Primary Hyperparathyroidism

Prevalence and Associated Morbidity in Middle-Aged Women and Elderly Men

HELENE SIILIN
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Abstract

Primary hyperparathyroidism (PHPT) is a common endocrine disease, existing in both genders and in all age groups. Postmenopausal women are at particular risk of developing the disease and estrogen decline after menopause is suggested to affect the progress. Although PHPT is mild in its presentation with subtle or no subjective symptoms, it is associated with an increased risk of associated morbidity and also mortality i.e cardiovascular complications, psychiatric instability, concomitant metabolic abnormalities, obesity and decrease in bone mineral density. The current cure is surgical removal of the diseased gland/s, but other medical alternatives have been investigated. The disease is thoroughly explored in postmenopausal women but less is known about other populations groups.

Since progression of the disease generally is slow, the underlying disturbance of the calcium homeostasis can be suspected to have been established a long time prior to diagnose with potential to affect associated morbidity. The general aim of this thesis is to clarify the expression of PHPT in premenopausal women and in elderly men and to explore how frequent the disease in these populations occurs. The women and men were investigated through population-based studies.

Baseline data and prevalence of PHPT in premenopausal women age 40-50 years were studied (Paper I), the prevalence was 5.1% in this population and was associated with decreased bone mineral density and associated obesity. In a three years follow up of the female cohort, the effects of menopausal transition and associated morbidity was investigated (Paper II). The prevalence and expression of PHPT in men between 69 and 81 years and impact on bone mineral density, physical performance, fall and fracture prevalence was explored through data from Mr Os Sweden (Papers III and IV). In this population prevalence of PHPT was 0.73% and associated with lower bone mineral density and inferior physical performance.

Keywords: Primary hyperparathyroidism, menopausal, bone mineral density, physical performance, parathyroid hormone

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Carpe Diem!
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals (I-IV).


IV **Siilin, H.**, Lundgren, E., Mallmin, H., Mellström, D., Ohlsson, C., Ribom, E., Ljunggren, Ö., Rosengren, B., Karlsson, M. K. (2011) Association of primary hyperparathyroidism and physical performance, fall and fracture risk in elderly men – Mr Os Sweden. *In manuscript*

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# Abbreviations

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<td>PHPT</td>
<td>Primary Hyperparathyroidism</td>
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<tr>
<td>iPTH</td>
<td>Intact Parathyroid Hormone</td>
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<tr>
<td>s-calcium</td>
<td>Serum Calcium</td>
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<td>BMD</td>
<td>Bone Mineral Density</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>sBMD</td>
<td>Standardized Bone Mineral Density</td>
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<td>LS</td>
<td>Lumbar Spine</td>
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<td>IEP</td>
<td>Inappropriately Elevated Parathyroid hormone</td>
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<td>Base-line</td>
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Introduction

Primary hyperparathyroidism (PHPT) is an endocrine disease, that primary disturbs the hormonal functioning of the parathyroid glands, which could lead to excessive serum calcium levels. From studies of embryogenesis, it is know the glands have a close relation to both the thyroid and thymus and share the same origin. The parathyroid glands develop and descend pairwise from the third (same as thymus) and fourth (same as thyroid gland) pharyngeal pouches, giving rise to the superior (fourth pharyngeal pouch) and inferior (third pharyngeal pouch) parathyroid glands located in near vicinity of the thyroid gland. Humans normally have four parathyroid glands, but there are individuals with supernumerous glands. The descent of the glands can go wrong and parathyroid tissue may be found anywhere along the pathway of descent of the pharyngeal pouches, from the pharynx down to the intra thoracic location and, as deep as around the aortic arc.

Histology and pathology

A normal parathyroid gland consists of two different types of cells, chief and oxyphil cells. In addition to these cells the glands have stromal fibrovascular- and adipose components. Chief cells are hormonally active and produce parathyroid hormone (PTH). The glands are tiny and an average normal gland is as small as a grain of rice and weighs around 40 mg.

There are three different pathological lesions inducing PHPT (Textbook of endocrine surgery; Clark, Duh, Kebebew 2nd edition 2005).

- Adenoma – benign neoplasm, usually affecting a single gland and the most common lesion in up to 85% of all cases of PHPT.
- Hyperplasia – an increase in hormonally active cells in all glands (multiglandular), in the absence of known stimulus for PTH hypersecretion. This condition represents approximately 15% of all PHPT cases and may be associated with genetic disturbance in inherited multiple endocrine neoplasia.
- Carcinoma – a rare condition representing less than 1% of all PHPT cases.
The etiology of PHTP is largely unknown but varies from a history of irradiation to the neck, hereditary disorders (MEN 1 and 2a) and, lithium treatment\textsuperscript{3} to sporadic cases. Different underlying genetic changes have been identified that may explain some of the pathogenesis of tumor development\textsuperscript{4}.

### Historical perspective

The first observation of the parathyroid gland was made by Richard Owen in 1849 when dissecting an Indian rhinoceros\textsuperscript{5}. However, glandula parathyroideae was first histologically described by Ivar V Sandström, a Swedish anatomist, in 1880. In 1891 a German pathologist, Friedrich Daniel von Recklinghausen, described the severe deterioration of the skeleton, \textit{i.e.} osteitis fibrosa cystica, but it was J Erdenheim who made the association between parathyroid gland and bone and, initially physicians considered the enlargement of the glands to be induced by the bone disease\textsuperscript{5}. The first patient successfully operated for PHPT was reported by Felix Mandl in 1925\textsuperscript{6}.

In 1935, Fuller Albright noted a majority of patients with PHPT, in addition to bone disease, had renal stones/calculi, and for decades this was regarded as the hallmark of PHPT\textsuperscript{7}. Up till the 1960s, patients were treated with surgery for obvious symptoms of PHPT, including marked hypercalcaemia, however, in mid-1960s, the operations increased in number and patients were treated earlier in the progression of the disease, thus, with fewer symptoms.

Another revolution came in the early 1970s with the introduction of automated measurement of serum calcium, which is now widely and liberally used. Henceforth, patients were frequently identified through their biochemical profile and the expression of PHPT changed from a disease with severe neuromuscular-, bone- and renal symptoms, with marked hypercalcemia, to a mild or even asymptomatic disease.

The surgical procedure for parathyroidectomy has also undergone changes – from a full exploration of the neck and a mandatory identification of all glands to minimal invasive surgery, with few complications and tolerability for most patients\textsuperscript{8,9}. Surgical development has been possible due to new techniques in the field of radiology that enable accurate pre-operative localizations diagnostics.
Calcium homeostasis

Calcium

Normal calcium concentration is crucial for normal body functioning and is involved in a variety of complex cellular processing\(^{10}\). Calcium homeostasis is strongly regulated by PTH and vitamin D in a negative feedback mechanism in order to maintain the concentration of calcium within a narrow physiologic range, therefore, minor alteration in concentration can trigger a substantial secretory response\(^{11}\), (Figure 1). The body of an adult consists of approximately 1 to 1.5 kg calcium, which is bound in the skeleton as hydroxylapatite which is substrate of calcium and phosphorus. Only 1% of the total calcium content circulates in the extracellular plasma, of which about half is bound to proteins such as albumin, the rest is stored intracellular. Calcium exerts its effects in ionized form (Ca\(^{2+}\)) through;

- Influencing neurons and muscle cells sensitivity and intracellular signaling
- Functioning as a co-factor in many enzymatic reactions
- Being the most potent inhibitor of PTH secretion by binding to the calcium sensor receptor in the membrane of parathyroid cells\(^{12}\)

![Figure 1](image_url)  
*Figure 1* A simplified schematic illustration of calcium homeostasis
Parathyroid hormone

When alterations in calcium concentration occur, the parathyroid cells are able to respond directly through activation of the calcium sensor receptor\textsuperscript{12}. PTH receptors are found in responsive target-organs, which are mainly the skeleton (osteoblasts) and the kidney, and enable a fast response and shift in calcium concentration\textsuperscript{13}.

In the kidney, activation of PTH-receptor leads to increased tubular re-absorption of calcium at the expense of increased phosphate ion excretion. Moreover, PTH up-regulates the enzyme responsible for conversion of inactive vitamin D into its active form. In the skeleton, the effects of the response are slower and the action is indirectly mediated via binding to osteoblasts leading to increased activity of the osteoclasts responsible for degradation of bone matrix and release of calcium bound in hydroxyapatite.

Intact PTH (iPTH) 1-84 is a polypeptide containing 84 amino acids which is released from the chief cells and is rapidly cleared (within a few minutes) from the blood circulation. The active part of the molecule is the amino group 1-34. Degradation of PTH occurs in the Kupffer cells in the liver and through renal filtration. The development of immunometric assay facilitates the diagnosis of PHPT through eradicating other causes of hypercalcaemia\textsuperscript{14}.

Vitamin D

Vitamin D is a group of substances with pro-hormone characteristics and vitamin D receptor is present in almost all cells in the body\textsuperscript{15}. An inert precursor form of vitamin D is either produced in the skin through ultra violet light or taken into the intestines from food. The activation of vitamin D is in two steps, the first transformation by hydroxylation occurs in the liver and results in 25(OH)D, which reflects a persons nutritional vitamin D status\textsuperscript{16}. The last step is processed in the kidney where 25(OH)D is further hydroxylated to its active form calcitriol (1,25 (OH)D) by influence of PTH. Calcitriol influences the levels of calcium via two principal ways but the effects are slower in contrast to the effects of PTH:

- Via a negative feed-back mechanism mediated by the intracellular vitamin D receptor (VDR) in the parathyroid cells. When VDR is activated by calcitriol it results in decreased PTH production through inhibition of PTH gene expression.

- Stimulates and increases up-take of calcium from food in the small intestine.
Vitamin D is vitally important for normal bone formation and bone remodulation and subnormal levels may lead to osteomalacia in adults and rickets in children.

**Diagnosis of primary hyperparathyroidism**

The recognition of PHPT is based on the disturbed balance between PTH and total s-calcium and Ca\(^{2+}\) \(^{17}\). Normally, PTH concentration is suppressed when s-calcium concentration is high and vice versa. At PHPT diagnosis, the s-calcium level is usually mildly to moderately elevated in combination with an inappropriately elevated intact parathyroid hormone (iPTH), in the absence of factors known to influence PTH/calcium concentration; i.e. medical treatment with tiazides or lithium \(^{3,18}\) or known benign familial hypocalciuric hypercalcaemia \(^{19}\).

Renal failure and vitamin D deficiency can induce secondary hyperparathyroidism but, these two variants of HPT can in general be distinguished from each other. The diagnosis of secondary HPT is based either on low levels of vitamin D or low estimated glomerular filtration rate (eGFR, renal failure) and presents with relatively low s-calcium and high phosphate ions levels in relation to sometimes extremely high values of iPTH in secondary HPT \(^{17}\). In all other causes of hypercalcemia such as malignancy, sarcoidosis, and multiple myeloma, PTH concentration is substantially suppressed.

Normocalcemic hyperparathyroidism, i.e. total s-calcium levels within but in the upper normal reference range combined with raised levels of PTH has been recognized \(^{20,21}\). Even preoperative normal levels of iPTH are reported as an entity in surgically treated patients and may occur at earlier stage, in younger patients with better renal function and lower bone turnover \(^{22,23}\). To identify patients with normocalcemic hyperparathyroidism, the measurement of ionized calcium has an important role \(^{24,25}\).

