT. The anti-resorptive effects of alendronate were rapidly seen within the first trimester of the study and they were sustained up to the sixth month, confirming the well known data that are available in post-menopausal osteoporosis [245].

Although treatment with vitamin D_3 [200,206] and vitamin D_2 [201,246] slightly decreases PTH levels in patients with vitamin D deficiency and primary hyperparathyroidism, it is not clear whether they can be considered a valuable and safe tool to manage NPHPT due to the lack of published data in this particular disease. Only a randomized placebo controlled [247] and an uncontrolled trial [248] have demonstrated a significant reduction of bone resorption markers and an increase of lumbar spine BMD in patients with primary hyperparathyroidism treated with vitamin D. Some doubts were raised on the safety of this treatment: in fact other investigators have reported increased plasma and urine calcium levels after treatment with vitamin D [200]. In our population, the administration of vitamin D was safe and it did not induce any cases of hypercalcemia or hypercalciuria. As shown above, both plasma and urinary

calcium did not significantly increase during the study and PTH was unaffected by the treatments. In contrast with patients treated with alendronate, the weekly treatment with 2800 IU of cholecalciferol in control group was not able to arrest bone loss, nor to decrease BMT.

Although an alendronate alone group was not included in our study, it may be presumed that the beneficial effect seen on the BMD was entirely due to alendronate. Indeed, Macdonald HM et al have demonstrated that, in postmenopausal women with vitamin D deficiency (at baseline), treatment with daily oral vitamin D3 at 400 IU (1 year) did not change markers of bone metabolism (CTX included) and was not able to significantly arrest the bone loss at hip compared to the treatment with 1000 IU/day [249]. Moreover, Recker et al showed that a once-weekly tablet containing both cholecalciferol (2800 IU) and alendronate (70 mg) didn't provided any further anti-resorptive improvement (evaluated by changes in bone bio-markers) compared to the single Alendronate (70mg) treatment [250].

To limit the potential difference in terms of bioavailability, all the subjects were instructed to take Fosavance and cholecalciferol once a week, at the same time (in the morning, before breakfast) in a fasting state. Indeed as vitamin D is liposoluble, its oral absorption could increase if ingested with a fat-rich meal [251] although there are contrasting evidence.

We found only a slight, not clinically significant change in 25-OH vitamin D levels after supplementation with cholecalciferol in the treated group. As we administered 2800 IU/week (400 IU/day) of cholecalciferol to our patients it is reasonable to believe that they should have raised their plasma vitamin D of a small amount that would have been difficult to detect in our small groups. Indeed it is described that 40 IU/day of vitamin D for 6 month can induce a rise in plasma 25(OH)D3 of about 0.7 ng/ml [252]. Holick et al demonstrated that ergocalciferol can produce a significant rise in plasma 25(OH) D3 only in patients with low basal vitamin D. Our patients had normal plasma vitamin D before the study and it is highly probable that their plasma 25(OH) D3 did not change over the study period [253]. It might be that in the control

group the compliance to the therapy was lower compared to the treated group. However at each visit we asked each subject to confirm they had taken the drugs as requested. Moreover the small sample size makes further interpretation difficult.

Our study has some limitations. It was not designed as a double-blind trial, the sample size was small (even if the prevalence and incidence of NPHPT are low) and we did not measure ionized calcium, nor the radius BMD. Moreover, we did not evaluate BMT at the end of the study as these measurements were only programmed after 3 and 6 months respectively. This decision was taken in advance, as we were interested in measuring early changes of bone turnover. After having seen the unexpected increase of BMD we realized that an evaluation of BMT at the end of the study could have added more power to our results.

In conclusion, for the first time the results of this study indicate that in postmenopausal women with NPHPT, treatment with alendronate together with vitamin D significantly increases BMD at the most clinically relevant skeletal sites. Moreover, treatment with alendronate and/or vitamin D does not affect calcium, phosphate and 25-OH vitamin D serum levels as well as urinary calcium excretion.

RESEARCH PROJECT N°2

NORMOCALCEMIC HYPOPARATHYROIDISM: PREVALENCE AND EFFECT ON BONE STATUS IN OLDER WOMEN. THE OPUS STUDY

CHAPTER 4: SPECIFIC BACKGROUND

4.1 Hypocalcemia and hypoparathyroidism

Hypocalcemia is frequently encountered in acute inpatient and intensive care unit (ICU) settings and less frequently in the outpatient clinic. Hypocalcemia is estimated to occur in approximately 15% and 85% of hospitalized and critically ill patients, respectively.1 True hypocalcemia, defined by a low ionized serum calcium concentration, is far less common in patients than is a depressed serum total Ca++ due to alterations in serum albumin or acid–base status [254]. For practical purposes, serum total Ca++ can be used to assess for hypocalcemia in outpatients as long as serum albumin is concomitantly measured to allow for calculating albumin-adjusted serum total Ca++ by the formulas in Table 6.

Table 6. Definition of Hypocalcemia and Formulas for Calculating Albumin Corrected Ca++

Parameter	Normal Range
Serum total Ca^{++}	Age 20–49 years: men, 8.6–10.3 mg/dL; women, 8.6–10.3 mg/dL Age >49 years: men, 8.6–10.3 mg/dL; women, 8.6–10.4 mg/dL
Serum ionized Ca^{++}	4.8–5.6 mg/dL 1.20–1.40 mM

Formulas for calculating albumin-corrected serum total Ca^{++}:
(I) albumin-corrected total Ca^{++} (mg/dL) = 0.8 [4-albumin (G/dL)] + measured total Ca^{++}
(II) albumin-adjusted Ca^{++} (mmol/L) = total Ca^{++} (mmol/L) + 0.016 [40-albumin]

Serum total Ca++ includes: ionized (45–50%); protein bound (45–50%); and anion-complexed (5–10%) fractions. In ICU patients, alterations in serum magnesium (Mg++), phosphate, citrate (due to multiple transfusions), and pH often complicate the picture of hypocalcemia. Because of these coincidental abnormalities, measuring the ionized Ca++ is strongly recommended in acutely ill patients who are hospitalized

in the ICU. The measurement of ionized Ca++ is often less readily available in outpatient clinical laboratories and is less essential because the circumstances are more straightforward. In postoperative patients, reduced oral intake, nutritional compromise, and hemodilution due to infusion of intravenous fluids may explain transient hypocalcemia, which is often mild and asymptomatic. Chronic nutritional deficiency, inflammation, and protein loss through the gastrointestinal tract or kidneys may depress serum albumin in hospitalized patients, but the serum total Ca++ generally corrects to the normal range. No specific treatment or detailed workup is usually needed.

In the patient who has undergone recent neck exploration for benign or malignant thyroid or parathyroid disease, with or without lymph node dissection, postoperative hypocalcemia is viewed quite differently. Patients who have undergone laryngeal surgery and/ or laryngectomy fall into this same category. All of these procedures, depending on the extent of the surgery, underlying disease, and surgeon's experience, can lead to acute hypocalcemia of either a transient or permanent nature. If the hypocalcemia causes clinical signs or symptoms and it is impressive from the laboratory standpoint, a comprehensive biochemical and hormonal evaluation must be undertaken. In such situations, the differential diagnosis ranges between hypocalcemia due to acute transient hypoparathyroidism (with recovery of function weeks to months later) to permanent hypoparathyroidism. The latter stems from inadvertent gland(s) removal or from devascularization of remaining parathyroid tissue, carrying with it a long-term need for treatment of the ensuing hypoparathyroid state. The "hungry bone syndrome" must also be considered in this setting because chronic hyperparathyroidism and thyrotoxicosis (two reasons for neck surgery) both can produce this postoperative hypocalcemic state. Hungry bone syndrome will resolve with restoration of bone Ca++ stores, but can require days to weeks of rather vigorous supplementation. The presence of an appropriately elevated PTH level and high alkaline phosphatase activity point the clinician to the hungry bone syndrome.

4.2 Diagnosis and causes of Hypocalcemia

In the approach to the evaluation of a low serum Ca++ confirmed twice by including the measurement of serum albumin or serum ionized Ca++ , the physician must first assess the patient clinically. Then, serum Mg++ , creatinine, intact PTH, and 25-Hydroxyvitamin D [25(OH)D] levels should be reviewed (figure 13 and table 7).

Figure 13. Clinical Flow chart for the evaluation of the patient with hypocalcemia[3]

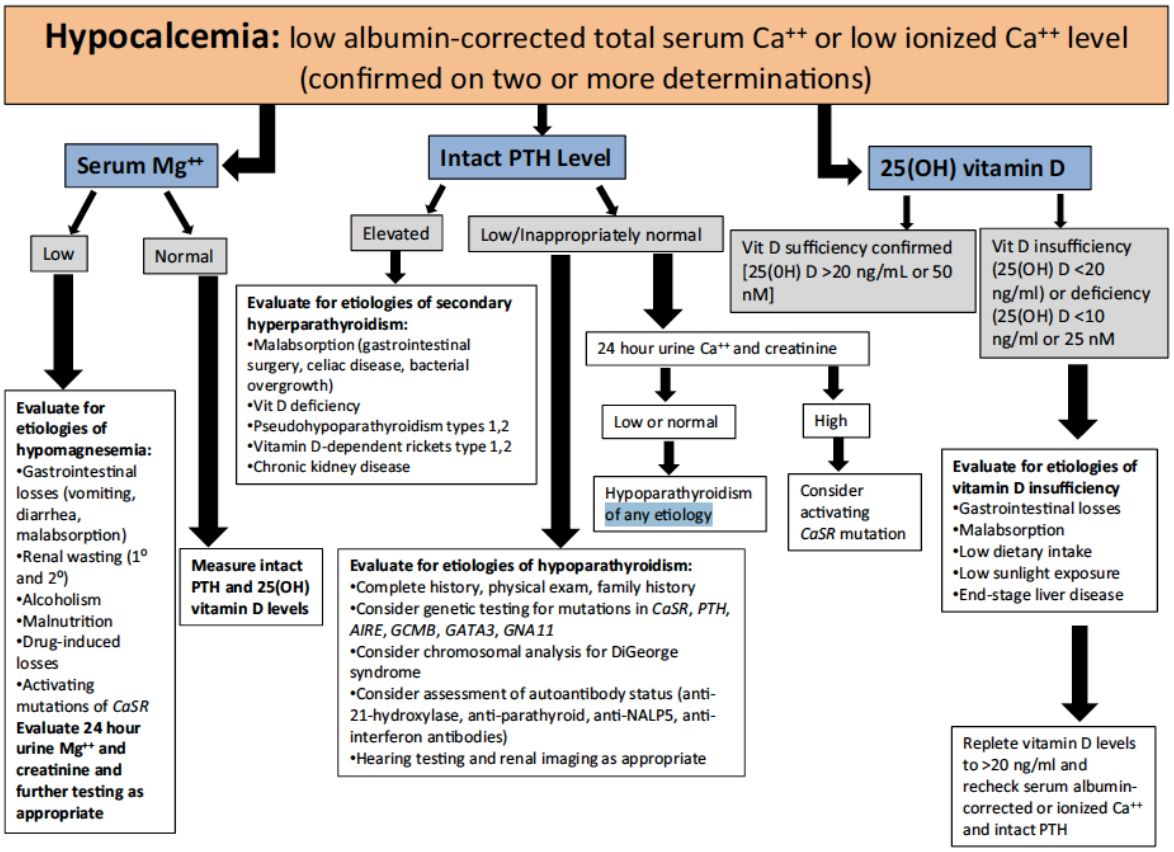


Table 7. Biochemical and Hormonal Profiles of Disorders Associated with Hypocalcemia[3]

Disorder	Ca^{++}	Phosphate	Mg^{++}	PTH	25(OH)D	$1,25(OH)_2D$
Hypoparathyroidism	low	high	nl or low	nl or low	nl	nl
Vitamin D deficiency	nl or low	low	nl	high	low	nl
Vitamin D-dependent rickets type 1	low	low	nl	high	nl	low
Vitamin D-dependent rickets type 2	low	low	nl	high	nl	high
Pseudohypoparathyroidism	low	high	nl	high	nl	nl or low
Mg^{++} depletion	low or nl	nl	low	nl, low, or high	nl	nl or low

In broad terms, these disorders can be classified as hypoparathyroid states, resistance

to PTH (pseudohypoparathyroidism) or vitamin D (vitamin D-dependent rickets), Vitamin D deficiency, hypomagnesemia, and miscellaneous disorders. In many cases, definitive diagnosis of the molecular etiology for hypoparathyroidism requires genetic testing and/or specific autoantibody assessment [255]. Clearly, however, the most common form of hypoparathyroidism is postsurgical [256]. A careful history and neck examination generally quickly confirms this diagnosis without the need to test any further. As one puts together the biochemical and hormonal profiles, clinical observations and historical details are extremely helpful. The astute clinician combines both to reach an accurate diagnosis efficiently. The following features are helpful in establishing the underlying diagnosis responsible for hypocalcemia:

The age of onset of the disorder. The earlier in life that hypocalcemia presents the more likely that a genetic disorder will be identified. Several reports document severe symptomatic presentations for patients with activating mutations in the extracellular Ca++ -sensing receptor (CaSR) in newborns manifesting with significant complications of hypocalcemia and hypomagnesemia [257]. These complications may include seizures, failure to thrive, growth retardation, and other problems. Most other patients with CaSR activating mutations however, are asymptomatic for years and are diagnosed incidentally when routine lab testing, for an unrelated problem, shows a low serum Ca++ [258]. Further testing then leads to the definitive diagnosis. Hypocalcemic hypoparathyroidism due to autoimmune polyglandular syndrome (APS1) may present in childhood, but often does not do so in the newborn period [259]. In the first years of life, children with mutations in AIRE (autoimmune regulator) and APS1 typically present with mucocutaneous candidiasis. This is usually the first clue to their underlying immune dysfunction. Other genetic forms of hypoparathyroidism, due to mutations in the genes encoding the transcription factors GCMB (glial cell missing b) [260] or GATA3 (GATA binding protein 3) [261] or in genes encoding PTH [262] or the G-protein alpha subunit GNA11 (G-protein alpha subunit 11) [263] can present at any age, depending on the severity of the hypocalcemia and the attendant symptoms. Mitochondrial disorders, although exceedingly rare,

often present in childhood before the age of 20.

Presence of renal anomalies and hearing loss. These are key findings in patients with GATA3 deficiency. Loss-of-function mutations in this transcription factor cause renal anatomic abnormalities and altered otic vesicle development associated with varying degrees of hearing impairment [264].

Mental retardation: This can be seen with the Sanjad–Sakati and Kenny–Caffey syndromes, extremely rare causes of hypoparathyroidism with cardiovascular and facial abnormalities. Mental retardation is also seen in pseudohypoparathyroidism type 1a.

Syndromic features. Manifestations of the DiGeorge, velocardiofacial or 22q11.2 deletion syndrome can include cleft palate, abnormal facies, hearing loss, feeding problems, developmental delay, delayed growth and speech, immunodeficiency, conotruncal cardiac abnormalities such as the tetralogy of Fallot, persistent truncus arteriosus, ventricular septal defect, behavioral problems, and hypocalcemic hypoparathyroidism. The extent and severity may depend on the extent of the genetic material deleted on chromosome 22q11, although the spectrum is quite variable [265]. Pseudohypoparathyroidism type 1a has a variety of phenotypic features (shortened metacarpal bones, obesity, ectopic ossifications, short stature, and mental retardation), although it is not often confused with hypoparathyroidism due to the elevated PTH value. Alopecia, delayed growth, and rickets are seen in vitamin D resistance due to mutations in the vitamin D receptor.50 This disorder should not be confused with hypoparathyroidism because the concomitant hypophosphatemia and elevated PTH and 1,25-dihydroxyvitamin D [1,25(OH)2 D] levels indicate the disturbance responsible for the hypocalcemia resides outside the parathyroid gland. The Kenny–Caffey syndrome includes short stature, thickening of bone cortices, and basal ganglia calcifications. Growth failure also complicates the Sanjad–Sakati syndrome, which includes microcephaly and cognitive impairment. Mitochondrial DNA defects causing syndromes that include hypoparathyroidism include the following [266]: (a) The Kearns–Sayre syndrome is accompanied by ophthalmologic

abnormalities (progressive ophthalmoplegia and pigmentary retinopathy) along with cardiomyopathy, heart block, and diabetes; (b) MELAS (mitochondrial encephalopathy, lactic acidosis, stroke-like episodes) involves spells, diabetes, and hypoparathyroidism; (c) The MTPDS (mitochondrial trifunctional protein deficiency syndrome) may encompass neuropathy, retinopathy, acute fatty liver in pregnancy, along with hypoparathyroidism. Even among the inherited causes of hypoparathyroidism, which are rare, these disorders are exceedingly uncommon.

Presentations dominated by Mg++ deficiency: Patients hospitalized for a variety of nonendocrinologic diagnoses often have low serum Mg++ levels (e.g., chronic diarrhea, malnutrition, acute pancreatitis, acute and chronic alcoholism, etc.) [255]. Because chronic Mg++ depletion impairs PTH secretion and action in target organs, serum Ca++ is often depressed, and PTH levels are inappropriately low. There is rarely a question that these medical illnesses are the ones responsible for the Mg++ and Ca++ disturbances. There is rarely confusion that the case is not one of hypoparathyroidism or any of the other endocrine disorders associated with hypocalcemia. The clinician must interpret the intact PTH level in the context of the prevailing hypomagnesemia and the often mild hypocalcemia in Mg++-deficient patients. It may be difficult to distinguish cases that are primarily Mg++ deficiency states that are also accompanied by some degree of hypoparathyroidism. Only with repletion of the body's Mg++ stores will intact PTH rise to normal or even modestly elevated levels, underscoring the idea that parathyroid function is indeed normal and only functionally impaired due to inadequate Mg++.

4.3 History and clinical evaluation

The patient with hypocalcemia can present in many ways ranging from a completely asymptomatic presentation to dramatic symptomatic and even life-threatening hypocalcemia (Table 8).

Table 8. Clinical Evaluations in Patients with Hypocalcemia[3]

Clinical Assessment	
Organ System	**Signs or Symptoms**
Neuromuscular	Tetany, muscle cramps, bronchospasm, stridor, laryngospasm, paresthesias, numbness, generalized weakness, abdominal cramps, positive Chvostek or Trousseau signs
Central nervous system	Altered mental status, seizures, coma, extrapyramidal signs
Ocular	Cataracts, papilledema; ophthalmoplegia and retinopathy (in specific syndromes)
Cardiovascular	Congestive heart failure
Auditory	Hearing impairment (HDR syndrome)

Both the severity of the disturbance and its acuity determine the clinical presentation. Neuromuscular and neurologic symptoms are often the most dramatic ones the clinician sees. They can include generalized seizures, loss of consciousness, altered mental status, paresthesias, muscle cramps, and tetany. If laryngeal and respiratory muscles are involved, laryngospasm, stridor, and wheezing can be seen and must be treated promptly. Chronic hypocalcemic cardiomyopathy leading to congestive heart failure can present with volume overload, pulmonary edema, arrhythmias, and profound dyspnea [255]. It is fortunately rare. Any cause of acute hypocalcemia can cause central nervous, neuromuscular, neurologic, or respiratory system complaints. Any etiology for severe chronic hypocalcemia can cause cardiac failure. Specific clinical risk factors or clinical precipitants have not been identified, but cardiac failure is typically reversible if treated aggressively. Postsurgical hypoparathyroidism, because of its onset postoperatively, often causes complaints that become associated with the lowered serum Ca++ values. Chronic states of PTH hyposecretion, however, even accompanied by dramatically low serum Ca++ values, may only present clinically after many years or after incidental lab testing for another

problem or coincidentally with another acute illness. A carefully taken history, review of old medical records, and physical examination will often clarify the diagnosis responsible for the hypocalcemia. Physical examination findings may include positive Chvostek's or Trousseau's signs indicative of neuromuscular irritability. These signs are fairly specific for tetany due to low Ca++ or Mg++ levels. Signs of cardiac failure, respiratory compromise, bronchospasm, stridor, or altered mental function, while not specific for hypocalcemia or hypoparathyroidism, support the severity of the Ca++ disturbance. Any of the classic findings of pseudohypoparathyroidism type 1a (short stature, obesity, brachydactyly, etc.) support that diagnosis and not one of true hypoparathyroidism. Bowing of the extremities and poor growth in childhood would support the diagnosis of hypocalcemia due to vitamin D resistance (along with alopecia if present) or chronic vitamin D deficiency. The DiGeorge or velocardiofacial syndrome can present with signs, symptoms, and/or findings referable to many systems (eye, cardiac, immunologic, facial development, behavioral, etc.). The presentation is most often in childhood, but cases with mild hypocalcemic hypoparathyroidism only have been described in adults who have no other gross developmental abnormalities. Fluorescence in situ hybridization (FISH) and other genetic analyses are done to confirm this diagnosis. Signs or other features of the APS1 should be sought in the patient with unexplained hypoparathyroidism. The other two diagnosis-defining features are adrenal insufficiency and candidiasis. Two of three classic disorders make this diagnosis definitively, but the presentation for them is usually asynchronous. Vitiligo, keratoconjunctivitis, type 1 diabetes, hepatitis, and gonadal failure may also occur in APS1. Other medical specialists play key roles. Ocular findings in many disorders that cause hypocalcemia will be confirmed by the ophthalmologist: retinopathy and ophthalmoplegia in mitochondrial disorders and premature cataracts in chronic hypoparathyroidism. Neurologists may refer patients with seizures, altered mental status, tetany, or basal ganglia calcifications on imaging. Many patients with syndromic features benefit from the diagnostic experience of a

genetics specialist who can also direct specific testing.

4.4 Hypoparathyroidism

4.4.1 Introduction

Hypoparathyroidism is a rare disorder characterized by low serum calcium and low or inappropriately low-normal serum parathyroid hormone. This condition may be acquired or inherited. The acquired form occurs due to the inadvertent removal of, or damage to, the parathyroid glands or their blood supply at the time of neck surgery for thyroid disease, head and neck cancer, or parathyroid disease in about 75% of cases. Of the remaining cases, the most common cause in adults is thought to be autoimmune disease, either affecting only the parathyroid glands, or multiple other endocrine organs. A variety of rare infiltrative disorders, metastatic disease, iron or copper overload, ionizing radiation exposure, or rare genetic disorders explains the remaining cases (table 9).

Table 9. Classification of Hypoparathyroid Disorders[3]

DESTRUCTION OR REMOVAL OF PARATHYROID TISSUE WITH INADEQUATE SECRETORY RESERVE
Postsurgical hypoparathyroidism
Autoimmune hypoparathyroidism
Deposition of heavy metals in parathyroid tissue
Radiation-induced destruction of parathyroid tissue
Metastatic infiltration of the parathyroid glands
REVERSIBLE IMPAIRMENT OF PTH SECRETION OR PTH ACTION WITH INTACT UNDERLYING SECRETORY ACTION
Severe magnesium depletion
Hypermagnesemia
GENETIC DISORDERS OF PTH BIOSYNTHESIS AND PARATHYROID GLAND DEVELOPMENT
Constitutively active CaSRs
PTH gene mutations
Mutations or deletions in transcription factors and other regulators of the development of the parathyroid glands
Mutations in mitochondrial DNA

4.4.2 Epidemiology

Postsurgical Hypoparathyroidism. Acquired hypoparathyroidism is most commonly the result of inadvertent removal or irreversible damage to the glands, usually to their blood supply, during thyroidectomy, parathyroidectomy, or radical neck dissection [267]. Rates of postoperative hypoparathyroidism vary across centers, the type of procedure, and surgical Larger series report total rates of 5.4–8.8%, although most cases are transient (4.9–7.3%) [268]. Other smaller studies report an incidence of temporary hypoparathyroidism of 25.4–83% [269]. Definitions of permanent postsurgical hypoparathyroidism vary, but the definition most generally accepted is insufficient parathyroid hormone (PTH) to maintain normocalcemia with adequate daily intake of calcium and vitamin D 6 months after surgery [255]. Permanent hypoparathyroidism occurs in 0.12–4.6% of cases [270]. Progression of transient to permanent hypoparathyroidism depends on several risk factors. The risk is greater if more than one parathyroid gland was inadvertently excised during thyroidectomy [271], serum calcium levels are low (≤ 8.0 mg/dL (≤ 2 mmol/L)) 1 week after surgery, or serum phosphorus is elevated while on oral calcium therapy (≥ 4 mg/dL; ≥ 1.29 mmol/L). Occurrence of postsurgical hypoparathyroidism depends on the experience of the surgeon. In one report, 32.8% of cases performed by surgical residents developed transient postoperative hypoparathyroidism, compared to 19.4% when cases were performed by an experienced endocrine surgeon [272]. In retrospective analyses of consecutive total thyroidectomy cases for thyroid cancer performed by a single surgeon, permanent hypoparathyroidism was more frequent during the earliest period of the surgeon's practice [273]. The type of underlying thyroid disease also affects the risk of postoperative hypocalcemia. Patients with advanced thyroid cancer, Graves' disease, or other manifestations of preoperative hyperthyroidism have significantly increased rates of postoperative hypocalcemia compared with patients with small cancers or benign euthyroid disease. The extent of surgery can also significantly increase the incidence of permanent hypocalcemia, with total

thyroidectomy, repeat thyroidectomy, and thyroidectomy with neck dissection being higher risk procedures [274]. Parathyroid hormone and calcium levels measured after surgery have been reported to be predictors of postoperative hypoparathyroidism. Patients with intact PTH levels below the reference range, and serum calcium levels of ≤ 8.0 mg/dL (2 mmol/L) are at increased risk of long-term hypoparathyroidism [275]. In a report of 170 postoperative study patients it was shown that measuring intact PTH 24 hours after total thyroidectomy, in combination with serum calcium levels on the second postoperative day, allowed the prediction of hypoparathyroidism with high sensitivity, specificity, and positive predictive value. The best sensitivity for predicting postoperative hypoparathyroidism was 97.7% for measurement of intact PTH level 24 hours after surgery, and the best specificity was 96.1% for measurement of serum calcium level on the first postoperative day. When using both intact PTH and serum calcium in a combined approach, the best result was with intact PTH values less than 15 pg/mL measured on the first postoperative day, and serum calcium values ≤ 7.6 mg/dL (1.9 mmol/L) measured on the second postoperative day. The combined approach demonstrated a sensitivity of 96.3%, with a specificity of 96.1%, a positive predictive value (PPV) of 86.0%, and a negative predictive value (NPV) of 99.0% [272]. Parathyroid injury may be caused by inadvertent removal of the parathyroid glands, ligation of the blood supply, or destruction secondary to capsular hematoma [276]. In order to prevent the development of permanent hypoparathyroidism, parathyroid autotransplantation is recommended in cases where it is suspected this may occur. While autotransplantation has been shown to be a risk factor for transient postoperative hypoparathyroidism due to the time required for the grafted parathyroid tissue to regain its function, rates of permanent hypoparathyroidism after autotransplantation are low [277]. One study showed that transient hypoparathyroidism increased with the number of parathyroid glands transplanted, from 9.8% if glands were not transplanted, to 11.9%, 15.1%, and 31.4% for one, two, and three glands transplanted, respectively ($p < 0.05$) [278]. The incidence of permanent hypoparathyroidism decreased with the number of glands transplanted at 0.98%,

0.77%, 0.97%, and 0% for one to four glands, respectively (p = NS).

Autoimmune hypoparathyroidism. Autoimmune hypoparathyroidism is thought to be the second most common cause of hypoparathyroidism in adults. Autoimmune isolated hypoparathyroidism may occur sporadically, in which case there may be a low remission rate of 3.8% [279]. Autoimmune hypoparathyroidism may also occur in combination with other autoimmune endocrine disorders as part of an autoimmune polyglandular syndrome type 1 (APS-1), otherwise known as autoimmune polyendocrinopathycandidiasis- ectodermal dystrophy (APECED) [280]. This disorder causes hypoparathyroidism, Addison's disease, and candidiasis, and at least two of the following: insulin-dependent diabetes mellitus, primary hypogonadism, autoimmune thyroid disease, pernicious anemia, chronic active hepatitis, steatorrhea, alopecia, or vitiligo. More than 80% of APS-1 patients have hypoparathyroidism, sometimes as the only manifestation of the disorder. APS-1 is usually an autosomal recessive disorder caused by mutations in the autoimmune regulator (AIRE) gene, although autosomal dominant versions have been reported. The AIRE gene product is a zinc-finger transcription factor found in thymus and lymph nodes, and is critical for mediating central tolerance by the thymus [281]. In contrast to other immune conditions, this disorder is monogenic, not associated with the major histocompatibility complex, and there does not appear to be a genotype– phenotype correlation. Most patients with APS-1 are diagnosed in childhood or adolescence, but these patients need to be followed long term for the gradual appearance of other conditions associated with the syndrome. The worldwide incidence of APS-1 is estimated to be 1 per 1,000,000 person-years, but the incidence is more common in three genetically distinct populations: 1:25,000 in Finns, 1:14,500 in Sardinians, and 1:9000 in Iranian Jews [282].

NACHT leucine-rich repeat protein 5 (NALP5) is an intracellular signaling molecule expressed in the parathyroid gland that may be a parathyroid-specific autoantigen present in APS-1 patients with hypoparathyroidism. Patients without APS-1 do not have antibodies to NALP5 [283]. The extracellular domain of the CaSR may also be an

autoantigen in patients with autoimmune hypoparathyroidism. Activating antibodies to this region of the receptor have been reported in both APS-1 and acquired hypoparathyroidism [284]. These findings suggest that, even though the majority of patients with APS-1 do not have CaSR antibodies, there may be a subset of patients who have hypoparathyroidism due to functional suppression of parathyroid gland activity, rather than irreversible destruction of the parathyroid glands [285]. The calcium-sensing receptor (CaSR) is a G-protein coupled receptor (GPCR) of the same family (family 3 or C) as those sensing glutamate, gamma-aminobutyric acid (GABA), odorants, sweet taste, and pheromones [286]. This family of receptors has large amino-terminal extracellular domains, comprising 612 amino acids in the human CaSR, and the seven membrane-spanning helices characteristic of the superfamily of GPCRs. The CaSR is heavily glycosylated and resides on the cell surface as a disulfide- linked dimer. The extracellular domain contains important determinants for binding calcium, the receptor's principal biologically relevant ligand, although there are additional calcium-binding sites within the seven membrane-spanning domain, since a "headless"receptor entirely lacking the extracellular domain still responds to calcium. The CaSR's best-established roles in calcium homeostasis are to inhibit parathyroid cellular proliferation, PTH secretion, and PTH gene expression, to stimulate calcitonin secretion, and to directly inhibit renal tubular calcium reabsorption [287]. Less well-documented actions are promoting proliferation, chemotaxis, differentiation of osteoblasts and their mineralization of bone, as well as inhibiting osteoclastic differentiation and activity. An early study reported the presence of anti-parathyroid gland antibodies in 38% of 75 patients with idiopathic hypoparathyroidism, 26% of 92 patients with idiopathic Addison's disease, 12% of 49 patients with Hashimoto thyroiditis, and 6% of 245 normal control patients [288]. Subsequent studies showed that some anti-parathyroid gland antibodies are specific for mitochondrial or endomysial antigens. Li et al. reported that sera from 20% of 25 patients with autoimmune hypoparathyroidism, idiopathic hypoparathyroidism, or autoimmune polyglandular syndrome type 1 contained

antibodies directed against the CaSR [289]. Patients with autoimmune hypoparathyroidism for less than 5 years were more likely to have anti-CaSR antibodies, whereas no anti-CaSR antibodies were found in 22 healthy control patients or 50 patients with autoimmune disorders without hypoparathyroidism. It is not yet clear whether anti-CaSR antibodies play a causal role in the disease or serve as markers of tissue injury [6]. Another report of two patients with activating anti-CaSR antibodies indicated that these antibodies inhibited PTH release by dispersed parathyroid adenoma cells, suggesting that their hypoparathyroidism resulted from an inhibitory effect of the antibodies on the CaSR and not irreversible parathyroid gland damage [290].

Normocalcemic hypoparathyroidism. The Osteoporotic Fractures in Men (MrOS) study and Dallas Heart Study (DHS) have been used to identify asymptomatic subjects with normocalcemic hypoparathyroidism [291]. Normocalcemic hypoparathyroidism is defined as occurring in patients with normal serum calcium with PTH level below the reference range. Cross-sectional data obtained showed that of 2364 men in MrOS, 26 had normocalcemic hypoparathyroidism, for a prevalence of 1.1%. Baseline data from the DHS showed that of 3450 men and women, 68 had normocalcaemic hypoparathyroidism, for a prevalence of 1.9%. Follow-up data of these patients over 8 years showed that none developed overt hypoparathyroidism, and that two (0.09%) had persistent evidence of normocalcaemic hypoparathyroidism.

Moreover, hypoparathyroidism may remain undiagnosed until a stressor such as a bisphosphonate is administered. Several case reports document patients with risk factors for hypoparathyroidism such as a history of thyroidectomy or iron overload becoming symptomatically hypocalcemic after bisphosphonate therapy [292]. A recent report investigating the etiology of bisphosphonate-associated atypical femur fractures showed an increased prevalence of hypocalcemia and a lower (though still robust) PTH level as compared with typical fracture patients, suggesting that deficient PTH production may predispose to this complication as well. An early or

subclinical form of hypoparathyroidism with normal serum calcium levels but low PTH levels was described in a small cohort of patients with thalassemia who had substantially lower nocturnal PTH levels and PTH/calcium ratios compared with controls.

4.4.3 Surgical Hypoparathyroidism

Surgical hypoparathyroidism is due to an insufficient production of PTH occurring after surgery. In almost all cases it is the consequence of either damage to or removal of parathyroid tissue during a surgical procedure on the neck, generally involving the thyroid or parathyroids. These glands are affected unintentionally at the time of thyroidectomy in most of the cases. Post-operative hypoparathyroidim can also occur after one or more parathyroids are removed during surgery for PHPT or a secondary–tertiary hyperparathyroidism. Surgical hypoparathyroidism can be divided in two main categories:

1) Following total or subtotal thyroidectomy.

2) Following parathyroid surgery, which can consist of the removal of a single parathyroid adenoma or removal of several affected glands (multiglandular disease) in either PHPT or SHPT.

The importance of occurring after thyroid surgery is more significant epidemiologically because so much more thyroid than parathyroid surgery is performed. Independent of the kind of neck surgery, this kind of hypoparathyroidism is usually evident within a few hours after the operation, but it can also occur later, such as 1 or 2 days postoperatively. Less commonly, it can occur even later. Postsurgical hypoparathyroidism can be transient or permanent. The transient one usually resolves within a few weeks, but may persist up to 6 months after surgery. After 6 months, if it persists, the clinical syndrome must be defined as permanent.

Hypoparathyroidism after thyroid surgery. During thyroid surgery, parathyroid glands can be devascularized by ligation of thyroid arteries proximal to the origin of the parathyroid arteries, thermally damaged by accidental electrocoagulation by heat induction using any coagulation energy device (radiofrequency or ultrasound), and,

rarely, by inadvertent removal, or damage. Even minor trauma such as parathyroid tissue being "nicked" can lead to hypoparathyroidism.

