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PRIMARY HYPERPARATHYROIDISM - COMORBIDITY AND OUTCOME AFTER PARATHYROID ADENOMECTOMY

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In memory of my grandfather Mille

ABSTRACT

Primary hyperparathyroidism (pHPT) is associated with increased mortality in certain malignant tumours. Breast cancer is the most common and a shared aetiology has been suggested. In a register-based nested case-control study, we compared breast cancer in patients with and without a previous operation for pHPT. Neither tumour size or stage, nor lymph node metastases differed, nor did breast cancer specific survival.

Longer life expectancy and a lower threshold for referral of pHPT patients to surgery have lead to an increasing proportion of elderly patients. In a large cohort study of the period 1961-2004, all-cause mortality within 30 days and one year after surgery for pHPT was analysed. The entire Swedish population, standardized for age, sex and time period, served as control. During the study period, 30-day mortality decreased from 4.2% to 0.4% and mean age increased by 11 years (53-64 years). Cardiovascular disease was the dominant cause of death in both sexes and all age groups.

Patients with pHPT have lower bone mineral density and display several risk factors of cardiovascular disease. Vitamin D deficiency is more common in pHPT and could aggravate the complications. In a randomized clinical trial, we examined the effect of vitamin D supplementation on bone mineral density, blood pressure and metabolic risk factors after curative surgery for pHPT. 150 patients were randomized to either calcium and vitamin D or calcium alone. Surgery had a positive effect on bone mineral density and insulin resistance and a small positive effect on systolic blood pressure. There was no obvious additive effect of vitamin D supplementation.

Conclusions: Breast cancer in pHPT patients seems to have the same characteristics and prognosis as in the general population. Parathyroidectomy is a safe operation, even in the elderly, and leads to improvements in bone mineral density, insulin resistance and to a lesser extent in systolic blood pressure. Vitamin D supplementation after surgical cure had no obvious beneficial effect.

LIST OF PUBLICATIONS

This thesis is based on the following original studies, which will be referred to in the text by their Roman numerals:

I. **Perioperative mortality in parathyroid surgery in Sweden during five decades – improved outcome despite older patients**
Norenstedt S., Ekbom A., Zedenius J., Nilsson I-L.
European Journal of Endocrinology (2009) 160; 295-299

II. **Breast cancer associated with primary hyperparathyroidism – a nested case control study**
Norenstedt S, Granath F, Ekbom A, Bergh J, Lambe M, Adolfsson J, Wärnberg F, Zedenius J, Nilsson I-L
Clinical Epidemiology (2011) 3; 103-106

III. **Primary hyperparathyroidism and metabolic risk factors: impact of parathyroidectomy and vitamin D supplementation; results of a randomized double-blind study**
Norenstedt S, Pernow Y, Brismar K, Sääf M, Ekip A, Granath F, Zedenius J, Nilsson I-L.
Submitted

IV. **Parathyroidectomy increases bone mineral density in primary hyperparathyroidism – no additive effect of vitamin D supplementation – a randomized double-blind study**
Norenstedt S, Pernow Y, Zedenius J, Nordenström J, Sääf M, Granath F, Nilsson I-L
Submitted

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

%CV	coefficient of variation in per cent
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
24h ABP	24-hour ambulatory blood pressure
25-OH-D	25-hydroxyvitamin D
βCTx	c-terminal telopeptide of type 1 collagen
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
BP	blood pressure
Ca^{2+}	serum ionized calcium
CI	confidence interval
D-	group treated with calcium carbonate
D+	group treated with cholecalciferol and calcium carbonate
DBP	diastolic blood pressure
DXA	dual x-ray absorptiometry
GFR	glomerular filtration rate
HDL	high density lipoprotein
HOMA-IR	the homeostatic model assessment insulin resistance
HR	heart rate
HR-pQCT	high-resolution peripheral quantitative computed tomography
ICD 7	International Classification of Diseases 7^{th} revision
IGF-I	insulin-like growth factor I
IGFBP-1	insulin-like growth factor binding protein 1
IQR	inter quartile range
IU	international units
LBM	lean body mass
LDL	low density lipoprotein
n	number
P	phosphate
P1NP	procollagen type 1 aminoterminal propeptide
pHPT	primary hyperparathyroidism
PTH	parathyroid hormone

PTX	parathyroid adenomectomy
RIA	radioimmunoassay
SBP	systolic blood pressure
SMR	standard mortality ratio
TG	triglycerides
UD	ultra distal

1 INTRODUCTION

1.1 PRIMARY HYPERPARATHYROIDISM

1.1.1 Clinical presentation of primary hyperparathyroidism

Primary hyperparathyroidism (pHPT) is a common endocrine disorder, characterized by elevated or "high normal" serum calcium in combination with an inappropriately high level of parathyroid hormone (PTH). It is caused by excessive, incompletely regulated secretion of PTH from one or more of the parathyroid glands. In more than 80% of the cases there is a single, benign parathyroid adenoma: in the remaining cases, multiglandular involvement is seen. Parathyroid cancer is rare (0.5% of the cases)[1]. PHPT is mostly a sporadic disease, but a small percentage of the cases is part of a hereditary multiple endocrine neoplasia type I or IIa, HPT-jaw tumour syndrome or other rare hereditary disorders[2]. The introduction of automated serum analyses of calcium in the early 1970s, led to a sharp increase in the observed prevalence of pHPT. Today the prevalence is around 1%, with a female:male ratio of 3:1[3-7]. It increases with age in both sexes, with a prevalence up to 3.4% or even higher, in postmenopausal women[3,5,8,9]. In a Swedish screening study on premenopausal women, the prevalence of assumed mild pHPT was as high as 5.1%, and 2.7% on repeated measures[10].

Besides the increased prevalence, the clinical picture has changed from the classic symptoms of severe osteoporosis or osteitis fibrosa cystica, gastrointestinal symptoms, muscle weakness, psychiatric symptoms, kidney stones and nephrocalcinosis[2] to a more or less asymptomatic disease in a majority of the patients[11]. By definition, asymptomatic pHPT presents without overt clinical signs. However, these patients often have reduced bone mineral density (BMD), preferentially in cortical bone[12-14], cardiovascular disturbances[15-17] and metabolic abnormalities[12,18,19]. Asymptomatic or normocalcaemic patients may have an early form of the disease, with smaller adenomas[20,21]. Long-term follow-up of conservatively treated pHPT patients shows that in most cases the disease is relatively stable. Progression has been documented in one fifth to one third of the patients[9,22-24]. Lowe et al. followed 37 normocalcaemic pHPT patients for 1-9 years and found that as many as 40% developed evidence of disease progression[25].

Patients with pHPT also have an increased risk of death from mainly cardiovascular disorders and certain malignancies, of which breast cancer is the most frequent[26,27,28-30].

1.1.2 Treatment

The only curative treatment of pHPT is surgical removal of the affected parathyroid gland(s). With improved techniques for preoperative localization, such as ultrasound and scintigraphy, and assays for intraoperative PTH monitoring, the surgery has become less extensive. Both the traditional four-gland exploration and minimally invasive surgery have a success rate of >95% and very low morbidity in the hands of experienced surgeons[31,32]. Since life expectancy and awareness of the disease have risen, an increasing proportion of elderly patients are referred to surgery.

The change in the disease profile has lead to controversies over the advisability of recommending surgery to all patients, especially if they are asymptomatic and the diagnosis is discovered incidentally. This has resulted in the development of international guidelines for the surgical treatment of asymptomatic pHPT, in order to select patients with an expected beneficial effect. The current criteria are: age < 50 years, serum calcium levels > 0.25 nmol/l above the upper limit of normal, creatinine clearance < 60 ml/min and BMD detected T-score < -2.5 at any site or previous fragility fracture[33].

After curative surgery, 9-62% of patients, depending on the time after surgery, have a persistently elevated PTH despite normocalcaemia[34,35]. Postoperative PTH elevation is associated with higher preoperative PTH, higher bone turnover markers and lower vitamin D levels[34,36]. The underlying aetiology is probably multifactorial. Possible causes are an increased need of calcium and phosphate in the remineralization of bone (hungry bone), vitamin D deficiency causing secondary hyperparathyroidism and reduced peripheral sensitivity to PTH[35,37,38]. The high preoperative PTH and low vitamin D levels suggest a beneficial effect of postoperative vitamin D supplementation in these patients, but there are no randomized trials which address this issue.

1.1.3 Calcium and parathyroid hormone

Calcium has several important physiological functions. One is to provide the mineral structure of bones and teeth and another is metabolic. Soluble calcium ions (Ca^{2+}) in the

extracellular fluid are essential in a large number of enzymatic reactions, cell signalling and electrical membrane potentials necessary for normal cellular function. The skeleton serves as a reservoir of calcium. Precise control of the calcium level is critical and involves the parathyroid glands, the kidneys, the skeleton and the gut. The principal regulators of calcium homeostasis are PTH and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). The free extracellular Ca^{2+} concentration is maintained within a narrow range (1.15-1.33 nmol/l). Approximately 50% of the total amount of circulating calcium is free: 40% is bound to proteins, mainly albumin, and about 10% to anions such as sulphate and citrate[39].

Parathyroid hormone is secreted by the chief cells of the parathyroid glands. It is initially synthesized as pre-pro-parathyroid peptide, which then undergoes post-translational modifications, resulting in the biologically active 84-aminoacid protein. PTH has a short half-life and is degraded by the liver and kidney. It exerts its action through binding to widely distributed PTH receptors, mainly expressed in bone and the kidneys[40,41].

PTH is a major regulator of calcium and phosphate homeostasis. Its main function is to maintain the extracellular calcium concentration within physiological limits through actions on bone metabolism, renal function, vitamin D activation and gastrointestinal absorption.

Ca^{2+} exerts its action on the parathyroid glands by binding to a surface-bound G-coupled receptor, the calcium sensing receptor[42]. A change in the secretory rate of PTH in response to low Ca^{2+} takes place in a matter of seconds and the net rate of PTH synthesis increases within 30 minutes[43]. PTH elevates serum Ca^{2+} concentrations by increasing calcium reabsorption in the loop of Henle and the distal tubule of the kidney and by stimulating the conversion of 25-hydroxyvitamin D (25-OH-D) to the active $1,25(OH)_2D$ in the proximal tubule. PTH also releases Ca^{2+} and phosphate from the skeleton. At the same time, PTH has a phosphaturic effect by stimulating the excretion of phosphate in the proximal tubule of the kidney (Figure 1). This is thought to compensate for the extra phosphate released from the skeleton.

PTH production is increased by phosphate and inhibited by Ca^{2+} and $1,25(OH)_2D$[44].

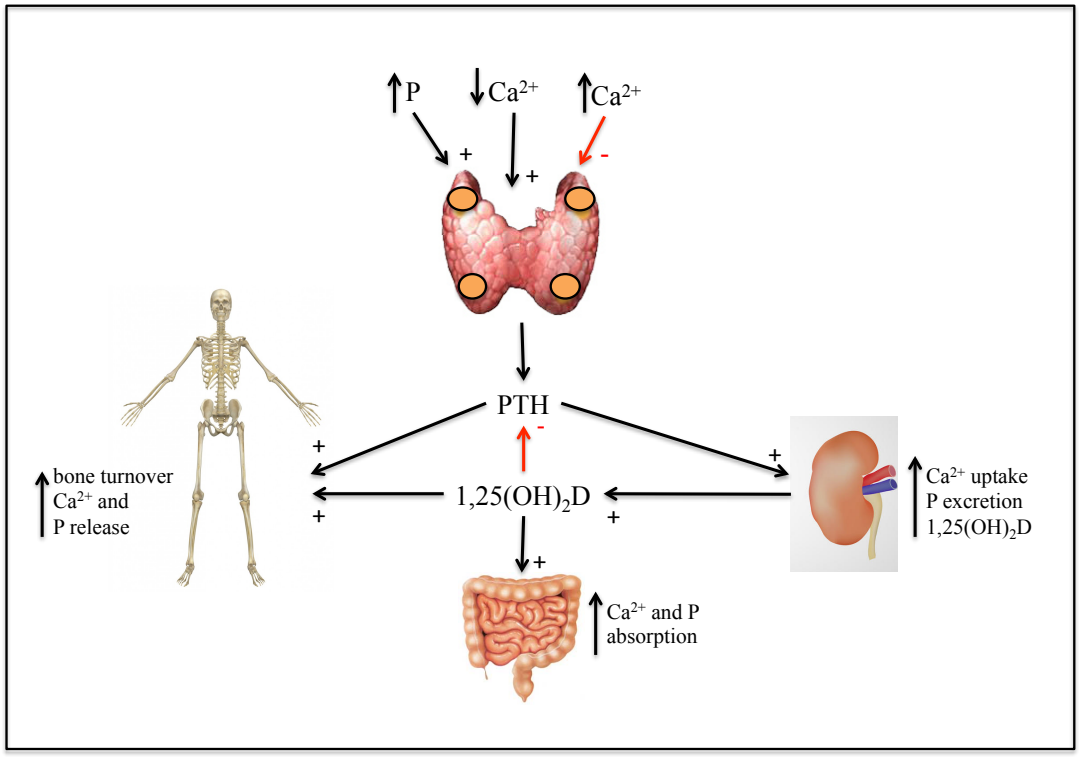


Figure 1. *Effects of PTH. PTH increases serum Ca^{2+} through the activation of vitamin D and reabsorption of Ca^{2} in the kidney, and through releasing Ca^{2} from the bone. Secretion is stimulated by low Ca^{2+}, high phosphate and low $1,25(OH)_2D$ and inhibited by high Ca^{2+} and $1,25(OH)_2D$. PTH=parathyroid hormone, P=phosphate, Ca^{2+}=ionized calcium, $1,25(OH)_2D$=1,25-dihydroxy-vitamin D*

1.2 ASSOCIATION TO BREAST CANCER

PHPT is associated with an increased risk of developing malignant disorders. Certain malignant tumours are overrepresented, for example breast cancer, colon cancer, cancer in the kidneys and non-melanotic skin cancer[8,27,29]. The increased risk of breast cancer persists for at least 15 years after parathyroid adenomectomy (PTX)[27,30]. Breast cancer patients have a high incidence of hypercalcaemia, and pHPT may be one of the causes apart from bone metastases[45]. PHPT was found to be more common in a breast cancer population than in patients with differentiated thyroid cancer and it was unrelated to clinical stage or anti-tumour therapy[46]. In a group of untreated breast cancer patients, the association was no longer significant when age was taken into account[47]. Serum calcium and 25-OH-D concentrations have also been associated with an increased risk of breast cancer, but data are not consistent[48-54]. Breast cancer and pHPT share several other characteristics: both typically affect postmenopausal women and both are associated with ionized radiation[55,56] and obesity[57,58].

These common features suggest potential shared etiological pathways or risk factors of pHPT and breast cancer, such as predisposing genetic or environmental factors. There could also be an increased susceptibility to one disease as a consequence of the other, but that is less likely because the risk of breast cancer persists after surgically treated pHPT[27]. Little is known about any possible causal relationship. Familial accumulations of pHPT and breast cancer, as well as isolated cases with high penetrance susceptibility genes, have been reported[59,60].

Whether pHPT affects the aggressiveness of breast cancer is not known. One study found an association between serum calcium levels and increased tumour aggressiveness in premenopausal and/or overweight women[61].

1.3 VITAMIN D

Vitamin D supplies in humans come from exposure to sunlight and dietary intake. Ultraviolet radiation from the sun converts 7-dehydrocholesterol to cholecalciferol (vitamin D3) in the skin. The quantity of vitamin D3 formed in the skin depends on the duration and intensity of sunlight exposure. Vitamin D3 can be stored in fat and in the liver. Vitamin D is hydroxylated twice, first in the liver to 25-hydroxyvitamin D (25-OH-D) and a second time in the kidney, where it is converted by 1α-hydroxylase to its biologically active form, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). 25-OH-D is used to determine a person's vitamin D status since it is more stable and its serum concentration is 500-1000 times higher than $1,25(OH)_2D$'s. $1,25(OH)_2D$ binds to the vitamin D receptor, an intracellular hormone receptor, to exert its effect. Vitamin D is involved in the regulation of cellular metabolism and differentiation, bone metabolism and inflammation. It plays a role in calcium homeostasis, where $1,25(OH)_2D$ increases serum calcium concentrations by enhancing absorption from the intestine and resorption of calcium from bone (Figure 1). PTH stimulates the conversion to $1,25(OH)_2D$, while $1,25(OH)_2D$ has a negative effect on PTH secretion. Other regulators of $1,25(OH)_2D$ are phosphorus, calcium and fibroblast growth factor 23[44,62,63].

There is no globally accepted cut-off defining vitamin D deficiency and insufficiency. The most widely used (recommended by the Institute of Medicine, USA[64] and the

Danish Sundhedsstyrelsen) is deficiency defined as a 25-OH-D concentration below 25 nmol/l and insufficiency as ≤ 50 nmol/l. Attempts to define vitamin D deficiency are often based on studies aimed at determining the point at which vitamin D cannot further suppress the PTH level, resulting in a wide range of values [65-67]. Other studies on 25-OH-D levels in relation to clinical outcomes, such as BMD, fractures and colorectal cancer, found advantages at 25-OH-D concentrations above 50-75 nmol/l [68,69]. A large cross-sectional analysis on more than 300,000 individuals showed a continuous decline in PTH with rising 25-OH-D and no inflection point or plateau [70].