**Epidemiology**

In many parts of the world, PHPT has evolved from a disease with severe symptoms of hypercalcaemia, i.e. overt bone disease, neuromuscular dysfunction and renal stones, towards a disorder that in many patients can be described as latent or subclinical with few or no symptoms \(^{26,27}\). However, the presentation of the disease is demographically different which has been proved in developing countries where cases with PHPT have more progressed symptomatology, and that could only partly be due to delayed diagnosis \(^{28}\).
Incidence and prevalence

The incidence and prevalence data of PHPT varies between different geographical areas and also between different periods (i.e. before and after introduction of automated s-calcium measurements in the early 1970s). In the mid 1960s Boonstra et al. identified 50 subjects with PHPT in a serum calcium survey of 50,330 individuals during a ten year period. In a longitudinal epidemiological study in Rochester, Minnesota, USA the incidence rate was evaluated during 36 years (1965 to 2001). Before the screening era (before mid 1974) the incidence was around 15 cases per 100,000 person-years with a peak rate observed in the second half of 1974 of 129/100,000 person-years. The incidence remained high for around eight years but then declined during the subsequent ten years, with a further decline in 2001 (16/100,000). In the same study the proportion of patients with symptomatic disease decreased from 20% to 5%. Initially, the peak rate detected was supposed to be explained by a catch-up effect after the 1970s due to the liberal use of the biochemical analysis, but the authors considered the possibility of other underlying etiologies for the sustained high incidence through 1982.

The occurrence of PHPT in Scandinavian countries displays a similar pattern. There are though reports on increasing prevalence of PHPT especially in females. The estimation of PHPT prevalence in different studies is strongly dependent on the biochemical criteria used for identifying the disease. In some health surveys attempting to recognize PHPT, only serum calcium concentration is used as an indicator of the disease and then often set at indisputable hypercalcaemia. Studies prior to the use of PTH assays generally display lower prevalence figures, which is probably due to the lack of identification of PHPT subjects with s-calcium just above or below the upper normal range.

Gender and age distribution

PHPT is found in both genders and in all age-groups, although the condition is rare and seldom seen in children. Incidence increases with age and women are more prone to develop the disease than men. The female/male ratio at older age is to be around 3:1. A study from the University of Michigan investigated the age- and sex-related incidence and distribution of PHPT in surgically treated patients and found the frequency of the disease begins to rise in both sexes at the age of eleven but with steeper/accelerating curve in females from their mid-twenties.

As postmenopausal women are at particular risk of developing PHPT, the highest prevalence is found in this sex/age group. The onset of menopause has been explored in women with hypercalcaemia that is probably due to...
PHPT, and these women had in average a 4.5 year younger menopausal age than eucalcemic controls\textsuperscript{38}. Although it can be hypothesized that estrogen deficiency occurring during menopausal transition in some way promotes the manifestation of PHPT and increases the risk for postmenopausal women to develop the disease. However, Miller et al\textsuperscript{37} has specifically evaluated the age and gender distribution in PHPT. The female:male ratio does not change during the peri- and postmenopausal years and is consistent across the age groups, with similar lifetime incidence curves, and this would question the theory of hormonal change at menopause being an underlying cause of increased prevalence in older women\textsuperscript{37}.

During the early 1990s a population based screening study in Uppsala, Sweden, attempted to identify post-menopausal women with PHPT\textsuperscript{39}. The women were recognized through the biochemical imbalance between s-calcium and PTH with pre-set criteria defining PHPT. The prevalence of post-menopausal women with PHPT was 2.1\% and the biochemical criteria utilized were verified surgically\textsuperscript{39}. The women were then revised eight years later and based on retrospective laboratory data of s-calcium and iPTH the prevalence in this particular group was adjusted to 3.4\%\textsuperscript{32}. In this thesis, the same biochemical pre-set criteria utilized for the population of postmenopausal women was used for identifying premenopausal women with assumed PHPT.

Associated morbidity

Early in the history of PHPT the patients suffered from very advanced symptoms described above, but these are rarely seen in industrial countries today. The disease now appears asymptomatic and, there has been substantial investigation of the clinical course and natural history of this “phenotype” and the changes in expression of the disease, but the reason has not been fully clarified. Although, PHPT often occurs in a mild form, it is evidently associated with increased mortality and morbidity in cardiovascular- neuromuscular- psychiatric- metabolic- renal- and diseases, and some malignancies\textsuperscript{40-43}. In addition, there is evidence of impact on the skeleton and a suggested increased in fracture risk for PHPT patients\textsuperscript{21,44}. PHPT is more common in postmenopausal women and is also best described in this population group.

Cardiovascular complications

Cardiovascular disease (CVD) and complications commonly occur in patients with symptomatic PHPT and increase the risk of premature death\textsuperscript{45-48}. The most common cause of death in patients with PHPT is cardiovascular complications, myocardial infarction; stroke; congestive heart failure and,
generalized atherosclerosis\textsuperscript{49,50}. Structural and functional changes in the vascular wall, as well as in the heart have been determined even in patients with normocalcemic PHPT, suggesting that there might be a combination of underlying metabolic disturbances that cause these alterations\textsuperscript{51}. Impaired renal function in patients with PHPT could trigger the development of hypertension, which \textit{per se} is a potential risk factor for coronary heart disease and stroke events. In a recently published study of 1020 surgically treated patients approximately 70\% had hypertension compared to 50\% of the controls\textsuperscript{52}. Structural cardiac changes, such as the development of left ventricular hypertrophy, that is a strong predictor of cardiovascular morbidity, may be partly explained by increased arterial vessel stiffness which leads to increased peripheral resistance. This is indirectly shown in a study of 21 subjects with PHPT, who had higher augmentation of the central aortic pressure than controls, which could be explained by a combination of both structural and functional changes in the vessels induced by PHPT\textsuperscript{53}. Also in subjects without manifest atherosclerosis, there is impairment of vessel function\textsuperscript{54}, which provides a natural hypothesis of raised blood pressure and the development of hypertension. In addition, CV risk factors including arterial rigidity, appears to be similar regardless the degree of hypercalcemia in patients with PHPT\textsuperscript{55}. However, the impacts on CV morbidity are higher in the hypercalcemic population\textsuperscript{55}.

\textbf{Metabolic disturbances}

The development of cardiovascular complications is probably multifactorial in origin, although the underlying pathophysiological mechanisms are not fully understood. Further, PHPT patients can also display subtle metabolic disturbances. Obesity and overweight as well as impaired glucose intolerance, diabetes mellitus and dyslipidemia have been detected as complications in patients with overt disease and in individuals with more subtle symptomatology including normocalcemic PHPT and hence increase the risk profile for CVD development\textsuperscript{43,56-58}.

In a cohort of postmenopausal\textsuperscript{56} women with increased body weight associated with PHPT, the increased body weight appears to precede the development of hypercalcaemia. A meta-analysis of seventeen studies (between 1975 to 2003)\textsuperscript{57} on body weight and its association with PHPT reveals that subjects with PHPT are consistently heavier than eucalcemic controls, analyzed in categories of pre-postmenopausal women and men.

The interaction of different underlying risk factors for CVD in PHPT is complex. Although obesity is associated with the disease, it may account for the associated metabolic abnormalities detected, thus PHPT might be one of the manifestations of the metabolic syndrome\textsuperscript{59}. 
Psychiatric disorders

Neuropsychiatric manifestations described in PHPT vary from condition similar to dementia in cases with severe disease, including psychotic symptoms^{60-62}, to anxiety, depression and, changed personality^{41}. In addition, there are a range of nonspecific psychiatric disturbances, such as subjective tiredness and cognitive impairment in mild cases^{63,64}. The severity of the psychiatric disturbances is correlated to the degree of hypercalcaemia^{61} of values well above normal. However, psychiatric symptoms of severe mental disturbance are recorded even in cases with mild hypercalcemia^{62}. Normally, subtle change in mental health is associated with mild disease, and subjects with extreme values of s-calcium values (>4 mmol/L) are at extremely high risk of developing severe conditions such as unconsciousness and hallucinations. The frequency of different psychiatric specific and nonspecific symptoms in associations with PHPT have been reported to range from around 10% to over 80%^{61,64}.

Psychometric tests are used to evaluate the impact of PHPT on quality of life (QoL)^{65} and appraise the effects of surgical treatment. Often these psychometrical tests are non disease specific, however, J Pasieka and associates have developed a disease specific tool to make it easier to evaluate symptoms affecting QoL in PHPT patients, which has shown to correlate well with short-form 36 questionnaire (SF-36)^{66}. Parathyroidectomy is beneficial for improving or curing associated psychiatric symptoms^{63,64,67,68}. In this thesis SF-36 was used to evaluate QoL^{69}.

Bone mineral density and fracture risk

The impact of PTH on bone remodeling is complicated and includes both catabolic and anabolic effects^{70}, with increased osteoclastic bone resorption and induced osteoblastic bone formation through different pathways, which is reviewed by Lee et al^{71}. In severe PHPT, there is strong association with skeletal morbidity, including fractures and, patients can suffer from nearly malignant skeletal detoriation (i.e osteitis fibrosa cystica).

With the acknowledgment of “mild PHPT”, the impact on skeletal health in association with this form of the disease has also been investigated^{21,72}. Decline in bone mineral density (BMD) associated with PHPT is primarily located at cortical sites / appendicular skeleton^{42,72} and may be associated with increased fracture risk at these locations. There are reports of manifest reduction of the trabecular bone in lumbar spine^{73} even though BMD is often preserved at this site.

The pathological outcome of excessive hormonal production on skeletal status may be partially dependent on the PTH secretory pattern^{70}. There is an association between PTH and osteoprotegerin (regulator of osteoclast
activity) secretion, and postmenopausal women with disturbed PTH circadian cycles, have deacreased osteoprotegerin, indicating that altered PTH secretion increases nocturnal bone resorption due to decreased inhibition of osteoclastic activity. It is suggested that in PHPT, the disturbed increase in PTH has diverse effects on trabecular and cortical bone formation. Studies on BMD in patients with mild PHPT indicate that elevated levels of PTH have anabolic effects in lumbar spine and appear to preserve bone mineral content better. Thus, postmenopausal women may be protected of the oestrogen deficiency osteoporosis observed after menopause.

Muscular functioning

Neuromuscular dysfunction is associated with PHPT. It appears as if it is particularly proximal muscles of the lower extremities that are affected and easy wearable. Other symptoms include paresthesias, bone or joint pain and unsteady walk. However, mental status such as depression and fatigue may contribute to the subjective feeling of muscular weakness. The use of electromyography, nerve conducting and muscle biopsies examination have been evaluated and minor changes to nerve and muscular functioning are documented but whether this can explain the symptomatology experienced by the patients has not been ascertained, as the studies were small, including only a few patients. Neuromuscular functioning has been evaluated before and after surgery and improvements in neuromuscular performance are often observed after restoration to normocalcemia.