In order for parathyroid tissue to be preserved during thyroid surgery, its anatomical integrity must be ensured. For this reason, the parathyroid glands should always be identified at the time of thyroidectomy. In order to maintain their blood supply, it is important to ligate thyroid arteries as close to the thyroid gland as possible, distal to the origin of parathyroid vessels. If parathyroid glands are accidentally devascularized, removed, or simply nicked, various grades of parathyroid insufficiency may develop [293]. An exact evaluation of the real impact of this complication of thyroid surgery is difficult to assess, since its incidence varies widely among the different surgical procedures and with the experience of the surgeon. The highly experienced surgeon, who could be defined by the number of total thyroidectomies performed annually, will typically report much lower rates of postoperative hypoparathyroidism than surgeons who perform thyroid surgery less often [294]. The rate of postoperative hypoparathyroidism largely depends upon surgical technique but also on both the extent of thyroid resection and the complexity and duration of the operation: extensive thyroid cancer surgery,radical neck lymph node dissections, and bulky and substernal goiters all are factors influencing the risk of postoperative hypoparathyroidism. Another aspect playing an important role is the underlying thyroid pathology. The risk of postoperative hypoparathyroidism is higher following thyroidectomy for Graves' disease [7]. Repeated surgery is often associated with a higher hypoparathyroidism risk forseveral reasons: it always implies a longer duration of the procedure, with more complex and difficult technical challenges. In some cases, the parathyroid glands have already been damaged in previous operations, and the extended dissection, which is inevitable during repeated surgery, may jeopardize the blood supply of the remaining parathyroid glands [295]. Pre-existing clinical situations also play an important role in determining the development of postoperative symptomatic hypocalcemia. Examples include low 25-hydroxyvitamin D, magnesium depletion, and high bone turnover rate, due to preoperative severe

hyperthyroidism. The latter example would be a "hungry bone syndrome" where resolution of the hyperthyroidism leads to rapid normalization of bone resorption. The drive towards bone formation without compensatory increases n bone resorption generates a calcium "sink" from blood into bone. Hungry bone syndrome can persist for several weeks [296]. This syndrome is associated with a precipitous drop in the serum calcium and phosphorus concentrations. Such patients, however, have normal or even high serum PTH concentrations, demonstrating a normal parathyroid state that is behaving in a compensatory manner.

Hypoparathyroidism after parathyroid surgery. In parathyroid surgery, the experience of the surgeon is important, but perhaps to a lesser extent than in thyroid surgery, in determining the risk of postoperative hypoparathyroidism. More important are issues related to the anatomic and physiologic integrity of the remaining parathyroid glands. Typically, the glands left behind in the course of parathyroid surgery are not functional because the overactive parathyroid tissue has physiologically suppressed them. If the PHPT or SHPT is severe, the remaining parathyroid tissue might require a short period of time before it recovers. The time of recovery is very much a function of the severity of the hyperparathyroid state. Hypoparathyroidism is a serious but relatively uncommon complication of parathyroid surgery [297]. It has to be differentiated from "hungry bone syndrome" as is the case following thyroid surgery. In this case, the PTH concentration will be elevated, while in transient or permanent hypoparathyroidism the PTH will be suppressed. Also similar to the situation after thyroid surgery, hungry bone syndrome is aided and abetted by increased influx of calcium into bone [298]. The incidence of postoperative hungry bone syndrome varies according to the severity of the parathyroid disease [299]. Various risk factors have been suggested for the development of this syndrome, including older age, the weight and volume of the resected parathyroid glands, radiographic evidence of bone disease, higher preoperative serum concentrations of calcium, alkaline phosphatase, circulating PTH, blood urea nitrogen values, and vitamin D deficiency. If radiologically overt parathyroid bone disease is

present, hungry bone syndrome should be anticipated in the early postoperative period. More commonly, though, there is no evidence for radiologically evident overtparathyroid bone disease, in which case hungry bone syndrome is unlikely and not anticipated. If present, treatment is aimed at maintaining the serum calcium levels with sufficient calcium and vitamin D supplementation. Intravenous calcium administration is often necessary. Adequate correction of magnesium deficiency is also important. In Sporadic Primary Hyperparathyroidism (S-PHPT) of renal origin, postoperative hungry bone syndrome after subtotal/total parathyroidectomy is more common than after surgery for PHPT, with incidence figures ranging from 27% [300] to 51.2% [301]. Symptoms are present typically within 18 hours after surgery; a prolonged hospital stay may be required. The only two identifiable preoperative risk factors were young age [300] and low preoperative calcium concentrations [301]. Such high risk patients require judicious preoperative calcium supplementation. Preoperative vitamin D therapy did prevent this complication and had no impact on the length of intravenous calcium supplementation that was required. Intensive monitoring of serum calcium concentrations is needed for at least 3 weeks after surgery [301].

Clinical aspects. The clinical pattern of surgical hypoparathyroidism is so characteristic that it is impossible to be missed, particularly when its onset occurs on the first postoperative day when the drop in calcium concentrations can be severe. Hypocalcemia, though, can also be asymptomatic if the decrease in serum calcium is mild. If the manifestations are dramatic, immediate treatment is mandatory. The surgeon must quickly recognize this complication and initiate "rescue" therapy with calcium. If this is not quickly recognized, postoperative hypoparathyroidism can be life-threatening. Acute hypocalcemia is associated with characteristic symptoms of neuromuscular irritability followed by neurological symptoms; also electrocardiographic alterations can sometimes be seen. Numbness and tingling in the fingertips and circumoral region generally appear first, followed by paresthesias in the extremities, which may be intense. As serum calcium concentrations decline, particularly if this occurs acutely, signs of tetany such as carpal or pedal spasm will

appear (the Trousseau sign is typical). Another manifestation that is typically reported is the Chvostek sign, consisting of a contraction of facial muscles provoked by light percussion on the facial nerve near its outlet close to the external auditory meatus. Two symptoms that are not common but may cause extreme concern for patients and their physicians are broncho- and laryngospasm: the latter of these must be carefully distinguished from bilateral recurrent nerve palsy, a very rare complication of thyroid surgery that mimics closely the manifestations of laryngospasm. Besides neuromuscular symptoms, there can be also central nervous system symptoms such as confusion, disorientation, and delirium. Cardiologic alterations induced by hypocalcemia are generally expressed by prolonged QT interval on electrocardiogram. Other cardiologic manifestations of the hypocalcemic syndrome include arrhythmias and, rarely, congestive heart failure [302]. Although symptoms of hypocalcemia may appear on the first postoperative day, the drop in calcium concentrations may not reach their nadir until 3 days or more after surgery. Thus, hypocalcemia can become clinically manifest several days after the operation in patients who were almost asymptomatic before [303]. In addition, totally asymptomatic hypocalcemia may develop. For this reason, all patients who have undergone a thyroid procedure should have their serum calcium concentration measured within a week or so after surgery, even in the absence of symptoms. The perioperative measurement of albumin-corrected serum calcium or ionized calcium, where available, is a reliable method to exclude or confirm postsurgical hypoparathyroidism. Measurement of intact PTH and phosphate can further help to establish parathyroid gland competency following surgery [304]. Low PTH and high phosphate levels are clues to the presence of a hypoparathyroidism state, while normal values for PTH and phosphate can be reassuring. The term parathyroid insufficiency [304] or partial hypoparathyroidism [305] has been used to characterize patients whose serum calcium is low with detectable PTH levels. While not clearly hypoparathyroidism (i.e., there is measurable PTH), the PTH is not sufficient to maintain normal serum calcium levels. Serum magnesium concentrations may also be

helpful in sorting out the presence of frank hypoparathyroidism [306]. With hypomagnesemia, PTH secretion is inhibited along with a peripheral PTH resistance, thus exacerbating and prolonging hypocalcemic symptoms. This effect of magnesium is reversible with magnesium replacement.

Prediction of surgical hypoparathyroidism. The onset of surgical hypoparathyroidism is one of the main factors limiting early hospital discharge for patients undergoing thyroid and parathyroid surgery, particularly when it occurs on the first or second postoperative day. Considering the present trend of performing these operations using same day surgery, which has been particularly favored by the adoption of minimally invasive techniques [307] and the use of local or loco-regional anesthesia [308], it would be highly advantageous to understand thoroughly the factors that can lead to surgical hypoparathyroidism after surgery, and to implement appropriate interventions prior to surgery to prevent this outcome. Measuring PTH serum concentrations during surgery or immediately thereafter seemed to constitute the most reliable and the earliest predictive factor for the onset of surgical HYPOPARA, mainly because of PTH's very short half-life in the circulation [303]. It is important though to distinguish between the use of an intraoperative rapid parathyroid hormone immunoassay and the standard measurement of PTH, which typically is available only several hours after the sample has been obtained.

Measurement of circulating PTH during or shortly after surgery, in particular if combined with serum calcium measurements at 6 or 12 hours, is generally agreed to be a useful approach for assessing the likelihood of postsurgical hypoparathyroidism [309]. Despite some controversy over this point, if the PTH concentration falls below 10 pg/mL shortly after surgery, a significant decrease in the serum calcium concentration on the first postoperative day might occur. Similarly, a drop of PTH greater than 75% intraoperatively or 65% 6 hours after the end of the operation is consistent with the onset of hypoparathyroidism in the first postoperative day [310,311]. In an observational study conducted among nine medical centers, a decrease in PTH greater than 65% after thyroidectomy showed a sensitivity of 96.4% and a specificity

of 91.4% in predicting postoperative hypoparathyroidism [312]. In summary, these data seem to support early PTH measurement in order to select which patients are prone to develop a postsurgical hypocalcemic syndrome. Such patients might be directed to take more calcium supplementation during this period. Surgery plays a primary role in preventing hypoparathyroidism. Accurate surgical technique constitutes the best prevention. This includes recognizing all parathyroid glands during thyroid surgery. If one or more parathyroid glands have been inadvertently removed, they should be promptly reimplanted. Close inspection of the thyroid specimen after its removal should be routinely carried out. Damaged parathyroid glands, in situ , also must be thoroughly examined and evaluated. A significant change in color indicates that the gland's blood supply might be compromised. A white or pale hue might mean damage to the arterial supply whereas a blue, dark color is consistent with insufficient venous drainage. Interruption of the arterial supply is a more urgent situation. Under these conditions, reimplantation of the damaged gland into the muscles or into the sternocleidomastoid muscle is advised [313]. The parathyroid glands generally receive their blood supply from the inferior thyroid vessels and for this reason a general rule is never to ligate the main trunk of the inferior artery but to ligate its branches very close to the thyroid capsule. In a few cases, though, the blood supply can derive also from the superior thyroid pedicle and this anatomical variant should be kept in mind by the surgeon. Despite these precautions, postoperative hypoparathyroidism can develop so an accurate work-up of the metabolic state of the patient prior to surgery is highly advisable, in particular vitamin D and magnesium state. Their deficiency can affect or worsen the hypocalcemia.2 In 2011, a Consensus Report [314] suggested that in the presence of 25-hydroxyvitamin D levels less than 20 ng/mL it is necessary to give an appropriate supplementation with cholecalciferol (vitamin D3), ergocalciferol (vitamin D2), or calcifediol (25-hydroxyvitamin D) in order to maintain a correct mineral ion homeostasis. In terms of magnesium deficiency, it should be remembered that several clinical or subclinical situations are characterized by mild or even severe hypomagnesemia: the use of chemotherapeutics, nutritional

deficiencies of various degrees, prolonged use of diuretics, and alcoholism can affect magnesium economy. Thus, it is important to optimize magnesium reserves before parathyroid or thyroid surgery. In patients with a higher risk of hypoparathyroidism, such as those who undergo repeated surgery, complex operations for severe neoplastic disease, or patients suffering from Graves' disease, it may prove advantageous to initiate treatment with calcitriol prior to and immediately after surgery [306].

4.4.4 Morbidity and mortality

In view of the fact that there are currently no formal guidelines, the management of hypoparathyroidism is based on experience and clinical judgment. The primary goals of management of chronic hypoparathyroidism include maintaining serum total calcium in the low-normal range, serum phosphorus in the highnormal range, 24-hour urine calcium less than 300 mg (7.5 mmol), and calcium–phosphate product less than 55 mg2 /dL2 (4.4 mmol2 /L2) [315]. The currently accepted standard treatment for hypoparathyroidism consists of supplementation with calcium and vitamin D, vitamin D metabolites, or vitamin D analogs, but does not employ replacement of the missing hormone, PTH. Complications of treatment of chronic hypoparathyroidism range from inadequate treatment to overtreatment. The rates of complications from hypoparathyroidism, or the management of hypoparathyroidism, however, have been difficult to estimate given the lack of large natural history studies.

Hypocalcemia. Inadequate treatment of hypoparathyroidism with suboptimal doses of calcium or vitamin D in the diet or with supplements may cause frequent symptomatic hypocalcemia. Current estimates project that 33% of patients with chronic hypoparathyroidism require at least one emergency department visit or hospital admission each year. Of hospital or emergency department visits, 62% are due to symptomatic hypocalcemia. Seizures may occur in up to 15% of patients with hypoparathyroidism each year. Dilated cardiomyopathy may also rarely occur due to prolonged or frequent hypocalcemia in these patients.

Hypercalcemia and Hypercalciuria. With the need for relatively high doses of supplementation with calcium and vitamin D and its analogs to maintain serum calcium levels close to the normal range, hypercalcemia is a frequent concern in patients with hypoparathyroidism [255]. The replacement regimen for hypoparathyroidism can, however, also lead to hypercalciuria, because PTH insufficiency impairs renal calcium reabsorption [316]. Hypercalciuria can lead to nephrolithiasis, nephrocalcinosis, and renal insufficiency [317]. The rates of nephrolithiasis reported in patients with hypoparathyroidism differ with the number of subjects studied. In one cross-sectional study of 25 patients with postsurgical hypoparathyroidism, 23% had 24-hour urine calcium excretion levels higher than 320 mg, and 8% had asymptomatic nephrolithiasis noted on renal ultrasound, with normal renal function in all patients [317]. In another cross-sectional study of 33 patients with hypoparathyroidism of diverse etiologies, 15% reported a history of nephrolithiasis [318]. A larger retrospective cohort of 120 patients reported nephrolithiasis and nephrocalcinosis in 31% of patients who were mostly asymptomatic. Rates of chronic kidney disease stage 3 or higher were two- to 17-fold greater than in age-matched controls [319]. Winer et al. have reported higher rates of renal complications in patients participating in clinical studies at the National Institutes of Health [320]. In a short-term randomized controlled trial comparing therapies for hypoparathyroidism, evidence of renal insufficiency was found in 80% of patients (n = 10). Four subjects had radiographic evidence of nephrocalcinosis, and two suffered from recurrent nephrolithiasis [321]. In another cohort of 17 patients, eight patients (47%) had evidence of nephrocalcinosis by renal computerized tomography scan, and 14 patients (80%) had renal insufficiency [322]. In a further randomized controlled trial that compared therapies for hypoparathyroidism for a longer period, 40% of 27 patients had nephrocalcinosis, and two-thirds had creatinine clearance values below the normal range [320].

Skeletal impairment. In the absence of PTH, bone remodeling is markedly reduced [323].Chronically low bone turnover in patients with hypoparathyroidism typically leads

to bone mass that is higher than in age- and sex-matched controls [324]. One study have investigate histomorphometric assessment of static and dynamic bone parameters in subjects with hypoparathyroidism compared to age and sex-matched control healthy subjects. The authors have recorded a greater cancellous bone volume, trabecular and cortical width with a significant suppresion of bone mineralizaton and formation rate in hypoparathyroidism compared to healthy subjects[318].

Cataracts. The presence of cataracts has long been associated with both postsurgical (55%)71 and idiopathic hypoparathyroidism (41–51%) [325]. Patients with cataracts tend to have a longer duration of hypoparathyroidism than those without (7.5 ± 11.0 vs. 4.8 ± 4.0 years, P = 0.49), and tend to be older (53.6 ± 15.3 vs. 43.2 ± 11.5 years, P = 0.11). Patients with idiopathic hypoparathyroidism with intracranial calcification had a higher frequency of occurrence of cataracts when compared with those without calcification (19/39, 48.7% vs. 2/12, 17.7%, P = 0.048) [326]. The mean duration of illness was longer in patients who had intracranial calcification or cataracts as compared to patients without these complications (9.0 ± 9.5 years vs. 2.4 ± 4.1 years, P = 0.002; and 11.6 ± 10.4 years vs. 4.4 ± 6.4 years, P = 0.01, respectively). Linear regression analysis of the data in models where age of onset of symptoms, duration of illness, and serum calcium levels were considered as independent variables showed that only the duration of the illness explained the presence of the variations observed in the frequency of occurrence of cataract or basal ganglia calcification. However, duration of the illness could explain the variation in the occurrence of intracranial calcification and cataracts in only 15–16% of patients. These findings suggest a role for other factors in their etiology.

Basal Ganglia Calcification. Basal ganglia calcification is a well-known complication of hypoparathyroidism,81 but it is not clear why the basal ganglia, among other tissues, should be prone to this. In patients with long-standing hypoparathyroidism, with duration of disease longer than 8–10 years, basal ganglia calcification, or even more diffuse brain calcification, may occur [327]. While this condition is generally asymptomatic, in some cases an association with cognitive

dysfunction [327], and even with organic mood disorder [328] has been reported. In the general population, basal ganglia calcification prevalence estimates are not well established, but have been reported to be low at 2–12.5% [329]. Reported rates of basal ganglia calcification in hypoparathyroidism vary, from 12% in a cohort of 33 patients75 to 36% of 25 patients with CaSR mutations [330]. In one cohort of mostly postsurgical hypothyroidism cases, 52% of 31 patients showed basal ganglia calcification on head CT scan.72 In contrast, in a cohort of 145 patients with idiopathic hypoparathyroidism, all of whom had head CT scans, 74% had basal ganglia calcification, and this correlated with the duration of hypocalcemia, choroid plexus calcification, seizures, and cataracts [325]. Familial idiopathic basal ganglia calcification has been shown to be caused by a mutation in a type III sodium–phosphate transporter leading to impaired cellular uptake of inorganic phosphate.This finding suggests that increased extracellular phosphate in the setting of chronic hyperphosphatemia may contribute to basal ganglia calcification in hypoparathyroidism. Serum phosphorus has been found to be quantitatively higher in patients with basal ganglia calcification compared to those without.

4.4.4.1 **Skeletal impairment**

Bone turnover markers. Low values of biochemical markers of bone turnover are a recognized feature of hypoparathyroidism [331,332]. Rubin et al. measured a panel of these markers in a histomorphometry study of 64 hypoparathyroid subjects (48 women and 16 men) [333]. The etiologies of hypoparathyroidism were post-thyroid surgery (n = 32), autoimmune disorder (n = 30), and DiGeorge syndrome (n = 2); and the mean duration of the disease was 15 ± 13 (SD) years. Subjects received vitamin D between 50 and 75,000 IU/d, and supplemental calcium between 0 and 9 g/d. Circulating markers of bone formation (P1NP, BAP, and osteocalcin) and of bone resorption (TRAP-5b and serum CTx) were in the lower half of the normal reference range [333]. Other studies have shown similarly low values for bone turnover markers [320]. Associated with reduced marker values, Rubin et al. demonstrated

reduced numbers of circulating osteogenic precursor cells [334] and increased circulating sclerostin concentrations [230].

Bone mineral density. Chronically low bone turnover in hypoparathyroidism leads to bone mass that is relatively higher than that of age- and sex-matched controls [335]. Bone mass, for example, was 21–28% higher in 13 women, 10–13 years after thyroidectomies complicated by hypoparathyroidism as compared to 13 women whose thyroidectomies were not complicated by hypoparathyroidism [335]. BMD in postmenopausal women with post-thyroidectomy hypoparathyroidism was found by DXA to be higher when compared with age-predicted means at the lumbar spine and proximal femur, although not at the distal radius [29]. Hypoparathyroidism was also found to retard the expected rate of postmenopausal bone loss as measured by DXA [336]. When a small number of hypoparathyroid subjects were compared to those with PHPT, hypoparathyroid subjects did not show the catabolic effects of PTH to reduce BMD at the femoral neck [337]. Greater insight into the architectural basis of the increase in bone mass can be obtained by peripheral quantitative computed tomography (pQCT). Using this technique, Chen and colleagues compared volumetric bone mineral density (vBMD) and geometry of the distal- and mid-radius among postmenopausal women with postoperative or idiopathic hypoparathyroidism, PHPT, and normal control individuals [338]. At the 4% distal radius site, which is enriched in cancellous bone, trabecular vBMD was higher in the rank order hypoparathyroidism > control > PHPT. At the 20% mid-radius site, cortical vBMD also was greater in the same rank order. The BMD differences among these three groups could be explained by differences in bone geometry. At both radial sites, total bone area and both periosteal and endosteal surfaces were greater in PHPT than in hypoparathyroid patients and controls, and cortical thickness and area were higher in the rank order hypoparathyroidism > control > PHPT.

Histomorphometry aspects. The most comprehensive information on the effects of hypoparathyroidism on the skeleton has come from histomorphometric analysis of iliac crest bone biopsies. Despite the marked reduction in bone remodeling activity,

variables reflecting the amount and microarchitecture of cancellous bone, such as cancellous bone volume, trabecular thickness, and marrow space star volume, were normal. A similarly profound suppression of bone turnover was reported in an earlier study on hypoparathyroid dogs, in which treatment with vitamin D was not able to restore normal bone turnover [339]. The more recent, larger histomorphometric study of Rubin et al. gave further insights [333]. In contrast to the earlier smaller study [41], cancellous bone volume was elevated in the hypoparathyroid subjects. The structural basis for the higher cancellous bone volume in hypoparathyroidism was an increase in trabecular width; trabecular number and trabecular spacing were both similar to control subjects. Cortical width also tended to be greater in the hypoparathyroid subjects, and cortical porosity was lower than in control subjects. Remodeling activity was assessed separately on cancellous, endocortical, and intracortical skeletal envelopes. Osteoid surface and width were reduced in the hypoparathyroid subjects in all three envelopes. The tetracycline-based bone formation rate (BFR) was significantly lower in all three envelopes in the hypoparathyroid subjects, with the most profound reduction seen in the cancellous envelope. The reduction in BFR was due to significant decreases in both mineralized surface and mineral apposition rate in all three envelopes. The eroded surface did not differ between the hypoparathyroid and normal subjects, but the bone resorption rate was significantly lower in the hypoparathyroid subjects in all three envelopes. These findings are all indicative of a profound reduction in the bone turnover rate in hypoparathyroidism accompanied by an increase in bone mass in both cancellous and cortical compartments.

The effects of PTH deficiency on cancellous and cortical bone mass, which were observed initially by non-invasive imaging and by two-dimensional (2D) histomorphometry, were confirmed by the threedimensional (3D) analytical capability of microcomputed tomography (μ CT) [340]. Results from this study corroborated the increase in cancellous bone volume and trabecular thickness in hypoparathyroid subjects and demonstrated higher trabecular number and trabecular connectivity in comparison with matched control subjects. In addition, the structural

model index was lower in hypoparathyroidism, indicating that the trabecular structure was more plate-like than rod-like.

Fracture risk. Prospective data on fracture risk in hypoparathyroidism are not available. In a retrospective cohort, 21/120 patients (18%) had fractures over 7 years of follow-up [319]. A simulated strength study with stress loading of images by finite element analysis suggested normal mechanical strength in hypoparathyroidism. the skeletal abnormalities that have been described, there is reason to be concerned about the fragility of bone in hypoparathyroidism [318], but further data are necessary to address this point.

4.4.5 Quality of life, well-being, mood

It has been shown that patients with chronic hypoparathyroidism receiving standard treatment with calcium and vitamin D suffer from a significant impairment of well-being and mood. Psychometric evaluation was performed in a cross-sectional controlled study of 25 unselected women with postsurgical hypoparathyroidism for 6.4 ± 8.0 years on stable treatment with calcium and vitamin D (or analogs) and 25 controls matched for sex, age, and time since surgery [315]. Three validated questionnaires were used, including the revised version Symptom Checklist-90 (SCL-90-R), the von Zerssen Symptom List (B-L Zerssen), and the short form of the Giessen Complaint List (GBB-24). The higher the score or subscale score in any of the three psychometrical instruments, the greater the impairment of well-being as assessed by the respective questionnaire. Compared with controls, hypoparathyroid patients in this study had significantly higher global complaint scores in SCL-90-R (P =0.020), B-L Zerssen (P=0.002), and GBB-24 (P=0.036), with predominant increases in the subscale scores for anxiety, phobic anxiety, and their physical equivalents. Aggarwal et al. showed that a significantly higher proportion of patients with idiopathic hypoparathyroidism showed neuropsychological dysfunctions than did controls [32.3% (95% CI: 20.9–45.3) vs. 5.7% (95% CI: 1.6–14.0), P < 0.001].79 Neurological signs were present in 35.5% of patients (extrapyramidal: 16.1%;

cerebellar: 20.9%). Volume of basal ganglia calcifications and number of sites with intracranial calcifications including the cerebellum and dentate nuclei were comparable in patients with and without neuropsychological, extrapyramidal, or cerebellar dysfunctions. Cognitive dysfunction score was lower by 1.7 points in males than in females (P =0.02) and increased by 0.21 and 5.5 for each year increase in the duration of illness (P=0.001) and one unit increase in serum calcium–phosphorus product (P=0.01), respectively. The scores improved by 0.27 for every 1.0 mg/dL increase in serum calcium (P=0.001). The study concluded that neuropsychological dysfunctions are present in up to one-third of patients with idiopathic hypoparathyroidism, and that these correlate with duration of illness, female gender, serum calcium, and calcium x phosphate product during follow-up, but not with intracranial calcification. These dysfunctions may affect their daily functions, safety, and drug compliance. Clarke et al. evaluated symptoms of patients with hypoparathyroidism aged 18 years or older who were diagnosed 6 months or more previously using an internetbased self-reported questionnaire [341]. The study population (N = 374) included 85% women with mean age 49 years. Surgery of the thyroid, parathyroid, or neck for cancer was the cause of hypoparathyroidism in 43%. Mean disease duration was 13 years, and moderate or severe disease was reported in 79%. Patients reported visiting an average of six different specialists or physicians before and after their diagnosis. More than 10 symptoms were experienced by 72% of patients in the preceding 12 months, despite standard symptomatic management with calcium and active vitamin D supplementation. Symptoms were experienced for an average 13 hours/day. Co-morbidities were experienced by 69% of patients. Disease-associated hospital stays or emergency department visits were required by 79% of patients. Fifty-six percent of subjects strongly agreed that they felt unprepared to manage their condition at diagnosis, 60% that controlling their hypoparathyroidism was harder than expected, and 75% were concerned about long-term complications of their current medications. Forty-five percent reported significant interference from hypoparathyroidism in their daily lives. The study concluded that patients with

hypoparathyroidism have a substantial multidimensional burden of illness, experiencing co-morbidities, acute episodes of hypocalcemia, and a nearly continuous presence of symptoms despite standard symptomatic management.

4.4.6 Cost and hospitalization

The population-based longitudinal medical-records linkage resources of the Rochester Epidemiology Project in Rochester, Minnesota, were used to assess the cost of caring for patients with hypoparathyroidism [342].All persons residing in Olmsted County in 2009 with any diagnosis of hypoparathyroidism ever assigned by a provider since 1945 were identified, and their detailed medical records reviewed to confirm their diagnosis of hypoparathyroidism and assign etiology. Two age- and sex-matched controls were assigned per confirmed case, and follow-up censored for every case-control set member at shortest follow-up for each member. Since 1987, Rochester Epidemiology Project resources also have included provider-linked line item billing data for essentially all medical services and procedures received by residents of Olmsted County, Minnesota, with the capacity to assign nationally standardized wage- and inflation-adjusted dollar estimates. Data on outpatient prescription costs are not included in these estimates. Using these resources, all medical care costs for each year 2006 through 2008 were obtained to compare cases with controls for 2009 estimated dollar costs. Results of cost comparisons showed that medical care of patients with hypoparathyroidism were about three times those of controls. These population-based data on medical care costs of patients with confirmed hypoparathyroidism reveal that, although a relatively rare condition, the burden of costs associated with HPT is substantial and consistent. Additional investigation is needed to elucidate the source of excess costs compared to controls.

Anyway, this kind of study [342] quantitated overall cost of medical care for patients with hypoparathyroidism, but did not separate out the individual costs related to, or the frequency of utilization of, outpatient clinic, hospital, emergency department, or pharmacy. No other studies have addressed the frequency of hospitalization of patients with hypoparathyroidism relative to normal controls, but it is assumed that

hospitalization for complications of hypoparathyroidism, such as bronchospasm, laryngospasm, seizures, or cardiac dysrhythmias, is increased.

CHAPTER 5

NORMOCALCEMIC HYPOPARATHYROIDISM: PREVALENCE AND EFFECT ON BONE STATUS IN OLDER WOMEN. THE OPUS STUDY*

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5.1 Introduction

Parathyroid hormone (PTH) is the major hormonal mediator of extracellular calcium homeostasis and regulates osteoclastic bone resorption, the renal excretion of calcium and synthesis of 1,25-dihydroxyvitamin D. Circulating PTH concentrations display a large inter-individual variability which has a strong heritable component [343]. However, the genetic factors governing serum PTH concentrations remain to be elucidated. PTH is an 84 amino-acid peptide, whose secretion by the parathyroid chief cells is regulated by the CaSR, which is expressed at the parathyroid cell-surface. Mutations of the CaSR gene lead to inherited forms of hypercalcaemia and hypocalcaemia [344], and common coding region CaSR single nucleotide polymorphisms (SNPs) have been revealed as determinants of serum calcium concentrations [345]. Cusano and colleagues recently reported the prevalence of normocalcaemic hyper- (NPHPT) and hypoparathyroidism (NHYPO) in two unselected, non-referral community-dwelling populations, identifying a prevalence of 0.4-3.1% and 1.1-1.9% respectively [291].

NPHPT is characterised by normal calcium levels with high PTH in the absence of secondary causes of hyperparathyroidism [346] and was officially recognised by the Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism [176]. These patients may develop a similar rate of low bone mineral density compared to subjects with PHPT [347] and have a trend towards a higher recurrence of NPHPT after parathyroidectomy [235].

While NPHPT is a well-documented diagnostic category, no consistent data are available for NHYPO. The diagnosis of NHYPO has been used for patients who develop hypocalcaemia in response to bisphosphonate therapy having had normal

calcium values and low PTH levels prior to starting therapy; such patients were considered to have inadequate parathyroid gland reserve [348]. We cannot yet be certain whether NHYPO is a real diagnostic category. Indeed, Cusano and colleagues identified 68 subjects with NHYPO, none of whom developed overt hypoparathyroidism on follow-up; persistent disease was noted in only 2 of 26 subjects that concluded the follow-up period [291].

It is possible that the prevalence of NHYPO by Cusano and colleagues [291] may have underestimated the true prevalence in the general population because they only studied men and young women. It is important to study older women as many parathyroid diseases such as PHPT have their peak incidence in the first decade after the menopause [349]. The primary end point of this study was to determine the prevalence and the metabolic bone profile of NHYPO in our population, assessing its evolution over the time. The secondary endpoint was to evaluate the prevalence of other calcium metabolic disorders.

5.2 Materials and methods

Study design and population

We recruited 2419 older women (age 55-79 years) and 258 younger women (age 30-40 years) from 5 European cities (the OPUS study) [350]. The OPUS study is a large population-based cohort study designed to compare quantitative ultrasound (QUS) performance with central DXA. The study design has previously been reported [350]. Of note, exclusion criteria were limited to disorders that precluded valid QUS measurements (i.e. bilateral fractures of the calcaneus, bilateral hip prostheses, disorders of the hand), general inability to undergo the specified exams, and cognitive limitations that precluded completion of self-administered questionnaires. Pregnant women were excluded because of potential risks associated with X-ray exposure. All investigations were conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from the local ethics committees and written informed consent was obtained from each subject.

The OPUS study became a longitudinal study when laboratory and other data were collected approximately 6 years later. Complete follow-up data are available in 1416 subjects. Each subject had a first visit (between 1999-2001) and then 6 years later was invited to attend for a second visit (between 2005-2007). At the time of each study visit a modified version of the European Vertebral Osteoporosis Study (EVOS) risk factor questionnaire [351] was administered to each subject. From this we were able to collect medical and lifestyle information. Medical history of diseases and treatments was recorded. Non-fasting venous blood samples were collected from each subject between 12:00 and 15:00 into serum separating tubes. The blood was left to clot for 30 minutes at room temperature and centrifuged at 2500 g for 10 minutes. The serum was then collected and stored at -80°C until analysis. Second morning void urine samples were collected and stored at -20°C until analysis.

To estimate reference intervals for adjusted calcium and PTH, 107 young women were eligible after excluding those with low 25-hydroxyvitamin D (25OHD) (< 50 nmol/L), eGFR < 60 ml/min per 1.73m^2, T-score at the lumbar spine or total hip equal to or less than -2.5 and those taking drugs or suffering from diseases known to affect bone. Both adjusted serum calcium and PTH were log10 transformed prior to analysis.

Biochemical measurements

Blood samples were drawn to measure serum:

1. Calcium and albumin, measured using a Cobas c701 (Roche Diagnostics, Germany) autoanalyser in the Chemical Pathology laboratory, Sheffield Teaching Hospitals, UK. The manufacturer's reported inter-assay precision was <2.0% for each test. We calculated adjusted calcium in all subjects (pre- and post-menopausal women were included in the analysis) based on the total calcium and albumin measurements according to James' formula[352]. As recommended in that report we excluded subjects with creatinine > 200 μmol/L and/or albumin <20 g/L or >50 g/L and/or total calcium >3 mmol/L. In particular, at baseline, 2638 women were included in the analysis and the local adjustment equation was expressed by the

following relationship: adjusted Calcium = Total Calcium - (0.015 x albumin) + 0.699. After six years follow up (1652 women were included in the analysis), the local adjustment equation was expressed by the following relationship: adjusted Calcium = Total Calcium - (0.018 x albumin) + 1.581

2. Collagen type 1 cross-linked C-telopeptide (CTX), intact procollagen type 1 N propeptide (PINP), bone alkaline phosphatase (bone ALP), 25OHD and PTHi. They were measured in serum using the IDS-iSYS automated immunoassays (Immunodiagnostic Systems, Boldon, United Kingdom). The inter assay coefficients of variation (CV) were 6.5%, 7.2%, 3.5%, 6.7% and 6.5% respectively.

3. Creatinine, measured using the Cobas c 311automated analyser (Roche Diagnostics, Germany). This was used to calculate the estimated glomerular filtration rate (eGFR) using the formula based on the modification of diet and renal disease (MDRD) [353].

We measured the baseline and 6 year samples at the same time.

Bone densitometry. At both visits, bone mineral density (BMD) was performed using dual-energy X-ray absorptiometry (DXA) of the lumbar spine and the proximal femur in posteroanterior projection (Hologic QDR-4500; Hologic, Bedford, MA, USA in the Kiel, Paris and Sheffield centres) or in anterior-posterior projection (Lunar Expert devices; GE Lunar, Madison, WI, USA in the Aberdeen and Berlin centres). Measurements were standardised and cross calibrated across centres.