Vitamin D insufficiency is one of the causes of secondary hyperparathyroidism. A chronic, low vitamin D concentration has an adverse effect on the skeleton, leading to rickets in children and osteoporosis/osteomalacia in adults. Many extra-skeletal conditions, such as autoimmune diseases, malignancies and cardiovascular morbidity and mortality, have been associated with low vitamin D levels in observational studies[51,71-73]. However, the existing data are inconclusive as to causality.

Supplementation with vitamin D and calcium has a putative positive effect on bone health and fracture prevention, but the evidence is inconsistent[74,75]. In a recent pooled analysis of eleven randomized clinical trials, looking at quartiles of actual intake of vitamin D, high-dose vitamin D supplementation (≥800 IU daily) was somewhat better for preventing hip fracture and any non-vertebral fracture in patients 65 years of age or older[76]. There is not enough evidence for benefits of vitamin D and/or calcium supplementation in extra-skeletal conditions and mortality[64,77,78]. In a pooled analysis, vitamin D and calcium reduced mortality, but data did not support an effect of vitamin D alone[79].

A low concentration of vitamin D is more prevalent in patients with pHPT than in geographically matched populations[80-83]. Vitamin D deficiency seems to be associated with more severe pHPT, in terms of higher PTH levels, larger adenomas and lower BMD[82,83]. It is also associated with a persistent PTH elevation after curative surgery[36]. Possible explanations for the relationship between pHPT and low vitamin D levels are stimulation of adenoma growth or inhibition of the production of vitamin D in skin and liver by the elevated level of $1,25(OH)_2D$, caused by an increased conversion to $1,25(OH)_2D$ in the kidney. Enhanced inactivation of 25-OH-D in the liver has also been suggested[84].

The Guidelines for management of asymptomatic pHPT recommend repletion to a 25-OH-D concentration > 50 nmol/l to distinguish primary from secondary hyperparathyroidism[33]. Supplementation with vitamin D in untreated pHPT patients may decrease PTH levels and bone turnover, but there are no randomized trials to prove any beneficial effects[85]. Since low vitamin D is common in pHPT patients and associated with a persistent elevation of PTH after surgery, postoperative vitamin D supplementation might have positive effects on bone health and metabolic disturbances associated with pHPT.

However, studies on repletion after parathyroid surgery are sparse and no randomized trial has been conducted. A reduction in PTH concentration has been shown, but no effect on BMD[86,87].

1.4 METABOLIC AND CARDIOVASCULAR COMPLICATIONS

1.4.1 Cardiovascular morbidity and mortality

Serum levels of PTH have been associated with cardiovascular morbidity and mortality in the general population[88-90]. An increase in cardiovascular mortality in patients with pHPT has been well documented in European studies[9,26,91-96]. North American studies are incongruent with these results but an association has been demonstrated between high calcium levels and increased cardiovascular mortality[97,98]. A possible explanation is that patients diagnosed in more recent years have less severe disease. However, Yu et al. found that patients with untreated mild pHPT, diagnosed between 1997 and 2006, had an increased cardiovascular morbidity and mortality compared to the general population, but mortality data in mild pHPT are scanty[99].

Most studies indicate that PTX results in a lower mortality[26,100,101]. The pathogenesis of the increased risk of cardiovascular disease in pHPT has not been established. PHPT has been associated with cardiac abnormalities in structure and function, such as left ventricular hypertrophy, diastolic dysfunction and conduction disturbances[28]. Several aspects of the metabolic syndrome have been linked to pHPT, such as increased body weight[57], hypertension, dyslipidaemia, glucose intolerance and insulin resistance[102-105].

PTH in normocalcaemic patients is also independently associated with hypertension, dyslipidemia, body mass index (BMI) and insulin sensitivity[106,107].

1.4.2 Hypertension and pHPT

Hypertension is common in pHPT even in its mild form[105,108,109]. The cause of this association is not entirely clear, but possible mechanisms are increased total peripheral resistance, disturbances in the renin-angiotensin-aldosteron axis[110] and endothelial dysfunction[16,111,112]. Associations with other cardiovascular risk factors such as diabetes complicate interpretations. Both prolonged elevation of calcium and PTH are associated with an increase in blood pressure[113-115]. Reversible endothelial dysfunction seems to precede structural vascular changes in pHPT[111,112]. Studies on PTX's effect on hypertension have produced contradictory results, where some report a decrease in blood pressure[102,105,108,116] and others show no effect[117,118]. Ambulatory monitoring of blood pressure (ABP) is superior to single office measurements in predicting the risk of cardiovascular complications[119]. Data on ABP in pHPT are scanty and not conclusive[108,117,118,120,121]. In view of the lack of agreement concerning PTX's effect on hypertension, the presence of hypertension in pHPT patients is currently not an indication for PTX[122].

1.4.3 Glucose metabolism and pHPT

Data on insulin resistance in pHPT, especially mild pHPT, are sparse and contradictory[123]. Increased incidences of non-insulin dependent diabetes mellitus, insulin resistance and decreased glucose tolerance have been reported[19,103,124-126]. PTH levels are associated with insulin sensitivity assessed by the hyperglycaemic clamp[107]. A plausible biological mechanism could be that PTH influences intracellular calcium levels and thereby insulin sensitivity. Both hypercalcaemia and hypophosphataemia have been linked to reduced insulin sensitivity[103,127]. PTX has been found to reduce abnormalities in glucose metabolism[125,126,128] but not in all studies[129]. In a study of patients with mild pHPT, PTX had a positive effect on BMD, but did not benefit insulin resistance and other metabolic risk factors[130].

Insulin-like growth factor I (IGF-I) plays an important role in the regulation of cell proliferation and differentiation. It is synthesized mainly in the liver and regulated by

growth hormone. It exerts its effect in most tissues, where it is involved in the pathogenesis of insulin resistance, metabolic syndrome and cardiovascular disease[131]. IGF binding protein 1 (IGFBP-1), a protein of predominantly hepatic origin, modulates the bioactivity of IGF-I. In addition, IGFBP-1 seems to have insulin-sensitizing, blood-pressure lowering and anti-atherosclerotic properties on its own[132,133]. IGFBP-1 is a marker of insulin secretion[134]. Increasing levels of IGFBP-1 seem to have favourable effects on insulin sensitivity, hypertension and other cardiovascular risk factors[133,135]. There are just a few studies on IGFBP-1 and pHPT. Jehle et al. reported higher concentrations of IGFBP-1 in pHPT patients than in healthy controls[136] but a smaller study found no difference[137]. In the latter study, on 13 patients and nine controls, the response of IFGBP-1 to oral glucose suggests an improvement in insulin sensitivity after PTX.

1.5 EFFECTS ON BONE

PTH has both anabolic and catabolic effects on bone, depending on whether the exposure to PTH is continuous as in pHPT (catabolic effects) or intermittent as during treatment with exogenous PTH in osteoporosis (anabolic effects). Intermittent exposure to PTH has an anabolic effect through enhanced osteoblast formation and survival whereas chronic PTH stimulation, as in pHPT, stimulates osteoclast formation, activity and survival[138]. The anabolic effects of PTH are at least partially mediated by a local synthesis of insulin-like growth factor I (IGF-I) in the osteoblasts[139]. In the Western world today, severe skeletal disease in pHPT, such as osteitis fibrosa cystica, is rare. However, many patients suffer from osteopenia or osteoporosis and the current guidelines cite the latter as an indication for surgery in asymptomatic pHPT[33].

In pHPT, there is a 50-60% increase in bone turnover and number of osteoclasts and osteoblasts, but decreased activity of the individual bone cells, leading to a prolonged active formation period and a tendency to a longer remodelling period, resulting in shallower resorption sites[140,141]. There is also a disturbed mineralization, which may be due to hypophosphataemia induced by PTH or low concentrations of 25-OH-D.

Most studies on BMD using dual X-ray absorptiometry (DXA), microcomputed tomography and analyses of iliac crest bone biopsies in patients with pHPT, show that

cortical bone undergoes reductions of cortical width and porosity that recover after PTX, while the cancellous bone is relatively preserved[14,142-144]. BMD measured by DXA, typically shows the greatest reduction in sites rich in cortical bone, such as the 1/3 proximal forearm, and a more modest reduction or even preserved BMD in the lumbar spine, dominated by cancellous bone[141]. Recently, studies using high-resolution peripheral quantitative computed tomography (HR-pQCT) have demonstrated both trabecular and cortical abnormalities at the radius and tibia, resulting in decreased whole bone and trabecular stiffness[145,146].

After PTX, bone turnover decreases, with an early fall in the concentration of resorption markers, while markers of bone formation decrease more slowly[147-149]. As a result, the BMD increases, predominantly in the lumbar spine and hip, and to a lesser extent in the forearm[130,146,150-153]. The greatest improvement occurs during the first postoperative year[154]. The majority of the patients in these studies have mild pHPT, and BMD improved after PTX even in patients who did not meet the criteria for surgical treatment[153]. Patients randomized to observation had a stable or slightly decreased BMD during follow-up (one or two years)[130,152,153,155]. In an observational study of patients with pHPT for 10 to 15 years, BMD decreased significantly after 5-10 years[23,24]. A quarter to one third of the patients met at least one criterion for surgery according to guidelines during the follow-up.

Bone mineral density is an important predictor of fracture risk. A number of cohort studies have reported an increased risk of fractures at several sites in patients with pHPT, even 10 years before diagnosis[156-158]. The fracture risk is increased not only at sites rich in cortical bone, as suggested by the DXA findings, but also in sites rich in cancellous bone, such as the lumbar spine and hip. This is in accordance with the above-mentioned recent finding of trabecular abnormalities.

No randomized studiy has been published on the effect of surgery on fracture risk in pHPT, but three cohort studies show a decreased risk of fractures of the hip, femur, forearm and upper arm[159-161].

2 AIMS OF THE THESIS

- To analyse all-cause mortality within 30 days and one year after parathyroid adenomectomy during five decades.

- To investigate whether a history of primary hyperparathyroidism affects the risk of mortality or factors predictive of prognosis and response to therapy in women with a subsequent breast cancer.

- To study the effects of surgery and postoperative vitamin D supplementation on insulin resistance, ambulatory blood pressure and other cardiovascular risk factors in patients with primary hyperparathyroidism.

- To study the effect of postoperative vitamin D supplementation on parathyroid hormone levels and bone mineral density in patients with primary hyperparathyroidism.

3 PATIENTS AND METHODS

3.1 STUDIES I AND II

3.1.1 Quality registers

The Swedish Cancer Registry is a well-validated register with 3-4% underreporting[162]. Since 1958, all malignant and a few benign tumours, including parathyroid adenomas, are reported to the register, by both the treating physician and the pathologist establishing the diagnosis. The registry includes date of diagnosis and type of tumour. Diagnoses are coded using the International Classification of Diseases 7th revision (ICD-7).

Causes of death are reported to the Cause of Death Registry at the National Board of Health and Welfare. The registry includes all deaths from 1952 onwards among registered Swedish residents. It also contains the underlying and contributory causes of death from the physician's death certificate in accordance with ICD-7, 8 and 9 and date of death. Underreporting is 0.5% and the proportion of misclassification was 1.2±0.3% (year 1998, www.socialstyrelsen.se).

Matching between registers can be achieved by means of the individual National Registration Number that is allocated to every Swedish resident.

3.1.2 Design and patients: Study I

In a cohort study of 14,635 patients subjected to PTX, generated from the Swedish Cancer Registry during January 1961 to December 2004, postoperative mortality within 30 days and one year was analysed. All patients had a histopathologically verified, single parathyroid adenoma. Neither cancer, nor hyperplasia were included. Date and cause of death were derived from the National Cause-of-Death Registry.

3.1.3 Statistical analysis: Study I

The person-year at risk was counted from the date of entry into the cohort until death, emigration or the end of the observation period, i.e. 31 December 2004. The entire

Swedish population, standardized for age, gender and time period was used as control to calculate standard mortality ratios (SMR). SMR were calculated as the ratio of the observed to the expected number of deaths and used as an indicator of risk. Nationwide statistics from the Causes-of-Death Registry include annual sex- and age-specific mortality rates for different ICD codes. The expected number of deaths in the observed population was calculated by multiplying the number of person-years at risk for each 5-year age group, gender and calendar year, by the corresponding age, gender and calendar year-specific mortality rates in the general population. The 95% confidence interval (CI) of SMR was calculated on the assumption that the number of deaths in various categories followed the Poisson distribution. Various stratification studies were conducted, using age and calendar year at entry, the duration of follow-up, attained age, gender and various combinations.

3.1.4 Design and patients: Study II

This was a nested case-control study comparing breast-cancer patients with and without a history of surgically cured pHPT. The study population was retrieved from the Swedish Cancer Registry. Requisites for inclusion of cases were parathyroid adenomectomy of a single parathyroid adenoma (ICD-7 1951) and a subsequent diagnosis of invasive breast cancer (ICD-7 170). For each patient, five control subjects with breast cancer but no history of pHPT, matched for age and time period, were enrolled. To minimize confounding by diagnosis, we excluded all cases with a breast cancer diagnosis discovered prior to primary hyperparathyroidism (n=59). All males were excluded, as were all women with a diagnosis of breast carcinoma *in situ*. The national registration number, a unique identifier for each Swedish resident, was used for linkage to the regional breast cancer registers in Stockholm and Uppsala and the Swedish Cause of Death Registry. Data on tumour size, stage, lymph node and hormonal receptor status, date and cause of death were retrieved from the registers.

Seventy-one women with breast cancer diagnosed after surgery for pHPT and 338 controls were identified during the period from January 1 1992 to December 31 2006. The American Joint Committee on Cancer's staging system for breast cancer was used[163].

3.1.5 Statistical analysis: Study II

Statistical analysis was performed with the PASW for Windows statistical package 18.0 (PASW Inc; Chicago, IL, USA). Student's two-tailed, unpaired *t*-test was used to compare mean tumour size between the cases and control subjects. The distribution of tumour characteristics of cases and controls was compared by Pearson's chi-square test. When cells had expected counts less than 5, a corresponding exact test was applied.

Survival time was calculated as the number of months between the date of diagnosis and whichever occurred first: date of death, date or end of follow-up. Breast cancer survival is presented in a Kaplan-Meier plot and tested with the Logrank test. $P < 0.05$ was considered to be statistically significant.

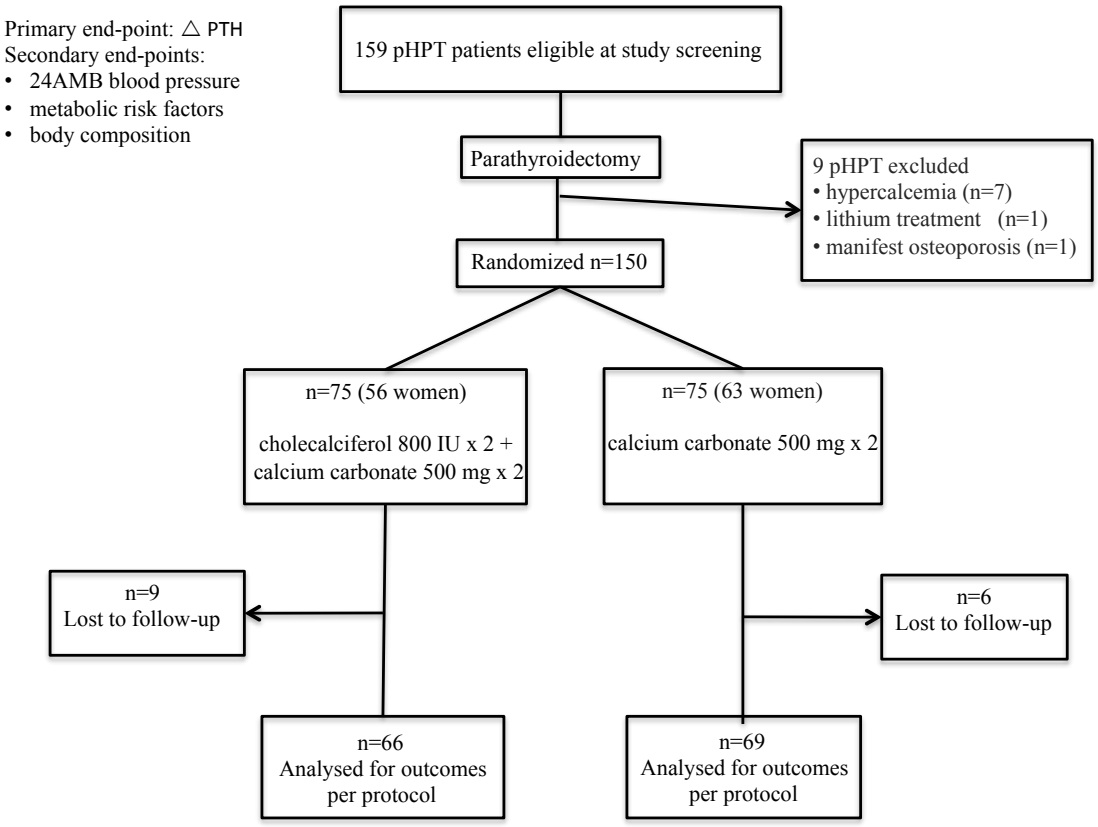
3.2 STUDIES III AND IV

3.2.1 Design

A randomized double-blind clinical trial (ClinicalTrials.gov Identifier: NCT00982722) to evaluate the effect of vitamin D supplementation after PTX was conducted at the Karolinska University Hospital during the period from April 2008 to November 2010. After successful PTX the patients were randomized to either one year of treatment with daily oral cholecalciferol 800 IU x 2 and calciumcarbonate 1 g x 2 (D+) or calciumcarbonate 1 g x 2 alone (D-) (Figure 2). The study was blinded for all the researchers, physicians, nurses and patients.

