Metabolic Disturbances in Relation to Serum Calcium and Primary Hyperparathyroidism

EMIL HAGSTRÖM
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Abstract

Primary hyperparathyroidism (pHPT), characterized by elevated serum levels of calcium and parathyroid hormone (PTH), is associated with a number of metabolic derangements causing secondary manifestations. These include osteoporosis and increased risk of fractures, but also risk factors for cardiovascular morbidity and mortality. These risk factors include impaired glucose tolerance (IGT), dyslipidemia, increased body mass index and hypertension. While the skeletal abnormalities are mainly due to elevated PTH, the latter disturbances are still unexplained. Non-insulin dependent diabetes mellitus (NIDDM), IGT, dyslipidemia and hypertension are all included in the metabolic syndrome, also associated with morbidity and mortality in cardiovascular diseases.

In this thesis, decreased bone mineral density (BMD) and variables of the metabolic syndrome are explored in patients with mild and normocalcemic pHPT before and after parathyroidectomy. To further investigate the relationship between insulin sensitivity and calcium, a community-based cohort was investigated.

In two different patient cohorts of pHPT, lipoprotein alterations with decreased levels of HDL-cholesterol and elevated triglycerides were found in association with a high frequency of IGT, NIDDM and decreased insulin sensitivity. Parathyroidectomy had effects on the dyslipidemia and in part on the glucose metabolism. The disturbed glucose metabolism in pHPT was substantiated by results from the general population by a negative association between insulin sensitivity, measured by hyperinsulinemic clamp, and serum calcium.

In conclusion, normocalcemic, mild and overt pHPT are associated with a range of risk factors for cardiovascular diseases, development of NIDDM and decreased BMD in cortical as well as trabecular bone. These findings explain, at least in part, the elevated morbidity and mortality from cardiovascular disease as well as fractures, reported in pHPT patients. Moreover, in the general population, serum calcium is associated with decreased insulin sensitivity. Parathyroidectomy has positive effects on several, but not all, of the investigated metabolic parameters.

Keywords: Primary hyperparathyroidism, Parathyroidectomy, Insulin sensitivity, Lipoproteins, Glucose metabolism disorders, Bone density, Calcium

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To my family
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


IV  Hagström E, Hellman P, Lundgren E, Lind L & Ärnlöv J. Serum calcium is independently associated with insulin sensitivity measured with euglycemic hyperinsulinemic clamp in a community-based cohort. Submitted for publication.

V  Hagström E, Lundgren E & Hellman P. Primary hyperparathyroidism is associated with low insulin sensitivity and the metabolic syndrome.

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<td>ANCOVA</td>
<td>analysis of covariance</td>
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<td>ANOVA</td>
<td>analysis of variance</td>
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<td>ATP III</td>
<td>Adult Treatment Panel III</td>
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<td>BMC</td>
<td>bone mineral content</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>CI</td>
<td>confidence interval</td>
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<td>CRP</td>
<td>c-reactive protein</td>
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<td>DXA</td>
<td>dual energy x-ray absorptiometry</td>
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<td>EIR</td>
<td>early insulin response</td>
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<td>free fatty acids</td>
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<td>HOMA</td>
<td>homeostasis model assessment</td>
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<td>HRT</td>
<td>hormone replacement therapy</td>
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<td>IFG</td>
<td>impaired fasting glucose</td>
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<td>impaired glucose tolerance</td>
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<td>IVGTT</td>
<td>intravenous glucose tolerance test</td>
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<td>LDL</td>
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<td>M/I</td>
<td>insulin sensitivity index</td>
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<td>oral glucose tolerance test</td>
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<td>P</td>
<td>plasma</td>
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<td>PAI-1</td>
<td>plasminogen activator inhibitor 1</td>
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<td>pHPT</td>
<td>primary hyperparathyroidism</td>
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<td>PTH</td>
<td>parathyroid hormone</td>
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<td>S</td>
<td>serum</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>ULSAM</td>
<td>Uppsala Longitudinal Study of Adult Men</td>
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<td>VDR</td>
<td>vitamin D receptor</td>
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<td>VLDL</td>
<td>very low-density lipoprotein</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>WHR</td>
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Introduction

Primary hyperparathyroidism (pHPT) is a commonly diagnosed endocrine disease. However, until the 1970’s the prevalence and incidence of pHPT was considered low (Cope 1966). When automated serum analyses began to be used in the early 1970’s, there was a sharp increase in diagnosed pHPT (Heath 1980, Wermers 1997, Silverberg 2001). The rise represented a large number of cases with sub-clinical or clinical but undiagnosed pHPT. After the introduction of improved laboratory equipment, a better appreciation of the true prevalence of the condition was reported by the Mayo Clinic, where a 4- to 5-fold elevation was detected (Heath 1980, Melton 1991). Since the 1970’s, reports on prevalence and incidence have varied widely, from low figures in American studies to high, up to 1%, in European studies (Christensson 1976, Heath 1980, Palmér 1988, Melton 1991, Lundgren 1997, Wermers 2006). Primary HPT is more common in females (3:1) and prevalence rises with increasing age in both sexes. There is a particularly sharp increase after female menopause with incidence up to 2-3% (Christensson 1976, Heath 1980, Palmér 1988, Lindstedt 1992, Sorva 1992, Jorde 2000, Åkerström 2004). In a post mortem study of parathyroid gland abnormalities, prevalence was reported to be as high as 10% (Åkerström 1986). In a report on incidental parathyroid tumors discovered during thyroid surgery, immunohistological investigation revealed that all but one of the parathyroid tumors had signs concomitant with functional abnormality. All cases were normocalcemic and only one had an abnormal level of parathyroid hormone (Hellman 1993). In data from several ongoing community-based studies in Sweden, prevalence is much higher than previously reported. This also includes pre-menopausal individuals (unpublished observation). In contrast to many previous prevalence studies, these studies include repeated investigations as well as intact PTH and vitamin D₃.

Diagnosis of primary hyperparathyroidism

Primary HPT is the most common cause of hypercalcemia. To distinguish pHPT from differential diagnoses, malignancies being the second most common cause, serum PTH determination establishes the diagnosis in most cases. In pHPT, PTH-levels are elevated in approximately 80-90% of cases (Ljunghall 1991b, Silverberg 1997). In cases with normal levels of PTH, the
value is usually in the upper normal range, with apparently dysregulated increased serum calcium (Silverberg 1997).

Although on most occasions pHPT is characterized by hypercalcemia and elevated PTH, the upper reference ranges of calcium and PTH are merely statistical cut-offs and not biological ones. Therefore, some cases should be expected to be normocalcemic but still have pHPT. However, the existence of normocalcemic and near normocalcemic pHPT is questioned (Monchik 2005) and exerts a diagnostic challenge versus normal calcium metabolism. Based on the assumption that the distribution of serum calcium in individuals with pHPT resembles a relatively normal distribution, a cohort of patients with pHPT would include a tail of normocalcemic individuals, which would overlap with the normal distribution of calcium of another cohort of individuals without pHPT (Figure 1). Indeed, some evidence of this has been presented (Ljunghall 1980, Hellman 1993, Lundgren 1996, Glendenning 1998, Bergenfelz 2003, Hagag 2003, Maruani 2003, Monchik 2004, Tordjman 2004, Monchik 2005), but fewer investigators have demonstrated histological evidence of its existence (Lundgren 1996, Bergenfelz 2003).

Only in a few other instances do individuals have increased serum PTH, i.e. secondary HPT due to renal insufficiency or treatment with lithium or occasionally in familial hypocaliuric hypercalcemia (FHH). FHH is caused by an inactivating mutation in the calcium sensing receptor gene, characterized by a family history of the disease, a mild, generally asymptomatic hypercalcemia and low urinary calcium excretion (Hellman 2000).
Etiology of and biochemical findings in primary hyperparathyroidism

In 80-85% of individuals with pHPT, the disease is caused by a single parathyroid adenoma, whereas multiglandular disease is present in 15-20% (Bilezikian 2000b, Åkerström 2004). The disease is generally a sporadic one, but in rare cases it is part of hereditary multiple endocrine neoplasia type I or IIa (MEN I, MEN IIa) (Bilezikian 2000b, Åkerström 2004). Furthermore, the increasing prevalence in aging individuals may be explained by a chronic low calcium intake in the elderly, decreased calcium absorption from the intestine due to low vitamin D intake or decreased reabsorption capacity in the kidneys, leading to subclinical secondary pHPT and development of parathyroid hyperplasia (Wermers 1997, Åkerström 2004). It has been hypothesized that elderly individuals with pHPT have a pathophysiological mechanism leading to subclinical renal impairment due to nephrosclerosis and to decreased levels and activity of 1-α-hydroxylase, resulting in reduced negative feedback from active vitamin D (1,25-(OH)₂ vitamin D₃) (Silverberg 1999a, Åkerström 2004). These findings are in part substantiated by results demonstrating an inverse relationship between creatinine clearance and serum calcium, and a positive association between creatinine clearance and both 1,25-(OH)₂ vitamin D₃ and urinary calcium excretion (Yamashita 2003). Another possible mechanism adding to the increase of pHPT prevalence with age may be presence of vitamin D₃ receptor gene polymorphism, which reduces the normal inhibitory effect of vitamin D₃ on parathyroid cell proliferation and PTH transcription (Carling 1995, Carling 1997).

Biochemical characteristics of pHPT include hypercalcemia as a result of elevated PTH levels. Serum phosphorus is usually at low normal levels with approximately one quarter of the patients having subnormal levels. Levels of inactive 25-OH vitamin D₃ are in the lower range and active 1,25-(OH)₂ vitamin D₃ levels are in the upper normal range, with one quarter having values above the upper normal limit, due to increased conversion of 25-OH to 1,25-(OH)₂ vitamin D₃ caused by the elevated PTH. Urinary calcium levels are usually in the high normal range, with up to 40% having hypercalcuria (Bilezikian 2000b, Lal 2005). Proliferation of parathyroid cells in pHPT is generally low, reflecting the slow development of the disease. At later stages, the proliferation rate may be higher, or at least, the effects of the abnormal cells may more be prominent. This concept can explain earlier reports of the disease being bi-phasic (Rao 1988, Silverberg 2003).
Calcium metabolism
Calcium and phosphate
The level of calcium in the human body is mainly controlled and adjusted by four organs: the parathyroids, the kidneys, the skeleton and the intestine. Extracellular calcium is regulated by parathyroid hormone (PTH) and 1,25-(OH)_{2} vitamin D_{3} (Jüppner 1999, Monchik 2005). The parathyroid glands express G protein-coupled calcium-sensing receptors (CaR) on the cell surface, which monitor the serum calcium concentration (Brown 1996). About 40% of the calcium is bound to plasma proteins, of which albumin is the most dominant. Sixty percent of the calcium is free circulating, of which 10% is bound to anions (phosphate, sulphate, citrate) and 50% is ionized. Approximately 35% of the ingested calcium is absorbed in the intestine, 15% of the absorbed calcium is secreted via pancreatic and mucosal secretion (Monchik 2005). Normal levels of calcium range from 2.20-2.60 mmol/L, with small intra-individual changes, but with variations during different seasons, depending on various dietary intakes and sun exposure levels.