***Statistical analysis*.** To classify the abnormalities of calcium homeostasis, we used the following approach: We calculated adjusted calcium in all subjects based on the total calcium and albumin measurements (as above). Reference intervals were calculated for adjusted serum calcium and PTH using data from the healthy pre-menopausal women. Both adjusted serum calcium and PTH were log10 transformed prior to analysis and the mean +/- 1.96SD calculated (Table 10). We allocated older women (baseline) into one of eight categories by using the ellipse defined by the Mahalanobis Distance Analysis (Figure 14) which measures how far each

observation is from the centre of a data cluster, taking into account the shape of the cluster. Observations are considered outliers if $MD^2 > \chi^2_{2;0.975} = 7.378$. We referred to 'high or low' if they were outside the ellipse and had values above or below those found in the young women. We referred to 'high or low normal' if they were outside the ellipse and had values above or below the mean found in the young women but within the reference interval. Normal was defined as anyone inside the ellipse at baseline. Hyperparathyroid hypercalcaemia (HH), that included both PHPT and familial hypocalciuric hypercalcaemia (FHH) was defined as anyone outside the ellipse with high adjusted calcium and high or high normal PTH. NPHPT was defined as anyone outside the ellipse with normal adjusted calcium, high PTH, 25OHD > 50 nmol/L and eGFR > 60 ml/min. Secondary hyperparathyroidism was defined as anyone outside the ellipse with low adjusted calcium and high-normal PTH, low adjusted calcium and high PTH, low-normal adjusted calcium and high PTH, high-normal adjusted calcium and high PTH with 25OHD < 50 nmol/L and/or eGFR < 60 ml/min. Hypoparathyroidism was defined as anyone outside the ellipse with low adjusted calcium and low or low normal PTH. NHYPO was defined as anyone outside the ellipse with normal adjusted calcium and low PTH. Non-PTH hypercalcaemia was defined as anyone outside the ellipse with high adjusted calcium and low or low normal PTH. Missing data patients were defined as anyone with missing calcium, PTH or albumin measurements.

We applied the same Mahalanobis Distance Analysis for the older women at follow up (figure 15). In this analysis subjects were included if they had measurements for PTH, calcium and albumin at baseline and follow-up (N = 1416).

Table 10. Reference Intervals for Adjusted Calcium and PTH Healthy Young Women.

	Geometric Mean	Reference Range	95% CI of lower limit of reference range	95% CI of upper limit of reference range	Mean (SD) log_{10}
Adjusted Serum Calcium (mmol/L) (N = 107)	2.359	2.123 – 2.620	2.086 – 2.160	2.575 – 2.667	0.373 (0.023)
PTH (ng/L) (N=107)	25.7	11.1 – 60.4	9.5 – 12.6	52.4 – 69.6	1.410 (0.19)

Figure 14. Baseline data results for adjusted calcium and PTH. The ellipse was derived using Mahalanobis distances method to define normal (black) and abnormal (red) values. The horizontal and vertical lines indicate the geometric mean, and reference intervals described in Table 10. The pink rectangle identifies subjects with normocalcemic hypoparathyroidism.

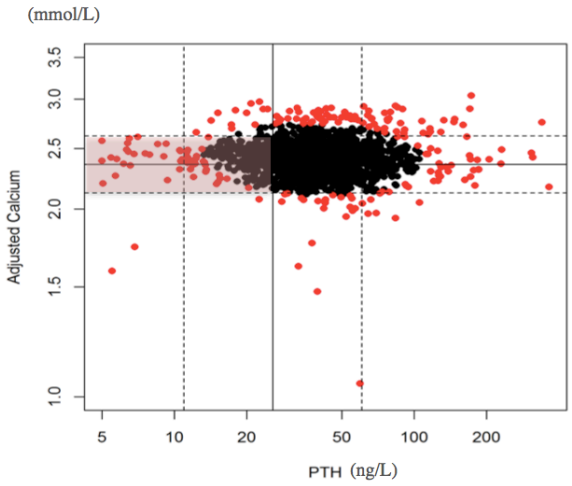
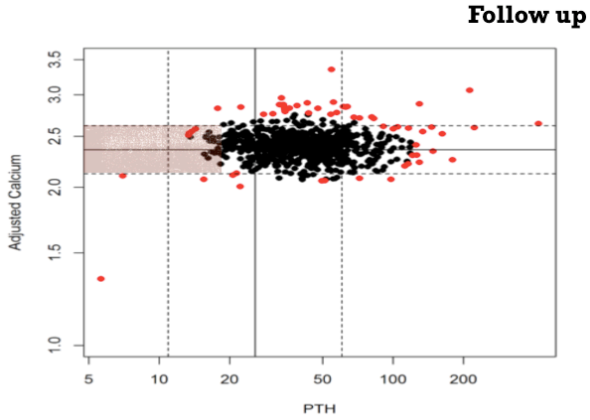


Figure 15. Follow-up results for adjusted calcium and PTH. The ellipse was derived using Mahalanobis distances method to define normal (black) and abnormal (red) values. The horizontal and vertical lines indicate the geometric mean, and reference intervals obtained from the healthy

young women. The pink rectangle identifies subjects with normocalcemic hypoparathyroidism.



Descriptive statistics. The subject characteristics were summarised using frequencies and percentages for categorical variables. For continuous variables, mean and standard deviation were calculated. Characteristics were compared between groups using ANOVA. Post-hoc tests compared all groups to the Normal group using the Dunnett method. All measurements with a skewed distribution were log10 transformed prior to analysis and differences between groups were back transformed and expressed as a percentage difference. We performed multiple regression analyses examining the relationship between measurements at baseline and BMD T-score at baseline; and measurements at baseline and change in BMD from baseline to year 6. We examined the change in PTH measurement from baseline to 6 years in the overall population using a paired t-test. The α-level was set at 0.05.

5.3 Results

Subject characteristics

The subject characteristics at baseline are shown in tables 11a and 11b.

Table 11a. Baseline characteristics of the older and younger women in the OPUS study

	Premenopausal			Postmenopausal		
	N	Mean	SD	N	Mean	SD
Age (years)	463	31.3	5.5	2419	67.1	7.1
Height (cm)	463	166.0	6.7	2418	160.3	6.3
Weight (kg)	463	66.3	13.0	2418	68.7	12.3
BMI (kg/m^2)	463	24.0	4.4	2418	26.7	4.5
Lumbar Spine BMD T-Score	462	0.09	1.12	2372	-0.94	1.51
Total Hip BMD T-Score	462	0.27	1.01	2396	-0.64	1.16
CTX (ng/mL)	423	0.25	0.17	2287	0.36	0.27
PINP (ng/mL)	435	39.7	20.6	2260	42.5	22.0
Bone ALP (ng/mL)	441	11.5	5.1	2313	15.1	6.4
Osteocalcin (ng/mL)	441	21.2	8.0	2303	24.5	13.9
25 (OH) Vitamin D (ng/mL)	440	25.4	12.6	2311	21.3	10.3
eGFR (mL/minute/1.73m^2)	439	86.1	12.3	2317	57.5	13.5
Calcium (mmol/L)	440	2.37	0.13	2320	2.39	0.16

Table 11b. Baseline characteristics for normal and NHYPO subjects.

	Age (year)		BMI (Kg/m^2)		Spine T-Score		Hip T-Score	
	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)	N	Mean (95% CI)
Normal	2063	67.0 (66.7, 67.3)	2062	26.6 (26.5, 26.8)	2024	-0.95 (-1.01, -0.88)	2043	-0.64 (-0.69, -0.59)
Normocalcaemic Hypoparathyroidism	57	66.3 (64.6, 67.9)	57	26.2 (25.1, 27.3)	57	-0.64 (-1.07, -0.21)	57	-0.47 (-0.78, -0.16)
P-Value[a] [a]P-Value from ANOVA testing for an overall difference in means	ns		ns		ns		ns	

Diagnostic categories according to calcium metabolism disorders at baseline

We identified (Tables 12 and 13) 2063 subjects (85.3%) with no calcium abnormalities (normal adjusted calcium levels [2.4 mmol/L, 95% CI 2.39 - 2.41] and normal PTH [39 ng/L, 95% CI 38.4 – 39.7]). 86 subjects (3.6%) were affected by HH (high calcium levels [2.79 mmol/L, 95% CI 2.78 – 2.80] and elevated PTH [58.8 ng/L, 95% CI 52.6 – 65.8]). At the second visit (after 6 years), of the 56 PHPT subjects with follow-up data, 47 (2%) had persistent evidence of PHPT. One subject (0.1%) was affected by NPHPT but none met the diagnostic criteria for NPHPT at the second visit. Sixty nine subjects (2.8%) were affected by secondary hyperparathyroidism (low calcium [2.16 mmol/L, 95% CI 2.09 – 2.23]) with high or high normal PTH (88.9 ng/L, 95% CI 75.8 – 104.3). Three subjects (0.1%) were affected by hypoparathyroidism**.** Fifty seven subjects (2.4%) were affected by NHYPO (normal calcium [2.39 mmol/L, 95% CI 2.36 – 2.42] with low PTH [10.1 ng/L, 95% CI 9.2 – 11.1]). Twelve subjects (0.5%) were affected by non-PTH hypercalcaemia (high calcium (2.82 mmol/L, 95% CI 2.75 – 2.89] with low or low normal PTH [18.5 ng/L, 95% CI 16.2 – 21.3]).

Normocalcaemic hypoparathyroidism

There were statistically significant differences when we compared the NHYPO group with the normal group in terms of BAP (difference = -15.4%, 95% CI: -26.2 to -3.0, p=0.007), CTX (difference = -66.3%, 95% CI: -74.0 to -56.4, p<0.001) and osteocalcin (difference = -36.8%, 95% CI: -45.6 to -26.6, p<0.001). Baseline BMD parameters, and the lumbar spine and hip change over time were not significantly different compared to the normal group. At the second visit (after 6 years), of the 35 NHYPO subjects with follow-up data, none developed overt hypoparathyroidism and only 15 (0.6%) subjects had persistent evidence of NHYPO.

Comparisons between the different categories and normal subjects (Tables 12 and 13)

At baseline, no statistically significant differences were found between the different groups compared to normal subjects with regards to age, BMI and lumbar spine BMD. As expected from its definition, secondary hyperparathyroidism showed a statistically significant reduction in 25 (OH) vitamin D and eGFR compared to the normal group (respectively, difference = -15.9%, 95% CI: -27.2 to -2.8, p=0.011; difference = -16.0%, 95% CI: -21.8 to -9.8, p<0.001).

In subjects with HH and non-PTH hypercalcaemia there was a statistically significant reduction in eGFR compared to the normal group (respectively, difference = -11.7%, 95% CI: -17.4 to -5.7, p<0.001; difference = -17.0%, 95% CI: -30.3 to -1.2, p=0.024).

Table 12. Baseline calcium metabolism characteristics for each category.

	Adjusted Calcium (mmol/L)		PTH (ng/L)		25(OH)D (ng/ml)		eGFR (ml/min)	
	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)
Normal	2063	2.40 (2.39, 2.41)	2063	39.0 (38.4, 39.7)	2058	19.2 (18.9, 19.6)	2060	56.7 (56.1, 57.2)
Primary Hyperparathyroidism	86	2.79*** (2.78, 2.80)	86	58.8*** (52.6, 65.8)	85	17.9 (16.1, 19.9)	85	50.0*** (47.8, 52.2)
Secondary Hyperparathyroidism	69	2.16*** (2.09, 2.23)	69	88.9*** (75.8, 104.3)	69	16.2* (14.1, 18.6)	69	47.6*** (44.1, 51.3)
Normocalcaemic Hypoparathyroidism	57	2.39 (2.36, 2.42)	57	10.1*** (9.2, 11.1)	57	20.7 (18.0, 23.8)	57	53.0 (50.3, 55.9)
Non-PTH Hypercalcaemia	12	2.82*** (2.75, 2.89)	12	18.6*** (16.2, 21.3)	12	19.9 (15.1, 26.2)	12	47.0* (40.8, 54.2)
	<0.001		<0.001		0.015		<0.001	

P-Value[a]				

[a]P-Value from ANOVA testing for an overall difference in means
Post-hoc testing for a difference in mean from the Normal group: * P-Value <0.050, ** P-Value < 0.010, *** P-Value < 0.001

Metabolic bone parameters

BMD

At baseline, no significant differences were found between the different groups compared to normal subjects at the lumbar spine. Post-hoc testing demonstrated a statistically significant difference in the mean hip T-Score between the normal and secondary hyperparathyroidism group (difference = -0.47, 95% CI: -0.83 to -0.11, p=0.004). The lumbar spine and hip BMD change over time (rate of bone loss per year from baseline) did not show any statistically significant differences between abnormal calcaemia categories compared to the normal group. Baseline total hip BMD was inversely related to serum PTH (R= -0.006, p<0.001) and age (R= -0.058, p<0.001), and positively associated with BMI (R= 0.105, p<0.001). The change in total hip BMD was inversely related to serum calcium (R= -27.567, p<0.001) and age (R= -0.425, p=0.029) (Table 14).

Bone turnover markers (table 13)

CTX

We found a statistically significant difference between the normal group and the PHPT (difference = 24.0%, 95% CI: 1.5 to 51.3, p=0.025), secondary hyperparathyroidism (difference = 28.1%, 95% CI: 2.8 to 59.5, p=0.02) and non-PTH hypercalcaemia (difference = -54.6%, 95% CI: -73.2 to -23.3, p=0.001) groups.

Osteocalcin

We found a statistically significant difference between the normal and secondary hyperparathyroidism groups (difference = 35.6%, 95% CI: 18.0 to 55.9, p<0.001).

Bone ALP

We found a statistically significant difference between the normal and non-PTH hypercalcaemia groups (difference = -27.2%, 95% CI: -45.80 to -2.3, p=0.023).

PINP

There were no statistically significant differences when we compared the normal group to other categories.

Table 13. Baseline bone turnover markers for each category.

	PINP (ng/mL)		Bone ALP (ng/mL)		CTX (ng/mL)		OC (ng/mL)	
	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)	N	Geometric Mean (95% CI)
Normal	2013	36.7 (35.7, 37.6)	2061	14.0 (13.7, 14.2)	2046	0.29 (0.28, 0.30)	2032	22.1 (21.7, 22.5)
Primary Hyperparathyroidism	85	41.0 (35.9, 46.9)	86	14.8 (13.4, 16.3)	86	0.35* (0.30, 0.41)	85	24.2 (21.1, 27.7)
Secondary Hyperparathyroidism	69	39.8 (33.9, 46.7)	69	14.7 (12.8, 17.0)	67	0.36* (0.29, 0.45)	63	30.0*** (25.4, 35.5)
Normocalcaemic Hypoparathyroidism	55	31.0 (26.6, 36.3)	57	11.8** (10.5, 13.3)	50	0.10*** (0.07, 0.13)	57	14.0*** (12.3, 16.0)
Non-PTH Hypercalcaemia	11	30.6 (20.7, 45.1)	12	10.2* (6.1, 16.8)	12	0.13*** (0.07, 0.24)	12	16.3 (13.3, 20.1)
P-Value[a]	0.048		<0.001		<0.001		<0.001	

Table 14a. Multiple regression analysis – do baseline measurements predict baseline BMD? Hip T-Score at Baseline = Intercept + Calcium + PTH + Age + BMI

	Coefficient	95% CI	P-Value
Adjusted Calcium	0.277	(0.017, 0.537)	0.037
PTH	-0.006	(-0.007, -0.004)	<0.001
Age	-0.058	(-0.063, -0.052)	<0.001
BMI	0.105	(0.096, 0.114)	<0.001

Values are per unit increase

Table 14b. Multiple regression analysis – do follow-up measurements predict change in BMD?
Change in Hip BMD = Intercept + Baseline BMD + Calcium + PTH + Age + BMI

	Coefficient	95% CI	P-Value
Baseline Hip BMD	-0.095	(-0.116, -0.075)	<0.001
Adjusted Calcium	-27.567	(-43.318, -11.815)	0.001
PTH	-0.081	(-0.195, 0.033)	0.164
Age	-0.425	(-0.806, -0.044)	0.029
BMI	0.455	(-0.140, 1.049)	0.134

Stability of PTH over time

In the overall population there was a statistically significant change in PTH measurement from baseline. Mean PTH measurement increased from 41.6 ng/L (SD = 21.1) at baseline to 45.5 ng/L (SD = 26.1) at six years (mean change = 3.9 ng/L, 95% CI: 2.79 to 4.98, p<0.001).

5.4Discussion

This is the first study to investigate the prevalence of NHYPO in a large cohort of postmenopausal women. Cusano et *al* previously conducted a large trial to evaluate the epidemiology of these subclinical conditions in an unselected community-based sample of old men (The Osteoporotic Fractures in Men study, MrOS study), and in young men and premenopausal women (Dallas Heart Study, DHS), revealing a prevalence respectively of 0.4% and 3.1% for NPHPT and of 1.1% and 1.9% respectively for NHYPO [291]. At baseline we identified 57 subjects (2.4%) affected by NHYPO and just 1 subject (0.1%) affected by NPHPT after ruling out the main causes of secondary hyperparathyroidism (vitamin D deficiency, eGFR < 60 ml/min;only 6 NHYPO subjectswere taking proton pump inhibitors). In our cohort

the prevalence of NHYPO is higher in comparison with the previous studies; this is probably due to differences in gender and age between the three populations.

Even if the baseline cross-sectional data indicated the existence of NHYPO as a new subclinical pathological category, the longitudinal data gave rise to many doubts. Indeed at the 6 year visit, of the 35 NHYPO subjects with follow-up data, none of them developed overt hypoparathyroidism and only 15 (0.6%) had persistent evidence of NHYPO. This finding is in keeping with that reported by others; Cusano and colleagues identified 68 subjects with NHYPO, none of whom developed overt hypoparathyroidism on follow-up and persistent disease noted in only 2 of the 26 subjects with follow-up data [291].

A few studies have shown that bone turnover marker levels are frankly low or low-normal in patients with hypoparathyroidism compared to normal subjects [230,354,355] and these findings are consistent with histomorphometric analysis. In particular, double-tetracycline labelling of bone biopsy specimens have demonstrated that dynamic skeletal indices are suppressed in hypoparathyroid patients [318]. According to these data, NHYPO seems to be characterised by a "low bone turnover" without a significant BMD change over time compared to normal individuals. In agreement, we found a significant reduction in serum levels of CTX, BAP and osteocalcin compared to subjects with no impairment of calcium metabolism.

Once again these findings confirm the key role of PTH in bone metabolism: a few studies have demonstrated that daily subcutaneous injections of PTH (1-84) result in a significant increase of bone formation and resorption markers (that were suppressed at baseline) in patients affected by hypoparathyroidism [356].

Our data on NHYPO raise questions on the appropriate management of these patients. Based on our findings a 'watch and wait' philosophy may be suitable but further data are needed before confirm recommendations can be made. If patients are taking a medication that might induce hypocalcaemia, such as an anti-resorptive drug (e.g. bisphosphonate, denosumab) or a loop diuretic (e.g. furosemide) then monitoring of serum calcium is recommended. This would include: annual

biochemical assessment of PTH, calcium and phosphate levels with kidney function; medical assessment; BMD evaluation every 18-24 months. The potential choice of treatment for subjects with NHYPO affected by osteoporosis is critical. Indeed powerful anti-resorptive drugs such as bisphosphonates and denosumab, could exacerbate the risk of adynamic bone disease by suppressing bone turnover [357]. Conversely, anabolic therapy could restore physiological bone turnover [358] but we are not yet able to predict if patients with NHYPO will experience an improvement in BMD. Indeed, in contrast to the effect of PTH (1–84) treatment in patients with osteoporosis, PTH (1-84) replacement therapy causes a general decrease in BMD at the hip, lumbar spine and whole body (apart from the forearm) in subjects with hypoparathyroidism[356]. Furthermore, despite significant changes in bone turnover, teriparatide did not provide any significant BMD improvement in hypoparathyroidism [359]. In particular, after 3 years of twice daily PTH (1–34) treatment, BMD and bone mineral content (BMC) at the lumbar spine, femoral neck and whole body remained stable although there was a non-significant downward trend in BMD at the distal radius.

It was noteworthy that the prevalence of PHPT was high in our study. The biochemical abnormality of high serum calcium and high or high-normal PTH is also found in familial hypocalciuric hypercalcaemia but we did not conduct any calcium excretion studies or gene testing to rule this out. Most of our cases were mild and the mean PTH was only at the upper limit of the reference interval for PTH; this might explain why most bone turnover markers were normal. The diagnosis of hypercalcaemia was made on samples after the second visit so this did not influence clinical management during the follow-up period.

It was also surprising that our rate of NPHPT was lower than in the MrOS study as many epidemiological studies have confirmed that hyperparathyroidism is less frequent in men [138,179]. Other large retrospective studies have attempted to evaluate the epidemiology of NPHPT but most did not rule out the main causes of secondary hyperparathyroidism [140]. The low prevalence of NPHPT in our population is most

likely due to the high rate of vitamin D insufficiency (52.3%, data not shown) and eGFR reduction (62.4 %, data not shown); the former due to geographical differences and the latter due to the higher mean age in comparison to Cusano's populations. The current definition of NPHPT probably tends to underestimate the prevalence of this category. Indeed, in agreement with Shibli-Rahhal *et al* [360], in our population only 10.7% (data not shown) of older women with 25OHD < 50 nmol/L had an elevated PTH.

Circulating PTH concentrations display a large inter-individual variability due to factors such as calcium intake and drugs such as lithium, $MgSO_4$ and diuretics which affect PTH levels. Moreover this variance has been shown to have a strong heritable component, although the genetic factors governing serum PTH concentrations remain unknown. Indeed, although it is well documented that loss of function and gain of function mutations of the CaSR lead respectively to hypercalcaemic and hypocalcaemic disorders [361], no studies have assessed the status of the CaSR in NPHPT and hypoparathyroidism subjects. Another interesting area of research could be the evaluation of autoantibodies directed against the extracellular domain of the CaSR and NALP5 in subjects with NHYPO. Previous studies have identified the CaSR and NALP5 as parathyroid autoantibody targets in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy and autoimmune hypocalciuric hypercalcaemia [362]. However, it is unclear whether these antibodies also contribute to the variability in serum PTH concentrations observed in normocalcaemic individuals.

Our study has a few limitations. We didn't measure ionised calcium and fasting phosphate, we didn't collect any biochemical data between the baseline visit and the second visit at 6 years, we only had a single value for each laboratory analyte at each time point, we lost a significant number of subjects over the course of the study and BMD analysis was performed by two different instruments. Moreover the prevalence of the different diagnostic categories might have been affected by long-term PTH storage. However, we do not consider it likely that the relatively high prevalence of

NHYPO could be attributable to degradation of PTH during storage. Indeed it has been estimated by some authors that there is about a 1% increase in intact PTH every year in women, with a faster rise after the menopause [363,364]; therefore the 9% increase we observed is likely to be age-related rather than resulting from minor degradation. Furthermore, if the low prevalence of SHPT and relatively high prevalence of NHYPO had been attributable to degradation of PTH during storage, we would have also expected a low prevalence of hyperparathyroid hypercalcaemia (PHPT and FHH). Instead we noted a high prevalence of hyperparathyroid hypercalcaemia.

In comparison to Cusano's findings, the prevalence of SHPT was quite low. This could be explained by the higher dietary calcium intake found in European countries compared to the US population. In one review, the mean calcium intake in the NHANES III study in older women was 600 mg/day, considerably lower than the estimates for the UK (800 mg), France (850 mg) and Germany (970 mg) [365].

This is the first large population based study to investigate the prevalence and metabolic bone status of NHYPO in postmenopausal women. It is fairly common, not always persistent and is characterised by low bone turnover.

CHAPTER 6: SPECIFIC BACKGROUND. TREATMENT OF HYPOPARATHYROIDISM

6.1 Calcium and vitamin D

The approach to treatment is based on the acuity and severity of the hypocalcemia. The goal is to render patients asymptomatic and for them to reach and maintain a serum calcium concentration in the low-normal reference range (8.0–8.5 mg/dL or 2.0–2.12 mmol/L). Another goal is to maintain 24-hour urinary calcium excretion at <250–300 mg or 10 mmol per day. It is important to maintain the calcium–phosphate product at less than 55 mg^2 /dL^2 or 4.4 $mmol^2$ /L^2. Serum phosphate should be kept at <1.93 mmol/L with a low phosphate diet and a phosphate binder if necessary [255]. Acutely symptomatic hypocalcemia requires intravenous administration of calcium, with intravenous calcium gluconate preferred. Calcium chloride should be avoided because it can be an intravascular irritant [366]. If the solution extravasates in the soft tissue, a painful sclerosing condition can result at the site. Intravenous calcium will transiently increase the serum calcium but continuous infusion is often necessary until the patient is stabilized and oral calcium and vitamin D supplementation have had their beneficial effects. When acute replacement of calcium intravenously is necessary, monitoring of the ECG is recommended. One gram of calcium gluconate provides 90 mg of elemental calcium and can be given over 10–20 minutes intravenously. Intravenous calcium should never be administered as an acute bolus. Such treatment, even in an overtly symptomatic patient, can lead to serious adverse consequences such as lethal cardiac asystole. After intravenous calcium replacement, a continuous infusion of calcium titrated to serum calcium can follow. A standard

protocol is 15 mg/kg of elemental calcium given intravenously over 4–8 hours. The serum calcium will typically increase by 0.5–0.75 mmol/L (2–3 mg/dL). If a patient has coexisting acidosis it is important to correct serum calcium before correcting the acidosis in order to prevent drastic falls in the ionized serum calcium concentration. This recommendation is based upon the fact that in acidosis, more calcium is in the free and physiological ionized form. If acidosis is corrected before calcium is replaced, ionized calcium will fall further leading to worsening symptoms of hypocalcemia. When hypocalcemia in the patient is due to severe magnesium deficiency, but in whom the parathyroids are intact, intravenous magnesium supplementation is used along with calcium [367]. Magnesium depletion is associated with impaired secretion of, and tissue resistance to PTH.

For the management of chronic hypoparathyroidism, oral calcium supplements and vitamin D are necessary (table 15).

Table 15. Vitamin D metabolites in the management of chronic hypoparathyroidism [368].

Medication	Typical dose	Time to onset of action (days)	Time to offset of action (days)
Calcitriol ($1,25(OH)_2D_3$)	0.25–2.0 μg once or twice daily	1–2	2–3
Alfacalcidiol[b] ($1\alpha(OH)D_3$)	0.5–4 μg once daily	1–2	5–7
Dihydrotachysterol[b]	0.3–1.0 mg once daily	4–7	7–21
Vitamin D_2 (ergocalciferol) or vitamin D_3 (cholecalciferol)[c]	25 000–200 000 IU daily	10–14	14–75

[a]Derived from Shoback (4).
[b]Alfacalcidiol and dihydrotachysterol are rapidly activated in the liver to $1,25(OH)_2D$ and 25(OH) dihydrotachysterol.
[c]These compounds could be used in a setting where active vitamin D metabolites are not available and/or too expensive.

Calcium carbonate and calcium citrate are the most suitable options. Calcium carbonate is approximately 40% elemental calcium, whereas calcium citrate is approximately 21% elemental calcium. Calcium citrate can be given without regard to meals, as absorption is not dependent on gastric pH. Conversely, calcium carbonate is best given with meals [267]. Elemental calcium needs may range from 500 to 1000 mg three to four times a day [367]. Active vitamin D metabolite therapy plays a key role in the management of hypoparathyroidism. Calcitriol (1,25-dihydroxyvitamin D3) enhances intestinal calcium absorption [369]. It also stimulates bone remodeling through the RANKL signaling pathway [369]. The range of calcitriol is

0.25 to 2.0 mcg/day. Calcitriol significantly increases serum calcium concentrations within 3 days [370]. Alfacalcidiol (1α -hydroxyvitamin D3) can also be administered although it is not as potent as calcitriol. Its onset is within 1–2 days and it has a longer half-life than calcitriol with an offset of action seen within 5–7 days [371]. Treatment with alfacalcidiol has been associated with decreases in GFR. These decreases are believed to be associated with the development of nephrocalcinosis [372]. Hypercalciuria has also been observed with calcitriol therapy. The hypercalciuria persists even with severe dietary calcium restriction and is believed to be secondary to bone resorption [35]. Long-term therapy with alfacalcidiol has been evaluated in 17 patients with hypoparathyroidism and prospectively followed over 10 years. Alfacalcidiol was initially given in doses of 2.0 mcg/day in addition to 0.5–1 g/day of calcium glubionate with subsequent dose adjustments based on clinical and laboratory data. Alfacalcidiol increased serum calcium concentrations, but also led to significant increases in urinary calcium excretion. Renal function remained stable. Long-term administration of calcitriol was evaluated in three patients with hypoparathyroidism, six patients with chronic kidney disease, and 10 patients with renal hypophosphatemic rickets [142]. Calcitriol increased serum calcium as well as urinary calcium excretion. It contributed to increased renal calcification as well as ectopic calcification. Ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) may also be used in addition to the active vitamin D metabolites. Their longer half-lives can contribute to improved control of serum calcium and are a valuable addition to therapy particularly if vitamin D concentrations are low and PTH is measurable. As PTH increases the conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, higher levels of PTH will be associated with a greater response to vitamin D2 or D3 therapy. Current management of hypoparathyroidism with calcium and vitamin D metabolite therapy remains suboptimal. The majority of patients continue to experience fluctuations in serum calcium and this impacts negatively on quality of life.

6.2 PTH replacement therapy

6.2.1PTH (1-34)

The first study to examine the efficacy of PTH replacement therapy in hypoparathyroidism enrolled 10 adults (40% female) who received PTH(1–34) or calcitriol in a 20-week randomized cross-over design [321]. The etiology of hypoparathyroidism was postsurgical in four patients, familial in three, polyglandular failure in two, and idiopathic in one. The age range in years was 18–70 and the duration of hypoparathyroidism was between 2 and 35 years. The study was divided into a 2-week inpatient dose adjustment phase and an 8-week outpatient maintenance phase. The dietary calcium intake was 1–2 grams supplemented by 1000 mg of calcium carbonate per day. Calcitriol was given twice daily and PTH once daily by subcutaneous injection. These doses were adjusted initially to achieve low-normal serum calcium concentrations (8.2 to 8.8 mg/dL) and then again to maintain a urine calcium to creatinine ratio of less than 0.25 g/day but not allowing serum calcium to fall below 7.6 mg/dL. The final dose of PTH(1–34) varied from 0.5 to 3 μ g/kg/day. Interestingly, during the first 10 days, the dose of PTH required to maintain normal calcium values increased dramatically but then decreased gradually over the next 9 weeks. Both calcitriol and PTH(1–34) treatment maintained serum calcium concentrations in the normal range while urinary calcium excretion was lower in the PTH(1–34) treated group. Diminishing effects of PTH were seen after about 12 hours with serum calcium falling below normal in some patients. Based on the above observation of the diminishing effect of PTH(1–34) over the course of the day, the same investigators conducted a study that compared once daily versus twice-daily PTH(1–34) [322]. The study enrolled 17 adults with hypoparathyroidism (76% female) who had not participated in the prior study. The etiology was postsurgical in nine patients, a mutation in the calcium sensing receptor (CaSR) in five, and familial, idiopathic, and autoimmune polyglandular syndrome type 1 (APS1) in one patient each. The design of the study was a randomized cross over trial, with each study period lasting 14 weeks. The PTH(1–34) dose was adjusted to maintain both serum

and urine calcium values as close to normal as possible. Calcitriol was stopped and dietary calcium ranged between 1 and 2 g (or was supplemented to 1 g if not obtained through diet). Twice-daily administration produced less variability in the serum calcium concentration with a lower daily dose of PTH (mean of 46 vs. 97 μ g/day). The benefit of a twice-daily dose was particularly pronounced in the five subjects who had hypoparathyroidism due to CaSR mutation. In these subjects serum calcium values remained close to the normal range with twice-daily injections and were clearly below normal with once daily regimen. Furthermore, twice daily administration was associated with higher mean serum calcium concentrations without increasing urinary calcium excretion, suggesting that PTH therapy is particularly useful in patients with the CaSR mutation. Several other reports have documented the beneficial effect of PTH(1–34) in patients with gain-of-function mutation in the CaSR [373]. One of these reports [374] describes a 20-year-old women who was treated with PTH(1–34) continuously from age 6. This child grew normally but continued to have hypercalciuria and hypermagnesuria despite having maintained low-normal or subnormal serum levels of calcium and magnesium. By the age of 19 she had developed nephrocalcinosis but had no impairment of renal function.

Efficacy and safety of PTH therapy in children with hypoparathyroidism was also investigated by Winer et al. [375,376]. In a randomized cross over trial lasting 28 weeks, once-daily and twice-daily administration of PTH(1–34) was compared in 14 children age 4–17 with the duration of hypoparathyroidism of 1 to 11 years (719). The causes of hypoparathyroidism were idiopathic in seven children, APS1 in five, and CaSR mutation and postsurgical in one each. Similar to the study in adults,6 each 14-week study arm consisted of a 2-week inpatient dose adjustment phase followed by a 12-week outpatient phase. A twice-daily regimen increased serum calcium and magnesium more effectively than once-daily dosing and this was particularly evident during the second half of the day. Both dosing regimens normalized urinary calcium excretion, but the total daily dose of PTH(1–84) given in the twice-daily dosing regimen amounted to less than one-half of that required for the once-daily

administration (mean of 25 vs. 58 μ g/day). Dietary calcium intake ranged between 800 and 1500 mg and most children required magnesium supplementation, although the levels of the latter were higher in the twice-daily regimen, particularly during the second half of the day. Long-term effects of PTH(1–34) administration to children was examined in comparison to conventional therapy with calcium and calcitriol in a 3-year study (720). In an open label parallel trial design, 12 children (eight males) ages 5–14 years, who had previously participated in the study described above (719), received twice-daily PTH(1–34) or calcitriol as well as 1200 mg of calcium and 800 IU of cholecalciferol per day. The doses of calcitriol and PTH were adjusted to maintain serum and urine calcium in or close to the normal range. All but two patients received magnesium supplementation. Mean pre-dose serum calcium levels were at or just below the normal range and urine calcium was in the normal range throughout the study with no significant difference between the groups. Height and weight percentiles also did not differ between the groups and remained normal for the duration of the study. Long-term safety and efficacy of PTH replacement therapy has also been investigated in 27 adults with hypoparathyroidism [377]. Among them, 21 had previously been exposed to PTH as reported in earlier publications [322]. This 3-year randomized, parallel group, open label trial compared twice-daily PTH(1–34) to calcitriol (conventional therapy). Subjects who had not participated in prior studies of PTH were first admitted to the Clinical Research Center (CRC) for a 2-week dose adjustment period during which the doses of PTH and calcitriol were titrated to maintain serum and urine calcium values as close to the normal range as possible. All subjects were encouraged to ingest 1000 mg of calcium from their diet. Fourteen subjects were randomly assigned to PTH and 13 subjects to calcitriol. Throughout the study, doses of both drugs were further adjusted to maintain a serum calcium concentration within or just below the normal range, but six subjects who had a gain-offunction mutation in the CaSR (four in the PTH and two in the calcitriol arm) had to maintain serum calcium values below normal to avoid marked elevation in urine calcium excretion. Magnesium was supplemented if below 0.7 mmol/L (normal

0.75–1.00) and 17 subjects required this supplementation (12 in the PTH and five in the calcitriol group). All subjects with CaSR mutation required magnesium. Throughout the 3 years of treatment, serum calcium was within or just below the normal range and did not differ between the two groups. Mean 24-hour urinary calcium excretion was within the normal range for the PTH group but remained above normal for the calcitriol group. Creatinine clearance was similar in the two groups and did not change over the course of the study, including in the five patients who had significant renal insufficiency at enrollment (creatinine clearance of 18–40 ml/min). Nephrolithiasis occurred in one subject treated by PTH who had long-standing nephrocalcinosis. Overall, there were no significant differences between groups in adverse events. Fatigue was a common complaint in calcitriol treated subjects and several patients described less fatigue and greater endurance with PTH therapy. However, a 9-minute walk–run test performed in seven patients did not show difference between the groups or between baseline and later values in the PTH-treated group (the data for this were not provided in the paper). However, two of the four patients in the PTH group showed a 50% improvement in selfreported fatigue after 6 months of therapy. Long-term (5-year) treatment with teriparatide was also described in a case report [378], where a 53-year-old woman with severe hypoparathyroidism refractory to conventional treatment was adequately controlled by multipulse subcutaneous administration of teriparatide by a pump. Continuous PTH(1–34) delivery by insulin pump was also compared to twice-daily injections in a 6-month randomized cross-over trial which enrolled eight patients with postsurgical hypoparathyroidism [358]. Pump therapy consisted of a basal rate that was set and adjusted by the investigator with an option of delivering a bolus for laboratory findings or symptoms of hypocalcemia. The pump reservoir held 1.5 cc (300 μ g) of PTH(1–34). Compared to the injections, pump therapy resulted in less pronounced fluctuations in serum calcium concentration >50% reduction in urine calcium, and a 65% reduction in daily PTH dose (13 vs. 37 μ g/day).