The primary end-point was the change in PTH after PTX and treatment with the study medication. For study III, secondary end-points were vitamin D levels, changes in metabolic risk factors, body composition and ambulatory blood pressure. Secondary end-points for study IV were vitamin D levels, biochemical markers of bone turnover and bone mineral density.

Figure 2 Flow chart of the study



3.2.2 Patients

Patients with pHPT planned for surgery were eligible for the study. Exclusion criteria were age under 18, manifest osteoporosis at pHPT diagnosis, persistent hypercalcaemia after surgery, postoperative hypocalcaemia requiring vitamin D treatment, glomerular filtration rate (GFR) <40 ml/min., pregnancy, breast-feeding or if the treating physician considered it unsuitable for the patient to participate for other reasons. Patients on vitamin D treatment, prescribed for medical reasons, were not included in the study.

A total of 159 consecutive patients were enrolled, but after PTX, nine of them met exclusion criteria; 150 patients were randomized, 75 patients in each arm. They were followed during one year. 135 patients had a complete follow-up: the fifteen who

dropped out were followed for median 6 months (min-max 1-9 months). Reasons for termination were patient's own will (n=11), emigration (n=1), deceased (n=2) and symptomatic vitamin D deficiency (n=1).
BMI was calculated at baseline as weight (kilograms) divided by the square of height (metres). Patients with insulin treatment (n=2) were excluded from the analyses of glucose, insulin and HOMA-IR.

All patients gave written consent to participation. The study complied with the Ethical Principles of the World Medical Association Declaration of Helsinki, and was approved by the Medical Products Agency in Sweden and by the Local Ethics Committee, Regionala etikprövningsnämnden, EPN, of Stockholm, Sweden.

3.2.3 Methods

3.2.3.1 Laboratory methods

Blood and urine samples were collected after an overnight fast at six ± two weeks before surgery, at randomization and after six and twelve months of treatment. Plasma concentrations of intact PTH, insulin-like growth factor I (IGF-I) and insulin and serum concentrations of procollagen type 1 aminoterminal propeptide (P1NP) and c-terminal telopeptide of type 1 collagen (βCTx) were determined with electrochemiluminescence immunoassay on the Modular E system (Roche Diagnostics GmbH, Mannheim, Germany). Serum ionized calcium (Ca^{2+}) was analysed on ABL 800 (Radiometer, Copenhagen, Denmark). Plasma concentrations of phosphate, creatinine, glucose, total cholesterol, triglycerides (TG), HDL and LDL were measured using the Synchron LX 20 system (Beckman Coulter Inc., Brea, CA). Serum concentrations of 25-OH-D were measured by chemiluminescence on Liason XL® (DiaSorin, Inc Stillwater, USA); values below 50 nmol/l were considered to represent vitamin D insufficiency[33]. The inter-assay coefficient of variation (%CV) is 4.6% at 15.5 nmol/L and 2.7% at 68.3 nmol/l; intra-assay %CV is 4.4% at 15.5 nmol/l and 2.6% at 68.3 nmol/l.

Estimated renal function (GFR ml/min/1.73 m^2) was derived by Cockroft-Gault's formula: GFR = (140-age in years) x (weight in kilograms/plasma creatinine) x (1.23 in men or 1.04 in women). An in-house radioimmunoassay (RIA) according to the method of Póvoa et al. determined IGFBP-1 concentrations in serum[164]. The sensitivity of the RIA was 3 μg/l and the intra- and inter-assay CVs were 3% and 10%, respectively.

Estimates of insulin resistance were calculated using the homeostatic model assessment (HOMA-IR): insulin resistance=fasting glucose x fasting insulin/22.5 after conversion of insulin levels from pmol/l to μU/ml by multiplication with a factor 6.945[165].

3.2.3.2 Bone mineral density and body composition

Areal bone mineral density (BMD, g/cm^2) of the total body, total hip, femoral neck, lumbar spine, non-dominant forearm (ultradistal (UD) and 1/3 proximal radius) and body composition was estimated using dual energy x-ray absorptiometry (DXA). The same instrument (Lunar Prodigy Advance, #PA+41562, GE Healthcare) was used for all the patients. Osteoporosis was defined as a T-score at any site -2.5 standard deviations below the value for white women aged 20-29 years. The precision error was 0.009 SD g/cm^2 in the lumbar spine, 0.010 SD g/cm^2 in the total hip and 0.007 SD g/cm^2 in the femoral neck. The precision error of the forearm was not measured.

3.2.3.3 Ambulatory blood pressure

Ambulatory blood pressure monitoring (24h ABP) was performed with a standardized ambulatory blood pressure device (Meditech ABPM-04 monitor (PMS Instruments, Maidenhead, United Kingdom) that was applied around the patient's non-dominant arm. Daytime was defined as the time from wakening to bedtime (07.00-23.00 in most cases) and night-time as the time the study participant spent in bed. The ambulatory device was set to record ABP and heart rate (HR) at 30-minute intervals during daytime and 60-minute intervals during night-time. If the recording failed, a new measurement was automatically done after 2 minutes. Patients were instructed to continue their usual daily activities while wearing the device and to continue any anti-hypertensive treatment. 125 patients completed pre-and postoperative AMB.

3.2.4 Statistical analysis and sample size calculation

Statistical analysis was performed with the IBM SPSS Statistics version 20. Since data did not follow a normal distribution, they were expressed as median and interquartile range. Intra-individual analyses were performed with the Wilcoxon signed rank sum test. Comparison between groups was performed with the Mann-Whitney U-test for unpaired data; the Kruskal-Wallis one-way analysis of variance was used for comparison of more than two independent continuous variables and the chi-square test was used for analysis of the distribution of categorical variables. Univariate analyses of relationships between variables were assessed with Spearman's ρ-correlation test.

Partial correlations were used to assess the relationship between delta BMD and PTH and 25-OH-D (controlling for age, gender, weight, smoking and creatinine).
All tests were done two-tailed, and $p<0.05$ was considered to be statistically significant.

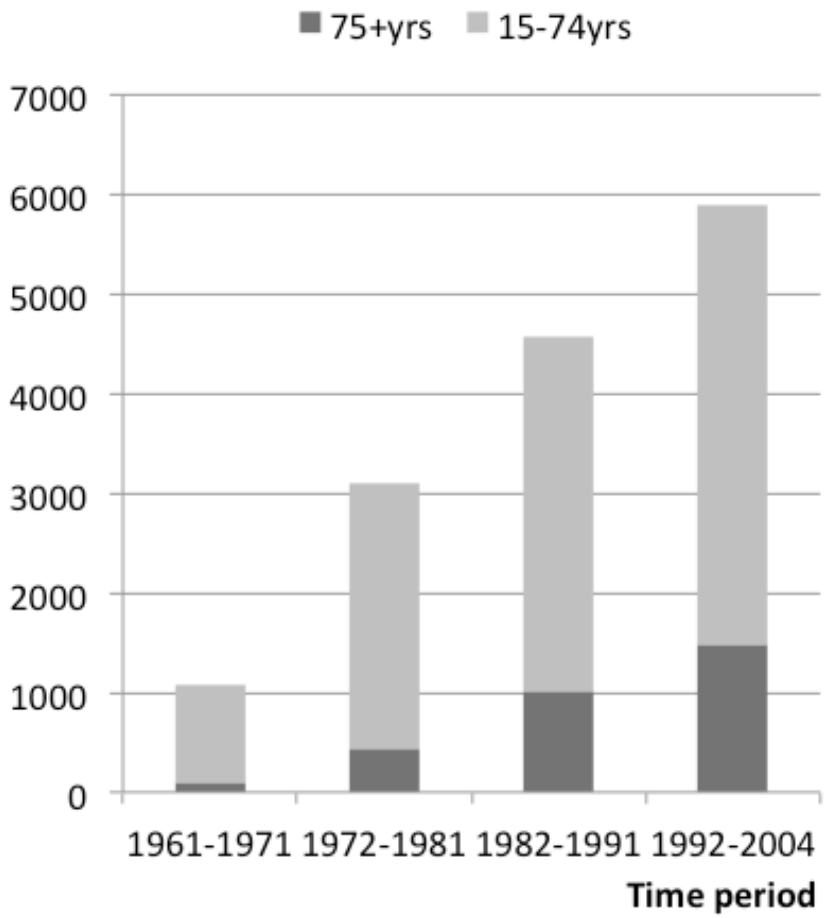
The size of the cohort was determined by a power analysis. Based on data from a European study showing that 90 % of a population of patients with pHPT had a vitamin D insufficiency[81] and a Swedish study where 28 % of the patients had an increased level of PTH 8 weeks after PTX[166], we expected PTH to be within the normal range, after PTX, in 72% of patients not receiving vitamin D and in 97 % of those treated with vitamin D. Since data on the effect of vitamin D on postoperative PTH levels are scarce, we assumed a normal PTH level in two-thirds of the patients with vitamin D supplementation. Thus, with a significance level of 0.05 and a power of 80%, we calculated a sample size of 71 patients in each group. To compensate for dropouts during the study, we chose to enrol 75 patients per group.

4 RESULTS

4.1 STUDY I

Of the 14,635 pHPT patients in the cohort, 79% were women. The observation time was more than 166,000 person-years. The mean age of the patients increased from 53 years in the period 1961–71 to 64 years in 1992–2004 ($p<0.0001$). Nearly 3000 of the pHPT patients were 75 years of age or more at the time of PTX and this age group constituted more than a quarter of the cases in the most recent period (1997-2004) (Figure 3).

Figure 3 Age distribution in different time periods



During the entire study period, 185 patients died within one month after PTX and 365 died during the next eleven months. An analysis of the 30-day mortality over time

showed a decrease from 4.2% during 1961-1976 to 0.4% 1997-2004. Mortality in the period from day 31 to day 365 after PTX ceased to be significantly increased from 1987 onwards (Table 1).

Table 1. Standard mortality ratio with 95% confidence interval in different time periods, after parathyroid adenomectomy.

	1st month				2-12 months		
	n	%	SMR	95% CI	n	SMR	95% CI
1961-1976	105	4.2 %	34.8	28.5-42.1	66	2.05	1.58-2.60
1977-1986	36	0.9 %	6.21	4.35-8.59	116	1.77	1.46-2.12
1987-1996	31	0.6 %	3.52	2.39-5.00	118	1.17	0.97-1.40
1997-2004	13	0.4 %	2.27	1.21-3.88	65	1.07	0.82-1.36
Total number	185	1.3 %	7.92	6.82-9.15	365	1.40	1.26-1.56

Table 2 shows the 30-day mortality in different age and calendar year groups. Mortality within 30 days after PTX in the period 1997–2004 among patients 75 years or older was 1.0%. The dominant causes of mortality during the first month after PTX were cardiovascular (37%), endocrine (32%) and malignancy-related (17%), demonstrable in both genders and in all the investigated age groups (Figure 4). Of the patients who died within the first year after PTX, 51% did so from a cardiovascular disorder.

Figure 4 Causes of death during the first month after parathyroidectomy.

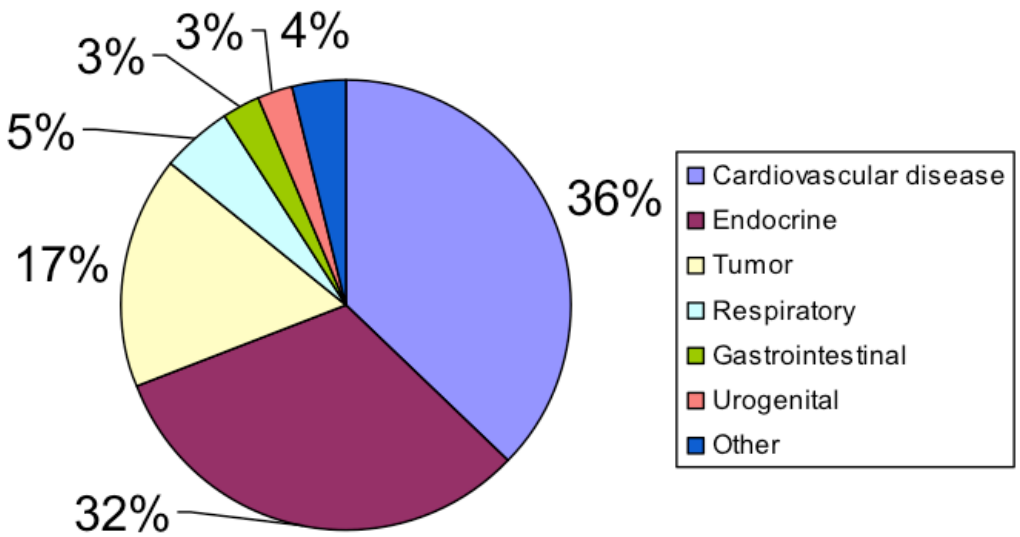


Table 2. Parathyroid adenoma cohort by calendar-year period, with the total number of registered individuals (n) and their age distribution.

	15-54 yrs				55-74 yrs				75+ yrs			
Calender years	n	╬	SMR	95%CI	n	╬	SMR	95%CI	n	╬	SMR	95%CI
1961–76	906	12	52.7	27.2-92.1	1390	49	27.8	20.5-36.7	214	44	42.9	31.2-57.6
1977–86	920	5	23.5	7.6-54.9	2289	12	4.2	2.2-7.4	609	19	6.9	4.2-10.8
1987–96	1119	0	0.0	0.0-17.8	2673	13	4.1	2.2-6.9	1240	18	3.3	2.0-5.3
1997–2004	796	0	0.0	0.0-27.8	1571	4	2.6	0.7-6.8	908	9	2.2	1.0-4.2
Total number	3741	17	21.8	12.7-34.9	7923	78	8.4	6.6-10.5	2971	90	6.8	5.5-8.4

╬ = number of deaths during the first postoperative month

4.2 STUDY II

The mean age at diagnosis of breast cancer was 69 years in both groups (standard deviation (SD) 11 years, 95% confidence interval (95%CI) 68-70 years). The mean interval between parathyroid adenoma operation and breast cancer diagnosis was 91 months (SD 68 months, 95%CI 72-111 months), ranging from 1 to 292 months. Tumour size, stage, axillary lymph node status and hormone receptor status are presented in Table 3.

Table 3 Tumour characteristics in women with pHPT + breast cancer (cases) and women with breast cancer only (controls).