Indications for surgical treatment – parathyroidectomy

Parathyroidectomy is the only definite therapy for PHPT and the surgical procedure with minimal invasive techniques is considered safe with few complications. However, the change in presentation of PHPT has required physicians and scientist to reconsider and discuss the treatment and the need of changing surgical indications. During the three international workshops (USA) on the management of asymptomatic PHPT surgical guidelines were revised. Consensus from the latest workshop (May 2008) provided updated guidelines for surgical intervention as follow:

1. Serum calcium concentration of 1 mg/dl (0.25 mmol/L) above the accepted normal reference range
2. Creatinine clearance reduced to <60 mL/min/1.73m²
3. Bone mineral density at any site reduced by more than 2.5 standard deviation below peak bone mass (t-score < 2.5) and/or previous fragility fractures.
4. Patients under 50 years of age.
5. Patients for whom medical surveillance was either not desirable (e.g. coexistent illness) or not possible.

In many of the asymptomatic patients who do not meet the guidelines of surgery, the progression of the disease appears to be slow\textsuperscript{81-84}. Around 30% of those who are diagnosed with PHPT are not treated surgically\textsuperscript{85}, but surveillance is necessary as progression of the disease is difficult to predict.
Hypothesis

Primary hyperparathyroidism is more common in post-menopausal women and the disease is best described in this population group, however, less is known about PHPT in pre-menopausal women and in men. The progress of the disease is usually very slow and any underlying disturbance in calcium/PTH balance can be assumed to have existed for a long time prior to diagnosis, with potential to affect associated morbidity. The general aim of this thesis was to clarify the expression of PHPT in premenopausal women and in older men and to explore the prevalence of the disease in these populations.

Specific aims of the studies

To investigate the prevalence of PHPT in pre-menopausal women and examine if PHPT in this particular group with assumed early PHTP was associated with morbidity. (Paper I)

To validate the changes of associated morbidity and bone mineralization in women with disturbed calcium homeostasis during menopausal transition. (Paper II)

To examine the prevalence of PHPT in older men and determine whether there were any associated changes in bone mineral density in this population group. (Paper III)

To explore the prevalence of associated muscular dysfunction and fall and fracture risk in older men with disturbed calcium homeostasis and established PHPT. (Paper IV)
Materials and Methods

Subjects

Papers I and II
Premenopausal women between 40 and 50 years of age were invited to participate in the study in connection with routine mammography health screening at the University Hospital of Uppsala, Sweden. Women taking hormonal replacement treatment (HRT) or contraceptives containing estrogens were excluded. The subjects included had to be clinically premenopausal at the time of inclusion, and were asked about menstrual flow during the last six months, and if in doubt follicle-stimulating hormone (FSH) was determined. The initial inclusion period was from 1 March 2002 until 28 February 28 2003. The participants were then re-examined at three years follow-up. A flow-chart illustrating the sampling process is presented (Figure 2)

Papers III and IV
Mr OS Sweden is a population-based database with the primary aim of investigating factors of importance for the development of osteoporosis in elderly men. Men aged between 69 and 81 years and living in three different geographical areas in Sweden (Gothenburg, Malmo, and Uppsala) were randomly selected from the background population and asked by telephone calls to participate in the study; 3014 men were enrolled with approximately 1000 men from each town. All had to be community dwellers and able to walk by themselves, with or without a stick, and not have bilateral hip prosthesis surgery done. The data from these studies displayed cross-sectional characteristics and base-line information, however, Mr OS Sweden has a prospective design and data are frequently collected, which is updated in the database.
Ethics
All studies were approved by local ethics committees and the participants were provided oral and written information and signed informed consent papers.

Methods

Paper I
Base-line (BL) data of premenopausal women with mild disturbances in calcium homeostasis comparable with PHPT are presented in Paper I.

Main study PHPT-cases and controls (Figure 2)

- A non-fasting venous blood sample was collected from all potential participants at the mammography centre (n=1866). The blood samples were analyzed for serum calcium and serum albumin, from which total s-calcium (albumin adjusted s-calcium) was calculated with equation: Albumin adjusted s-calcium=analyzed s-calcium – (analyzed s-albumin-46)*0.02.
- All women with total s-calcium ≥2.50 mmol/L (n=540) were asked to continue in the trial.
- A second fasting venous blood sample (n=535; five dropouts) was obtained within a few month of the first occasion and analysed for: s-albumin; s-creatinine; p-iPTH; and s-calcium. Women with inappropriately elevated levels of iPTH in relation to albumin adjusted s-calcium (according to preset diagnostic criteria – see below) were invited for further investigations (n=232 minus 18 dropouts).
- At the third sampling occasion (between March 2003 and November 2004) controls (n=214) were chosen from women with total s-calcium below 2.50 mmol/l at first screening. The cases (n=214) were matched to controls with respect to age and date of initial investigation.
- Fasting blood samples were analyzed for cases (n=214) and controls (n=214) and included: Hemoglobin; leukocytes; blood platelets; s-calcium; iPTH; s-phosphate; s-albumin; s-creatinine; s-potassium; s-sodium; blood glucose; and 25-hydroxyvitamin D (25(OH)D) and FSH.
- In conjunction with the third occasion, the participants were examined by dual X-ray absorptiometry (DXA) from which body mass index (BMI) was determined. Finally, SF-3669 questionnaires were completed.
Participants with suspected vitamin D deficiency (values below 20 µg/L), indicating possible “secondary” PHPT, were excluded (n=41 cases and n=21 controls) from the analysis.

Paper II

The participants in the BL study were reevaluated at a three-year follow-up (FU) between March 2006 to April 2008. Fasting blood samples were collected and analyzed for parameters of calcium homeostasis. FSH was tested to establish menopausal status.

The subjects were divided into three groups; defining their menopausal status, as determined by FSH measurements at BL and FU.

- Group PRE - premenopausal at BL and premenopausal at FU.
- Group PRE-POST - premenopausal at BL and postmenopausal at FU.
- Group POST - perimenopausal* at BL and postmenopausal at FU (*i.e women who were clinically premenopausal at inclusion but on first examination had an elevated FSH level indicating ovarian failure).

Bone mineral density (BMD) of the lumbar spine, total hip, femoral neck and distal third of radius was measured by DXA. The BMI for each subject was determined and waist circumference measured.

The participants also completed SF-36, and provided information on associated morbidity and intake of medicine and/or diverse health products (*i.e vitamins, calcium substitute).
1866 WOMEN SCREENED FOR S-CALCIUM

1) FIRST ANALYSIS
540 HAD S-CA > 2.50 MMOL/L

5 DROP-OUTS

2) SECOND BLOOD ANALYSIS
18 DROP-OUTS

232 FULFILLED PRE-SET CRITERIA FOR PHPT (CASES)

21 VITAMIN D LEVELS < 20NG/L

173 CASES INCLUDED
193 CONTROLS INCLUDED

AT BASE LINE IN TOTAL 366 WOMEN

BMD ANALYSIS
147 CASES VS 174 CONTROLS

BMD
26 CASES
19 CONTROLS

SF-36
35 CASES
22 CONTROLS

SF-36 MEASUREMENT
138 CASES VS 171 CONTROLS

Figure 2 Flow-chart of the sampling selection of subjects in Papers I and II

Papers III and IV

Blood samples were analyzed for parameters of calcium homeostasis, 25(OH)D and cystatin-C from which estimated glomerular functioning (eGFR) was calculated. To calculate albumin adjusted s-calcium (total s-calcium) this equation was used for correction; (s-calcium-(0.018(albumin-42)). Subjects were examined by DXA measurements and their age, height and weight were recorded (Paper III), they performed different physical functioning tests to evaluate muscular functioning and provided information on previous falls and fractures (Paper IV). The tests and questions included;

- Timed stands test was measured with a straight-backed chair without arm-supports, and a seat height of 45 cm. Five repeated chair stands from sitting position to standing and back to sitting again was performed. Completion time was recorded in seconds.
- Grip strength was measured in both hands using a Jamar® hydraulic hand dynamometer (5030J1), Jackson, MI, USA. There were two trials from each hand and the best value was used in the data analysis.
- Walking six meters at usual pace; time (seconds) and number of steps were measured.
Walking six meters inside a narrow 20 cm line. The time (seconds) and number of deviations were recorded; more than three was considered as failure.

All participants answered a health questionnaire were they reported if they had sustained any fall during the 12 month preceding the measurements or if they had had any fractures and if so; at what age.

Subjects with missing laboratory values were excluded and so was men with reduced renal function, GFR (<21mL/min/1.73m²) and vitamin D insufficiency i.e. 25(OH)D values < 50 nmol/L.

Diagnostics of PHPT in the premenopausal cohort

**Main study (Papers I and II)**

The diagnostic criteria used to define women with assumed PHPT (cases) were the same criteria as previously established for surgically verified PHPT in postmenopausal women³⁹, with the exception that the initial screening threshold for s-calcium was lowered from; at 2.55 mmol/L or above to 2.50 mmol/L. As in the previous study, one of the following conditions had to be fulfilled at subsequent testing;

- Fasting total s-calcium <2.50 mmol/L and iPTH ≥55ng/L
- Fasting total s-calcium 2.50-2.60 mmol/L and iPTH ≥35ng/L
- Fasting total s-calcium >2.60 mmol/L and iPTH ≥25ng/L

The rationale for including women with lower screening s-calcium (value at or above 2.50 mmol/L) was based on revised data presented several years after the initial study of postmenopausal women³².

**Subgroup study (Paper I)**

With the intention of methodically investigate the impact of minor disturbances of calcium homeostasis on metabolic morbidity, bone mineralization, physical and mental health, the cohort (n=366) was divided into four groups depending on the relation between s-calcium and iPTH, based on the median values.
This is presented as the subgroup analysis. The median values for total s-calcium (2.41 mmol/L) and for iPTH (49 ng/L) were set as cut-off for division into four groups:

- **Group A (low-low):** s-calcium $\leq 2.41$ and iPTH $\leq 49$ (n=90)
- **Group B (low-high):** s-calcium $\leq 2.41$ and iPTH $> 49$ (n=93)
- **Group C (high-low):** s-calcium $\geq 2.41$ and iPTH $\leq 49$ (n=98)
- **Group D (high-high):** s-calcium $\geq 2.41$ and iPTH $>49$ (n=85)

Group D (high-high) consisted of women with the most abnormal biochemical pattern; i.e. inappropriately elevated iPHT in relation to total s-calcium.

**Diagnostics of PHPT in the male cohort**

*Main study PHPT-group (Paper III and IV)*

Men with values above normal range of total s-calcium (>2.5 mmol/L) and iPTH (>6.9 pmol/L) were considered to fulfill criteria for PHPT (PHPT-group; n=22).

*Subgroup study IEP-group (Papers III and IV)*

To evaluate mild disturbances in calcium homeostasis suggesting “normocalcemic HPT” the participants were divided into two groups based on the relations between total s-calcium and iPHT. Median values for both parameters were calculated and set as cut-off value for each group (s-calcium $>2.34$ mmol/L and iPTH $>4.24$ pmol/L). The group with inappropriately elevated iPHT in relation to total s-calcium is referred to as “inappropriately elevated parathyroid hormone-group” (IEP-group n=387) (*Paper III*) (*Figure 3*). In Paper IV subjects with defined PHPT was excluded from analysis, leaving 365 subjects in the IEP-group.

*Control subjects*

In *Paper III* the PHPT-controls were defined as the rest of the cohort (2235 minus 22 = 2213) the same was applied for the IEP-controls (2235 minus 387 =1848).