There was no difference in well-being as assessed by the fatigue index, 6-minute

walk test and Biodex muscle indices. Nevertheless, at the end of the study, seven out of eight patients preferred the pump to twice-daily injections. Pump administration of PTH has also been successful in controlling hypocalcemia in patients refractory conventional therapy or PTH injections [379,380].

6.2.2 PTH (1-84)

In an investigator-initiated study conducted at Columbia University in New York, Rubin and colleagues enrolled 30 adults (73% female) with hypoparathyroidism that had been present for 3–45 years [381]. The etiology of hyperparathyroidism was surgical in 15, idiopathic in 11, DiGeorge in two, and autoimmune and autosomal dominant hypoparathyroidism (ADH) in one patient each. The patients had to be on stable doses of calcium and calcitriol for 6 months prior to enrollment. All subjects received 100 μ g of PTH(1–84) (NPS Pharmaceuticals, Bedminster NJ, USA) given by a subcutaneous injection every other day [381]. The study was continued for 24 months with blood sampling at baseline and then 48 hours after the last PTH dose at months 1, 2, 3, 4, 5, 6, 9, 12, 18, and 24. Twenty-four-hour urinary calcium excretion was measured at baseline and at 3, 6, 9, and 12 months with urine collections starting 24 hours after the last PTH dose. The design of the study was to reduce the calcium dose by 500 mg until the daily dose reached 1500 mg and then reduce the calcitriol dose to a goal of 0.25 μ g while maintaining serum calcium at the target range (low-normal). During the study, the requirements for calcium fell significantly with the percentage of subjects requiring over 1500 mg calcium per day decreasing from 73% at the beginning to 40% at the end of the study.

Two subjects were able to discontinue calcium supplements altogether. Calcitriol requirements fell as well, with the percentage of subjects requiring more than 0.25 μ g/day decreasing from 83% in the beginning to 50% at the conclusion of the study, and the number of subjects not needing any calcitriol increasing from one to eight. Serum calcium concentrations remained similar to baseline values despite the large reduction in supplement dose. Hypercalcemia was rare (only 4% of all calcium

measurements). Serum phosphate concentrations fell significantly as did the 25-hydroxyvitamin D level, while the concentration of 1,25-dihydroxyvitamin D did not change. Urinary calcium excretion did not show a consistent change, with only some of the time points showing levels that were slightly higher (during the initial time when the calcium doses were being reduced) or lower than the baseline (latter part of the study). It should be noted, however, that calciuria was assessed during the day when PTH was not given. As a result, it is possible that the known effect of PTH to reduce urinary calcium excretion was not detected. Long-term results of PTH(1–84) therapy were investigated in a subset of 27 patients from the above study who were followed for 4 years [382]. PTH treatment reduced the requirements for supplemental calcium and 1,25-dihydroxyvitamin D by 37% and 45%, respectively, and 12 subjects (44%) were able to reduce both calcium and vitamin D supplements by at least 50%. Seven subjects (26%) were able to discontinue all active vitamin D supplements. Some patients required dose modification from the original 100 μ g every other day to 100 μ g daily in five subjects, 100 μ g every third day in two subjects and after a 50 μ g dose became available nine subjects were switched to that dose, generally after month 42. Serum calcium was maintained at or above baseline, while serum phosphate fell as did urinary calcium excretion, at some time points. Hypercalcemia was rare—only 11 episodes occurred in eight subjects over the 4-year span of the study, which is 1.9% of all values. Most of the hypercalcemia episodes occurred in the first 6 months and were resolved with reduction in calcium and/or calcitriol dosing. One subject developed nephrolithiasis despite having normal measured serum calcium and a reduction in urinary calcium excretion while in the study. Cusano and colleagues also examined quality of life (QOL) during PTH(1–84) therapy in 54 hypothyroid subjects (40 female) of a Columbia (New York) cohort,19 some of whom had participated in the prior studies from the same group [382]. The etiology was postsurgical in 27 patients, autoimmune in 26, and DiGeorge syndrome in one patient. In this group of patients, PTH(1–84) reduced calcium and calcitriol supplement requirements by 52% and 51%, respectively. RAND 36-Item Health

Survey [383] was administered at baseline and at 1, 2, 6, and 12 months after starting PTH(1–84). At baseline, subjects scored lower than the normative reference range in all eight domains with a T-score between −1.35 and −0.78. With PTH therapy, the scores improved after 1 month and remained higher throughout the study. The improvement was observed for the overall mental component score, as well as the three mental health domains (vitality, social functioning, and mental health), the overall physical domain score, and two physical health domains (physical functioning and general health). Patients with postsurgical and other etiologies did not differ in QOL changes, and there was no correlation between improved QOL scores and reduction in calcium supplements. There were, however, moderate correlations between the decrease in calcitriol dose and bodily pain and mental health scores. In the 34 subjects who were followed for 2 years the improvement persisted for all domains that were significant at 1 year. The effect of PTH(1–84) was also evaluated in a Danish investigator-initiated study of Sikjaer et al. [356]. This was a 24-week double-blind placebo-controlled study where 100 μ g of PTH or placebo (Preotact™ from Nycomed, Zurich, Switzerland) were given daily to 62 patients with hypoparathyroidism. The patients (85% female) had the disease for 1 to 37 years with 94% having surgical etiology and the remaining being idiopathic. The design was such that PTH was added to conventional therapy with calcium and active vitamin D (one patient on calcitriol and 59 on alfacalcidol, the preferred form of active vitamin D in Europe). The supplement doses were reduced only if subjects developed hypercalcemia (plasma calcium >1.4 mmol/L) or increased 24-hour urinary calcium excretion (>7.5 mmol). In the PTH-treated group the requirements for calcium and active vitamin D decreased by 75% and 73%, respectively, while no change was observed in the placebo group. Among the PTH-treated patients, 15 (47%) were able to discontinue calcium supplements and seven stopped calcitriol as well. Among these, five remained hypercalcemic and required a reduction in PTH dosing frequency from daily to every other day (one subject), every third day (one subject), or five times per week (three subjects). Plasma calcium increased in the PTH-treated

group with 19% of calcium measurements falling above the upper limit of normal. There were 17 episodes of symptomatic hypercalcemia (one requiring hospitalization) in 11 PTH-treated patients compared to one such episode in the placebo group. PTH treatment significantly decreased serum phosphate. Urinary calcium excretion was significantly higher in the PTH group during the first half of the study with no significant differences between the groups after 12 weeks. Among the adverse events, the only one that was significantly higher in the treated patients was nausea and it was believed to be related to hypercalcemia. There was no assessment of wellbeing or QOL but patients were asked at the end of the study whether they believed that they were receiving the active drug or placebo. In the PTH group, 79% (23 of the 29 responders) thought they were receiving the drug while in the placebo group 43% (12 of 28 responders) thought that they were receiving the drug. Sikjaer et al. [247] also reported a 24-hour study of pharmacokinetics and pharmacodynamics in 38 patients who completed the above study. The pharmacokinetics study was conducted at the end of their 6 months' exposure to PTH(1–84) or placebo while maintaining the blind [247]. PTH was injected into the thigh, as it was previously shown for other hormones that this site provides a longer duration of action than injection into the abdomen [384]. Similarly, it was reported in abstract form that in healthy postmenopausal women the duration of the effect of PTH(1–84) was longer after an injection into the thigh than into the abdomen.

In the study of Sikjaer, there were 21 subjects in the active group and 17 in the placebo group. Blood and urine were sampled frequently during the 24-hour inpatient hospital stay. PTH concentration reached the maximum of 26.5 pmol/l after the median time of 15 minutes after the injection. Interestingly, the authors report that many subjects had a biphasic increase in PTH concentration in the plasma with the first peak at 15 minutes, presumably reflecting rapid absorption of the drug directly into the circulation, and the second peak at 120 minutes, which may be the result of lymphatic transport. The median half-life of PTH was 2.2 hours and the levels returned to baseline after approximately 16 hours. Plasma calcium concentration

reached a peak at the median of 7 hours while phosphate concentration reached the nadir at 3 hours. This suggests that the effect of PTH on plasma phosphate is mediated by increased renal phosphate excretion, while the increase in calcium is mediated not just by renal effects but also by increased intestinal absorption through increase in 1,25-dihydroxyvitamin D and possibly by the effects of PTH on bone resorption, which were not reported in this study. Of note is that 71% of patients in the PTH group had a serum calcium level above the upper limit of the normal reference range compared to 12% in the placebo group. Subjects who developed hypercalcemia did not differ from those who did not in supplement dose, body weight, gender, age or maximum PTH concentration. However, the number of measurements with hypercalcemia did correlate with the area under the curve for PTH measurements. Urinary calcium excretion was not different between the two groups for the overall 24-hour value. However, there was a clear difference in the pattern of urinary calcium excretion, with a decrease observed between 2 and 8 hours in the PTH-treated patients but not in placebo group. The opposite trend was noted for urine phosphate with the phosphaturic effect of PTH observed in the first 6–8 hours after its administration. The observation that urine calcium was lowest during the time when serum calcium was highest, i.e., 2–8 hours after PTH injection, suggests that the PTH effect on urinary calcium excretion corresponds to its presence in the circulation. Later on, when circulating PTH concentration in the plasma is falling, there is an increase in urinary calcium excretion because high serum calcium results in an increased filtered load. As a result, the 24-hour urine calcium measurements did not differ between the placebo and PTH-treated patients and failed to reflect the direct renal calcium conserving effect of PTH. This pharmacodynamics study has important implications for eventual clinical use of PTH in hypoparathyroid subjects. It is clear that at its peak (about 7–10 hours after the injection of PTH) serum calcium is significantly higher, by about 1 mg/dL, than at its nadir prior to injection. Consequently, adjusting the dose of the PTH or supplements in response to a calcium measurement in the blood should take into account the time elapsed

between the administration of PTH and time of the blood test. In addition, it is not clear whether transiently elevated calcium levels resulting from PTH administration have the same undesirable consequences as when hypercalcaemia occurs during treatment with high doses of calcium and calcitriol. It may be that PTH-induced hypercalcemia is not as detrimental since the serum phosphate, the calcium–phosphate product, and urinary calcium excretion are all likely to be lower in the presence of PTH. Finally, the observation that the decrease in urinary calcium excretion is only observed for the first 4–6 hours after the administration of PTH(1–84) suggests that a twice-daily administration may result in lower 24-hour urinary calcium excretion as well as smaller excursions in serum calcium levels. An international, multicenter, placebo-controlled phase 3 study assessed safety and efficacy of PTH(1–84) (Natpara, NPS Pharmaceuticals, Bedminster NJ, USA) in patients with hypoparathyroidism over a 24-week period [385]. The study included 134 patients (78% female) with 74% having surgical etiology, 16% idiopathic, and the remainder autoimmune, genetic, or radiation causes. During the initial 2–16-week optimization phase the doses of calcium and active vitamin D were adjusted to achieve consistent serum calcium levels in the normal range. The subjects were then randomized (2:1 ratio) to daily injection of PTH(1–84) at a dose of 50 μg or the placebo equivalent. Over the next 12 weeks (titration phase) the doses of calcium and active vitamin D were reduced while the dose of PTH was uptitrated to 75 and then 100 μ g to maintain serum calcium at or above baseline level. During the remaining 12 weeks of the study (maintenance phase) the PTH dose could not be increased further but could be decreased and the supplement dose could be adjusted to maintain normocalcemia. The primary endpoint was at least 50% reduction in the dose of both calcium and active vitamin D supplements while maintaining normal serum calcium. This composite primary endpoint was achieved by 53% of the PTH-treated patients compared to 2% of the placebo group. Independence from active vitamin D and reduction in oral calcium to ≤ 500 mg/day, a secondary endpoint, was achieved by 41% of patients in the PTH-treated and 2% in the placebo-treated group. At the end

of the study, 52% (47/90) of patients in the rhPTH(1–84) group were on 100 μ g/day, 27% (24/90) were on 75 μ g/ day, and 21% (19/90) were on 50 μ g/d. It is possible that a dose of PTH(1–84) higher than 100 μ g would have resulted in primary and secondary endpoints being achieved in additional patients but the study design did not allow for this option. Serum phosphate was lower in the PTH-treated patients and calcium–phosphate product decreased more in the PTH- than in the placebo-treated patients (from about 40 mg^2 /dl^2 in both groups at baseline to 35 mg^2 /dl^2 and 39 mg^2/dl^2 at the end of the study in PTH and placebo groups respectively). Urinary calcium excretion decreased in both groups but did so through different mechanisms: in the placebo group there was a significant reduction in serum calcium, particularly during the titration phase. In contrast, in the PTH-treated group a modest decrease in urine calcium occurred despite maintenance of or even increase in serum calcium, consistent with the known PTH effect on urinary calcium conservation. The overall incidence of adverse events was similar in the two groups and during the maintenance phase there were proportionally fewer reports of the clinical symptoms of hypocalcemia in the PTH-treated than in placebo-treated subjects. During the 4-week follow-up phase, after PTH treatment ended and the patients returned to baseline calcium and calcitriol doses, there was a significantly higher proportion of hypocalcemia reported by patients who discontinued PTH than in the control group. The above study is important for several reasons. First, it is quite large considering that hypoparathyroidism is a rare disease. The flexible dosing regimen generated data that provide clear evidence that the PTH requirements differ between individuals and this will be important for the eventual development of commercially available preparations and their use in the clinic. In addition, the study employed a precise titration algorithm that can be used as a practical guide to physicians who may not be familiar with the rapidity and magnitude of PTH effects on calcium levels in hypoparathyroid patients. Finally, the 4-week wash out period after stopping the PTH illustrated that hypocalcemia can be quite significant if PTH replacement therapy is interrupted indicating that discontinuation of therapy should be done carefully with

aggressive increase in supplement dosing and frequent monitoring of serum calcium. In a smaller study with the same preparation reported in an abstract form, an even lower dose of PTH (25 μ g per day) was effective in some patients. Among 22 patients on this dose, 18% were able to reduce calcium supplements to ≤ 500 mg/day and calcitriol to ≤ 0.25 mcg per day, and 9% were able to reduce both the calcium and the calcitriol dose by at least 50% while maintaining serum calcium in the target range. Based on the above studies it is likely that if or when PTH is approved for clinical use, flexible dosing options will be important to allow adjustment of PTH dose to each patient's requirements.

6.2.3 Advantages and weak points.

There is now a substantial body of knowledge regarding replacement therapy for hypoparathyroidism using PTH(1–34) and PTH(1–84). Both preparations have been shown to be effective in maintaining serum calcium in the normal range while reducing calcium and/or active vitamin D requirements. Several studies have reported efficacy over 3–4 years (see above) with case reports documenting favorable effects for even longer periods of time [378]. There have been no reports of loss of efficacy over time or development of antibodies to PTH, suggesting that long-term therapy with PTH is feasible. When using PTH for osteoporosis the duration of treatment is limited to 2 years. The reason for the limitation relates in part to the short-term nature of the pivotal clinical trials as well as to studies in rats in which osteosarcoma is regularly seen [386]. However, the rat model is unlikely to be related to the human skeleton for several reasons. Rats were treated with much higher doses and for a much longer period of time than would be the case in human subjects (2 years of a rat's life is equivalent to 75 years of human life). Even in rats, a safe, non-oncogenic dose of PTH has been identified [387]. Furthermore, rats respond to PTH more exuberantly than humans and non-human primates, the latter of which do not develop osteosarcoma after exposure to high doses of PTH(1–34) or PTH(1–84) [388,389]. In addition, there has been no osteosarcoma reported in clinical trials with PTH(1–34)

or (1–84), or in observational studies totaling more than 16,000 patients treated for up to 3 years [390]. The three reports of osteosarcoma that have been reported among over 1.5 million subjects treated with teriparatide [391] are below what one might expect on a coincidental basis. There have been no reports of osteosarcoma in human subjects treated with PTH(1–84), constituting well over 57,000 patient-years of exposure to PTH(1–84).

One of the potential advantages of PTH replacement therapy, compared to high doses of calcium and active vitamin D, is the promise of decreased urinary calcium excretion resulting from the known hypocalciuric effect of PTH. This effect has been observed in some but not all studies. The pharmacokinetic study of Sikjaer [392] may provide some explanation for the discrepancy among the studies. Decreased urinary calcium excretion seems to occur only when PTH is present in the circulation, while the calcium-maintaining effect of PTH lasts longer, resulting in a higher calcium filtered load and increased urinary calcium excretion [392]. Consistent with this point, a dramatic reduction in urinary calcium excretion was seen in patients receiving PTH(1–34) as a continuous infusion via a pump. In this setting, PTH was present throughout the day exerting its hypocalciuric effects while serum calcium was not allowed to reach high levels, thus preventing a high filtered calcium load. Further studies are needed to design the ideal dosing regimen and mode of administration for PTH in order to achieve better control of hypercalciuria. It should be noted that the treatment goals are quite different when using PTH as anabolic therapy for osteoporosis as compared to PTH replacement therapy for hypoparathyroidism. Anabolic effect of PTH occurs when the bone is exposed to PTH briefly, usually once a day. In contrast, treating hypoparathyroidism requires longer duration of PTH action. Consequently, PTH(1–34), which has shorter duration of action, is well suited for anabolic therapy when given by a daily injection. However, treating hypoparathyroidism with PTH(1–34) requires multiple daily doses or continuous administration by a pump. PTH(1–84) has a longer duration of action and thus can be given as a daily injection even when treating hypoparathyroidism, although the

pharmacokinetic data described above suggest that even this preparation may afford a better control of blood and urine calcium when administered twice a day or by a pump. Since many patients with hypoparathyroidism treated with calcium and active vitamin D report a less than optimal sense of well-being, energy, strength, and cognition [315], improved quality of life has been another important goal of PTH replacement therapy. Anecdotally, participants in clinical trials with PTH report improved well-being and exercise tolerance, and readily extend their participation in long-term trials involving one or multiple daily injections. However, measured improvements in quality of life have been difficult to document in studies, partly because the instruments used to assess these subjective variables are likely not sensitive enough, or because the changes were not examined systematically or vigorously. Recently, however, a clear benefit in SF36 was observed when studied systematically in a sufficiently large number of patients as reported by Cusano et al.[393]. Although this was an open label study with only pre-therapy measurements as the control, the results are consistent with anecdotal observations and suggest that PTH does positively affect well-being. Interestingly, in a study that compared twice-daily injection of PTH(1–34) to pump therapy [358], patients preferred pump therapy, suggesting that the improved well-being may be due to decreased fluctuations in serum calcium and/or PTH levels. Further studies are needed to elucidate the biochemical basis for the reduced quality of life in hypoparathyroidism and its improvement with PTH treatment. Due to differences in study design, a direct comparison between the effects of PTH(1–34) and (1–84) is difficult. Most of the studies with PTH(1–34) employed an initial dose-finding inpatient stay followed by a maintenance period on a fixed dose. In contrast, the studies of PTH(1–84) used fixed doses, with only one study using dose titration as a part of the design [385]. In most studies, PTH(1–34) was given in a twice-daily dosing regimen while 1–84 was given in a once-daily or even every other day regimen, based on the assumption that PTH(1–84) has a longer duration of effect [394]. However, the pharmacokinetic study of Sikjaer suggests that even 1–84 may perform better in a twice-daily regimen. With

such an approach, it is likely that the total daily dose of the drug, the excursions in serum calcium, and the total urinary calcium excretion would be lower. Further studies examining use of PTH(1–84) in a twice-daily regimen or via a pump are needed to answer these questions. Another intriguing possibility is the use of transdermal or other depot preparations of PTH [395,396]. While these approaches are challenging when treating osteoporosis, where PTH effect needs to be transient, they may be much more suitable for PTH replacement therapy for hypoparathyroidism where longer duration of action would be beneficial.

In summary, great advances have been made in treating hypoparathyroidism with PTH. Further refinement in dosing regimens and modes of delivery, as well as tailoring therapy to individual patients' needs, are likely to improve the quality of life and long-term prognosis for the patients with this challenging disease.

CHAPTER 7:

PTH(1-34) FOR THE PRIMARY PREVENTION OF POST-THYROIDECTOMY HYPOCALCEMIA: THE THYPOS TRIAL*

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7.1 Introduction

A recent meta-analysis has estimated that the prevalence of post-thyroidectomy hypocalcemia may range from 19% up to 38% [397]. In particular, transient hypoparathyroidism after neck surgery is relatively common, ranging from 6.9% to 46% [398]. However, its true prevalence is probably underestimated for many reasons: lack of clear definitions of hypocalcemia, variety of laboratory ranges for normocalcemia and reference values, timing of blood sampling in the postoperative period and short or incomplete follow-up [399]. Post-surgical hypocalcemia can lead to a high risk of arrhythmias and symptomatic patients often require extended hospitalizations following thyroid surgery with increased healthcare costs [400]. Predicting the risk of developing hypoparathyroidism is a challenge for the best care offered to patients. In particular, some studies indicate that iPTH levels measured shortly after thyroidectomy have a high predictive value: iPTH levels below the normal range (<10 pg/mL) at 4 and 6 hours after the operation may correctly predict postoperative hypocalcemia [401,402]. Indeed, Grodski *et al*. have concluded that iPTH levels <10 pg/mL, at 4 hours after total thyroidectomy, had a good precision to predict hypocalcemia (serum albumin-adjusted calcium level < 8 mg/dL) 24 hours after surgery, with a positive predictive value of 90%, sensitivity 94% and specificity 100% with overall accuracy 98% [403].

PTH 1-34 has been successfully tested in permanent chronic hypoparathyroidism. In particular, Winer et al have demonstrated that the administration of once-daily PTH(1–34) can restore normocalcemia for 12 hours [404], whereas twice-daily administrations or pump infusion is able to reduce the required total daily dose of

calcium and bone turnover markers together with restoring normocalcemia [358,405]. Recently, it has been clearly demonstrated that replacement therapy using twice-daily 20-mcg subcutaneous injection of PTH(1–34) was able to maintain serum calcium and phosphate levels and to improve quality of life in adult subjects with post-surgical hypoparathyroidism with no serious side effects [406]. A very recent pilot study has also shown that teriparatide therapy, in patients with post-thyroidectomy hypoparathyroidism, can both control symptomatic hypocalcemia and reduced the duration of hospitalization [407].

No data are available on the effect of teriparatide as a primary prevention for post-surgical hypocalcemia. The aim of this Prospective Phase II Randomized Open Label Trial was to evaluate whether teriparatide can prevent post-surgical hypocalcemia in high risk subjects after thyroid surgery. We also investigated the effect of teriparatide on the duration of hospitalization and on the need for calcium and vitamin D supplementation after the discharge.

7.2 Materials and methods

7.2.1 Study design and population

This is a Monocentric Prospective Phase II Randomized Open Label Trial (**T**eriparatide for **HY**pocalcemia in **PO**st-surgical **S**ubjects: Thypos Trial).

Screening. At Surgical ward, at University Campus Bio-Medico (Rome), preoperatively, all patients with formal surgical indication for thyroidectomy (thyroid cancer or Grave's disease or multinodular goiter) were screened for the study. During the screening visit, we have evaluated patients' baseline status and clinical history. Physical examination was performed and height and body weight (body mass index kg/m2), were recorded. At 8.00 AM, in a fasting state, a blood sample was drawn and calcium, phosphate, albumin, magnesium, iPTH, 25 OH vitamin D, kidney and liver function were measured. Exclusion criteria were:

- Age younger than 18 years

- Pregnancy
- Renal failure (glomerular filtration rate < 30 mL/min)
- Hypersensitivity to the active substance or excipients
- Any prior parathyroid pathology
- Preexisting hypercalcemia
- Metabolic bone disease other than osteoporosis
- Ongoing therapy for osteoporosis
- Administration of calcitonin, systemic corticosteroids, estrogens, raloxifene, fluoride, lithium, loop or thiazide diuretics, aromatase inhibitors or other drugs that could interfere with calcium metabolism in the last 12 months
- History of skeletal malignancies (primary or metastatic)
- Active or recent urolithiasis
- Unexplained elevation of serum alkaline phosphatase levels
- Prior radiation therapy involving the skeleton
- Serum magnesium levels below the lower limits or above the upper limits of normal

All the screened patients (seventy-two subjects) underwent total thyroidectomy. All surgical procedures were performed by two experienced endocrine surgeons working at the Neck and Chest Surgery department, University Campus Bio-Medico (Rome). Total thyroidectomy was defined as total bilateral extracapsular thyroidectomy. Operative time was registered and surgical procedures lasted from 70 to 90 minutes. iPTH was measured at 4 hours after the end of the surgical procedure. Every surgical procedure was performed between 8.00 AM and 10.00 AM.

Enrollment. Twenty-six subjects (26/72) experienced iPTH levels at 4 hours after thyroidectomy $\leq$ 10 pg/ml therefore they were enrolled in the present study. Enrolled subjects were randomized (1:1) to receive treatment with teriparatide (treatment group) or following the standard clinical care ("wait and see") [408]. Indeed, up to now there are no clinical guidelines that recommend supplements and/or drugs to prevent post surgical hypocalcemia. A blocked randomization scheme was generated by a

software algorithm ("blockrand" package for R). A nurse was educated to the subcutaneous abdominal administration of 20 mcg of teriparatide using an injection pen (Forsteo® Eli Lilly Nederland B.V.). The first administration was done immediately after the randomization. Subsequent administrations were done every 12 hours until discharge. Therefore, subjects belonging to the treatment group received 4 subcutaneous administrations of teriparatide according to the mean duration of hospitalization after surgery (2 days).

At 8.00 AM on postoperative days 1 and 2, in a fasting state, a blood sample was drawn and calcium, phosphate, albumin, magnesium, were measured. A clinical evaluation and ECG were performed in order to exclude signs or symptoms of hypocalcemia. In the presence of any sign or symptom of hypocalcemia, a new blood sample was drawn in order to confirm the hypocalcemic state. According to the previous studies, we defined Hypocalcemia as a serum calcium concentration <8.0 mg/dL in at least one measurement. The presence and type of symptoms of hypocalcemia was registered by a surgeon or by a nurse, together with the evaluation of Chvostek and Trousseau's signs, twice a day, from the day of surgery to hospital discharge. Hypocalcemic patients received supplementation therapy, even if asymptomatic. Supplementation therapy included 1g every 12 hours of oral calcium (Metocal 1250 mg tablets, Artropharm A.P.S.; 1 tablet contains 500 mg elemental calcium) and 0.25 mcg every 12 hours of 1,25(OH)vitamin D (calcitriol [Rocaltrol] 0.25 mcg tablets; Roche SpA, Milan, Italy). If symptoms persisted following oral therapy or in the presence of electrocardiographic abnormalities or calcium concentration <7 mg/dL, intravenous calcium gluconate was administered. Supplementation therapy was adjusted on the basis of serum calcium measurements. If a patient belonging the treatment group developed hypocalcemia, teriparatide was stopped and calcium and vitamin D supplementation therapy was started.

Discharge. At the discharge, all the subjects were prescribed 2 g elemental calcium per day together with 0.5 mcg per day of 1,25(OH)vitamin D. In order to balance the treatment with calcium and vitamin D after the discharge, measurement of blood

calcium, phosphate and albumin was scheduled every week for 1 month, for each subject. In particular, if serum albumin-adjusted calcium remained stable above the 8.0 mg/dL without any symptoms, calcium supplement was reduced by 1000 mg decrements until the potential withdrawal was reached. If calcium supplementation was stopped, calcitriol was reduced by 0.25 mcg (weekly) decrements until the potential withdrawal was reached maintaining stable serum calcium.

Assays: iPTH was measured by an immunochemiluminometric assay using the automatic analyzer Modular E170 (Roche Diagnostics, Indianapolis, Ind, USA). Normal serum iPTH levels ranged between 10 and 65 pg/ml. Serum calcium was measured by automated techniques. Serum calcium was adjusted for albumin by the following formula: (0.8 [4.0 - patient's albumin] + serum calcium)[352]. Serum phosphate, magnesium and creatinine were also measured by automated techniques. Fasting blood sampling was obtained in the morning (from 8:00 to 8:30 AM).

7.2.2 Sample size calculation and statistical analysis

Previous studies have shown that measurement of iPTH levels < 10 pg/ml at 4 hours after thyroidectomy has a positive predictive value of 90% [310], sensitivity 94% and specificity 100% with an overall accuracy of 98% in predicting hypocalcemia [401]. According to these findings, our hypothesis is that in patients with low PTH levels after thyroidectomy a reduction of <60% incidence of hypocalcemia can be achieved in the group treated with teriparatide (a value considered to be clinically relevant). Therefore the required sample size to achieve 80% statistical power at two side significant p value of 0.05 is of 11 subjects for each group.

The two groups were compared using descriptive statistics, differences were evaluated using the t-test for independent groups or the chi-square test, as appropriate. The variation of serum calcium concentration over time in the two groups was analyzed using ANOVA for repeated measures including a time group interaction. The risk of developing hypocalcemia in the treatment group compared to the control group was estimated by calculating the relative risk with 95% confidence

intervals. Finally, longitudinal changes in serum calcium concentration and dose of calcium and vitamin D supplementation were evaluated using ANOVA for repeated measures.

7.2.3 Ethics

All investigations were conducted in accordance with the Declaration of Helsinki. The study was approved by our local Ethical Committee and all the patients signed an informed consent statement allowing their anonymized information to be used for data analysis. Patient's records were anonymized and de-identified prior to analysis. ISRCTN registry (reference number: ISRC TN74486450).

7.3 Results

Baseline features

Twenty-six subjects were recruited for this study (6 males, 20 females, mean age 53.4, SD 17.0). Baseline patient characteristics for both groups are summarized in Table 16. No differences among the factors known to negatively affect calcium balance after thyroidectomy were found between the two groups (Table 16).

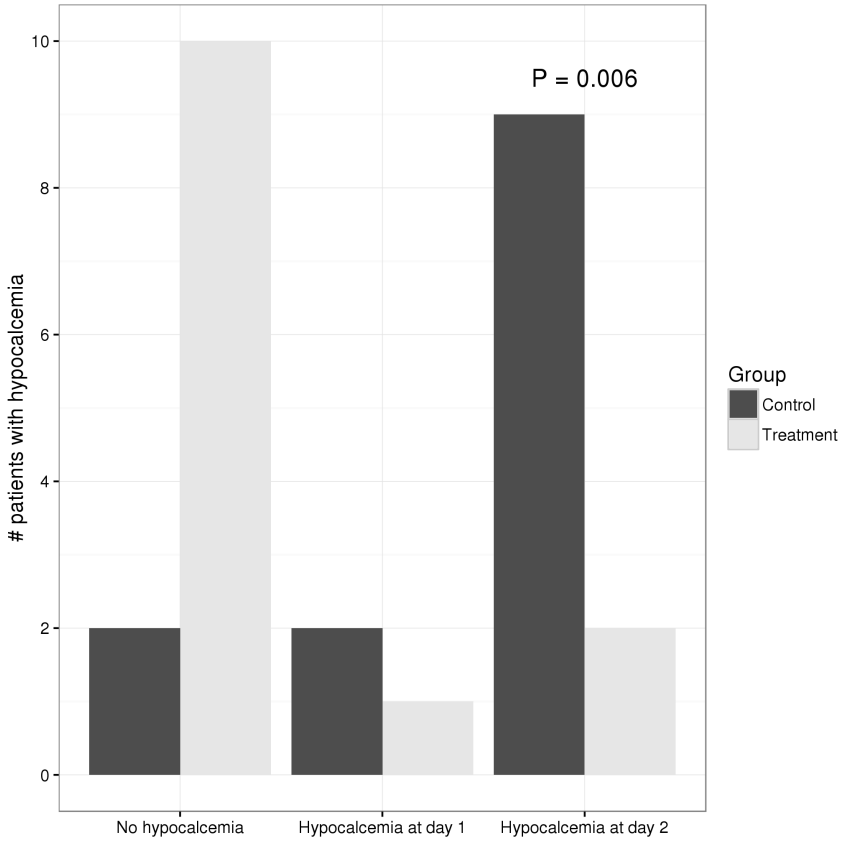
Table 16. Baseline Characteristics of the study population.

	CONTROL GROUP (n=13)	TREATMENT GROUP (n=13)	P-value
M (%)	25	18	1
Age (yrs)	51.1 (19.7)	55.9 (13.8)	0.506
BMI (Kg/m^2)	26.1 (4.1)	25 (4.5)	0.529
Adjusted serum calcium (mg/dl)	9.1 (0.3)	9.1 (0.4)	0.925
Serum Phosphate (mg/dl)	3.4 (0.5)	3.2 (0.7)	0.457
Basal iPTH (pg/ml)	57.9 (20.4)	61.1 (24)	0.74
25 (OH)Vitamin D (ng/ml)	22.9 (7)	24.3 (5.9)	0.596
Serum creatinine (mg/dl)	0.7 (0.2)	0.7 (0.1)	0.953
Magnesium (mg/dl)	1.9 (0.3)	1.9 (0.2)	0.847
PTH at 4 hours (pg/ml)	6 (3.7)	6.5 (3.1)	0.701

Biochemical and clinical evaluation

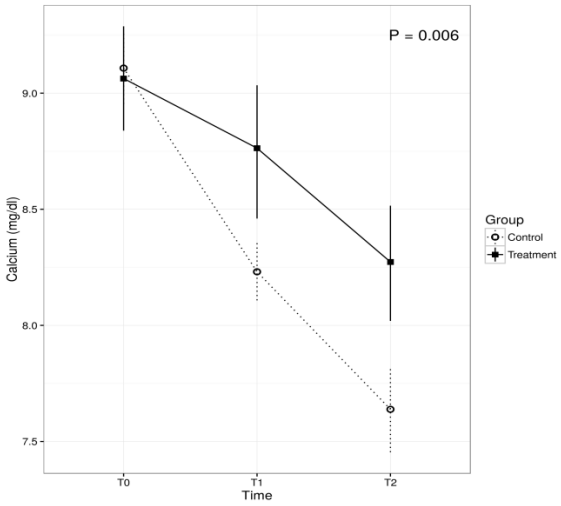
Overall, the incidence of hypocalcemia was 3/13 in treatment group and 11/13 in the control group (P = 0.006) (Figure 16). At day one, we observed one hypocalcemic event in the treatment group and two events in control group. The relative risk for hypocalcemia in the treatment group compared to the control group was 0.26 (95% CI: 0.09 -- 0.723).

Figure 16. Incidence of hypocalcemia during the first and second post-operative day. Overall incidence of hypocalcemia between the groups, P= 0.006



In the treatment group, mean serum albumin-adjusted calcium concentration on post-operative days 1 and 2 were 8.8 mg/dl and 8.3 mg/dl, respectively. The corresponding figures in the control group were 8.2 mg/dl (P = 0.006 vs. treatment group) and 7.6 mg/dl (P=0.001 vs. treatment group) (ANOVA for repeated measures P = 0.006) (Figure 17).

Figura 17. Serum albumin-adjusted calcium trend during the first and second post-operative day. Top solid line is the treatment group. Solid and dotted lines are for mean values. Bars are for SD. ANOVA for repeated measures P= 0.006.



