Parathyroid hormone as an outcome indicator in old age

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ACADEMIC DISSERTATION

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Helsinki 2010
“Stillness, insight, and wisdom arise only when we can settle into being complete in this moment, without having to seek or hold on to or reject anything.”

- Jon Kabat-Zinn
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Abstract

Serum parathyroid hormone (PTH) and vitamin D are the major regulators of extracellular calcium homeostasis. The inverse association between PTH and vitamin D and the common age-related elevation of the PTH concentration are well-known phenomena. However, the confounding or modifying factors of this relationship and their impact on the response of PTH levels to vitamin D supplementation need further investigation.

Clinical conditions such as primary hyperparathyroidism (PHPT), renal failure and vitamin D deficiency, characterized by an elevation of the PTH concentration, have been associated with impaired long-term health outcomes. Curative treatments for these conditions have also been shown to decrease PTH concentration and attenuate some of the adverse health effects. In PHPT it has also been commonly held that hypercalcaemia, the other hallmark of the disease, is the key mediator of the adverse health outcomes. In chronic kidney disease the systemic vascular disease has been proposed to have the most important impact on general health. Some evidence also indicates that vitamin D may have significant extraskeletal actions.

However, the frank elevation of PTH concentration seen in advanced PHPT and in end-stage renal failure have also been suggested to be at least partly causally related to an increased risk of death as well as cognitive dysfunction. However, the exact mechanisms have remained unclear. Furthermore, the predictive value of elevated PTH in unselected older populations has been less well studied.

The studies presented in this thesis investigated the impact of age and mobility on the responses of PTH levels to vitamin D deficiency and supplementation. Furthermore, the predictive value of PTH for long-term survival and cognitive decline was addressed in an unselected population of older people. The hypothesis was that age and chronic immobility are related to a persistently blunted elevation of PTH concentration, even in the presence of chronic vitamin D deficiency, and to attenuated responses of PTH to vitamin D supplementation. It was also further hypothesized that a slightly elevated or even high-normal PTH concentration is an independent indicator of an increased risk of death and cognitive decline in the general aged population.

The data of this thesis are based on three samples: a meta-analysis of published vitamin D supplementation trials, a randomized placebo controlled six-month vitamin D supplementation trial, and a longitudinal prospective cohort study on a general aged population. Based on a PubMed search, a meta-analysis of 52 clinical trials with 6290 adult participants was performed to evaluate the impact of age and immobility on the responses of PTH to 25-OHD levels and vitamin D supplementation. A total of 218 chronically immobile, very old inpatients were also enrolled into a vitamin D supplementation trial. Mortality data for these patients was also collected after a two-year follow-up. Finally, data from the Helsinki Aging Study, which followed three random age cohorts (75, 80 and 85 years) until death in almost all subjects, was used to evaluate the predictive value of PTH for long-term survival and cognitive decline.

This series of studies demonstrated that in older people without overt renal failure or severe hypercalcaemia, serum 25-OHD and PTH were closely associated, but this relationship was also affected by age and immobility. Furthermore, a substantial
proportion of old chronically bedridden patients did not respond to vitamin D deficiency by elevating PTH, and the effect of a high-dose (1200 IU/d) six-month cholecalciferol supplementation on the PTH concentration was minor. This study demonstrated longitudinally for the first time that the blunted PTH also persisted over time. Even a subtle elevation of PTH to high-normal levels predicted impaired long-term health outcomes. Slightly elevated PTH concentrations indicated an increased risk of clinically significant cognitive decline and death during the last years of life in a general aged population. This association was also independent of serum ionized calcium (Ca\textsuperscript{2+}) and the estimated glomerular filtration rate (GFR). A slightly elevated PTH also indicated impaired two-year survival during the terminal years of frail elderly subjects independently of Ca\textsuperscript{2+}, GFR, and of 25-OHD levels.

The interplay between PTH and vitamin D in the regulation of calcium homeostasis is more complex than has been generally considered. In addition to musculoskeletal health parathyroid hormone is also related to the maintenance of other important domains of health in old age. Higher PTH concentrations, even within conventional laboratory reference ranges, seem to be an independent indicator of an increased risk of all-cause and of cardiovascular mortality, independently of established cardiovascular risk factors, disturbances in mineral metabolism, and renal failure. Limited and inconsistent evidence supports the role of vitamin D deficiency-related lack of neuroprotective effects over the causal association between PTH and impaired cognitive functions. However, the causality of these associations remains unclear. The clinical implications of the observed relationships remain to be elucidated by future studies interfering with PTH concentrations, especially by long-term interventions to reduce PTH.
Tiivistelmä

Parathormoni (PTH) eli lisäkilpirauhashormoni ja D-vitamiini ovat keskeisiä solun ulkoisen kalsiumtasapainon säätelijöitä. PTH:n ja D-vitamiinin välinen käänteinen yhteys sekä ikääntymiseen liittyvä PTH-pitoisuuksien nousu ovat hyvin tunnettuja ilmiöitä. PTH:n ja D-vitamiinin välistä yhteyttä sekoittavia ja muokkaavia tekijöitä on systemaattisesti tutkittu kuitenkin varsin vähän, eikä näiden tekijöiden merkitystä D-vitamiinikorvaushoidon PTH-vasteisiin tunneta kunnolla.


Tämän väitöskirjan osatutkimukset tarkastelivat iän ja liikuntakyvyn vaikutusta ikääntyneiden PTH-vasteeseen sekä D-vitamiinin puutteen ja D-vitamiinikorvaushoidon aikana. Myös PTH:n ennustevaikutus lisääntyneen kuolleisuuden ja kognition heikentymisen riskille selvitettiin valikoimattomassa ikääntyneiden ihmisten aineistossa. Tutkimusten hypoteeseina olivat: 1.) Ikääntyminen ja Krooninen liikuntakyvyttömyys ovat yhteydessä PTH-pitoisuuden nousun pysyvään vaimentumiseen D-vitamiinin puutteessa. 2.) Ikääntyminen ja Krooninen liikuntakyvyttömyys vaimentavat D-vitamiinikorvaushoidon PTH-pitoisuutta laskevaa vaikutusta. 3.) Lievästi kohonnut tai jopa vain viitekeleen ylärajoilla oleva PTH-pitoisuus on yhteydessä lisääntyneeseen kuoleman ja kognition heikentymisen riskiin ikääntyneessä väestössä.


List of original publications

This thesis is based on the following publications:


The publications are referred to in the text by their Roman numerals.
## Abbreviations

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<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>1.25-OHD</td>
<td>1.25-hydroxyvitamin D = calcitriol</td>
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<td>1α-OHase</td>
<td>1-alpha hydroxylase</td>
</tr>
<tr>
<td>24-OHase</td>
<td>Vitamin D-24-hydroxylase</td>
</tr>
<tr>
<td>25-OHase</td>
<td>Vitamin D-25-hydroxylase</td>
</tr>
<tr>
<td>25-OHD</td>
<td>25-hydroxyvitamin D = calcidiol</td>
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<tr>
<td>95%CI</td>
<td>95 percent confidence interval</td>
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<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>Ca2+</td>
<td>Serum ionized calcium</td>
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<tr>
<td>CaSR</td>
<td>Calcium-sensing receptor</td>
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<tr>
<td>CaT</td>
<td>Serum total calcium</td>
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<tr>
<td>CDR</td>
<td>Clinical dementia rating</td>
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<tr>
<td>CPS</td>
<td>Cognitive performance scale</td>
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<tr>
<td>CV</td>
<td>Coefficient of variation</td>
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<tr>
<td>e.g.</td>
<td>exempli gratia (for example)</td>
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<tr>
<td>etc.</td>
<td>et cetera</td>
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<tr>
<td>FGF-23</td>
<td>Fibroblast growth factor 23</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
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<tr>
<td>GPCR</td>
<td>G protein-coupled receptors</td>
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<tr>
<td>HR</td>
<td>Cox proportional hazard ratio</td>
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<tr>
<td>i.e.</td>
<td>id est (that is)</td>
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<tr>
<td>ICTP</td>
<td>Carboxyl-terminal telopeptide of type I collagen</td>
</tr>
<tr>
<td>IU</td>
<td>International units</td>
</tr>
<tr>
<td>LURIC</td>
<td>Ludwigshafen risk and cardiovascular health study</td>
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<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
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<tr>
<td>N</td>
<td>Number</td>
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<tr>
<td>Na</td>
<td>Sodium</td>
</tr>
<tr>
<td>NHANES</td>
<td>National health and nutrition examination survey</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>P</td>
<td>Phosphate</td>
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<td>PHPT</td>
<td>Primary hyperparathyroidism</td>
</tr>
<tr>
<td>PINP</td>
<td>Amino-terminal propeptide of type I procollagen</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
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<tr>
<td>PTH1R</td>
<td>Parathyroid hormone receptor 1</td>
</tr>
<tr>
<td>PTH2R</td>
<td>Parathyroid hormone receptor 2</td>
</tr>
<tr>
<td>r</td>
<td>Pearson’s bivariate correlation coefficient</td>
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<tr>
<td>RAS</td>
<td>renin-angiotensin system</td>
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<tr>
<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>SHPT</td>
<td>Secondary hyperparathyroidism</td>
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<tr>
<td>SMR</td>
<td>Standardized mortality ratio</td>
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<tr>
<td>TIP39</td>
<td>Tuberoinfundibular peptide of 39 residues</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
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<tr>
<td>TRPV5</td>
<td>Transient receptor potential vanilloid 5 channel</td>
</tr>
<tr>
<td>TRPV6</td>
<td>Transient receptor potential vanilloid 6 channel</td>
</tr>
<tr>
<td>UV-B</td>
<td>Ultraviolet B radiation</td>
</tr>
<tr>
<td>VDBP</td>
<td>Vitamin D-binding protein</td>
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<tr>
<td>VDR</td>
<td>Nuclear vitamin D receptor</td>
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1. INTRODUCTION

Parathyroid hormone (PTH) is a circulating hormone comprised of 84 amino acids. It is produced in the parathyroid glands and primarily acts on bone and kidney to maintain extracellular calcium levels within normal limits. Its release from the parathyroid glands is triggered by hypocalcaemia and hyperphosphataemia. PTH stimulates osteoblast activity and increases renal tubular reabsorption of calcium. It also promotes the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-dihydroxyvitamin D (1,25-OHD) by 1-alpha hydroxylase in the kidney, leading to the increased gastrointestinal absorption of calcium. Classically, the biological activity has been thought to be carried out by 1,25-OHD, but 25-OHD may also have direct effects. Hypercalcaemia and hypophosphataemia are the major suppressors of PTH synthesis and secretion. However, vitamin D also exerts negative feedback on PTH synthesis.

Parathyroid disorders include increased activity, i.e. hyperparathyroidism, and decreased activity, i.e. hypoparathyroidism. Hyperparathyroidism has been classically classified as primary (PHPT) or secondary (SHPT), depending whether the cause of increased PTH secretion is found in the parathyroid glands themselves (hyperplasia or adenoma) or externally (chronic kidney disease or vitamin D deficiency). Long-standing SHPT may also lead to a PHPT-like condition, namely tertiary hyperparathyroidism, if the regulatory mechanisms of PTH synthesis and secretion are disrupted. Parathyroid destruction caused by thyroid surgery is a well documented cause of hypoparathyroidism. In contrast to an elevated PTH concentration, hypoparathyroidism is not a common finding in the elderly. SHPT due to chronic kidney disease and PHPT are important causes of hyperparathyroidism. Thus, progressing renal dysfunction and the development of hypercalcaemia in PHPT are thought to be closely involved in mediating the health outcomes of elevated PTH in these patients. However, elevated PTH levels are even more common in normocalcaemic older patients with a relatively well preserved renal function. Thus, vitamin D deficiency seems to be the most common cause of elevated PTH concentrations in the elderly.

Hyperparathyroidism, namely SHPT due to chronic kidney disease and PHPT in particular, has been associated with several adverse health outcomes, including osteoporotic fractures, all cause mortality as well as cognitive dysfunction. Typically, the PTH concentrations have been very high or accompanied by hypercalcaemia or uraemia in these patients. However, the prognostic significance of subtle hyperparathyroidism has been less well established.

This thesis addresses the predictive value of parathyroid hormone concentrations in older persons. This thesis also aims at investigating the factors confounding the responses of parathyroid hormone to vitamin D supplementation.
2. REVIEW OF THE LITERATURE

2.1 Parathyroid hormone physiology

There are usually four parathyroid glands that are typically located on the posterior surface of the left and right lobes of the thyroid, weighing approximately 500 mg together. The chief cells of the parathyroid glands are highly specialized to synthesize, process, and secrete the 84 amino acid PTH, i.e. intact PTH or PTH (1-84).

2.1.1 Classical regulation of circulating parathyroid hormone

The primary function of PTH is to maintain the calcium concentration of the extracellular fluid within a narrow normal range. PTH acts directly on bone and kidney and indirectly on the intestine through its effects in promoting the renal synthesis of calcitriol to increase serum calcium concentrations (Figure 1). The synthesis and secretion of PTH and the subsequent serum levels are tightly regulated by a negative feed-back loop. Serum ionized calcium (Ca$^{2+}$), acting through the calcium-sensing receptor (CaSR), and vitamin D, acting through its nuclear receptor (VDR), inhibit PTH synthesis and release (Theman & Collins 2009, Lehmann & Meurer 2010). Parathyroid synthesizes and secretes PTH unless it is restrained by CaSRs, whereas the binding of the vitamin D-VDR complex to the upstream regulatory element of the PTH gene decreases the rate of PTH gene transcription and parathyroid cell proliferation (Nagpal et al. 2005, Naveh-Many 2010). Serum P has also been recognized as an important regulator of circulating parathyroid hormone levels. An increase in P levels leads to increased PTH secretion, PTH mRNA stability, and parathyroid cell proliferation, similarly to low calcium (Naveh-Many 2010).

Both the rate and magnitude of changes in the Ca$^{2+}$ concentration are detected by extracellular CaSRs expressed on parathyroid cells. The CaSR is a member of family C G-protein-coupled receptors and bears both structural homology and some degree of ligand crossover with the metabotropic glutamate receptors and $\gamma$-aminobutyric acid receptors (Theman & Collins 2009). CaSRs are also expressed in target tissues for PTH, in addition to chief cells of the parathyroid glands (Su et al. 2004). These target tissues of PTH regulate Ca$^{2+}$ by translocating calcium ions into or out of the bodily fluids, and include the kidney, which expresses the CaSR at robust levels in certain nephron segments, and bone and intestine, which express the receptor at lower levels.

Once secreted, PTH circulates freely in plasma and is rapidly metabolized in the liver and kidneys, the half-life being approximately four minutes. The parathyroid glands usually only contain enough PTH to maintain an increased stimulatory response for several hours. However, the synthesis of new PTH is also enhanced by hypocalcaemia, in addition to secretion, also ensuring the long-term requirements of increased parathyroid activity (Barret & Barret 2009). Furthermore, the rate of clearance of the secreted 84-amino-acid peptide from blood is more rapid than the rate of clearance of the fragments
corresponding to the middle and carboxyl-terminal regions of PTH (Figure 2), which have been considered biologically inactive (Murray et al. 2005).

Figure 1  Control of mineral metabolism by parathyroid hormone (Shoback 2008).  Copyright © [2008], Massachusetts Medical Society. All rights reserved.
2.1.2 Novel parathyroid-endocrine axis

Recent discoveries have revealed that PTH mRNA stability is regulated by the balanced interactions of protective and the decay-promoting proteins (Naveh-Many 2010). The key regulator of these interactions has been identified as the peptidyl-prolyl cis/trans isomerase Pin1 (Figure 3). It has also been shown that Pin1 knockout mice have increased parathyroid gland PTH levels and circulating serum PTH concentrations without changes in serum calcium or P levels (Nechama et al. 2009). However, human studies are lacking.