Parathyroid hormone
The dominant cell type in the parathyroid glands, the chief cells, synthesize, store and secrete PTH. Initially, the polypeptide preproPTH consisting of 115 amino acids is produced. After cleavage of a 25 amino acid signal peptide, proPTH is formed. The process of forming active PTH includes removal of another six amino acids. The remaining polypeptide consists of 84 amino acids where the 34 amino acids in the N-terminal portion attach to the PTH receptor, and thus is the biologically active domain (Bilezikian 2005). Serum PTH is routinely analyzed with a double antibody immunoradiometric assay (IRMA), or an immunocheluminometric assay (ICMA), measuring the entire, intact, PTH molecule. Recently, measurement of biointact PTH has become available, using even more selective aminoterminal antibodies directed against PTH 1-34 not interacting with PTH 7-34 fragments, which may be recognized in the intact PTH method (Bilezikian 2005, Lal 2005). PTH is constantly secreted at a low basal rate (Wallfelt 1988a, Wallfelt 1988c), and if there is a change in the extracellular calcium level, i.e. ionized calcium, the response in PTH secretion is immediate (Habener 1984). The vector is steep in the middle section of the sigmoid curve describing the relationship between calcium and PTH, i.e. a small decrease in calcium results in a large increase in PTH secretion within the normal range (Figure 2) (Brown 1983). Primary HPT develops in individuals where clones of abnormal parathyroid cells have multiplied into hyperplasia or into an adenoma. These abnormal cells have a right-shifted set-point of the sigmoid curve, with decreased calcium sensitivity. The lower sensitivity is due to
resistance to extracellular calcium, and a resulting increased PTH secretion (Wallfelt 1988b).

![Figure 2: Sigmoid curve of relationship between calcium and PTH secretion. Grey line indicates right-shifted set-point in pHPT.](image)

PTH exerts its calcium modulating effects on the skeleton and on the kidney. PTH has an immediate effect on osteocytes, resulting in a surface bone osteolysis. In this process calcium is transferred from the bone to the extracellular fluid. At a slower rate, osteoclasts are stimulated by both PTH and 1,25-(OH)₂ vitamin D₃ to reabsorb mineralized bone, releasing not only phosphate and calcium into the extra-cellular fluid, but also organic material of the bone matrix, mainly collagen. If the elevated stimulus from PTH persists, osteoblasts, the bone forming cells, are inhibited in their actions. Due to the lack of PTH receptors, the PTH effect on osteoclasts is mediated through paracrine signaling from other cells in the bone (Jüppner 1999, Monchik 2005).

In the kidney, PTH inhibit phosphate reabsorption, stimulate calcium re-uptake and activates vitamin D₃ 1-α-hydroxylase to increase levels of 1,25-(OH)₂ vitamin D₃ (Jüppner 1999, Monchik 2005).

**Vitamin D**

Vitamin D and its metabolites are cholesterol derivates. Inactive vitamin D, cholecalciferol, is absorbed from the diet or is synthesized in the skin by ultra-violet light from its precursor 7-dehydrocholesterol. Cholecalciferol is converted by hydroxylation in the liver to inactive 25-hydroxycholecalciferol (25-OH vitamin D₃). 25-OH vitamin D₃ is bound to alpha-globulin (vitamin D binding protein, DBP) in serum and is the quantitatively largest form of circulating vitamin D. If there is an increase in the serum level of PTH, decrease in 1,25-(OH)₂ vitamin D₃ or ionized calcium, 25-OH vitamin D₃ 1-α-hydroxylase activity in the kidney is increased, hydroxylating the substrate 25-OH vitamin D₃ to active 1,25-(OH)₂ vitamin D₃. If there is an excess of ionized calcium, 25-OH vitamin D₃ can be hydroxylated to inac-
active 24,25-(OH)2 vitamin D3, by 24-hydroxylase, an ubiquitously expressed enzyme, which also inactivates excess 1,25-(OH)2 vitamin D3 (Holick 1999). Active vitamin D3 stimulates absorption of calcium from the small intestine, from the kidneys and resorption from bone (Bouillon 1998, Holick 1999). The effects of vitamin D are mediated via the vitamin D receptor (VDR). After the binding of active vitamin D3 to VDR it is transported to the nucleus where it acts as a transcription factor of the steroid, thyroid and retinoic acid receptors gene family. Active vitamin D3 also has an effect in the parathyroid cells, by inhibiting PTH transcription, secretion and cell proliferation (Hellman 1999, Silver 1999, Hellman 2000).

Classical manifestations of primary hyperparathyroidism
Prior to the widespread use of automated analyses of serum calcium, pHPT was often diagnosed when apparent symptoms appeared such as kidney stones, osteitis fibrosa cystica or severe osteoporosis (Parisien 1990, Bilezikian 2005). Today, these manifestations are rare and the dominant patient category includes patients with mild pHPT with few, if any, apparent symptoms of their disease. However, in the third world severe manifestations are still common and represent the symptoms seen at diagnosis in the majority of cases (Bilezikian 2000a, Mithal 2001). In pHPT, the raised plasma calcium concentration exceeds the kidney calcium absorption capacity, despite increased PTH levels promoting calcium reabsorption and leading to hypercalciuria (Silverberg 1990). This increased urinary calcium concentration together with decreased phosphate reabsorption are the major causes for nephrolithiasis.

Non-classical manifestations of primary hyperparathyroidism
Today, patients in the Western world often lack the “classical” symptoms and manifestations of pHPT and when diagnosed, are unaware of having the disease (Lundgren 1998b). However, if scrutinized thoroughly, neuromuscular symptoms including muscle tiredness, especially in proximal muscle groups, psychological manifestations including fatigue and depression are often present (Joborn 1988, Joborn 1989b, Solomon 1994, Silverberg 2001). Many patients describe a “cloud” surrounding them, leading to a general psychosocial weakness which disappears after surgery, thus making it recognizable usually only after cure of the disease. The majority of the symptoms are discrete early in the path of the disease. However, the secondary effects of pHPT (described below), which we have only come to appreciate more recently, are most likely not discrete in the later phases. The secondary
effects encompass increased morbidity and mortality in cardiovascular diseases, elevated risk of diabetes and prediabetic states, proatherosclerotic lipoprotein pattern and raised body weight. Many of these variables are included in the cluster of disturbances denoted as metabolic syndrome, a major cause of morbidity and mortality in cardiovascular diseases (CVD). Furthermore, the extent of the disease does not necessarily correlate to the secondary symptoms and manifestations (Harrison 1991).

The secondary effects of pHPT, sometimes included in the term “non-classical manifestations”, will be described in the following sections.

**Mechanisms for non-classical metabolic disturbances in primary hyperparathyroidism**

Prior studies have reported that many components of metabolic syndrome are frequently observed in pHPT patients. One mechanism for this association may be the presence of impaired glucose metabolism in both pHPT and in metabolic syndrome. Altered glucose metabolism is, at least in part, responsible for the development of many of the components in metabolic syndrome (Reaven 2003). In pHPT, lower insulin sensitivity due to decreased insulin receptor binding capacity or fewer insulin receptors has been reported (Prager 1984), in part corroborated by *in vivo* investigation with decreased insulin sensitivity as measured with euglycemic hyperinsulinemic clamp (Prager 1990).

The impaired glucose metabolism in pHPT may be mediated by elevated levels of PTH which increase calcium influx through calcium channels, hence raising intracellular calcium levels (Borle 1978, Hvarfner 1988, Fardella 1995, Schiffl 1997). Raised levels of intracellular calcium, as measured in e.g. adipocytes, platelets and leukocytes, have been related not only to insulin resistance, but to the metabolic syndrome as a whole (Reusch 1991, Byyny 1992, Barbagallo 1993, Baldi 1996, Barbagallo 1999, Levy 1999, Resnick 1999). Findings of elevated levels of PTH are negatively associated with insulin sensitivity and glucose tolerance (Wareham 1997, Chiu 2000) support the associations between pHPT and impaired glucose metabolism.

In addition to the relationship between pHPT and impaired glucose metabolism, pHPT patients have been reported to have increased BMI and fat mass (Grey 1994, Bolland 2005), one of the largest contributors for the development of CVD and components of metabolic syndrome. Serum levels of PTH have been reported as strong predictors for increased body weight in the general population (Kamycheva 2004, Parikh 2004), and corroborated by a reduction in PTH after weight loss. With elevated body weight, the 25-OH and 1,25-(OH)₂ vitamin D₃ level declines, perhaps due to dispersion in fat,
promoting PTH release and parathyroid gland proliferation with development of chief cell hyperplasia (Parikh 2004).

Moreover, serum phosphate tends to be in the lower normal range in pHPT and in several reports, low levels are associated with impaired glucose metabolism (Marshall 1978, DeFronzo 1980, Haap 2006).

Increased intake of dietary calcium has been reported to improve insulin sensitivity and reduce blood pressure (Colditz 1992, Bucher 1996, Sanchez 1997, Pereira 2002), and low calcium intake is associated with an elevated blood pressure (McCarron 1984, Cappuccio 1995). A possible explanation for this may be that in individuals without primary hyperparathyroidism, high levels of serum PTH constitute a marker of calcium deficit. Likewise, positive associations between serum PTH and blood pressure have been reported (Young 1990, St John 1994, Morfis 1997, Jorde 1999a) and after chronic infusion of PTH, the development of hypertension has been reported (Hulter 1986). Also vitamin D supplementation seems to improve insulin sensitivity, decrease body weight and blood pressure (Lind 1987, Lind 1988b, Mak 1989, Lind 1992, Kautzky-Willer 1995, Mak 1998). However, these relationships are not entirely clear since endogenous 25-OH vitamin D₃ levels are reported to have an inverse correlation with development of diabetes mellitus (Scrugg 1995) and 1,25-(OH)₂ vitamin D₃ may increase intracellular calcium levels via elevated calcium influx (Shan 1993).

Cardiac and vascular diseases in primary hyperparathyroidism

Patients with mild and moderate pHPT have an increased risk of morbidity (Lundgren 1998b) and premature death from diseases of the cardiovascular system as described in large population-based and longitudinal patient cohort studies (Ronni-Sivula 1985, Palmér 1987b, Sivula 1987, Hedbäck 1990, Udén 1990, Ljunghall 1991a, Hedbäck 1998, Walgenbach 2000, Lundgren 2001, Ogard 2004). The increased morbidity and mortality rate is mainly due to myocardial infarction, stroke and heart failure, but also from urogenital diseases and diabetes mellitus (Palmér 1987a, Palmér 1987b, Sivula 1987, Hedbäck 1990, Udén 1990, Öhrvall 1994, Hedbäck 1998, Wermers 1998, Nilsson 2002) and seems to be independent of age and gender. However, American studies of mortality have not been able to confirm the results over the entire range of calcium (Søreide 1997), only in cases with the most severe pHPT (Wermers 1998).