Two out of three hypocalcemic subjects belonging to the treatment group experienced minor signs or symptoms of hypocalcemia such as perioral tingling with positive Chvostek's sign that disappeared after calcium/vitamin D administration.

In the control group, all the subjects with hypocalcemia (11/13) experienced minor symptoms such as perioral tingling with positive Chvostek's sign. In only 3 subjects, Trousseau's sign and carpopedal spams were both positive. Oral calcium and calcitriol administration led to full resolution of symptoms. No subject required intravenous calcium administration.

The median duration of hospitalization was 3 days (IQR: 1) in control subjects and 2 days (IQR: 0) in treated subjects ($P = 0.012$). At hospital discharge, patients treated with teriparatide had a median calcium level of 8.5 mg/dL (SD: 0.5) while control subjects had a median calcium level of 7.8 mg/dL (SD: 0.4) ($P < 0.001$).

Safety data

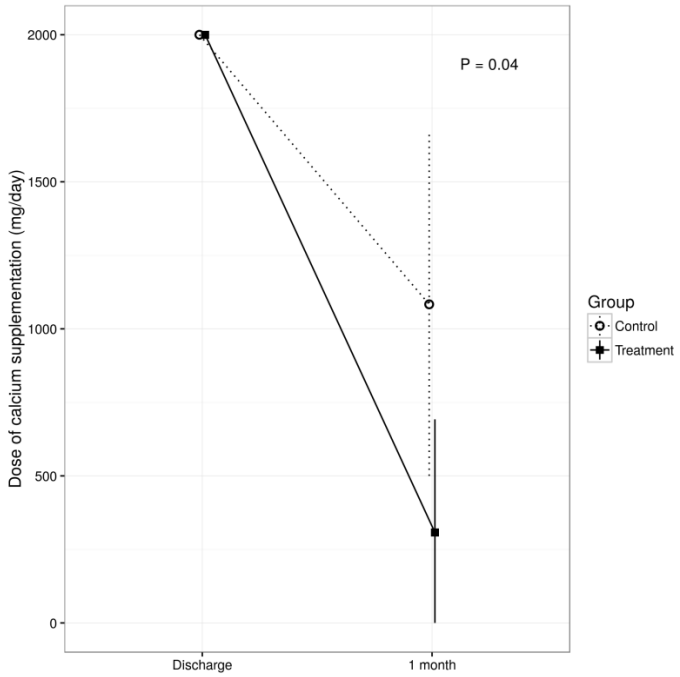
No serious adverse events occurred during the study period. Only 2 subjects experienced nausea (both in treatment group) but the symptom was mild and they did not require any drugs. The 2 subjects belong to treatment group but the symptom was mild and they did not require any drugs to control it.

According to the study protocol, 3 subjects discontinued teriparatide treatment due to hypocalcemia onset. No other adverse events requiring discontinuation of teriparatide treatment were observed.

Calcium and Calcitriol supplementation at 30 days after the discharge.

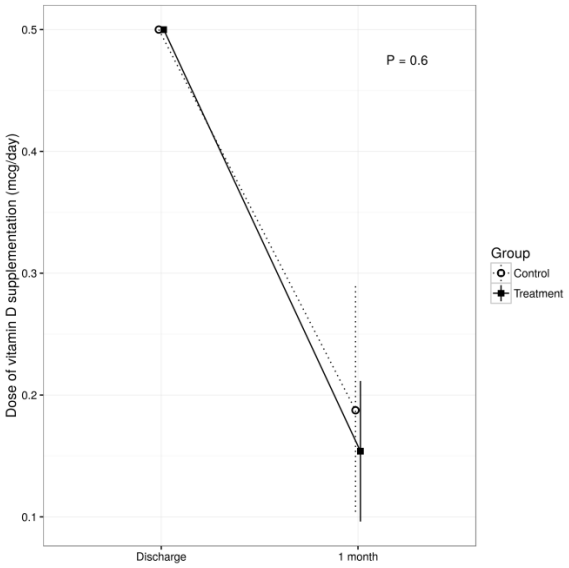
All patients were prescribed calcium (2000 mg/day) and calcitriol (0.5 mcg/day) at discharge. One month after discharge, 10 out of 13 subjects in the treatment group had stopped calcium carbonate supplements (mean dose of calcium supplementation in the whole treatment group = 308 mg/day), while only 5 subjects out of 13 in the control group had discontinued calcium (mean dose of calcium supplementation in the whole control group = 1083 mg/day). The ANOVA for repeated measures showed a significant difference in calcium supplements between groups at one month visit (P=0.04) as well as a significant difference between discharge and one month visit in the treatment group (P for interaction time group= 0.04) (Figure 18).

Figure 18. Calcium supplementation at the discharge and 1 month after discharge. Continuos and dotted lines are for mean values. Bars are for SD. ANOVA for repeated measures showed a significant difference in calcium supplements between groups at one month visit (P= 0.04) as well as a significant difference between discharge and one month visit in the treatment group (P for interaction time group= 0.04).



In the treatment group, 5 out of 13 subjects had discontinued calcitriol therapy and the mean dose of vitamin D supplementation at one month visit was 0.153 mcg/day. In the control group, 5 out of 13 subjects had stopped calcitriol therapy and the mean dose of vitamin D supplementation at one month visit was 0.187 mcg/day (P= 0.472, interaction time group P= 0.603) (Figure 19).

Figure 19. Calcitriol supplementation at the discharge and 1 month after discharge. Continuous and dotted lines are for mean values. Bars are for SD. The ANOVA for repeated measures did not show a significant difference in calcitriol supplements between groups at one month visit. There was not a significant difference between discharge and one month visit in the treatment group (P= 0.472, interaction time group P= 0.603).



In treatment group, the mean serum albumin-adjusted calcium concentration on post-operative days 30 was 8.7±0.4; in control group, the mean serum albumin-adjusted calcium concentration at 1 month after discharge was 8.7±0.3.

7.4 Discussion

We have demonstrated that teriparatide may prevent the onset of post-surgical hypocalcemia in subjects with high risk of hypocalcemia after thyroidectomy.

It has been estimated that hypocalcemia may occur in approximately 15% and 85%

of hospitalized and critically ill patients, respectively [409]. In particular, in postoperative subjects, reduced oral intake, nutritional compromise, and hemodilution due to infusion of intravenous fluids could explain transient hypocalcemia, which is often asymptomatic and mild [410]. Instead, subjects who undergo neck surgery such as thyroidectomy can experience severe hypocalcemia due to the devascularization of parathyroid tissue and/or inadvertent gland removal [408]. It has been well established that the most common form of transient or permanent hypoparathyroidism is post-surgical. Moreover, neck exploration for thyroid cancer, Graves' disease, large multinodular goiter, together with poor surgeon's experience, vitamin D deficiency, age and rate of PTH decline may represent important factors that will negatively affect the calcium and phosphate balance [411].

As shown above, our study population was homogeneous relative to the potential risk factors for hypocalcemia: in fact, at baseline, the above-mentioned parameters did not significantly differ between treatment and control groups. In particular plasma vitamin D was similar in the two groups thus avoiding the risk of a pre- and post-surgical different stimulation of PTH secretion. This hypothesis is confirmed by iPTH level at 4 hours after thyroidectomy, that did not show any significant difference between the two groups, but as far as all the subjects enrolled in our study had blood vitamin D levels lower than normal, the doubt of an altered calcium balance may still arise. As a matter of fact, low vitamin D represents a deficient substrate that, in turn, may reduce the conversion of vitamin D into calcitriol even under the effect of supplemental exogenous PTH. Although a recent paper by Raffaell et al have clearly shown that vitamin D deficiency is not a risk factor for post-surgical hypocalcemia [411] we cannot exclude that the achievement of normal pre-surgical plasma values of vitamin D could have changed the rates of hypocalcemia onset in both groups. On the other hand we must observe that teriparatide-treated patients showed higher values of post-surgical plasma calcium than controls thus suggesting that exogenous PTH may enhance 25(OH)vitamin D conversion into calcitriol even in a condition of vitamin D deficiency. Moreover,

PTH may have increased plasma calcium independently of calcitriol by directly acting on distal tubular reabsorption of calcium and maybe on bone resorption. Before January 2015, the only therapies that had been approved by the US Food and Drug Administration (FDA) for hypoparathyroidism (from any cause) were calcium and magnesium supplements variably associated with an active forms of vitamin D (calcitriol). In the last 15 years, several authors have described the ability of PTH(1-34) [358,404–406] and PTH(1-84) [381,385,393] to restore and maintain normocalcemia together with improving the quality of life [393] in subjects with chronic hypoparathyroidism. These evidence have led to the FDA approval for the use of recombinant PTH (1-84) in hypoparathyroidism. Although there are authors that have investigate the safety and efficacy of PTH treatment for the management of chronic hypoparathyroidism, poor and low quality data are available for the therapy of acute hypocalcemia. Severe hypocalcemia may lead to cardiac arrhythmias and tetany with an increase of morbidity rate and duration of hospitalization. Raffaelli et al have demonstrated that the most important determinant of acute hypocalcemia is a post-surgical iPTH decline higher than 50% with respect to baseline values [411]. Therefore, early PTH administration after neck surgery appears to be an etiological approach to acute post-surgical hypocalcemia. Recently, Shah et al have shown in a small open label trial that teriparatide administration to hypocalcemic hospitalized patients following thyroidectomy is safe and it can rapidly eliminate symptoms linked to low calcium levels; furthermore, this treatment has been associated with reduced duration of hospitalization [407]. Our results allow going further in the treatment of post-surgical hypocalcemia. In fact in our study teriparatide was administered if iPTH went under 10 pg/ml at 4 hours after surgery thus configuring an etiological therapy rather than a rescue treatment for acute hypocalcemia. Teriparatide allowed a good control of hypocalcemia in treated patients, while standard clinical care ("wait and see") was not able to achieve a similar result in the control group. Moreover our patients had been taking teriparatide every 12 hours only for the duration of hospital staying (2-3 days), while patients in Shah's study were on teriparatide for at least 1 week, with

the option of continuing it for up to 3 weeks. While our data do not allow us to draw any definitive conclusion, it may be hypothesized that by rapidly counteracting iPTH post-surgical fall with teriparatide, the stress on parathyroid glands is lower as calcium homeostasis is exogenously maintained. Therefore, a less urgent "functioning request" can give enough time for a more physiological recovery to the sub-ischemic parathyroid gland. Whatever the case, the difference in therapeutic schedules between our study and Shah's study represent and undeniable economic advantage as a shorter period of teriparatide administration leads to a significant reduction in the overall costs and particularly it may positively counterbalance the expenses for teriparatide purchase with a shorter hospital stay.

Another important result, confirming the usefulness of early teriparatide administration after neck surgery, is the lower need of long-term calcium supplements in treated patients than in controls. In fact, 30 days after surgery, only 3 patients out of 13 of those who received teriparatide were taking calcium carbonate, while 8 out of 13 in the control group still needed calcium supplements. Moreover, daily mean calcium carbonate intake was significantly reduced from discharge to 30-days-follow-up-visit in treated patients, while the subjects in the control group were still taking almost the same amount of calcium they were prescribed after discharge. A rapid tapering of calcium carbonate is certainly positive for patients as far as calcium supplements are known to induce gastro-intestinal discomfort and may somehow increase urinary supersaturation with respect to calcium salts that in turn, may induce a higher risk for urolithiasis.

Finally, we were able to confirm that teriparatide treatment is safe and well tolerated. Two patients treated with teriparatide experienced minor symptoms of hypocalcemia such as perioral tingling and Chvostek's sign compared to 11 out of 13 subjects in the control group. Moreover, in control group severe symptoms and signs of hypocalcemia such as Trousseau's sign and carpopedal spams have been recorded.

This study has some important limitations. First of all, it is lacking of placebo controlled group. As far as symptoms, but not signs, of hypocalcemia may be

emphasized by individual perception, the presence of a placebo group could have given a better definition of their entity. Secondly, we enrolled a small group of patients in this study and even though we reached the pre-calculated sample size it cannot be excluded that different results can be obtained from a larger population. Third we did not measure PTH at 30 days after discharge and, therefore, we do not have any data on parathyroid glands functional recovery even though the lower need of calcium supplements in teriparatide-treated patients seems to suggest an endogenous PTH production.

In summary, to our knowledge this is the first study that has evaluated that PTH (1-34) treatment may prevent hypocalcemia in subjects at high risk of post-surgical hypocalcemia. Teriparatide might be associated both with a reduction of hospitalization duration and with a lower need of calcium carbonate supplements after the discharge. Larger and more robust randomized placebo controlled trials are needed in order to confirm our findings.

CHAPTER 8

PTH(1-34) FOR SURGICAL HYPOPARATHYROIDISM: A PROSPECTIVE OPEN LABEL INVESTIGATION ON EFFICACY AND QUALITY OF LIFE*

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8.1 Introduction

Hypoparathyroidism is a rare mineral metabolism disorder characterized by hypocalcemia, hyperphosphatemia and deficient parathyroid hormone (PTH). It can be both congenital or due to the accidental removal or irreversible damage of the parathyroid glands after thyroidectomy. Before January 2015, the only therapies that had been approved by the US Food and Drug Administration (FDA) for hypoparathyroidism (from any cause) were calcium and magnesium supplements variably associated with an active form of vitamin D (calcitriol). However this kind of therapy implies long-term treatment with large quantities of calcium supplements and vitamin D and it may often cause hypercalciuria and sometimes ectopic soft tissue calcification [255]. Moreover some patients with severe chronic hypoparathyroidism are not able to achieve normal serum calcium levels, notwithstanding they are treated with large amounts of calcium and vitamin D supplements. In 2002 synthetic human PTH(1-34) was approved as a treatment for severe osteoporosis [32].

A few years before some authors had shown that PTH(1-38) was able to restore normocalcaemia in two adolescents affected by autoimmune hypoparathyroidism [412]. In 2010 Bilezikian et al published an important study on 30 patients suffering from chronic hypoparathyroidism of various aetiologies treated for two years with PTH(1-84) [381]. They demonstrated that 100 micrograms of PTH(1-84) given every other day by subcutaneous injection for 24 months significantly reduced the demand for calcium and calcitriol as well as it maintained stable serum calcium concentration and reduced urinary excretion of calcium. A few years later, in an uncontrolled study, Cusano et al showed that PTH(1–84) treatment can improve physical and mental

functioning in patients with hypoparathyroidism [393]. Moreover, in a double-blind placebo-controlled randomised phase 3 study, Mannstadt M. et al demonstrated that 50 µg, 75 µg, or 100 µg per day of PTH(1-84), administered subcutaneously is efficacious and well tolerated as replacement therapy for patients with hypoparathyroidism [385]. All this evidence led to approval of PTH (1-84) for the treatment of hypoparathyroidism by the US FDA on January 23, 2015. Other studies also demonstrated that the administration of once-daily PTH(1-34) can restore normocalcaemia for 12 hours [404], while twice daily administration or pump infusion were able to reduce the required total daily dose of calcium, bone turnover markers and finally to restore normocalcaemia [322,358]. As both trials were conducted on small cohorts including subjects with hypoparathyroidism of various aetiology [394], the aim of our study was to investigate the effects of 6 months PTH(1-34) treatment in a homogeneous cohort of adult subjects with post-operative hypoparathyroidism. Furthermore for the first time we tested the hypothesis that PTH(1–34) therapy can improve quality of life (QOL) in these subjects.

8.2 Materials and methods

8.2.1 Study population

From June 2013 to September 2014, forty-two subjects (34 females, 8 males) with documented post-surgical hypoparathyroidism were recruited. Nine Italian centers participated in this study.

The diagnosis of hypoparathyroidism was established by the presence of serum calcium and PTH concentrations below the lower limits of normal on at least two prior occasions separated by an interval of at least 30 days.

To be enrolled in this trial, subjects should have met all the following inclusion criteria:

- Hypoparathyroidism should have been present for at least 1 year so as to establish a chronic state of PTH deprivation;

- Each subject should have taken ≥ 2 g of elemental calcium (as calcium carbonate) together with ≥ 0.5 mcg of calcitriol every day or they should have been intolerant to any or high dosage of calcium carbonate;
- All patients had to be on stable regimens of supplemental calcium carbonate and vitamin D for at least 3 months prior to enrolment.

Exclusion criteria.

Patients were excluded if:

- They suffered from diabetes mellitus, severe chronic liver or renal (GFR <30 mL/min) diseases, Cushing's syndrome, sarcoidosis, multiple myeloma, Paget's disease or rheumatic diseases such as systemic lupus erythematosus and rheumatoid arthritis;
- They had been on a bisphosphonate therapy within 2 years prior to study entry;
- They had taken in the last 6 months calcitonin, systemic corticosteroids, estrogens, raloxifene, fluoride, lithium, loop or thiazide diuretics, aromatase inhibitors or other drugs that could interfere with calcium metabolism;
- They had been taken PTH(1-34) or PTH(1-84) in the past;
- They had serum magnesium levels below the lower limits or above the upper limits of normal on at least two prior occasions.

Study design

Outpatient admissions occurred at 0, 3, and 6 months. At the first admission, the baseline status while receiving calcium carbonate plus calcitriol was evaluated. Data about dietary calcium intake were acquired and height and body weight were measured. Body mass index (BMI) was also calculated. Dietary calcium intake was ascertained by the use of a questionnaire [413] and it was approximately 800 mg/day elemental calcium during outpatient admissions. Subjects were instructed to self-administer a subcutaneous twice-daily 20 mcg injection of PTH(1-34) (Teriparatide, Forsteo, Eli Lilly) in the abdomen at 08.00 and 20.00 h and to rotate sites after each

injection. At each visit, blood sampling and 24-h urinary collection were obtained. The average value of the two pre-treatment serum calcium determinations was used as baseline calcium value. In addition to the three time points, serum calcium was measured 15 days after commencement of PTH(1–34) treatment in order to evaluate the opportunity to decrease the calcium/calcitriol supplementation. In particular, if serum calcium was stable or above the pre-treatment level, supplemental calcium was reduced by 500 mg decrements until a goal of 1000 mg calcium supplementation was reached. After the calcium supplementation had been reduced to 1000 mg daily, calcitriol was reduced by 0.25 mcg decrements until the goal of stable serum calcium was reached. After the calcium and vitamin D supplementation had been reduced to 1000 mg and 0.25 mcg daily respectively, in those subjects with serum calcium levels still $\geq$ 9 mg/dl, we continued to reduce the supplementation until the potential withdrawal. Serum calcium was measured 4 days after each supplementation therapy change to ensure stability of the serum calcium concentration.

As this one is a real-world trial, we didn't establish an exact target value for serum calcium. The main aim was to bring back the adjusted serum calcium inside its normal range avoiding hypercalcemia.

Assays

Serum calcium was measured by automated techniques, with a normal range of 8.4–10.2 mg/dl. Serum calcium was adjusted for albumin by the following formula: [0.8*(4.0 – patient's albumin) + serum calcium]. Serum phosphate, magnesium, creatinine, alkaline phosphatase, uric acid were also measured by automated techniques. Blood sampling was performed 12 h after the last PTH(1-34) injection and immediately before the next injection, in fasting state, in the morning (from 8.00 to 08.30 am).

Urinary calcium was measured by a colorimetric method and the 24-h urinary calcium collection was begun 10-12 h after the last PTH injection.

QOL

Following previous published studies [414], we used the RAND 36-Item Short Form (SF-36) Health Survey (version 1.0) to evaluate the quality of life in subjects with surgical hypoparathyroidism before and after 6 months of treatment with PTH(1-34). It taps 36 items covering 8 domains of physical and mental health: physical functioning (PF), role limitations caused by physical health problems (RF), bodily pain (BP), perception of general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional health problems (RE) and mental health (MH).

Each item was scored on a 0 to 100 range so that the lowest and highest possible scores were 0 and 100, respectively; higher scores indicated more favourable physical functioning and psychological well-being. Following to previous studies [414], we also grouped the 8 domains into 2 summary measures: the physical component summary (PCS) comprised of PF, RF, BP and GH; the mental component summary (MCS) comprised of VT, SF, RE and MH.

Ethics

All investigations were conducted in accordance with both the Declaration of Helsinki and the Italian Drug Agency (AIFA) regulation for the use of PTH(1-34) for the treatment of severe chronic hypoparathyroidism resistant to the calcium and/or vitamin D supplements. According to the AIFA instructions, all the patients were required to sign an informed consent statement and to follow the prearranged time-points follow up with physical and biochemical investigations (see the supplementary materials). All the patients signed an informed consent statement allowing their anonymized information to be used for data analysis. Patient records were anonymized and de-identified prior to analysis.

8.2.2 Statistical analysis

Values are expressed as mean ± standard deviation (SD) for continuous variables and as proportions for categorical variables. Normal distribution was tested with the

Shapiro-Wilk normality test. Paired *t*-test and Wilcoxon signed-rank test were used to analyze changes between two time points for parametric and non-parametric continuous variables, respectively. Multilevel mixed-effects linear regression models for repeated measures were used to evaluate linear trends for measures with more than 2 observations over the time. Briefly, repeated observations were nested within individuals (random effect) and time was entered as an independent variable (fixed effect). Similarly, multilevel mixed-effects logistic regression was used to evaluate changes in proportions over the time. A value of $p<0.05$ was considered significant at 80% power level. All statistical analyses were performed using *Stata/IC 12.1* software for Mac (StataCorp, College Station, TX, USA).

8.3 Results

Baseline features

Baseline features of the 42 subjects with surgical hypoparathyroidism enrolled in the study (90.5% female) are shown in table 17. According to the inclusion criteria, the severity of hypoparathyroidism resulted in 100% subjects showing serum calcium levels below the lower normal value at baseline, despite the ongoing replacement therapy with calcium carbonate and calcitriol (mean±SD [range] daily supplementation before teriparatide treatment: 4.1±1.7g [1.5-8] and 0.8±0.2mcg [0.5-1.25], respectively).

Table 17. Baseline Characteristics of the Study Population

	Mean	SD	Median	Range
Age, y	55.8	10.4	54	34–77
Duration of hypoparathyroidism, y	7.3	5.1	6.5	1–20
BMI, kg/m^2	28.8	6.7	28.5	17–43
Serum calcium, mg/dL	7.6	0.6	7.8	6.1–8.5
Serum phosphate, mg/dL	4.3	1.1	4.0	1.4–6.7
25-OH vitamin D, ng/mL	31.4	15.6	28.5	7.6–70
Uric acid, mg/dL	4.3	1.4	4.0	2–7.5
Serum creatinine, mg/dL	0.8	0.2	0.8	0.5–1.4
Alkaline phosphatase, U/L	75.9	38.5	64.5	0.81–191
Urine calcium, mg per 24 h	219.6	146.4	205.0	37–696
Calcitriol supplementation, μg/d	0.8	0.3	0.75	0.5–1.25
Calcium supplementation, g/d	4.0	1.7	4.0	2–8

Biochemical evaluation and calcium and vitamin D supplementation over the time

The percentage of subjects with hypocalcaemia decreased to 26.2%, 38.1% and 42.9% after 15 days, 3 and 6 months of teriparatide treatment, respectively ($p<0.001$ vs baseline at all time points). Accordingly, mean serum calcium levels significantly increased from baseline to 15 days (7.6 ± 0.6 vs 9.1 ± 0.9 mg/dl, $p<0.001$) and then remained stable until the end of the observational period (8.9 ± 1.1 mg/dl at three months; $p<0.001$ vs baseline, $p=0.227$ vs 15 days; 8.8 ± 0.7 mg/dl at six months; $p<0.001$ vs baseline, $p=0.940$ vs 3 months) (figure 20), despite a significant reduction in calcium (4.0 ± 1.1 g/day at baseline vs 1.7 g/day at six months, $p<0.001$) and calcitriol supplementation (0.8 ± 0.3 mcg/day at baseline vs 0.2 ± 0.2 mcg/day at six months, $p<0.001$) (figure 21).

Figure 20. Calcium (A), phosphate (B) and calcium * phosphate product (C) levels before (T0), 15 days, 3 and 6 months after teriparatide treatment . Triangles and dotted lines are for mean values. * $p<0.001$ vs T0; # $p<0.05$ vs T0

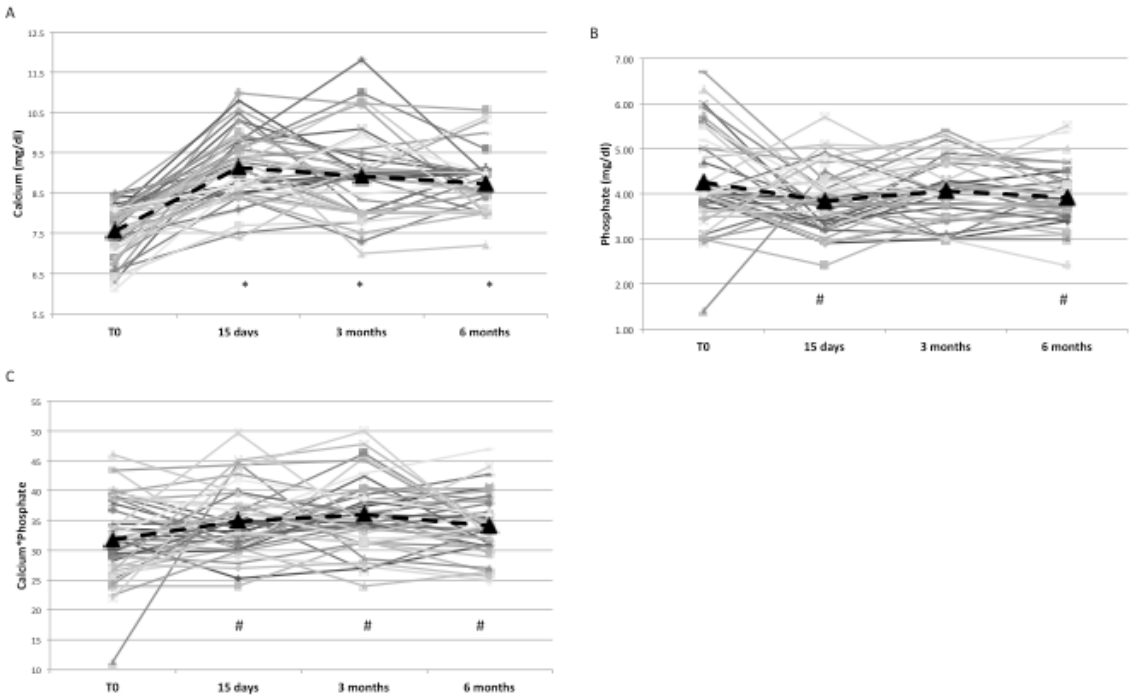
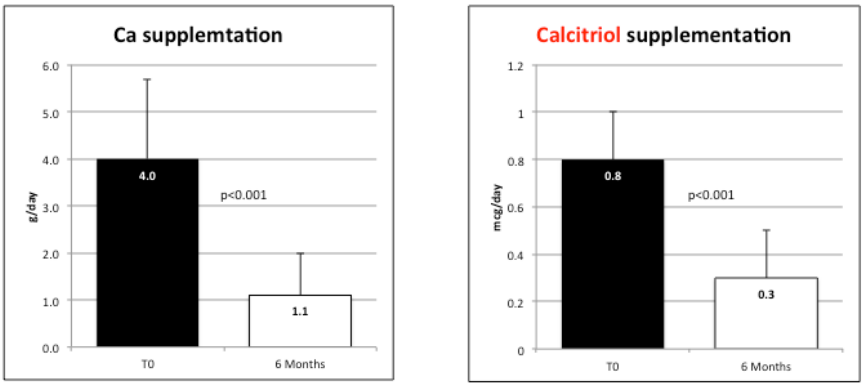


Figure 21. Calcium and calcitriol supplementation before (T0) and 6 months after teriparatide treatment. Bars are for standard deviation.



Similarly, serum phosphate levels significantly decreased after 15 days of therapy (4.3 ± 1.1 vs 3.8 ± 0.7, p=0.03) and remained stable after 3 months (4.1 ± 0.7; p=0.081 vs 15 days; p=0.120 vs baseline) and 6 months (3.9 ± 0.6; p=0.411 vs 15 days; p=0.019 vs baseline) (figure 20b). A slight but significant increase was also found in the calcium phosphate product (Ca*P) after 15 days of therapy (31.8 ± 1.1 at baseline vs 34.9 ± 5.8 after 15 days, p=0.029), while levels remained then stable towards the remaining study period (36.0 ± 0.9 at 3 months, p=0.303 vs 15 days, p<0.001 vs baseline; 34.1 ± 0.8 at 6 months, p=0.458 vs 15 days, p=0.023 vs

baseline) (figure 20C). Over the treatment period no significant differences in serum creatinine and 25-OH vitamin D were found (table 18).

Table 18. Biochemical changes over the time.

	Baseline	3 Months	6 Months	*P* Value[a]
25-OH vitamin D	31.4 ± 15.6		31.5 ± 11.3	.957
Serum creatinine, mg/dL	0.8 ± 0.2		0.8 ± 0.2	.771
Alkaline phosphatase, U/L	75.9 ± 38.5	111.2 ± 64.2	132.7 ± 78.5	<.001
Uric acid, mg/dL	4.3 ± 1.4		5.2 ± 1.6	<.001
Urinary calcium, mg per 24 h	219.6 ± 146.4	253.5 ± 119.7	219.6 ± 124.4	.874

[a] For the trend.

A transient trend towards a modest increase in urinary calcium levels was registered at 3 months (219.6 ± 146.4 at baseline vs 253.5 ± 119.7 mg/24h at 3 months, p= 0.192), reverting to baseline values at 6 months (219.5 ±124.4 mg/24h; p=0.008 vs 3 months, p=0.984 vs baseline). After 6 months from the initiation of teriparatide, both alkaline phosphatase and uric acid levels significantly increased from baseline to 6 months (p<0.001) (table 18).

Quality of Life Evaluation

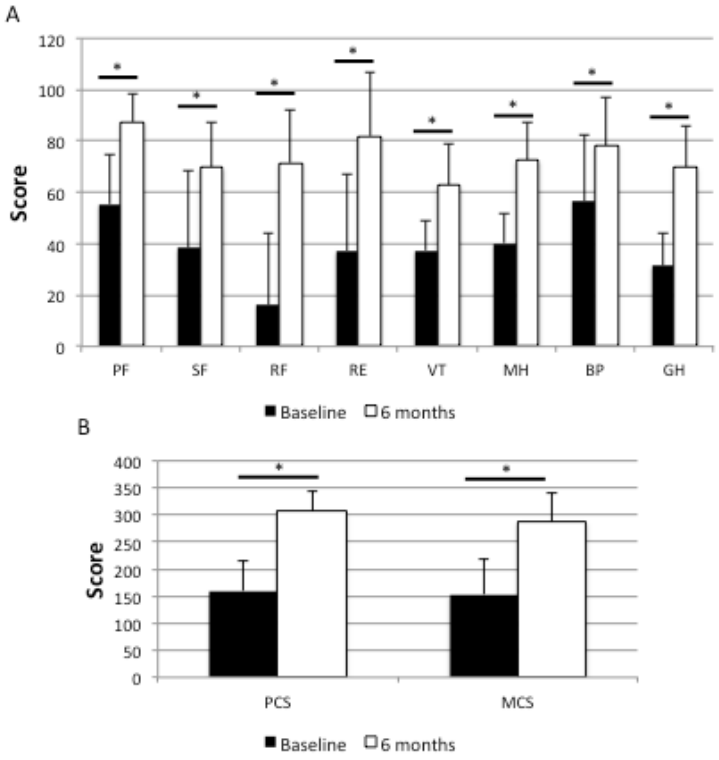
The results of the RAND36-Item Health Survey data at baseline and after 6 months treatment with teriparatide showed a significant improvement in the mean scores of all 8 domains. Consequently, both the physical component summary (PCS) score and the mental component summary (MCS) score also significantly increased after 6 months of treatment (table 19 and figure 22).

Table 19. Quality of Life evaluation

	Baseline	6 months	p-value
Physical Functioning	55.5 ± 19.6	87.5 ± 10.6	<0.001
Social Functioning	38.4 ± 30.0	69.8 ± 17.2	<0.001
Role limitation caused by physical health	16.3 ± 27.5	71.6 ± 20.9	<0.001

problems			
Role limitations due to Emotional	37.3 ± 29.8	82.0 ± 25.0	<0.001
Vitality	37.4 ± 11.7	62.9 ± 16.3	<0.001
Mental Health	40.3 ± 11.6	72.4 ± 14.6	<0.001
Bodily Pain	56.5 ± 25.8	78.3 ± 18.9	<0.001
Perception of General Health	31.6 ± 12.3	69.8 ± 16.3	<0.001
Physical Component Summary	159.9 ± 56.4	307.2 ± 38.1	<0.001
Mental Component Summary	153.4 ± 63.7	287.1 ± 54.6	<0.001

Figure 22. Changes in the RAND36-Item Health Survey before and after 6 months of treatment with teriparatide. A) Each one of the 8 domains examined and B) physical and mental component summary. PF: physical functioning; SF: social functioning; RF: role limitations caused by physical health problems; RE: role limitations due to emotional and health problems; VT: vitality; MH: mental health; BP: bodily pain; GH: perception of general health; PCS: physical component summary; MCS: mental component summary. Bars are for standard deviation. *p<0.001



Safety data

No serious adverse events occurred during the study period. Transient injection-site erythema was observed in 3 patients. Other mild adverse events such as nausea or gastrointestinal illness (n=2) and headache (n=1) were recorded. No subjects developed nephrolithiasis. No adverse events led to the discontinuation of the treatment. Only one subject developed an asymptomatic mild-moderate hypercalcemia (11.6 mg/dl at three month time-point) due to the missed reduction of calcium carbonate and calcitriol supplements as the protocol suggested.

8.4 Discussion

Hypoparathyroidism is the only endocrine deficiency disease that is not currently treated with the missing hormone; in fact in this disorder the substitution is performed administering the end products of PTH action, i.e. calcium and calcitriol. Even though this kind of therapy can account for an acceptable serum calcium level, the supraphysiological doses of calcium and calcitriol needed to achieve this result have raised many concerns about the risk of hypercalciuria, hyperphosphatemia, nephrocalcinosis, urolithiasis and ectopic soft tissue calcification. Moreover the daily ingestion of large amounts of calcium is very uncomfortable for the patients [415].

Some authors have tested the effect of PTH(1-84) on biochemical and QOL parameters in patients with hypoparathyroidism of various aetiologies and they have found consistent results in terms of both lowering the need of supplemental calcium and calcitriol and improving QOL functioning although the authors did not take into consideration a placebo control group. On the contrary, in a 6 months randomized control trial, Sikjaer et al did not support an immediate beneficial effect of PTH(1-84) replacement therapy on muscle function or QoL probably due to a high frequency of hypercalcemia among the study population [416].

Other studies have shown the effectiveness of PTH(1-34) in the management of patients with hypoparathyroidism although their sample sizes were small [382,385](7-10), and most of them took into consideration pediatric populations.

Our results can add relevant information to former literature as, to our knowledge, we treated with PTH(1-34) the largest number of adult subjects that were exclusively affected by post-surgical hypoparathyroidism. Moreover, for the first time, we showed that PTH(1–34) improves mental and physical health in these patients.