Fibroblast growth factor 23 (FGF-23) is also a newly identified bone-derived hormone that decreases PTH gene expression and secretion (Ben-Dov et al. 2007). In addition to the inhibition of expression of the renal sodium phosphate transporter, FGF-23 reduces renal 1-alpha-hydroxylase expression leading to impaired production of 1.25-OHD, stimulates 24-hydroxylase expression causing accelerated elimination of vitamin D, and decreases both PTH mRNA expression and PTH secretion from the parathyroid gland (Razzaque 2009). FGF-23 signalling is also supported by an enhancing co-factor, klotho, a membrane protein involved in the renal regulation of calcium absorption (Razzaque 2009).
2.1.3 Actions of parathyroid hormone

PTH is a true hormone, because it is produced by a gland and then travels through the bloodstream and binds to its receptor to act on its target tissues. It has been commonly held that all of the major biological activities of PTH are mediated by the N-terminal hormone residues within the (1–34) region. The classical actions of PTH on bone and kidney are aimed at the preservation of the calcium concentration and elimination of phosphorus.

2.1.3.1 Classical actions on bone and kidney

PTH binds to the parathyroid hormone receptor 1 (PTH1R), which has a high affinity for the N-terminal hormone residues within the (1–34) region of PTH. Synthetic PTH including the first 34 N-terminal residues, i.e. PTH(1-34), has also been shown to have a high affinity for PTH1R and the capacity to reproduce all the major effects of intact PTH, aiming at the maintenance of calcium homeostasis (Murray et al. 2005). PTH1R is a member of the class II family of G protein-coupled receptors (GPCR). Like other members of this family (including secretin, calcitonin, vasoactive intestinal peptide, glucagon-like peptide-1, growth hormone-releasing hormone, corticotropin-releasing factor, and glucagon receptor), it is capable of coupling to several different G proteins,

In addition to vitamin D and calcitonin, PTH acts as a major regulator of calcium homeostasis. In bone, the effects of PTH are complex and lead to the stimulation of bone resorption, which delivers calcium and P into the circulation. According to the prevailing view, persistently elevated PTH acts indirectly on bone resorption via PTH1Rs expressed on osteoblasts and stromal cells, causing an increased production of cytokines and reduced production of the antiresorptive protein, osteoprotegerin (Murray et al. 2005). This further leads to the differentiation of osteoclast precursors and to stimulation of the resorbing activity of mature differentiated osteoclasts. In contrast to the effects of persistently elevated PTH on bone health, intermittently elevated PTH has been shown to result in the increased formation of trabecular bone (Neer et al. 2001, Lotinun et al. 2002).

PTH has three major actions in the kidneys. In the distal part of the nephron, PTH stimulates active Ca\textsuperscript{2+} reabsorption. The transient receptor potential vanilloid 5 (TRPV5) channel constitutes the luminal gate for Ca\textsuperscript{2+} entry in the distal convoluted tubule. PTH activates the adenyl cyclase-cAMP-protein kinase A signalling cascade, which rapidly phosphorylates threonine-709 of TRPV5, increasing the open probability of the channel and promoting Ca\textsuperscript{2+} reabsorption in the distal nephron (de Groot et al. 2009). The excretion of P is also enhanced by PTH in concert with fibroblast growth factor 23 (Schiavi 2006), through the inhibition of renal type II sodium-phosphate cotransporter activity, leading to phosphaturia (Fukumoto et al. 2002). The third renal action of PTH is to promote vitamin D 1-hydroxylase responsible for 25-OHD hydroxylation to 1.25-OHD (Loré & Di Cairano 1986).

Thus, when hypocalcaemia or hyperphosphataemia threatens to develop, these steps are able to restore the ionized Ca\textsuperscript{2+} levels to the physiological limits, and through the actions of PTH and other factors in the kidney, reset the level of serum P within the normal range. Finally, the increasing circulating Ca\textsuperscript{2+} and decreasing P induce a negative feedback effect on PTH synthesis and secretion (Figures 1 and 3). During recent decades, the differences in the skeletal effects of continuously elevated PTH and intermittent treatment with PTH have been well established (Neer et al. 2001). Continuous elevation of PTH results in decreased bone mineral density and an increased risk of osteoporotic fractures, but intermittent treatment has a strong anabolic effect on bone.

**2.1.3.2 Nonclassical actions**

In addition to the classical actions of PTH on bone and kidney aimed at the preservation of calcium homeostasis, some evidence also suggests PTH actions in other organ systems, since PTH1Rs are expressed throughout the human tissues. In addition to bone and kidney, the PTH1Rs have at least been identified in human heart, skeletal muscle and several regions of the brain (Reppe et al. 2007, Monego et al. 2009, Lupp et al. 2010), indicating possible actions of PTH in these tissues.
Experimental animal models of placebo-controlled PTH treatment have shown direct cardiovascular effects of parathyroid hormone, including myocardial damage (Bogin et al. 1981), a prosclerotic effect on vascular smooth muscle cells (Perkovic et al. 2003), and accelerated decompensation following left ventricular hypertrophy (Cha et al. 2010). Thirdly, human studies of chronic (12 days) continuous intravenous PTH (1–34) infusion in healthy subjects (N = 4) have resulted in persistent hypercalcaemia and hypertension, reversible during a 4- to 8-day recovery period (Hulter et al. 1986). However, animal models have also associated PTH treatment with the inhibition of osteogenic vascular calcification (Shao et al. 2003), improved cardiac repair by enhanced neovascularization and cell survival after myocardial infarction (Zaruba et al. 2008), an improved mechanical response of cardiomyocytes to electrical stimulation (Tastan et al. 2009) and protective effects through the activation of bone marrow-derived stem cells, resulting in reduction of perfusion defects in experimental animal model of cardiac ischemia (Huber et al. 2010).

It has also been reported that when high levels of PTH are sustained in rat hippocampal organotypic cultures, a toxic increase in intracellular calcium results (Hirasawa et al. 2000). At 1 week of exposure, the toxic effects were dose-dependent over the range of 0.1 pM to 0.1 microM, the minimum effective dose being 10 pM. Furthermore, the PTH (1-34)-induced adverse effects were significantly inhibited by co-administration of Ca\textsuperscript{2+} channel blocker.

Parathyroid hormone may also play a role in the maintenance of body composition. A prospective population-based study has associated higher PTH levels with decreased muscle mass and impaired muscle strength (Visser et al. 2003). Furthermore, PTH levels have been associated with increased body fat in the elderly (Pitroda et al. 2009). Parathyroid hormone receptor 2 (PTH2R) has also been characterized in humans. However, a tuberoinfundibular peptide of 39 residues (TIP39) instead of PTH has been found as the primary ligand for PTH2R. PTH2R and TIP39 constitute a neuromodulator system implicated in endocrine and nociceptive regulation (Bagó et al. 2009). Other receptors for PTH have also been characterised in other species, the importance of which to human physiology has remained uncertain (Murray et al. 2005).

Carboxyl (C)-terminal PTH fragments have also been identified in the circulation in large amounts originating from direct secretion by the parathyroid glands as well as hepatic proteolysis of intact PTH. These fragments have been presumed to be biologically inactive, because they have no activity at the PTH1R, but compelling evidence suggests that the physiological parathyroid regulation of calcium and bone metabolism may additionally involve receptors for circulating C-terminal PTH ligands (Murray et al. 2005). There is no evidence to suggest that regions of PTH (1–84) located C-terminal to residue 34 contribute to ligand binding or activation of the PTH1R. On the other hand, large portions of the C terminus of PTH (1–84) are highly conserved across species. For example, the sequences PTH (53–61) and PTH (65–75) are 80% identical and otherwise differ only by conservative substitutions across mammalian species. This high degree of evolutionary conservation strongly suggests the possibility of an additional, independent biological function for this region of the PTH molecule (Divieti et al. 2005). Receptors specific for the C-terminal region of parathyroid hormone have also been identified in bone-derived cells (Divieti et al. 2005, Banerjee et al. 2006), but the clinical relevance of
these observations is largely unknown. However, accumulation of the C-terminal fragments of PTH has been associated with decreasing renal function in humans (Patel et al. 2010). Furthermore, in experimental animal models, the administration of C-terminal PTH(7–84) has been shown to act as an antagonist to the effects of intact PTH, independent of PTHR1 in both bone and kidney (Divieti et al. 2002, Langub et al. 2003, Nakajima et al. 2009).

2.2 Vitamin D physiology

Vitamin D is a group of fat-soluble prohormones, the two major forms of which are vitamin D3, i.e. cholecalciferol, and vitamin D2, i.e. ergocalciferol. Cholecalciferol is formed in the skin after exposure to sunlight. Vitamin D can be also obtained from nutrients and supplements, but the formation of cholecalciferol in the skin is the most important source of vitamin D. Ergocalciferol is obtained by irradiation of plants or plant materials or foods. Even though vitamin D is not produced by a gland, it travels through the bloodstream and binds to a receptor to act on its target tissues like a hormone.

2.2.1 Synthesis and metabolism of vitamin D

Photochemical synthesis of vitamin D3 occurs cutaneously, where pro-vitamin D3 (7-dehydrocholesterol) is converted to pre-vitamin D3 in response to sunlight (ultraviolet B radiation 280–315 nm) exposure. Vitamin D3, obtained from the isomerization of pre-vitamin D3 in the epidermal basal layers or intestinal absorption of natural and fortified foods and supplements, binds to vitamin D-binding protein (VDBP) in the bloodstream, and is transported to the liver (Figure 4) (Barret & Barret 2009).

In the liver, vitamin D-25-hydroxylase metabolizes vitamin D3 and D2 to calcidiol (25-OHD), which is then transformed to calcitriol (1.25-OHD) by 1-alpha hydroxylase (1α-OHase) in the kidney under the stimulation of PTH. The 1-alpha hydroxylation of calcidiol to calcitriol is tightly regulated by PTH (Lehmann & Meurer 2010). Other regulators are calcium, P, calcitonin, fibroblast growth factor 23, and 1.25-OHD itself. Increases in serum Ca\(^{2+}\), P and 1.25-OHD levels downregulate renal 1α-OHase expression, resulting in the suppression of 1.25-OHD synthesis.

Following hydroxylation in the kidneys, calcitriol is released into the circulation, and again by binding to the VDBP carrier protein in the plasma it is transported to various target organs (Figure 4). The circulatory levels of 1.25-OHD are also kept relatively stable in physiological and most pathological conditions, whereas the vitamin D stores are sensitively reflected by the 25-OHD concentration. Many extrarenal cells and tissues have been shown to express 1α-OHase, and thus under the influence of cytokines 25-OHD can also be hydroxylated to 1.25-OHD in the target tissues themselves (Lips 2006). This extrarenal 1.25-OHD production is important for the paracrine and autocrine regulation of cell differentiation and function (Peterlik & Cross 2005, Oudshoorn et al. 2009). It has
been also suggested that 25-OHD may have additional direct effects on target tissues (Lehmann & Meurer 2010).

The elimination of both 1.25-OHD and 25-OHD is regulated by the kidneys through the actions of the mitochondrial enzyme vitamin D-24-hydroxylase (24-OHase), encoded by the cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1) gene. Expression of the 24-OHase in the kidney is stimulated by 1.25-OHD, and 24-OHase converts 1.25-OHD and 25-OHD into less active metabolites, which are then excreted to urine. These feedback mechanisms play an important role in the protection against hypercalcaemia and hyperphosphataemia during vitamin D sufficiency (Christakos et al. 2007).
2.2.2 Actions of vitamin D

Calcitriol can enter the cell and bind to the VDR, and subsequently to a responsive gene. Vitamin D also has rapid non-genomic effects through a membrane receptor and second messengers (Lips 2006).

Upon binding to calcitriol, the VDR is phosphorylated and recruits one of the three 9-cis-retinoid X receptors. Regulation of gene expression is then dependent on the ability of these heterodimers to build co-regulatory protein complexes, including the steroid receptor coactivators and the VDR interacting protein. These complexes bind to specific genomic sequences in the promoter region named vitamin D response elements. The VDR not only directly upregulates gene transcription but also directly downregulates the transcription of several genes such as those encoding parathyroid hormone (Lehmann & Meurer 2010).

Calcitriol can also activate a variety of non-genomic signal transduction systems including Ca²⁺ influx; release of Ca²⁺ from intracellular stores; modulation of adenylate cyclase, phospholipase C, and protein kinases C and D; as well as mitogen-activated protein and rapidly growing fibrosarcoma kinase pathways. These activities have been found in many cells, including keratinocytes, enterocytes (intestinal absorptive cells), muscle cells, osteoblasts, and chondrocytes. VDR seems to be necessary for some of these nongenomic transduction processes; however, another protein named 1-alpha-25-dihydroxy-membrane associated rapid response steroid binding protein is also seemingly involved in these rapid nongenomic actions (Lehmann & Meurer 2010).

The well documented actions of vitamin D include the increased absorption of calcium from intestines and enhanced reabsorption of calcium in the kidneys. One of the main mechanisms for Ca²⁺ absorption is the transcellular (active) route, which involves the entry of Ca²⁺ into the cell via Ca²⁺ channels, diffusion of Ca²⁺ through the cytosol bound to calbindins and active extrusion of Ca²⁺ via a Ca²⁺ pump or a Na/Ca exchanger (Perez et al. 2008). The epithelial Ca channels are members of the transient receptor potential superfamily, and more precisely, the vanilloid subfamily (TRPV). The TRPV5 channel is the major isoform in the kidney, while the TRPV6 channel is highly expressed in the intestine. Interestingly, animal models have shown close regulation of TRPV6 expression by vitamin D. Both in VDR and in 1-alpha hydroxylase knockout mice, intestinal TRPV6 expression is strongly reduced, which impairs intestinal Ca²⁺ absorption (Song et al. 2003, Hoenderop et al. 2004). Less is known about the precise mechanisms of renal actions, but vitamin D may also be involved in the regulation of TRPV5 expression (Renkema et al. 2005).

The musculoskeletal effects of vitamin D supplementation have mostly been studied during recent decades. It appears that vitamin D supplementation improves neuromuscular functions, such as balance and reaction time (Dhesi et al. 2004, Lips et al. 2010), but has no major impact on muscle strength. However, the improved neuromuscular functions are thought to lead to a decreased risk of falls and subsequent fractures. Two recent meta-analyses by Bischoff-Ferrari et al. have shown that supplemental vitamin D at a dose of 700-1000 IU per day reduces the risk of falling among older individuals by 19%, and a dose higher than 400 IU a day should reduce nonvertebral fractures by at least 20% in individuals aged 65 years or older (Bischoff-Ferrari et al. 2009a, Bischoff-Ferrari et al. 2010).
Thus, vitamin D has been classically considered as counteracting PTH in the regulation of calcium homeostasis and bone health.

Vitamin D receptors are found in most tissues and vitamin D is involved in the regulation of over 200 genes (Holick 2007). In fact, vitamin D seems to have a role in several different and important physiological processes such as cell differentiation and immune function (Figure 4). In observational studies, vitamin D deficiency has also been associated with many chronic diseases, including osteoporosis, cancer, cardiovascular diseases, diabetes, and dementia. These observations have led to recommendations to increase the intake of vitamin D of specific patients groups at risk, such as the elderly, by supplementation or fortification of foods, in order to reach 25-OHD levels of at least 75-100 nmol/l (Souberbielle et al. 2010), even though conflicting results are also emerging (Bolland et al. 2010). Furthermore, high-quality large-scale randomized controlled trials addressing these putative pleiotropic effects of vitamin D are lacking and many of the performed trials have so far shown negative results.

2.3 Parathyroid disorders in old age and their prognostic significance

Parathyroid disorders include hyper- and hypoparathyroidism. Hypoparathyroidism refers to insufficient production of PTH in order to maintain calcium balance. When the actions of PTH are reduced or lost, all subsequent steps in the maintenance of calcium homeostasis are impaired, resulting in hypocalcaemia, hyperphosphataemia, and hypercalciuria. Post-surgical tetany as a cause of death was already observed in the 19th century in thyroidectomy patients. The early research also focused on the prevention of these deaths and led to the discovery of the parathyroid glands and hormone at the beginning of the 20th century.

Hyperparathyroidism is due to increased activity of the parathyroid glands, either from autonomous hypersecretion (primary or tertiary hyperparathyroidism) or from a secondary increase in PTH secretion in response to hypocalcaemia (secondary hyperparathyroidism). An uncontrolled and nonsuppressed increase in parathyroid activity leads to hypercalcaemia and hyperphosphataemia, in addition to increased secretion of calcium to urine. Hyperparathyroidism was first described in the 1930s, and during the 1980s the introduction of automated calcium measurements revealed the common mild PHPT in the elderly. Because of the large number of heterogeneous conditions associated with parathyroid function, this chapter focuses on the existing data on parathyroid disorders as an outcome indicator in older persons, particularly in terms of mortality and cognition.