Parathyroidectomy has had diverging results concerning the incidence of premature death. Some studies have demonstrated no effect of surgery (Vestergaard 2003b), while others show decreased mortality (Palmér 1987b, Hedbäck 1991, Nilsson 2002, Vestergaard 2003a).

The metabolic syndrome


One of the main abnormalities in metabolic syndrome is insulin resistance, caused at least in part by an increased body fat mass, especially of easily mobilized visceral fat (Palaniappan 2004). Normal weight individuals can also be affected by the MeS, especially those having fat disproportionately stored in the abdomen (Grundy 2004). Non-insulin dependent diabetes mellitus (NIDDM) develops in insulin resistant individuals who cannot
overcome the resistance with greater production of insulin (Warram 1990, Lillioja 1993). Before hyperglycemia becomes manifest these individuals are usually increasingly insulin resistant in parallel to their weight gain over years to decades. This leads to hyperinsulinemia and several associated metabolic abnormalities. The condition is commonly characterized by some degree of glucose intolerance, high plasma triglycerides, low HDL-cholesterol concentration and essential hypertension (Reaven 1993a). This cluster of metabolic abnormalities associated with insulin resistance was first described in the late 1980’s (Reaven 1988). Studies generally give different prevalence data, but incidence increases with age and is more common in men than in women (Balkau 2002). According to WHO or ATP III criteria (Table 1), metabolic syndrome is present in 16-25% of the population, if diabetics are excluded (Ferrannini 1996, Hu 2004, Miccoli 2005). The prevalence in middle-aged individuals ranges from 20-40% in men and 10-20% in women, increasing to above 40% and 25% respectively in individuals over the age of 55 (Balkau 2002, Ford 2002, Alexander 2003, Ford 2003). Approximately 5% of normal-weight adults, 20% of overweight adults, and 60% of obese adults have metabolic syndrome (Park 2003).

Definitions
Several empirical definitions of the syndrome have been established and the most frequently used are the definitions by the World Health Organization (WHO) and the Adult Treatment Panel III (ATP III) of the National Cholesterol Education Program (Table 1) (WHO Diabetes Mellitus Guidelines 1999, NCEP Guidelines 2001).

Insulin sensitivity can be investigated in several ways, but euglycemic hyperinsulinemic clamp is the most accurate and regarded as the gold standard method. During the investigation, insulin is infused and the level is “clamped” at a supranormal level. Glucose is simultaneously infused and the metabolized amount of glucose per unit of insulin is used as a measurement of insulin sensitivity. Impaired insulin sensitivity can also be assessed in indirect ways, such as raised levels of fasting insulin or by the homeostasis model assessment (HOMA) at an oral glucose tolerance test (OGTT). Both fasting insulin and HOMA insulin sensitivity are limited as indicators of insulin sensitivity as they are also highly influenced by beta cell function, i.e. insulin secretion.
Mechanisms in the development of metabolic syndrome

Several metabolic changes occur in response to the constant hyperinsulinemia. Insulin resistance is mainly located in adipose and muscle tissue, whereas other tissues are usually more insulin sensitive. In addition, muscle tissue is much less insulin sensitive than adipose tissue. The resulting hyperinsulinemia makes other than muscle and adipose tissues vulnerable, leading to expression of the metabolic syndrome (Bernstein 1978, Skott 1991, Facchini 1996, Reaven 1997).

Clinical manifestations of metabolic syndrome

Besides the development of impaired glucose tolerance and impaired fasting glucose, several other changes occur, often directly due to hyperinsulinemia. The increment in serum levels of FFA, secreted at elevated levels due to insulin stimulation of adipose tissue lipolysis, and hyperinsulinemia enhance the liver secretion of triglycerides and other proatherosclerotic lipoproteins (Reaven 1993a, NCEP Guidelines 2001, Reaven 2003). HDL-cholesterol levels are usually decreased, postprandial levels of triglyceride-rich lipoproteins are elevated and low-density lipoproteins are reduced in size and become denser (Reaven 1993b). Disturbances of endothelial function and enhanced ability of monocytes to adhere to the endothelium are also associated
with MeS (Ross 1986, Baron 1999). A chronic inflammatory state is described whereby insulin sensitivity is further decreased. It is characterized by elevated levels of acute phase reactants, such as C-reactive protein (CRP), elevated white blood cell count (Facchini 1991, McLaughlin 2002) and secretion of pro-inflammatory chemokines such as IL-6 and TNF alpha from fat tissue (Yudkin 2000). Furthermore, in MeS a procoagulant state is reported with increased levels of both fibrinogen and plasminogen activator inhibitor-1 (PAI-1) (Juhan-Vague 1993, Abbasi 1999, Meigs 2000). The kidneys are capable of maintaining insulin sensitivity, and as a response to the chronic hyperinsulinemia, uric acid clearance is reduced and sodium absorption elevated, resulting in elevated plasma uric acid concentration, water retention and raised blood pressure (Facchini 1991, Skott 1991, Reaven 1997, Facchini 1999).

Metabolic syndrome in primary hyperparathyroidism

In several studies pHPT has been associated with the different components of MeS, but never to the syndrome as a whole. Peripheral insulin resistance is described in some reports, most using surrogate markers of insulin sensitivity assessed by HOMA, intravenous glucose tolerance test (IVGTT) and serum insulin levels (Kim 1971, Prager 1983, Prager 1984, Kautzky-Willer 1992, Kumar 1994, Procopio 2002). Only one study has used the gold standard method, euglycemic hyperinsulinemic clamp, for investigation of insulin sensitivity (Prager 1990). In prior investigations the prevalence of NIDDM in pHPT has been 7-8%, 2 to 4 times the prevalence in the general population (Ljunghall 1983, Taylor 1991, Taylor 1997, Valdemarsson 1998a, Procopio 2002). Impaired glucose tolerance and impaired fasting glucose are also reported by some investigators (Prager 1984, Kumar 1994, Smith 2000, Procopio 2002). The associations between pHPT and abnormalities in glucose metabolism and diabetes are reported to be uninfluenced by BMI or the extent of pHPT (Taylor 1991, Kumar 1994). Reversal of diabetes, improved diabetic control or improvement of impaired glucose metabolism after parathyroidectomy have been reported from some studies (Birge 1969, Werner 1974, Walsh 1975, Akgun 1978, Prager 1983, Cheung 1986, Prager 1990, Kautzky-Willer 1992, Richards 1999) but not all (Ljunghall 1983, Bannon 1988).

Microalbuminuria has not previously been associated with pHPT. Other variables associated with MeS and CVD, which are also reported in individuals with pHPT, are hyperuricemia (Pepersack 1989, Lundgren 1998a), increased inflammatory parameters, IL-6, CRP and TNF alpha (Grey 1996, Guo 2000, Ridker 2000, Cesari 2003, de Lemos 2003, Ruskoaho 2003, Safley 2004, Øgard 2005) and elevated sympathetic activity (Barletta 2000).
Lipoprotein disturbances in primary hyperparathyroidism

Dyslipidemia with elevated LDL-cholesterol, triglycerides and decreased HDL-cholesterol levels are major causes of morbidity and mortality in CVD (NCEP Guidelines 2001, Baigent 2005). Several subclasses of particles are included within the LDL-cholesterol fraction, of which small dense LDL has the highest predictive association with CVD. Often associated with the increased concentration of small dense LDL and also with the MeS is a decreased level of HDL-cholesterol, an independent predictor for CVD (Gordon 1977). Preferably, HDL-cholesterol should be as high as possible, since it is involved in the transport of non-beneficial lipoproteins from peripheral tissues to clearance in the liver. A rise in triglyceride levels, also highly associated with CVD, is often linked to the changes in levels of LDL- and HDL-cholesterol described above (NIH Dyslipidemia Guidelines 1993, Hokanson 1996, Stampfer 1996, Austin 1998).

In a few studies, overt pHPT has been associated with non-beneficial levels of lipoproteins. Elevated concentrations of total and VLDL-triglycerides, VLDL-cholesterol and decreased HDL-cholesterol have been reported (Vaziri 1983, Lacour 1986, Lundgren 1998a, Smith 2000). However, some older studies have reported contrary findings with lower total triglycerides and total cholesterol compared to controls (Christensson 1977, Ljunghall 1978).

Improvement of the lipid disturbances by parathyroidectomy has been substantiated in a few small studies with reduction of VLDL-cholesterol and total triglycerides levels (Ljunghall 1978, Lacour 1986, Valdemarsson 1998a) while the opposite or no effect also has been reported (Christensson 1977, Vaziri 1983).

Body weight in primary hyperparathyroidism

In some prior studies of pHPT, body weight and BMI were reported to be elevated and these findings are often regarded as coincidental. In some early studies of pHPT, cases were as much as mean 9 kg heavier than euclidean controls due to increased mass of fat. Furthermore, the cases had a more android fat distribution (Grey 1994), although the majority of pHPT cases were females. The raised body weight is reported to precede the development of pHPT by at least 27 years (Grey 1995), and thereby possibly influences the development of pHPT. In a meta-analysis of weight and pHPT from 18 studies, cases with calcium below 2.84 mmol/L were 3.1 kg heavier or had 1.1 kg/m² elevated BMI compared to euparathyroid controls. The 1.1 kg/m² increase in BMI per se could explain a 155% increase in the risk of diabetes mellitus; 15-25% of hypertension; 6-11% of CVD and an increased

**Normal serum calcium levels as risk factor for cardiovascular diseases and diabetes**

In individuals with normal calcium levels in the general population, calcium *per se* is associated with risk factors for and with increased incidence of CVD. Three prior epidemiological studies have investigated the CVD frequency in relation to serum calcium. In 21,131 men below 50 years of age, with a mean follow-up of 10.8 years, a calcium level of 2.51-2.55 mmol/L compared to 2.31-2.45 mmol/L was associated with a 50% increased risk of death, mainly due to cardiovascular diseases (Leifsson 1996). Furthermore, in a previous investigation of the ULSAM cohort (n=2,183), starting at the age of 50 and followed for 18 years, normal serum calcium levels were an independent risk factor of myocardial infarction. Individuals who developed myocardial infarction had a higher mean serum calcium level compared with individuals who did not (Lind 1997). In men with prior myocardial infarction, serum calcium levels were increased and levels were predictive for myocardial infarction with odds ratio of 1.21 per 0.1 mmol/L serum calcium elevation (Jorde 1999b).

A few previous large scale studies have investigated the relationship between serum calcium and insulin sensitivity and insulin secretion (Lind 1997, Wareham 1997, Sun 2005). In these studies, increasing levels of calcium were associated with decreasing insulin sensitivity as assessed by fasting insulin or HOMA. HOMA estimated beta cell function was negatively associated with calcium in females, but not in males (Sun 2005). In the ULSAM study, no association was found between serum calcium and insulin secretion as estimated by the intravenous glucose tolerance test (Lind 1997).