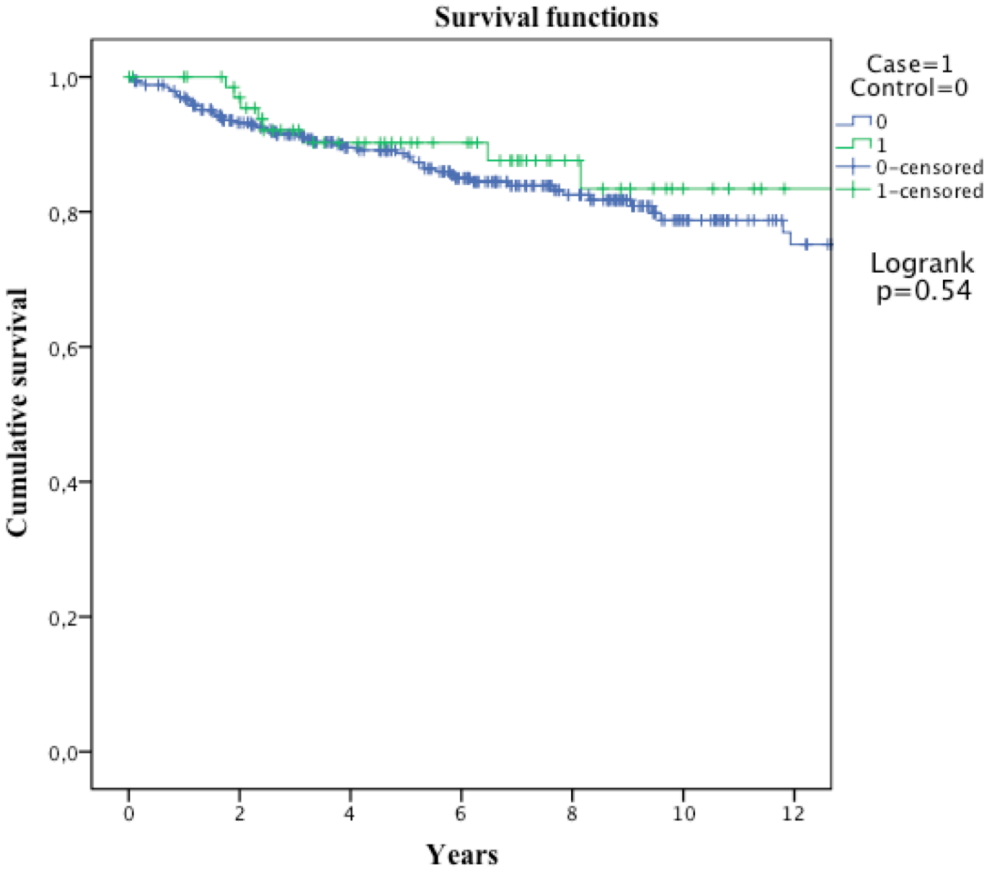
	Cases (n=71)	**Controls** (n=338)	**p-value**
Tumour size (mm±SD)	18±10	20±14	0.27
Missing	4	29	
Axillary lymph node status			
Negative	35 (59%)	176 (65%)	
1-3 positive nodes	11 (19%)	63 (23%)	
≥4 positive nodes	13 (22%)	32 (12%)	0.11
Missing	*12 (17%)*	*67 (20%)*	
Tumour stage			
I (T1+N0)	29 (46%)	149 (51%)	
IIa (T1+N1 or T2+N0)	25 (40%)	77 (27%)	
IIb (T2+N1 or T3+N0)	9 (14%)	49 (17%)	
III (T3+N1 or T4)	0 (0%)	4 (1%)	
IV (M1)	0 (0%)	11 (4%)	0.13[c]
Undefined	*8 (11%)*	*48 (14%)*	
Hormone receptor status			
Positive[a]	46 (88%)	217 (84%)	
Negative[b]	6 (12%)	42 (16%)	0.38
Missing	*19 (27%)*	*79 (23%)*	

[a]ER positive, PR positive or negative according to local laboratory and clinical standards
[b]ER and PR negative
[c]Exact Pearson Chi-Square test

None of the prognostic factors analysed in this study differed between the women with and those without a history of pHPT. Mean time of follow-up was 80 months (SD 59 months, 95%CI 74-86). At December 31 2009, 29 (41%) cases and 150 (44%) controls

had died. There was no statistically significant difference between the two groups in the cumulative breast cancer specific survival (Figure 5).

Figure 5 Kaplan-Meier plot of breast cancer specific survival in women with pHPT+breast cancer (cases) and women with breast cancer only (controls).



No. at risk:						
Cases						
71	62	46	37	22	13	8
Controls						
338	279	214	170	116	66	42

4.3 STUDIES III AND IV

Patient characteristics and biochemical data at baseline and after PTX are shown in Tables 4 and 5. The calcium level was normalized six weeks after PTX in all patients, but 50% had a persistently high PTH (>65 ng/l). Vitamin D levels were lower in patients with a high postoperative PTH (25-OH-D 39 nmol/l (31-44) vs. 42 nmol/l (32-52), p=0.027) and they had larger adenomas (534 g (310-1038) vs. 333 g (204-819), p=0.003) and their preoperative PTH levels were higher (141 ng/l (119-169) vs. 94 (82-108), p<0.001) but creatinine did not differ (data not shown). The incidence of 25-OH-D below 50 nmol/l at baseline was 76% and was similar in men and women.

Table 4. Clinical characteristics

	n=150
Age (years, median (min-max))	60 (30-80)
Women / men (n)	119 / 31
Women ≤50 yrs / >50 yrs (n)	19 / 100
BMI (kg/m2, median (min-max))	26 (17-44)
Weight of adenoma (mg, median (min-max)	450 (75-27800)
Multiglandular disease (n)	4
Vitamin D < 50 nmol/l (n (%))	114 (76%)
Osteoporosis (n (%))	69 (46%)
Smokers (n (%))	23 (15%)
Diabetes (n (%))	8 (5%)
Antihypertensive treatment	67 (45%)
Loop diuretics	26 (17%)
ACE inhibitors	31(21%)
Betablockers	32 (21%)
Calcium channel blockers	16 (11%)
Other relevant medication	
Statins	24 (16%)
Steroids	3 (2%)
Oestrogen, systemic	6 (4%)
Insulin	2 (1%)
Oral antidiabetics	6 (4%)

BMI=body mass index

Table 5. Biochemistry before and after parathyroid adenomectomy (PTX)

	Baseline		After PTX		
	Median	IQR	Median	IQR	p (W)
S-25-OH-D (75-250 nmol/l)	**40**	31-49	**42**	33-54	0.004
P-PTH (10-65 ng/l)	**116**	89-145	**65**	53-68	<0.001
S-Ca^{2+} (1.15-1.33 mmol/l)	**1.43**	1.39-1.43	**1.25**	1.22-1.27	<0.001
P-Phosphate (0.75-1.4 mmol/l)	**0.83**	0.74-0.92	**1.0**	0.92-1.1	<0.001
P-Creatinine (♀<90, ♂<100 µmol/l)	**65**	56-76	**67**	58-75	0.400
GFR Creatinine (ml/min)	**97**	79-117	**95**	79-115	0.900
P-Glucose (4.0-6.0 mmol/l)[a]	**5.2**	4.9-5.6	**5.2**	4.8-5.6	0.022
S-Insulin (18-173 pmol/l)[a]	**66**	43-97	**58**	37-95	<0.001
HOMA-IR[a]	**2.2**	1.4-3.3	**1.8**	1.2-3.2	<0.001
S-IGF-I (110–270 µg/l)	**144**	117-179	**138**	115-172	<0.001
S-IGFBP1	**30**	21-49	**37**	21-54	0.046
S-Cholesterol (3.3-7.8 mmol/l)	**5.6**	4.8-6.1	**5.5**	4.9-6.3	0.134
P-HDL (♀01.0-2.7, ♂0.8-2.1 mmol/l)	**1.4**	1.2-1.8	**1.4**	1.2-1.8	0.825
P-LDL (1.4-5.3 mmol/l)	**3.5**	2.8-4.1	**3.5**	2.8-4.1	0.161
S-Triglycerides (0.45–2.6 mmol/l)	**0.98**	0.75-1.40	**0.96**	0.70-1.52	0.256
S-P1NP (µg/l)	**62**	47-89	**57**	42-78	<0.001
S-βCTx (ng/l)	**545**	388-707	**318**	216-455	<0.001

[a] patients with insulin treatment were excluded from the analysis.
W=Wilcoxon signed rank sum test for paired data
25-OH-D=25-hydroxyvitamin D, PTH=parathyroid hormone, Ca^{2+}=ionized calcium, GFR=glomerular filtration rate, HOMA-IR=homeostatic model assessment insulin resistance, IGF-1=insulin-like growth factor 1, IGFBP1=IGF binding protein1, HDL=high density lipoprotein, LDL=low density lipoprotein

At follow-up after twelve months, the D+ group had a significantly higher level of vitamin D and lower PTH (Table 6). 19 % (n=26) had a persistently high concentration of PTH (D+ n=9, D- n=17). Only two patients had 25-OH-D below 50 nmol/l in the D+ group, compared to 36 patients in the D- group. 12 patients, all in the D- group, had a combination of high PTH and 25-OH-D < 50 nmol/l.

Table 6. Biochemistry at randomization and after one year of study medication

		D+ (n=66; 48 women)					D- (n=69; 57 women)					D+ vs D-	
		Randomization		One year			Randomization		One year			p (M-W)	
		Median	IQR	Median	IQR	*P*(W)	Median	IQR	Median	IQR	*P*(W)	Random.	One year
S-25-OH-vitD (nmol/l)		**40**	33-52	**76**	65-93	<0.001	**45**	35-54	**49**	40-62	<0.001	0.285	<0.001
P-PTH (ng/L)		**67**	52-88	**40**	34-52	<0.001	**64**	56-80	**49**	38-66	<0.001	0.610	0.011
S-Ca2+ (mmol/l)		**1.24**	1.21-1.27	**1.26**	1.23-1.28	0.07	**1.25**	1.22-1.27	**1.26**	1.22-1.28	0.028	0.872	0.928
P-Phosphate (mmol/l)		**1.00**	0.94-1.10	**1.00**	0.90-1.10	0.469	**1.00**	0.90-1.10	**1.00**	0.94-1.20	0.017	0.625	0.606
P-Creatinine (µmol/l)		**66**	58-74	**66**	59-78	0.07	**67**	58-77	**68**	58-80	0.011	0.462	0.536
P-Glucose (mmol/l)[a]		**5.2**	4.8-5.7	**5.1**	4.8-5.6	0.891	**5.1**	4.9-5.5	**5.2**	4.8-5.5	0.526	0.386	0.747
S-Insulin (pmol/l)[a]		**58**	40-106	**53**	33-81	0.807	**53**	33-88	**51**	35-82	0.114	0.216	0.852
HOMA-IR[a]		**1.9**	1.3-1.9	**1.9**	1.2-3.6	0.512	**1.8**	1.1-3.1	**1.7**	1.1-3.0	0.445	0.214	0.314
S-IGF-I (µg/l)		**132**	109-157	**129**	109-160	0.690	**139**	119-174	**149**	124-183	0.370	0.237	0.047
S-IGFBP-1 (µg/l)		**33**	20-51	**34**	22-54	0.720	**43**	24-61	**38**	26-67	0.176	0.100	0.099
P-HDL (mmol/l)		**1.4**	1.2-1.9	**1.5**	1.2-1.8	0.546	**1.4**	1.2-1.8	**1.7**	1.3-1.9	0.038	0.512	0.115
P-LDL (mmol/l)		**3.7**	3.0-4.3	**3.6**	2.9-4.2	0.478	**3.4**	2.8-4.0	**3.3**	2.5-4.1	0.118	0.216	0.270
S-Triglycerides (mmol/l)		**1.00**	0.74-1.70	**0.95**	0.82-1.40	0.926	**0.94**	0.60-1.45	**0.91**	0.66-1.25	0.381	0.175	0.076
S-P1NP (µg/l)		**56**	33-52	**25**	20-36	<0.001	**57**	39-73	**25**	20-36	<0.001	0.407	0.806
S- βCTx (ng/l)		**314**	213-460	**193**	130-286	<0.001	**318**	221-466	**193**	130-286	<0.001	0.924	0.539
Ambulatory BP (mm Hg)				n=65					n=66				
24h	SBP	**132**	124-145	**131**	124-141	0.087	**124**	117-131	**122**	114-130	0.322	<0.001	<0.001
	DBP	**78**	69-82	**75**	71-80	0.293	**73**	67-78	**72**	67-77	0.512	0.009	0.014
Daytime	SBP	**139**	128-152	**135**	129-144	0.036	**130**	123-140	**126**	120-135	0.004	0.001	<0.001
	DBP	**81**	75-88	**80**	75-84	0.246	**79**	72-83	**77**	71-84	0.198	0.031	0.040
Nighttime	SBP	**121**	111-134	**122**	111-133	0.172	**110**	103-123	**111**	103-120	0.651	<0.001	<0.001
	DBP	**68**	60-72	**66**	62-73	0.987	**63**	57-69	**63**	58-68	0.310	0.011	0.025

[a] patients with insulin treatment were excluded from the analysis.
W=Wilcoxon signed rank sum test for paired data
M-W=Mann-Whitney *U*-test for unpaired data

4.3.1 Study III

Patients with 25-OH-D in the lowest quartile at baseline (< 31 nmol/l) had higher levels of fP-glucose (median 5.4 (IQR 5.1-6.3) vs. 5.2 (4.9-5.5) mmol/l); insulin (79.4 (53.5-129.0) vs. 60.5 (43.1-87.9), HOMA-IR (2.7 (1.7-5.2) vs. 2.0 (1.4-3.1) and triglycerides (1.3 (0.9-1.8) vs.0.9 (0.7-1.2); $p<0.05$ for all parameters. Plasma glucose, insulin, HOMA-IR and IGF-I decreased after PTX, while IGFBP1 increased. △IGFBP-1 correlated to △PTH (r=0.18; P=0.03) and was inversely correlated to △insulin (r=-0.26; p=0.002) and △HOMA-IR (r=-0.25; p=0.002).

After one year of study medication, the D+ group had a lower serum concentration of IGF-1 than the D- group (Table 6). All other biochemistry (except PTH and vitamin D, as mentioned above) was unchanged compared with six weeks after surgery.

Ambulatory blood pressure

Data on 24h ABP are presented in Table 6. Median 24h SBP at baseline was significantly correlated to baseline PTH (r=0.24), serum insulin (r=0.29) and TG (r=0.37), $p<0.01$, and inversely to IGFBP-1 (r=-0.19; $P<0.005$). 24h SBP decreased in both groups. The change in 24h SBP was not correlated to changes in PTH, ionized calcium or 25-OH-D (data not shown). Eleven patients, equally distributed between the D+ and the D- group, were able to either cease or reduce their antihypertensive treatment. Vitamin D supplementation did not give any additive effect.

Body composition

Total body BMC increased in both D+ and D- (△BMC: D+ 68 g (-16-127), $p<0.001$; D- 56 g (-32-108), p=0.013). The changes in BMC, LBM and fat mass did not correlate to the change in either 25-OH-D or Ca^{2+}, but there was an inverse correlation between delta PTH and delta BMC (r=-0.30, p=0.002).

4.3.2 Study IV

Bone mineral density

BMD at baseline was similar in the D+ and D- groups. After twelve months of study medication, median BMD had increased significantly in the lumbar spine, the total hip and the femoral neck in both the D+ and the D- group (Table 7). Patients in the D+

group also increased their BMD of the ultra-distal forearm. The increase in BMD did not differ either between patients with or without osteoporosis, or between men and women (data not shown).

BMD at baseline and the change in BMD did not differ between patients with or without vitamin D insufficiency (25-OH-D<50nmol/l). In both groups BMD improved in the lumbar spine and hips; the insufficient patients had an increased BMD in the ultra-distal forearm as well. No significant additive effect of vitamin D supplementation was observed. For patients with insufficient vitamin D levels after 1 year, BMD was lower in the lumbar spine, 1/3 proximal forearm and ultra-distal forearm ($p<0.05$).

The changes in BMD, especially in the hips, were correlated to the baseline concentrations of PTH, ionized calcium and bone turnover markers, but not to vitamin D (Figure 6). This correlation remained significant when controlling for age, gender, smoking, weight and creatinine ($r=0.38$, $p<0.001$).

Patients with PTH > 65 ng/l 6±2 weeks after PTX had a greater improvement in BMD in the total hip, femoral neck and distal forearm than patients with normalized PTH levels. In patients with PTH > 65 ng/l after PTX, BMD increased at all measured sites in the D+ group, but not in the forearm (ultra-distal and 1/3 proximal forearm) in the D- group, without regard to vitamin D status at baseline.

Table 7. Bone mineral density sex weeks before surgery (baseline) and after one year of study medication.