2.3.1 Hypoparathyroidism

Hypoparathyroidism is a rare disease and refers to reduced or lacking activity of the parathyroid glands, resulting in insufficient production of parathyroid hormone to maintain calcium homeostasis (Potts 2001). Hypoparathyroidism is most commonly seen
as a complication of thyroid surgery (Shoback 2008). Other causes are divided between a range of hereditary and acquired diseases such as DiGeorge syndrome (Kobrynski & Sullivan 2007), autoimmunity, and increased function of the CaSRs by either activating mutations (Pollak et al. 1994) or stimulatory antibodies (Kifor et al. 2004). In Finland, in particular, hereditary autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a common cause of hypoparathyroidism (Perheentupa 2002).

The parathyroid secretory reserve is ample, so considerable damage must occur before hypoparathyroidism develops (Shoback 2008). It is estimated that one normal gland is sufficient for maintaining PTH levels and serum calcium homeostasis. Definitions of permanent postsurgical hypoparathyroidism vary, but the definition is generally accepted to be insufficient PTH to maintain normocalcaemia 6 months after surgery. Hypoparathyroidism is estimated to occur after approximately 0.5 to 6.6% of total thyroidectomies; the rates of this complication are even higher in some case series, whereas reported rates at endocrine surgical centres with high volumes are 0.9 to 1.6% (Thomusch et al. 2003, Zarnegar et al. 2003, Page & Strunski 2007, Asari et al. 2008). Hypoparathyroidism is found in approximately 4 out of 100 000 patients at the population level. Thus, hypoparathyroidism is far less common than hyperparathyroidism in the elderly.

Acute hypoparathyroidism can cause hypocalcaemia with consequent paresthesias, muscle spasms, seizures, and even tetany and death. In contrast, chronic hypoparathyroidism generally causes hypocalcaemia so gradually that the only symptom may be visual impairment from cataracts caused by years of hypoparathyroidism (Marx 2000). It is also well known that the hypoparathyroidism related to APECED requires careful treatment and monitoring (Perheentupa 2002). However, there are no available data from clinical trials to show whether the complications of mild chronic hypocalcaemia related to mild hypoparathyroidism are preventable with aggressive therapy, or whether these patients derive benefits from therapy. Furthermore, the long-term prognostic significance of chronic hypoparathyroidism has not been systematically studied.

2.3.2 Primary hyperparathyroidism

Primary hyperparathyroidism is caused by adenoma, hyperplasia or carcinoma of one or more of the four parathyroid glands. Parathyroid adenoma is the most common cause of PHPT, whereas parathyroid carcinoma is rare. In PHPT, the hyperplastic or adenomatous glands are not able to correctly sense the circulating calcium concentration, and the physiological negative feedback between serum calcium and PTH secretion is thus disrupted. This disruption results in autonomous hypersecretion of PTH, and worsening hypercalcaemia develops in the course of the disease. The classical signs and symptoms of primary hyperparathyroidism are mainly attributable to hypercalcaemia, hypophosphataemia, hypercalciuria and increased bone resorption, including such clinical manifestations as renalcalculi, overt bone disease, neuromuscular dysfunction, gastrointestinal complaints as well as neuropsychological dysfunction (Bilezikian et al. 2002). Patients lacking these clinical manifestations are termed “asymptomatic” in the
older literature. This definition is now ambiguous or inappropriate. Due to frequent measurements of calcium and PTH, the screening and diagnostic awareness of PHPT has improved significantly during recent decades, and the newly diagnosed PHPT patients have been found to have more vague symptoms (weakness and easy fatigability in the absence of overt muscle weakness). Some patients also seem to be truly asymptomatic (Bilezikian et al. 2009).

The detection of hypercalcaemia has been the cornerstone in the diagnosis of primary hyperparathyroidism (Figure 5). The population-based sex-adjusted incidence of primary hyperparathyroidism in U.S. whites (Rochester, Minnesota) was 21.6 per 100,000 person years during a 9-year follow-up, with a two-fold higher incidence in females (28.4 per 100,000 person years) compared with males (13.8 per 100,000 person years) (Wermers et al. 2006). The incidence increased with age, reaching a peak of 63.2 per 100,000 person years at ages 65–74 yr. Furthermore, the prevalence of PHPT requiring clinical attention has been shown to be approximately 3% in older females and less than 1% in older men in Helsinki, Finland (Sorva et al. 1992).

All symptomatic patients are usually treated by parathyroidectomy. However, the guidelines for surgical treatment of asymptomatic primary hyperparathyroidism patients have been changing during the last 20 years (Table 1). Furthermore, the neuropsychological symptoms have been ruled out in the consensus statements as an indication for surgery in asymptomatic patients. However, at Helsinki University Central Hospital, rapid development (within 1–2 years) of neuropsychological symptoms in a patient with a biochemically confirmed PHPT is also considered as an indication for parathyroidectomy. Medical management of asymptomatic PHPT is a promising option for those who are not candidates for parathyroidectomy. Bisphosphonates and hormone replacement therapy provide skeletal protection in patients with PHPT (Sankaran et al. 2010). Calcimimetics favourably alter serum calcium and PTH in PHPT, but do not significantly affect either bone turnover or BMD (Khan et al. 2009).

<table>
<thead>
<tr>
<th>Table 1. Guidelines for parathyroid surgery in asymptomatic primary hyperparathyroidism</th>
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<tr>
<td><strong>Criterion</strong></td>
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<tr>
<td>1. Extent of hypercalcaemia</td>
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<tr>
<td>2. 24 hr urinary calcium</td>
</tr>
<tr>
<td>3. Creatinine clearance</td>
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<tr>
<td>4. Bonde mineral density</td>
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<tr>
<td>5. Age</td>
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<td>6. Cognitive symptoms</td>
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Figure 5  Biochemical investigation of hypercalcaemia should result in the measurement of intact parathyroid hormone, with the initial classification into parathyroid or non-parathyroid causes on the basis of the combination of concentrations of parathyroid hormone and calcium. Further tests might be needed to establish the precise cause of hypercalcaemia in some patients. FBHH = familial benign hypocalciuric hypercalcaemia.

2.3.2.1 Primary hyperparathyroidism and survival

Increased cardiovascular mortality in patients with severe and moderately severe PHPT has been well documented (Palmer et al. 1987, Ronni-Sivula 1985, Hedbäck et al. 1991, Ljunghall et al. 1991). Classically, the increased mortality and morbidity has been associated with higher total calcium (CaT) levels (Wermers et al. 1998, Lundgren et al. 2001, Nilsson et al. 2002), and the beneficial effects of parathyroidectomy have been thought to be causally related to decreasing CaT levels (Hedbäck & Odén 1998). Nilsson
et al. analyzed mortality over a 30-year period in 10,995 Swedish patients who underwent parathyroidectomy. Although an increased risk of cardiovascular mortality was observed in the overall cohort, this risk dissipated in those enrolled later in the study, when the patients had lower levels of serum CaT. Another Swedish study on 4,461 patients operated for primary hyperparathyroidism reported that the decline in the death risk paralleled the decrease in the mean preoperative serum CaT level over time (Hedbäck & Odén 1998). Although hypercalcaemia has been associated with such powerful cardiovascular risk factors as carotid plaque thickness (Rubin et al. 2007) and vascular calcification, the exact mechanisms underlying the increased risk of death related to hypercalcaemia have remained unclear. Nevertheless, cohort studies have also demonstrated that in symptomatic patients, bone density improves and the fracture rate declines after parathyroidectomy. Cognitive function appears to improve. The incidence of kidney stones declines after surgery in patients who had kidney stones before surgery. Cardiovascular disease and premature death also appear to decrease after surgery in symptomatic subjects (Udelsman et al. 2009). Thus, almost all patients with biochemically confirmed symptomatic PHPT are referred for parathyroid surgery in clinical practice. However, even though the higher mortality rate declines with time from parathyroidectomy, it persists long after the surgical cure, suggesting that PHPT may cause enduring damage to the cardiovascular system (Hedbäck et al. 1991, Nilson et al. 2002).

Studies from the US also including asymptomatic patients have shown different results (Wermers et al. 1998). Wermers et al. followed all residents with primary hyperparathyroidism in Rochester, Minnesota, for 15 years. A total of 309 cases of primary hyperparathyroidism (CaT = 2.67 ± 0.10 mmol/l, age = 56 ± 17 years) without surgery were identified during study period. The survival of patients was better than expected for white Minnesota residents of a similar age and gender, but higher CaT levels emerged as an independent predictor of mortality in these patients. The authors concluded that overall survival was not adversely affected among unselected patients with mild PHPT in the community, although patients with more severe disease indicated by higher serum CaT levels may have an increased risk of death (Wermers et al. 1998). Furthermore, according to a population-based US study, the survival of PHPT patients was also comparable to age- and sex-matched controls after parathyroidectomy (Søreide et al. 1997), not supporting the hypothesis of enduring damage of the cardiovascular system. However, researchers from Europe have shown an increased risk among mild to moderate PHPT patients who did not undergo parathyroidectomy (Palmer et al. 1987, Lundgren et al. 2001). Lundgren et al. followed 55 patients with similarly mild hypercalcaemia (CaT 2.67 ± 0.07 mmol/l, mean age 51 years) to those investigated by Wermers et al. in a case-control study for 25 years. In contrast to the findings of Wermers et al., mortality was higher than expected in patients younger than 70 years and cardiovascular diseases were over-represented causes of death. However, Lundgren et al. also showed that hypercalcaemia independently indicated an increased risk of cardiovascular death (HR = 1.72, 95%CI = 1.24–2.37) in these patients. The existing incongruent evidence from mortality data, in particular cardiovascular mortality, in mild PHPT patients has been
explained by the variation in disease severity between study regions and the absence of population studies of mortality in a genuine mild PHPT cohort (Silfverberg et al. 2009).

Within the last decade, a large body of the PHPT literature has focused on the proper management of mild and asymptomatic disease (Eastell et al. 2009, Khan et al. 2009, Silverberg et al. 2009, Udelsman et al. 2009, Sankaran et al. 2010). However, population-based long-term follow-up studies and large-scale randomized controlled trials addressing the prognostic significance of asymptomatic PHPT and the effects of active treatment of these patients are still lacking. Recently, Yu et al. studied for the first time a representative population-based sample of 1683 (69.1% female) patients identified with mild PHPT (CaT = 2.58 mmol/l, mean PTH = 99 ng/l) during 1997–2006 in Tayside, Scotland (Yu et al 2009). The authors showed that mortality and morbidity were increased for patients with mild untreated PHPT (standardized mortality ratio (SMR)-all cause = 2.62, 95%CI 2.39–2.86; SMR-cardiovascular = 2.68, 95%CIs 2.34–3.05). The increases were similar to more severe PHPT. Furthermore, patients with mild PHPT have a significantly increased risk of developing cardiovascular and cerebrovascular disease, renal dysfunction and fractures compared to the age- and sex-adjusted general population. It should also be noted that the severity or mildness of PHPT in the studies referred to above has been defined by the degree of hypercalcaemia. Similar survival analyses based on PTH levels in PHPT have not been reported.

2.3.2.2 Primary hyperparathyroidism and cognitive functions

Current data on the association between primary hyperparathyroidism and cognitive functions are even less clear. It is well known that classical primary hyperparathyroidism was characterized by prominent psychological and neurological manifestations. The elevated serum calcium and PTH concentrations closely related to advanced primary hyperparathyroidism clearly have the potential to produce neuropsychiatric symptoms, and case reports have also shown that these cognitive symptoms are dramatically improved after parathyroidectomy (Logullo et al. 1998, Papageorgiou et al. 2008). However, it remains uncertain to what extent neuropsychiatric symptoms are present in the mild form of primary hyperparathyroidism commonly seen today. Symptoms such as fatigue, lassitude, mood swings, irritability, anxiety, depression, difficulty concentrating, memory loss, and increased sleep requirements have been studied in the PHPT patient population in a variety of case-control, cohort, and case series studies, but the results have been inconsistent (Caron & Pasieka 2009). The observational nature, small sample sizes, the inclusion of subjects with advanced symptomatic hyperparathyroidism, a lack of appropriate control groups, and testing at short intervals after parathyroidectomy have limited the conclusions that can be drawn from these studies.

According to a recent systematic review of all data on neuropsychological assessments in PHPT, the data collectively suggest that the neurobehavioral syndrome associated with PHPT is ambiguous, and the impact of parathyroidectomy on differential improvement in cognitive functioning has been inadequately examined and may be confounded by the
presence of depression (Coker et al. 2005). In fact, Coker et al. found only six studies in which cognitive function was measured with formal neuropsychological tests.

Recently, Perrier et al. performed a prospective randomized controlled trial of parathyroidectomy vs. observation in asymptomatic patients (N = 18, CaT = 2.58 mmol/l, mean PTH = 122 ng/l) (Perrier et al. 2009). They used functional magnetic resonance imaging together with sleep assessment and comprehensive formal neuropsychological testing and found an association between sleep and brain function. Sleep seemed to be an indicator of brain activation in the anterior cingulate gyrus and precentral cortex. Subjective sleepiness also decreased in the surgically treated patients, whereas the observed control patients reported increased sleepiness, resulting in a statistically significant difference between groups. However, this significance disappeared (P = 0.090) at the six-month re-evaluation. Interestingly, subjective sleepiness was also associated with executive functions, the impairment of which has been related to early stages of Alzheimer’s disease (Dickerson et al. 2007). Pilot data have also suggested that that a decrease in regional blood flow in patients with primary hyperparathyroidism can be demonstrated with single photon emission computed tomography of the brain (Mjåland et al. 2003, Cermik et al. 2007), and this decrease also seems to normalize after parathyroidectomy (Mjåland et al. 2003). However, the results of studies using formal neuropsychological testing have remained inconsistent during recent years (Benge et al. 2009).

Cognition has a major impact on the quality of life. Thus, it is noteworthy that Pasieka et al. have recently demonstrated the long-term benefit of parathyroidectomy in primary HPT patients (Pasieka et al. 2009). In this prospective study, the pre-operative symptoms of patients decreased after parathyroidectomy and contributed to the improved quality of life at 10 years, when compared to controls undergoing thyroid surgery. The pre-operative CaT decreased from a mean of 2.76 mmol/l to 2.30 mmol/l during the ten-year follow-up, the respective figures being 107 and 35 for parathyroid hormone. The positive effect of parathyroidectomy on the quality of life is supported by three other studies with shorter follow-up periods (Pasieka et al. 2002, Sheldon et al. 2002, Quiros 2003).

Although patients with mild PHPT clearly have neuropsychological complaints, available data remain incomplete on their precise nature and their reversibility with surgery. However, there are some data supporting a modest beneficial effect of parathyroidectomy on the quality of life and psychological functioning. Further efforts to define neuropsychological and cognitive deficits that are specific to PHPT are needed (Silverberg et al. 2009).

2.3.3 Secondary hyperparathyroidism

Secondary hyperparathyroidism is a heterogeneous group of medical conditions that leads to a secondary increase in parathyroid secretion of PTH in response to hypocalcaemia, the major causes being vitamin D deficiency and chronic kidney disease. In secondary hyperparathyroidism, the cause of parathyroid gland overactivity is outside the glands themselves, and parathyroid function is thought to normalize if the external
cause is treated. The prevalence of an elevated PTH concentration has varied between 20% and 60% in different elderly populations (Jensen 1999, Harris et al. 2001, Giusti et al. 2006, Saraiva et al. 2007, Papavasiliou et al. 2009). The most frequent single cause of secondary hyperparathyroidism seems to be vitamin D deficiency and/or low calcium intake (Saleh et al. 2006). Furthermore, vitamin D deficiency and chronic kidney disease have been two areas of major clinical and scientific interest during recent years. However, in most subjects with secondary hyperparathyroidism, the cause is probably a combination of several factors.

2.3.3.1 Vitamin D deficiency

Vitamin D deficiency has been found to be very common globally (Holick 2007, Lips 2010). Depending on the cut-point of adequacy, the reported prevalence of vitamin D deficiency has varied widely. It has been suggested that a 25-OHD level of 50 nmol/l would be sufficient and levels below 25 nmol/l would be deficient for older adults (Mosekilde 2005). Levels of 25-OHD between 25 and 50 nmol/l would correspond to vitamin D insufficiency. However, the evidence is accumulating to support the elevation of target levels of 25-OHD to 75 nmol/l (Bischoff-Ferrari et al. 2009a, Bischoff-Ferrari et al. 2009b, Souberbielle et al. 2010).