However, in Paper IV we modified the groups by excluding PHPT-subjects from IEP-group (*i.e.* 387 minus 22 = 365) and IEP-group from controls in comparison with PHPT-group (*i.e* 2213 minus 365 =1848). Therefore all who had normal calcium homeostasis were defined as controls (n=1848) to both PHPT-group (n=22) and IEP-group (n=365) (*Paper IV*).
Figure 3 Scatter plot of s-calcium and iPTH of the 2235 men in Mr OS Sweden including the definition of the “inappropriately elevated PTH in relation to s-calcium group” (i.e. in the square). ©Springer Science and Business Media

Biochemistry

Papers I and II
Intact serum PTH (iPTH normal range, 12-65 ng/L) was measured with the ADVIA Centuar two-site immuno-chemiluminometric assay (Bayer, ADVIA Centaur) with an inter assay coefficient of variation of less than 8.1% (Paper I). At follow-up the analysis of iPTH had changed to the Immulite 2000 Intact PTH Assay (Diagnostic Products Corporation, Los Angeles, CA, USA), and values for normal range were 1.1-6.9 pmol/L. Nichols Advantage Speciality System was used for measurements of 25-hydroxyvitamin D (normal range, 10-70 ug/L). The rest of blood chemistry were analysed at the Uppsala University Hopitals’ by clinical routine analysis but the reference range for normal s-calcium was lowered from BL to FU from 2.20-2.60 mmol/L (Paper I) to 2.15-2.50 mmol/L (Paper II).

Paper III and IV
The plasma and serum samples were collected locally. Samples were frozen immediately and stored at -80°C. Serum PTH was analyzed by Immulite 2000 Intact PTH Assay (Diagnostic Products Corporation, Los Angeles, CA, USA). Calcium and albumin were measured by routine techniques at each site. Cystatin C was analyzed with polyclonal antibodies against human cystatin C and measured by immunoturbidimetry (Cystatin C Immunoparticles, Dako Denmark A/S, Glostrup, Denmark).
Estimated glomerular filtration rate (eGFR) was estimated by: 

\[ GFR = 79.901 \times (cystatin \ C)^{-1.4389} \]

This proxy for GFR has good precision, good linearity and strong correlation with iohexol clearance \((R^2 = 0.956)\).

25(OH) D was measured on the Nichols Advantage automated assay system (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Dual energy X-ray absorptiometry

**Papers I and II**

Bone mineral density (BMD, g/cm\(^2\)) of the lumbar spine, femoral neck and distal third of radius was measured with dual energy X-ray absorptiometry (DXA; Lunar Prodigy DXA; GE Lunar Corp.) The CVs for the BMD measurements ranged from 0.5 to 3%, depending on application.

**Papers III**

Areal BMD (aBMD, g/cm\(^2\)) of the hip and lumbar spine were assessed using the Lunar Prodigy DXA \((n = 2004\) from the Uppsala and Malmö cohorts; GE Lunar Corp., Madison, WI, USA) or Hologic QDR 4500/A-Delphi \((n = 1010\) from the Gothenburg cohort; Hologic, Waltham, MA, USA). The CVs for the aBMD measurements ranged from 0.5% to 3%. To be able to use the DXA measurements performed with equipment from two different manufacturers, standardised BMD (sBMD) was calculated as previously described\(^{86}\).

Health questionnaire and SF-36

**Papers I and II**

All participants answered questions on previous and present health problems. The questions included prevalence of diabetes mellitus (DM), hypertension, cardiac complications, different fractures and year of injury, thyroid or parathyroid diseases, nephrolithiasis, and malignancies (**Paper I and II**). They also reported the number of children they had given birth to, for how long they had breastfed them, and on smoking and exercise habits (**Paper I**). SF-36- was used to evaluate quality of life \(^{87}\).

Statistics

Statistical analyses were made with STATISTICA (version 7 and 8; StatSoft Inc, Tulsa, Oklahoma, USA) (**Paper I, II and III**), SPSS (version 16) (SPSS Incorporation Chicago, Illinois, USA **Paper II**) and SAS 9.2(SAS Institute Inc., Cary, NC, USA ). Comparisons were made between cases and controls as well as between the different defined groups (**Paper I-IV**).
Normal distribution was controlled and skewed variables were logarithmically transformed or if not appropriate, suitable non-parametric tests were performed. For continuous variables, ANOVA and Student T-test were used. Mann-Whitney’s U-test and Kruskal-Wallis Anova were used to analyze SF-36, and Chi-square test, and Fisher’s exact test were used for frequency distribution for answers (categorical variables) in the health questionnaires (Paper I and II). General linear model were used for adjusting for co-variates i.e. weight and 25(OH)D for BMD (Paper I); in addition age, and renal function (Paper IV); and the different domains in SF-36 was adjusted for weight (Paper I). Analysis of fracture incidence was made both for overall fracture rate and fractures sustained within ten years preceding the measurement, logistic regression was used for adjustment of covariates. As the PHPT-group consisted of only 22 subjects the assumptions for multivariate methods are questionable, and no adjustment was made (Paper IV).

Linear mixed model with restricted maximum likelihood mean was used in the multivariate analysis of BMD at FU: compound symmetry fitted the data best (lowest BIC). The multivariate model exposed effects of case/control, menopausal status, time (base-line/follow-up), weight, vitamin-D level (at BL), and renal function (creatinine), and the assumed interaction between them (Paper II).

Differences in sum score of SF-36 at FU were tested by non-parametric tests, Mann-Whitney U-test and with paired tests, as they were their own controls over time. Some of the scores in the different domains were skewed, thus, the values were recoded to either 0 (value=100= highest score possible) or 1 (values below 100, i.e some impact on QoL), the data were then processed and analysed with Generalized Estimating Equations (GEE) in a model exposing case/control status, weight and time (Paper II). Statistical data are presented as mean and standard deviation in addition to confidence intervals and p-value in illustrations. A p-value below 0.05 was considered statistically significant.
Results

Paper I

Main study

From the initial 1866 subjects included, 232 women had screening total s-calcium $\geq$2.50 mmol/L in combination with one of the preset biochemical criteria for PHPT $^{39}$ of which 18 individuals declined further testing. In the remaining 214 subjects the mean value of 25(OH)D was 28±9.7 ug/L (7-54 ug/L) and of those 19.5% (n=41 cases) were excluded due to vitamin-D levels under 20 ug/L. According to the diagnostic criteria stipulated $^{39}$, the prevalence of assumed mild primary hyperparathyroidism was estimated to 5.1% (n=96), (Table 1, column 1). The variables are presented in Table 2.

When body weight was adjusted, lower bone mineral density in proximal femur and femoral neck, (Figure 4) and lower score for vitality and general health in SF-36 were confirmed for cases. There were no differences between cases and controls comparing previous and present health problems. Obese women (BMI $>$30kg/m$^2$) had significantly higher mean iPTH than all other BMI groups (i.e. underweight, normal weight, overweight), and weight and iPTH were positively correlated (p$<$0.05).
Table 1 Influence of different s-calcium cut-off levels at screening. Total s-calcium is expressed as s-calcium (s-Ca). * Cases with total s-calcium ≥2.55 mmol/L at screening © Copyright 2008, The Endocrine Society

<table>
<thead>
<tr>
<th></th>
<th>*No (percent) of cases with s-Ca ≥2.55 mmol/L at screening</th>
<th>No (percent) of cases with s-Ca ≥2.50 mmol/L at screening</th>
<th>*Mean (range) of s-Ca in the three diagnostic criteria groups (mmol/L)</th>
<th>*Mean (range) of iPTH in the three diagnostic criteria groups (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Ca &lt;2.50 mmol/L and iPTh ≥55 ng/L</td>
<td>36 (1.9%)</td>
<td>66 (3.5%)</td>
<td>2.41±0.06 (2.22-2.49)</td>
<td>76.7±23.1 (56-164)</td>
</tr>
<tr>
<td>S-Ca 2.50-2.60 mmol/L and iPTh ≥35 ng/L</td>
<td>47 (2.5%)</td>
<td>85 (4.6%)</td>
<td>2.54±0.03 (2.50-2.60)</td>
<td>52.9±18.6 (35-126)</td>
</tr>
<tr>
<td>S-Ca &gt;2.60 mmol/L and iPTh ≥25 ng/L</td>
<td>13 (0.7%)</td>
<td>22 (1.2%)</td>
<td>2.67±0.05 (2.61-2.77)</td>
<td>52.0±30.5 (25-142)</td>
</tr>
</tbody>
</table>

Table 2 Different variables analyzed for cases and controls. *Total s-calcium range in cases (2.24-2.83 mmol/L) and controls (2.01-2.67 mmol/L). ¤ iPTh range in cases (16-329 ng/L) and controls (12-220 ng/L) © Copyright 2008, The Endocrine Society.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=173)</th>
<th>Controls (n=193)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/L)</td>
<td>137±9.0</td>
<td>135±8.14</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>S-calcium (mmol/L)*</td>
<td>2.44±0.09</td>
<td>2.38±0.08</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>P-iPTH (ng/L)</td>
<td>58.6±31.1</td>
<td>47.5±21.4</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>S-phosphate (mmol/L)</td>
<td>1.05±0.16</td>
<td>1.09±0.22</td>
<td>p=0.079</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.27±4.6</td>
<td>24.16±3.5</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.06±13.7</td>
<td>67.19±11.1</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>SF-36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical functioning</td>
<td>89±15</td>
<td>93±12</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Vitality</td>
<td>62±23</td>
<td>67±21</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>General health</td>
<td>76±19</td>
<td>81±16</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>
Figure 4 Differences in bone mineral density femoral neck (g/cm²) between cases and controls. Adjustment were made for vitamin D and weight, (p=0.022). Vertical bars denote 95% confidence interval © Copyright 2008, The Endocrine Society.

Subgroup study

In the “four groups” (with different calcium/iPTH combination), significant differences for BMD in L2-L4, proximal femur and femoral neck as well as for body weight and BMI (p<0.05) were determined (Figure5). Group A (low-low) had significantly higher BMD (p<0.01) in all locations, and Group D (high-high) had higher mean body weight (p<0.01) and hence higher mean BMI (Table 3). No differences among “the four groups” were found in the analysis of SF-36 or self reported associated morbidity in the health questionnaire.
Table 3 Data and statistical outcome of the “four groups” © Copyright 2008, The Endocrine Society.