**Bone disease and primary hyperparathyroidism**

Osteoporosis is a general disorder of the skeleton characterized by low bone mineral density (BMD) and micro-architectural deterioration of the bone tissue leading to increased bone fragility and increased risk of fractures (NIH Osteoporosis Guidelines 1991). In the general population, the majority of
fractures occur at sites prone to having low BMD in osteoporosis, i.e. sites rich in trabecular bone such as the spine and femoral neck. The fracture risk increases between 1.5 to 3 fold for every standard deviation decline in BMD (Kanis 1994, Schuit 2004). The development of osteoporosis is especially pronounced in postmenopausal females, but is also found in older men (van Staa 2001). At the age of 80, 27% of women are osteopenic and 70% osteoporotic (Dennison 2005).

Diagnosis of osteoporosis or severe bone loss is made when the T-score (BMD measured with dual energy X-ray absorptiometry, DXA) is below 2.5 standard deviations compared with BMD of a young adult reference population matched for sex and weight. Osteopenia is defined as a T-score between −1 to −2.5 SD (WHO Osteoporosis Guidelines 1994, NIH Osteoporosis Guidelines 2001). With DXA, two X-ray beams are emitted with different energy levels through the investigated site. With this method, in contrast to the previously used single X-ray absorptiometry (SXA), soft tissue absorption is more easily adjusted for. Previously, Z-score was used, comparing BMD values with an age, sex and weight matched reference population.

An apparent classical manifestation of pHPT, osteitis fibrosa cystica, a severe disease with fractures, cystic formations in the skeleton and with a very typical radiographical appearance, was prior to the use of multichannel screening tests more common (Parisien 1990). In the Western world today, cases with pHPT seldom develop the severe bone disease, but many suffer from osteopenia (Abdelhadi 1998).

PTH has both anabolic and catabolic effects on the bone, depending on the serum level and duration of raised hormone and bone type (Jüppner 1999). Moderately increased continuous levels of PTH, as part of a therapeutic regime for postmenopausal osteoporosis or as an in mild pHPT, increase BMD mainly on cortical sites. Higher levels of PTH, as in moderate pHPT or secondary HPT, increase bone turnover and bone resorption and may lead to osteoporosis. In pHPT cortical bone, e.g. the distal one third of the radius, is mainly affected (Silverberg 1995a). In contrast to bone loss in pHPT, postmenopausal bone disease is mainly associated with BMD decrease in trabecular bone (Christiansen 1992, Parisien 1995). Classically, sites with large proportion of trabecular bone seems to be better preserved in pHPT, e.g. lumbar spine and femoral neck (Silverberg 1995a). However, it seems as if pHPT cases also suffer from increased fracture risk at sites with a large proportion of trabecular bone, regardless of the DXA-findings that suggest that the only increased risk of fractures occurs at sites with mainly cortical bone (Khosla 1999). The presence of increased fracture risk in pHPT is controversial. Several authors report increased frequency both in mild and overt pHPT (Dauphine 1975, Melton 1992, Kenny 1995, Vestergaard 2000, Khosla 2002, Vestergaard 2003c, Vestergaard 2004), while others present contradictory findings (Larsson 1993). One explanation for the divergent findings of BMD and fracture risk is that pHPT may have a bi-phasic course,
initially subclinically with increased PTH affecting bone and causing increased turnover and risk of fractures. The second phase, characterized by hypercalcemia, is stable regarding the skeleton and without increased risk of fractures (Rao 1988). This hypothesis is corroborated by studies in which cases had an increased fracture incidence before diagnosis (Melton 1992) and that the bone disease seems not to progress over time after the initial drop in BMD (Silverberg 1999b).

Recent studies indicate improvement after surgery with rapid BMD gain, also in individuals with mild pHPT and osteopenia (Silverberg 1995b, Guo 1996, Silverberg 1999b, Sitges-Serra 2004) and possibly also reduced fracture risk (Vestergaard 2003c).

Treatment of primary hyperparathyroidism

Parathyroidectomy

Long-term studies performed of untreated pHPT rarely reported any serious complications such as renal impairment or progressive hypercalcemia (Palmér 1987a, Rao 1988). According to these reports, the 1990 National Institutes of Health (NIH) treatment guidelines for pHPT were rather conservative in their recommendations for parathyroidectomy (NIH pHPT Guidelines 1991). The report stated that conservative surveillance was safe for the majority of patients and surgery should only be considered in individuals with symptoms and biochemical variables which in the Western world are nowadays considered as relatively severe pHPT. Patients to be conservatively followed should lack “significant bone, renal, gastrointestinal or neuromuscular symptoms typical of pHPT”. In 2002, a consensus development conference revised the previous guidelines, taking into consideration some, but far from all, of the gathered knowledge of secondary manifestations in pHPT (Bilezikian 2002b). Treatment guidelines from 1990 and 2002 are presented in Table 2.

Individuals with mild and normocalcemic pHPT with symptoms may also fulfill criteria for surgery, according to the latest guidelines. Furthermore, not only are improvements in many of the non-classical metabolic alterations discussed in previous sections seen after surgery, but there is also relief of the classical symptoms and signs (Joborn 1989a, Lundgren 1998a, Valdemarsson 1998b, Bergenfelz 2003).
Conservative observation and medical treatment of primary hyperparathyroidism

According to the pHPT guidelines, conservative surveillance is preferable in the majority of individuals with mild pHPT. However, conservative management has been reported to maintain and also exaggerate several non-beneficial secondary manifestations of pHPT described in previous sections. In addition, the calcium level may not necessarily reflect the severity of the symptoms (Bergenfelz 2003). Alternative treatments besides surgery have been studied and are suggested as preferable for older individuals, for patients with mild disease or with contraindications to surgery (Slatopolsky 2003). The pharmacological treatment strategies evaluated in pHPT are vitamin D, vitamin D analogues and calcimimetics. Active vitamin D and vitamin D analogues are used with good results in secondary HPT. Via the VDR, vitamin D and analogues decrease transcription of the PTH gene in the parathyroid glands. However, there is a risk of developing hypercalcemia with vitamin D treatment and frequent monitoring is needed. Calcimimetics are substances that increase the effect of the calcium ion by interacting with the calcium sensing receptor (CaR) on the parathyroid cell surface. One substance, cinacalcet, is currently registered for treatment of secondary HPT, but some initial reports have reported promising effects on levels of calcium and PTH in pHPT (Shoback 2003, Peacock 2005).

<table>
<thead>
<tr>
<th></th>
<th>1990 treatment guidelines</th>
<th>2002 treatment guidelines</th>
</tr>
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<tbody>
<tr>
<td>Serum calcium (mmol/L)</td>
<td>&gt;3.0</td>
<td>&gt;2.85</td>
</tr>
<tr>
<td>Urinary calcium (mmol/24h)</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>Reduced by 30%</td>
<td>Reduced by 30%</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>No indication for surgery</td>
<td>If persistently abnormal</td>
</tr>
<tr>
<td>Bone mineral density</td>
<td>Z-score &lt;– 2 SD at distal radius</td>
<td>T-score &lt;– 2.5 SD at the distal radius, lumbar spine or femoral neck</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&lt;50</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Nephrolithiasis</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Osteitis fibrosa cystica</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Neuromuscular symptoms</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Previous episode of life-threatening hypercalcemia</td>
<td>✓</td>
<td>✓</td>
</tr>
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Table 2. Treatment guidelines for parathyroidectomy in pHPT.
Aims of the thesis

The aims of this thesis are:

- to investigate lipoprotein patterns and disturbances in glucose metabolism in patients with mild and normocalcemic pHPT.

- to study bone mineral density in patients with mild and normocalcemic pHPT.

- to assess whether parathyroidectomy or conservative observation improve the lipoprotein pattern, glucose level and bone mineral density in patients with mild and normocalcemic pHPT.

- to investigate whether serum calcium in the normal range in a community-based cohort is associated with insulin sensitivity and insulin secretion.

- to study insulin sensitivity and other variables associated with the metabolic syndrome in patients with pHPT.
Main hypothesis

Primary HPT has in a large number of studies been associated with increased morbidity and mortality in cardiovascular diseases and with increased fracture risk. The mechanisms are largely unknown but a number of theories have recently been raised, where both serum calcium and PTH have been suggested as mediators. The main hypothesis of the present thesis concludes that increased serum levels of calcium and PTH induce impaired glucose metabolism with primary and/or secondary effects on lipoprotein pattern, adipose tissue and the cardiovascular system creating a state similar to the metabolic syndrome. In addition the deranged mineral homeostasis also causes early reductions in BMD in both trabecular and cortical bone. If these hypotheses are supported, patients with pHPT would suffer from a state similar to the metabolic syndrome, and their derangements including risk of morbidity and mortality in cardiovascular diseases, NIDDM as well as the increased fracture risk may be reduced after parathyroidectomy.
Subjects and methods

Study samples

Studies I and II

At a community-based health screening of 5,202 post-menopausal women, 87 were identified as having pHPT (2.1% of screened population, Figure 3). Diagnosis criteria for pHPT were (I) hypercalcemia (>2.60 mmol/L) with serum parathyroid hormone (PTH) ≥25 ng/L (normal range 12–55 ng/L), (II) serum calcium 2.50–2.60 mmol/L and PTH ≥35 ng/L, or (III) serum calcium <2.50 mmol/L and PTH >55 ng/L (Lundgren 1997). Serum calcium and PTH were investigated on four consecutive occasions to rule out a temporary false value. All of the cases had serum creatinine <160 µmol/L, a urine calcium:creatinine ratio well above 0.01, excluding familial hypocalciuric hypercalcemia (FHH) (Bilezikian 2002a), and none had a family history of hypercalcemia. An initial attempt to randomize the patients to either conservative observation or parathyroid surgery failed because of contraindications for parathyroidectomy or lack of interest in undergoing surgery. Therefore, parathyroidectomy was performed on all cases that accepted surgery and had no general contraindications. This led to the division of the remaining patients into two subgroups, 49 parathyroidectomized and 17 conservatively observed patients. The diagnosis was histologically confirmed in the parathyroidectomized patients after removal of a single parathyroid adenoma. These individuals normalized their serum calcium and PTH values. Serum calcium at study entry was equal in both groups. Control individuals were chosen from the screening population and matched for age and quarter of year for biochemical analysis. Control individuals had serum calcium <2.55 mmol/L, PTH level appropriate for the calcium level and serum creatinine <160 µmol/L. Controls were excluded from further follow-up when the cases left the study. Cases and controls had similar smoking and daily exercise habits. Difference in menopausal age averaged 5.0 (±4.05) years within the case-control pairs. No cases or controls had vitamin D or calcium supplementation during the study. At baseline, one case and no controls had lipid-lowering treatment. At the five-year follow-up, two parathyroidectomized cases, two conservatively followed cases and four controls had lipid-lowering treatment. Over different periods during follow-up, eleven cases and four controls were treated with hormone replacement ther-
apy (HRT, 2 mg daily oral estradiol and 1 mg noretisterone). Investigations were performed at inclusion, after one year (study I only) and after five years. All participants gave informed consent, and the ethics committee of Uppsala University approved the study protocol.