The results of this study clearly show that subcutaneous twice daily PTH(1-34) injections can both restore serum calcium levels and reduce the need for calcium and calcitriol supplements in patients with post-surgical hypoparathyroidism. The increase of serum calcium is very rapid and occurs within the first 15 days of treatment. The achieved level is therefore maintained stable at 3 and 6 months after initiation of the therapy. Simultaneously serum phosphate significantly decreased at each time point of the study. Both these results may reflect the natural action of PTH on renal tubules, with a concomitant increase of distal calcium and inhibition of proximal phosphate reabsorption. If this was true, one would have expected that the calcium-phosphate product (Ca*P) remain stable or, possibly, decreased throughout the study. On the contrary, the Ca*P increased slightly but significantly, soon after the initiation of therapy, and it remained higher than baseline values for the entire study period. It is noteworthy that the tapering of calcium therapy began within the first two weeks of PTH(1-34) treatment by reducing the intake by 500 mg/day at each measurement of a normal serum calcium, until the goal of a maximum 1000 mg daily intake of calcium was reached. Soon afterwards calcitriol reduction was initiated. Even though the requirement for calcium supplements rapidly and significantly decreased, it is possible that the delayed reduction of calcitriol may have maintained an enhanced intestinal absorption of alimentary phosphate, thus resulting in a slight increase of Ca*P. Alternatively, the Ca*P product may have increased because baseline serum calcium was very low and increased during the study period. Despite the lower phosphate, the higher serum calcium may have driven the increase in the Ca*P. Indeed, at the end of the study the administered dose of calcium and calcitriol supplements was dramatically reduced compared to pre-enrolment values.

Similarly to what was previously found with PTH (1-84) treatment [381], the daily urinary excretion of calcium increased slightly, but not significantly, after 3 months of therapy and then it returned to baseline values. A possible explanation is that PTH treatment has induced higher serum levels of calcium with a subsequent increase of renal calcium excretion. A second possibility takes into consideration bone turnover. In fact we observed a significant, progressive increase of alkaline phosphatase that reflects an acceleration of bone formation and even though we did not measure a marker of bone resorption, it is reasonable that it has increased as well. As PTH favours bone resorption, mostly by affecting the RANKL/osteoprotegerin ratio [417], it is possible that a larger amount of calcium has been acutely removed from inside the bones, thus increasing the renal load of calcium and ultimately renal calcium excretion. By this time, together with the progressive reduction of calcium carbonate supplements, calcium excretion may have returned to baseline values. Whatever the case, PTH treatment induced only a small, transient, increase of renal calcium excretion thus confirming previous findings that this therapy does not produce any additional renal risk.

According to Rubin et al, the significant progressive increase of alkaline phosphatase may indirectly reflect a recovery of osteoblasts function by means of both increased cell differentiation and decreased pre-osteoblasts and osteoblasts apoptosis [418]. The marked increase in bone turnover is very important as it may induce a renewal of the overmineralized bone that is typically seen in hypoparathyroidism. Therefore, in contrast to the effect of PTH treatment in patients with osteoporosis, PTH-replacement therapy in hypoparathyroidism may cause a decrease in BMD and possibly a more physiological bone metabolism [419].

We also observed a slight rise in serum uric acid that confirms the well known median average increase of 0.9 mg/dl described in the literature. As increased uric acid in blood is a risk factor for gout some concerns may exist as to a possible occurrence of this disease, even though the mean uric acid of our patients did not exceed the upper normal limit after teriparatide treatment. Noteworthy in the Fracture

Prevention Trial serum uric acid rose above the upper limit of normal, but no cases of gout have been reported. Given that it is not the standard care to monitor uric acid levels even in patient treated with teriparatide for osteoporosis, we believe that hypoparathyroid subjects do not encounter any additional risk of gout when treated with exogenous PTH.

Another relevant result of this study is the significant improvement in all the physical and mental QOL functioning aspects. Patients with long-standing post-surgical hypoparathyroidism can be a good model for studying QOL, particularly because they were used to living with normal serum calcium before surgery. The need for large amounts of calcium and calcitriol is felt as a dependence on drugs without achieving an acceptable quality of life. PTH replacement therapy is also felt as dependence but patients got aware that this treatment allows them to normally carry out the daily activities without any significant symptoms. Moreover, fluctuations in serum calcium, above or below the normal range, can exacerbate symptoms and require temporary dose adjustments. Calcitriol toxicity can also develop, manifesting as hypercalcemia or hypercalciuria, with its adverse effects on the renal, central nervous and cardiovascular systems. The stable increase of serum calcium and the dramatic reduction of supplemental calcium and calcitriol daily doses may represent the main reason for both the physical and mental QOL improvements, confirming previous results by Cusano etal. in post-surgical and idiopathic hypoparathyroidism[393]. Actually we are not able to confirm whether the increase in serum calcium or decrease in supplementation requirements is strictly associated with the QOL improvement because the study population filled in the questionnaire only at baseline and at 6 months after treatment and we can't exclude that PTH itself improves the QOL indices. Moreover, as this is an uncontrolled study, we cannot be sure that the QOL improvement is due to the attention given to trial subjects. No serious adverse events were recorded during the study period and, in particular, no subjects developed nephrolithiasis although subcutaneous (sc) PTH(1-34) therapy did not produce a significant decline in urinary calcium. Indeed, as demonstrated in

Winer's pump study, sc replacement therapy did not improve the urinary calcium compared to continuous infusion of PTH(1-34) [358]. This trial was short and further studies of long-term renal complications are needed. This study has a few important limitations. First of all, it is lacking in a control group of subjects with hypoparathyroidism who continued calcium and calcitriol treatment. Although this point represents a limitation, the subjects served as their own control. Moreover, we only enrolled patients that met the AIFA criteria of "being resistant to the calcium and/or vitamin D supplements" in order to obtain refunding of PTH(1-34) therapy by the Italian Government. Therefore a control group would have been composed of patients with a less severe hypoparathyroidism thus reducing the validity of group comparisons. However the great decreases in oral calcium and calcitriol supplementation supports the effect of PTH(1-34) in our patients, and it is unlikely that such a big effect could be obtained without PTH administration. Moreover we did not evaluate the presence and severity of symptoms related to hypocalcemia with validated scales, so we are not able to show the eventual improvement of symptoms and their relationship with the improvement in quality of life we reported. We also didn't measure the ionized calcium. Our study design may have led us to miss the detection of peak serum and urinary calcium levels if these occurred within 24 h of PTH administration. Another important limitation is that the results are only over a 6 month period and long-term data are needed as PTH therapy would be used as a chronic treatment. For this reason, this study will be continuing through 2 years. Despite these limitations, this is the largest study that demonstrates the effectiveness and safety of PTH(1-34) in the treatment of patients with post-surgical hypoparathyroidism. For the first time it has been shown that PTH(1–34) improves mental and physical health in adult hypoparathyroid subjects.

Further placebo-controlled studies are necessary in order to confirm our results although we might be confident to encourage the use of teriparatide as a substitution therapy due to its important effects on biochemical and QOL parameters.

CHAPTER 9

WHAT IS THE IMPACT OF MY RESEARCH WORKS AND WHICH ARE THE MAIN LIMITATIONS? WHICH ARE THE FUTURE PERSPECTIVES?

1)Effects of alendronate and vitamin D in patients with normocalcemic primary hyperparathyroidism

Although the Fourth International workshop for the management of asymptomatic PHPT tries to clarify its diagnostic criteria and suggests the way to manage subjects with NPHPT, no prospective trials have evaluated the impact of the anti-resorptive therapy in this kind of population. This is the first study evaluating the effect of alendronate and vitamin D on patients affected by NPHPT. Our data have demonstrated the ability of alendronate to increase BMD in osteoporotic patients with NPHPT. Moreover, we have demonstrated that the administration of vitamin D was safe but it did not positively affect BMD or BTM.

As far as osteoporosis representing an indication for PTX, these findings might raise some doubts about the real need of parathyroidectomy in this group of patients even if we do not have any data about the impact of alendronate on fracture risk.

Main Limitations

-It was not designed as a double-blind trial

-The sample size was small (even if the prevalence and incidence of NPHPT are low)

-We did not measure ionized calcium, nor the radius BMD.

Future perspectives

a) According to the previous findings, we would like to design a multicentric double-blind placebo-controlled trial to evaluate the effect alendronate on BMD, bone turnover, ionised and albumin adjusted serum calcium, and PTH in individuals with NPHPT. To overcome the previous limitations, enrolled subjects will be allocated to

the active treatment group receiving weekly 70 mg of alendronate for 2 years. The control group will receive placebo in the first year and will be switched to active therapy with alendronate in the second year. BMD was monitored every 12 months, and bone turnover markers, serum calcium, and PTH were monitored every 3 months.

b)We would like to design a new trial aimed to evaluate the bone material properties assessed by micro-indentation technique and the trabecular bone score in NPHPT compared to PHPT and healthy subjects.

2) Normocalcemic Hypoparathyroidism: Prevalence and Effect on Bone Status in Older Women. The OPUS Study.

Cusano et *al* previously conducted a large trial to evaluate the epidemiology of these subclinical conditions in an unselected community-based sample of old men (The Osteoporotic Fractures in Men study, MrOS study), and in young men and premenopausal women (Dallas Heart Study, DHS) but this is the first study to investigate the prevalence of NHYPO in a large cohort of postmenopausal women. Our data gave rise to many doubts about the real existence of NHYPO as a new subclinical pathological category because at the 6 yrs visit, of the 35 NHYPO subjects with follow-up data, none of them developed overt hypoparathyroidism and only 15 (0.6%) had persistent evidence of NHYPO. Anyway, our findings support the hypothesis that NHYPO subjects are characterised by a "low bone turnover" without a significant BMD change over time compared to normal individuals. It could be very important to identify these subjects because the potential choice of treatment for subjects with NHYPO affected by osteoporosis is critical. Indeed powerful anti-resorptive drugs such as bisphosphonates and denosumab, could exacerbate the risk of adynamic bone disease by suppressing bone turnover.

Main Limitations

-We didn't measure ionised calcium and fasting phosphate;

-We didn't collect any biochemical data between the baseline visit and the second visit at 6 years;

-We only had a single value for each laboratory analyte at each time point;

-We lost a significant number of subjects over the course of the study;

-BMD analysis was performed by two different instruments;

Future perspectives

We would like to address the following questions: "Is this a real subclinical pathological category? What is the cause of NHYPO?".

Therefore, we have already performed some genetic analysis in order to determine the prevalence of CaSR variants in subjects with normocalcaemic forms of hypoparathyroidism and to investigate whether common coding region CaSR SNPs are associated with serum PTH concentrations in these subject groups. Anyway, we need to enlarge the sample size to be sure about our findings.

Moreover, we have also investigate the prevalence of NALP5 and calcium-sensing receptor autoantibodies in subjects with normocalcaemic hypoparathyroidism because we aimed to test if the identification of NALP5 and calcium-sensing receptor autoantibodies help us to predict the evolution of normocalcaemic hypoparathyroidism.

3) PTH(1–34) for the Primary Prevention of Post-thyroidectomy Hypocalcemia: The THYPOS Trial

Although there are authors that have investigated the safety and efficacy of PTH treatment for the management of chronic hypoparathyroidism, poor and low-quality data are available for the therapy of acute hypocalcemia. Moreover, no studies have investigated teriparatide for the primary prevention of post-surgical hypocalcemia. For the first time, we have demonstrated that teriparatide may prevent the onset of postsurgical hypocalcemia in subjects with high risk of hypocalcemia after thyroidectomy. This finding may be very important because severe hypocalcemia

may lead to cardiac arrhythmias and tetany with an increase of morbidity rate and duration of hospitalization. Indeed, teriparatide treatment was able to shorten the duration of hospitalization in our subjects.

Another important result, confirming the usefulness of early teriparatide administration after neck surgery, is the lower need of long-term calcium supplements in treated patients than in controls. Moreover, daily mean calcium carbonate intake was significantly reduced from discharge to the 30-days' follow-up visit in treated patients, whereas the subjects in the control group were still taking almost the same amount of calcium they were prescribed after discharge. A rapid tapering of calcium carbonate is certainly positive for patients as far as calcium supplements are known to induce gastrointestinal discomfort and may somehow increase urinary supersaturation with respect to calcium salts that in turn, may induce a higher risk for urolithiasis.

Main Limitations

-Lack of placebo controlled group.

-Small groups of subjects even though we reached the pre-calculated sample size

-We did not measure PTH at 30 days after discharge

Future perspectives

We are designing a prospective multicentric randomized double-blind controlled trial to evaluate the ability of teriparatide to prevent the post-surgical hypocalcemia in subjects with high risk to develop it. Subjects will be randomized (1:1:1) to receive:

- Subcutaneous administration of teriparatide (20 mcg) every 12 hours until the discharge
- Carbonate Calcium;
- Carbonate Calcium plus calcitriol.

Main outcome measures: Adjusted serum calcium, duration of hospitalization, and calcium/calcitriol supplementation

4) PTH(1–34) for Surgical Hypoparathyroidism: A Prospective, Open-Label Investigation of Efficacy and Quality of Life

Other studies have shown the effectiveness of PTH(1–34) in the management of patients with hypoparathyroidism, although their sample sizes were small, and most of them considered only pediatric populations. Our results can add relevant information to former literature because we treated the largest number, to our knowledge, of adult subjects with PTH(1–34) that were exclusively affected by postsurgical hypoparathyroidism. The results of this study clearly show that sc twice daily PTH(1–34) injections can both restore serum calcium levels and reduce the need for calcium and calcitriol supplements in patients with postsurgical hypoparathyroidism. The increase of serum calcium is very rapid and occurs within the first 15 days of treatment. The achieved level is then maintained at a stable level at 3 and 6 months after the initiation of the therapy.

Furthermore, patients with long-standing postsurgical hypoparathyroidism can be a good model for studying QOL because they were used to living with normal serum calcium before surgery. The need to take large amounts of calcium and calcitriol made the patient feel dependent on drugs and without the benefit of achieving an acceptable QOL. For the first time, we have demonstrated that PTH(1–34) treatment might improve mental and physical health in adult hypoparathyroid subjects.

Main Limitations

- Lack of placebo controlled group (calcium and vitamin D supplementation).
- We did not evaluate the presence and severity of symptoms related to hypocalcemia.
- We didn't measure the ionized calcium

Future perspectives

We are collecting the data at 24 months because this is a 2-year, prospective, open-label study, and we have only reported the results after the first 6 months of PTH(1–

34) treatment. Indeed, up to now, outpatient admissions occurred at 0, 3, 6, 12, 18 and 24 months. At 24 months, it has been performed also a DXA scan evaluation at lumbar spine, femoral neck and total hip.

OTHER PEER-REVIEWED PAPERS PUBLISHED DURING MY PhD

1) Vescini F, Attanasio R, Balestrieri A, Bandeira F, Bonadonna S, Camozzi V, Cassibba S, Cesareo R, Chiodini I, Francucci CM, Gianotti L, Grimaldi F, Guglielmi R, Madeo B, Marcocci C, **Palermo A**, Scillitani A, Vignali E, Rochira V, Zini M. *Italian association of clinical endocrinologists position statement: drug therapy of osteoporosis.* J Endocrinol Invest. 2016 Jul;39(7):807-34

2) **Palermo A,** Tuccinardi D, Defeudis G, Watanabe M, D'Onofrio L, Lauria Pantano A, Napoli N, Pozzilli P, Manfrini S. *BMI and BMD: The Potential Interplay between Obesity and Bone Fragility.* Int J Environ Res Public Health. 2016 May 28;13(6).

3) Musumeci M*, **Palermo A***, D'Onofrio L, Vadalà G, Greto V, Di Stasio E, Maddaloni E, Di Rosa M, Tibullo D, Angeletti S, Napoli N, Denaro V, Manfrini S. *Serum chitotriosidase in postmenopausal women with severe osteoporosis.*Osteoporos Int. 2016 Feb;27(2):711-6 * these authors contributed equally to this work

4) Defeudis G, Gianfrilli D, Di Emidio C, Pofi R, Tuccinardi D, **Palermo A**, Lenzi A, Pozzilli P. *Erectile dysfunction and its management in patients with diabetes mellitus.* Rev Endocr Metab Disord. 2015 Oct 26

5) Cesareo R, Iozzino M, D'onofrio L, Terrinoni I, Maddaloni E, Casini A, Campagna G, Santonati A, **Palermo A.** *Effectiveness and safety of calcium and vitamin D treatment for postmenopausal osteoporosis.* Minerva Endocrinol. 2015 Sep;40(3):231-7.

6) **Palermo A,** D'Onofrio L, Eastell R, Schwartz AV, Pozzilli P, Napoli N.*Oral anti-diabetic drugs and fracture risk, cut to the bone: safe or dangerous? A narrative review.* Osteoporos Int. 2015 Aug;26(8):2073-89

7) **Palermo A**, Strollo R, Maddaloni E, Tuccinardi D, D'Onofrio L, Briganti SI, Defeudis G, De Pascalis M, Lazzaro MC, Colleluori G, Manfrini S, Pozzilli P, Napoli N. *Irisin is associated with osteoporotic fractures independently of bone mineral density, body composition or daily physical activity.* Clin Endocrinol (Oxf). 2015 Apr;82(4):615-9

8) Maddaloni E, Sabatino F, Del Toro R, Crugliano S, Grande S, Lauria Pantano A, Maurizi AR, **Palermo A**, Bonini S, Pozzilli P, Manfrini S. *In vivo corneal confocal microscopy as a novel non-invasive tool to investigate cardiac autonomic neuropathy in Type 1 diabetes.* Diabet Med. 2015 Feb;32(2):262-6

9) Nusca A, Lauria Pantano A, Melfi R, Proscia C, Maddaloni E, Contuzzi R, Mangiacapra F, **Palermo A**, Manfrini S, Pozzilli P, Di Sciascio G. *Glycemic Variability Assessed by Continuous Glucose Monitoring and Short-Term Outcome in Diabetic Patients Undergoing Percutaneous Coronary Intervention: An Observational Pilot Study.* J Diabetes Res. 2015;2015:250201.

10) **Palermo A**, Strollo R, Papalia R, D'Onofrio L, Maddaloni E, Briganti SI, Napoli N, Vespasiani U, Costantino S, Pozzilli P, Denaro V, Manfrini S. *Severe hypophosphatemic osteomalacia secondary to fanconi syndrome due to adefovir: a case report.* Endocr Pract. 2014 Dec;20(12):e246-9

11) Maddaloni E, D'Onofrio L, Lauria A, Maurizi AR, Strollo R, **Palermo A**, Napoli N, Angeletti S, Pozzilli P, Manfrini S. *Osteocalcin levels are inversely associated with Hba1c and BMI in adult subjects with long-standing type 1 diabetes.* J Endocrinol Invest. 2014 Jul;37(7):661-6.

12) **Palermo A**, Maggi D, Maurizi AR, Pozzilli P, Buzzetti R. *Prevention of type 2 diabetes mellitus: is it feasible?* Diabetes Metab Res Rev. 2014 Mar;30 Suppl 1:4-12.

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REFERENCE

1. Goltzman, M. J. F. and D. in *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism* 171–177 (2013).
2. WALSER, M. Ion association. VI. Interactions between calcium, magnesium, inorganic phosphate, citrate and protein in normal human plasma. *J. Clin. Invest.* **40,** 723–30 (1961).
3. Bilezikian J.P., Marcus R, Levine M.A., Marcocci Claudio, Silverberg S.J., P. J. T. *The Parathyroids: basic and clinical concepts.* (2015).
4. Fraser, D. R. & Kodicek, E. Regulation of 25-hydroxycholecalciferol-1-hydroxylase activity in kidney by parathyroid hormone. *Nat. New Biol.* **241,** 163–6 (1973).
5. Lieben, L. *et al.* Trpv6 mediates intestinal calcium absorption during calcium restriction and contributes to bone homeostasis. *Bone* **47,** 301–8 (2010).
6. Brown, E. M. Clinical lessons from the calcium-sensing receptor. *Nat. Clin. Pract. Endocrinol. Metab.* **3,** 122–33 (2007).
7. Shoback, D. Clinical practice. Hypoparathyroidism. *N. Engl. J. Med.* **359,** 391–403 (2008).
8. Huan, J., Martuseviciene, G., Olgaard, K. & Lewin, E. Calcium-sensing receptor and recovery from hypocalcaemia in thyroparathyroidectomized rats. *Eur. J. Clin. Invest.* **37,** 214–21 (2007).
9. Neuman, M. W. Blood:bone equilibrium. *Calcif. Tissue Int.* **34,** 117–20 (1982).
10. Ward BK, Magno AL, Walsh JP, R. T. The role of the calcium-sensing receptor in human disease. - PubMed - NCBI. *Clin Biochem.* **45,** 943–53 (2012).
11. Schwarz, P., Sørensen, H. A., McNair, P. & Transbøl, I. Cica-clamp technique: a method for quantifying parathyroid hormone secretion: a sequential citrate and calcium clamp study. *Eur. J. Clin. Invest.* **23,** 546–53 (1993).
12. Jung, A., Mayer, G. P., Hurst, J. G., Neer, R. & Potts, J. T. Model for parathyroid hormone secretion and metabolism in calves. *Am. J. Physiol.* **242,** R141-50 (1982).
13. Morrissey, J. J., Hamilton, J. W., MacGregor, R. R. & Cohn, D. V. The secretion of parathormone

fragments 34-84 and 37-84 by dispersed porcine parathyroid cells. *Endocrinology* **107,** 164–71 (1980).

14. Brown, E. M., Leombruno, R., Thatcher, J. & Burrowes, M. The acute secretory response to alterations in extracellular calcium concentration and dopamine in perifused bovine parathyroid cells. *Endocrinology* **116,** 1123–32 (1985).

15. Naveh-Many, T. & Silver, J. Regulation of parathyroid hormone gene expression by hypocalcemia, hypercalcemia, and vitamin D in the rat. *J. Clin. Invest.* **86,** 1313–9 (1990).

16. Naveh-Many, T., Friedlaender, M. M., Mayer, H. & Silver, J. Calcium regulates parathyroid hormone messenger ribonucleic acid (mRNA), but not calcitonin mRNA in vivo in the rat. Dominant role of 1,25-dihydroxyvitamin D. *Endocrinology* **125,** 275–80 (1989).

17. Denda M, Finch J, S. E. Phosphorus accelerates the development of parathyroid hyperplasia and secondary hyperparathyroidism in rats with renal failure. - PubMed - NCBI. *Am J Kidney Dis.* **28,** 596–602 (1996).

18. Goodman WG, Q. L. Development and progression of secondary hyperparathyroidism in chronic kidney disease: lessons from molecular genetics. - PubMed - NCBI. *Kidney Int.* **74,** 276–88 (2008).

19. Schaffler, M. B., Cheung, W.-Y., Majeska, R. & Kennedy, O. Osteocytes: master orchestrators of bone. *Calcif. Tissue Int.* **94,** 5–24 (2014).

20. Liu, S. & Quarles, L. D. How fibroblast growth factor 23 works. *J. Am. Soc. Nephrol.* **18,** 1637–47 (2007).

21. Hu, M. C., Shiizaki, K., Kuro-o, M. & Moe, O. W. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu. Rev. Physiol.* **75,** 503–33 (2013).

22. Silver, J., Rodriguez, M. & Slatopolsky, E. FGF23 and PTH--double agents at the heart of CKD. *Nephrol. Dial. Transplant* **27,** 1715–20 (2012).

23. Lavi-Moshayoff, V., Wasserman, G., Meir, T., Silver, J. & Naveh-Many, T. PTH increases FGF23 gene expression and mediates the high-FGF23 levels of experimental kidney failure: a bone parathyroid feedback loop. *Am. J. Physiol. Renal Physiol.* **299,** F882-9 (2010).

24. Miao, D., He, B., Karaplis, A. C. & Goltzman, D. Parathyroid hormone is essential for normal fetal bone formation. *J. Clin. Invest.* **109,** 1173–82 (2002).

25. Ren, Y. *et al.* Endogenous PTH deficiency impairs fracture healing and impedes the fracture-healing efficacy of exogenous PTH(1-34). *PLoS One* **6,** e23060 (2011).

26. Andreassen, T. T., Ejersted, C. & Oxlund, H. Intermittent parathyroid hormone (1-34) treatment increases callus formation and mechanical strength of healing rat fractures. *J. Bone Miner. Res.* **14,** 960–8 (1999).

27. Aspenberg, P. *et al.* Teriparatide for acceleration of fracture repair in humans: a prospective, randomized, double-blind study of 102 postmenopausal women with distal radial fractures. *J. Bone Miner. Res.* **25,** 404–14 (2010).

28. Peichl, P., Holzer, L. A., Maier, R. & Holzer, G. Parathyroid hormone 1-84 accelerates fracture-healing in pubic bones of elderly osteoporotic women. *J. Bone Joint Surg. Am.* **93,** 1583–7 (2011).

29. Duan, Y., De Luca, V. & Seeman, E. Parathyroid hormone deficiency and excess: similar effects on trabecular bone but differing effects on cortical bone. *J. Clin. Endocrinol. Metab.* **84,** 718–22 (1999).

30. Mendonça, M. L. *et al.* Increased vertebral morphometric fracture in patients with postsurgical hypoparathyroidism despite normal bone mineral density. *BMC Endocr. Disord.* **13,** 1 (2013).

31. Dempster, D. W. *et al.* Preserved three-dimensional cancellous bone structure in mild primary hyperparathyroidism. *Bone* **41,** 19–24 (2007).

32. Neer, R. M. *et al.* Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis. *N. Engl. J. Med.* **344,** 1434–41 (2001).

33. Greenspan, S. L. *et al.* Effect of recombinant human parathyroid hormone (1-84) on vertebral fracture and bone mineral density in postmenopausal women with osteoporosis: a randomized trial. *Ann. Intern. Med.* **146,** 326–39 (2007).

34. Recker, R. R. *et al.* Cancellous and cortical bone architecture and turnover at the iliac crest of postmenopausal osteoporotic women treated with parathyroid hormone 1-84. *Bone* **44,** 113–9 (2009).

35. Stewart, A. F. *et al.* Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and nonhumoral groups. *N. Engl. J. Med.* **303,** 1377–83 (1980).

36. Datta, N. S. & Abou-Samra, A. B. PTH and PTHrP signaling in osteoblasts. *Cell. Signal.* **21,** 1245–54 (2009).

37. Miao, D. *et al.* Severe growth retardation and early lethality in mice lacking the nuclear localization sequence and C-terminus of PTH-related protein. *Proc. Natl. Acad. Sci. U. S. A.* **105,** 20309–14 (2008).

38. Chen X, Macica CM, Ng KW, B. A. Stretch-induced PTH-related protein gene expression in osteoblasts. - PubMed - NCBI. *J Bone Min. Res.* **20,** 1454–61 (2005).

39. Klopocki, E. *et al.* Deletion and point mutations of PTHLH cause brachydactyly type E. *Am. J. Hum. Genet.* **86,** 434–9 (2010).

40. Gupta, A. *et al.* Variable number of tandem repeats polymorphism in parathyroid hormone-related protein as predictor of peak bone mass in young healthy Finnish males. *Eur. J. Endocrinol.* **158,** 755–64 (2008).

41. Estrada, K. *et al.* Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture. *Nat. Genet.* **44,** 491–501 (2012).

42. Zhu, Q. *et al.* Endogenous parathyroid hormone-related protein compensates for the absence of parathyroid hormone in promoting bone accrual in vivo in a model of bone marrow ablation. *J. Bone Miner. Res.* **28,** 1898–911 (2013).

43. McCauley, L. K. & Martin, T. J. Twenty-five years of PTHrP progress: from cancer hormone to multifunctional cytokine. *J. Bone Miner. Res.* **27,** 1231–9 (2012).

44. Dobnig, H. *et al.* Early changes in biochemical markers of bone formation correlate with improvements in bone structure during teriparatide therapy. *J. Clin. Endocrinol. Metab.* **90,** 3970–7 (2005).

45. Girotra, M., Rubin, M. R. & Bilezikian, J. P. The use of parathyroid hormone in the treatment of osteoporosis. *Rev. Endocr. Metab. Disord.* **7,** 113–21 (2006).

46. Lindsay, R. *et al.* A novel tetracycline labeling schedule for longitudinal evaluation of the short-term effects of anabolic therapy with a single iliac crest bone biopsy: early actions of teriparatide. *J. Bone Miner. Res.* **21,** 366–73 (2006).

47. Compston, J. E. Skeletal actions of intermittent parathyroid hormone: effects on bone remodelling and structure. *Bone* **40,** 1447–52 (2007).

48. Rhee, Y. *et al.* Resorption controls bone anabolism driven by parathyroid hormone (PTH) receptor signaling in osteocytes. *J. Biol. Chem.* **288,** 29809–20 (2013).

49. Pierroz, D. D. *et al.* Are osteoclasts needed for the bone anabolic response to parathyroid hormone? A study of intermittent parathyroid hormone with denosumab or alendronate in knock-in mice expressing humanized RANKL. *J. Biol. Chem.* **285,** 28164–73 (2010).

50. Gasser, J. A., Kneissel, M., Thomsen, J. S. & Mosekilde, L. PTH and interactions with bisphosphonates. *J. Musculoskelet. Neuronal Interact.* **1,** 53–6 (2000).

51. Demiralp, B., Chen, H.-L., Koh, A. J., Keller, E. T. & McCauley, L. K. Anabolic actions of parathyroid hormone during bone growth are dependent on c-fos. *Endocrinology* **143,** 4038–47 (2002).

52. Samadfam, R., Xia, Q. & Goltzman, D. Co-treatment of PTH with osteoprotegerin or alendronate increases its anabolic effect on the skeleton of oophorectomized mice. *J. Bone Miner. Res.* **22,** 55–63 (2007).

53. Kostenuik, P. J. *et al.* OPG and PTH-(1-34) have additive effects on bone density and mechanical strength in osteopenic ovariectomized rats. *Endocrinology* **142,** 4295–304 (2001).

54. Samadfam, R., Xia, Q. & Goltzman, D. Pretreatment with anticatabolic agents blunts but does not eliminate the skeletal anabolic response to parathyroid hormone in oophorectomized mice. *Endocrinology* **148,** 2778–87 (2007).

55. Drake, M. T. *et al.* Effects of intermittent parathyroid hormone treatment on osteoprogenitor cells in

postmenopausal women. *Bone* **49,** 349–55 (2011).

56. Black, D. M. *et al.* The effects of parathyroid hormone and alendronate alone or in combination in postmenopausal osteoporosis. *N. Engl. J. Med.* **349,** 1207–15 (2003).

57. Finkelstein, J. S. *et al.* The effects of parathyroid hormone, alendronate, or both in men with osteoporosis. *N. Engl. J. Med.* **349,** 1216–26 (2003).

58. Finkelstein, J. S., Wyland, J. J., Lee, H. & Neer, R. M. Effects of teriparatide, alendronate, or both in women with postmenopausal osteoporosis. *J. Clin. Endocrinol. Metab.* **95,** 1838–45 (2010).

59. Cosman, F. *et al.* Effects of intravenous zoledronic acid plus subcutaneous teriparatide [rhPTH(1-34)] in postmenopausal osteoporosis. *J. Bone Miner. Res.* **26,** 503–11 (2011).

60. Tsai, J. N. *et al.* Teriparatide and denosumab, alone or combined, in women with postmenopausal osteoporosis: the DATA study randomised trial. *Lancet (London, England)* **382,** 50–6 (2013).

61. Miao, D. *et al.* Skeletal abnormalities in Pth-null mice are influenced by dietary calcium. *Endocrinology* **145,** 2046–53 (2004).

62. Hansen, S., Beck Jensen, J.-E., Rasmussen, L., Hauge, E. M. & Brixen, K. Effects on bone geometry, density, and microarchitecture in the distal radius but not the tibia in women with primary hyperparathyroidism: A case-control study using HR-pQCT. *J. Bone Miner. Res.* **25,** 1941–7 (2010).

63. Postlethwait, J. H. *et al.* Zebrafish comparative genomics and the origins of vertebrate chromosomes. *Genome Res.* **10,** 1890–902 (2000).

64. Albright, F. & Ellsworth, R. STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS: I. Calcium and Phosphorus Studies on a Case of Idiopathic Hypoparathyroidism. *J. Clin. Invest.* **7,** 183–201 (1929).

65. Murray, R. D. *et al.* Parathyroid hormone (PTH) decreases sodium-phosphate cotransporter type IIa (NpT2a) mRNA stability. *Am. J. Physiol. Renal Physiol.* **304,** F1076-85 (2013).

66. Lanzano, L. *et al.* Differential modulation of the molecular dynamics of the type IIa and IIc sodium phosphate cotransporters by parathyroid hormone. *Am. J. Physiol. Cell Physiol.* **301,** C850-61 (2011).

67. Nagai, S. *et al.* Acute down-regulation of sodium-dependent phosphate transporter NPT2a involves predominantly the cAMP/PKA pathway as revealed by signaling-selective parathyroid hormone analogs. *J. Biol. Chem.* **286,** 1618–26 (2011).

68. Guo, J. *et al.* Activation of a non-cAMP/PKA signaling pathway downstream of the PTH/PTHrP receptor is essential for a sustained hypophosphatemic response to PTH infusion in male mice. *Endocrinology* **154,** 1680–9 (2013).

69. Déliot, N. *et al.* Parathyroid hormone treatment induces dissociation of type IIa Na+-P(i) cotransporter-Na+/H+ exchanger regulatory factor-1 complexes. *Am. J. Physiol. Cell Physiol.* **289,** C159-67 (2005).

70. Wang, B. *et al.* Ezrin-anchored protein kinase A coordinates phosphorylation-dependent disassembly of a NHERF1 ternary complex to regulate hormone-sensitive phosphate transport. *J. Biol. Chem.* **287,** 24148–63 (2012).

71. Capuano, P. *et al.* Defective coupling of apical PTH receptors to phospholipase C prevents internalization of the Na+-phosphate cotransporter NaPi-IIa in Nherf1-deficient mice. *Am. J. Physiol. Cell Physiol.* **292,** C927-34 (2007).

72. Khundmiri, S. J. *et al.* Novel regulatory function for NHERF-1 in Npt2a transcription. *Am. J. Physiol. Renal Physiol.* **294,** F840-9 (2008).

73. Karim, Z. *et al.* NHERF1 mutations and responsiveness of renal parathyroid hormone. *N. Engl. J. Med.* **359,** 1128–35 (2008).

74. Hoenderop, J. G. J. *et al.* Renal Ca2+ wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. *J. Clin. Invest.* **112,** 1906–14 (2003).

75. de Groot, T. *et al.* Molecular mechanisms of calmodulin action on TRPV5 and modulation by parathyroid hormone. *Mol. Cell. Biol.* **31,** 2845–53 (2011).

76. Kim, S. W. *et al.* Intermittent parathyroid hormone administration converts quiescent lining cells to active osteoblasts. *J. Bone Miner. Res.* **27,** 2075–84 (2012).

77. Magyar, C. E., White, K. E., Rojas, R., Apodaca, G. & Friedman, P. A. Plasma membrane Ca2+-ATPase and NCX1 Na+/Ca2+ exchanger expression in distal convoluted tubule cells. *Am. J. Physiol. Renal Physiol.* **283,** F29-40 (2002).

78. van Abel, M. *et al.* Coordinated control of renal Ca(2+) transport proteins by parathyroid hormone. *Kidney Int.* **68,** 1708–21 (2005).

79. Yang, W. *et al.* Expression of 25(OH)D3 24-hydroxylase in distal nephron: coordinate regulation by 1,25(OH)2D3 and cAMP or PTH. *Am. J. Physiol.* **276,** E793-805 (1999).

80. Brenza, H. L. & DeLuca, H. F. Regulation of 25-hydroxyvitamin D3 1alpha-hydroxylase gene expression by parathyroid hormone and 1,25-dihydroxyvitamin D3. *Arch. Biochem. Biophys.* **381,** 143–52 (2000).