<table>
<thead>
<tr>
<th></th>
<th>low-low (n=90)</th>
<th>low-high (n=93)</th>
<th>high-low (n=98)</th>
<th>high-high (n=85)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes Mellitus</td>
<td>n=0</td>
<td>n=1 (1%)</td>
<td>n=1 (1%)</td>
<td>n=4 (5%)</td>
<td>n.s</td>
</tr>
<tr>
<td>Hypertension</td>
<td>n=4 (4%)</td>
<td>n=6 (6.5%)</td>
<td>n=5 (5%)</td>
<td>n=10 (12%)</td>
<td>n.s</td>
</tr>
<tr>
<td>BMI&gt;30 (kg/m²)</td>
<td>n=6 (7%)</td>
<td>n=14 (15%)</td>
<td>n=7 (7%)</td>
<td>n=23 (27%)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5±3.3</td>
<td>25.0±4.2</td>
<td>24.3±3.7</td>
<td>27.0±5.1</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.2±12.8</td>
<td>69.3±12.6</td>
<td>67.8±11.5</td>
<td>75.1±15.5</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Tot s-ca (mmol/L)</td>
<td>2.35±0.06</td>
<td>2.34±0.05</td>
<td>2.48±0.07</td>
<td>2.48±0.07</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>iPTH (ng/L)</td>
<td>36.8±8.2</td>
<td>66.9±21.9</td>
<td>36.2±8.2</td>
<td>73.2±35.4</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>P-25-(OH) D(µg/L)</td>
<td>32.9±8</td>
<td>32.3±8.5</td>
<td>32.9±9.6</td>
<td>29.8±7.4</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Figure 5 BMD femoral neck (g/cm²), of “the four groups” with different s-calcium and iPTH relations. Vertical bars denote 95% confidence interval © Copyright 2008, The Endocrine Society.
Paper II

The mean time to follow-up was 2.98±0.19 years. The subjects (n=366) were invited for new assessments but, 27 subjects (7.4%) did not come to DXA measurements (of which 23 had no measure of FSH) and in addition another 22 subjects (6.0%) had no FSH evaluation and therefore their menopausal status could not be determined and they were excluded from analysis of BMD. Altogether, 57 subjects (15.5%) were excluded for QoL analysis as they did not complete SF-36.

Skeletal health

No difference in fracture rate was determined (radius, hip or lumbar spine). BMD in total hip and femoral neck was significantly lower for cases (p<0.05) and factors of importance of BMD in the final statistical model were case/control (lower for cases), menopausal status (lower BMD for group POST: p<0.01), time (lower at follow-up: p<0.01), weight (positively correlated: p<0.01), and interaction between menopausal status and time (p<0.01) (Table 4).

BMD in distal third of radius was significantly lower for cases (p<0.05). The factors of importance were the same as for total hip and femoral neck, except for menopausal status (ns). However, there was an interaction between menopausal status and time; i.e postmenopausal women had an accelerated BMD loss in distal radius during the three-year period (p<0.05) (Table 4). No significant difference in BMD for lumbar spine (LS) was determined between cases and control, but as expected menopausal status influenced the decrease in BMD over time (p<0.05).

Associated morbidity and QoL

The analysis of SF-36 revealed lower scoring for general health over time for both cases and controls (p<0.05); in addition, the controls scored lower for vitality and physical functioning over time (p<0.05). However, there was good comparison between the mean values and the validated normal population, matched for age and gender. In the non-parametric evaluation between cases and controls, the cases had lower rank value for physical functioning. However, adjustment of weight and time in the multivariate analyses with recoded values could not reveal any difference in QoL. Differences in associated morbidity between PHPT subjects and controls were found for PPI-consumption and obesity.

At FU, cases were still heavier than controls (p<0.01), and both groups gained weight during the follow-up period (p<0.05) with a mean of 1.8 kg for cases and 1.6 kg for controls, however the difference in weight gain between cases and controls was not significant. The cases were on average 5.7 kg heavier than controls and had 6.3 cm wider waist circumference (cross-sectional data from FU: p<0.05). Baseline data of QoL and BMD of missing subjects at follow-up did not differ from the rest of the cohort.
Table 4 Factors of importance influencing BMD in total hip and distal third of radius

<table>
<thead>
<tr>
<th></th>
<th>BMD total hip</th>
<th>BMD distal third of radius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>95% CI</td>
</tr>
<tr>
<td>Case/Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case</td>
<td>1.000±0.009</td>
<td>0.982 - 1.017</td>
</tr>
<tr>
<td>Control</td>
<td>1.025±0.008</td>
<td>1.009 - 1.041</td>
</tr>
<tr>
<td>MS status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre/pre</td>
<td>1.030±0.008</td>
<td>1.014 - 1.045</td>
</tr>
<tr>
<td>Pre/post</td>
<td>1.026±0.010</td>
<td>1.007 - 1.046</td>
</tr>
<tr>
<td>Peri/post</td>
<td>0.981±0.013</td>
<td>0.955 - 1.007</td>
</tr>
<tr>
<td>Time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base-line</td>
<td>1.021±0.006</td>
<td>1.009 - 1.033</td>
</tr>
<tr>
<td>Follow-up</td>
<td>1.004±0.006</td>
<td>0.992 - 1.016</td>
</tr>
<tr>
<td>MS status *time</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Paper III

**PHPT-group**

The pre-set criteria of PHPT were fulfilled by 22 subjects: a prevalence of 0.73%. There was a weak negative correlation between s-calcium and iPTH \((r = -0.27; p<0.05)\). The PHPT-group had significant lower sBMD in total hip, femoral neck and trochanter area compared with the PHPT-controls \((n=2213)\). Mean values for different parameters are presented in Table 5. There was also a weak negative correlation between age and sBMD in total hip \((r = -0.12)\), femoral neck \((r = -0.13)\) and trochanter area \((r = -0.08)\) \((p<0.05)\), but no correlation between age and sBMD in lumbar spine. Weight correlated positively with sBMD over all \((p<0.05)\). However, no significant difference in mean BMI between the groups was seen.

**IEP-group**

Median value for s-calcium and p-iPTH in the total cohort were 2.34 mmol/L, and 4.24 pmol/L, respectively, with 387 individuals matching the IEP- criteria with median s-calcium >2.34 mmol/L in combination with median p-iPTH >4.24 pmol/L. In the IEP-group, a significant lower mean sBMD \((p<0.05)\) was found in total hip, the trochanter area, and also in lumbar spine in a model controlling for weight, age, eGFR, 25(OH)D level \((\log 25(OH)D)\) and study site (Figure 6).

Renal function differed between the groups and subjects in the IEP-group had significant lower mean eGFR \((p<0.01)\) compared to their controls. Median eGFR value in IEP-group was 68.7 mL/min/1.73 m² (inter quartile (IQ) range 25-75 = 54-83 mL/min/1.73 m²) and eGFR correlated negatively.
with iPTH ($r = -0.19$, $p < 0.05$). However, renal function did not significantly influence sBMD in the multivariate analysis.

Table 5 Clinical characteristics for PHPT group and their controls © Springer Science and Business Media

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumbar spine</td>
<td>1136.2±207</td>
<td>1142.3±203</td>
<td>n.s</td>
</tr>
<tr>
<td>Tot hip left</td>
<td>897.2±162</td>
<td>964.3±147</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Tot hip right</td>
<td>886.4±163</td>
<td>952.1±145</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Femoral neck left</td>
<td>784.3±132</td>
<td>845.8±132</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Femoral neck right</td>
<td>768.3±138</td>
<td>835.7±133</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Trochanter left</td>
<td>712.0±153</td>
<td>804.1±142</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Trochanter right</td>
<td>701.1±147</td>
<td>793.3±142</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Age (year)</td>
<td>74.8±3.5</td>
<td>74.9±3.1</td>
<td>n.s</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.2±12.1</td>
<td>80.2±11.7</td>
<td>n.s</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3±3.9</td>
<td>26.3±3.4</td>
<td>n.s</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>65.5±22.1</td>
<td>72.6±19.7</td>
<td>n.s</td>
</tr>
</tbody>
</table>

Figure 6 Comparison between IEP subjects and controls of sBMD in left hip. IEP-group defined from above median s-calcium (2.34 mmol/L) and median iPTH (4.24 pmol/L) and controls. Vertical bars denote 95% confidence interval © Springer Science and Business Media
Paper IV

Men with PHPT performed inferior in the time stands tests than men with normal calcium homeostasis (p<0.05). No group differences were found in the other performance tests, the one year fall prevalence, the overall or the 10 year fracture prevalence (Table 6).

Men with IEP performed inferior in time stands tests, six meters walking test and 20 cm narrow walking test than men with normal calcium homeostasis (all p<0.01, respectively) (Table 6). No group difference was found in grip strength test and all results remained after adjusting for described covariates (Table 6). Furthermore, there were no group differences in one year fall prevalence, the overall or the ten year fracture prevalence.

Table 6 Results of different physical functioning tests for subjects with PHPT (n=22) and inappropriately elevated parathyroid hormone (IEP-group n=365) compared to the controls (n=1848). The data is presented as mean with ± standard deviations (SD) or as proportions (%). (*p-value adjusted for covariates; age, renal function, weight, height, vitamin D level)

<table>
<thead>
<tr>
<th>Variables</th>
<th>P-value (PHPT)</th>
<th>PHPT (n=22)</th>
<th>Controls (n=1848)</th>
<th>IEP-group (IEP)</th>
<th>P-value (IEP)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grip strength right hand (kg)</td>
<td>0.432</td>
<td>39.0±8.5</td>
<td>40.3±8.1</td>
<td>40.0±8.1</td>
<td>0.711</td>
</tr>
<tr>
<td>Grip strength left hand (kg)</td>
<td>0.072</td>
<td>36.4±8.7</td>
<td>39.4±7.8</td>
<td>39.1±8.0</td>
<td>0.489</td>
</tr>
<tr>
<td>Five repeated chair stands completing time (seconds)</td>
<td>0.007</td>
<td>15.5±4.5</td>
<td>13.0±4.1</td>
<td>14.2±4.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Walking six meters (seconds)</td>
<td>0.120</td>
<td>5.4±1.2</td>
<td>5.0±1.3</td>
<td>5.4±1.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Walking six meters (number of steps)</td>
<td>0.153</td>
<td>9.5±1.6</td>
<td>9.1±1.3</td>
<td>9.6±1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Walking inside a narrow line 20 cm (*deviation)</td>
<td>0.105</td>
<td>6.1±1.9</td>
<td>5.5±1.7</td>
<td>5.9±2.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Falls past 12 month</td>
<td>0.856</td>
<td>13.6%</td>
<td>15.3%</td>
<td>16.3%</td>
<td>0.588</td>
</tr>
<tr>
<td>Fractures up till now</td>
<td>0.221</td>
<td>45.5%</td>
<td>33.1%</td>
<td>37.1</td>
<td>0.113</td>
</tr>
<tr>
<td>Fractures the last ten years</td>
<td>0.294</td>
<td>9.1%</td>
<td>4.4%</td>
<td>5.5%</td>
<td>0.418</td>
</tr>
</tbody>
</table>
Discussion

Prevalence

The prevalence of PHPT varies in different epidemiological studies. In different populations including both genders and all age groups, the prevalence of the disease is reported to be from 0.17% to 13.9% \(^{32,33,36,82,88}\). However, estimation of prevalence is generally lower in studies undertaken prior to the introduction of iPTH measures and before acknowledgment of normocalcemic primary hyperparathyroidism\(^{33}\). There is no current consensus about uniform biochemical criteria for defining PHPT, including also normocalcemic PHPT; therefore comparison and evaluation of different figures of incidence rate and prevalence is complex. This is illustrated in this thesis by the two cohorts; PHPT in the premenopausal cohort and the cohort of older men is defined by different biochemical recognition; therefore long-term evaluation is required so as to establish the diagnostics set-ups used (Paper I and III).

In the cross-sectional study of premenopausal women, the prevalence of assumed PHPT was 5.1%, based on cut-off value of s-calcium \(\geq 2.55\) mmol/L at screening (Paper I). This result suggested, early PHPT could be more prevalent than previously recognized in this population. However, the criteria used have not been surgically verified in this cohort of premenopausal women, but are based on histopathological verification of biochemical criteria in postmenopausal women.