There has been growing interest in the putative pleiotropic effects of vitamin D during recent years, despite the lack of evidence on causality. Autier and Gandini published a meta-analysis on the effect of vitamin D supplementation on total mortality in 2007 (Autier & Gandini 2007). They identified 18 independent randomized controlled trials, including 57 311 participants, that had also assessed mortality. However, none of the trials had mortality as a primary endpoint. Nevertheless, vitamin D supplementation indicated a relative risk reduction of mortality from any cause by 7% (Hazard ratio (HR) = 0.93, 95%CIs 0.87–0.99). Since the publication of this meta-analysis, several epidemiological studies (Dobnig et al. 2008, Melamed et al. 2008, Ginde et al. 2009, Kuroda et al. 2009, Zittermann et al. 2009, Pilz et al. 2009, Semba et al. 2009, Semba et al. 2010, Hutchinson et al. 2010, Annweiler et al. 2010a) and one intervention trial (LaCroix et al. 2009) have addressed the association between vitamin D deficiency and survival.

In a prospective cohort study of patients (N = 3258, mean = 62 years, follow-up 7.7 years) referred for angiography within the LURIC study, all-cause multivariable-adjusted mortality was significantly increased by 53% (HR = 1.53, 95%Cia 1.17-2.01) in patients with a 25-OHD level below 32.5 nmol/l (Dobnig et al. 2008). Melamed et al. (N = 13 331, mean age = 43 years) and Ginde et al. (N = 3408, age ≥ 65 years) also performed nested prospective cohort studies within NHANES III with follow-ups of 8.7 and 7.3 years, respectively (Melamed et al. 2008, Ginde et al. 2009). In line with Dobnig et al., both studies reported an increased risk of all-cause mortality among patients with a low 25-OHD level (< 45 nmol/l for Melamed et al., < 50 nmol/l for Ginde et al.). The increase in the risk of all-cause mortality was also significant after multivariable adjustment in both studies, and emphasized (HRs 1.86 vs. 1.26) among the older patients studied by Ginde et al.
Kuroda et al. have reported an even higher increase in all-cause mortality in a prospective 6.9-year observational study of 1232 postmenopausal volunteers (mean age = 63.9 years) from Japan. The multivariable-adjusted hazard ratio for death was 2.17 (95% CIs 1.27–3.72) in patients with 25-OHD level below 50 nmol/l when compared to patients with a 25-OHD concentration 50 nmol/l or greater. Furthermore, Pilz et al. reported a multivariable-adjusted hazard ratio of 1.97 (95% CIs 1.08–3.58) in a population-based sample (N = 614, age = 50–75 years). Semba et al. also reported high multivariable-adjusted hazard ratios for all-cause mortality in two prospective population-based studies of older community-dwelling US women (N = 714, age = 70–79, HR = 2.87, 95% CIs 1.10–7.4) (Semba et al. 2009) and Italians (N = 1009, median age = 74 years, HR = 1.97, 95% CIs 1.11–3.47) (Semba et al. 2010). However, both studies by Semba et al. compared the lowest 25-OHD quartile to the highest. Furthermore, the most recent work by Hutchinson et al. using a single cut-point of 50 nmol/l for the 25-OHD level again showed a more modest 32% increase in the risk of all-cause mortality (multivariable-adjusted HR = 1.32, 95% CIs 1.07–1.62) in a non-smoking sample (N = 4751, mean age = 60 years) of the longitudinal population-based Tromso study focusing on lifestyle-related diseases.

Interestingly, Annweiler et al. found that lower 25-OHD levels were also associated with increasing risk of short-term (within hospital stay) mortality in an acute geriatric setting (N = 399, mean age 85 years). This study recruited all the patients admitted to the geriatric acute care unit of Angers University Hospital, France, between January and October 2009 who were able to give their informed consent. Furthermore, Zittermann et al. showed in a prospective cohort study of end-stage heart failure patients (N = 510, mean age = 53 years) that the 1-year survival of patients is impaired in patients with low levels of 1,25-OHD.

However, only some of the studies reviewed above measured parathyroid hormone concentrations (Pilz et al. 2009, Zittermann et al. 2009, Semba et al. 2009, Semba et al. 2010, Hutchinson et al. 2010). Only Pilz et al. and Semba et al. also adjusted the results for parathyroid hormone levels. Furthermore, Hutchinson et al. did not include PTH in their “fully adjusted” model because PTH was not measured in all patients (approximately 50%), but when PTH was entered into the models the significance of 25-OHD as a mortality indicator was lost. However, this was most probably due to the collinearity between PTH and 25-OHD.

To date, there has been just one large-scale randomized controlled vitamin D supplementation trial having all-cause mortality as a primary endpoint (LaCroix et al. 2009). LaCroix et al. randomized 36 282 women aged 50–79 years from 40 US centres to 400 IU of vitamin D3 with 1000 mg elemental calcium carbonate daily or placebo for 7 years. They found an almost significant benefit of the supplementation in terms of all-cause mortality (HR = 0.91, 95% CIs 0.83–1.01). The study had problems with a high dropout rate and intervention contamination. Furthermore, the dose of vitamin D supplementation was relatively low for older adults, perhaps resulting in the larger range of 95% CIs in patients aged 70 years or older (HR = 0.95, 95% CIs 0.80–1.12) compared to the younger patients (HR = 0.89 95% CIs 0.79–1.01).

The suggested mechanisms for the causal association between vitamin D and mortality have included hypertension through the activation of the renin-angiotensin system (RAS)
(Zittermann et al. 2006) and atherosclerosis through immune dysfunction (Saggese et al. 1989). 1.25-OHD is a negative endocrine regulator of the RAS (Li 2003), and experiments on VDR knockout mice have suggested that cardiac hypertrophy is a consequence of activation of both the systemic and cardiac RAS (Xiang et al. 2005). In addition, the immune system appears to be involved in the pathogenesis of atherosclerosis (Libby 2002), and 1.25-OHD seems to have immunosuppressive effects, with a reduction in lymphocyte proliferation and the production of cytokines (Saggese et al. 1989).

Studies evaluating the effect of 25-OHD concentrations on cognition have reported mixed results, in part depending on which tests were used to assess cognitive function as well as differences in sample sizes. Overall, it appears that cognitive function, as assessed by the MMSE, is not associated with 25-OHD concentrations. However, other instruments that assess executive functioning have associated better performance with higher 25-OHD concentrations. Most of the studies have also been limited by cross-sectional methodology and a single measurement of 25-OHD, which does not allow control for seasonal fluctuation (Barnard K & Colón-Emeric 2010).

2.3.3.2 Chronic kidney disease

Secondary hyperparathyroidism occurs in chronic kidney disease as an adaptive response to deteriorating renal function. A combination of factors contribute to the increase in parathyroid hormone that are additive as the glomerular filtration rate (GFR) decreases. Circulating 1.25-OHD begins to decrease in stage 2, and continues to fall as the renal mass decreases and renal 1α-hydroxylase enzyme is inhibited by hyperphosphataemia, hyperuricaemia, metabolic acidosis, and vitamin D deficiency. As GFR decreases below 60 ml/min/1.73m², P is retained and stimulates synthesis and secretion of parathyroid hormone. Hypocalcaemia, the hallmark of renal failure at the beginning of the disease, develops as the GFR decreases below 50 mL/min/1.73m², further stimulating the release of the hormone. As GFR decreases further, the half-life of intact parathyroid hormone (1–84) increases and C-terminal fragments of the hormone accumulate. A relative state of end-organ resistance to the hormone exists, but chronic elevation of it has major consequences, resulting in bone loss (particularly cortical bone), fractures, cardiovascular disease, and increased mortality (Fraser 2009).

The impaired survival associated with progressive chronic kidney disease has been well documented (Weiner et al. 2004a, Weiner et al. 2004b, Tonelli et al. 2006, Chronic Kidney Disease Prognosis Consortium 2010). Tonelli et al. performed a meta-analysis of 39 studies that followed a total of 1 371 990 participants. They reported that during a median follow-up ranging from 1 to 16 years, the unadjusted relative risk for mortality in participants (mean age from 34 to 79 years) with reduced kidney function compared with those without ranged from 0.94 to 5.0 and was significantly more than 1.0 in 93% of cohorts. Among the 16 studies that provided suitable data, the absolute risk for death increased exponentially with decreasing renal function. The prevalence and severity of chronic kidney disease has been shown to increase with age, but this increase may be exaggerated by the current use of estimated GFR in CKD classification (Abdelhafiz et al. 2010).
In fact, nearly half of the consecutive oldest old patients (≥ 80 years, N = 138, mean GFR = 32 ml/min/1.73m²) referred to a nephrology centre were classified to suffer from stage 4 or higher CKD (El-Ghoul et al. 2010). However, in one-third of the patients the renal dysfunction was found to be nonprogressive and associated with a lower mortality rate than in those with progressive kidney dysfunction. Simple covariates (low proteinuria, lack of hypertension, low cardiovascular comorbidity) predicted nonprogression of CKD in these patients.

There are several possibilities for the mechanisms by which CKD might mediate the increased risk of death. First, CKD often coexists with other cardiovascular risk factors, including dyslipidaemia, hypertension, smoking, and diabetes (Hannedouche et al. 1993). Second, patients with evidence of renal disease are less likely to receive proven efficacious therapies (Tonelli et al. 2001). Third, impaired kidney function may merely be a marker for the severity of a vascular disease, including atherosclerosis, that is not yet clinically evident (Abdelhafiz et al. 2010). Fourth, impaired kidney function is associated with markers of inflammation and other putative risk factors of cardiovascular events and subsequent death (Garg et al. 2001, Stenvinkel et al. 2002, Stuveling et al. 2004, Knight et al. 2004). Finally, vascular and soft-tissue calcification, including the rare but often fatal calcific uraemic arteriopathy, is strongly linked with frank elevations in serum calcium, P, and parathyroid hormone in the setting of chronic kidney failure (Kestenbaum & Belozeroff 2007).

The association of the parameters of mineral metabolism with excess mortality seems to be U-shaped in patients with advanced chronic kidney disease. In a follow-up (median 21 months) study of 7970 random haemodialysis patients from 11 European countries, both low and high levels of total CaT (< 2.10 and > 2.75 mmol/l), P (< 1.31 and > 1.78 mmol/l), and parathyroid hormone (< 150 ng/l and > 300 ng/l) were associated with increased risk of all-cause mortality in a fully adjusted model (Floege et al. 2010). Furthermore, Floege et al. used a time-dependent model, which also evaluates any potential effects of updating exposure and selected covariates over time. The U-shaped association has also been supported by a previous systematic review reporting a similar relationship between PTH and mortality (Kestenbaum & Belozeroff 2007). However, Floege et al. used two different methods for PTH analysis, which may result in misclassification of patients (Souberbielle et al. 2006), even though the updating of data over time reduces the possibility of such bias. Other limitations of the study by Floege et al include common missing data and a lack of 25-OHD measurements.

Observational studies have also associated parathyroidectomy with improved survival of patients suffering from renal failure related secondary hyperparathyroidism (Costa-Hong et al 2007, Dussol et al. 2007, Trombetti et al. 2007, Kestenbaum et al. 2004). The median survival of dialysis patients (N = 9116) was over six months longer during a median follow-up of 3 years in patients undergoing first parathyroidectomy compared to individually matched control patients who did not undergo parathyroidectomy (Kestenbaum et al. 2004).

Cross-sectional studies have also suggested that impaired kidney function is associated with cognitive impairment in the elderly (Hailpern et al. 2007, Kurella Tamura et al. 2008, Barzilay et al. 2008) However, there have been few longitudinal studies examining the
association of impaired kidney function with the course of cognitive decline, and results from studies that have been reported are conflicting (Kurella et al. 2005, Slinin et al. 2008). Recently, in a cohort of more than 850 community-dwelling older adults without dementia (GFR = 59.0 ± 15.8 ml/min/1.73 m², MMSE = 27.9 ± 2.1), a decreased level of GFR or the presence of impaired kidney function at baseline was associated with a more rapid rate of cognitive decline (Buchman et al. 2009). This association between impaired kidney function and cognitive decline persisted after excluding participants with severely impaired renal function. The association of kidney function and cognition persisted even after controlling for several potential confounders such as BMI, serum haemoglobin, physical activity, social activity, vascular risk factors, vascular diseases, and depressive symptoms. Further analyses showed that kidney function was related to specific cognitive abilities, including episodic memory, semantic memory, and working memory, but not perceptual speed or visuospatial abilities. The systemic microvascular disease involving both renal and cerebral vasculature has been proposed as a potential mechanism of cognitive impairment in patients with chronic kidney disease (Murray 2008), but further studies are needed to determine the detailed biological basis for the association of kidney function and cognition.

2.4 Parathyroid hormone as an outcome indicator in old age

The prognostic significance of the parathyroid hormone concentration has been extensively studied in patients suffering from PHPT or SHPT due to renal failure. However, the significance of the parathyroid hormone concentration as an independent outcome indicator has been less well investigated. The following chapter focuses on studies that have addressed the association of PTH with mortality and cognition in other populations than selected hypercalcaemic PHPT patients or SHPT patients due to chronic kidney disease. Furthermore, studies published simultaneously with the present research are reviewed in the discussion.

2.4.1 Mortality

The predictive value of elevated PTH levels for increased mortality has been addressed in prospective studies in critically ill emergency room patients (Carlstedt et al. 1997, Carlstedt et al. 1998), in operatively treated hip fracture patients (Fischer et al. 2007) and in nursing home residents (Sambrook et al. 2004, Chen et al. 2008).

The predictive role of PTH was first reported by Carlstedt et al. in 1997. The authors investigated the occurrence of hypocalcaemia and elevated PTH levels and their relationship with mortality and the severity of disease in a broad spectrum of 140 acutely ill patients suffering from common diseases such as stroke, acute abdominal disorders, obstructive lung diseases, heart failure, acute myocardial infarction, angina pectoris, trauma and infectious diseases (Carlstedt et al 1997). PTH was significantly elevated in non-survivors compared with survivors, and was found to be a stronger predictor of
mortality than the severity of disease score. In accordance with the early results of Carlstedt et al. in 238 consecutive older operatively treated hip fracture patients (mean age 81.9 ± 7.8 years; 72% women), an elevated serum PTH level also emerged as a strong predictor of in-hospital all-cause mortality (HR = 18.5; 95% CI 2.0–72.3; p = 0.010) independently of age, sex, the 25-OHD level, and comorbidities (Fisher et al. 2007). However, the calcium concentration was not included in the multivariate model, even though the patients with higher PTH also had slightly higher levels of albumin-adjusted calcium (2.29 vs. 2.24 mmol/l, p = 0.001). Nevertheless, PTH levels also predicted perioperative myocardial injury indicated by TnI elevation above 0.006 µg/l in the study by Fisher et al. (2007).

The predictive value of elevated PTH levels for impaired survival extends beyond the short follow-up of studies performed by Carlstedt et al. (1997) and Fisher et al. (2007). This was shown during a mean follow-up of 31 months in a cohort study of older people (N = 842, mean age 85 years) living in residential care facilities (Sambrook et al. 2004). Furthermore, in this study PTH, but not 25-OHD, remained a significant predictor of mortality in the multivariate analyses corrected for health status, nutritional status, and renal function or bone ultrasound attenuation, in addition to serum calcium and P. In an extended follow-up study on the same study population, the absence of secondary hyperparathyroidism in the presence of hypovitaminosis D was common in the frail elderly and was associated with longer survival, similar to that observed in vitamin D-replete elderly subjects (Chen et al. 2008). Their sample included 1280 older men and women. The prevalence of hypovitaminosis D (25OHD < 39 nmol/l) and absence of secondary hyperparathyroidism (PTH > 64 ng/l) in the presence of hypovitaminosis D was 77.5% and 53.3%, respectively.

Taken together, these epidemiological studies indicate an increased risk of mortality in selected patients with elevated serum PTH levels. The association seems to be independent of serum calcium levels, renal function and vitamin D status, and also appears to apply in older patients with different medical backgrounds.