Figure 3. Flow chart of patients and controls in studies I and II.
Study III
Same initial cohort as studies I and II. This study was comprised of a subsample of 30 (0.6% of the screened population) individuals with normocalcemic pHPT (Figure 4). At four consecutive analyses the individuals had normal serum calcium and an inappropriately high serum PTH. The diagnostic criteria were: (I) serum calcium 2.50–2.60 mmol/L and PTH ≥35 ng/L (normal range 12–55 ng/L), or (II) serum calcium <2.50 mmol/L and PTH >55 ng/L. Fifteen cases were parathyroidectomized and nine were conservatively observed. Investigations were performed at inclusion and after five years.

![Flow chart of patients and controls in study III.](image-url)
Study IV
This study includes individuals from the Uppsala Longitudinal Study of Adult Men (ULSAM). All men born between 1920 and 1924 who were residents of the community of Uppsala, Sweden, were invited to participate in a longitudinal health study at the age of 50. The study included 2,322 individuals. In this study, at the second follow-up at age 70, 1,221 individuals attended. Of these, 104 individuals were excluded for the following reasons: unavailable albumin-corrected serum calcium data (n=45) and unavailable euglycemic hyperinsulinemic clamp data (n=59). Thus, the investigated sample comprised 1,117 individuals (Figure 5). All participants gave written informed consent, and the ethics committee of Uppsala University approved the study protocol.

Figure 5. Flow chart of patients in study IV.
Study V
Consecutive cases with pHPT accepted for parathyroidectomy at Uppsala University Hospital’s surgical clinic were enrolled in the study. Twenty-three cases (19 women) were investigated before parathyroidectomy. The cases had plasma creatinine <160µmol/L, did not have a family history of hypercalcemia and had a urine calcium:creatinine ratio well above 0.01 excluding FHH (Bilezikian 2002a). All individuals had one solitary enlarged parathyroid gland removed and the diagnosis of parathyroid adenoma was histologically confirmed. Some cases were treated with antihypertensive drugs (n=11), lipid lowering therapy (n=2) or oral antidiabetics (n=2), respectively. Two patients had NIDDM diagnosed prior to the investigation. All participants gave written informed consent, and the ethics committee of Uppsala University approved the study protocol.
Investigations

Biochemical Evaluation

In studies I, II, III, IV and V, laboratory analyses were performed after an overnight fast as follows: serum/plasma (normal range 2.20-2.60 mmol/L) and urine calcium (normal range 0.6-5.0 mmol/24 h) were measured spectrophotometrically with a compleximetric method using orthocresolphthalein dye binding. Serum/plasma albumin was measured with spectrophotometry using bromine cresol green (normal range 37-48 g/L). Albumin-corrected serum/plasma calcium was calculated as [serum/plasma calcium + 0.019 x (42–plasma/plasma albumin)] and urine calcium was standardized by urine creatinine. Ionized plasma calcium (normal range 1.10–1.30 mmol/L) was determined with an ion sensitive electrode (I, II, III: Kone Instruments, Espoo, Finland, V: Radiometer, Copenhagen, Denmark). Intact serum/plasma PTH (normal range 12-55 ng/l or 1.1–6.9 pmol/L) was determined with immunochemiluminometric or radioimmunometric assay (Nichol’s Institute, San Juan Capistrano, CA). Serum/plasma phosphate was determined with spectrophotometry and a compleximetric method using ammonium molybdenum (normal range 0.74-1.54 mmol/L), and serum/plasma creatinine was calculated after using Jaffé's reaction (normal range 60-106 µmol/L). Serum/plasma glucose was measured using the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany) or hexokinase method (normal range 3.3-5.7 mmol/L). B-hemoglobin A1c (HbA1c) was evaluated with high-pressure liquid chromatography (Bio-Rad Laboratories, Hercules, CA, USA, normal range 3.9-5.3%). Plasma insulin was assayed by using chemiluminiscence (Bio-Rad Laboratories, Hercules, CA, USA, or Roche, Basel, Switzerland). Cystatin C and high sensitivity C-reactive protein (CRP) was measured with a turbidimetric method. Fibrinogen was measured with a clotting assay (Diagnostica Stago, Parsippany, NJ, USA). Plasminogen activator inhibitor I was measured with an immunochemical method and proinsulin with a solid base ELISA (Wallac, Perkin Elmer, Wellesley, MA, USA). 1,25-(OH)2 and 25-OH vitamin D3 were measured with a chemiluminiscence method. Fasting total serum/plasma cholesterol (normal range 2.6–7.1 mmol/L), HDL-cholesterol (normal range 0.8–1.9 mmol/L), triglycerides (normal range 0.23–1.70 mmol/L) and lipoprotein fractions were measured with an enzymatic method (I, II, III: Technicon Auto Analyzer II). LDL-cholesterol was calculated with Friedewald’s formula: [total cholesterol] – [HDL-cholesterol] – [total triglycerides x 0.45]. The atherogenic index was calculated as [total cholesterol – HDL-cholesterol]/[HDL-cholesterol]. The instruments used for biochemical analysis were Hitachi 717 or 911 (study IV Hitachi, Japan) or Architect (Abbott, Abbot Park, IL, USA) unless otherwise stated.
Anthropometry

Body mass index (BMI) was calculated as body weight/(body height)² (kg/m²). Standing height was measured to the nearest whole 0.5 cm with a Harpender Stadiometer (Holtain Ltd, Crymych, UK) and body weight to the nearest 0.1 kg. The height instrument was calibrated daily. The waist and hip circumferences were measured in standing position. The waist was measured midway between the lowest rib and the iliac crest and the hip over the widest part. The waist/hip ratio was calculated.

Office blood pressure

In study V, blood pressure was measured in the right arm with the subject in the supine position after a 10 minute rest. Systolic and diastolic blood pressures were defined as Korotkoff phases I and V, respectively.

Insulin Sensitivity and Secretion

In studies IV and V, the in vivo sensitivity to insulin was assessed with DeFronzo’s euglycemic hyperinsulinemic clamp technique (DeFronzo 1979), with a slight modification to suppress hepatic glycogen output. Insulin (Actrapid Human®, Novo, Copenhagen, Denmark) was infused in a primary dose for the first 10 minutes and then as a continuous infusion (56 mU/min per body surface area (m²)) for two hours. The rate of infused glucose during the last hour was used as a measure of insulin sensitivity (M-value). The insulin sensitivity index (M/I ratio) was calculated by dividing M by the mean insulin concentration during the same period of the clamp. M/I thus represents the amount of glucose metabolized per unit of plasma insulin and is given in mg/kg bw/min per mU/L of insulin multiplied by 100.

Glucose tolerance was assessed with the oral glucose tolerance test (OGTT), performed by measuring the concentrations of plasma glucose and insulin immediately before and 120 minutes after ingestion of 75 g of anhydrous dextrose. The OGTT and the clamp procedure were performed at least 1 week apart. Beta-cell function was calculated with early insulin response (EIR) at OGTT ([insulin 30 min] – [insulin 0 min]) / ([glucose 30 min] – [glucose 0 min]). Assessment of insulin sensitivity was performed at OGTT with the homeostasis model assessment (HOMA) ([fasting glucose] x [fasting insulin]/22.5) (Matthews 1985).

Dietary data and physical activity

In study IV, alcohol, coffee and tea consumption were recorded using a 7-day pre-coded food diary according to the instructions of a dietician. Daily intakes were calculated using computer software and the Swedish National...
Food Administration database (SLV Database, 1990). Participants also reported their leisure time physical activity on a standardized questionnaire.

Bone mineral density
In study II, dual energy x-ray absorptiometry, DXA (DPX-L, Lunar Co, Madison, WI, USA) was used to measure bone mineral density (BMD) in lumbar spine (L2-L4), left proximal femur and total body. Longitudinal precision of measurements on a spine phantom estimated a coefficient of variation of <1% during the study period.

Definitions
In studies I, III, IV and V, hypertriglyceridemia was defined as fasting serum values >2.3 mmol/L, marked hypercholesterolemia as >8.0 mmol/L and hypercholesterolemia as > 5.18 mmol/L (NIH Dyslipidemia Guidelines 1993, Arnett 2005). Diabetes mellitus was diagnosed as a fasting plasma glucose level ≥7.0 mmol/L, two hours glucose level ≥11.1 mmol/L at OGTT, or by the use of oral hypoglycemic agents or insulin. Impaired glucose tolerance (IGT) was defined as two hours value ≥7.8 to <11.1 mmol/L at OGTT. Impaired fasting glucose (IFG) was defined as fasting glucose ≥6.1 to <7.0 mmol/L (ADA Diabetes Mellitus Guidelines 2005). BMI >25 was regarded as overweight and BMI >30 as obesity (NIH Obesity Guidelines 1998). In study II, the T-score was calculated as the BMD difference in standard deviation from a young adult female reference population matched for weight. T-score ≤1 to – 2.5 SD was regarded as osteopenia and below –2.5 SD as osteoporosis (WHO Osteoporosis Guidelines 1994). Z-score was calculated as the difference in standard deviation from an age and weight matched female reference population.

Statistical analyses
Data were given as mean ± standard deviation (SD). Two-tailed 95% confidence intervals and p values were given, with \( P <0.05 \) regarded as significant. Statistical software package StatView 5.0 (SAS Institute, Cary, NC, USA) was used in study I. JMP 5.1 (SAS Institute, Cary, NC, USA) was used in studies II, III and V, and STATA 8.2 (Stata Corp., College Station, Texas, USA) was used in study IV. Shapiro-Wilk’s test was used to assess normality in continuous variables. Logarithmic transformation was performed to achieve normal distribution for skewed variable.

In study II, paired and unpaired T-test (normal distributed variables) were used. In studies I, III and V, Wilcoxon’s signed ranks test was used. In study I, the Mann-Whitney test (skewed continuous variables), and in study III the
chi square test (categorical variables) were used. In studies I, II and III, Spearman’s rank correlation was used.