In the postmenopausal cohort by Lundgren et al; the prevalence of PHPT was 3.4% adjusted after eight years of surveillance and including women with s-calcium \(\geq 2.50\) mmol/L on screening occasion\(^{32}\). If the cut-off value of s-calcium of \(\geq 2.50\) mmol/L was applied and evaluated in the study of the younger population the prevalence rose to 9.3% (Table 1 Paper I). However, on repeated testing (two different fasting examinations) of diagnostic criteria fulfilment, only 2.7% of the cases fulfilled the same or another criterion for PHPT, which was more comparable with figures estimated in the postmenopausal cohort. This indicated calcium and iPTH varies over time and, for younger women the progress appears slow, but probably also indicate truly early biochemical disturbances in the natural progression of PHPT.

In a comparison between the two age cohorts of women, premenopausal (Paper I) versus postmenopausal\(^{39}\) s-calcium levels and degree of severity of
disturbance in calcium homeostasis differ in presentation. At screening, the population of premenopausal women had higher non-fasting mean s-calcium value compared to postmenopausal women (2.45 mmol/L vs 2.37 mmol/L). Of the women fulfilling the diagnostic criteria, 86% of younger women were normocalcemic at diagnosis and 66% of the elderly women. In the Tromsø study, comprising also younger women, the prevalence of PHPT in women between 40 and 49 years of age was 1.2‰; however, the biochemical analysis was based on non-fasting values of two examinations and the diagnostic criteria differed slightly from ours (Paper I). The cut-off limit at screening occasion in the Tromsø study (>2.59 mmol/L) exemplified impact of the cut-off value on prevalence estimation.

The women participating in the study represented around 10% of the underlying female population of both pre- and postmenopausal females between 40 and 50 years of age in the Uppsala County (www.scb.se) (Paper I). About 80% of all women invited to the mammography health survey program actually undergo mammograms, which might affect the estimation. In a study of reasons for not attending mammogram screening program displayed that the majority of women who decline participation (59.1%) had no interest, other health problems (i.e. malignancies), or perform the mammography by private consultants due to an easy access to private healthcare in the area. Health problems, such as malignancies are associated with PHPT, thus, this bias might increase the prevalence calculated. The effects of exclusion from mammography for other reasons are more difficult to predict.

The prevalence of PHPT including male populations has been estimated. In Swedish men aged 69 to 81 years, a prevalence of PHPT of 0.73% was determined (Paper III), which was in agreement with the population-based survey by Jorde et al. where a similar prevalence of PHPT (0.95%) in Norwegian men between 70 and 75 years of age was found. In an earlier study (1969-1971) the prevalence in men was less than 0.7% in all age groups.

The criteria used for identifying PHPT in this study (Paper III) were entirely based on above normal values of both s-calcium and p-iPTH derived from a normal adult male population. In this respect, the prevalence of disease was probably underestimated as individuals with normocalcemic hyperparathyroidism were not considered.

Vitamin D

Another important factor in prevalence estimation of PHPT is the level of vitamin D concentration. Vitamin D is essential for normal calcium homeostasis and has an important function in regulating PTH production. Research on the vitamin D receptor has given new insight into the pathophysiological mechanism of cell functioning in PHPT. A certain
genotype of VDR receptor is overrepresented in PHPT and the different genotypes correlates with the parathyroid cell function and its responses to calcium concentration\(^{91}\). The frequency of vitamin-D insufficiency in patients with PHPT ranges from 50\% to 80\% and there is often more symptomatic symptoms expressed in these cases\(^{92-95}\). Thus, an additional explanation of the diverse symptoms and grade of severity in patients with PHPT could be considered.

The ongoing debate on the inconsistent cut-off limit for vitamin D deficiency/insufficiency also concerns the level at which vitamin-D inhibits further increase of PTH and its impact on severity grade of PHPT\(^{80,93}\). There is evidence that PTH concentration does not decrease if vitamin D supplement is given for vitamin D concentration over 50 nmol/L\(^{96}\).

In this thesis, subjects with assumed vitamin D insufficiency were excluded due to the risk of including subjects with secondary HPT. The prevalence of vitamin D insufficiency in the male cohort was estimated to 17.7\% (Paper III). In the female population; 19\% of the women with alleged PHPT had vitamin D values below 20 µg/L. However, frequency of vitamin D insufficiency in the female population did not reflect the true prevalence, as subjects had been included due to inappropriate high level of iPTH (Paper I).

In a series of 229 consecutive patients evaluated for osteoporosis, 15 had vitamin D levels below 15 pg/mL of which a third were alleged to have PHPT after restoration of vitamin D concentration, which render a prevalence of 2.2\% in these patients\(^{97}\). Therefore, vitamin D insufficiency could have revealed PHPT in the two cohorts (Paper I and III), and a bias in this respect would increase the prevalence of disease estimates.

### Associated morbidity

#### Bone mineral density

In the premenopausal cohort the cases had lower BMD than controls in all measured sites, except for lumbar spine both at base-line and at follow-up (Paper I and II). Menopausal status was important and had decreasing effect on BMD in sites rich of trabecular bone \textit{i.e.} BMD of lumbar spine and women in the early postmenopausal phase had a steeper deterioration of the skeleton curve. In sites rich of cortical bone such as the distal third of radius, menopausal status alone did not significantly affect BMD, although the women who had been in menopausal transition the longest had greater bone loss over time (Paper II).

Cortical sites are prone to decrease in BMD in patients with PHPT\(^{83}\), which was also observed in this cohort (Paper II). But, at no sites, women
with mild disturbances in calcium homeostasis (alleged PHPT) had accelerated decrease in BMD compared to controls during the three years of follow-up. The estrogen deficiency occurring after menopause and the risk of osteoporosis and decline in BMD, particularly in late perimenopausal phase, is a well evaluated factor. At the workshop meeting of asymptomatic PHPT in 2002 the implication of onset of menopause was discussed. The panel highlighted women in early post-menopausal state who do not fulfill surgical guidelines but should still be evaluated and considered for surgical treatment; as estrogen deficiency increase BMD decline. Another aspect is the actual age of onset of menopause, which was explored in mid 1970s by Christensen et al. and, women with PHPT have in average 4.5 years earlier onset of menopause which evidently could impact bone density over time because of earlier decrease in estrogens level.

The decline in sBMD were the same in the cohort of the elderly men, including both the hypercalcemic group as well as those with mild disturbance in calcium homeostasis compared to controls (Paper III). In addition, sBMD in lumbar spine was lower in the iEP group compared to controls. Vertebral osteopenia investigated in association with PHPT by Silverberg et al. in 143 patients who fulfilled the surgical guidelines for PTx; of those 14 had lumbar spine osteopenia and the eight who underwent surgery improved BMD post-operatively. The lower sBMD in lumbar spine in the cohort of elderly men did not have any impact on fracture risk either for long-term risk (i.e evaluated up till the age of inclusion) or within the previous ten years, when comparing reported fractures (Paper IV).

There are reports of increased fracture risk in patients with PHPT. An increased rate of mild morphometric vertebral fractures (25% cases vs 4% controls), is found in a case-control study of 150 postmenopausal women with sporadic PHPT, but the indication for surgery is uncertain. In a cross-sectional study of 116 female PHPT patients in Japan, the threshold for the risk of vertebral fracture was evaluated in distal radius, the author found that the threshold was lower in PHPT patients compared to controls especially at distal radius and therefore radial examinations as predictive of vertebral fractures should not be used. Women with PHPT could have higher BMI and this may compensate for the lower radial threshold in prediction of osteoporotic vertebral fractures. In the population of middle-aged women, no difference in reported fractures was determined (Paper II). However, the follow-up time was relatively short and only 50% of the women were at postmenopausal state atFU.

Parathyroidectomy appears to increase BMD in cortical site but preferentially in sites rich of trabecular bone – probably because of increased bone turn over and is suggested to decrease fracture risk. However, in a large study from Denmark during 1982-1996 no differences in fracture risk in PHPT patients could be determined between those who were surgically treated (n= 841) and non-surgically treated (n= 360). There was
though an increased fracture risk for all diagnosed with PHPT compared to controls.

In the study of perimenopausal women no major changes in BMD occurred during the years around menopausal transition (Paper II), and mere surveillance of this particular group could be advocated in this respect.

Impact of ageing on skeleton and renal functioning
Ageing influences BMD and with older age, reduction of mineralization of the skeleton is expected. The men participating in the study were between aged 69 and 81, and age was an independently predictor of low sBMD of the hip (Paper III). In elderly people the irreversible component of bone remodelling (i.e remodelling balance) is usually negative; this means the catabolic effects on the skeleton are greater than the anabolic effects.

Age is a strong predictor of decline in BMD of femoral neck in men. However the cohort in Blain et al’s study was on average 54 years of age and, in the statistical analysis of factors influencing bone formation, age accounted for 29.5% of variance in femoral neck of the hip and, after further evaluation of significant factors the age-related increase in PTH is one of the explanations of the decline in bone formation. The men in the IEP group were on average 5.5 months older than the men in the control group, but this did not impact the results as the age differences were controlled for in the multivariate analysis of group differences in sBMD (Paper III).

Ageing is also a strong negative predictor for renal function and, GFR declines continuously and almost halves from the age of 30 (120 mL/min/1.73 m²) to the age of 90 (65 mL/min/1.73 m²). Impaired GFR increases the levels of circulating PTH and, for patients with PHPT, an impaired renal function can result in a high bone turnover, increased remodelling space and subsequently, greater bone loss. The inclusion cut-off limit for GFR in the study of elderly men was set low (Paper III), and the risk of including men with possible secondary HPT were concerned but estimated to be small as PHPT was defined as above normal value of both iPTH and s-calcium with no significant difference of GFR compared to controls.

There were statistical differences in mean GFR in the subgroup study of IEP-subjects with higher iPTH and lower GFR. As expected GFR correlated negatively with iPTH, but mean values in IEP-group and controls were above values indicating clinically important impaired renal function and significant group difference in s-phosphate level was not detected (Paper III). Nor was there any correlation between sBMD and GFR.
Neuromuscular functioning and impact on fall and fracture risk

Normal neuromuscular functioning is complex and dependent on a variety of factors that regulate the functions of neuromuscular system. Apart from obvious neuromuscular diseases, such as multiple scleroses, stroke events, brain tumors, spine disorders etc, impairment of the neuromuscular system is also associated with PHPT. The underlying mechanisms are largely unknown, but are probably multifactorial in origin. The positive effect of parathyroidectomy on subjectively described symptoms is studied with different QoL evaluation tests. However, there have been studies in which patients have been examined with objective measurements of muscular performance and, even though there are few participants included, improvement of neuromuscular symptoms is noticed post-operatively\(^{77,108-113}\) in some but, not in all studies\(^{114}\).

Formal testing of muscular strength is not normally available at routine assessment of patients with PHPT; therefore conclusions from QoL investigation are limited due to the lack of objective measurement\(^{115}\). Studies of formal muscular testing can be biased through the lack of control groups and due to learning the tests over time\(^{77}\). Nevertheless, in a small study by Deutch et al\(^{113}\), 19 females having PTx for PHPT underwent formal muscular testing to evaluate the effects of surgery, and patients going through thyroidectomy for non-toxic goitre were chosen as controls. Preoperatively, the PHPT patients had lower maximal voluntary knee extension strength than controls but, increased their muscular strength in the quadriceps muscle after operation. This correlated positively with increasing age at operation. No effects on handgrip strength, quadriceps endurance, or general work capacity were seen, but the PHPT patients experienced less fatigue postoperatively\(^{113}\). Although this finding is difficult to analyze, the decrease in fatigue after surgery may be responsible for the increased muscular strength or vice versa.