### 2.4.2 Cognitive functions

The literature on the independent association between PTH and cognitive functions outside PHPT and renal failure patients is scarce. To date, there have only been three cross-sectional studies partly addressing this issue (Jorde et al. 2006, Hoogendijk et al. 2008, Braverman et al. 2009).

Jorde et al. investigated 21 normocalcaemic SHPT patients without renal failure (CaT < 2.40 mmol/L, serum PTH > 58 ng/l, and normal serum creatinine) and compared them with 63 control subjects using tests for cognitive and emotional function (Jorde et al. 2006). Those in the SHPT group had significantly impaired performance in 3 of 14 cognitive tests (Digit span forward, Stroop test part 1 and 2, and Word association test) as compared with the controls, and also had a significantly higher Beck Depression Inventory score. In a multiple linear regression model, a high serum PTH level was significantly associated with low performance in the Digit span forward, Stroop test part 1 and 2, and
Digit Symbol tests. A low level of serum 25-OHD was significantly associated with a high depression score.

Since depressed mood is a well-known confounder of cognitive functions, it is worthwhile to also present the data from another large population-based cross-sectional study of older persons (1282 community residents aged 65 to 95 years) reporting a significant association between depression status and severity with decreased serum 25-OHD levels ($P = 0.03$) and increased serum PTH levels ($P = 0.008$) (Hoogendijk et al. 2008). Furthermore, some evidence also suggests that parathyroidectomy alleviates cognitive symptoms independently of changes in calcium levels or renal function in patients suffering from PHPT or SHPT due to renal failure (Chou et al. 2008, Burney et al. 1999), supporting the independent role of PTH. However, the current data are scarce and lack consistency.

Considering the fact that there are receptors for PTH and 1,25-dihydroxyvitamin D in the brain, and that there are clinical and experimental data indicating that PTH and vitamin D may affect cerebral function, future studies will be important in this field.
3. AIMS OF THE STUDY

The studies presented in this thesis aimed at evaluating PTH as an outcome indicator in older people. The specific aims of the studies were:

(1) To investigate the relationship between PTH and serum 25-OHD levels.

(2) To evaluate the factors affecting the PTH response to vitamin D supplementation.

(3) To explore the prognostic significance of serum PTH levels in older adults during the last years of life.
4. MATERIALS AND METHODS

This thesis is based on six original publications: one meta-analysis of previous clinical trials (I), one cross-sectional study (II), one clinical vitamin D supplementation trial (III), and three prospective follow-up studies (IV–VI). The aims, study type, sample size, inclusion criteria, and primary endpoints of each study are shown in Table 2.

<table>
<thead>
<tr>
<th>Study</th>
<th>Aims</th>
<th>Study type</th>
<th>N</th>
<th>Inclusion criteria</th>
<th>End points</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Relationship between PTH and vitamin D</td>
<td>Meta-analysis</td>
<td>6290</td>
<td>All clinical trials published until February 2007</td>
<td>Correlation and associating factors</td>
</tr>
<tr>
<td>II</td>
<td>Relationship between PTH and vitamin D</td>
<td>Cross-sectional analysis</td>
<td>218</td>
<td>Long-term inpatients in Helsinki; age ≥ 65 years; chronic immobility; stable general condition; no medication affecting calcium homeostasis</td>
<td>Correlation and associating factors</td>
</tr>
<tr>
<td>III</td>
<td>PTH response to vitamin D supplementation</td>
<td>Clinical trial</td>
<td>218</td>
<td>Long-term inpatients in Helsinki; age ≥ 65 years; chronic immobility; stable general condition; no medication affecting calcium homeostasis</td>
<td>Changes in PTH concentration</td>
</tr>
<tr>
<td>IV</td>
<td>Independent prognostic significance of PTH</td>
<td>Prospective follow-up of a population-based cohort</td>
<td>567</td>
<td>Complete data on PTH and mortality at baseline</td>
<td>All-cause mortality</td>
</tr>
<tr>
<td>V</td>
<td>Independent prognostic significance of PTH</td>
<td>Prospective follow-up</td>
<td>218</td>
<td>Long-term inpatients in Helsinki; age ≥ 65 years; chronic immobility; stable general condition; no medication affecting calcium homeostasis</td>
<td>All-cause mortality</td>
</tr>
<tr>
<td>VI</td>
<td>Independent prognostic significance of PTH</td>
<td>Prospective follow-up of a population-based cohort</td>
<td>514</td>
<td>Complete data on PTH and cognition excluding very severe dementia at baseline</td>
<td>Cognitive decline</td>
</tr>
</tbody>
</table>
4.1 Meta-analysis of data on the relationship between PTH and 25-OHD

The meta-analysis of the published vitamin D supplementation trials (Study I) was based on a PubMed search performed in February 2007. The primary search was limited to clinical or randomized controlled trials, and the terms “vitamin D” AND “parathyroid hormone” were used. The primary search included 519 articles. A total of 102 articles that addressed the effects of vitamin D2 or D3 supplementation were selected for closer review. Trials not reporting both pre- and post-trial PTH and 25-OHD levels or focusing on PHPT or SHPT due to renal failure were excluded. The detailed flow-chart for the trial selection is shown in Figure 3. Furthermore, 11 other articles were included in the meta-analysis through the review of the reference lists.

![Flowchart of article selection for the meta-analysis](image)

4.2 Subjects

Two study populations consisted of the long-term care inpatients from municipal hospitals in Helsinki, Finland, participating in a vitamin D supplementation trial (Studies II, III, and V) and a follow-up of a random cohort of older people living in Helsinki (Studies IV and VI).

The long-term inpatients (N = 1215) from four municipal hospitals in Helsinki, Finland, were screened between September and December 2005 to participate in a vitamin D
supplementation trial. The inclusion criteria were age over 65 years, chronically impaired mobility, and stable general condition. However, a large majority of the patients were excluded during screening because of their present or recent (1 year) medication affecting calcium metabolism (vitamin D supplements, antiepileptics, glucocorticoids, osteoporosis medication). After baseline laboratory analyses, patients with markedly elevated creatinine (> 125 µmol/l), hypercalcaemia (Ca^2+ > 1.32 mmol/l), hypo- (thyrotropin > 5.3 mU/l) or hyperthyroidism (thyrotropin < 0.2 mU/l) were also excluded. The flow chart of the vitamin D supplementation trial is shown in Figure 2.

![Flowchart of the vitamin D supplementation trial](image)

**Figure 7**  
*Flowchart of the vitamin D supplementation trial*

In the prospective follow-up study called the Helsinki Aging Study, a random sample of persons born in 1904 (N = 300), 1909 (N = 300), and 1914 (N = 300) was selected from the census register in Helsinki, Finland (Tilvis et al. 2004). The participants alive at entry (N = 795) were mailed a questionnaire including several items about illnesses, medications, functioning, mood, and attitudes towards life. A total of 144 persons refused
to answer. At entry, participants (N = 651) were examined clinically by a nurse, general practitioner, neurologist, and cardiologist, and the patient records were collected.

The characteristics of the three different study populations are shown in Table 3. The patients in long-term care were the oldest. The PTH levels were also highest and 25-OHD levels lowest in these patients. The youngest patients with the lowest PTH levels were found among the data collected for the meta-analysis.

Table 3. Characteristics of patients by study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Meta-analysis of clinical trials</th>
<th>Long-term care inpatients</th>
<th>General aged population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>6290</td>
<td>218</td>
<td>567</td>
</tr>
<tr>
<td>Age, years</td>
<td>71.5 ± 10.7</td>
<td>84.5 ± 7.5</td>
<td>80.4 ± 4.2</td>
</tr>
<tr>
<td>Women, %</td>
<td>NA</td>
<td>81.7</td>
<td>73.7</td>
</tr>
<tr>
<td>Co-morbidity, %</td>
<td>NA</td>
<td>NA</td>
<td>74.3</td>
</tr>
<tr>
<td>Severe dementia, %</td>
<td>NA</td>
<td>74.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Chronic immobility, %</td>
<td>2.7</td>
<td>100.0</td>
<td>NA</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>NA</td>
<td>22.5 ± 4.7</td>
<td>25.6 ± 4.2</td>
</tr>
<tr>
<td>25-hydroxyvitamin D, nmol/l</td>
<td>49.5 ± 22.4</td>
<td>22.8 ± 10.0</td>
<td>NA</td>
</tr>
<tr>
<td>25-OHD &lt; 50 nmol/l, %</td>
<td>51.7</td>
<td>98.2</td>
<td>NA</td>
</tr>
<tr>
<td>Parathyroid hormone, ng/l</td>
<td>43.8 ± 16.6</td>
<td>60.9 ± 35.7</td>
<td>54.4 ± 64.7</td>
</tr>
<tr>
<td>Hyperparathyroidism*, %</td>
<td>17.3</td>
<td>23.4</td>
<td>32.6</td>
</tr>
<tr>
<td>Ionised calcium, mmol/l</td>
<td>NA</td>
<td>1.22 ± 0.39</td>
<td>1.26 ± 0.05</td>
</tr>
<tr>
<td>Total calcium, mmol/l</td>
<td>NA</td>
<td>2.32 ± 0.10</td>
<td>2.41 ± 0.11</td>
</tr>
<tr>
<td>Creatinine μmol/l</td>
<td>NA</td>
<td>60.9 ± 19.7</td>
<td>94.4 ± 20.5</td>
</tr>
<tr>
<td>Glomerular filtration rate, ml/min/1.73m2</td>
<td>NA</td>
<td>64.5 ± 26.3</td>
<td>48.2 ± 12.9</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>NA</td>
<td>33.4 ± 3.3</td>
<td>37.5 ± 3.4</td>
</tr>
</tbody>
</table>

* PTH > 55 ng/l in the general aged population and systematic review; PTH > 73 ng/l in bedridden older inpatients
NA = data not available

4.3 Interventions

The average daily doses of cholecalciferol or ergocalciferol were 853 ± 814 IU and 3602 ± 4366 IU, respectively, in the 72 vitamin D supplementation groups included in the meta-analysis (Study I). The average daily calcium dose was 564 ± 503 mg. The length of
supplementation was described as the time interval between laboratory analyses, the shortest being three weeks and the longest three years. However, 71.5% of the patients were supplemented for six months.

The eligible long-term care inpatients patients (N = 218) were randomized into three treatment groups (Study III) receiving cholecalciferol (Vigantol®, Merck KGaA, Darmstadt, Germany 20 000 IU/ml in Migliol® oil) in doses of 0 µg, 140 µg, or 420 µg (groups I, II, and III) every two weeks, corresponding to daily intakes of 0 IU, 400 IU, or 1200 IU, respectively (Figure 6). All three groups received identical volumes (26 drops = 0.84 ml) of the medication oil. Furthermore, the oil was swallowed entirely in the presence of the nurse and given with a small amount of food or drink, if necessary. Each bottle was individually coded to blind the patients and ward nurses of both the content and the group labels of the bottles. A daily calcium carbonate substitution of 500 mg was given to patients during the intervention in case of insufficient use of dairy products (N = 40). The duration of the intervention was six months.

4.4 Data collection

Table 4 provides a summary of the laboratory analyses, clinical assessments and mortality data collected from the 52 trials included in the meta-analysis, from the long-term care inpatients participating in the vitamin D supplementation trial, and from the general aged population. It should be noted that data from placebo or control groups were not included in the meta-analysis.

<table>
<thead>
<tr>
<th>Table 4. Summary of data collection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard laboratory analyses</strong></td>
</tr>
<tr>
<td>Meta-analysis</td>
</tr>
<tr>
<td>Long-term care inpatients</td>
</tr>
<tr>
<td>General aged population</td>
</tr>
</tbody>
</table>
4.4.1 Laboratory analyses

Two different methods for PTH analyses were used in this series of studies. In the general aged population (Studies IV and VI), an immunoradiometric method (AllegroR Intact PTH kit, Nichols Institute, San Juan Capistrano, CA, USA) was used to determine serum PTH (Ratcliffe et al. 1989). All PTH analyses were performed simultaneously. The sensitivity of the assay was 5 ng/l, the intra-assay CV was 3.6% at 45 ng/l and 6.1% at 390 ng/l, and the inter-assay CV was 7.9% at 22 ng/l and 7.7% at 273 ng/l. The reference range was 10–55 ng/l. In the long-term care in patients, the PTH concentrations were determined with a solid-phase, two-site chemiluminescent enzyme-labelled immunometric assay (IMMULITE 2000 Intact PTH). The intra-assay coefficient of variation (CV) (N = 20) was 5.7% at 72 ng/l and 4.3% at 258 ng/l (Kao et al. 1992). The interassay CV (N = 10) was 6.3% at 54 ng/l and 8.8% at 387 ng/l. It is well known that the method of determination has a significant impact on the observed PTH concentration, and the IMMULITE 2000 Intact PTH has been shown to yield approximately 30 to 40% higher values for the PTH concentration (Souberbielle et al. 2006).

Serum total calcium (CaT) was determined by the method of Gindler (Gindler et al. 1972) in an automatic analyser (Hitachi model 705, Naka Woeks, Hitachi Ltd, Katsuta Japan). The inter-assay CV was 1.4% (long-term serum control) and the intra-assay CV was 0.7% in a subject serum sample with CaT 1.27 mmol/l, and 1.1% in a sample with CaT 3.15 mmol/l. The reference range was 2.20–2.60 mmol/l. Ionized calcium (Ca\(^{2+}\)) was measured with an ion-selective electrode at pH 7.40 (Bowers et al. 1986). The inter-assay CV was 1.7% at a Ca\(^{2+}\) level of 0.75 mmol/l and 0.8% at a Ca\(^{2+}\) level of 1.75 mmol/l (long-term aqueous controls). The intra-assay CV was 0.5% both in a subject serum with 0.70 mmol/l Ca\(^{2+}\) and in one with 1.74 mmol/l Ca\(^{2+}\). The reference range was 1.17–1.29 mmol/l.

Additional analyses in the general aged population included apolipoprotein E (APOE) allele phenotyping, which was done according to Menzel, Kladetzky, and Assmann (Menzel et al. 1982) with some modifications (Enhholm et al. 1986).

Additional analyses in the long-term care patients included vitamin D status by 25-OHD and bone turnover by amino-terminal propeptide of type I procollagen (PINP), carboxyl-terminal telopeptide of type I collagen (ICTP). High performance liquid chromatography was used to measure plasma 25-OHD levels (Turpeinen et al. 2003). The method’s limit of quantification, defined as the lowest concentration with a signal-to-noise ratio of 10:1, was 10 nmol/L. The within-assay CV was 5.6% at 21.6 nmol/l (N = 14) and 3.7% at 138 nmol/l (N = 15). The total CV was 7.3% at 16.4 nmol/l (N = 12) and 5.7% at 167 nmol/l (N = 15). Similar to PTH measurement, the observed concentration of 25-OHD also depends on the method used, but to a lesser extent (Carter 2010). However, the HPLC method used in the present study has been considered as one of the most reliable methods. Plasma ICTP levels were determined by radioimmunoassay (RIA) (Risteli et al. 1993). The method yielded CVs between 3 and 8% for a wide range of concentrations. The levels of PINP were also analysed using RIA (Melkko et al. 1996). The inter- and intra-assay CVs ranged from 3.1 to 9.3% (N = 191) for values within the reference intervals (mean ±
2SD) for intact PINP in serum, which ranged from 19 to 84 g/l for women and from 20 to 76 g/l for men.

### 4.4.2 Clinical assessments

All clinical assessments of the long-term care inpatients were based on the Resident Assessment Instrument Minimum Data Set 2.0 assessments (Morris et al. 1990). Cognition of the patients was assessed using the cognitive performance scale (CPS) (Morris et al. 1994). The CPS is divided into seven categories of cognitive impairment (0 = none, 1 = borderline, 2 = mild, 3 = moderate, 4 = moderate to severe, 5 = severe and 6 = very severe), and the scores closely correspond with those generated by the Mini-Mental State Examination (24.9, 21.9, 19.2, 15.4, 6.9, 5.1, and 0.4, respectively).