In studies I and III, multiple linear regression models were used to assess the relationship between lipoprotein fractions or serum glucose (dependent variables) and the serum levels of calcium and PTH (continuous variables). The models were adjusted for BMI. In study IV, multiple linear regression models were used to assess the relationship between insulin sensitivity and secretion (dependent variables) and the serum levels of calcium (continuous variable). ANOVA and ANCOVA were used to assess the relationship between insulin sensitivity and secretion and the quartiles of serum calcium. The following models were used: A, unadjusted analyses. B, analyses adjusted for BMI, physical activity, smoking, consumption of tea, alcohol and coffee. C, as model B but also adjusted for phosphate and creatinine. All models evaluating the relationship between EIR and serum calcium were also adjusted for M/I, as previous studies have shown that EIR does not seem to provide clinically relevant information except when adjusted for insulin sensitivity (Haffner 1996a, Haffner 1996b). In order to maximize the statistical power, only participants with missing covariates needed for that particular model were excluded from the analyses. In exploratory analyses the association of non-corrected serum calcium to M/I in order to exclude the possibility that the association between albumin corrected calcium and M/I was driven by a relation between albumin and M/I were also analyzed.
Results studies I-V

Study I: Mild pHPT is associated with increased levels of triglyceride rich lipoproteins and decreased HDL-cholesterol. Parathyroidectomy has beneficial effects.

Baseline
A total of 87 cases with mild pHPT were enrolled in this study together with controls. Cases had higher levels of serum calcium and a disproportionately elevated PTH level. At baseline the cases had increased levels of total triglycerides, VLDL-triglycerides, VLDL-cholesterol and atherogenic index, and a lower HDL-cholesterol level ($P=0.001-0.05$, Figure 6). Hypertriglyceridemia was more prevalent in the cases than in the controls (20 cases vs. 6 controls, $P=0.0001$), but hypercholesterolemia was not (16 cases vs. 10 controls, not significant). Furthermore, there was an inverse correlation (of unknown importance) at baseline between serum PTH and the level of total cholesterol, VLDL-cholesterol and total triglycerides.

Five-year examination
At the five year follow-up, 11 of the parathyroidectomized cases had received additional HRT and 38 cases had not, but were treated as one group (n=49) since stratification for HRT treatment did not demonstrate any confounding effect on calcium homeostasis or lipoprotein fractions. Serum levels of calcium and PTH had normalized. All lipoprotein variables had returned to the levels of controls. Further, parathyroidectomized cases also had lower total triglycerides than the cases that were followed conservatively ($P<0.05$).

In the conservatively treated cases, ionized plasma calcium and PTH values remained higher than in controls. In addition, total triglycerides and atherogenic index were higher, and the HDL-cholesterol lower, than in matched controls. Over time, the conservatively treated cases improved their
HDL- and LDL-cholesterol levels as well as atherogenic index. The 62 controls followed for five years decreased in ionized, but not in total serum calcium, increased in serum PTH, total triglycerides and HDL-cholesterol, decreased in LDL-cholesterol and the atherogenic index.

**Figure 6.** Baseline differences in some lipoprotein fractions in pHPT cases (n=87) and controls (n=87). * P<0.05, ** P<0.01
Study II: Patients with mild pHPT have increased frequency of osteoporosis and lower levels of BMD in both trabecular and cortical bone. Parathyroidectomy has beneficial effects.

Baseline
At baseline, the 87 cases had lower BMD and T-scores in the lumbar spine, femoral neck and total body than their matched controls, with differences between −5.3 and −5.9% (P=0.0006-0.03, Figure 7). T-scores for the cases were lower than −1.0 SD in all investigated areas. The proportion of individuals with T-scores lower than −2.5 SD, i.e. osteoporosis, in the lumbar spine and femoral neck were higher in cases than in controls. However, this was only statistically different in the femoral neck (P<0.05). Osteoporosis was present in 45% of cases and 35% of controls. In multiple regression analysis, the differences between cases and controls persisted also when BMI was included as a confounder. Cases with total serum calcium above the upper reference limit (>2.60 mmol/L; n=21) had lower BMD and T-score in the lumbar spine and total body than cases with serum calcium in the normal range (n=58; P=0.0004-0.01). A corresponding difference in BMD for serum PTH above and below the upper reference limit (>55 ng/L) could not be identified. There were no associations between age and BMD in any of the investigated areas.

Follow-up
At the 5-year follow-up, parathyroidectomized cases (n=49) had no biochemical signs of pHPT and controls (n=68) had slightly decreased ionized calcium and increased PTH values. Cases had increased BMD by 2.9% (P=0.002) in the lumbar spine. Femoral neck BMD remained at the same level and total body BMD had decreased by 1.8% (P=0.02). Parathyroidectomized cases decreased 0.2 cm in height over time (ns). At follow-up there were no longer any differences in BMD or T-score between parathyroidectomized cases and their matched controls (Figure 8). The controls had lost 0.6 cm in height, and had decreased in BMD in L2-L4, neck and total body by 0.6-3.6% (P<0.0001-0.05). The change in BMD in parathyroidectomized cases was positive and differed significantly from the negative change seen in the controls, both in L2-L4 (P=0.02) and the femoral neck (P=0.04, Figure 9).
Figure 7. Baseline differences in BMD at lumbar spine (L2-L4), femoral neck and total body in patients (n=87) and controls (n=87). * $P<0.05$, ** $P<0.01$

Figure 8. T-score in parathyroidectomized cases (n=49) and controls (n=68) at follow up for lumbar spine (L2-L4), femoral neck and total body. ns= not significant
In parathyroidectomized cases, age correlated with change in BMD over time in the femoral neck ($r=-0.326, P=0.025$), but not in L2-L4 or total body. BMD increase was more pronounced in parathyroidectomized cases below 67 years of age at study entry (mean and median age parathyroidectomized cases 66 years, n=48; 25 cases <67 years, 23 cases ≥67 years), comprising 4.1% ($P=0.007$) in L2-L4 and 2.5% ($P=0.013$) in the femoral neck, compared to base line, reaching the same level as the controls. The corresponding difference was not seen in total body BMD. Cases ≥67 years of age did not change postoperatively in L2-L4 or in the femoral neck, but increased in total body by 2.0% ($P=0.013$). There were no differences in BMD change in controls stratified for age.

Figure 9. Difference in change of BMD during the study period at lumbar spine (L2-L4) and femoral neck of parathyroidectomized (PTx) cases and controls. * $P<0.05$
Study III: Patients with normocalcemic pHPT express changes in lipoprotein and glucose metabolism.

Baseline
Thirty individuals were diagnosed with normocalcemic pHPT based on the dysregulation between calcium and PTH values. The cases had higher serum calcium and PTH values compared with matched controls (n=30). The cases also had higher glucose, BMI, urate, VLDL-cholesterol, LDL/HDL-cholesterol ratio, total and VLDL-triglycerides, and lower HDL-cholesterol than the controls ($P$=<0.0001-0.035, Figure 10). Ten cases had hypertriglyceridemia (triglycerides >2.3 mmol/L) compared to one control ($P$<0.001). Four cases had a marked hypercholesterolemia (>8.1 mmol/L) versus two controls (ns). In multiple regression analysis including the metabolic variables, the differences between cases and controls persisted when excluding confounding from BMI and age.

**Figure 10.** Biochemical variables at baseline in cases with normocalcemic pHPT (n=30) and controls (n=30). *$P$<0.05**  **$P$<0.01**
Follow-up of parathyroidectomized cases

Follow-up examinations were performed on 15 parathyroidectomized cases and 15 controls five years after inclusion in the study. The parathyroidectomized cases had no biochemical signs of pHPT. Of the other variables, none of the previously noted differences between cases and controls were present at this investigation, except for a lower HDL-cholesterol level and a higher serum urate level ($P=0.026-0.0499$). Over time, the parathyroidectomized cases improved their total and LDL-cholesterol and the LDL/HDL-cholesterol ratio ($P=0.008-0.02$). Serum urate and glucose increased over time ($P<0.05$). The increment in glucose was mainly due to one case with normal values at inclusion, but who developed diabetic glucose values during the time to follow-up.

Follow-up of conservatively observed cases

The nine cases followed conservatively slightly increased their levels of serum calcium and PTH over time (ns). These variables were significantly higher in the conservatively treated cases compared with parathyroidectomized cases ($P=<0.0001-0.028$). The other investigated metabolic variables did not change over time, except LDL-cholesterol, which decreased ($P=0.043$). When comparing conservatively observed cases with controls, total triglycerides, HbA1c and BMI were increased ($P=0.025-0.028$), as were several other of the investigated variables. However, these were not statistically significant.
Study IV: Increasing levels of serum calcium in the general population are associated with decreasing insulin sensitivity.

Baseline characteristics of the individuals are presented in Table 3. In the whole cohort (n=1,117), four subjects had hypercalcemia (>2.60 mmol/L), and 63 subjects had hypocalcemia (<2.20 mmol/L). Median M/I in the hypercalcemic subjects was 4.50 ± 1.4 100 x mg/kg body weight/min/mU/L and median M/I in hypocalcemic subjects was 4.73 ± 2.4 100 x mg/kg body weight/min/mU/L.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71.0 ± 0.60</td>
</tr>
<tr>
<td>Albumin-corrected serum calcium (mmol/L)</td>
<td>2.33 ± 0.09</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>26.3 ± 3.4</td>
</tr>
<tr>
<td>Fasting serum glucose (mmol/L)</td>
<td>5.78 ± 1.5</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/L)</td>
<td>12.9 ± 8.3</td>
</tr>
<tr>
<td>M/I (100 x mg/kg body weight/min/mU/L)</td>
<td>5.78 ± 1.5</td>
</tr>
<tr>
<td>Early insulin response</td>
<td>13.7 ± 11</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>93.7 ± 15</td>
</tr>
</tbody>
</table>

Table 3. Baseline characteristics of 1,117 men, 70 years old.

Insulin sensitivity

In the linear regression models A-C, one standard deviation increase of serum calcium was associated with 0.15-0.22 units decrease in M/I in the whole sample (P=0.001-0.047). This association remained essentially the same in the sub-sample with normal glucose (0.21-0.23 units decrease in M/I per SD increase in calcium, P=0.01-0.028) and became even stronger in the sub-sample with serum calcium within the normal range (0.35-0.53 units decrease in M/I per SD increase in calcium, P<0.001-0.01, Figure 11).

In the analyses of quartiles of serum calcium in the whole cohort, the subjects in the lowest quartile of calcium had significantly higher insulin sensitivity as compared to quartile 4 in all models (beta -0.58 to -0.69, P<0.001-0.006). This relationship became stronger in the sub-sample with normal glucose and glucose tolerance, as well as the sub-sample with normal glucose, glucose tolerance and normal calcium (beta -0.74 to -1.2, P<0.001-0.0012). In the latter sub-sample, insulin sensitivity in model B, quartiles 2, 3 and 4 and in model C, quartiles 3 and 4, were lower than in quartile 1. Mean M/I in the quartiles of serum calcium are shown in Figure 12.
The results remained essentially the same when using non-albumin corrected serum calcium in the linear regression models and analyses of quartiles of serum calcium (data not shown).

Figure 11. Schematic graph of M/I change in relation with increase in calcium level. The line for the whole sample and the subsample with normal glucose metabolism are identical.