81. Kim, M. *et al.* 1Alpha,25(OH)2D3-induced transrepression by vitamin D receptor through E-box-type elements in the human parathyroid hormone gene promoter. *Mol. Endocrinol.* **21,** 334–42 (2007).

82. Armbrecht, H. J., Hodam, T. L. & Boltz, M. A. Hormonal regulation of 25-hydroxyvitamin D3-1alpha-hydroxylase and 24-hydroxylase gene transcription in opossum kidney cells. *Arch. Biochem. Biophys.* **409,** 298–304 (2003).

83. Weaver, V. M., Franks, D. J. & Welsh, J. E. Activation of protein kinase C modulates dihydroxycholecalciferol synthesis in rat renal tubules. *Cell. Signal.* **4,** 293–301 (1992).

84. Janulis, M., Wong, M. S. & Favus, M. J. Structure-function requirements of parathyroid hormone for stimulation of 1,25-dihydroxyvitamin D3 production by rat renal proximal tubules. *Endocrinology* **133,** 713–9 (1993).

85. Healy, K. D., Vanhooke, J. L., Prahl, J. M. & DeLuca, H. F. Parathyroid hormone decreases renal vitamin D receptor expression in vivo. *Proc. Natl. Acad. Sci. U. S. A.* **102,** 4724–8 (2005).

86. Sociedade Brasileira de Endocrinologia e Metabologia *et al.* Diagnosis and management of primary hyperparathyroidism--a scientific statement from the Department of Bone Metabolism, the Brazilian Society for Endocrinology and Metabolism. *Arq. Bras. Endocrinol. Metabol.* **57,** 406–24 (2013).

87. Cusano, N. E., Silverberg, S. J. & Bilezikian, J. P. Normocalcemic primary hyperparathyroidism. *J. Clin. Densitom.* **16,** 33–9

88. Lassen, T., Friis-Hansen, L., Rasmussen, A. K., Knigge, U. & Feldt-Rasmussen, U. Primary hyperparathyroidism in young people. When should we perform genetic testing for multiple endocrine neoplasia 1 (MEN-1)? *J. Clin. Endocrinol. Metab.* **99,** 3983–7 (2014).

89. Groussin, L. Genetic evaluation in primary hyperparathyroidism: What investigation? For which patients? *Ann. Endocrinol. (Paris).* **76,** 142–4 (2015).

90. Lafferty, F. W. Differential diagnosis of hypercalcemia. *J. Bone Miner. Res.* **6 Suppl 2,** S51–9; discussion S61 (1991).

91. Yu, N., Donnan, P. T., Murphy, M. J. & Leese, G. P. Epidemiology of primary hyperparathyroidism in Tayside, Scotland, UK. *Clin. Endocrinol. (Oxf).* **71,** 485–93 (2009).

92. Sociedade Brasileira de Endocrinologia e Metabologia *et al.* Diagnosis and management of primary hyperparathyroidism--a scientific statement from the Department of Bone Metabolism, the Brazilian Society for Endocrinology and Metabolism. *Arq. Bras. Endocrinol. Metabol.* **57,** 406–24 (2013).

93. Eastell, R. *et al.* Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J. Clin. Endocrinol. Metab.* **94,** 340–50 (2009).

94. Kerschan-Schindl, K. *et al.* Bone metabolism in patients with primary hyperparathyroidism before and after surgery. *Horm. Metab. Res.* **44,** 476–81 (2012).

95. Bandeira, F. *et al.* Vitamin D deficiency: A global perspective. *Arq. Bras. Endocrinol. Metabol.* **50,** 640–6 (2006).

96. Bandeira, F. A. F., Oliveira, R. I. R. B., Griz, L. H. M., Caldas, G. & Bandeira, C. Differences in accuracy of 99mTc-sestamibi scanning between severe and mild forms of primary hyperparathyroidism. *J. Nucl. Med. Technol.* **36,** 30–5 (2008).

97. Christensson, T., Hellström, K., Wengle, B., Alveryd, A. & Wikland, B. Prevalence of hypercalcaemia in a health screening in Stockholm. *Acta Med. Scand.* **200,** 131–137 (1976).

98. Jorde, R., Bønaa, K. H. & Sundsfjord, J. Primary hyperparathyroidism detected in a health screening.

The Trømsø study. *J. Clin. Epidemiol.* **53,** 1164–9 (2000).

99. Akerström, G. *et al.* Histologic parathyroid abnormalities in an autopsy series. *Hum. Pathol.* **17,** 520–7 (1986).

100. Heath, H., Hodgson, S. F. & Kennedy, M. A. Primary hyperparathyroidism. Incidence, morbidity, and potential economic impact in a community. *N. Engl. J. Med.* **302,** 189–93 (1980).

101. Melton, L. J. History of the Rochester Epidemiology Project. *Mayo Clin. Proc.* **71,** 266–74 (1996).

102. Wermers, R. A. *et al.* The rise and fall of primary hyperparathyroidism: a population-based study in Rochester, Minnesota, 1965-1992. *Ann. Intern. Med.* **126,** 433–40 (1997).

103. Palumbo, P. J., Elveback, L. R., Chu, C. P., Connolly, D. C. & Kurland, L. T. Diabetes mellitus: incidence, prevalence, survivorship, and causes of death in Rochester, Minnesota, 1945-1970. *Diabetes* **25,** 566–73 (1976).

104. Mundy, G. R., Cove, D. H. & Fisken, R. Primary hyperparathyroidism: changes in the pattern of clinical presentation. *Lancet (London, England)* **1,** 1317–20 (1980).

105. Adami, S., Marcocci, C. & Gatti, D. Epidemiology of primary hyperparathyroidism in Europe. *J. Bone Miner. Res.* **17 Suppl 2,** N18-23 (2002).

106. Wermers, R. A. *et al.* Incidence of primary hyperparathyroidism in Rochester, Minnesota, 1993-2001: an update on the changing epidemiology of the disease. *J. Bone Miner. Res.* **21,** 171–7 (2006).

107. Mollerup, C. L., Bollerslev, J. & Blichert-Toft, M. Primary hyperparathyroidism: incidence and clinical and biochemical characteristics. A demographic study. *Eur. J. Surg.* **160,** 485–9 (1994).

108. Derby, C. A. *et al.* Correlates of postmenopausal estrogen use and trends through the 1980s in two southeastern New England communities. *Am. J. Epidemiol.* **137,** 1125–35 (1993).

109. Vestergaard, P. & Mosekilde, L. Cohort study on effects of parathyroid surgery on multiple outcomes in primary hyperparathyroidism. *BMJ* **327,** 530–4 (2003).

110. Abood, A. & Vestergaard, P. Increasing incidence of primary hyperparathyroidism in Denmark. *Dan. Med. J.* **60,** A4567 (2013).

111. Yeh, M. W. *et al.* Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. *J. Clin. Endocrinol. Metab.* **98,** 1122–9 (2013).

112. Schneider, A. B. *et al.* Dose-response relationships for radiation-induced hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **80,** 254–7 (1995).

113. Cohen, J., Gierlowski, T. C. & Schneider, A. B. A prospective study of hyperparathyroidism in individuals exposed to radiation in childhood. *JAMA* **264,** 581–4 (1990).

114. Fujiwara, S. *et al.* Hyperparathyroidism among atomic bomb survivors in Hiroshima. *Radiat. Res.* **130,** 372–8 (1992).

115. Khosla, S., Melton, L. J. & Riggs, B. L. The unitary model for estrogen deficiency and the pathogenesis of osteoporosis: is a revision needed? *J. Bone Miner. Res.* **26,** 441–51 (2011).

116. Heshmati, H. M. *et al.* Role of low levels of endogenous estrogen in regulation of bone resorption in late postmenopausal women. *J. Bone Miner. Res.* **17,** 172–8 (2002).

117. Wermers, R. A. *et al.* Survival after the diagnosis of hyperparathyroidism: a population-based study. *Am. J. Med.* **104,** 115–22 (1998).

118. Vestergaard, P. *et al.* Cardiovascular events before and after surgery for primary hyperparathyroidism. *World J. Surg.* **27,** 216–22 (2003).

119. Söreide, J. A. *et al.* Survival after surgical treatment for primary hyperparathyroidism. *Surgery* **122,** 1117–23 (1997).

120. Udén, P. & Tibblin, S. Mortality in patients surgically treated for primary hyperparathyroidism due to solitary adenoma. *Ann. Chir. Gynaecol.* **79,** 123–8 (1990).

121. Akerström, G., Grimelius, L., Johansson, H., Pertoft, H. & Lundqvist, H. Estimation of the parathyroid parenchymal cell mass by density gradients. *Am. J. Pathol.* **99,** 685–94 (1980).

122. Yu, N., Leese, G. P. & Donnan, P. T. What predicts adverse outcomes in untreated primary hyperparathyroidism? The Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin. Endocrinol. (Oxf).* **79,** 27–34 (2013).

123. Yu, N., Donnan, P. T. & Leese, G. P. A record linkage study of outcomes in patients with mild primary hyperparathyroidism: the Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin. Endocrinol. (Oxf).* **75,** 169–76 (2011).

124. Salinas, M. *et al.* Serum calcium (S-Ca), the forgotten test: preliminary results of an appropriateness strategy to detect primary hyperparathyroidism (pHPT). *Bone* **56,** 73–6 (2013).

125. Clarke, B. L. Epidemiology of primary hyperparathyroidism. *J. Clin. Densitom.* **16,** 8–13

126. Wu, B. *et al.* Underutilization of parathyroidectomy in elderly patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **95,** 4324–30 (2010).

127. Sosa, J. A. *et al.* Cost implications of different surgical management strategies for primary hyperparathyroidism. *Surgery* **124,** 1028-35–6 (1998).

128. Boudou, P., Ibrahim, F., Cormier, C., Sarfati, E. & Souberbielle, J.-C. Potential utility of high preoperative levels of serum type I collagen markers in postmenopausal women with primary hyperparathyroidism with respect to their short-term variations after parathyroidectomy. *J. Bone Miner. Metab.* **27,** 240–6 (2009).

129. Madorin, C. A. *et al.* Comparison of radiation exposure and cost between dynamic computed tomography and sestamibi scintigraphy for preoperative localization of parathyroid lesions. *JAMA Surg.* **148,** 500–3 (2013).

130. Morris, L. F. *et al.* The value of intraoperative parathyroid hormone monitoring in localized primary hyperparathyroidism: a cost analysis. *Ann. Surg. Oncol.* **17,** 679–85 (2010).

131. Norman, J., Chheda, H. & Farrell, C. Minimally invasive parathyroidectomy for primary hyperparathyroidism: decreasing operative time and potential complications while improving cosmetic results. *Am. Surg.* **64,** 391-5-6 (1998).

132. Espiritu, R. P. *et al.* Depression in primary hyperparathyroidism: prevalence and benefit of surgery. *J. Clin. Endocrinol. Metab.* **96,** E1737-45 (2011).

133. Beyer, T. D., Solorzano, C. C., Starr, F., Nilubol, N. & Prinz, R. A. Parathyroidectomy outcomes according to operative approach. *Am. J. Surg.* **193,** 368-72–3 (2007).

134. Doherty, G. M., Weber, B. & Norton, J. A. Cost of unsuccessful surgery for primary hyperparathyroidism. *Surgery* **116,** 954-7-8 (1994).

135. Bollerslev, J. *et al.* Current evidence for recommendation of surgery, medical treatment and vitamin D repletion in mild primary hyperparathyroidism. *Eur. J. Endocrinol.* **165,** 851–64 (2011).

136. Zanocco, K., Heller, M. & Sturgeon, C. Cost-effectiveness of parathyroidectomy for primary hyperparathyroidism. *Endocr. Pract.* **17 Suppl 1,** 69–74

137. Silverberg, S. J. & Bilezikian, J. P. "Incipient" primary hyperparathyroidism: a "forme fruste" of an old disease. *J. Clin. Endocrinol. Metab.* **88,** 5348–52 (2003).

138. Lowe, H., McMahon, D. J., Rubin, M. R., Bilezikian, J. P. & Silverberg, S. J. Normocalcemic primary hyperparathyroidism: further characterization of a new clinical phenotype. *J. Clin. Endocrinol. Metab.* **92,** 3001–5 (2007).

139. Eufrazino, C., Veras, A. & Bandeira, F. Epidemiology of Primary Hyperparathyroidism and its Non-classical Manifestations in the City of Recife, Brazil. *Clin. Med. Insights. Endocrinol. Diabetes* **6,** 69–74 (2013).

140. Lundgren, E., Rastad, J., Thrufjell, E., Akerström, G. & Ljunghall, S. Population-based screening for primary hyperparathyroidism with serum calcium and parathyroid hormone values in menopausal women. *Surgery* **121,** 287–94 (1997).

141. Palmér, M., Jakobsson, S., Akerström, G. & Ljunghall, S. Prevalence of hypercalcaemia in a health survey: a 14-year follow-up study of serum calcium values. *Eur. J. Clin. Invest.* **18,** 39–46 (1988).

142. Oliveira, U. E. M. *et al.* Analysis of the diagnostic presentation profile, parathyroidectomy indication and bone mineral density follow-up of Brazilian patients with primary hyperparathyroidism. *Braz. J. Med. Biol. Res.* **40,** 519–26 (2007).

143. Amaral, L. M., Queiroz, D. C., Marques, T. F., Mendes, M. & Bandeira, F. Normocalcemic versus Hypercalcemic Primary Hyperparathyroidism: More Stone than Bone? *J. Osteoporos.* **2012,** 128352 (2012).

144. Zhao, L. *et al.* The changing clinical patterns of primary hyperparathyroidism in Chinese patients: data from 2000 to 2010 in a single clinical center. *J. Clin. Endocrinol. Metab.* **98,** 721–8 (2013).

145. Eufrazino, C., Veras, A. & Bandeira, F. Epidemiology of Primary Hyperparathyroidism and its Non-classical Manifestations in the City of Recife, Brazil. *Clin. Med. Insights. Endocrinol. Diabetes* **6,** 69–74 (2013).

146. Lo, C.-Y. *et al.* Surgical treatment for primary hyperparathyroidism in Hong Kong: changes in clinical pattern over 3 decades. *Arch. Surg.* **139,** 77–82; discussion 82 (2004).

147. Bandeira, F. *et al.* Prevalence of cortical osteoporosis in mild and severe primary hyperparathyroidism and its relationship with bone markers and vitamin D status. *J. Clin. Densitom.* **12,** 195–9

148. Silva, B. C. *et al.* Trabecular bone score (TBS)--a novel method to evaluate bone microarchitectural texture in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **98,** 1963–70 (2013).

149. Kulak, C. A. *et al.* Marked improvement in bone mass after parathyroidectomy in osteitis fibrosa cystica. *J. Clin. Endocrinol. Metab.* **83,** 732–5 (1998).

150. Vestergaard, P. *et al.* Cohort study of risk of fracture before and after surgery for primary hyperparathyroidism. *BMJ* **321,** 598–602 (2000).

151. Hisa, I. *et al.* Parathyroid hormone-responsive Smad3-related factor, Tmem119, promotes osteoblast differentiation and interacts with the bone morphogenetic protein-Runx2 pathway. *J. Biol. Chem.* **286,** 9787–96 (2011).

152. Vignali, E. *et al.* Morphometric vertebral fractures in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **94,** 2306–12 (2009).

153. Rossini, M. *et al.* Effects of oral alendronate in elderly patients with osteoporosis and mild primary hyperparathyroidism. *J. Bone Miner. Res.* **16,** 113–9 (2001).

154. Fitzpatrick, L. A., Bilezikian, J. P. & Silverberg, S. J. Parathyroid hormone and the cardiovascular system. *Curr. Osteoporos. Rep.* **6,** 77–83 (2008).

155. Starup-Linde, J., Waldhauer, E., Rolighed, L., Mosekilde, L. & Vestergaard, P. Renal stones and calcifications in patients with primary hyperparathyroidism: associations with biochemical variables. *Eur. J. Endocrinol.* **166,** 1093–100 (2012).

156. Diniz, E. T. *et al.* Primary hyperparathyroidism is associated with subclinical peripheral neural alterations. *Endocr. Pract.* **19,** 219–25

157. Talpos, G. B. *et al.* Randomized trial of parathyroidectomy in mild asymptomatic primary hyperparathyroidism: patient description and effects on the SF-36 health survey. *Surgery* **128,** 1013-20–1 (2000).

158. Veras, A., Maia, J., Mesquita, P., Eufrazino, C. & Bandeira, F. Lower quality of life in longstanding mild primary hyperparathyroidism. *Arq. Bras. Endocrinol. Metabol.* **57,** 139–43 (2013).

159. Bess, M. A., Edis, A. J. & van Heerden, J. A. Hyperparathyroidism and pancreatitis. Chance or a causal association? *JAMA* **243,** 246–7 (1980).

160. Walker, M. D. *et al.* Cardiac structure and diastolic function in mild primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **95,** 2172–9 (2010).

161. Iwata, S. *et al.* Aortic valve calcification in mild primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **97,** 132–7 (2012).

162. Rao, D. S., Phillips, E. R., Divine, G. W. & Talpos, G. B. Randomized controlled clinical trial of surgery versus no surgery in patients with mild asymptomatic primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **89,** 5415–22 (2004).

163. Bollerslev, J. *et al.* Medical observation, compared with parathyroidectomy, for asymptomatic primary hyperparathyroidism: a prospective, randomized trial. *J. Clin. Endocrinol. Metab.* **92,** 1687–92 (2007).

164. Ambrogini, E. *et al.* Surgery or surveillance for mild asymptomatic primary hyperparathyroidism: a prospective, randomized clinical trial. *J. Clin. Endocrinol. Metab.* **92,** 3114–21 (2007).

165. Bilezikian, J. P., Khan, A. A., Potts, J. T. & Third International Workshop on the Management of Asymptomatic Primary Hyperthyroidism. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. *J. Clin. Endocrinol. Metab.* **94,** 335–9 (2009).

166. Pasieka, J. L., Parsons, L. & Jones, J. The long-term benefit of parathyroidectomy in primary hyperparathyroidism: a 10-year prospective surgical outcome study. *Surgery* **146,** 1006–13 (2009).

167. Mihai, R. & Sadler, G. P. Pasieka's parathyroid symptoms scores correlate with SF-36 scores in patients undergoing surgery for primary hyperparathyroidism. *World J. Surg.* **32,** 807–14 (2008).

168. Amstrup, A. K., Rejnmark, L. & Mosekilde, L. Patients with surgically cured primary hyperparathyroidism have a reduced quality of life compared with population-based healthy sex-, age-, and season-matched controls. *Eur. J. Endocrinol.* **165,** 753–60 (2011).

169. Tsukahara, K., Sugitani, I., Fujimoto, Y. & Kawabata, K. Surgery did not improve the subjective neuropsychological symptoms of patients with incidentally detected mild primary hyperparathyroidism. *Eur. Arch. Otorhinolaryngol.* **265,** 565–9 (2008).

170. Maruani, G., Hertig, A., Paillard, M. & Houillier, P. Normocalcemic primary hyperparathyroidism: evidence for a generalized target-tissue resistance to parathyroid hormone. *J. Clin. Endocrinol. Metab.* **88,** 4641–8 (2003).

171. Martinez, I., Saracho, R., Montenegro, J. & Llach, F. The importance of dietary calcium and

phosphorous in the secondary hyperparathyroidism of patients with early renal failure. *Am. J. Kidney Dis.* **29,** 496–502 (1997).

172. Paloyan, E., Farland, M. & Pickleman, J. R. Hyperparathyroidism coexisting with hypertension and prolonged thiazide administration. *JAMA* **210,** 1243–5 (1969).

173. Mallette, L. E., Khouri, K., Zengotita, H., Hollis, B. W. & Malini, S. Lithium treatment increases intact and midregion parathyroid hormone and parathyroid volume. *J. Clin. Endocrinol. Metab.* **68,** 654–60 (1989).

174. Balsa JA, Botella-Carretero JI, Peromingo R, Zamarrón I, Arrieta F, Muñoz-Malo T, V. C. Role of calcium malabsorption in the development of secondary hyperparathyroidism after biliopancreatic diversion. - PubMed - NCBI. *JEI* **31,** 845–50 (2008).

175. Wills, M. R., Pak, C. Y., Hammond, W. G. & Bartter, F. C. Normocalcemic primary hyperparathyroidism. *Am. J. Med.* **47,** 384–91 (1969).

176. Bilezikian, J. P., Khan, A. A., Potts, J. T. & Third International Workshop on the Management of Asymptomatic Primary Hyperthyroidism. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. *J. Clin. Endocrinol. Metab.* **94,** 335–9 (2009).

177. Rao, D. S., Wilson, R. J., Kleerekoper, M. & Parfitt, A. M. Lack of biochemical progression or continuation of accelerated bone loss in mild asymptomatic primary hyperparathyroidism: evidence for biphasic disease course. *J. Clin. Endocrinol. Metab.* **67,** 1294–8 (1988).

178. Charopoulos, I. *et al.* Effect of primary hyperparathyroidism on volumetric bone mineral density and bone geometry assessed by peripheral quantitative computed tomography in postmenopausal women. *J. Clin. Endocrinol. Metab.* **91,** 1748–53 (2006).

179. Tordjman, K. M., Greenman, Y., Osher, E., Shenkerman, G. & Stern, N. Characterization of normocalcemic primary hyperparathyroidism. *Am. J. Med.* **117,** 861–3 (2004).

180. García-Martín, A., Reyes-García, R. & Muñoz-Torres, M. Normocalcemic primary hyperparathyroidism: one-year follow-up in one hundred postmenopausal women. *Endocrine* **42,** 764–6 (2012).

181. Bilezikian, J. P. *et al.* Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the Fourth International Workshop. *J. Clin. Endocrinol. Metab.* **99,** 3561–9 (2014).

182. Moure, D. *et al.* 99MTc-sestamibi as sole technique in selection of primary hyperparathyroidism patients for unilateral neck exploration. *Surgery* **144,** 454–9 (2008).

183. Lindqvist, V. *et al.* Preoperative 99Tc(m)-sestamibi scintigraphy with SPECT localizes most pathologic parathyroid glands. *Langenbecks. Arch. Surg.* **394,** 811–5 (2009).

184. Fraker, D. L., Harsono, H. & Lewis, R. Minimally invasive parathyroidectomy: benefits and requirements of localization, diagnosis, and intraoperative PTH monitoring. long-term results. *World J. Surg.* **33,** 2256–65 (2009).

185. Irvin, G. L., Solorzano, C. C. & Carneiro, D. M. Quick intraoperative parathyroid hormone assay: surgical adjunct to allow limited parathyroidectomy, improve success rate, and predict outcome. *World J. Surg.* **28,** 1287–92 (2004).

186. Rubin, M. R. *et al.* The natural history of primary hyperparathyroidism with or without parathyroid surgery after 15 years. *J. Clin. Endocrinol. Metab.* **93,** 3462–70 (2008).

187. Silverberg, S. J., Shane, E., Jacobs, T. P., Siris, E. & Bilezikian, J. P. A 10-year prospective study of primary hyperparathyroidism with or without parathyroid surgery. *N. Engl. J. Med.* **341,** 1249–55 (1999).

188. Silverberg, S. J., Brown, I. & Bilezikian, J. P. Age as a criterion for surgery in primary hyperparathyroidism. *Am. J. Med.* **113,** 681–4 (2002).

189. Grey, A. B., Stapleton, J. P., Evans, M. C., Tatnell, M. A. & Reid, I. R. Effect of hormone replacement therapy on bone mineral density in postmenopausal women with mild primary hyperparathyroidism. A randomized, controlled trial. *Ann. Intern. Med.* **125,** 360–8 (1996).

190. Rubin, M. R., Lee, K. H., McMahon, D. J. & Silverberg, S. J. Raloxifene lowers serum calcium and markers of bone turnover in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 1174–8 (2003).

191. Silverberg, S. J. Vitamin D deficiency and primary hyperparathyroidism. *J. Bone Miner. Res.* **22 Suppl 2,** V100-4 (2007).

192. Tassone, F. *et al.* Vitamin D status in primary hyperparathyroidism: a Southern European perspective. *Clin. Endocrinol. (Oxf).* **79,** 784–90 (2013).

193. Zierold, C., Mings, J. A. & DeLuca, H. F. Regulation of 25-hydroxyvitamin D3-24-hydroxylase mRNA by 1,25-dihydroxyvitamin D3 and parathyroid hormone. *J. Cell. Biochem.* **88,** 234–7 (2003).

194. Bolland, M. J., Grey, A. B., Gamble, G. D. & Reid, I. R. Association between primary hyperparathyroidism and increased body weight: a meta-analysis. *J. Clin. Endocrinol. Metab.* **90,** 1525–30 (2005).

195. Rao, D. S. *et al.* Effect of vitamin D nutrition on parathyroid adenoma weight: pathogenetic and clinical implications. *J. Clin. Endocrinol. Metab.* **85,** 1054–8 (2000).

196. Silverberg, S. J., Shane, E., Dempster, D. W. & Bilezikian, J. P. The effects of vitamin D insufficiency in patients with primary hyperparathyroidism. *Am. J. Med.* **107,** 561–7 (1999).

197. Stein, E. M. *et al.* Vitamin D deficiency influences histomorphometric features of bone in primary hyperparathyroidism. *Bone* **48,** 557–61 (2011).

198. Farahnak, P., Lärfars, G., Sten-Linder, M. & Nilsson, I.-L. Mild primary hyperparathyroidism: vitamin D deficiency and cardiovascular risk markers. *J. Clin. Endocrinol. Metab.* **96,** 2112–8 (2011).

199. Persson, A. *et al.* Effect of surgery on cardiac structure and function in mild primary hyperparathyroidism. *Clin. Endocrinol. (Oxf).* **74,** 174–80 (2011).

200. Grey, A. *et al.* Vitamin D repletion in patients with primary hyperparathyroidism and coexistent vitamin D insufficiency. *J. Clin. Endocrinol. Metab.* **90,** 2122–6 (2005).

201. Tucci, J. R. Vitamin D therapy in patients with primary hyperparathyroidism and hypovitaminosis D. *Eur. J. Endocrinol.* **161,** 189–93 (2009).

202. Velayoudom-Cephise, F.-L. *et al.* [Half of the patients with primary hyperparathyroidisms have a vitamin D deficiency: aggravating the osseous attack]. *Press. médicale (Paris, Fr. 1983)* **40,** e120-7 (2011).

203. Rao, R. R. *et al.* Prolonged treatment with vitamin D in postmenopausal women with primary hyperparathyroidism. *Endocr. Connect.* **1,** 13–21 (2012).

204. Wagner, D., Xia, Y. & Hou, R. Safety of vitamin D replacement in patients with primary hyperparathyroidism and concomitant vitamin D deficiency. *Endocr. Pract.* **19,** 420–5

205. Isidro, M. L. & Ruano, B. Biochemical effects of calcifediol supplementation in mild, asymptomatic, hyperparathyroidism with concomitant vitamin D deficiency. *Endocrine* **36,** 305–10 (2009).

206. Isidro, M. L. & Ruano, B. Biochemical effects of calcifediol supplementation in mild, asymptomatic, hyperparathyroidism with concomitant vitamin D deficiency. *Endocrine* **36,** 305–10 (2009).

207. Stewart, Z. A. *et al.* 25-hydroxyvitamin D deficiency is a risk factor for symptoms of postoperative hypocalcemia and secondary hyperparathyroidism after minimally invasive parathyroidectomy. *Surgery* **138,** 1018-25–6 (2005).

208. Shane, E., Baquiran, D. C. & Bilezikian, J. P. Effects of dichloromethylene diphosphonate on serum and urinary calcium in primary hyperparathyroidism. *Ann. Intern. Med.* **95,** 23–7 (1981).

209. Reasner, C. A., Stone, M. D., Hosking, D. J., Ballah, A. & Mundy, G. R. Acute changes in calcium homeostasis during treatment of primary hyperparathyroidism with risedronate. *J. Clin. Endocrinol. Metab.* **77,** 1067–71 (1993).

210. Schmidli, R. S., Wilson, I., Espiner, E. A., Richards, A. M. & Donald, R. A. Aminopropylidine diphosphonate (APD) in mild primary hyperparathyroidism: effect on clinical status. *Clin. Endocrinol. (Oxf).* **32,** 293–300 (1990).

211. Chow, C. C. *et al.* Oral alendronate increases bone mineral density in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 581–7 (2003).

212. Khan, A. A. *et al.* Alendronate in primary hyperparathyroidism: a double-blind, randomized, placebo-controlled trial. *J. Clin. Endocrinol. Metab.* **89,** 3319–25 (2004).

213. Khan, A. A. *et al.* Alendronate therapy in men with primary hyperparathyroidism. *Endocr. Pract.* **15,** 705–13

214. Orellana, J. M. *et al.* Use of cinacalcet for the management of hyperparathyroidism in patients with different degrees of renal failure. *Nefrol. publicación Of. la Soc. Española Nefrol.* **36,** 121–5 (2016).

215. Christiansen, P. *et al.* Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. *Bone* **21,** 93–9 (1997).

216. Silverberg, S. J. *et al.* Longitudinal measurements of bone density and biochemical indices in untreated primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **80,** 723–8 (1995).

217. Silverberg, S. J. *et al.* Longitudinal measurements of bone density and biochemical indices in untreated primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **80,** 723–8 (1995).

218. Christiansen, P. *et al.* Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. *Bone* **21,** 93–9 (1997).

219. Thorsen, K., Kristoffersson, A. O. & Lorentzon, R. P. Changes in bone mass and serum markers of bone metabolism after parathyroidectomy. *Surgery* **122,** 882–7 (1997).

220. Christiansen, P. *et al.* Primary hyperparathyroidism: short-term changes in bone remodeling and bone mineral density following parathyroidectomy. *Bone* **25,** 237–44 (1999).

221. Steiniche, T. *et al.* Primary hyperparathyroidism: bone structure, balance, and remodeling before and 3 years after surgical treatment. *Bone* **26,** 535–43 (2000).

222. Garton, M. *et al.* Changes in bone mass and metabolism after surgery for primary hyperparathyroidism. *Clin. Endocrinol. (Oxf).* **42,** 493–500 (1995).

223. Christiansen, P. *et al.* Primary hyperparathyroidism: short-term changes in bone remodeling and bone mineral density following parathyroidectomy. *Bone* **25,** 237–44 (1999).

224. Tamura, Y. *et al.* Remarkable increase in lumbar spine bone mineral density and amelioration in biochemical markers of bone turnover after parathyroidectomy in elderly patients with primary hyperparathyroidism: a 5-year follow-up study. *J. Bone Miner. Metab.* **25,** 226–31 (2007).

225. Tamura, Y. *et al.* Remarkable increase in lumbar spine bone mineral density and amelioration in biochemical markers of bone turnover after parathyroidectomy in elderly patients with primary hyperparathyroidism: a 5-year follow-up study. *J. Bone Miner. Metab.* **25,** 226–31 (2007).

226. Nakchbandi, I. A. *et al.* Circulating levels of interleukin-6 soluble receptor predict rates of bone loss in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **87,** 4946–51 (2002).

227. Guo, C. Y. *et al.* Immediate changes in biochemical markers of bone turnover and circulating interleukin-6 after parathyroidectomy for primary hyperparathyroidism. *Eur. J. Endocrinol.* **142,** 451–9 (2000).

228. Nakchbandi, I. A. *et al.* Circulating levels of interleukin-6 soluble receptor predict rates of bone loss

in patients with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **87,** 4946–51 (2002).

229. Keller, H. & Kneissel, M. SOST is a target gene for PTH in bone. *Bone* **37,** 148–58 (2005).

230. Costa, A. G. *et al.* Circulating sclerostin in disorders of parathyroid gland function. *J. Clin. Endocrinol. Metab.* **96,** 3804–10 (2011).

231. van Lierop, A. H., Witteveen, J. E., Hamdy, N. A. T. & Papapoulos, S. E. Patients with primary hyperparathyroidism have lower circulating sclerostin levels than euparathyroid controls. *Eur. J. Endocrinol.* **163,** 833–7 (2010).

232. Viapiana, O. *et al.* Sclerostin and DKK1 in primary hyperparathyroidism. *Calcif. Tissue Int.* **92,** 324–9 (2013).

233. Ardawi, M.-S. M., Al-Sibiany, A. M., Bakhsh, T. M., Rouzi, A. A. & Qari, M. H. Decreased serum sclerostin levels in patients with primary hyperparathyroidism: a cross-sectional and a longitudinal study. *Osteoporos. Int.* **23,** 1789–97 (2012).

234. Cusano, N. E., Silverberg, S. J. & Bilezikian, J. P. Normocalcemic primary hyperparathyroidism. *J. Clin. Densitom.* **16,** 33–9

235. Udelsman, R. *et al.* The surgical management of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. *J. Clin. Endocrinol. Metab.* **99,** 3595–606 (2014).

236. Sankaran, S., Gamble, G., Bolland, M., Reid, I. R. & Grey, A. Skeletal effects of interventions in mild primary hyperparathyroidism: a meta-analysis. *J. Clin. Endocrinol. Metab.* **95,** 1653–62 (2010).

237. Rolighed, L. *et al.* Vitamin D treatment in primary hyperparathyroidism: a randomized placebo controlled trial. *J. Clin. Endocrinol. Metab.* **99,** 1072–80 (2014).

238. Kantorovich, V., Gacad, M. A., Seeger, L. L. & Adams, J. S. Bone mineral density increases with vitamin D repletion in patients with coexistent vitamin D insufficiency and primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **85,** 3541–3 (2000).

239. Grubbs, E. G. *et al.* Preoperative vitamin D replacement therapy in primary hyperparathyroidism: safe and beneficial? *Surgery* **144,** 852-8-9 (2008).

240. Montomoli, M. *et al.* Validation of a food frequency questionnaire for nutritional calcium intake assessment in Italian women. *Eur. J. Clin. Nutr.* **56,** 21–30 (2002).

241. Khan, A. A. *et al.* Alendronate in primary hyperparathyroidism: a double-blind, randomized, placebo-controlled trial. *J. Clin. Endocrinol. Metab.* **89,** 3319–25 (2004).

242. Chow, C. C. *et al.* Oral alendronate increases bone mineral density in postmenopausal women with primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **88,** 581–7 (2003).

243. Sankaran, S., Gamble, G., Bolland, M., Reid, I. R. & Grey, A. Skeletal effects of interventions in mild primary hyperparathyroidism: a meta-analysis. *J. Clin. Endocrinol. Metab.* **95,** 1653–62 (2010).

244. Khan, A. A. *et al.* Alendronate therapy in men with primary hyperparathyroidism. *Endocr. Pract.* **15,** 705–13

245. Black, D. M. *et al.* Effects of continuing or stopping alendronate after 5 years of treatment: the Fracture Intervention Trial Long-term Extension (FLEX): a randomized trial. *JAMA* **296,** 2927–38 (2006).

246. Grubbs, E. G. *et al.* Preoperative vitamin D replacement therapy in primary hyperparathyroidism: safe and beneficial? *Surgery* **144,** 852-8-9 (2008).

247. Rolighed, L. *et al.* Vitamin D treatment in primary hyperparathyroidism: a randomized placebo controlled trial. *J. Clin. Endocrinol. Metab.* **99,** 1072–80 (2014).

248. Kantorovich, V., Gacad, M. A., Seeger, L. L. & Adams, J. S. Bone mineral density increases with vitamin D repletion in patients with coexistent vitamin D insufficiency and primary hyperparathyroidism. *J. Clin. Endocrinol. Metab.* **85,** 3541–3 (2000).

249. Macdonald, H. M. *et al.* Hip bone loss is attenuated with 1000 IU but not 400 IU daily vitamin D3: a 1-year double-blind RCT in postmenopausal women. *J. Bone Miner. Res.* **28,** 2202–13 (2013).