Among the random age cohorts, all subjects (N = 567) were clinically examined and assessed for co-morbidity. Subjects were identified as healthy (no co-morbidity) if their subjective and objective (according to the examining physicians) health was good or moderate, they had no hypertension, diabetes, dementia, or symptoms of cardiovascular, cerebrovascular, or pulmonary diseases, cancer or other disabling diseases, and had a normal exercise tolerance in relation to their history. Complete assessment of cognition, including the Mini-Mental State Examination (MMSE) (Folstein et al. 1975) and Clinical Dementia Rating (CDR) (Hughes et al. 1982), was performed in 514 subjects at baseline. Cognitive impairment was defined as an MMSE score below 24 and CDR class one or higher. Patients with a baseline MMSE score below 4 and patients in the highest CDR class were excluded from follow-up analyses for cognitive decline in study II, because cognitive decline was defined as at least a 4-point decrease in the MMSE score or increase in the CDR class. Both CPS and MMSE are validated and widely accepted tools for the assessment of cognitive function in the elderly.

The body mass index (BMI) and glomerular filtration rate (GFR) estimated by the Cockcroft-Gault equation (Levey et al. 2003) were also calculated in both study populations.

### 4.4.3 Mortality data

Two-year mortality data on the long-term care inpatients were collected from patient records in January 2008, whereas among the random age cohorts the date of death for each diseased subject was drawn from the National Census Records in July 2007. The collection was complete for the long-term care patients, but five subjects were excluded from the survival analysis among the random age cohorts, because the mortality data were not obtained due to an incomplete social security number.
4.5 Statistics

The data were analysed using Windows SPSS (SPSS for Windows, Chicago: SPSS Inc.). All variables were examined for outliers, missing data, and entry error. Differences in proportions were tested with a chi-squared test, and continuous measurements with analysis of variance. The independent samples T-test was used to test the equality of means between the two populations.

In the meta-analysis, linear and logarithmic curve estimation regression models for baseline and post-trial levels of PTH as well as for changes in PTH were produced. Series of multivariate linear regression models were also created, respectively, to determine the changes in $R^2$ and their levels of significance by entering each of the potential determinants stepwise into the models. All variables were weighted by the number of patients in each intervention group.

For the follow-up studies, Kaplan-Meier analysis was used to determine the median survival times for patients with varying PTH levels. Series of Cox regression models were created in order to calculate the hazard ratios and their level of significance for the predictive value of PTH on survival. Series of multivariate logistic regression models were also created to calculate the risk ratios and their level of significance for the predictors of cognitive decline. Age and gender were used as standard covariates. Other confounders were retained in the model based upon their established association with the mortality rate. Covariates were entered one by one into the model. Logarithmic transformation of a variable was used in the calculation of P-values if the distribution was not normal. P-values below 0.05 were considered statistically significant.
5. RESULTS

5.1 Relationship between PTH and 25-OHD levels (Studies I-III)

In the meta-analysis of the published clinical vitamin D supplementation trials (Study I), the relationship between PTH and 25-OHD levels and the factors affecting the effects of vitamin D supplementation on the PTH response were addressed. These issues were also investigated in a randomized controlled trial of 218 older chronically bedridden inpatients (Studies II and III).

5.1.1 Cross-sectional observations

A significant negative correlation was found between baseline PTH and 25-OHD levels \( (r = -0.593, p < 0.001) \) in the meta-analysis. This held also true when all pre- and post-trial values were scattered (Figure 8).

![Figure 8](image_url)

**Figure 8** Relationship between serum levels of 25-hydroxyvitamin D and parathyroid hormone in 72 intervention groups of 52 vitamin D supplementation trials \( (N = 6290) \) when all pre- and post-trial values were scattered. \( R \)-square for the linear correlation is 0.294 \( (p < 0.001) \). Parathyroid hormone levels are means and the standard deviation is shown by error bars.
The correlation between baseline PTH and 25-OHD levels ($r = -0.126$, $p = 0.064$) was substantially weaker in the chronically immobile long-term care inpatients in Study III (Table 5). Furthermore, in Study II the PTH levels were elevated above the laboratory reference (72 ng/l) in 25% of the long-term care inpatients, despite widespread (99%) vitamin D deficiency. The proportion of low PTH levels, i.e. blunted PTH responses, was also higher in the long-term care patients than in the meta-analysis (Table 5).

<table>
<thead>
<tr>
<th>Table 5. Relationship between PTH and 25-OHD levels.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline Pearson’s correlation coefficient</strong></td>
</tr>
<tr>
<td>Metanalysis</td>
</tr>
<tr>
<td>Long-term care inpatients</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* Percentage of PTH < 40 ng/l in patients/intervention groups with 25-OHD < 50 nmol/l

In fact, in addition to their chronic immobility, the long-term-care inpatients were markedly older (84.5 ± 7.5 years, range 65.0–104.3 years) than the patients included in the meta-analysis (Table 3). In the multiple regression analyses, age and chronic immobility also emerged as independent predictors of the baseline PTH concentration (Table 6).

<table>
<thead>
<tr>
<th>Table 6. Multiple linear regression analyses of average pre- and post-trial parathyroid hormone levels and their changes in 72 treatment groups of 52 vitamin D supplementation trials (N = 6 290)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change statistics</strong></td>
</tr>
<tr>
<td><strong>Dependent variable: Pre-trial PTH</strong>*</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Dependent variable: Post-trial PTH†</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Dependent variable: Changes in PTH‡</strong></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

* Adjusted for pre-trial 25-OHD, immobility, and age.
† Adjusted for post-trial 25-OHD, pre-trial PTH, immobility, and.
‡ Adjusted for changes in 25-OHD, pre-trial PTH, immobility, and age.
Since low PTH levels, i.e. blunted PTH responses, were prevalent in the long-term care inpatients, the factors associating with the lowest PTH quartile (PTH < 38 ng/l) were further addressed (Study II). Patients with low PTH were characterised by a history of hip fracture, low BMI, higher bone resorption marker ICTP, and shorter length of stay. In the logistic multiple regression analysis, a history of hip fracture, BMI, and ICTP also emerged as independent associates of the lowest PTH quartile (Table 7). Blunted PTH also proved to be persistent over time. At six-month re-examination, a blunted PTH response was recorded in 59.3% (35/59) of the surviving patients. Closer analysis also revealed that in 32 (76.2%) of the 42 survivors with a blunted baseline PTH response, the PTH level remained within reference values even after 6 months, despite persistent vitamin D deficiency. SHPT developed in only eight patients (19.0%) with blunted PTH at baseline. Furthermore, all patients remained bedridden.

**Table 7. Independent associates of the lowest quartile of plasma intact parathyroid hormone (PTH) at baseline (N = 52)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% Confidence Intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>History of hip fracture</td>
<td>2.893</td>
<td>1.239 6.754</td>
</tr>
<tr>
<td>Body mass index</td>
<td>0.910</td>
<td>0.841 0.984</td>
</tr>
<tr>
<td>ICTP†</td>
<td>1.120</td>
<td>1.012 1.240</td>
</tr>
</tbody>
</table>

* Length of stay, history of hip fracture, body mass index, and ICTP were entered.
† Carboxy-terminal telopeptide of type I collagen.

### 5.1.2 Responses of parathyroid activity to vitamin D supplementation

A strong negative linear (r = -0.620, p < 0.001) correlation between the changes in 25-OHD and PTH levels was found in the meta-analysis (Study I, Figure 9). Closer analysis revealed that PTH decreased consistently in most (63/72) of the intervention groups, the average decrease being 22.0 ± 15.7% in the responding groups. Again, the correlation between changes in PTH and 25-OHD was clearly weaker (r = -0.209, P = 0.005) in the chronically immobile older inpatients. Furthermore, in line with the cross-sectional observations, age and chronic immobility emerged as independent predictors of changes in PTH concentrations in the multiple regression analyses of the meta-analysis (Table 6). In fact, together with pre-trial PTH and changes in 25-OHD, age and chronic immobility explained 53.2% (R-square = 0.532) of the variation in the responses of PTH to vitamin D supplementation. Closer analysis also demonstrated that vitamin D supplementation resulted in a smaller decrease in PTH levels (-8.4% vs. -17.4%, p <0.001), despite a larger increase in 25-OHD levels (187.2% vs. 109.8%, p < 0.001), in the chronically immobile patients.

However, despite the older age and chronic immobility of the long-term care inpatients, both the six-month 400 IU/d and 1200 IU/d vitamin D supplementations were able to modestly reduce the PTH concentration (Study III, Table 8). Furthermore, the ratio of PINP to ICTP decreased significantly (0.2% with placebo, -11.8% with 400 IU/d,
-19.0% with 1200 IU/d, *P = 0.025*), providing further evidence for decreased bone turnover.

![Graph showing correlations between changes in the levels of 25-hydroxyvitamin D and parathyroid hormone](image)

**Figure 9** Correlations between changes in the levels of 25-hydroxyvitamin D and parathyroid hormone in 72 intervention groups of 52 vitamin D supplementation trials (*N* = 6290). R-square for the linear correlation is 0.385 (*p* < 0.001).

**Table 8. Changes in biochemical variables after six months of vitamin D supplementation**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th></th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I Placebo</td>
<td>II 400 IU/d</td>
<td>III 1200 IU/d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-hydroxyvitamin D nmol/l</td>
<td>1.9±10.2</td>
<td>26.5±11.8</td>
<td>49.1±19.5</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Parathyroid hormone ng/l</td>
<td>5.2±20.0</td>
<td>-4.2±20.1</td>
<td>-4.7±24.1</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>ICTP* μg/l</td>
<td>-0.60±2.48</td>
<td>-0.08±2.25</td>
<td>1.06±6.23</td>
<td>0.078</td>
<td></td>
</tr>
<tr>
<td>PINP† μg/l</td>
<td>-1.80±21.07</td>
<td>-7.51±24.86</td>
<td>-7.78±13.62</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>Ratio of PINP to ICTP‡</td>
<td>0.00±0.36</td>
<td>-0.14±0.50</td>
<td>-0.19±0.33</td>
<td>0.025</td>
<td></td>
</tr>
<tr>
<td>Ionized calcium mmol/l</td>
<td>0.017±0.034</td>
<td>0.024±0.073</td>
<td>0.032±0.038</td>
<td>0.061</td>
<td></td>
</tr>
</tbody>
</table>

* Carboxyl-terminal telopeptide of type I collagen (ICTP)
† Amino-terminal propeptide of type I procollagen (PINP)
‡ PINP / (7 x ICTP)
5.2 Predictive value of an elevated PTH concentration

The role of PTH as an outcome indicator was evaluated in both a general aged population (Studies IV and VI) and in long-term care inpatients (Study V).

A large majority of the patients were women, and the mean age was above 80 years in both study populations (Table 3). Old age was associated with impaired renal function, lower serum albumin, and an increased prevalence of secondary hyperparathyroidism, in particular, in both study populations. Co-morbidity was found in 74.3% of patients in the general aged population, whereas all of the older inpatients were considered co-morbid. Cognitive function was relatively well preserved (MMSE ≥ 24) in most (60.5%) persons of the general aged population, and severe dementia (MMSE < 7) was found in only 1.5% of patients. However, up to 74.5% of the older inpatients suffered from severe cognitive impairment (CPS > 3; comparable with MMSE < 7), and none of the inpatients had preserved cognitive functions (CPS = 0; comparable with MMSE > 22) at baseline.

The PTH concentrations ranged from 3 to 1394 ng/l and 12 ng/l to 268 ng/l in the general population and older inpatients, the cut-points for quartile IV being 63 ng/l, and 72 ng/l, respectively. Hyperparathyroidism was found in 32.6% (PTH > 55 ng/l, laboratory reference value) of those in the general aged population, the respective figure being 25.2% (PTH > 73 ng/l, laboratory reference value) in the older inpatients. Age and renal function associated with PTH levels in both study populations (Tables 9 and 10). High PTH concentrations were also significantly associated with impaired cognitive function in the general population. Patients with PTH in quartile IV were also characterized by co-morbidity and impaired renal function (both study populations), as well as a higher BMI (older inpatients).

<table>
<thead>
<tr>
<th>Variables mean ± SD</th>
<th>Quartile I</th>
<th>Quartile II</th>
<th>Quartile III</th>
<th>Quartile IV</th>
<th>P-value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>134</td>
<td>151</td>
<td>140</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>79.0±3.9</td>
<td>80.0±4.3</td>
<td>80.5±3.9</td>
<td>82.0±4.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Women, %</td>
<td>57.6</td>
<td>74.5</td>
<td>78.1</td>
<td>84.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Co-morbidity, %</td>
<td>69.2</td>
<td>73.8</td>
<td>80.1</td>
<td>74.4</td>
<td>0.215</td>
</tr>
<tr>
<td>Severe dementia, %</td>
<td>3.9</td>
<td>0.8</td>
<td>0.7</td>
<td>0.9</td>
<td>0.103</td>
</tr>
<tr>
<td>MMSE</td>
<td>23.8±5.4</td>
<td>24.2±5.4</td>
<td>23.8±5.5</td>
<td>22.4±5.6</td>
<td>0.048</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.1±4.0</td>
<td>25.4±3.9</td>
<td>25.8±4.1</td>
<td>25.0±5.0</td>
<td>0.270</td>
</tr>
<tr>
<td>Ionised calcium, mmol/l</td>
<td>1.26±0.04</td>
<td>1.25±0.04</td>
<td>1.25±0.04</td>
<td>1.26±0.08</td>
<td>0.397</td>
</tr>
<tr>
<td>Total calcium, mmol/l</td>
<td>2.40±0.08</td>
<td>2.39±0.08</td>
<td>2.39±0.10</td>
<td>2.42±0.14</td>
<td>0.217</td>
</tr>
<tr>
<td>Phosphate, mmol/l</td>
<td>1.03±0.13</td>
<td>1.03±0.14</td>
<td>1.00±0.14</td>
<td>1.02±0.16</td>
<td>0.206</td>
</tr>
<tr>
<td>Creatinine μmol/l</td>
<td>89.6±15.6</td>
<td>91.1±16.5</td>
<td>96.6±17.8</td>
<td>100.1±28.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GFR, ml/min/1.73m²</td>
<td>52.4±12.3</td>
<td>49.0±12.6</td>
<td>47.7±12.2</td>
<td>43.5±13.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>37.2±3.0</td>
<td>37.4±3.6</td>
<td>37.7±3.4</td>
<td>37.6±3.5</td>
<td>0.695</td>
</tr>
</tbody>
</table>

Table 9. Baseline characteristics of the general aged population by quartiles of PTH (cut-points for quartiles 33, 45, and 63 ng/l, respectively).
### Table 10. Baseline characteristics of chronically immobile older inpatients by quartiles of PTH (cut-points for quartiles 38, 58, and 72 ng/l, respectively).