Insulin secretion

There were no associations between EIR and serum calcium in any linear regression models or in the quartile analyses, either in the whole study sample or in the subgroups (not shown). Mean EIR in the quartiles of serum calcium are shown in Figure 12.
Figure 12. Insulin sensitivity and early insulin response in different serum calcium quartiles.
Study V: Patients with pHPT have impaired glucose metabolism and other abnormalities associated with the metabolic syndrome.

The cases had mild to moderate pHPT with mean plasma calcium of 2.73 (± 0.1) mmol/L and a mean plasma PTH of 12.6 (± 6) pmol/L.

At baseline investigation, half of the cases (10/21) fulfilled the criteria for the metabolic syndrome (NCEP Guidelines 2001). Six cases were insulin resistant. Four of these also had diabetic fasting glucose and IGT. Two of the insulin resistant cases had normal glucose tolerance and fasting glucose. Furthermore, 5 additional cases had IGT (one with diabetic values) and/or IFG. NIDDM was diagnosed in two of these five cases prior to the study. Glucose metabolism was disturbed in 11 of the 21 cases. Several of the investigated parameters in the ATP III criteria for the metabolic syndrome were abnormal; IGT, increased waist circumference and BMI, urine albumin excretion and systolic blood pressure. Fasting glucose and diastolic blood pressure were borderline elevated. Furthermore, PAI-1 and high sensitive CRP were elevated.

Comparison with control populations revealed a higher rate of metabolic syndrome in the pHPT patients. Six controls (4 women), matched for age and BMI, underwent the same investigations as the cases (control group 1). Another 18 (2 men) healthy individuals (age 49.8 ± 6 years) were characterized for parameters of the metabolic syndrome to allow comparison (control group 2). One of the 6 controls in group 1 fulfilled the criteria for the metabolic syndrome, while all others (group 1 and 2) had normal glucose metabolism. No control had pHPT. Compared with control group 1, the cases had numerically, but not significantly reduced, insulin sensitivity and HDL-cholesterol, elevated fasting glucose and glucose at OGTT, triglycerides, systolic blood pressure, PAI-1 and high sensitive CRP. Compared with control group 2 the cases had significantly elevated fasting glucose and insulin, triglycerides, systolic and diastolic blood pressures, waist circumference and a lower HDL-cholesterol and insulin sensitivity (HOMA) (P<0.001-0.05), while BMI was similar. In addition, as further comparison data from 1,117 euglycemic hyperinsulinemic clamps obtained in studies of the ULSAM cohort at the age of 70 was used. In these individuals M/I was higher (5.78 ± 1.5) than in the pHPT cases. The other variables of the metabolic syndrome were more beneficial in the ULSAM cohort than in the pHPT cohort (not shown).
Discussion

In this thesis three different populations have been used to characterize certain metabolic derangements in pHPT. Results from two cohorts of patients with pHPT and one general population have clarified that 1, pHPT is associated with metabolic alterations with increased risk of NIDDM and cardiovascular diseases, along with BMD loss in trabecular as well as cortical bone, 2, pHPT patients seem to benefit from surgical intervention regarding the investigated variables, and 3, increasing calcium levels which lie within the normal range in the general population are also associated with decreasing insulin sensitivity.

Lipoprotein metabolism in primary hyperparathyroidism

It is demonstrated that the investigated patients with pHPT have unfavorable alterations in the lipoprotein fractions, consistent with an increased risk of cardiovascular complications, as compared with controls (studies I, III and IV). Moreover, this is also present in cases with mild and even normocalcemic pHPT, indicating that dyslipidemia may be an early manifestation in the course of the disease. The changes included decreased HDL-cholesterol level, increased total and VLDL-triglycerides, VLDL-cholesterol and LDL/HDL-cholesterol ratio. The alterations were not due to different ages or BMI between cases and controls. The results corroborate some previous studies of pHPT (Ljunghall 1978, Lacour 1986), while others have either found a different pattern of dyslipidemia or have been unable to substantiate any influence of pHPT in this respect (Christensson 1977, Vaziri 1983). The extent of the changes in lipoproteins coincided with conventional indications for treatment of dyslipidemia (NIH Dyslipidemia Guidelines 1993, NCEP Guidelines 2001), but were not regarded as an issue to be taken into account in the guidelines on pHPT treatment (NIH pHPT Guidelines 1991, Bilezikian 2002a). An increased morbidity and mortality from cardiovascular diseases is reported in pHPT cases. It is not far fetched to suggest that a part of this excess may be due to an altered lipoprotein profile. In fact, increased levels of total triglycerides and low levels of HDL-cholesterol per se are strong risk factors for cardiovascular diseases (Stampfer 1996, Krauss 1998, NCEP Guidelines 2001, Baigent 2005), and improvement has a proven, beneficial effect on survival (NCEP Guidelines 2001, Baigent 2005). The mechanistic relationship between pHPT and the disturbances in the
lipoproteins is unknown. One possible mechanism is that elevated calcium and PTH levels amplify the activity of lipolysis in adipose tissue, elevating secretion of triglycerides to the circulation with a secondary increase of other lipoprotein fractions (Ljunghall 1978). This has been corroborated by experimental studies demonstrating stimulative effect by PTH on lipolysis in human adipocytes resulting in increased triglyceride secretion (Sinha 1976, Taniguchi 1987). In secondary HPT due to uremia, increased levels of triglycerides and depressed HDL-cholesterol have been reported (Lacour 1986). Furthermore, hyperinsulinemia may be responsible for a large proportion of the dyslipidemia seen in conditions with elevated insulin levels such as NIDDM or the metabolic syndrome, but possibly also in pHPT (see below). The pattern in the hyperinsulinemic states is similar to what was noted in the patients in the present studies with raised triglyceride-rich lipoproteins and decreased HDL-cholesterol levels (WHO Diabetes Mellitus Guidelines 1999, NCEP Guidelines 2001). Altered levels of intracellular calcium, e.g. in adipocytes, platelets and leukocytes, have also been hypothesized to be involved in lipoprotein disturbances (Resnick 1999, McCarty 2006). Moreover, as noted in some of the investigated patients and reported from the literature, there is an association between pHPT and increased BMI (Bolland 2005). Elevated BMI is closely related to altered lipoprotein metabolism. The relationship between dyslipidemia and pHPT in the present studies was substantiated by the improving effect of parathyroidectomy. Moreover, disturbance of the lipid variables in the conservatively observed cases persisted.

Glucose metabolism in primary hyperparathyroidism

In this thesis, glucose metabolism has been investigated in cases with normocalcemic to overt pHPT. The normocalcemic pHPT cases had a raised glucose level compared with the controls (study III), as did the entire cohort of cases with mild pHPT (Lundgren 1998a). Furthermore, in the consecutive pHPT cases (study V), glucose metabolism was abnormal. In this study, insulin sensitivity was investigated with the gold standard method, the euglycemic hyperinsulinemic clamp, a method used in only one prior study which included few cases with pHPT (Prager 1990). Other previous studies have all used indirect methods of assessing the insulin sensitivity such as HOMA or the fasting insulin level. However, both these methods are confounded by insulin secretion. The present results are consistent with a substantial number of previous studies demonstrating an increased frequency of IFG, IGT and decreased insulin sensitivity in pHPT (Kim 1971, Prager 1983, Prager 1984, Prager 1990, Kautzky-Willer 1992, Kumar 1994, Procopio 2002). Supporting the results from the present studies are a reported comorbidity between NIDDM and pHPT, with a 7-8% prevalence of NIDDM in pHPT, two to four-fold the prevalence in the general population (Ljunghall 1983, Taylor 1991, Taylor 1997, Valdemarsson 1998a, Procopio
The results from the current study are in part corroborated by other studies associating serum calcium with glucose tolerance and insulin secretion (Wareham 1997, Sun 2005), by the study of calcium and insulin sensitivity in the general population (study IV) and by reports of decreasing insulin sensitivity with increasing levels of PTH (Chiu 2000). Several other investigators have reported reversal of diabetes or improved metabolic control, and improvements in glucose tolerance and insulin sensitivity after parathyroidectomy (Birge 1969, Werner 1974, Walsh 1975, Akgun 1978, Prager 1983, Cheung 1986, Prager 1990, Kautzky-Willer 1992, Richards 1999) but not all (Ljunghall 1983, Bannon 1988).

Insulin sensitivity and calcium in general population

In the community-based cohort of elderly men (ULSAM), increasing levels of serum calcium were associated with decreasing insulin sensitivity as measured by euglycemic hyperinsulinemic clamp, independent of lifestyle or dietary factors, serum phosphate or serum creatinine (study IV). The results remained consistent in individuals with normal fasting glucose and glucose tolerance and became stronger in individuals with serum calcium within the normal range. There was no relationship between serum calcium and insulin secretion, as measured by the early insulin response at an OGTT. These results in part substantiate the findings of affected glucose metabolism from the pHPT cohorts.

Three previous large scale studies have investigated the relationship between serum calcium and insulin sensitivity and secretion (Lind 1997, Wareham 1997, Sun 2005). In these studies, increasing serum levels of calcium were associated with decreased insulin sensitivity as assessed by fasting insulin or HOMA. In one prior study (Lind 1997), insulin secretion and calcium were not associated but the relationship were found to be present in women in another study (Sun 2005). The association between insulin sensitivity and calcium in the healthy subgroup with normocalcemia, normal fasting glucose and glucose tolerance suggests that calcium may be involved early in the development of diabetes mellitus and that the association is not driven by a few individuals with supra- or sub-normal levels of calcium due to other disorders such as pHPT, secondary HPT or malignancies. The noted association may indicate a causal role for calcium in the regulation of insulin sensitivity. However, the association may be confounded by other causal factors related to the serum levels of calcium and insulin sensitivity such as BMI, physical activity or dietary factors. However, serum calcium was consistently related to insulin sensitivity independently of these confounders in all sub-samples, arguing against confounding as a sole explanation of the findings.

It is not clear why the relationship between serum calcium and insulin sensitivity is stronger in cases with normal serum calcium levels. The mean
M/I seems to be lower in both hypo- and hypercalcemic individuals, possibly indicating a U-shaped relationship. As there were only four hypercalcemic individuals, the exclusion of these subjects is unlikely to cause the increased strength in the association between calcium and insulin sensitivity. A possible mechanistic explanation may be that increased levels of PTH are likely to be found in a majority of subjects with hypocalcemia. PTH may influence intracellular calcium levels and thereby reduce insulin sensitivity (Baldi 1996, Resnick 1999).