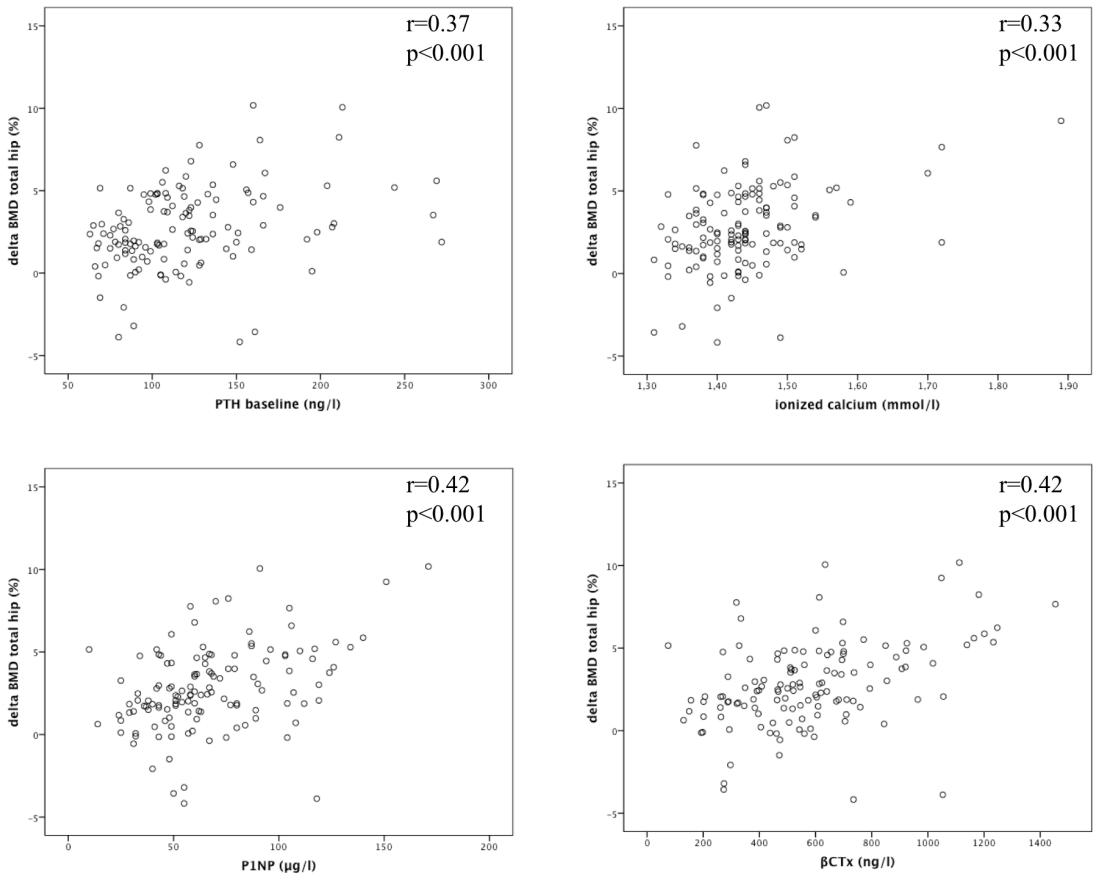
	Vitamin D+					Vitamin D-					D+ vs D-	
	Baseline		Change at 1 year			Baseline		Change at 1 year			Baseline	1 year
	Median	IQR	%	IQR	p[a]	Median	IQR	%	IQR	p[a]	p[b]	p[b]
BMD lumbar spine (g/cm2)	**1.067**	0.951-1.242	**3.6**	0.5-6.0	<0.001	**1.042**	0.938-1.182	**3.0**	0.5-6.2	<0.001	0.180	0.839
Z-score	**0.1**	-0.8-0.8				**-0.4**	-1.2-0.3					
T-score	**-0.9**	-1.9-0.3				**-1.1**	-2.2-0					
BMD hip, total (g/cm2)	**0.915**	0.823-1.018	**2.8**	1.5-4.7	<0.001	**0.889**	0.797-0.971	**2.1**	1.2-4.3	<0.001	0.194	0.376
Z-score	**-0.2**	-0.9-0.5				**-0.4**	-1.0-0.3					
T-score	**-0.9**	-1.6-(-0.1)				**-1.1**	-1.9-(-0.4)					
BMD femoral neck (g/cm2)	**0.852**	0.773-0.948	**3.2**	1.0-4.9	<0.001	**0.845**	0.749-0.940	**2.3**	0.3-4.0	<0.001	0.549	0.092
Z-score	**-0.3**	-0.9-0.2				**-0.5**	-1.0-0.2					
T-score	**-1.4**	-1.9-(-0.6)				**-1.3**	-2.0-(-0.7)					
BMD radius UD (g/cm2)	**0.405**	0.330-(-0.458)	**2.0**	-1.7-5.4	0.013	**0.371**	0.331-0.440	**1.1**	-2.2-5.1	0.091	0.192	0.449
Z-score	**-1.0**	-1.9-0.3				**-1.3**	-2.1-(-0.7)					
T-score	**-1.7**	-3.0-(-0.5)				**-2.2**	-3.1-(-1.3)					
BMD radius 33% (g/cm2)	**0.801**	0.694-0.898	**0.2**	-2.0-3.2	0.529	**0.766**	0.656-0.901	**0.3**	-1.7-2.7	0.381	0.391	0.911
Z-score	**-0.2**	-1.2-0.5				**-0.5**	-1.5-0.0					
T-score	**-1.2**	-2.2-(-0.2)				**-1.5**	-2.6-(-0.5)					

IQR=inter quartile range

[a] Wilcoxon sign rank sum test, paired data

[b] Mann-Whitney U-test, unpaired data

Figure 6 Correlations between delta BMD total hip and baseline PTH, Ca^{2+} and bone turnover markers.



Biochemical markers of bone turnover

In 79 patients, P1NP and βCTx were within the normal range at baseline, while 70 patients had an increased level of P1NP (n=46), βCTx (n=1) or both (n=23). Patients with bone turnover markers above the normal range at baseline had higher PTH, ionized calcium and ALP (PTH: 120 (101-152) vs. 105 (84-133), ionized calcium: 1.46 (1.41-1.51) vs. 1.42 (1.38-1.44), ALP: 1.3 (1.1-1.6) vs. 1.2 (0.9-1.4), $p<0.05$).

βCTx and P1NP decreased significantly in both groups from baseline to six weeks and one year after PTX (Table 5 and 6). βCTx changed most after PTX, while the decrease in P1NP was more pronounced after one year. Both bone turnover markers were correlated to the change in BMD (Figure 6). There was no correlation between the bone turnover markers and 25-OH-D, except for a weak inverse correlation six weeks after PTX between 25-OH-D and βCTx ($r=-0.21$, $p<0.05$).

5 DISCUSSION

5.1 STUDY I

Postoperative mortality after parathyroid adenomectomy in Sweden has decreased from 4.2 % to 0.4 % since 1961, notwithstanding a simultaneous 11-year increase in patients' mean age. Similar postoperative mortality data have been reported[167]. There are several plausible explanations. Measurements of serum calcium became routine in the 1970's and lead to earlier diagnosis and less severe disease at the time of surgery. Modern anaesthetic procedure and better postoperative care may also affect the postoperative mortality. Better preoperative localization techniques have paved the way for focused surgery, even under local anaesthesia. However, in our country, 99% of PTX are still performed under general anaesthesia[168]. Improved medical treatment of a number of chronic diseases has made it possible to operate patients despite a certain degree of comorbidity. Surgery in the elderly seems to be safe and beneficial[169-171].

The dominant cause of death, for all ages and both genders, within the first month and the following year after PTX was cardiovascular disease. This is in line with the 30-day mortality of other elective surgical procedures, such as groin hernia repair[172]. Several risk factors for cardiovascular disease are overrepresented in pHPT[28] and an increased mortality in cardiovascular disorders has been demonstrated[26,95,96]. It may be assumed that the duration of the disease is important for the prognosis, since mortality risk has been associated with the degree of hypercalcaemia and the weight of the adenoma[95].

Postoperative mortality after PTX was higher than for other elective surgical procedures, such as inguinal hernia repair, thyroidectomy for benign goitre and cataract[173-175]. After PTX, an increased mortality persists for at least 15 years[26].

Hypercalcaemic crisis could also affect mortality rates, since it is associated with a significant mortality, especially in patients with extremely high serum calcium levels[176,177]. In a study of 1055 patients who underwent PTX from 1969 to 2004, the prevalence of hypercalcaemic crisis was estimated to be 4%[176].

5.1.1 Strengths and limitations

The registries used are well validated and the size of the investigated cohort provides good statistical power. A limitation is that the cohort does not represent the entire pHPT population in Sweden, since the registry does not include either conservatively treated patients or patients with multiglandular disease, comprising approximately 15% of the pHPT population. Neither are there any data on the degree of hypercalcaemia, symptoms, surgical procedure or postoperative serum calcium levels. In a long-term follow-up of Swedish patients, 95% had reversed hypercalcaemia after PTX[178,179].

5.2 STUDY II

The results in this nested case-control study indicate that factors predictive of prognosis and response to therapy did not differ between patients with breast cancer and previous surgery for pHPT and matched controls without previous PTX, although none of the cases had stage III or IV disease..

PHPT and breast cancer have several characteristics in common. Both mainly affect postmenopausal women and have been associated with obesity and increased calcium and 25-OH-D levels[48-51,57,58,180]. The mammary gland has receptors for both calcium[181], PTH[182] and vitamin D [183]. A causal relationship between calcium and/or PTH levels and breast cancer seems less likely, since unlike cardiovascular mortality, the risk of breast cancer remains unchanged at least 15 years after PTX[26,27]. Neither did Almquist et al. find any association between PTH level and breast cancer in a nested case-control study of 764 patients with breast cancer[48].

Vitamin D deficiency is a factor that could contribute to the aetiology of both breast cancer and pHPT. 1,25-$(OH)_2$-D has the ability to inhibit proliferation, invasion and angiogenesis and promote differentiation and apoptosis[184,185]. There is a potential link between vitamin D deficiency and both the development and prognosis of breast cancer[185-187] as well as an aggravated clinical presentation of pHPT and larger parathyroid adenomas[82,188]. Two meta-analyses of serum vitamin D and breast cancer risk found an inverse association with 25-OH-D measured after diagnosis of breast cancer. However, this could not be confirmed in prospective studies with measurements of 25-OH-D years before diagnosis[50,51]. Neither have studies on a possible association

between vitamin D intake and breast cancer risk yielded consistent results. A meta-analysis of observational studies found a possible positive effect of higher intakes of vitamin D[189] but a nested case-control study of calcium and vitamin D supplementation versus placebo during seven years (mean) did not show any protective effect[190]. However, 57% of the subjects in the placebo arm took personal supplements, and a re-analysis restricted to the women not taking any extra calcium and/or vitamin D, showed a 14-20% decrease in the risk of breast cancer in the calcium and vitamin D arm[191]. Many factors may complicate the interpretation of data on vitamin D status, such as age, BMI, liver and kidney function, chronic illness and sun exposure[192].

Obesity is another factor associated with both pHPT[57], an increased risk of postmenopausal breast cancer[180,193] and vitamin D deficiency[194]. In addition to oestrogen, hyperinsulinaemia is suggested to be a contributory risk factor for breast cancer in obese postmenopausal women[193,195] and is also associated with pHPT[19].

5.2.1 Strengths and limitations

The strengths of the study are the use of well-validated registers, including two of the most important prognostic factors – tumour size and lymph node involvement. The risk of confounding by diagnosis was minimized by excluding cases with a breast cancer diagnosis before pHPT. Other strengths are the relatively large number of observations with five controls per case and the long follow-up.

The main weakness lies in the scope of the data in the registers. Data on HER-2/neu and the cell proliferation marker Ki67 were incomplete. Elston-Ellis tumour grading could not be properly analysed because of too many missing data, especially in the early period. Neither did the register include any information on risk factors for breast cancer, treatment modalities and biochemical data in the register. However, bias because of differences in treatment is likely to be negligible, since the compliance with the regional and national treatment guidelines is excellent and controls were matched for region. Misclassification of controls may affect isolated cases, based on the comparatively low incidence of pHPT in the population[9].

5.3 STUDIES III AND IV

The major findings in this randomized, double-blind study were that PTX had beneficial effects on insulin resistance, blood pressure and bone mineral density. However, despite a high prevalence of vitamin D insufficiency (76 % < 50 nmol/l) in the cohort, postoperative supplementation with vitamin D had no obviously beneficial effect. Preoperative SBP and the increase in BMD correlated with the preoperative PTH concentration.

Vitamin D supplementation did lower the level of PTH in the D+ group. At follow-up this group had a significantly higher concentration of 25-OH-D, indicating an adequate dose of vitamin D.

50 % of the patients had a persistently high concentration of PTH six weeks after PTX. The clinical importance of persistent PTH elevation after curative PTH is still an open question[36]. Several factors are probably causally involved. One is the interval after PTX and another is secondary hyperparathyroidism due to vitamin D deficiency, which was the case in some, but not all patients. High postoperative levels of PTH have been associated with larger adenomas and high preoperative PTH, as in the patients in the present study, and may be due to an increased need of calcium in the remineralization of the bone or to an increased peripheral resistance to PTH [37,38].

The cause and clinical importance of the persistent PTH elevation in nearly 20% of the patients more than one year after PTX are more complicated. Not all of them had a low vitamin D concentration and only one showed obvious signs of recurrent disease. Our results are in line with other long term follow-up studies after PTX[36,196,166].

Vitamin D supplementation resulted in a lower PTH concentration at follow-up after one year. It cannot be excluded that the higher PTH concentration in the D- group has negative effects in the long term. In the general population, PTH in the upper normal range has been associated with an increased risk of cardiovascular complications[88,89,197], increased blood pressure[198,199] and decreased insulin sensitivity[107,200]. Furthermore, a

high PTH level in combination with low vitamin D has been associated with an increased risk of fractures[201].

Among patients with pHPT, those with low vitamin D levels and a higher PTH concentration have also been found to have greater catabolic effects in cortical bone and greater anabolic effects in cancellous bone[202].

5.3.1 Insulin resistance and pHPT

Available reports on the relationship between pHPT and insulin resistance and the effect of PTX are contradictory[123,124,128,130]. The simultaneous reduction of HOMA-IR, glucose, insulin and IGF-I and the increase in IGFBP-1 seen postoperatively and remaining at follow-up, support a possible reversibility of the impaired glucose metabolism coupled to pHPT. The underlying mechanism is not clear, since both PTH and calcium are associated with insulin sensitivity[200,203].

In a randomized study on vitamin D supplementation to women with insulin resistance and vitamin D deficiency, vitamin D had a positive effect on insulin resistance and sensitivity; the optimal vitamin D concentration was ≥ 80 nmol/l[204] (von Hurst 2010). In the present study, the median 25-OH-D concentration at one year was 76 nmol/l. Even so, effects of vitamin D on insulin resistance could not be seen, beyond the positive effect of PTX.

5.3.2 Blood pressure and pHPT

Control of hypertension appears to be crucial for the prevention of cardiovascular complications. The Framingham Study has confirmed that the risk of cardiovascular complications increases incrementally with blood pressure even within the normal range and that SBP is a more important risk factor than DBP[205,206]. It is also well established that 24h ABP is superior to single office measurements in predicting a risk of cardiovascular morbidity and mortality[119]. The available information on 24h ABP in pHPT is limited to a few studies, with contradictory results[108,117,118,120,121]. Recently, Luigi et al. compared patients with pHPT to patients with essential hypertension and to normal subjects, 30 in each group[108]. They found a strong correlation between PTH and SBP and a high prevalence of the metabolic syndrome in patients with pHPT, with

significant improvements after parathyroid surgery. Others have also reported a high percentage of alterations in the normal circadian rhythm of 24h ABP in pHPT[117,120]. Our patients had a small but significant decrease in SBP, regardless of vitamin D levels. The results may be biased by the high proportion of patients with hypertensive medication, but similar findings were obtained in another study with selected patients without known cardiovascular risk factors[80]. A positive effect on blood pressure after surgery has been demonstrated among patients with pHPT and hypertension[105]. However, few studies have been able to show any effect of PTX on blood pressure. Partially irreversible vascular changes may be a possible explanation[122].

The coexistence with other risk factors seems to be important and the combination of high PTH, hypertension and insulin resistance could potentiate the risk of cardiovascular complications in pHPT.

5.3.3 Bone and pHPT

Vitamin D supplementation had no obviously beneficial effect on bone recovery after PTX. The increase in BMD did not differ either between patients with or without vitamin D insufficiency or between patients with or without osteoporosis or between genders. Instead, the change in BMD correlated with the preoperative concentrations of PTH, ionized calcium and bone turnover markers. This is in accordance with other studies[146,147,151,207].

The results of the present study are comparable to previously reported effects of parathyroid surgery only, confirming the positive effects on BMD in sites rich in cancellous bone, such as the lumbar spine and total hip[130,150,154,208,209].

Studies using DXA and analyses of iliac crest bone biopsies in patients with pHPT, show a reduction of cortical width and cortical bone porosity, while the cancellous bone is relatively preserved[14,143]. Recent studies using HR-pQCT found both trabecular and cortical abnormalities, resulting in decreased whole bone and trabecular stiffness[145] and improvements in the microarchitecture after PTX in both cortical and trabecular bone[146]. The potential for improvements in the microarchitecture and bone strengths was related to the baseline levels of PTH and bone turnover markers. These findings

are consistent with the strong correlation between △BMD and the baseline concentration of PTH and bone turnover markers in our patients.

There is a possibility that vitamin D supplementation has a beneficial effect in certain subgroups, for example those with a high PTH level after PTX; they showed a greater improvement in BMD and a beneficial effect in the forearm from vitamin D supplementation. The entire group with vitamin D supplementation also had a positive effect on BMD of the ultra-distal forearm. This raises the question of whether vitamin D and/or PTH have differential effects on different skeletal compartments, for example weight-bearing and non-weight-bearing skeletal sites[41] or stage of maturation of the bone cells[210]. In a Danish study on patients with pHPT, high levels of $1,25(OH)_2D$ were inversely correlated to BMD in the distal radius[211].

5.3.4 Strengths and limitations

To the best of my knowledge, this is the first randomized study on vitamin D supplementation after PTX in patients with pHPT. The strengths of this interventional study are the prospective randomized design, the close and standardized follow-up with good compliance and the achievement of adequate vitamin D levels in the D+ group. Furthermore, the diagnosis was verified by PTX in all cases and the loss to follow-up was 10%. The advantage of a proper randomization is that it could eliminate bias in treatment assignment, especially selection bias and confounding. In this study, the groups were comparable in all studied parameters except for the 24h ABP, which was higher in the D+ group. However, the number of patients with medication for hypertension was the same in D- and D+ (n=33 vs 34) and both groups showed the same decrease in SBP. This is in accordance with a study on selected patients with pHPT but without known cardiovascular risk factors[80] and another study with a high proportion of patients with hypertension[108].

The study was blinded to patients, investigators and assessors to further minimize the risk of bias (information bias).