<table>
<thead>
<tr>
<th>Variables mean ± SD</th>
<th>Quartile I</th>
<th>Quartile II</th>
<th>Quartile III</th>
<th>Quartile IV</th>
<th>P-value for Trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>52</td>
<td>54</td>
<td>57</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>84.1±7.5</td>
<td>82.9±6.9</td>
<td>83.7±7.7</td>
<td>87.4±7.0</td>
<td>0.007</td>
</tr>
<tr>
<td>Women, %</td>
<td>78.8</td>
<td>79.6</td>
<td>82.5</td>
<td>85.5</td>
<td>0.807</td>
</tr>
<tr>
<td>Severe dementia, %</td>
<td>71.2</td>
<td>75.9</td>
<td>75.0</td>
<td>75.9</td>
<td>0.934</td>
</tr>
<tr>
<td>CPS</td>
<td>4.8±1.5</td>
<td>4.9±1.5</td>
<td>5.0±1.3</td>
<td>4.8±1.3</td>
<td>0.950</td>
</tr>
<tr>
<td>History of hip fracture, %</td>
<td>25.0</td>
<td>9.3</td>
<td>12.3</td>
<td>9.1</td>
<td>0.056</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.0±4.1</td>
<td>22.4±5.4</td>
<td>22.5±4.2</td>
<td>23.9±4.7</td>
<td>0.016</td>
</tr>
<tr>
<td>25-OHD, nmol/l</td>
<td>24.3±8.8</td>
<td>23.7±10.2</td>
<td>22.1±10.7</td>
<td>21.2±10.2</td>
<td>0.374</td>
</tr>
<tr>
<td>ICTP</td>
<td>9.3±4.6</td>
<td>8.2±2.7</td>
<td>7.1±2.8</td>
<td>7.5±2.4</td>
<td>0.003</td>
</tr>
<tr>
<td>PINP</td>
<td>52.3±20.2</td>
<td>53.2±20.5</td>
<td>52.6±25.5</td>
<td>54.3±22.9</td>
<td>0.967</td>
</tr>
<tr>
<td>Ionised calcium, mmol/l</td>
<td>1.23±0.04</td>
<td>1.22±0.04</td>
<td>1.23±0.04</td>
<td>1.22±0.04</td>
<td>0.587</td>
</tr>
<tr>
<td>Total calcium, mmol/l</td>
<td>2.33±0.10</td>
<td>2.31±0.09</td>
<td>2.33±0.09</td>
<td>2.32±0.11</td>
<td>0.825</td>
</tr>
<tr>
<td>Phosphate, mmol/l</td>
<td>1.03±0.13</td>
<td>1.04±0.13</td>
<td>0.98±0.15</td>
<td>0.98±0.12</td>
<td>0.018</td>
</tr>
<tr>
<td>Creatinine μmol/l</td>
<td>57.8±17.6</td>
<td>57.8±18.8</td>
<td>58.7±20.9</td>
<td>69.0±19.3</td>
<td>0.005</td>
</tr>
<tr>
<td>GFR, ml/min/1.73m²</td>
<td>61.7±20.5</td>
<td>71.5±27.9</td>
<td>70.9±31.5</td>
<td>53.8±19.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>33.4±3.3</td>
<td>32.9±3.0</td>
<td>33.6±3.6</td>
<td>33.6±3.5</td>
<td>0.641</td>
</tr>
</tbody>
</table>

### 5.2.1 Survival prognosis

Up to 93% of the random persons aged 75, 80, and 85 years at entry had died during the long 17-year follow-up. The mean follow-up time of the older bedridden inpatients was not more than 25.3 months (range 24.4–26.5 months). However, up to 56.9% of the inpatients died during the follow-up.

Elevated serum PTH levels (IV quartile) were significantly associated with an impaired survival prognosis in both the general aged population and the older inpatients (Figures 10 and 11). In the general aged population, PTH levels above 63 ng/l predicted a 1.51-fold (95% CI: 1.25–1.86) over-mortality, resulting in a 2.3-year shorter median life expectancy. The predictive value of elevated PTH (quartile IV) was very similar in the bedridden inpatients, with a 1.58-fold (95%CIs 1.08–2.32) over-mortality and a 9.1-month shortening of the median life expectancy. It should be noted, however, that the cut-point for quartile IV of the PTH concentration that was used to stratify patients for comparison was slightly higher in the older inpatients than in the general aged population (72 ng/l vs. 63 ng/l).
Figure 10  Survival prognosis according to PTH quartiles in the general aged population. Median survival shown by lines for the first and fourth quartile.

Figure 11  Survival prognosis according to PTH quartiles in bedridden older inpatient. Median survival shown by lines.
The predictive value of elevated PTH also remained significant after adjusting for confounders in both patient groups (Table 11). Closer analysis revealed that over-mortality was consistent in all subgroups with one exception (Figure 12). The prognostic significance of elevated PTH for impaired survival was not observed in a subgroup of the older inpatients with preserved renal function. However, after controlling for GFR alone, the predictive value of elevated PTH also remained marginally significant (HR = 1.47, 95%CIs 0.99-2.17, p = 0.055) in the older inpatients.

<table>
<thead>
<tr>
<th>Table 11. Predictive value of elevated parathyroid hormone in the aged general population and chronically bedridden older inpatients.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>17-year follow-up of the general aged population (PTH ≥ 63 ng/l)</td>
</tr>
<tr>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Controlled for</td>
</tr>
<tr>
<td>Two-year follow-up of chronically bedridden older inpatients (PTH ≥ 72 ng/l)</td>
</tr>
<tr>
<td>Uncontrolled</td>
</tr>
<tr>
<td>Controlled for</td>
</tr>
</tbody>
</table>

* Adjusted for age, gender, co-morbidity, ionized calcium, and creatinine.
† Adjusted for age, gender, BMI, creatinine, 25-OHD, six-month vitamin D supplementation group, ionized calcium, and albumin.

Figure 12  The hazard ratios (95% confidence intervals by T-bars) for the predictive value of elevated PTH (≥ 63 ng/l) in relation to mortality by age cohort, gender, the presence of co-morbidity, the median of the estimated glomerular filtration rate (eGFR), and ionized calcium (Ca2+).
5.2.2 Cognitive decline

The predictive value of PTH for cognitive decline was evaluated in the general aged population alone, because the oldest inpatients were already severely demented at baseline.

Cognitive decline was assessed three times during the 10-year follow-up in the general aged population. Cognitive decline (MMSE-score decrease ≥ 4 points) was found in 15.5% of these subjects within the first year of follow-up, when cognition was assessed with the MMSE. The respective figure was 31.1% for an increase in the CDR class. Elevated PTH indicated a 2-fold higher risk for at least a 4-point decrease in the MMSE and a 3-fold risk for increase in the CDR class within the first year of follow-up (Figure 13). The risk remained significantly elevated even after controlling for age, gender, baseline cognition, Ca^{2+}, creatinine and APOE4.

![Figure 13](image_url) *Risk ratios (RR) and 95% confidence intervals (T-bars) for cognitive decline in patients with elevated parathyroid hormone (≥ 62 ng/l, quartile IV) in a series of logistic regression models. • RR for cognitive decline within the first year of follow-up with the MMSE, × RR for cognitive decline within the first year of follow-up with the CDR, ♦ RR for cognitive decline within five years of follow-up with the CDR. Model 1 = Unadjusted. Model 2 = Adjusted for age, gender, baseline MMSE or CDR, apolipoprotein E allele 4, ionized calcium, and creatinine.*

The five-year CDR assessment was completed in 56.9% of persons, in whom 28% had experienced cognitive decline. The MMSE was not used for the assessment of cognition at the five-year examination. Again, an elevated baseline PTH concentration predicted a 3-fold higher risk for cognitive decline (Figure 13). The significance (P = 0.025) also
remained after controlling for confounders. Furthermore, the predictive value of elevated PTH was consistent in subgroup analysis (Figure 14).

Figure 14  Unadjusted risk ratios (95% CIs with error bars) for cognitive decline according to the Mini-Mental State Examination (MMSE, decrease in score ≥ 4) and Clinical Dementia Rating (CDR, increase in class ≥ 1) within one- and five-year follow-ups in patients with an elevated PTH concentration (≥ 62 ng/l). Patients were stratified by baseline age, gender, the presence of apolipoprotein E allele 4 (APOE4), median levels of ionized calcium (Ca²⁺) and the estimated glomerular filtration rate (GFR). ● RR for cognitive decline within the first year of follow-up with the MMSE, × RR for cognitive decline within the first year of follow-up with the CDR, ● RR for cognitive decline within five years of follow-up with the CDR.
6. DISCUSSION

6.1 Main observations

This series of studies revealed that in older people without overt renal failure or severe hypercalcaemia, serum 25-OHD and PTH were closely associated, but this relationship was also affected by age and chronic immobility. Furthermore, a substantial proportion of old chronically bedridden patients did not respond to vitamin D deficiency by elevating PTH, and the effect of a high-dose six-month cholecalciferol supplementation on the PTH concentration was minor. This study demonstrated longitudinally for the first time that the absence of secondary hyperparathyroidism also persisted over time.

The second major finding of the present study was that even a subtle elevation of PTH to high-normal levels predicted impaired long-term health outcomes. Slightly elevated PTH concentrations indicated an increased risk of clinically significant cognitive decline and death during the last years of life in a general aged population. This association was also independent of serum ionized calcium (Ca\(^{2+}\)) and the estimated glomerular filtration rate (GFR). A slightly elevated PTH also indicated impaired two-year survival, even during the terminal years of frail elderly subjects, independent not only of Ca\(^{2+}\) and GFR, but even of 25-OHD levels.

6.2 Relationship between PTH and 25-OHD concentrations

According to the systematic meta-analysis of the present study (Study I), both older age and immobility associated with a blunted increase in the PTH concentration during vitamin D deficiency and with an attenuated response of PTH levels to vitamin D supplementation. These results accord well with previous cross-sectional studies showing associations between immobility and blunted PTH (Sorva et al. 1994, Bischoff et al. 1999, Sato et al. 1999, Chen et al. 2006), as well as age and attenuated elevation of PTH levels (Reginster et al. 1998). However, previous studies have not addressed the confounding role of age and immobility on the responses of PTH to vitamin D supplementation. The results of the present study suggest that the assumed health benefits of vitamin D supplementation mediated by lowering of PTH may also be compromised in disabled older persons. However, vitamin D supplementation should be considered, even in these patients, due to the well documented muscle effects of vitamin D, which are assumed to be independent of PTH (Bischoff-Ferrari et al. 2009b, Ceglia 2009).

This study also revealed that normal or low-normal PTH levels, i.e. blunted PTH, were persistent over time. Blunted PTH was originally described in cross-sectional studies of aged hip fracture patients, and prevalences of 53.9% and 52.5% were observed (Sahota et al. 2001, Fisher & Davies 2006). Recently, observations of blunted PTH have been reported in relatively large samples of postmenopausal women (N = 405, median age 50 years) and also in vitamin D insufficient subgroups (N = 516, mean age 54 years) of...
population-based cohorts (Rejnmark et al. 2008, Gunnarsson et al. 2009). Rejnmark et al. observed that PTH levels were below approximately 40 ng/l in 63.4% of patients with 25-OHD below 50 nmol/l. The prevalence of blunted PTH (~ 75%) was even higher in the present study (Study II). However, the upper reference limit for PTH was 72 ng/l in the long-term care inpatients (Study II). Nevertheless, the high prevalence of blunted PTH is well in line with another Finnish study on institutionalized middle-aged patients (N = 138, mean age 47 years) with intellectual disability (Kilpinen-Loisa et al. 2009). The blunted PTH response has additionally been demonstrated in the oldest of the elderly. Up to 35% of Italian centenarians (N = 104) had PTH levels within laboratory reference levels, despite severe vitamin D deficiency (25-OHD levels below 5 nmol/l in 95% of cases) (Passeri et al. 2003). It can be argued that blunted PTH could be a short-term phenomenon due to recently and/or suddenly impaired vitamin D stores. However, blunted PTH emerged as a persistent phenomenon in the present study (Study II), and to the best of our knowledge, this is the only study so far that has addressed the stability of blunted PTH over time. Furthermore, the rapid regulation of PTH concentrations in physiological conditions does not support the delayed response to vitamin D deficiency. Thus, it is clear that the blunted PTH response to vitamin D deficiency is a common and persistent phenomenon in elderly patients with different medical conditions.

The associations of low PTH with a lower BMI, elevated ICTP and with a tendency for lower creatinine observed in the present study also suggest that low PTH in hypovitaminosis D is characteristic of frail and immobile patients in a catabolic state. Unloading of bones has additionally been shown to result in the release of calcium into circulation from bones (Chen et al. 2006, Bischoff et al. 1999), which may partly explain the blunted PTH of these patients. In fact, hyperparathyroidism has been associated with sarcopenia and falls, as PTH may stimulate muscle protein breakdown (Visser et al. 2003). Furthermore, high PTH has been associated with increased adiposity, and a causal relationship has been suggested through decreased lipolysis caused by an increased PTH-related flux of calcium into adipocytes (Rejnmark et al. 2008). Moreover, vitamin D status, osteocalcin, calcium, P, and insulin have been reported to explain only a small proportion (18%) of this association (Pitroda et al. 2009). Thus, chronic elevation of parathyroid function may increase the risk of disability and ultimately chronic immobility, partly through the stimulation of muscle protein breakdown leading to decreased muscle strength and through the accumulation of body fat, causing an increased burden to weakening muscles. Finally, chronic immobility results in excess calcium flux from bones, causing suppression of the originally elevated PTH levels.

In addition to chronic immobilisation, smoking and alcohol intake have been associated with blunted PTH (Fisher et al. 2010). However, among the long-term inpatients (Study II) there were no active smokers or drinkers. Several age-dependent alterations in vitamin D and PTH metabolism have also been presented (Oudshoorn et al. 2009, Fisher et al. 2010). These may include age-related impairment of renal 1-alpha hydroxylation of 25-OHD to calcitriol, abnormalities of the 1.25-OHD receptor, and intracellular magnesium depletion leading to impaired PTH synthesis. Furthermore, sex hormone differences have been suggested as confounding factors of the PTH-vitamin D axis (Gunnarsson et al. 2009). A blunted PTH response to vitamin D deficiency during
winter was also found to be more common in a small sample of multiple sclerosis patients (N = 23) than in healthy controls (Soilu-Hänninen et al. 2009).

In recent years, new proteins involved in PTH regulation and actions have been discovered, such as fibroblast growth factor 23 (FGF-23), klotho, and Pin1 (Oudshoorn et al. 2009, Razzaque 2009, Nechama et al. 2009, Naveh-Many 2010). These proteins, particularly Pin1, appear to have key regulatory roles in PTH synthesis and secretion. Thus, age and/or disease-related changes in the functioning of these novel regulators of PTH may also result in an altered relationship between vitamin D and PTH.

6.3 Parathyroid hormone as an indicator of impaired survival

In this study, elevated PTH consistently predicted impaired survival in both a general aged population and in frail older long-term care inpatients (Studies IV and V). Furthermore, the predictive value of elevated PTH proved to be more important than 25-OHD in the older inpatients with a high prevalence of vitamin D deficiency. In comparison with previous trials on selected populations (Carlstedt et al 1997, Sambrook et al. 2004, Fisher et al. 2007, Chen et al. 2008), the present study expands the results from a general aged population to severely disabled long-term inpatients.

Interestingly, the independent predictive value has also recently been emphasized by others (Premao et al. 2009, Hagström et al. 2009, Pilz et al. 2010). In the recent work by Pilz et al., PTH levels emerged as an independent predictor of mortality and cardiovascular events during a median follow-up time of 7.7 years in 3 232 Caucasian patients who had undergone coronary angiography at baseline (Pilz et al. 2010). Unadjusted Cox proportional HRs (with 95% confidence intervals) in the fourth when compared to the first PTH quartile were 2.13 (1.75-2.60) for all-cause and 2.47 (1.92-3.17) for cardiovascular mortality. After adjustments for common cardiovascular risk factors, these HRs remained significant with 1.71 (1.39-2.10) for all-cause and 2.02 (1.55-2.63) for cardiovascular mortality. Among specific cardiovascular events, a particularly strong association of PTH with sudden cardiac death was observed. These results are also supported by another recent study in a community-based cohort of elderly men (mean age, 71 years; N = 958) (Hagström et al. 2009). In Cox proportional-hazards models adjusted for established cardiovascular risk factors (age, systolic blood pressure, diabetes, smoking, body mass index, total cholesterol, high-density lipoprotein cholesterol, antihypertensive treatment, lipid-lowering treatment, and a history of cardiovascular disease), higher plasma PTH was associated with a higher risk of cardiovascular mortality (HR for a 1-SD increase in PTH, 1.38; 95% confidence interval, 1.18 to 1.60; P < 0.001) during a median follow-up of 9.7 years. This association remained essentially unaltered in participants without previous cardiovascular disease and in those with normal PTH (< 62 ng/l), with no other signs of a disturbed mineral metabolism (normal CaT, 2.2 to 2.6 mmol/l; normal glomerular filtration rate, >50 ml/min/1.73m2; no vitamin D deficiency, plasma 25-OH vitamin D > 37.5 nmol/l). Interestingly, elevated plasma PTH (> 48 ng/l) was estimated to account for 20% (95% confidence interval, 10 to 26) of the population-attributable risk proportion for cardiovascular mortality.
In accordance with our observations in long-term care inpatients, the independent prognostic significance of PTH in the institutionalized elderly has recently also been demonstrated by another research group (Premaor et al. 2009). Premaor et al. prospectively evaluated the association of SHPT with mortality and hospitalization during a 6-month period in a cohort of 100 individuals aged between 65 and 102 years living in geriatric institutions. Patients with PHPT or in haemodialysis were excluded. Fifty-eight percent of the individuals had SHPT, defined as serum PTH > 48 ng/l and normal or low CaT. The mean baseline serum 25-OHD level was 31.3 nmol/l. The odds of an individual with SHPT dying or being hospitalized was 6.6 (95%CI 0.8–54.6; p = 0.07) and 10.7 (95%CI 1.3–85.9; p = 0.007), respectively. SHPT and BMI were independently associated with the combined outcome, after correction for the GFR and 25-OHD.