The metabolic syndrome

Although many prior studies of pHPT have reported components of the metabolic syndrome being present in pHPT, no previous study has investigated all the parameters at once. In the study of consecutive cases with mild to moderate pHPT accepted for parathyroidectomy (study V), 48% fulfilled the criteria for having metabolic syndrome according to ATP III (NCEP Guidelines 2001). As a group, the cases had elevated waist circumference, increased systolic and borderline high diastolic blood pressure as well as a raised urine albumin. In addition, the cases had increased levels of markers of inflammation and impaired coagulation, PAI-1 and high sensitive CRP. Technical reasons delaying the investigation in controls resulted in only six control individuals undergoing insulin clamp and OGTT. The controls had a mean age and BMI similar to the investigated pHPT cases. One individual fulfilled the criteria for metabolic syndrome. This individual also had IGT, but with normal fasting glucose. The rest of the controls had normal glucose metabolism. Since normal populations investigated with clamp are rare, insulin sensitivity was also compared with 1,117 men of age 70 from the UL-SAM cohort (study IV). In these individuals M/I was higher than in the pHPT cases. In the investigated patients, secondary HPT or vitamin D malabsorption seemed an unlikely explanation for the alterations in mineral metabolism or other metabolic variables since glomerular filtration rate and creatinine were normal and the vitamin D metabolites did not indicate hypovitaminosis.

Experimental and clinical data suggest that intra-cellular calcium disturbances may be the linking factor between the wide range of abnormalities of the metabolic syndrome, such as hypertension, insulin resistance and obesity (Reusch 1991, Byyny 1992, Barbagallo 1993, Baldi 1996, Barbagallo 1999, Levy 1999, Resnick 1999). In pHPT, lower insulin sensitivity due to a reduced number of glucose transporters (GLUT-4), decreased insulin receptor binding capacity or a lower number of insulin receptors have been reported (Prager 1984, Taylor 2001). This may be mediated by elevated levels of PTH increasing calcium influx through calcium channels, hence raising intracellular calcium levels with secondary effects on insulin sensitivity (Borle 1978, Hvarfner 1988, Fardella 1995, Schiff 1997).
Beside the discussed derangement in glucose metabolism, several studies of pHPT patients have reported the presence of classical cardiovascular risk factors included in the metabolic syndrome: hypertension (Lind 1991), elevated body weight (Bolland 2005), dyslipidemia with increased levels of triglycerides and decreased HDL-cholesterol (Smith 2000) and also associated components such as hyperuricemia and increased inflammatory parameters (Øgard 2005).

The presence of multiple cardiovascular risk factors as well as the metabolic syndrome in 48% of the patients with pHPT described in the present study may constitute a possible explanation for the increased morbidity and mortality from cardiovascular diseases in pHPT (Hedbäck 1998). This is supported by the data on adverse metabolic variables in patients with pHPT and by the associations between serum calcium and insulin sensitivity (studies I, III and IV).

Components of the metabolic syndrome found in this thesis are presented in table 4.

<table>
<thead>
<tr>
<th>Component</th>
<th>Thesis results</th>
<th>pHPT</th>
<th>Metabolic syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased mortality from CVD</td>
<td>-</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Hyperinsulinemia</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Glucose intolerance</td>
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<td>√</td>
<td>√</td>
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<tr>
<td>Impaired fasting glucose</td>
<td>√</td>
<td>√</td>
<td>√</td>
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<tr>
<td>Increased risk of NIDDM</td>
<td>√</td>
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<td>√</td>
</tr>
<tr>
<td>Elevated triglyceride levels</td>
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<tr>
<td>Decreased HDL-cholesterol levels</td>
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</tr>
<tr>
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<tr>
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**Table 4.** Comparison between the literature and present studies on components of the metabolic syndrome and pHPT.
Bone
In study III, decreased BMD in the lumbar spine, femoral neck and total body in untreated mild and normocalcemic pHPT is described. This is consistent with findings in other series with much higher serum calcium (Guo 1996, Silverberg 1996, Christiansen 1999). The results also substantiate the theory that pHPT affects the skeleton adversely despite the fact that serum calcium is within the normal reference range or only slightly raised together with inappropriately serum PTH levels. The gain in BMD after parathyroidectomy was limited to the lumbar spine in the total cohort, whereas other studies have also documented recovery in the femoral neck (Christiansen 1999, Silverberg 1999b, Nomura 2004, Rao 2004). If the material was dichotomized by age, BMD also increased in the femoral neck reaching the same levels as the controls in the younger half of the cohort. While the parathyroidectomized cases lost only 0.2 cm in height, the controls lost 0.6 cm. This finding supports the positive effects of parathyroidectomy on lumbar spine BMD.

More explicitly, these results substantiate the causal coupling between bone abnormalities and mild parathyroid disorder and the suggestion that the difference between cases and controls should not be considered as fortuitous. The findings corroborate previous reports on the existence of bone loss early and in very mild stages of pHPT (Rao 1988, Christiansen 1997) and underline the potential gain in both cortical and trabecular bone from active treatment in mild pHPT in post-menopausal females too.

Clinical Implications
If there is a causal relationship between pHPT and this wide range of metabolic changes, it is possible that cases with pHPT would benefit from early intervention, although according to pHPT treatment guidelines, surgery may not be indicated. Since indications that non-classical metabolic alterations appear early in the disease, also in normocalcemic pHPT, surgical intervention may also be beneficial at this stage. Furthermore, both lipoprotein fractions and glucose metabolism should be investigated in pHPT, since without surgical treatment regression of the non-classical manifestations seems unlikely.

A potential approach to medical treatment is calcimimetics. This may have beneficial effects on calcium and PTH and possibly on secondary effects in pHPT. However, these have hitherto been used mostly in secondary HPT and only initial reports on pHPT are available (Shoback 2003, Peacock 2005).
Limitations

In studies I, II and III, a contribution of secondary hyperparathyroidism to the noted disturbances in calcium homeostasis could be argued, since the criteria for diagnosis in the cases extended into the normocalcemic range; 1,25-(OH)₂ and 25-OH vitamin D₃ were not measured and no parameters of kidney function other than serum creatinine were documented. However, secondary HPT seems unlikely due to the cut-off for serum creatinine, that a single parathyroid adenoma was found at surgery in a proportion similar to most series of pHPT (Lundgren 1996) and that the relation and levels of calcium and PTH were normalized after parathyroidectomy. Furthermore, an important limitation was the inability to randomize the treatments of pHPT caused by the relatively frequent contraindications to parathyroidectomy. In many of the cases, this was due to the mild extent of pHPT and the exploratory nature of the study. In study II, BMD assessment was not performed at the distal one third of the radius, a site recommended for investigation in the latest treatment guidelines for pHPT (Bilezikian 2002a). In study IV, limitations included the limited applicability to women, other age and ethnic groups, the lack of measurement of PTH, vitamin D and the cross-sectional study setting. In study V, limitations included the recruitment of cases consisting of consecutive patients and the lack of a sufficient number of matched controls.
General summary

Patients with mild and normocalcemic pHPT had a non-beneficial lipoprotein pattern, characterized by increased levels of triglyceride-rich lipoproteins and decreased HDL-cholesterol levels, as well as an elevated fasting glucose.

Cases with mild and normocalcemic pHPT had decreased BMD in total body indicating loss of predominantly cortical bone and also in the lumbar spine and the femoral neck representing a reduction of trabecular bone.

Parathyroidectomy improved the dyslipidemia and the decreased BMD in both normo- as well as hypercalcemic patients with pHPT.

Serum levels of calcium were negatively associated with M/I in the general population and stronger in the healthy sub-sample of individuals with both normocalcemia and normal glucose metabolism.

Patients with pHPT had several non-beneficial levels of variables associated with the metabolic syndrome. Almost half of the cases fulfilled the criteria for the metabolic syndrome and half had impaired glucose metabolism.
Bisköldkörtlarna, fyra till antalet och vardera av ett risgryns storlek, är belägna på halsen. De reglerar kalknivån i kroppen med hjälp av hormonet parathormon. En normal kalknivå är nödvändig för att upprätthålla kroppens alla funktioner, t.ex. muskelaktivitet, hjärtslag och nervimpulser. Avhandlingen handlar om primär hyperparathyreoidism (pHPT), som är en sjuklig överfunktion i en eller flera av körtlarna med för hög hormoninsöndring till blodbanan. Sjukdomen är vanligt förekommande i befolkningen (ca 1%), ökar med stigande ålder och är vanligast hos kvinnor efter klimakteriet (ca 3%). Klassiska följder av pHPT, såsom njurstenar och frakturer p.g.a. benkärl, blir allt mer sällsynta i och med en ökad diagnostisk säkerhet och tidig behandling. Sjukdomen botas med borttagande av den sjuka körteln vid operation. Det har på senare tid uppmärksammats ett antal "icke klassiska" yttringar vid pHPT, en kraftigt ökad risk för död i hjärtärs sjukdomar och störningar i ämnesomsättningen av bl.a. blodsocker och blodfetter.

Riskfaktorer för hjärtärs sjukdomar och avvikelser i ämnesomsättningen undersöks i avhandlingens delarbeten. Hypotesen är att pHPT leder till förändringar i ämnesomsättningen som liknar det metabola syndromet (MeS), vilket ökar risk för hjärtärs sjukdomar. MeS karaktäriseras av diabetes eller av förhöjda blodsockernivåer, påverkade blodfetter med låga nivåer av det goda kolesterol och höga nivåer av fetten triglycerider. Vidare ingår bukfetma med manlig fettdistribution (till skillnad från den mer gynnsamma kvinnliga fetman) och högt blodtryck i syndromet. Flera av avvikelserna i MeS kan bero på försämrad insulinläckighet, samt nedsatt förmåga att tillgodogöra sig insulin och förbränna socker. Ett ökat insjuknande i benskörhet vid pHPT, även vid relativt mild sjukdom, har också uppmärksammat. Wilken del av skelettet som påverkas mest och effekten av operativ behandling är ännu inte fullständigt klarlagt.

Avhandlingen påvisar att patienter med mild pHPT har förändringar som vid MeS med bl.a. avvikande blodfetter, ökade sockernivåer och sänkt insulinläckighet. Efter operativ bot av en grupp patienter minskar en del riskfaktorer; detta till skillnad från icke opererade patienter, som fortsätter att ha ögynnsamma värden. Ökande kalkvärden inom normala gränser är kopplade till minskande insulinläckighet i en manlig population från normalbefolkningen. Det innebär att ett högre kalkvärde är sämre än ett lägre. Det stäm-
mer också med resultaten från patienter med pHPT och förhöjda kalkvärden. Dessutom klargörs att minskad bentäthet i rygg och höft kvarstår om patienter med mild pHPT inte opereras.

Sammanfattningsvis visar avhandlingen att patienter med pHPT har ökade riskfaktorer för hjärtkärlsjukdom, sockersjuka och benskörhet, samt att framgångsrik kirurgi har gynnsamma effekter på riskerna. Resultaten stämmer med och stärks av att även normala kalknivåer är associerade till insulinkänslighet i normalbefolkning.
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The cover illustration is a $^{99m}$Tc-sestamibi SPECT-CT-image fusion (single photon emission computed tomography and a standard computed tomography) visualizing a single parathyroid adenoma on the neck. The heart, also loading with tracer, is visualized to right. Image reproduced with kind permission from dr Garske, Department of Nuclear medicine, University Hospital, Uppsala, Sweden.
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