In the cohort of men only 22 subjects were diagnosed with PHPT (Paper IV), and they had a significant lower performance in repeated knee flexion test compared to controls, which was in accordance with the findings of Deutch et al in their female subjects\(^{113}\). However, one reason for the insignificant results for the other of the physical performance tests may be the small number of men with PHPT, as the differences in mean values in some of the tests were greater than the values in the IEP-group compared to controls (Table 6). In men with minor disturbances in calcium homeostasis which could be suggested to be comparable to “normocalcemic HPT”, there was lower physical performance in most of the tests, except hand grip strength (Paper IV). Histological evaluation of muscle biopsies in PHPT patients can show sign of muscular atrophy preferably in type II fibres\(^{76}\) and this could be one explanation for the differing results between the IEP-group and controls in the study (Paper IV). Other reasons or confounding factors
for the difference in performance between the groups could be explained by co-morbidity as PHPT is associated with cardiac disorders and also DM, which could affect peripheral nerve functions and general performance. This association with CVD, DM and PHPT could provide an alternative explanation for poorer outcome of muscular performance in the men. Co-morbidity in this group of elderly men has not been investigated in context with muscle performance. However, our results underline the conclusion that PHPT has an impact on muscular functioning also in extremely mild cases, but the mechanism is still not completely understood.

Maintaining normal neuromuscular functioning is crucial for avoiding falls. Fall incidence increases progressively with age and is of major importance for injury rate in the older population, including increased fracture risk\(^1\). Lower standardized bone mineral density was evident in the male population (Paper III) with as much as 6.9 to 11.6% lower sBMD in regions of interests; this finding indicated an increased risk of fractures. Nevertheless, even though the subjects with disturbance in calcium homeostasis performed poorer than controls it did not reflect an impact on fall or fracture prevalence. However, in PHPT subjects there were over 30% higher overall fractures and doubled fracture prevalence during the ten years preceding diagnosis, but we failed to show significant differences. This raises the question whether this population in particular (men between 69 to 81 years of age) would benefit from surgery in this respect. However, subjective measurements of self-rated disability and fatigue (i.e. quality of life evaluation) and effects on daily activity have not yet been explored in this context.

Cardiovascular morbidity

PHPT is associated with a range of cardiovascular disorders including vessel stiffness, altered arterial reactivity, hypertension, structural changes in the myocardial structure, heart failure and stroke incidence\(^1\). The pre-menopausal women included in the study (Paper I and II) were between 40 and 50 years of age at inclusion, and prevalence of heart/vascular diseases was not expected to be high in this relatively young population. In the total cohort, the proportion of subjects with hypertension was seven to eight percentage (Papers I and II), and even fewer suffered from any heart condition. This is far lower than in another study in which 70% of PHPT patients and 50% of controls were determined to be hypertensive\(^5\). In this respect, a bias could be introduced at inclusion level. The study design possibly appealed more to women with some sort of “health awareness” and consequently did not have same attraction for people already being taken care of in the general health system. However, in “the four groups” (Paper I) there were nominal differences regarding hypertension, although this was not statistically significant; twelve percentages (n=10) of those with minor
calcium disturbances and inappropriately elevated iPTH (group high-high) had medication for hypertension compared to approximately 4-6% in the other groups. Hypertension is one underlying factor inducing cardiac complications over time, and the female cohort was probably too young to exhibit any long-term cardiac effects.

Metabolic disturbances and obesity

Individuals with increased body mass index (i.e. obese BMI>30 kg/m²) often have increased BMD, compared to individuals of normal weight. In the population of elderly men weight correlated significantly with sBMD at all measured sites (Paper III). The correlation between obesity and effect on bone mass is reviewed by Zhao et al118, who consider it questionable if fat mass is beneficial for bone formation as in statistical analysis adjusting for mechanical loading reveals an inverse relation with fat and BMD. In addition, exercise improves bone mass but at the same time can cause weight loss.

There is an association with obesity and PHPT also in male population57. However, this association was not confirmed in the cohort of men, as no significant difference in BMI was detected between the groups (Paper III).

The prevalence of obesity has increased remarkably since the 1970s especially in all Western countries119,120. In Sweden, approximately 20% of the general population is overweight, and 10% of the adult population is obese (www.scb.se). In the cohort of pre-menopausal women, noticeable differences in body weight and, accordingly, BMI were detected between cases and controls (Paper I). The results were reliable even when comparing women with the most atypical biochemical patterns of s-calcium and iPTH levels and those with more normal s-calcium and iPTH distribution (i.e. the four groups) (Paper I). The association between mild PHPT and obesity was consistent with previously published articles, and increased body weight appears to be a risk factor preceding calcium/iPTH disturbances43,57,121. Nevertheless, it is still unclear whether increased body weight induces calcium disorders or vice versa. Adipocytes and osteoblasts stem from the same mesenchymal precursor cell and at least osteoblasts are under influence of PTH. In addition to storing energy, adipocytes secrete estrogens, which have positive effects on bone formation but; the question is raised whether obesity is beneficial for bone formation118. Vitamin D insufficiency more often occurs in subjects with obesity and could increase the negative effect and decline in BMD122. In the premenopausal cohort (Paper I), iPTH and body weight were positively correlated; this is established for postmenopausal women43,121. At the three-year FU, the women had gained weight; 1.8 kg in cases and 1.6 kg in controls, but the difference was not significant (Paper I and II). An association between obesity and calcium disturbance was established and the high prevalence of assumed early PHPT
in premenopausal women might be a direct consequence of the epidemic increase of obesity in the Western world today.

Psychiatric morbidity and Quality of Life

Psychiatric disturbances are other associated complications in PHPT. Even if patients are considered asymptomatic, there is evidence of impacted QoL in this disease group\(^\text{62,123}\). The symptoms experienced by patients are often vaguely described, but the majority of disturbances are depressive in character, including anxiety, fatigue, and cognitive dysfunction\(^\text{41,63,64}\). Surgical treatment with restoration of normal calcium homeostasis appears to improve those symptoms\(^\text{64,123}\).

Short-form 36 (SF-36) is a psychometric test tool specifically developed for interpreting health status. SF-36 contains eight different domains reflecting the most frequently measured concepts of health affected by disease or treatment. It does not target a specific disease or age group. It also summarise a comparative component score for general physical and general mental health by an algorithm from the scores of the domains and is used to compare different populations\(^\text{69}\). The instrument is widely used for different diseases and treatments including psychometric measurement before and after surgery for PHPT\(^\text{66}\).

At BL, there were demonstrable significant differences in life quality between cases and controls in the female cohort with reference to lower scores for vitality and general health for cases even after adjustment for obesity \((\text{Paper I})\). At FU the cases had lower physical functioning compared to the controls; but it did not differ from the normative population \((\text{Paper II})\).

In the statistical context, there is concern and objection that ordinal data are treated as continuous variables, and often statisticians suggest only non-parametric tests are applicable\(^\text{124}\).

The comparison of score analyses from BL to FU was not directly comparative (other than nominal) because of the choise of different statistical approach \((\text{Paper I and II})\). At BL, the data were treated as continuous variables and, at FU non-parametric tests were applied and the data regarded as ordinal. To enable longitudinal statistical analysis of the domains in SF-36, the scores were recoded into binominal values to discriminate between subjects who scored less than maximum (1) with those who scored maximum (0) on the questions, thus, time (BL to FU) could be applied as a factor and adjustment for weight. Even so, there were no differences between cases and controls and no difference in the physical and mental component summary score.

Therefore, it could be concluded either the mild disturbance in calcium homeostasis, suggesting early PHPT, had no impact on QoL or, the SF-36 questionnaire might not have been sufficiently sensitive to discriminate between subtle changes of QoL in this apparently healthy population.
Conclusions

In this thesis, two different cohorts representing underlying populations were investigated in relation to prevalence and impact on associated morbidity in PHPT. The cohorts differed from each other regarding gender and age and the data collected presented further understanding of the disease in these populations groups.

- The biochemical disturbance between s-calcium and iPTH appears to be established several years before menopause.

- Prevalence of PHPT in premenopausal women is higher than previous considered.

- Mild PHPT is associated with lower BMD in hip for both genders.

- Old men with minor disturbances in calcium/iPTH balance - which could be representative for normocalcemic PHPT - had lower sBMD in lumbar spine.

- Mild PHPT is associated with overweight and obesity in younger women but no such association is apparent in older men.

- Minor disturbance in calcium homeostasis decreases muscular functioning in men, but does not have any impact on fall or fracture risk.
Populärvetenskaplig sammanfattning
(Summery in Swedish)

Primär hyperparathyroidism (PHPT) är en vanlig endokrin sjukdom som drabbar bisköldkörtarnas förmåga att reglera produktionen av bisköldkörtelhormon (parathyroideahormon - PTH). PTH tillsammans med D-vitamin utgör en stark regulator av kalcium i kroppen och regleras i sin tur av kalciumnivåer i blodet via en negativ återkopplingsmekanism. Målorgan för PTH är skelett och njurar och vid insöndring av hormonet påverkas både uppbyggnad och nedbrytning av skelett, och vid höga nivåer av PTH får man en ökning av kalcium i blodet. I njurarna leder förhöjda värden av PTH till ökat återupptag av kalcium på bekostnad av fosfat, och vidare triggas också aktiveringen av inaktivt D-vitamin till aktivt vitamin igång. D-vitaminet i sin tur har förmåga att öka kalkupptaget i tarmen och fungerar dessutom som en ”dämpare” av PTH produktionen.

Sjukdomen återfinns i alla åldrar, men är ovanlig hos yngre individer. Kvinnor verkar mer benägna än män (3:1) att utveckla rubbningen och man har noterat att det i synnerhet är kvinnor som passerat klimakteriet som drabbas. Det finns en del studier där man försökt kartlägga förekomsten av sjukdomen i olika populationer och där noterar man att siffrorna varierar mycket, från så få som 0.3% till upp emot 13.9%. Skillnaden som framträder i olika populationer beror dels på underlaget, dvs vilken population som legat till grund för undersökningen, men också hur man definierat diagnoskriterier för att kunna identifiera sjukdomen. Före introduktionen av screening av kalcium, som kom i början av 1970-talet, var sjukdomen ovanlig och kännetecknades av uttalad hyperkalcemi. Individer som insjuknade hade ofta uttalade symptom på kalkrubningar i form av grava skelettförändringar, neuromuskulära symptom, njurstensproblematik och psykiska rubningar när de fick sin diagnos.