250. Recker, R. *et al.* Alendronate with and without cholecalciferol for osteoporosis: results of a 15-week randomized controlled trial. *Curr. Med. Res. Opin.* **22,** 1745–55 (2006).

251. Raimundo, F. V., Faulhaber, G. A. M., Menegatti, P. K., Marques, L. da S. & Furlanetto, T. W. Effect of High- versus Low-Fat Meal on Serum 25-Hydroxyvitamin D Levels after a Single Oral Dose of Vitamin D: A Single-Blind, Parallel, Randomized Trial. *Int. J. Endocrinol.* **2011,** 809069 (2011).

252. Heaney, R. P., Davies, K. M., Chen, T. C., Holick, M. F. & Barger-Lux, M. J. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am. J. Clin. Nutr.* **77,** 204–10 (2003).

253. Holick, M. F. & Chen, T. C. Vitamin D deficiency: a worldwide problem with health consequences. *Am. J. Clin. Nutr.* **87,** 1080S–6S (2008).

254. Kelly, A. & Levine, M. A. Hypocalcemia in the critically ill patient. *J. Intensive Care Med.* **28,** 166–77

255. Bilezikian, J. P. *et al.* Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J. Bone Miner. Res.* **26,** 2317–37 (2011).

256. Khan, M. I., Waguespack, S. G. & Hu, M. I. Medical management of postsurgical hypoparathyroidism. *Endocr. Pract.* **17 Suppl 1,** 18–25

257. Mittelman, S. D. *et al.* A hypocalcemic child with a novel activating mutation of the calcium-sensing receptor gene: successful treatment with recombinant human parathyroid hormone. *J. Clin. Endocrinol. Metab.* **91,** 2474–9 (2006).

258. Pearce, S. H. *et al.* A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. *N. Engl. J. Med.* **335,** 1115–22 (1996).

259. Michels, A. W. & Gottlieb, P. A. Autoimmune polyglandular syndromes. *Nat. Rev. Endocrinol.* **6,** 270–7 (2010).

260. Thomée, C. *et al.* GCMB mutation in familial isolated hypoparathyroidism with residual secretion of parathyroid hormone. *J. Clin. Endocrinol. Metab.* **90,** 2487–92 (2005).

261. Ali, A. *et al.* Functional characterization of GATA3 mutations causing the hypoparathyroidism-deafness-renal (HDR) dysplasia syndrome: insight into mechanisms of DNA binding by the GATA3 transcription factor. *Hum. Mol. Genet.* **16,** 265–75 (2007).

262. Ertl, D.-A., Stary, S., Streubel, B., Raimann, A. & Haeusler, G. A novel homozygous mutation in the parathyroid hormone gene (PTH) in a girl with isolated hypoparathyroidism. *Bone* **51,** 629–32 (2012).

263. Nesbit, M. A. *et al.* Mutations affecting G-protein subunit α11 in hypercalcemia and hypocalcemia. *N. Engl. J. Med.* **368,** 2476–86 (2013).

264. Upadhyay, J., Steenkamp, D. W. & Milunsky, J. M. The syndrome of hypoparathyroidism, deafness, and renal anomalies. *Endocr. Pract.* **19,** 1035–42

265. Sullivan, K. E. Chromosome 22q11.2 deletion syndrome: DiGeorge syndrome/velocardiofacial Syndrome. *Immunol. Allergy Clin. North Am.* **28,** 353–66 (2008).

266. Labarthe, F. *et al.* Partial hypoparathyroidism associated with mitochondrial trifunctional protein deficiency. *Eur. J. Pediatr.* **165,** 389–91 (2006).

267. Shoback, D. Clinical practice. Hypoparathyroidism. *N. Engl. J. Med.* **359,** 391–403 (2008).

268. Thomusch, O. *et al.* The impact of surgical technique on postoperative hypoparathyroidism in bilateral thyroid surgery: a multivariate analysis of 5846 consecutive patients. *Surgery* **133,** 180–5 (2003).

269. Asari, R., Passler, C., Kaczirek, K., Scheuba, C. & Niederle, B. Hypoparathyroidism after total thyroidectomy: a prospective study. *Arch. Surg.* **143,** 132–7; discussion 138 (2008).

270. Powers, J., Joy, K., Ruscio, A. & Lagast, H. Prevalence and incidence of hypoparathyroidism in the United States using a large claims database. *J. Bone Miner. Res.* **28,** 2570–6 (2013).

271. Wingert, D. J. *et al.* Post-thyroidectomy hypocalcemia. Incidence and risk factors. *Am. J. Surg.* **152,** 606–10 (1986).

272. Asari, R., Passler, C., Kaczirek, K., Scheuba, C. & Niederle, B. Hypoparathyroidism after total thyroidectomy: a prospective study. *Arch. Surg.* **143,** 132–7; discussion 138 (2008).

273. Toniato, A. *et al.* Complications in thyroid surgery for carcinoma: one institution's surgical experience. *World J. Surg.* **32,** 572–5 (2008).

274. Toniato, A. *et al.* Complications in thyroid surgery for carcinoma: one institution's surgical experience. *World J. Surg.* **32,** 572–5 (2008).

275. Lindblom, P., Westerdahl, J. & Bergenfelz, A. Low parathyroid hormone levels after thyroid surgery: a feasible predictor of hypocalcemia. *Surgery* **131,** 515–20 (2002).

276. Thomusch, O. *et al.* The impact of surgical technique on postoperative hypoparathyroidism in bilateral thyroid surgery: a multivariate analysis of 5846 consecutive patients. *Surgery* **133,** 180–5 (2003).

277. Palazzo, F. F., Sywak, M. S., Sidhu, S. B., Barraclough, B. H. & Delbridge, L. W. Parathyroid autotransplantation during total thyroidectomy--does the number of glands transplanted affect outcome? *World J. Surg.* **29,** 629–31 (2005).

278. Palazzo, F. F., Sywak, M. S., Sidhu, S. B., Barraclough, B. H. & Delbridge, L. W. Parathyroid autotransplantation during total thyroidectomy--does the number of glands transplanted affect outcome? *World J. Surg.* **29,** 629–31 (2005).

279. Ahonen, P., Myllärniemi, S., Sipilä, I. & Perheentupa, J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N. Engl. J. Med.* **322,** 1829–36 (1990).

280. Ahonen, P., Myllärniemi, S., Sipilä, I. & Perheentupa, J. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N. Engl. J. Med.* **322,** 1829–36 (1990).

281. Björses, P. *et al.* Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am. J. Hum. Genet.* **66,** 378–92 (2000).

282. Lankisch, T. O., Jaeckel, E. & Strassburg, C. P. The autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy or autoimmune polyglandular syndrome type 1. *Semin. Liver Dis.* **29,** 307–14 (2009).

283. Alimohammadi, M. *et al.* Autoimmune polyendocrine syndrome type 1 and NALP5, a parathyroid autoantigen. *N. Engl. J. Med.* **358,** 1018–28 (2008).

284. Husebye, E. S., Perheentupa, J., Rautemaa, R. & Kämpe, O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J. Intern. Med.* **265,** 514–29 (2009).

285. Kemp, E. H. *et al.* Activating autoantibodies against the calcium-sensing receptor detected in two patients with autoimmune polyendocrine syndrome type 1. *J. Clin. Endocrinol. Metab.* **94,** 4749–56 (2009).

286. Bräuner-Osborne, H., Wellendorph, P. & Jensen, A. A. Structure, pharmacology and therapeutic prospects of family C G-protein coupled receptors. *Curr. Drug Targets* **8,** 169–84 (2007).

287. Hauache, O. M. Extracellular calcium-sensing receptor: structural and functional features and association with diseases. *Brazilian J. Med. Biol. Res. = Rev. Bras. Pesqui. médicas e biológicas / Soc. Bras. Biofísica ... [et al.]* **34,** 577–84 (2001).

288. Blizzard, R. M., Chee, D. & Davis, W. The incidence of parathyroid and other antibodies in the sera of patients with idiopathic hypoparathyroidism. *Clin. Exp. Immunol.* **1,** 119–28 (1966).

289. Li, Y. *et al.* Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *J. Clin. Invest.* **97,** 910–4 (1996).

290. Kifor, O. *et al.* Activating antibodies to the calcium-sensing receptor in two patients with autoimmune hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **89,** 548–56 (2004).

291. Cusano, N. E. *et al.* Normocalcemic hyperparathyroidism and hypoparathyroidism in two community-based nonreferral populations. *J. Clin. Endocrinol. Metab.* **98,** 2734–41 (2013).

292. Schussheim, D. H., Jacobs, T. P. & Silverberg, S. J. Hypocalcemia associated with alendronate. *Ann. Intern. Med.* **130,** 329 (1999).

293. Zarnegar, R., Brunaud, L. & Clark, O. H. Prevention, evaluation, and management of complications following thyroidectomy for thyroid carcinoma. *Endocrinol. Metab. Clin. North Am.* **32,** 483–502 (2003).

294. Zarnegar, R., Brunaud, L. & Clark, O. H. Prevention, evaluation, and management of complications following thyroidectomy for thyroid carcinoma. *Endocrinol. Metab. Clin. North Am.* **32,** 483–502 (2003).

295. Sousa, A. de A. *et al.* Predictors factors for post-thyroidectomy hypocalcaemia. *Rev. do Colégio Bras. Cir.* **39,** 476–82 (2012).

296. Welch, K. C. & McHenry, C. R. Total thyroidectomy: is morbidity higher for Graves' disease than nontoxic goiter? *J. Surg. Res.* **170,** 96–9 (2011).

297. Witteveen, J. E., van Thiel, S., Romijn, J. A. & Hamdy, N. A. T. Hungry bone syndrome: still a challenge in the post-operative management of primary hyperparathyroidism: a systematic review of the literature. *Eur. J. Endocrinol.* **168,** R45-53 (2013).

298. Khan, M. I., Waguespack, S. G. & Hu, M. I. Medical management of postsurgical hypoparathyroidism. *Endocr. Pract.* **17 Suppl 1,** 18–25

299. Witteveen, J. E., van Thiel, S., Romijn, J. A. & Hamdy, N. A. T. Hungry bone syndrome: still a challenge in the post-operative management of primary hyperparathyroidism: a systematic review of the literature. *Eur. J. Endocrinol.* **168,** R45-53 (2013).

300. Goldfarb, M. *et al.* Postoperative hungry bone syndrome in patients with secondary hyperparathyroidism of renal origin. *World J. Surg.* **36,** 1314–9 (2012).

301. Latus, J. *et al.* Incidence of and risk factors for hungry bone syndrome in 84 patients with secondary

hyperparathyroidism. *Int. J. Nephrol. Renovasc. Dis.* **6,** 131–7 (2013).

302. Cooper, M. S. & Gittoes, N. J. L. Diagnosis and management of hypocalcaemia. *BMJ* **336,** 1298–302 (2008).

303. Hermann, M. *et al.* Kinetics of serum parathyroid hormone during and after thyroid surgery. *Br. J. Surg.* **95,** 1480–7 (2008).

304. Promberger, R. *et al.* Normal parathyroid hormone levels do not exclude permanent hypoparathyroidism after thyroidectomy. *Thyroid* **21,** 145–50 (2011).

305. Prichard, R. S., Edhouse, P. J., Sidhu, S. B., Sywak, M. S. & Delbridge, L. Post-operative partial hypoparathyroidism: an under-recognized disorder. *ANZ J. Surg.* **81,** 524–7

306. Wilson, R. B., Erskine, C. & Crowe, P. J. Hypomagnesemia and hypocalcemia after thyroidectomy: prospective study. *World J. Surg.* **24,** 722–6 (2000).

307. Seybt, M. W. & Terris, D. J. Outpatient thyroidectomy: experience in over 200 patients. *Laryngoscope* **120,** 959–63 (2010).

308. Miccoli, P., Barellini, L., Monchik, J. M., Rago, R. & Berti, P. F. Randomized clinical trial comparing regional and general anaesthesia in minimally invasive video-assisted parathyroidectomy. *Br. J. Surg.* **92,** 814–8 (2005).

309. Wong, C., Price, S. & Scott-Coombes, D. Hypocalcaemia and parathyroid hormone assay following total thyroidectomy: predicting the future. *World J. Surg.* **30,** 825–32 (2006).

310. Grodski, S. & Serpell, J. Evidence for the role of perioperative PTH measurement after total thyroidectomy as a predictor of hypocalcemia. *World J. Surg.* **32,** 1367–73 (2008).

311. Grodski, S., Lundgren, C. I., Sidhu, S., Sywak, M. & Delbridge, L. Postoperative PTH measurement facilitates day 1 discharge after total thyroidectomy. *Clin. Endocrinol. (Oxf).* **70,** 322–5 (2009).

312. Noordzij, J. P. *et al.* Early prediction of hypocalcemia after thyroidectomy using parathyroid hormone: an analysis of pooled individual patient data from nine observational studies. *J. Am. Coll. Surg.* **205,** 748–54 (2007).

313. Darr, E. A. & Randolph, G. W. Management of laryngeal nerves and parathyroid glands at thyroidectomy. *Oral Oncol.* **49,** 665–70 (2013).

314. Ross, A. C. *et al.* The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J. Clin. Endocrinol. Metab.* **96,** 53–8 (2011).

315. Arlt, W. *et al.* Well-being, mood and calcium homeostasis in patients with hypoparathyroidism receiving standard treatment with calcium and vitamin D. *Eur. J. Endocrinol.* **146,** 215–22 (2002).

316. Mitchell, D. M. *et al.* Long-term follow-up of patients with hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **97,** 4507–14 (2012).

317. Weber, G. *et al.* Nephrocalcinosis in children and adolescents: sonographic evaluation during long-term treatment with 1,25-dihydroxycholecalciferol. *Child Nephrol. Urol.* **9,** 273–6

318. Rubin, M. R. *et al.* Dynamic and structural properties of the skeleton in hypoparathyroidism. *J. Bone Miner. Res.* **23,** 2018–24 (2008).

319. Mitchell, D. M. *et al.* Long-term follow-up of patients with hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **97,** 4507–14 (2012).

320. Winer, K. K. *et al.* Long-term treatment of hypoparathyroidism: a randomized controlled study comparing parathyroid hormone-(1-34) versus calcitriol and calcium. *J. Clin. Endocrinol. Metab.* **88,** 4214–20 (2003).

321. Winer, K. K., Yanovski, J. A. & Cutler, G. B. Synthetic human parathyroid hormone 1-34 vs calcitriol and calcium in the treatment of hypoparathyroidism. *JAMA* **276,** 631–6 (1996).

322. Winer, K. K., Yanovski, J. A., Sarani, B. & Cutler, G. B. A randomized, cross-over trial of once-daily versus twice-daily parathyroid hormone 1-34 in treatment of hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **83,** 3480–6 (1998).

323. Langdahl, B. L., Mortensen, L., Vesterby, A., Eriksen, E. F. & Charles, P. Bone histomorphometry in hypoparathyroid patients treated with vitamin D. *Bone* **18,** 103–8 (1996).

324. Touliatos, J. S. *et al.* Hypoparathyroidism counteracts risk factors for osteoporosis. *Am. J. Med. Sci.* **310,** 56–60 (1995).

325. Goswami, R. *et al.* Prevalence and progression of basal ganglia calcification and its pathogenic mechanism in patients with idiopathic hypoparathyroidism. *Clin. Endocrinol. (Oxf).* **77,** 200–6 (2012).

326. Goswami, R. *et al.* Prevalence of calcium sensing receptor autoantibodies in patients with sporadic idiopathic hypoparathyroidism. *Eur. J. Endocrinol.* **150,** 9–18 (2004).

327. Kowdley, K. V, Coull, B. M. & Orwoll, E. S. Cognitive impairment and intracranial calcification in chronic hypoparathyroidism. *Am. J. Med. Sci.* **317,** 273–7 (1999).

328. López-Villegas, D. *et al.* Neuropsychological alterations in patients with computed tomography-detected basal ganglia calcification. *Arch. Neurol.* **53,** 251–6 (1996).

329. Fénelon, G. *et al.* A prospective study of patients with CT detected pallidal calcifications. *J. Neurol. Neurosurg. Psychiatry* **56,** 622–5 (1993).

330. Raue, F. *et al.* Activating mutations in the calcium-sensing receptor: genetic and clinical spectrum in 25 patients with autosomal dominant hypocalcaemia - a German survey. *Clin. Endocrinol. (Oxf).* **75,** 760–5 (2011).

331. Kruse, K., Kracht, U., Wohlfart, K. & Kruse, U. Biochemical markers of bone turnover, intact serum parathyroid horn and renal calcium excretion in patients with pseudohypoparathyroidism and

hypoparathyroidism before and during vitamin D treatment. *Eur. J. Pediatr.* **148,** 535–9 (1989).

332. Mizunashi, K. *et al.* Effects of active vitamin D3 and parathyroid hormone on the serum osteocalcin in idiopathic hypoparathyroidism and pseudohypoparathyroidism. *J. Clin. Invest.* **82,** 861–5 (1988).

333. Rubin, M. R. *et al.* PTH(1-84) administration reverses abnormal bone-remodeling dynamics and structure in hypoparathyroidism. *J. Bone Miner. Res.* **26,** 2727–36 (2011).

334. Rubin, M. R. *et al.* Parathyroid hormone stimulates circulating osteogenic cells in hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **96,** 176–86 (2011).

335. Abugassa, S., Nordenström, J., Eriksson, S. & Sjödén, G. Bone mineral density in patients with chronic hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **76,** 1617–21 (1993).

336. Fujiyama, K. *et al.* Attenuation of postmenopausal high turnover bone loss in patients with hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **80,** 2135–8 (1995).

337. Duan, Y., De Luca, V. & Seeman, E. Parathyroid hormone deficiency and excess: similar effects on trabecular bone but differing effects on cortical bone. *J. Clin. Endocrinol. Metab.* **84,** 718–22 (1999).

338. Chen, Q. *et al.* Effects of an excess and a deficiency of endogenous parathyroid hormone on volumetric bone mineral density and bone geometry determined by peripheral quantitative computed tomography in female subjects. *J. Clin. Endocrinol. Metab.* **88,** 4655–8 (2003).

339. Malluche, H. H. *et al.* 1,25-Dihydroxyvitamin D maintains bone cell activity, and parathyroid hormone modulates bone cell number in dogs. *Endocrinology* **119,** 1298–304 (1986).

340. Rubin, M. R. *et al.* Three dimensional cancellous bone structure in hypoparathyroidism. *Bone* **46,** 190–5 (2010).

341. Hadker, N., Egan, J., Sanders, J., Lagast, H. & Clarke, B. L. Understanding the burden of illness associated with hypoparathyroidism reported among patients in the PARADOX study. *Endocr. Pract.* **20,** 671–9 (2014).

342. Leibson C, Clarke BL, Ransom JE, L. H. Medical care costs for persons with and without prevalent hypoparathyroidism: a populationbased study. *JBMR* **26,** 182 (2011).

343. Hunter, D. *et al.* Genetic contribution to bone metabolism, calcium excretion, and vitamin D and parathyroid hormone regulation. *J. Bone Miner. Res.* **16,** 371–8 (2001).

344. Hannan, F. M. *et al.* Identification of 70 calcium-sensing receptor mutations in hyper- and hypo-calcaemic patients: evidence for clustering of extracellular domain mutations at calcium-binding sites. *Hum. Mol. Genet.* **21,** 2768–78 (2012).

345. Kapur, K. *et al.* Genome-wide meta-analysis for serum calcium identifies significantly associated SNPs near the calcium-sensing receptor (CASR) gene. *PLoS Genet.* **6,** e1001035 (2010).

346. Eastell, R. *et al.* Diagnosis of asymptomatic primary hyperparathyroidism: proceedings of the Fourth International Workshop. *J. Clin. Endocrinol. Metab.* **99,** 3570–9 (2014).

347. Monchik, J. M. & Gorgun, E. Normocalcemic hyperparathyroidism in patients with osteoporosis. *Surgery* **136,** 1242–6 (2004).

348. Schussheim, D. H., Jacobs, T. P. & Silverberg, S. J. Hypocalcemia associated with alendronate. *Ann. Intern. Med.* **130,** 329 (1999).

349. Oltmann, S. C., Rajaei, M. H., Sippel, R. S., Chen, H. & Schneider, D. F. Primary hyperparathyroidism across the ages: presentation and outcomes. *J. Surg. Res.* **190,** 185–90 (2014).

350. Glüer, C. C. *et al.* Association of five quantitative ultrasound devices and bone densitometry with osteoporotic vertebral fractures in a population-based sample: the OPUS Study. *J. Bone Miner. Res.* **19,** 782–93 (2004).

351. O'Neill, T. W. *et al.* Reproducibility of a questionnaire on risk factors for osteoporosis in a multicentre prevalence survey: the European Vertebral Osteoporosis Study. *Int. J. Epidemiol.* **23,** 559–65 (1994).

352. James, M. T., Zhang, J., Lyon, A. W. & Hemmelgarn, B. R. Derivation and internal validation of an equation for albumin-adjusted calcium. *BMC Clin. Pathol.* **8,** 12 (2008).

353. Levey, A. S. *et al.* Expressing the Modification of Diet in Renal Disease Study equation for estimating glomerular filtration rate with standardized serum creatinine values. *Clin. Chem.* **53,** 766–72 (2007).

354. Minisola, S. *et al.* Clinical validation of a new immunoradiometric assay for intact human osteocalcin. *Calcif. Tissue Int.* **64,** 365–9 (1999).

355. Okabe, R. *et al.* Clinical evaluation of the Elecsys beta-CrossLaps serum assay, a new assay for degradation products of type I collagen C-tlopeptides. *Clin. Chem.* **47,** 1410–4 (2001).

356. Sikjaer, T. *et al.* The effect of adding PTH(1-84) to conventional treatment of hypoparathyroidism: a randomized, placebo-controlled study. *J. Bone Miner. Res.* **26,** 2358–70 (2011).

357. Frazão, J. M. & Martins, P. Adynamic bone disease: clinical and therapeutic implications. *Curr. Opin. Nephrol. Hypertens.* **18,** 303–7 (2009).

358. Winer, K. K. *et al.* Synthetic human parathyroid hormone 1-34 replacement therapy: a randomized crossover trial comparing pump versus injections in the treatment of chronic hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **97,** 391–9 (2012).

359. Cusano, N. E., Rubin, M. R., Sliney, J. & Bilezikian, J. P. Mini-review: new therapeutic options in hypoparathyroidism. *Endocrine* **41,** 410–4 (2012).

360. Shibli-Rahhal, A. & Paturi, B. Variations in parathyroid hormone concentration in patients with low 25 hydroxyvitamin D. *Osteoporos. Int.* **25,** 1931–6 (2014).

361. Hannan, F. M. & Thakker, R. V. Calcium-sensing receptor (CaSR) mutations and disorders of calcium, electrolyte and water metabolism. *Best Pract. Res. Clin. Endocrinol. Metab.* **27,** 359–71

(2013).

362. Kemp, E. H. *et al.* Prevalence and clinical associations of calcium-sensing receptor and NALP5 autoantibodies in Finnish APECED patients. *J. Clin. Endocrinol. Metab.* **99,** 1064–71 (2014).

363. Riggs, B. L., Khosla, S. & Melton, L. J. A unitary model for involutional osteoporosis: estrogen deficiency causes both type I and type II osteoporosis in postmenopausal women and contributes to bone loss in aging men. *J. Bone Miner. Res.* **13,** 763–73 (1998).

364. Khosla, S., Atkinson, E. J., Melton, L. J. & Riggs, B. L. Effects of age and estrogen status on serum parathyroid hormone levels and biochemical markers of bone turnover in women: a population-based study. *J. Clin. Endocrinol. Metab.* **82,** 1522–7 (1997).

365. AC, L. *Dietary calcium: recommendations and intakes around the world (Chapter 8)*. (2006).

366. Reber, P. M. & Heath, H. Hypocalcemic emergencies. *Med. Clin. North Am.* **79,** 93–106 (1995).

367. Al-Azem, H. & Khan, A. A. Hypoparathyroidism. *Best Pract. Res. Clin. Endocrinol. Metab.* **26,** 517–22 (2012).

368. Bollerslev, J. *et al.* European Society of Endocrinology Clinical Guideline: Treatment of chronic hypoparathyroidism in adults. *Eur. J. Endocrinol.* **173,** G1-20 (2015).

369. Holick, M. F. Vitamin D deficiency. *N. Engl. J. Med.* **357,** 266–81 (2007).

370. Quack, I. *et al.* Dihydrotachysterol therapy for hypoparathyroidism: consequences of inadequate monitoring. Five cases and a review. *Exp. Clin. Endocrinol. Diabetes* **113,** 376–80 (2005).

371. Onyia, J. E. *et al.* In vivo demonstration that human parathyroid hormone 1-38 inhibits the expression of osteoprotegerin in bone with the kinetics of an immediate early gene. *J. Bone Miner. Res.* **15,** 863–71 (2000).

372. Halabe, A., Arie, R., Mimran, D., Samuel, R. & Liberman, U. A. Hypoparathyroidism--a long-term follow-up experience with 1 alpha-vitamin D3 therapy. *Clin. Endocrinol. (Oxf).* **40,** 303–7 (1994).

373. Shiohara, M. *et al.* Effect of parathyroid hormone administration in a patient with severe hypoparathyroidism caused by gain-of-function mutation of calcium-sensing receptor. *Endocr. J.* **53,** 797–802 (2006).

374. Theman, T. A. *et al.* PTH(1-34) replacement therapy in a child with hypoparathyroidism caused by a sporadic calcium receptor mutation. *J. Bone Miner. Res.* **24,** 964–73 (2009).

375. Winer, K. K., Sinaii, N., Peterson, D., Sainz, B. & Cutler, G. B. Effects of once versus twice-daily parathyroid hormone 1-34 therapy in children with hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **93,** 3389–95 (2008).

376. Winer, K. K. *et al.* Long-term treatment of 12 children with chronic hypoparathyroidism: a randomized trial comparing synthetic human parathyroid hormone 1-34 versus calcitriol and calcium. *J. Clin. Endocrinol. Metab.* **95,** 2680–8 (2010).

377. Winer, K. K. *et al.* Long-term treatment of hypoparathyroidism: a randomized controlled study comparing parathyroid hormone-(1-34) versus calcitriol and calcium. *J. Clin. Endocrinol. Metab.* **88,** 4214–20 (2003).

378. Díaz-Soto, G. *et al.* Efficacy and safety of long term treatment of unresponsive hypoparathyroidism using multipulse subcutaneous infusion of teriparatide. *Horm. Metab. Res. = Horm. und Stoffwechselforsch. = Horm. métabolisme* **44,** 708–10 (2012).

379. Linglart, A. *et al.* Long-term results of continuous subcutaneous recombinant PTH (1-34) infusion in children with refractory hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **96,** 3308–12 (2011).

380. Puig-Domingo, M. *et al.* Successful treatment of vitamin D unresponsive hypoparathyroidism with multipulse subcutaneous infusion of teriparatide. *Eur. J. Endocrinol.* **159,** 653–7 (2008).

381. Rubin, M. R., Sliney, J., McMahon, D. J., Silverberg, S. J. & Bilezikian, J. P. Therapy of hypoparathyroidism with intact parathyroid hormone. *Osteoporos. Int.* **21,** 1927–34 (2010).

382. Cusano, N. E. *et al.* Therapy of hypoparathyroidism with PTH(1-84): a prospective four-year investigation of efficacy and safety. *J. Clin. Endocrinol. Metab.* **98,** 137–44 (2013).

383. Ware, J. E. & Sherbourne, C. D. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med. Care* **30,** 473–83 (1992).

384. Laursen, T., Jørgensen, J. O. & Christiansen, J. S. Metabolic effects of growth hormone administered subcutaneously once or twice daily to growth hormone deficient adults. *Clin. Endocrinol. (Oxf).* **41,** 337–43 (1994).

385. Mannstadt, M. *et al.* Efficacy and safety of recombinant human parathyroid hormone (1-84) in hypoparathyroidism (REPLACE): a double-blind, placebo-controlled, randomised, phase 3 study. *lancet. Diabetes Endocrinol.* **1,** 275–83 (2013).

386. Watanabe, A. *et al.* Osteosarcoma in Sprague-Dawley rats after long-term treatment with teriparatide (human parathyroid hormone (1-34)). *J. Toxicol. Sci.* **37,** 617–29 (2012).

387. Vahle, J. L. *et al.* Bone neoplasms in F344 rats given teriparatide [rhPTH(1-34)] are dependent on duration of treatment and dose. *Toxicol. Pathol.* **32,** 426–38

388. Vahle, J. L. *et al.* Lack of bone neoplasms and persistence of bone efficacy in cynomolgus macaques after long-term treatment with teriparatide [rhPTH(1-34)]. *J. Bone Miner. Res.* **23,** 2033–9 (2008).

389. Fox, J. *et al.* Treatment of skeletally mature ovariectomized rhesus monkeys with PTH(1-84) for 16 months increases bone formation and density and improves trabecular architecture and biomechanical properties at the lumbar spine. *J. Bone Miner. Res.* **22,** 260–73 (2007).

390. Andrews, E. B. *et al.* The US postmarketing surveillance study of adult osteosarcoma and teriparatide: study design and findings from the first 7 years. *J. Bone Miner. Res.* **27,** 2429–37 (2012).

391. Subbiah, V., Madsen, V. S., Raymond, A. K., Benjamin, R. S. & Ludwig, J. A. Of mice and men:

divergent risks of teriparatide-induced osteosarcoma. *Osteoporos. Int.* **21,** 1041–5 (2010).

392. Sikjaer, T. *et al.* PTH(1-84) replacement therapy in hypoparathyroidism: a randomized controlled trial on pharmacokinetic and dynamic effects after 6 months of treatment. *J. Bone Miner. Res.* **28,** 2232–43 (2013).

393. Cusano, N. E. *et al.* The effect of PTH(1-84) on quality of life in hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **98,** 2356–61 (2013).

394. Sikjaer, T., Rejnmark, L. & Mosekilde, L. PTH treatment in hypoparathyroidism. *Curr. Drug Saf.* **6,** 89–99 (2011).

395. Ameri, M., Fan, S. C. & Maa, Y.-F. Parathyroid hormone PTH(1-34) formulation that enables uniform coating on a novel transdermal microprojection delivery system. *Pharm. Res.* **27,** 303–13 (2010).

396. Ameri, M., Daddona, P. E. & Maa, Y.-F. Demonstrated solid-state stability of parathyroid hormone PTH(1-34) coated on a novel transdermal microprojection delivery system. *Pharm. Res.* **26,** 2454–63 (2009).

397. Edafe, O., Antakia, R., Laskar, N., Uttley, L. & Balasubramanian, S. P. Authors' reply: Systematic review and meta-analysis of predictors of post-thyroidectomy hypocalcaemia (Br J Surg 2014; 101: 307-320). *Br. J. Surg.* **101,** 883–4 (2014).

398. See, A. C. & Soo, K. C. Hypocalcaemia following thyroidectomy for thyrotoxicosis. *Br. J. Surg.* **84,** 95–7 (1997).

399. Lorente-Poch, L., Sancho, J. J., Muñoz-Nova, J. L., Sánchez-Velázquez, P. & Sitges-Serra, A. Defining the syndromes of parathyroid failure after total thyroidectomy. *Gland Surg.* **4,** 82–90 (2015).

400. Shaha, A. R. & Jaffe, B. M. Parathyroid preservation during thyroid surgery. *Am. J. Otolaryngol.* **19,** 113–7

401. Lombardi, C. P. *et al.* Early prediction of postthyroidectomy hypocalcemia by one single iPTH measurement. *Surgery* **136,** 1236–41 (2004).

402. Richards, M. L., Bingener-Casey, J., Pierce, D., Strodel, W. E. & Sirinek, K. R. Intraoperative parathyroid hormone assay: an accurate predictor of symptomatic hypocalcemia following thyroidectomy. *Arch. Surg.* **138,** 632-5-6 (2003).

403. Grodski, S. & Serpell, J. Evidence for the role of perioperative PTH measurement after total thyroidectomy as a predictor of hypocalcemia. *World J. Surg.* **32,** 1367–73 (2008).

404. Winer, K. K., Yanovski, J. A. & Cutler, G. B. Synthetic human parathyroid hormone 1-34 vs calcitriol and calcium in the treatment of hypoparathyroidism. *JAMA* **276,** 631–6 (1996).

405. Winer, K. K., Fulton, K. A., Albert, P. S. & Cutler, G. B. Effects of pump versus twice-daily injection

delivery of synthetic parathyroid hormone 1-34 in children with severe congenital hypoparathyroidism. *J. Pediatr.* **165,** 556–63.e1 (2014).

406. Santonati, A. *et al.* PTH(1-34) for surgical hypoparathyroidism: A prospective, open-label investigation of efficacy and quality of life. *J. Clin. Endocrinol. Metab.* **100,** 3590–3597 (2015).

407. Shah, M. *et al.* Teriparatide Therapy and Reduced Postoperative Hospitalization for Postsurgical Hypoparathyroidism. *JAMA Otolaryngol. Head Neck Surg.* **141,** 822–7 (2015).

408. Brandi, M. L. *et al.* Management of Hypoparathyroidism: Summary Statement and Guidelines. *J. Clin. Endocrinol. Metab.* **101,** 2273–83 (2016).

409. Hannan, F. M. & Thakker, R. V. Investigating hypocalcaemia. *BMJ* **346,** f2213 (2013).

410. Steele, T., Kolamunnage-Dona, R., Downey, C., Toh, C.-H. & Welters, I. Assessment and clinical course of hypocalcemia in critical illness. *Crit. Care* **17,** R106 (2013).

411. Raffaelli, M. *et al.* Post-thyroidectomy hypocalcemia is related to parathyroid dysfunction even in patients with normal parathyroid hormone concentrations early after surgery. *Surgery* **159,** 78–84 (2016).

412. Stögmann, W., Bohrn, E. & Woloszczuk, W. [Initial experiences with substitution treatment of hypoparathyroidism with synthetic human parathyroid hormone]. *Monatsschrift Kinderheilkd. Organ der Dtsch. Gesellschaft für Kinderheilkd.* **138,** 141–6 (1990).

413. Montomoli, M. *et al.* Validation of a food frequency questionnaire for nutritional calcium intake assessment in Italian women. *Eur. J. Clin. Nutr.* **56,** 21–30 (2002).

414. Cusano, N. E. *et al.* PTH(1-84) is associated with improved quality of life in hypoparathyroidism through 5 years of therapy. *J. Clin. Endocrinol. Metab.* **99,** 3694–9 (2014).

415. Bilezikian, J. P. *et al.* Hypoparathyroidism in the adult: epidemiology, diagnosis, pathophysiology, target-organ involvement, treatment, and challenges for future research. *J. Bone Miner. Res.* **26,** 2317–37 (2011).

416. Sikjaer, T. *et al.* The effect of adding PTH(1-84) to conventional treatment of hypoparathyroidism: a randomized, placebo-controlled study. *J. Bone Miner. Res.* **26,** 2358–70 (2011).

417. Rubin, M. R. *et al.* Parathyroid hormone stimulates circulating osteogenic cells in hypoparathyroidism. *J. Clin. Endocrinol. Metab.* **96,** 176–86 (2011).

418. Miller, P. D. Safety of parathyroid hormone for the treatment of osteoporosis. *Curr. Osteoporos. Rep.* **6,** 12–6 (2008).

419. Sikjaer, T. *et al.* Effects of PTH(1-84) therapy on muscle function and quality of life in hypoparathyroidism: results from a randomized controlled trial. *Osteoporos. Int.* **25,** 1717–26 (2014).