The use of calcium carbonate instead of placebo could be a limitation, since one cannot definitely exclude the possibility that the calcium supplementation interfered with the results. However, the changes in insulin resistance were detected before the start of

study medication and remained stable during the study period and the positive effects of PTX on BMD and blood pressure have been reported by others[108,130,209].

Another limitation may be the time interval between operation and randomization. It is our clinical routine to check biochemical parameters after six weeks and we chose to randomize the patients at this time point to ensure that they were cured before starting the study medication. In some patients, for example those with significant vitamin D deficiency or hungry bone, a shorter interval might have been favourable for the early mineralization of the bone.

There is a potential risk of type II error, considering the precision errors of the DXA and blood pressure devices and the %CV of the assays of the biochemical parameters. The sample size was based on the change in PTH (primary end-point), and could therefore be too small to detect differences in secondary end-points.

6 CONCLUSIONS

- Parathyroid adenomectomy is a safe procedure today, even in older patients. Chronological age *per se* is no reason for abstaining from surgical treatment of pHPT.

- A history of primary hyperparathyroidism does not seem to affect factors predictive of prognosis and response to therapy in women with subsequent breast cancer.

- Parathyroid adenomectomy has beneficial effects on insulin resistance, blood pressure and bone mineral density.

- Supplementation with vitamin D and calcium after parathyroid adenomectomy lowers the levels of parathyroid hormone, but no obviously beneficial effect was found on blood pressure, metabolic factors or bone mineral density.

7 SAMMANFATTNING PÅ SVENSKA (SWEDISH SUMMARY)

Primär hyperparatyreoidism (pHPT) är en sjukdom där en eller flera av de vanligen fyra bisköldkörtlarna producerar för mycket av sitt hormon, parathormon (PTH). Det leder till för hög koncentration av kalcium i blodbanan. Sjukdomen medför bl. a. urkalkning av skelettet, ökad risk för njursten och hjärt- kärlsjukdomar samt ökad dödlighet. Orsaken till sjukdomen är vanligtvis okänd. PHPT drabbar ca. 1% av den vuxna befolkningen och är vanligast hos kvinnor efter klimakteriet. Den upptäcks ofta genom rutinblodprov. Sjukdomen kan botas med kirurgi, där man tar bort den/de sjuka körteln/körtlarna.

Medelåldern i befolkningen ökar och därmed även de patienter med pHPT som blir aktuella för opereration. Man får då väga riskerna med en operation mot nyttan. I en stor kohortstudie på 14 635 patienter som opererats p.g.a. pHPT under perioden 1961 till 2004, jämfördes dödligheten inom 30 dagar och 31-365 dagar efter operation med dödligheten i hela den svenska befolkningen, standardiserad för ålder kön och tidsperiod.

Medelåldern hos patienterna steg från 53 till 64 år, samtidigt som dödligheten inom 30 dagar efter operation sjönk från 4.2% till 0.4%. Under senare år var en fjärdedel av patienterna 75 år och äldre. Den vanligaste dödsorsaken var hjärt- och kärlsjukdom.

Patienter med pHPT har också en ökad risk att drabbas av vissa cancerformer, varav bröstcancer är den vanligaste. I en registerstudie jämfördes bröstcancer hos kvinnor med och utan tidigare operation p.g.a. pHPT avseende prognostiska faktorer och dödlighet. Grupperna skilde sig inte åt avseende tumörstorlek, hormonreceptorstatus, lymfkörtelengagemang eller dödlighet i bröstcancer.

En hög andel av patienter med pHPT har brist på D-vitamin, vilket kan förvärra sjukdomens komplikationer. Efter botande operation av pHPT återhämtar sig bentätheten till viss del. Huruvida riskfaktorerna för hjärt- kärlsjukdom påverkas eller ej är inte helt klarlagt. De studier som finns är motstridiga. I avhandlingen undersöks om man kan påskynda och/eller förbättra återhämtningen i skelett och metabola

faktorer som blodtryck och blodsockerreglering, genom att ge D-vitamintillskott efter operationen. Av 150 patienter fick hälften kalcium och D-vitamin och hälften enbart kalcium under ett år efter botande operation för pHPT. Själva operationen hade en gynnsam effekt på bentäthet, blodtryck och blodsockerreglering men D-vitamin verkar inte tillföra någon ytterligare effekt, utom möjligen på bentätheten. D-vitamintillskott sänkte nivån av PTH, vilket i sig skulle kunna vara gynnsamt på lång sikt, då PTH åt det högre hållet är kopplat till t. ex. ökad risk för frakturer och hjärt- kärlsjukdom.

Sammanfattningsvis har vi sett att prognosen hos patienter med bröstcancer inte verkar skilja sig mellan de som har eller inte har haft pHPT. Nuförtiden är operation av primär hyperparatyreoidism säker, även hos äldre personer. Ålder i sig bör inte utesluta att patienten får genomgå en potentiellt botande behandling av pHPT. Kirurgisk behandling av pHPT har en gynnsam effekt på PTH-nivå, bentäthet och blodsockerreglering samt till viss del blodtryck. Någon säker vinst av D-vitamintillskott efter operation har inte kunnat påvisas, mer än en sänkning av PTH, vilket skulle kunna ha positiva effekter i sig.

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9 REFERENCES

1. Akerstrom G, Hellman P. Primary hyperparathyroidism. *Curr Opin Oncol.* Jan 2004;16(1):1-7.

2. Bilezikian JP, Brandi ML, Rubin M, Silverberg SJ. Primary hyperparathyroidism: new concepts in clinical, densitometric and biochemical features. *J Intern Med.* Jan 2005;257(1):6-17.

3. Jorde R, Bonaa KH, Sundsfjord J. Primary hyperparathyroidism detected in a health screening. The Tromso study. *J Clin Epidemiol.* Nov 2000;53(11):1164-1169.

4. Heath H, 3rd, Hodgson SF, Kennedy MA. Primary hyperparathyroidism. Incidence, morbidity, and potential economic impact in a community. *N Engl J Med.* Jan 24 1980;302(4):189-193.

5. Lundgren E, Rastad J, Thrufjell E, Akerstrom G, Ljunghall S. Population-based screening for primary hyperparathyroidism with serum calcium and parathyroid hormone values in menopausal women. *Surgery.* Mar 1997;121(3):287-294.

6. Siilin H, Lundgren E, Mallmin H, et al. Prevalence of primary hyperparathyroidism and impact on bone mineral density in elderly men: MrOs Sweden. *World J Surg.* Jun 2011;35(6):1266-1272.

7. Sorva A, Valvanne J, Tilvis RS. Serum ionized calcium and the prevalence of primary hyperparathyroidism in age cohorts of 75, 80 and 85 years. *J Intern Med.* Mar 1992;231(3):309-312.

8. Palmer M, Jakobsson S, Akerstrom G, Ljunghall S. Prevalence of hypercalcaemia in a health survey: a 14-year follow-up study of serum calcium values. *Eur J Clin Invest.* Feb 1988;18(1):39-46.

9. Lundgren E, Hagstrom EG, Lundin J, et al. Primary hyperparathyroidism revisited in menopausal women with serum calcium in the upper normal range at population-based screening 8 years ago. *World J Surg.* Aug 2002;26(8):931-936.

10. Siilin H, Rastad J, Ljunggren O, Lundgren E. Disturbances of calcium homeostasis consistent with mild primary hyperparathyroidism in premenopausal women and associated morbidity. *J Clin Endocrinol Metab.* Jan 2008;93(1):47-53.

11. Wermers RA, Khosla S, Atkinson EJ, et al. Incidence of primary hyperparathyroidism in Rochester, Minnesota, 1993-2001: an update on the changing epidemiology of the disease. *J Bone Miner Res.* Jan 2006;21(1):171-177.

12. Lundgren E, Ljunghall S, Akerstrom G, Hetta J, Mallmin H, Rastad J. Case-control study on symptoms and signs of "asymptomatic" primary hyperparathyroidism. *Surgery.* Dec 1998;124(6):980-985; discussion 985-986.

13. Silverberg SJ, Shane E, de la Cruz L, et al. Skeletal disease in primary hyperparathyroidism. *J Bone Miner Res.* Jun 1989;4(3):283-291.

14. Dempster DW, Muller R, Zhou H, et al. Preserved three-dimensional cancellous bone structure in mild primary hyperparathyroidism. *Bone.* Jul 2007;41(1):19-24.

15. Lundgren E, Szabo E, Ljunghall S, Bergstrom R, Holmberg L, Rastad J. Population based case-control study of sick leave in postmenopausal women before diagnosis of hyperparathyroidism. *Bmj.* Sep 26 1998;317(7162):848-851.

16. Rubin MR, Maurer MS, McMahon DJ, Bilezikian JP, Silverberg SJ. Arterial stiffness in mild primary hyperparathyroidism. *J Clin Endocrinol Metab.* Jun 2005;90(6):3326-3330.
17. Walker MD, Fleischer JB, Di Tullio MR, et al. Cardiac structure and diastolic function in mild primary hyperparathyroidism. *J Clin Endocrinol Metab.* May 2010;95(5):2172-2179.
18. Hagstrom E, Lundgren E, Lithell H, et al. Normalized dyslipidaemia after parathyroidectomy in mild primary hyperparathyroidism: population-based study over five years. *Clin Endocrinol (Oxf).* Feb 2002;56(2):253-260.
19. Procopio M, Magro G, Cesario F, et al. The oral glucose tolerance test reveals a high frequency of both impaired glucose tolerance and undiagnosed Type 2 diabetes mellitus in primary hyperparathyroidism. *Diabet Med.* Nov 2002;19(11):958-961.
20. Bergenfelz A, Lindblom P, Lindergard B, Valdemarsson S, Westerdahl J. Preoperative normal level of parathyroid hormone signifies an early and mild form of primary hyperparathyroidism. *World J Surg.* Apr 2003;27(4):481-485.
21. Lundgren E, Ridefelt P, Akerstrom G, Ljunghall S, Rastad J. Parathyroid tissue in normocalcemic and hypercalcemic primary hyperparathyroidism recruited by health screening. *World J Surg.* Sep 1996;20(7):727-734; discussion 735.
22. Ljunghall S, Jakobsson S, Joborn C, Palmer M, Rastad J, Akerstrom G. Longitudinal studies of mild primary hyperparathyroidism. *J Bone Miner Res.* Oct 1991;6 Suppl 2:S111-116; discussion S121-114.
23. Silverberg SJ, Shane E, Jacobs TP, Siris E, Bilezikian JP. A 10-year prospective study of primary hyperparathyroidism with or without parathyroid surgery. *N Engl J Med.* Oct 21 1999;341(17):1249-1255.
24. Rubin MR, Bilezikian JP, McMahon DJ, et al. The natural history of primary hyperparathyroidism with or without parathyroid surgery after 15 years. *J Clin Endocrinol Metab.* Sep 2008;93(9):3462-3470.
25. Lowe H, McMahon DJ, Rubin MR, Bilezikian JP, Silverberg SJ. Normocalcemic primary hyperparathyroidism: further characterization of a new clinical phenotype. *J Clin Endocrinol Metab.* Aug 2007;92(8):3001-3005.
26. Nilsson IL, Yin L, Lundgren E, Rastad J, Ekbom A. Clinical presentation of primary hyperparathyroidism in Europe--nationwide cohort analysis on mortality from nonmalignant causes. *J Bone Miner Res.* Nov 2002;17 Suppl 2:N68-74.
27. Nilsson IL, Zedenius J, Yin L, Ekbom A. The association between primary hyperparathyroidism and malignancy: nationwide cohort analysis on cancer incidence after parathyroidectomy. *Endocr Relat Cancer.* Mar 2007;14(1):135-140.
28. Andersson P, Rydberg E, Willenheimer R. Primary hyperparathyroidism and heart disease--a review. *Eur Heart J.* Oct 2004;25(20):1776-1787.
29. Pickard AL, Gridley G, Mellemkjae L, et al. Hyperparathyroidism and subsequent cancer risk in Denmark. *Cancer.* Oct 15 2002;95(8):1611-1617.
30. Michels KB, Xue F, Brandt L, Ekbom A. Hyperparathyroidism and subsequent incidence of breast cancer. *Int J Cancer.* Jun 20 2004;110(3):449-451.
31. McGill J, Sturgeon C, Kaplan SP, Chiu B, Kaplan EL, Angelos P. How does the operative strategy for primary hyperparathyroidism impact the findings and cure rate? A comparison of 800 parathyroidectomies. *J Am Coll Surg.* Aug 2008;207(2):246-249.
32. Mihai R, Barczynski M, Iacobone M, Sitges-Serra A. Surgical strategy for sporadic primary hyperparathyroidism an evidence-based approach to surgical

strategy, patient selection, surgical access, and reoperations. *Langenbecks Arch Surg.* Sep 2009;394(5):785-798.
33. Bilezikian JP, Khan AA, Potts JT, Jr. Guidelines for the management of asymptomatic primary hyperparathyroidism: summary statement from the third international workshop. *J Clin Endocrinol Metab.* Feb 2009;94(2):335-339.
34. Biskobing DM. Significance of elevated parathyroid hormone after parathyroidectomy. *Endocr Pract.* Jan-Feb 2010;16(1):112-117.
35. Oltmann SC, Maalouf NM, Holt S. Significance of elevated parathyroid hormone after parathyroidectomy for primary hyperparathyroidism. *Endocr Pract.* Mar-Apr 2011;17 Suppl 1:57-62.
36. Carsello CB, Yen TW, Wang TS. Persistent elevation in serum parathyroid hormone levels in normocalcemic patients after parathyroidectomy: does it matter? *Surgery.* Oct 2012;152(4):575-581; discussion 581-573.
37. Nordenstrom E, Westerdahl J, Isaksson A, Lindblom P, Bergenfelz A. Patients with elevated serum parathyroid hormone levels after parathyroidectomy: showing signs of decreased peripheral parathyroid hormone sensitivity. *World J Surg.* Feb 2003;27(2):212-215.
38. Westerdahl J, Valdemarsson S, Lindblom P, Bergenfelz A. Postoperative elevated serum levels of intact parathyroid hormone after surgery for parathyroid adenoma: sign of bone remineralization and decreased calcium absorption. *World J Surg.* Nov 2000;24(11):1323-1329.
39. Bushinsky DA, Monk RD. Electrolyte quintet: Calcium. *Lancet.* Jul 25 1998;352(9124):306-311.
40. Friedman PA, Goodman WG. PTH(1-84)/PTH(7-84): a balance of power. *Am J Physiol Renal Physiol.* May 2006;290(5):F975-984.
41. Bellido T, Saini V, Pajevic PD. Effects of PTH on osteocyte function. *Bone.* Jun 2013;54(2):250-257.
42. Brown EM, Pollak M, Hebert SC. Sensing of extracellular Ca2+ by parathyroid and kidney cells: cloning and characterization of an extracellular Ca(2+)-sensing receptor. *Am J Kidney Dis.* Mar 1995;25(3):506-513.
43. Mihai R. The calcium sensing receptor: from understanding parathyroid calcium homeostasis to bone metastases. *Ann R Coll Surg Engl.* May 2008;90(4):271-277.
44. Dumitru CA. Disorders of calcium metabolism. In: Giebisch Sa, ed. *The Kidney*. Fifth Edition ed: Elsevier Inc.; 2013:2273-2309.
45. Hickey RC, Samaan NA, Jackson GL. Hypercalcemia in patients with breast cancer. Osseous metastases, hyperplastic parathyroid tissue, or pseudohyperparathyroidism? *Arch Surg.* May 1981;116(5):545-552.
46. Fierabracci P, Pinchera A, Miccoli P, et al. Increased prevalence of primary hyperparathyroidism in treated breast cancer. *J Endocrinol Invest.* May 2001;24(5):315-320.
47. Belardi V, Fiore E, Giustarini E, et al. Is the risk of primary hyperparathyroidism increased in patients with untreated breast cancer? *J Endocrinol Invest.* Aug 29 2012.
48. Almquist M, Bondeson AG, Bondeson L, Malm J, Manjer J. Serum levels of vitamin D, PTH and calcium and breast cancer risk-a prospective nested case-control study. *Int J Cancer.* Nov 1 2010;127(9):2159-2168.
49. Almquist M, Manjer J, Bondeson L, Bondeson AG. Serum calcium and breast cancer risk: results from a prospective cohort study of 7,847 women. *Cancer Causes Control.* Aug 2007;18(6):595-602.
50. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: serum vitamin D and breast cancer risk. *Eur J Cancer.* Aug 2010;46(12):2196-2205.