Furthermore, in addition to mortality, Fisher et al. detected an association between elevated PTH and multiple poor short-term health outcomes in a follow-up study of their original hip fracture population (Fisher et al. 2009). They found that SHPT, but not vitamin D deficiency, was associated with older age, a higher prevalence of trochanteric fractures, coronary artery disease, hypertension, previous stroke and renal impairment, increased levels of serum osteocalcin, bone-specific alkaline phosphatase and adiponectin, as well as a significantly higher in-hospital mortality (11.8 vs. 0.54%, p = 0.001), perioperative myocardial injury (32.7% vs. 22.5%, p = 0.043), a length of stay (LOS) of 20 days or more (40.2% vs. 26.9%, P = 0.017), and being discharged to institutional care (29.5 vs. 14.6%, P = 0.019). In multivariate regression analyses, SHPT strongly associated with in-hospital mortality and a LOS of 20 days or more. The conclusion of Fisher et al. was that vitamin D deficiency was common, but did not per se predict the poor health outcomes of patients, further supporting the independent role of PTH.

Two new papers based on large cohorts of community dwelling elderly with PTH and 25-OHD measurements and with long follow-up times have also been published very recently (Jassal et al. 2010, Cawthon et al. 2010). In line with the results of this thesis, Cawthon et al. reported an increased risk of mortality with increasing PTH concentration in both a crude and fully adjusted models, but not with decreasing 25-OHD in older men (N = 1490, mean age = 73.7 years) participating in an observational osteoporosis study. In contrast, Jassal et al. found no relationship between either PTH or 25-OHD and survival in a prospective study of Caucasian, middle-income, community-dwelling older men and women (N = 1073, mean age = 74 years) living in sunny southern California. The main difference between these studies was in the vitamin D status. Cawthon et al. (2010) reported a 64% prevalence of 25-OHD values below 75 nmol/l, the respective figure being only 14% in the study of Jassal et al. (2010).

6.4 Role of parathyroid hormone in cognitive decline

The literature concerning the association of cognition and PTH levels has been scarce and mostly concentrated on mental disorders related to vitamin D deficiency, PHPT or renal failure. The elevated serum calcium and PTH concentrations closely related to advanced primary hyperparathyroidism have been observed to associate with neuropsychiatric
symptoms, and case reports have further demonstrated that these cognitive symptoms are dramatically improved after parathyroidectomy (Logullo et al. 1998, Papageorgiou et al. 2008). Recently, parathyroidectomy has additionally been shown to improve the cognition of SHPT patients with renal failure (Chou et al. 2008). Interestingly, the association between increased parathyroid hormone levels and cognitive decline was not explained by hypercalcaemia or impaired renal function in the present study (Study VI), again suggesting a more independent role of PTH in the pathophysiology of memory loss. However, the present study lacked data on 25-OHD.

In a cross-sectional study of 752 French women, a subset of an observational prospective multicentre cohort study designed to evaluate risk factors for hip fractures among community-dwelling women aged 75 years and older, vitamin D levels below 25 nmol/l, but not PTH concentrations, independently associated with poorer cognitive performance (Annweiler et al. 2010b). Furthermore, in another cross-sectional investigation of vitamin D stores, dementia, and MRI measures of cardiovascular disease (white matter hyperintensity volume, grade, and prevalence of large vessel infarcts) in elderly subjects receiving home care (N = 318, aged 65–99 years), vitamin D deficiency (25-OHD < 20 ng/ml) was significantly associated with more than twice the odds of all-cause dementia (OR = 2.3), Alzheimer disease (OR = 2.5), and stroke (with and without dementia symptoms, OR = 2.0) (Buell et al. 2010). Finally, in a cohort study of 1 604 men enrolled in the Osteoporotic Fractures in Men Study, with an average follow-up of 4.6 years, Slinin et al. reported weak independent associations between lower 25-hydroxyvitamin D levels and baseline global and executive cognitive function or incident cognitive decline (Slinin et al 2010). Thus, the association between PTH and cognitive decline in the present study could be explained by the neuroprotective effects of vitamin D, such as antioxidative mechanisms, neuronal calcium regulation, immunomodulation, enhanced nerve conduction and detoxification mechanisms (Buell & Dawson-Hughes 2008). However, according to a recent systematic review, the data on this topic lack consistency (Annweiler et al. 2009). It should also be noted that Buell et al. and Slinin et al. did not measure parathyroid hormone levels in their studies.

Just recently, the association between 25-OHD levels and the risk of substantial cognitive decline was investigated in a secondary analysis of the InCHIANTI population-based study conducted in Italy between 1998 and 2006 (Llewellyn et al. 2010). The multivariate adjusted relative risk of substantial cognitive decline in the MMSE in participants who were severely serum 25-OHD deficient (<25 nmol/L) in comparison with those with sufficient levels of 25-OHD (≥75 nmol/L) was 1.60 (95%CIs 1.19–2.00). The MMSE scores of participants who were severely vitamin D deficient declined by an additional 0.3 points per year more than those with sufficient levels of 25-OHD. Llewellyn et al. also observed that the relative risk for a substantial decline in Trail-Making Test B, another formal measure of cognition, was again consistently increased (RR = 1.31, 95%CIs 1.03–1.51) among those who were severely 25-OHD deficient compared with those with sufficient levels of 25-OHD. The long follow-up of this study and the evaluation of cognitive decline with two different formal tools strengthen the current data on this association. However, Llewellyn et al. also failed to quantify the effect of PTH levels in their observations.
6.5 Is an elevated PTH concentration a risk indicator or a risk factor?

The question now arises whether an elevated PTH level is a risk indicator or a risk factor of poor health outcomes. In other words, 1) does PTH directly cause the poor health outcomes, 2) is the elevation of PTH secondary to underlying disease that is also responsible for the outcomes, or 3) are PTH levels and a deterioration in health parallel phenomena not causally related to each other?

Even though the evidence is accumulating from epidemiological studies that PTH acts as an independent predictor for impaired survival and increased cardiovascular mortality, in particular, it may well be that the variables used to adjust for the underlying diseases are not sufficiently accurate. For example, all of the studies reporting PTH as an independent predictor of mortality (Carlstedt et al. 1997, Sambrook et al. 2004, Fisher et al. 2007, Hagström et al. 2009, Premaor et al. 2009, Pilz et al. 2010), except the present study (Studies IV-VI), have used albumin-adjusted calcium or total calcium instead of the ionized fraction to control for calcaemic disorders. However, it is well known that such methods do not match the accuracy of direct measurement of Ca²⁺ (Björkman et al. 2009). Similarly, the epidemiological studies, including the present study, use estimated instead of directly measured GFR to adjust for renal function. Furthermore, few studies have provided data on 25-OHD or 1.25-OHD levels. Thus, it is possible that the impaired health outcomes statistically associated with elevated PTH levels are nevertheless clinically explained by the age-related loss of renal function, vitamin D deficiency and disturbances in mineral metabolism, such as PHPT. In fact, Zittermann et al. recently demonstrated that the survival of end-stage heart failure patients was more closely related to 1.25-OHD concentrations than PTH levels (Zittermann et al. 2009). Semba et al. also observed no relationship between PTH and mortality in community dwelling elderly women, whereas a significant association was found with 25-OHD (Semba et al. 2009). Furthermore, despite the fact that many of the factors related to metabolic syndrome and cardiovascular disease have been associated with PTH (Ahlström et al. 2009), evidence of a causal relationship remains scarce. PTH has also been associated with an age-related loss of muscle mass and strength (Visser et al. 2003), but again the interaction with the muscle effects of vitamin D (Bischoff-Ferrari et al. 2009b, Ceglia 2009) makes interpretations difficult.

Recent studies have additionally revealed that factors other than renal function, Ca²⁺ and vitamin D are involved in the regulation of parathyroid function. These include such factors as FGF-23, klotho and Pin1. FGF-23 has been demonstrated to predict mortality independently of PTH levels in haemodialysis patients (Gutierrez et al. 2008), but yet again the authors failed to quantify the possible effect of klotho, a co-receptor of FGF-23, and a high dietary intake of P, which has also been associated with impaired survival in renal failure patients. In addition, Pin1 is an important regulator of PTH gene expression (Naveh-Many 2010). Moreover, increased parathyroid gland PTH levels and circulating serum PTH concentrations have been recorded in Pin1-knockout mice without changes in serum Ca²⁺ and P levels (Nechama et al. 2009). Most interestingly, Pin1 has been associated with telomere maintenance (Lee et al. 2009) and neurodegenerative disorders, including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and
frontotemporal dementia associated with parkinsonism linked to chromosome 17 (Rudrabhatla & Pant 2010). However, further studies are required to determine whether these associations also explain the predictive value of elevated PTH for mortality and cognitive decline in the present study.

Despite these concerns, some evidence supports the direct involvement of PTH in the pathophysiology of the poor health outcomes. Firstly, PTH receptors are abundantly expressed in the heart and brain (Reppe et al. 2007). Secondly, experimental studies on both animals and humans have reported direct cardiovascular effects of parathyroid hormone, including myocardial damage (Bogin et al. 1981), hypertension (Hulter et al. 1986), a pro sclerotic effect on vascular smooth muscle cells (Perkovic et al. 2003) and accelerated decompensation following left ventricular hypertrophy (Cha et al. 2010). On the other hand, PTH has been associated with an improved mechanical response of cardiomyocytes to electrical stimulation (Tastan et al. 2009) and protective effects through the activation of bone marrow derived stem cells resulting in a reduction in perfusion defects in an experimental animal model of cardiac ischemia (Huber et al. 2010). However, these results may not necessarily be in contradiction with each other, because in bone physiology and in osteoporotic bone, PTH exerts both anabolic and catabolic effects (Neer et al. 2001, Lotinun et al. 2002).

Because of the close relationship between increased parathyroid function and cardiovascular mortality (Hagström et al. 2009, Pilz et al. 2010), it is very interesting that PHPT has been associated with several abnormalities that characterize the metabolic syndrome, including dyslipidaemia, obesity, insulin resistance and hypertension (Kumar et al. 1994, Hagström et al. 2002, Procopio et al. 2003, Bolland et al. 2005). These abnormalities are also well established risk factors for cardiovascular diseases and subsequent cardiovascular mortality. Furthermore, these metabolic abnormalities have been reported to associate more closely with PTH than with CaT levels in a random cohort of normocalcaemic 70-year-old community-dwelling people, even after controlling for renal function (Ahlström et al. 2009). In fact, according to another previous hypothesis, elevated PTH rather than elevated calcium is the main explanation for the association between PHPT and the increased risk of cardiovascular diseases (Gotoh et al. 2005, Jorde et al. 2005, Hagström et al. 2007, Snijder et al. 2007). Most importantly, the results of Ahlström et al. did not change when PHPT patients were excluded. This observation also supports the independent role for even subtle elevation of PTH levels as a cardiovascular outcome indicator. It should be noted, however, that Ahlström et al. measured the calcaemic status by albumin adjustment of CaT, which is well known for its inaccuracy compared to the direct measurement of Ca$^{2+}$ (Björkman et al. 2009). Nevertheless, in our random sample of older persons, directly measured Ca$^{2+}$ concentrations did not correlate with the characteristics of the metabolic syndrome addressed by Ahlström et al., whereas PTH concentrations correlated inversely with BMI and showed a reverse U-shaped relationship with both diastolic and systolic blood pressure (unpublished univariate analysis). Although this observation is not fully consistent with Ahlström et al., possibly because of the markedly older subjects, it does, however, emphasize the possible role of parathyroid function over calcium concentrations in the pathophysiology of the metabolic syndrome.
Interestingly, it has also been hypothesized that since the cardiac effects of PTH are mediated by GPCRs that activate PLC, it is reasonable to presume that a sufficient concentration of PTH can be arrhythmogenic in the ischemic heart (McCarty et al. 2009). This hypothesis is based on evidence from animal studies showing catecholamines, angiotensin II, and endothelin to be arrhythmogenic for ischemic myocardium through the activation of receptors linked to the activation of PLC (McCarty et al. 2009). Furthermore, in a recent prospective large cohort study on angiography patients (N = 3232) controlling for common cardiovascular risk factors and disorders of mineral metabolism (GFR, CaT, 25-OHD, 1.25-OHD, diuretic use), PTH levels above 40 ng/l significantly predicted increased all-cause mortality and cardiovascular events (Pilz et al. 2010). Interestingly, in line with the hypothesis of McCarty et al. (2009), Pilz et al. (2010) also observed a strong correlation between PTH and sudden cardiac deaths. However, even though the study by Pilz et al. (2010) was highly detailed in comparison to all previous studies, it lacked data on directly measured Ca\textsuperscript{2+}, FGF-23, and Pin1 activity.

In summary, the causal relationship between PTH and different clinical outcome measures remains putative. Intuitively, it also seems unlikely that a single hormone could play a substantial role in preventing or ameliorating a diverse range of diseases. Experience from other outcome indicators that have been more thoroughly investigated also attenuates the enthusiasm to suggest a direct causal role of PTH levels in the pathophysiology of increased mortality and cognitive decline at this time. However, studies on the effects of PTH-lowering treatments should be vigorously pursued in order to further elucidate these associations.

6.6 Strengths and limitations of the study

The major strength of this thesis is the study design of each of the substudies. The meta-analysis, randomized controlled trial and long-term prospective follow-up of a general aged population give a strong base for the validity of the results. The consistency between the results of the meta-analysis (Study I) and the clinical trial (Study III), as well as between the general aged population and long-term inpatients further emphasizes the credibility of this thesis. In contrast to previous research, Ca\textsuperscript{2+} was directly measured, the golden standard in the assessment of calcemic status, in all the follow-up studies (Studies IV-VI). Furthermore, the intact PTH levels were determined with the gold standard Nichols Institute diagnostic method in the prospective follow-up studies on a general aged population (Studies IV and VI). The method used to measure 25-OHD in the chronically immobile older inpatients is also one of the most precise methods available. These studies have numerous additional strengths, including the exceptionally long follow-up at the end of life, with the vast majority of the subjects being followed until death, the complete data on end points from the daily updated National Census Records, the strict age limits of the three cohorts and the large amount of baseline data, including thorough clinical examinations at entry, enabling the results to be controlled for co-morbidity. Furthermore, all the analyses of the clinical trial (Study III) were performed according to intention to treat.
A major limitation of this thesis is the lack of vitamin D data for the general aged population. The measurement of the novel regulators of parathyroid function, such as FGF-23, klotho and Pin1 activity, in addition to direct measurement of GFR also would have considerably strengthened the data. However, there was no access to such measurements during data collection due to the lack of analytical methods and resources. Despite the observed minor decrease in PTH levels among the long-term care inpatients after vitamin D supplementation, the severe dementia and homogeneity of these patients is also a limitation for any further investigations on the causal relationship between the outcomes and elevated PTH. It could also have been worthwhile to include other databases in addition to PubMed in the search for trials to include in the meta-analysis. Furthermore, it should be noted that the results of the meta-analysis were driven by the inclusion of Study III, which was the largest vitamin D supplementation trial on immobilized elderly subjects. It is also possible that the methodological differences in 25-OHD and PTH measurement between the trials included in the meta-analysis may have confounded the results. However, these limitations are not likely to have a major effect on the conclusions of this thesis.

6.7 Conclusions and future perspectives

In light of the data presented in thesis, it appears that the interplay between PTH and vitamin D in the regulation of calcium homeostasis is more complex than has generally been considered. The concurrent results of other research groups also accord well with the present study, suggesting that in addition to musculoskeletal health, PTH is also related to the maintenance of other important domains of health in old age. Higher PTH concentrations, even within conventional laboratory references, seem to be an independent indicator of an increased risk of all-cause and of cardiovascular mortality independently of established cardiovascular risk factors, disturbances in mineral metabolism, and renal failure. Limited and inconsistent evidence supports the role of a vitamin D deficiency-related lack of neuroprotective effects over the causal association between PTH and impaired cognitive functions. However, the causality of these associations remains unclear. The clinical implications of the relationships found remain to be elucidated by future studies interfering with PTH concentrations, especially by long-term interventions to reduce PTH.
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