De senaste 30-40 åren har dock uttrycket av sjukdomen förändrats från uttalade symptom, till att bli närmast asymptomatisk med endast milda rubbningar i kalkbalansen. Diagnosen bygger på relationen mellan PTH och kalk och deras speciella feedbackinverkan på varandra. Vid alla andra tillstånd med hyperkalcemi tex maligniteter med skelettaffektioner, sarkoidos, hypertyhreos mm är PTH nedpressat, ofta till subnormala nivåer. Det är endast vid PHPT som man ser förhöjda kalknivåer i kombination med oproportionerligt höga PTH-värden och diagnosen synes därför enkel.
Nuförtiden har man även accepterat varianten ”normokalcemisk” hyperparathyroidism. Även om sjukdomen blivit mild i sin karaktär, har rubbningen visat sig påverka flera av kroppens funktioner och organsystem. Nyare forskning har kunnat visa på ett samband mellan PHPT och ett ökat insjuknande och ökad dödlighet i bland annat hjärt/kärlsjukdom. Man har även noterat påverkan på skelettet med försämrad mineralisering, diffusa psykiska besvär som trötthet, försämrad livskvalitet och nedsatt kognitiv förmåga, samt en del metabola rubbningar som ökad insulinresistens, hyperlipidemi och fetma.

Sjukdomen är som tidigare nämnts vanligast hos äldre kvinnor och där misstänks östrogennedgången efter klimakteriet som en möjlig bidragande orsak till utvecklingen, även om de bakomliggande mekanismerna är höljda i dunkel. PHPT är bäst beskrivet i denna sub-population men mindre är känt om sjukdomen och uttrycket av densamma hos premenopausala kvinnor och hos män. I detta epidemiologiska avhandlingsarbete har just dessa grupper undersömts med målet att;

- uppskatta förekomsten av sjukdomen i dessa grupper (delarbete I och III)
- kartlägga eventuell associerad sjuklighet (delarbete I-IV)
- samt undersöka eventuella förändringar i sjukdomsuttryck i samband med klimakterieövergången (delarbete II)

Delarbete I

I delarbete I har förekomsten av PHPT och associerad sjuklighet i en cohort av premenopausala kvinnor i ålder 40 till 50 år studerats. Kvinnorna tillfrågades om deltagande i studien i samband med mammografiscreening. På de individer som accepterade medverkan (n=1866) och som inte nyttjade östrogeninnehållande läkemedel, genomfördes en initial screening av parametrar för kalciumomsättning. De kvinnor som uppfyllde föruppsatta kriterier för PHPT kallades åter för förnyad provtagning och matchades även med kontroller (n=193). Totalt identifierades 173 personer med kalciumrubbning tydande på PHPT (fall), där prevalensen således kunde uppskattas till 5.1%.

Alla inkluderade genomgick därefter mätning av bentsäthet (BMD) i höft, ländrygg och radius med dual-X-ray absorptiometry (DXA) från vilket body mass index (BMI) kunde räknas fram. De fick också svara på frågor i ett omfattande hälsosformulär som bland annat inkluderade frågor om tidigare sjukdomar, mediciner, antal graviditeter, antal amningsmånader, motionsvanor, tidigare frakturer, rökvanor mm samt fylla i ett psykometriskt valideringsinstrument (SF-36) för att möjliggöra utvärdering av sjukdoms-/hälsorelaterad livskvalitet. I detta arbete presenteras base-line (BL) data från kvinnorna.
I den statistiska analysen gjordes jämförelser mellan fall och kontroller för att kunna utvärdera skillnader i eventuellt associerad sjuklighet. Fallen hade lägre bentäthet i höften jämfört med kontrollerna, men skillnader i fråga om frakturer eller bentäthet i ländrygg framkom inte. Individer med PHPT var tyngre och hade följaktligen ett högre medelvärde på BMI. Flera individer uppfyllde också kriterierna för övervikt och fetma. Det fanns en positiv korrelation mellan BMD och vikt men skillnaden i bentäthet i höften stod sig även efter korrigering för detta. Vad gäller andra associerade sjukdomar såsom diabetes mellitus, hjärt-kärlsjukdom, njurstensproblem sågs inga skillnader, men vid analys av livskvalitet med utvärdering av SF-36 skattade fallen lägre för vitalitet, allmän hälsa och fysisk funktion. När vikt lades in som co-variabel försvann dock skillnaden i fysisk funktion men kvarstod i vitalitet och allmän hälsa.

Delarbete II

I detta arbete presenteras data från tre års uppföljning av kvinnorna i delarbete I. Återigen lämnade de inkluderade blodprover för analys med bland annat follicelstimulerande hormon (FSH) för att kunna avgöra vilka kvinnor som gått in i klimakteriet. Liksom vid det tidigare tillfället undersöktes de med DXA-mätning, hälsoböcker och fick också upprepa livskvalitetsskattning.

För att kunna ta hänsyn till menopausstatus delades kvinnorna in i tre olika grupper utifrån värdet på FSH vid BL och uppföljningen (FU);

1. premenopausala – de som fortfarande inte kommit in i klimakteriet
2. perimenopausala – de som gått in i klimakteriet från BL till FU
3. postmenopausala – de som var i perimenopaus vid BL

Medelåldern för kvinnorna vid uppföljningen var 50,1 år. Vid statistisk analys av bentäthetsmätningen framkom flera faktorer som påverkade BMD. För BMD i höften gällde att fallen hade lägre värden än kontrollerna; högre vikt inverkade positivt. BMD minskade för både grupper over tid och menopausstatus hade betydelse dvs, de som hade varit postmenopausala längst hade lägre BMD och det fanns också en koppling över tid som innebar att kvinnor i klimakteriet förlorade snabbare i bentäthet över tid jämfört med menstruerande kvinnor. För BMD i handled detsamma förutom att menopausstatus inte påverkade bentätheten signifikant. Ingen skillnad förelåg i frakturincidens.

Vad gäller associerad morbiditet så var det fler fall som behandlades med protonpumpshämmare än kontroller. Både fall och kontroller ökade i vikt under UF, 1.8 resp 1.6 kg men som tidigare var fallens medelvikt högre än kontrollerna i snitt 5.7 kg och fler uppfyllde kriterierna för fetma. Ingen
skillnad mellan grupperna vid analys av sjukdomsrelaterad livskvalitet kunde ses.

Delarbete III

I denna epidemiologiska studie har prevalensen av PHPT och effekt på BMD hos äldre män undersöks. Detta delarbete är ett delprojekt i den större internationella studien Mr Os som samlat en cohort av män mellan 69-81 år. Grundtanken för Mr Os i stort är att belysa faktorer av betydelse för benskörhetsutveckling i denna population. För just denna delstudie var målet att dels beräkna förekomst av PHPT i äldersgruppen samt att undersöka om det fanns associerad skelett påverkan.

I Mr Os Sweden har totalt 3014 män inkluderats från tre regioner, Malmö, Göteborg och Uppsala. Samtliga medverkande har lämnat blodprover för analyser. Bentäthet i höft och ländrygg undersöktes med DXA och vikt och vikt beräknades.

För att identifiera män med PHPT exkluderades individer med tecken på njurinsufficiens (eGFR (<21mL/min/1.73m²) och D-vitaminbrist (<50 nmol/l) (totalt n=490). Ytterligare 289 personer exkluderades på grund av saknade eller felaktigt analyserades blodprover. I denna studie kvarstod därför 2235 män att utvärdera.

Individer med albuminkorrigerat kalcium och PTH över normalgräns (>2.50 mmol/L kalcium och >6,9 pmol/L PTH) bedömdes ha PHPT (fall) och jämfördes med dem som hade normala värden dvs alla andra inkluderade (kontroller).

För att också kunna utvärdera milda rubbningar i kalciumbalansen sannolikt beroende på "normokalcemisk" hyperparathyroidism användes medianvärde för kalcium och iPTH i cohorten som cut-off värde för indelning i två grupper. Män med patologiskt förhöjdt iPTh (> 4.24 pmol/L) i förhållande till kalcium (>2.34 mmol/L) (n=387) jämfördes sedan med kvarvarande invidiver (n=1848) som hade en mer normal kalcium/iPTH spegel.

I den statistiska analysen av sBMD korrigerades utfallet för vikt, njurfunktion, vitamin-D nivå, ålder samt geografisk ort.

Medelvärde för kalcium var 2.34±0.16 mmol/L och för iPTh 4.66±2.8 pmol/L i hela cohorten. Det fanns en svag negativ korrelation mellan kalcium och PTH samt mellan ålder och BMD i höften, men inte i ländryggen.

Prevalensen av PHPT i denna population beräknades till 0,73%. Hos män med PHPT var BMD i höften signifikant lägre jämfört med kontrollerna. Beroende på vilken skelettregion som mättes varierade BMD mellan 6,9 % och 11,6 %. Det fanns dock ingen skillnad i bentäthet vad gäller ländryggen. Även för gruppen med patologiskt förhöjdt iPTh i förhållande till kalcium noterades en lägre bentäthet i total hip, och denna kvarstod också efter att de
22 män med PHPT exkluderats i analysen. I denna grupp noterades också en signifikant skillnad i sBMD för ländryggen. Äldre män förefaller ha en lägre risk att drabbas av PHPT jämfört med kvinnor i samma ålder. Den patologiska effekten av rubbad kalciumbalans var dock tydlig även i denna grupp.

Delarbete IV

I delarbete IV har vi utvärderat männen i Mr Os med avseende på kalciumrubbning och associerad risk för fall och frakturer. Vi har analyserat alla frakturer som de drabbats av under livet men också gjort analys av frakturer som inträffat de senaste tio åren. Vidare har fysisk funktion utvärderats mellan fall/kontroller och män med mild rubbning i kalciumhomeostasen/ kontroller.

Fyra olika fysiska test som mäter muskulär funktion användes för att utvärdera fysisk förmåga;

- Tiden det tog att upprepa fem benböjningar i rad (mätt i sekunder)
- Gripstyrka i bågge händer (kilo); två försök per hand varav det bästa försöket räknades
- Sex meters promenad i normal takt med mätning av både tid (sekunder) och antal steg
- Gång längs en tunt linje (20 cm bred); antal snedsteg från linjen mätttes och mer än tre avsteg betraktades som ett misslyckande
- De fick också fylla i ett omfattande hälsoformulär där de bland annat fick svara på frågan;
  - Har du någon gång fått en fraktur? Om ja - vilket år?
  - Har du någon gång fallit/ramlat under de senaste 12 månaderna? Om ja – hur många gånger?

Män med PHPT hade jämfört med sina kontroller väsentligen lika fysisk funktion förutom vid upprepade benböjningar, där PHPT gruppen upprisade sämre resultat än kontrollerna. Däremot var det ingen skillnad i vare sig fall- eller frakturincidensen.

Män med mild kalciumrubbning (dvs patologiskt förhöjt iPTH i relation till s-kalcium) upprisade dock i tre av fyra funktionstest en lägre fysisk kapacitet i jämförelse med kontroller. Ingen skillnad kunde noteras för gripstyrka och resultatet kvarstod även efter korrigering för vikt, ålder, njurfunktion och D-vitaminnivå. Det framkom inte heller för denna grupp någon skillnad i fall- eller frakturincidens.
Konklusion
I detta epidemiologiska avhandlingsarbete har ny kunskap om förekomst av PHPT och effekter av sjukdomen hos medelålders kvinnor samt hos äldre män beskrivits.

- Den biokemiska obalansen mellan kalk och PTH vid mild PHPT förefaller vara etablerad hos många kvinnor flera år före menopaus.
- Förekomsten av PHPT hos premenopausal kvinnor är vanligare än vad man skattat tidigare.
- Även milda kalkrubbningar beroende på dysfunktion i parathyroideakörtlarna påverkar bentätheten negativt i höften för båda könen men även i ländryggen hos män.
- PHPT associeras med övervikt och fetma hos yngre kvinnor men inte hos äldre män.
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