51. Gandini S, Boniol M, Haukka J, et al. Meta-analysis of observational studies of serum 25-hydroxyvitamin D levels and colorectal, breast and prostate cancer and colorectal adenoma. *Int J Cancer.* Mar 15 2011;128(6):1414-1424.
52. Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. *Cancer Epidemiol Biomarkers Prev.* Aug 2006;15(8):1427-1437.
53. Edvardsen K, Veierod MB, Brustad M, Braaten T, Engelsen O, Lund E. Vitamin D-effective solar UV radiation, dietary vitamin D and breast cancer risk. *Int J Cancer.* Mar 15 2011;128(6):1425-1433.
54. Garland CF, Gorham ED, Mohr SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *J Steroid Biochem Mol Biol.* Mar 2007;103(3-5):708-711.
55. Eidemuller M, Holmberg E, Jacob P, Lundell M, Karlsson P. Breast cancer risk among Swedish hemangioma patients and possible consequences of radiation-induced genomic instability. *Mutat Res.* Oct 2 2009;669(1-2):48-55.
56. Holmberg E, Wallgren A, Holm LE, Lundell M, Karlsson P. Dose-response relationship for parathyroid adenoma after exposure to ionizing radiation in infancy. *Radiat Res.* Oct 2002;158(4):418-423.
57. Bolland MJ, Grey AB, Gamble GD, Reid IR. Association between primary hyperparathyroidism and increased body weight: a meta-analysis. *J Clin Endocrinol Metab.* Mar 2005;90(3):1525-1530.
58. Lahmann PH, Hoffmann K, Allen N, et al. Body size and breast cancer risk: findings from the European Prospective Investigation into Cancer And Nutrition (EPIC). *Int J Cancer.* Sep 20 2004;111(5):762-771.
59. Pal SK, Blazer K, Weitzel J, Somlo G. An association between invasive breast cancer and familial idiopathic hyperparathyroidism: a case series and review of the literature. *Breast Cancer Res Treat.* May 2009;115(1):1-5.
60. Ghataorhe P, Kurian AW, Pickart A, et al. A carrier of both MEN1 and BRCA2 mutations: case report and review of the literature. *Cancer Genet Cytogenet.* Dec 2007;179(2):89-92.
61. Almquist M, Anagnostaki L, Bondeson L, et al. Serum calcium and tumour aggressiveness in breast cancer: a prospective study of 7847 women. *Eur J Cancer Prev.* Sep 2009;18(5):354-360.
62. Glade MJ. Vitamin D: health panacea or false prophet? *Nutrition.* Jan 2013;29(1):37-41.
63. Holick MF. Vitamin D deficiency. *N Engl J Med.* Jul 19 2007;357(3):266-281.
64. Ross AC, Manson JE, Abrams SA, 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.* Jan 2011;96(1):53-58.
65. Thomas MK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med.* Mar 19 1998;338(12):777-783.
66. Ooms ME, Lips P, Roos JC, et al. Vitamin D status and sex hormone binding globulin: determinants of bone turnover and bone mineral density in elderly women. *J Bone Miner Res.* Aug 1995;10(8):1177-1184.
67. Ginde AA, Wolfe P, Camargo CA, Jr., Schwartz RS. Defining vitamin D status by secondary hyperparathyroidism in the U.S. population. *J Endocrinol Invest.* Jan 2012;35(1):42-48.
68. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr.* Jul 2006;84(1):18-28.
69. Kuchuk NO, Pluijm SM, van Schoor NM, Looman CW, Smit JH, Lips P. Relationships of serum 25-hydroxyvitamin D to bone mineral density and

serum parathyroid hormone and markers of bone turnover in older persons. *J Clin Endocrinol Metab.* Apr 2009;94(4):1244-1250.

70. Valcour A, Blocki F, Hawkins DM, Rao SD. Effects of age and serum 25-OH-vitamin D on serum parathyroid hormone levels. *J Clin Endocrinol Metab.* Nov 2012;97(11):3989-3995.

71. Leu M, Giovannucci E. Vitamin D: epidemiology of cardiovascular risks and events. *Best Pract Res Clin Endocrinol Metab.* Aug 2011;25(4):633-646.

72. Yang CY, Leung PS, Adamopoulos IE, Gershwin ME. The Implication of Vitamin D and Autoimmunity: a Comprehensive Review. *Clin Rev Allergy Immunol.* Jan 29 2013.

73. Kendrick J, Targher G, Smits G, Chonchol M. 25-Hydroxyvitamin D deficiency is independently associated with cardiovascular disease in the Third National Health and Nutrition Examination Survey. *Atherosclerosis.* Jul 2009;205(1):255-260.

74. Lips P, van Schoor NM. The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab.* Aug 2011;25(4):585-591.

75. Turner AG, Anderson PH, Morris HA. Vitamin D and bone health. *Scand J Clin Lab Invest Suppl.* Apr 2012;243:65-72.

76. Bischoff-Ferrari HA, Willett WC, Orav EJ, et al. A pooled analysis of vitamin D dose requirements for fracture prevention. *N Engl J Med.* Jul 5 2012;367(1):40-49.

77. Elamin MB, Abu Elnour NO, Elamin KB, et al. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab.* Jul 2011;96(7):1931-1942.

78. Bolland MJ, Grey A, Avenell A, Gamble GD, Reid IR. Calcium supplements with or without vitamin D and risk of cardiovascular events: reanalysis of the Women's Health Initiative limited access dataset and meta-analysis. *Bmj.* 2011;342:d2040.

79. Rejnmark L, Avenell A, Masud T, et al. Vitamin D with calcium reduces mortality: patient level pooled analysis of 70,528 patients from eight major vitamin D trials. *J Clin Endocrinol Metab.* Aug 2012;97(8):2670-2681.

80. Farahnak P, Larfars G, Sten-Linder M, Nilsson IL. Mild primary hyperparathyroidism: vitamin D deficiency and cardiovascular risk markers. *J Clin Endocrinol Metab.* Jul 2011;96(7):2112-2118.

81. Boudou P, Ibrahim F, Cormier C, Sarfati E, Souberbielle JC. A very high incidence of low 25 hydroxy-vitamin D serum concentration in a French population of patients with primary hyperparathyroidism. *J Endocrinol Invest.* Jun 2006;29(6):511-515.

82. Moosgaard B, Vestergaard P, Heickendorff L, Melsen F, Christiansen P, Mosekilde L. Vitamin D status, seasonal variations, parathyroid adenoma weight and bone mineral density in primary hyperparathyroidism. *Clin Endocrinol (Oxf).* Nov 2005;63(5):506-513.

83. Silverberg SJ, Shane E, Dempster DW, Bilezikian JP. The effects of vitamin D insufficiency in patients with primary hyperparathyroidism. *Am J Med.* Dec 1999;107(6):561-567.

84. Silverberg SJ. Vitamin D deficiency and primary hyperparathyroidism. *J Bone Miner Res.* Dec 2007;22 Suppl 2:V100-104.

85. Rolighed L, Bollerslev J, Mosekilde L. Vitamin D treatment in primary hyperparathyroidism. *Curr Drug Saf.* Apr 2011;6(2):100-107.

86. Beyer TD, Solorzano CC, Prinz RA, Babu A, Nilubol N, Patel S. Oral vitamin D supplementation reduces the incidence of eucalcemic PTH elevation after surgery for primary hyperparathyroidism. *Surgery.* Jun 2007;141(6):777-783.

87. Nordenstrom E, Westerdahl J, Bergenfelz A. Effect on bone density of postoperative calcium and vitamin-D supplementation in patients with primary hyperparathyroidism: A retrospective study. *Langenbecks Arch Surg.* May 2009;394(3):461-467.
88. Kamycheva E, Sundsfjord J, Jorde R. Serum parathyroid hormone levels predict coronary heart disease: the Tromso Study. *Eur J Cardiovasc Prev Rehabil.* Feb 2004;11(1):69-74.
89. Hagstrom E, Hellman P, Larsson TE, et al. Plasma parathyroid hormone and the risk of cardiovascular mortality in the community. *Circulation.* Jun 2 2009;119(21):2765-2771.
90. Pilz S, Tomaschitz A, Drechsler C, et al. Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography. *Eur Heart J.* Jul 2010;31(13):1591-1598.
91. Walgenbach S, Hommel G, Junginger T. Outcome after surgery for primary hyperparathyroidism: ten-year prospective follow-up study. *World J Surg.* May 2000;24(5):564-569; discussion 569-570.
92. Hedback G, Tisell LE, Bengtsson BA, Hedman I, Oden A. Premature death in patients operated on for primary hyperparathyroidism. *World J Surg.* Nov-Dec 1990;14(6):829-835; discussion 836.
93. Ronni-Sivula H. Causes of death in patients previously operated on for primary hyperparathyroidism. *Ann Chir Gynaecol.* 1985;74(1):13-18.
94. Palmer M, Adami HO, Bergstrom R, Akerstrom G, Ljunghall S. Mortality after surgery for primary hyperparathyroidism: a follow-up of 441 patients operated on from 1956 to 1979. *Surgery.* Jul 1987;102(1):1-7.
95. Hedback G, Oden A. Increased risk of death from primary hyperparathyroidism--an update. *Eur J Clin Invest.* Apr 1998;28(4):271-276.
96. Bergenfelz A, Bladstrom A, Their M, Nordenstrom E, Valdemarsson S, Westerdahl J. Serum levels of uric acid and diabetes mellitus influence survival after surgery for primary hyperparathyroidism: a prospective cohort study. *World J Surg.* Jul 2007;31(7):1393-1400; discussion 1401-1392.
97. Soreide JA, van Heerden JA, Grant CS, Yau Lo C, Schleck C, Ilstrup DM. Survival after surgical treatment for primary hyperparathyroidism. *Surgery.* Dec 1997;122(6):1117-1123.
98. Wermers RA, Khosla S, Atkinson EJ, et al. Survival after the diagnosis of hyperparathyroidism: a population-based study. *Am J Med.* Feb 1998;104(2):115-122.
99. Yu N, Donnan PT, Flynn RW, et al. Increased mortality and morbidity in mild primary hyperparathyroid patients. The Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin Endocrinol (Oxf).* Jul 2010;73(1):30-34.
100. Hedback G, Oden A, Tisell LE. The influence of surgery on the risk of death in patients with primary hyperparathyroidism. *World J Surg.* May-Jun 1991;15(3):399-405; discussion 406-397.
101. Vestergaard P, Mollerup CL, Frokjaer VG, Christiansen P, Blichert-Toft M, Mosekilde L. Cardiovascular events before and after surgery for primary hyperparathyroidism. *World J Surg.* Feb 2003;27(2):216-222.
102. Farahnak P, Ring M, Caidahl K, Farnebo LO, Eriksson MJ, Nilsson IL. Cardiac function in mild primary hyperparathyroidism and the outcome after parathyroidectomy. *Eur J Endocrinol.* Sep 2010;163(3):461-467.
103. Kumar S, Olukoga AO, Gordon C, et al. Impaired glucose tolerance and insulin insensitivity in primary hyperparathyroidism. *Clin Endocrinol (Oxf).* Jan 1994;40(1):47-53.

104. Hagstrom E, Lundgren E, Rastad J, Hellman P. Metabolic abnormalities in patients with normocalcemic hyperparathyroidism detected at a population-based screening. *Eur J Endocrinol.* Jul 2006;155(1):33-39.
105. Broulik PD, Broulikova A, Adamek S, et al. Improvement of hypertension after parathyroidectomy of patients suffering from primary hyperparathyroidism. *Int J Endocrinol.* 2011;2011:309068.
106. Ahlstrom T, Hagstrom E, Larsson A, Rudberg C, Lind L, Hellman P. Correlation between plasma calcium, parathyroid hormone (PTH) and the metabolic syndrome (MetS) in a community-based cohort of men and women. *Clin Endocrinol (Oxf).* Nov 2009;71(5):673-678.
107. Chiu KC, Chuang LM, Lee NP, et al. Insulin sensitivity is inversely correlated with plasma intact parathyroid hormone level. *Metabolism.* Nov 2000;49(11):1501-1505.
108. Luigi P, Chiara FM, Laura Z, et al. Arterial Hypertension, Metabolic Syndrome and Subclinical Cardiovascular Organ Damage in Patients with Asymptomatic Primary Hyperparathyroidism before and after Parathyroidectomy: Preliminary Results. *Int J Endocrinol.* 2012;2012:408295.
109. Lind L, Jacobsson S, Palmer M, Lithell H, Wengle B, Ljunghall S. Cardiovascular risk factors in primary hyperparathyroidism: a 15-year follow-up of operated and unoperated cases. *J Intern Med.* Jul 1991;230(1):29-35.
110. Tomaschitz A, Ritz E, Pieske B, et al. Aldosterone and parathyroid hormone: a precarious couple for cardiovascular disease. *Cardiovasc Res.* Apr 1 2012;94(1):10-19.
111. Kosch M, Hausberg M, Vormbrock K, et al. Impaired flow-mediated vasodilation of the brachial artery in patients with primary hyperparathyroidism improves after parathyroidectomy. *Cardiovasc Res.* Sep 2000;47(4):813-818.
112. Nilsson IL, Aberg J, Rastad J, Lind L. Endothelial vasodilatory dysfunction in primary hyperparathyroidism is reversed after parathyroidectomy. *Surgery.* Dec 1999;126(6):1049-1055.
113. Nilsson IL, Rastad J, Johansson K, Lind L. Endothelial vasodilatory function and blood pressure response to local and systemic hypercalcemia. *Surgery.* Dec 2001;130(6):986-990.
114. Hulter HN, Melby JC, Peterson JC, Cooke CR. Chronic continuous PTH infusion results in hypertension in normal subjects. *J Clin Hypertens.* Dec 1986;2(4):360-370.
115. Snijder MB, Lips P, Seidell JC, et al. Vitamin D status and parathyroid hormone levels in relation to blood pressure: a population-based study in older men and women. *J Intern Med.* Jun 2007;261(6):558-565.
116. Ring M, Farahnak P, Gustavsson T, Nilsson IL, Eriksson MJ, Caidahl K. Arterial structure and function in mild primary hyperparathyroidism is not directly related to parathyroid hormone, calcium, or vitamin D. *PLoS One.* 2012;7(7):e39519.
117. Feldstein CA, Akopian M, Pietrobelli D, Olivieri A, Garrido D. Long-term effects of parathyroidectomy on hypertension prevalence and circadian blood pressure profile in primary hyperparathyroidism. *Clin Exp Hypertens.* May 2010;32(3):154-158.
118. Rydberg E, Birgander M, Bondeson AG, Bondeson L, Willenheimer R. Effect of successful parathyroidectomy on 24-hour ambulatory blood pressure in patients with primary hyperparathyroidism. *Int J Cardiol.* Jun 25 2010;142(1):15-21.

119. Staessen JA, Asmar R, De Buyzere M, et al. Task Force II: blood pressure measurement and cardiovascular outcome. *Blood Press Monit.* Dec 2001;6(6):355-370.
120. Letizia C, Ferrari P, Cotesta D, et al. Ambulatory monitoring of blood pressure (AMBP) in patients with primary hyperparathyroidism. *J Hum Hypertens.* Nov 2005;19(11):901-906.
121. Nilsson IL, Aberg J, Rastad J, Lind L. Circadian cardiac autonomic nerve dysfunction in primary hyperparathyroidism improves after parathyroidectomy. *Surgery.* Dec 2003;134(6):1013-1019; discussion 1019.
122. Silverberg SJ, Lewiecki EM, Mosekilde L, Peacock M, Rubin MR. Presentation of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metab.* Feb 2009;94(2):351-365.
123. Rubin MR, Silverberg SJ. Glucose intolerance and primary hyperparathyroidism: an unresolved relationship. *Endocrine.* Oct 2012;42(2):231-233.
124. Ayturk S, Gursoy A, Bascil Tutuncu N, Ertugrul DT, Guvener Demirag N. Changes in insulin sensitivity and glucose and bone metabolism over time in patients with asymptomatic primary hyperparathyroidism. *J Clin Endocrinol Metab.* Nov 2006;91(11):4260-4263.
125. Prager R, Schernthaner G, Niederle B, Roka R. Evaluation of glucose tolerance, insulin secretion, and insulin action in patients with primary hyperparathyroidism before and after surgery. *Calcif Tissue Int.* Jan 1990;46(1):1-4.
126. Kautzky-Willer A, Pacini G, Niederle B, Schernthaner G, Prager R. Insulin secretion, insulin sensitivity and hepatic insulin extraction in primary hyperparathyroidism before and after surgery. *Clin Endocrinol (Oxf).* Aug 1992;37(2):147-155.
127. DeFronzo RA, Lang R. Hypophosphatemia and glucose intolerance: evidence for tissue insensitivity to insulin. *N Engl J Med.* Nov 27 1980;303(22):1259-1263.
128. Valdemarsson S, Lindblom P, Bergenfelz A. Metabolic abnormalities related to cardiovascular risk in primary hyperparathyroidism: effects of surgical treatment. *J Intern Med.* Sep 1998;244(3):241-249.
129. Ljunghall S, Palmer M, Akerstrom G, Wide L. Diabetes mellitus, glucose tolerance and insulin response to glucose in patients with primary hyperparathyroidism before and after parathyroidectomy. *Eur J Clin Invest.* Oct 1983;13(5):373-377.
130. Bollerslev J, Rosen T, Mollerup CL, et al. Effect of surgery on cardiovascular risk factors in mild primary hyperparathyroidism. *J Clin Endocrinol Metab.* Jul 2009;94(7):2255-2261.
131. Abbas A, Grant PJ, Kearney MT. Role of IGF-1 in glucose regulation and cardiovascular disease. *Expert Rev Cardiovasc Ther.* Sep 2008;6(8):1135-1149.
132. Rajwani A, Ezzat V, Smith J, et al. Increasing circulating IGFBP1 levels improves insulin sensitivity, promotes nitric oxide production, lowers blood pressure, and protects against atherosclerosis. *Diabetes.* Apr 2012;61(4):915-924.
133. Petersson U, Ostgren CJ, Brudin L, Brismar K, Nilsson PM. Low levels of insulin-like growth-factor-binding protein-1 (IGFBP-1) are prospectively associated with the incidence of type 2 diabetes and impaired glucose tolerance (IGT): the Soderakra Cardiovascular Risk Factor Study. *Diabetes Metab.* Jun 2009;35(3):198-205.

134. Brismar K, Hilding A, Lindgren B. Regulation of IGFBP-1 in humans. *Prog Growth Factor Res.* 1995;6(2-4):449-456.
135. Heald AH, Cruickshank JK, Riste LK, et al. Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia.* Mar 2001;44(3):333-339.
136. Jehle PM, Ostertag A, Schulten K, et al. Insulin-like growth factor system components in hyperparathyroidism and renal osteodystrophy. *Kidney Int.* Feb 2000;57(2):423-436.
137. Diez JJ, Grande C, Mendez J, Gonzalez-Gancedo P, Iglesias P. Growth hormone and insulin-like growth factor binding protein-1 responses to oral glucose in patients with primary hyperparathyroidism. *Clin Endocrinol (Oxf).* Jul 2006;65(1):27-34.
138. Lewiecki EM, Miller PD. Skeletal effects of primary hyperparathyroidism: bone mineral density and fracture risk. *J Clin Densitom.* Jan-Mar 2013;16(1):28-32.
139. Aslan D, Andersen MD, Gede LB, et al. Mechanisms for the bone anabolic effect of parathyroid hormone treatment in humans. *Scand J Clin Lab Invest.* Feb 2012;72(1):14-22.
140. Eriksen EF, Mosekilde L, Melsen F. Trabecular bone remodeling and balance in primary hyperparathyroidism. *Bone.* 1986;7(3):213-221.
141. Mosekilde L. Primary hyperparathyroidism and the skeleton. *Clin Endocrinol (Oxf).* Jul 2008;69(1):1-19.
142. Grey AB, Evans MC, Stapleton JP, Reid IR. Body weight and bone mineral density in postmenopausal women with primary hyperparathyroidism. *Ann Intern Med.* Nov 15 1994;121(10):745-749.
143. Steiniche T, Christiansen P, Vesterby A, et al. Primary hyperparathyroidism: bone structure, balance, and remodeling before and 3 years after surgical treatment. *Bone.* May 2000;26(5):535-543.
144. Charopoulos I, Tournis S, Trovas G, 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.* May 2006;91(5):1748-1753.
145. Stein EM, Silva BC, Boutroy S, et al. Primary hyperparathyroidism is associated with abnormal cortical and trabecular microstructure and reduced bone stiffness in postmenopausal women. *J Bone Miner Res.* Dec 7 2012.
146. Hansen S, Hauge EM, Rasmussen L, Jensen JE, Brixen K. Parathyroidectomy improves bone geometry and microarchitecture in female patients with primary hyperparathyroidism: a one-year prospective controlled study using high-resolution peripheral quantitative computed tomography. *J Bone Miner Res.* May 2012;27(5):1150-1158.
147. Christiansen P, Steiniche T, Brixen K, et al. Primary hyperparathyroidism: short-term changes in bone remodeling and bone mineral density following parathyroidectomy. *Bone.* Aug 1999;25(2):237-244.
148. Boudou P, Ibrahim F, Cormier C, Sarfati E, Souberbielle JC. 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.* 2009;27(2):240-246.
149. Costa AG, Bilezikian JP. Bone turnover markers in primary hyperparathyroidism. *J Clin Densitom.* Jan-Mar 2013;16(1):22-27.
150. Silverberg SJ, Gartenberg F, Jacobs TP, et al. Increased bone mineral density after parathyroidectomy in primary hyperparathyroidism. *J Clin Endocrinol Metab.* Mar 1995;80(3):729-734.

151. Dy BM, Grant CS, Wermers RA, et al. Changes in bone mineral density after surgical intervention for primary hyperparathyroidism. *Surgery.* Dec 2012;152(6):1051-1058.
152. Rao DS, Phillips ER, Divine GW, Talpos GB. Randomized controlled clinical trial of surgery versus no surgery in patients with mild asymptomatic primary hyperparathyroidism. *J Clin Endocrinol Metab.* Nov 2004;89(11):5415-5422.
153. Ambrogini E, Cetani F, Cianferotti L, et al. Surgery or surveillance for mild asymptomatic primary hyperparathyroidism: a prospective, randomized clinical trial. *J Clin Endocrinol Metab.* Aug 2007;92(8):3114-3121.
154. Christiansen P, Steiniche T, Brixen K, et al. Primary hyperparathyroidism: effect of parathyroidectomy on regional bone mineral density in Danish patients: a three-year follow-up study. *Bone.* Nov 1999;25(5):589-595.
155. Sankaran S, Gamble G, Bolland M, Reid IR, Grey A. Skeletal effects of interventions in mild primary hyperparathyroidism: a meta-analysis. *J Clin Endocrinol Metab.* Apr 2010;95(4):1653-1662.
156. Vestergaard P, Mosekilde L. Cohort study on effects of parathyroid surgery on multiple outcomes in primary hyperparathyroidism. *Bmj.* Sep 6 2003;327(7414):530-534.
157. Nordenstrom E, Westerdahl J, Lindergard B, Lindblom P, Bergenfelz A. Multifactorial risk profile for bone fractures in primary hyperparathyroidism. *World J Surg.* Dec 2002;26(12):1463-1467.
158. Khosla S, Melton LJ, 3rd, Wermers RA, Crowson CS, O'Fallon W, Riggs B. Primary hyperparathyroidism and the risk of fracture: a population-based study. *J Bone Miner Res.* Oct 1999;14(10):1700-1707.
159. Vestergaard P, Mollerup CL, Frokjaer VG, Christiansen P, Blichert-Toft M, Mosekilde L. Cohort study of risk of fracture before and after surgery for primary hyperparathyroidism. *Bmj.* Sep 9 2000;321(7261):598-602.
160. Vestergaard P, Mosekilde L. Parathyroid surgery is associated with a decreased risk of hip and upper arm fractures in primary hyperparathyroidism: a controlled cohort study. *J Intern Med.* Jan 2004;255(1):108-114.
161. VanderWalde LH, Liu IL, O'Connell TX, Haigh PI. The effect of parathyroidectomy on bone fracture risk in patients with primary hyperparathyroidism. *Arch Surg.* Sep 2006;141(9):885-889; discussion 889-891.
162. Barlow L, Westergren K, Holmberg L, Talback M. The completeness of the Swedish Cancer Register: a sample survey for year 1998. *Acta Oncol.* 2009;48(1):27-33.
163. Singletary SE, Allred C, Ashley P, et al. Revision of the American Joint Committee on Cancer staging system for breast cancer. *J Clin Oncol.* Sep 1 2002;20(17):3628-3636.
164. Povoa G, Roovete A, Hall K. Cross-reaction of serum somatomedin-binding protein in a radioimmunoassay developed for somatomedin-binding protein isolated from human amniotic fluid. *Acta Endocrinol (Copenh).* Dec 1984;107(4):563-570.
165. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* Jul 1985;28(7):412-419.
166. Nordenstrom E, Westerdahl J, Bergenfelz A. Long-term follow-up of patients with elevated PTH levels following successful exploration for primary hyperparathyroidism. *World J Surg.* Jun 2004;28(6):570-575.

167. Chigot JP, Menegaux F, Achrafi H. Should primary hyperparathyroidism be treated surgically in elderly patients older than 75 years? *Surgery.* Apr 1995;117(4):397-401.
168. Bergenfelz A, Jansson S, Martensson H, et al. Scandinavian Quality Register for Thyroid and Parathyroid Surgery: audit of surgery for primary hyperparathyroidism. *Langenbecks Arch Surg.* Jul 2007;392(4):445-451.
169. Dobrinja C, Silvestri M, de Manzini N. Primary hyperparathyroidism in older people: surgical treatment with minimally invasive approaches and outcome. *Int J Endocrinol.* 2012;2012:539542.
170. Kebebew E, Duh QY, Clark OH. Parathyroidectomy for primary hyperparathyroidism in octogenarians and nonagenarians: a plea for early surgical referral. *Arch Surg.* Aug 2003;138(8):867-871.
171. Egan KR, Adler JT, Olson JE, Chen H. Parathyroidectomy for primary hyperparathyroidism in octogenarians and nonagenarians: a risk-benefit analysis. *J Surg Res.* Jun 15 2007;140(2):194-198.
172. Bay-Nielsen M, Kehlet H. Anaesthesia and post-operative morbidity after elective groin hernia repair: a nation-wide study. *Acta Anaesthesiol Scand.* Feb 2008;52(2):169-174.
173. Seagroatt V, Goldacre M. Measures of early postoperative mortality: beyond hospital fatality rates. *Bmj.* Aug 6 1994;309(6951):361-365; discussion 365-366.
174. Thomusch O, Machens A, Sekulla C, et al. Multivariate analysis of risk factors for postoperative complications in benign goiter surgery: prospective multicenter study in Germany. *World J Surg.* Nov 2000;24(11):1335-1341.
175. Nilsson H, Stylianidis G, Haapamaki M, Nilsson E, Nordin P. Mortality after groin hernia surgery. *Ann Surg.* Apr 2007;245(4):656-660.
176. Lew JI, Solorzano CC, Irvin GL, 3rd. Long-term results of parathyroidectomy for hypercalcemic crisis. *Arch Surg.* Jul 2006;141(7):696-699; discussion 700.
177. Harjit K, Zanariah H, Hisham AN. Hypercalcaemic crisis: immediate parathyroidectomy and intraoperative intravenous calcium infusion improves outcome. *Asian J Surg.* Jul 2007;30(3):173-177.
178. Lundgren E, Rastad J, Ridefelt P, Juhlin C, Akerstrom G, Ljunghall S. Long-term effects of parathyroid operation on serum calcium and parathyroid hormone values in sporadic primary hyperparathyroidism. *Surgery.* Dec 1992;112(6):1123-1129.
179. Kjellman M, Sandelin K, Farnebo LO. Primary hyperparathyroidism. Low surgical morbidity supports liberal attitude to operation. *Arch Surg.* Mar 1994;129(3):237-240.
180. Carmichael AR, Bates T. Obesity and breast cancer: a review of the literature. *Breast.* Apr 2004;13(2):85-92.
181. Cheng I, Klingensmith ME, Chattopadhyay N, et al. Identification and localization of the extracellular calcium-sensing receptor in human breast. *J Clin Endocrinol Metab.* Feb 1998;83(2):703-707.
182. Downey SE, Hoyland J, Freemont AJ, Knox F, Walls J, Bundred NJ. Expression of the receptor for parathyroid hormone-related protein in normal and malignant breast tissue. *J Pathol.* Oct 1997;183(2):212-217.
183. Eisman JA, Martin TJ, MacIntyre I. Presence of 1,25-dihydroxy vitamin D receptor in normal and abnormal breast tissue. *Prog Biochem Pharmacol.* 1980;17:143-150.
184. Leyssens C, Verlinden L, Verstuyf A. Antineoplastic effects of 1,25(OH)2D3 and its analogs in breast, prostate and colorectal cancer. *Endocr Relat Cancer.* 2013;20(2):R31-47.

185. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control.* Mar 2005;16(2):83-95.
186. Palmieri C, MacGregor T, Girgis S, Vigushin D. Serum 25-hydroxyvitamin D levels in early and advanced breast cancer. *J Clin Pathol.* Dec 2006;59(12):1334-1336.
187. Goodwin PJ, Ennis M, Pritchard KI, Koo J, Hood N. Prognostic effects of 25-hydroxyvitamin D levels in early breast cancer. *J Clin Oncol.* Aug 10 2009;27(23):3757-3763.
188. Ozbey N, Erbil Y, Ademoglu E, Ozarmagan S, Barbaros U, Bozbora A. Correlations between vitamin D status and biochemical/clinical and pathological parameters in primary hyperparathyroidism. *World J Surg.* Mar 2006;30(3):321-326.
189. Gissel T, Rejnmark L, Mosekilde L, Vestergaard P. Intake of vitamin D and risk of breast cancer--a meta-analysis. *J Steroid Biochem Mol Biol.* Sep 2008;111(3-5):195-199.
190. Chlebowski RT, Johnson KC, Kooperberg C, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. *J Natl Cancer Inst.* Nov 19 2008;100(22):1581-1591.
191. Bolland MJ, Grey A, Gamble GD, Reid IR. Calcium and vitamin D supplements and health outcomes: a reanalysis of the Women's Health Initiative (WHI) limited-access data set. *Am J Clin Nutr.* Oct 2011;94(4):1144-1149.
192. Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle: physiology and biomarkers. *Am J Clin Nutr.* Aug 2008;88(2):500S-506S.
193. Rose DP, Vona-Davis L. Interaction between menopausal status and obesity in affecting breast cancer risk. *Maturitas.* May 2010;66(1):33-38.
194. Hamoui N, Anthone G, Crookes PF. Calcium metabolism in the morbidly obese. *Obes Surg.* Jan 2004;14(1):9-12.
195. Gunter MJ, Hoover DR, Yu H, et al. Insulin, insulin-like growth factor-I, and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst.* Jan 7 2009;101(1):48-60.
196. Bergenfelz A, Valdemarsson S, Tibblin S. Persistent elevated serum levels of intact parathyroid hormone after operation for sporadic parathyroid adenoma: evidence of detrimental effects of severe parathyroid disease. *Surgery.* Jun 1996;119(6):624-633.
197. Anderson JL, Vanwoerkom RC, Horne BD, et al. Parathyroid hormone, vitamin D, renal dysfunction, and cardiovascular disease: dependent or independent risk factors? *Am Heart J.* Aug 2011;162(2):331-339 e332.
198. Jorde R, Sundsfjord J, Haug E, Bonaa KH. Relation between low calcium intake, parathyroid hormone, and blood pressure. *Hypertension.* May 2000;35(5):1154-1159.
199. Jorde R, Svartberg J, Sundsfjord J. Serum parathyroid hormone as a predictor of increase in systolic blood pressure in men. *J Hypertens.* Sep 2005;23(9):1639-1644.
200. Soares MJ, Ping-Delfos WC, Sherriff JL, Nezhad DH, Cummings NK, Zhao Y. Vitamin D and parathyroid hormone in insulin resistance of abdominal obesity: cause or effect? *Eur J Clin Nutr.* Dec 2011;65(12):1348-1352.
201. Rejnmark L, Vestergaard P, Brot C, Mosekilde L. Increased fracture risk in normocalcemic postmenopausal women with high parathyroid hormone levels: a 16-year follow-up study. *Calcif Tissue Int.* Mar 2011;88(3):238-245.

202. Stein EM, Dempster DW, Udesky J, et al. Vitamin D deficiency influences histomorphometric features of bone in primary hyperparathyroidism. *Bone.* Mar 1 2011;48(3):557-561.
203. Hagstrom E, Hellman P, Lundgren E, Lind L, Arnlov J. Serum calcium is independently associated with insulin sensitivity measured with euglycaemic-hyperinsulinaemic clamp in a community-based cohort. *Diabetologia.* Feb 2007;50(2):317-324.
204. von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr.* Feb 2010;103(4):549-555.
205. Kannel WB, Vasan RS, Levy D. Is the relation of systolic blood pressure to risk of cardiovascular disease continuous and graded, or are there critical values? *Hypertension.* Oct 2003;42(4):453-456.
206. Vasan RS, Larson MG, Leip EP, et al. Impact of high-normal blood pressure on the risk of cardiovascular disease. *N Engl J Med.* Nov 1 2001;345(18):1291-1297.
207. Leopaldi E, Paolino LA, Bevilacqua M, et al. Prediction of bone mass gain by bone turnover parameters after parathyroidectomy for primary hyperparathyroidism: neural network software statistical analysis. *Surgery.* Jun 2006;139(6):827-832.
208. Almqvist EG, Becker C, Bondeson AG, Bondeson L, Svensson J. Early parathyroidectomy increases bone mineral density in patients with mild primary hyperparathyroidism: a prospective and randomized study. *Surgery.* Dec 2004;136(6):1281-1288.
209. Sitges-Serra A, Garcia L, Prieto R, Pena MJ, Nogues X, Sancho JJ. Effect of parathyroidectomy for primary hyperparathyroidism on bone mineral density in postmenopausal women. *Br J Surg.* Jul 2010;97(7):1013-1019.
210. Yang D, Atkins GJ, Turner AG, Anderson PH, Morris HA. Differential effects of 1,25-dihydroxyvitamin D on mineralisation and differentiation in two different types of osteoblast-like cultures. *J Steroid Biochem Mol Biol.* Dec 7 2012.
211. Christensen MH, Apalset EM, Nordbo Y, Varhaug JE, Mellgren G, Lien EA. 1,25-dihydroxyvitamin D and the vitamin D receptor gene polymorphism Apa1 influence bone mineral density in primary hyperparathyroidism. *PLoS One.* 2013;8(2):e56019.