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CARDIOVASCULAR ASPECTS IN MILD PRIMARY HYPERPARATHYROIDISM AND THE OUTCOME AFTER PARATHYROIDECTOMY

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توانا بود هر که دانا بود

Knowledge is power

Ferdowsi (Persian poet, 940-1020)

To Kamal and Sheida

ABSTRACT

Data on the extent and clinical significance of cardiovascular abnormalities in primary hyperparathyroidism (PHPT) are conflicting. The main objective of this thesis was to evaluate the cardiovascular function in patients with mild PHPT without other known risk factors and to analyze the effect of parathyroid adenomectomy. Our second aim was to analyze whether an elevated parathyroid hormone level induces any acute effect on endothelial function.

In a prospective case-control design, 51 consecutive PHPT patients without any known cardiovascular risk factor were compared to 51 randomly enrolled healthy controls. The patients were re-examined 15±4 months after parathyroid adenomectomy, when parathyroid hormone and calcium levels had normalized. Vitamin D deficiency, defined as 25-OH-D < 50 nmol/L, was found in 77% of the patients compared to 20% of the controls; 40% of the PHPT patients were still vitamin D deficient at follow-up. Systolic blood pressure and triglyceride levels were higher in cases compared to controls and decreased after parathyroid adenomectomy. Cardiac morphology and function, evaluated by echocardiography and Doppler tissue imaging, were normal in both groups but the systolic myocardial velocities were higher in the PHPT group at baseline. Both systolic and diastolic blood pressure correlated to the PTH and calcium levels. Structural properties and function of the carotid and radial arteries, evaluated by ultrasound and pulse-wave analysis, were normal in both groups and did not change after parathyroid adenomectomy. Biomarkers of inflammation, coagulation and endothelial function were normal in both groups. The forearm blood flow response to metacholin and nitroprusside, analyzed in young healthy subjects, was not altered during parathyroid hormone infusion.

In conclusion, patients with mild PHPT without known cardiovascular risk factors had normal cardiovascular structure and function but a higher systolic myocardial performance at baseline which decreased after parathyroid adenomectomy. Vitamin D deficiency was more common in cases. Parathyroid adenomectomy had an overall positive effect on blood pressure, vitamin D status and triglyceride level. However, based on our results, we have no evidence that cardiovascular complications can be prevented by parathyroid surgery in PHPT patients without known cardiovascular diseases and risk factors. We registered no vasoactive effect mediated by PTH alone.

LIST OF PUBLICATIONS

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

I. Farahnak P, Lind L, Marttala K, Nilsson I-L. Parathyroid Hormone's Acute Effect on Vasodilatory Function. *Clinical Medicine Insights: Endocrinology and Diabetes 2010;3:37–42.*

II. Farahnak P, Ring M, Caidahl K, Farnebo L-O, Eriksson M J, Nilsson I-L. Cardiac function in mild primary hyperparathyroidism and the outcome after parathyroidectomy. *European Journal of Endocrinology 2010;163: 461–467.*

III. Ring M, Farahnak P, Gustavsson T, Nilsson I-L, Eriksson M J, Caidahl K. Arterial structure and function in mild primary hyperparathyroidism before and after parathyroidectomy. *Manuscript.*

IV. Farahnak P, Lärfars G, Sten-Linder M, Nilsson I-L. Mild primary hyperparathyroidism: Vitamin D deficiency and cardiovascular risk markers. *Accepted.*

CONTENTS

LIST OF ABBREVIATIONS

1,25(OH)2D	1,25 dihydroxyvitamin D
25-OH-D	25-hydroxyvitamin D
A	Peak transmitral diastolic flow velocity during atrial contraction
A´	Peak late diastolic velocity recorded by DTI
AI	Augmentation index
ALP	Alkaline phosphatase
Apo	Apolipoprotein
AV-plane	Atrioventricular plane
BMI	Body mass index
BMD	Bone mineral density
BP	Blood pressure
BSA	Body surface area
Ca++	Ionized calcium
CaSR	Calcium sensing receptors
CCA	Common carotid artery
CV	Cardiovascular
DBP	Diastolic blood pressure
DTI	Doppler tissue imaging
DT	Deceleration time
E	Early transmitral diastolic flow
E´	Peak early diastolic velocities recorded by DTI
EF	Ejection fraction
EFI	Endothelial function index
EDV	Endothelium-dependent vasodilation
EIDV	Endothelium-independent vasodilation
FHH	Familial hypocalciuric hypercalcemia
FBF	Forearm blood flow
FS	Fractional shortening
GFR	Glomerular filtration rate
HDL	High density lipoprotein
hs-CRP	High sensitive C-reactive protein
IGF-1	Insulin-like growth factor 1
IM-GSM	Intima media grey scale median
IMT_{cca}	Intima media thickness in common carotid artery
IMT_{rad}	Intima media thickness in the radial artery
IRMA	Immunoradiometric assay
IVRT	Isovolumic relaxation time
LV	Left ventricular
LVDd	Left ventricular end-diastolic diameter
LVDs	Left ventricular end-systolic diameter
LVH	Left ventricular hypertrophy
LVMI	Left ventricular mass index
IVSd	End-diastolic interventricular septum thickness
LDL	Low density lipoprotein

MCh	Metacholine
PAI-1	Plasminogen activator inhibitor-1
PHPT	Primary hyperparathyroidism
PTH	Parathyroid hormone
PTHrP	PTH-related protein
PTX	Parathyroid adenomectomy
PWA	Pulse-wave analysis
PWTd	End-diastolic left ventricular posterior wall thickness
RV	Right ventricle
RVDd	Right ventricular end-diastolic diameter
S´	Peak systolic velocities recorded by DTI
SBP	Systolic blood pressure
SD	Standard deviation
SNP	Sodium nitroprusside
TG	Triglycerides
VWF	Von Willebrand factor

1 INTRODUCTION

1.1 BACKGROUND

Primary hyperparathyroidism (PHPT) is a common endocrine disorder, characterized by a high-normal serum calcium concentration and an inappropriately increased parathyroid hormone (PTH) level. PHPT is caused by a single adenoma in 80–85% of the patients, while multiglandular parathyroid disease (hyperplasia or multiple adenomas) is found in 15–20 % and carcinoma is rare, less than 1% [1]. The introduction of automated methods for the determination of serum calcium in recent decades has led to a dramatic increase in the number of diagnosed cases of PHPT. The prevalence of PHPT is about 1% in the adult population, with a female dominance of 3–4:1; it increases with age in both sexes and is highest in postmenopausal women, 3–4% [2, 3].

PHPT was classically known as a disease of "bones, stones, abdominal groans and psychiatric moans" and was associated with severe skeletal and renal complications and apparent mortality. Today, the majority of patients with newly diagnosed PHPT show none of the classic symptoms or signs traditionally associated with the disease and the condition is often defined as a mild asymptomatic disorder. PHPT has been associated with impaired glucose tolerance, lipid metabolic disturbances, increased cardiovascular (CV) morbidity and premature death in CV and malignant disorders [4-8]. It has been argued that the risk of CV complications is coupled to more severe disease and the extent and the nature of CV involvement in those with mild disease are still unclear [9]. Our knowledge of CV disturbances in PHPT is mostly based on data from cohorts with varying disease severity and pre-existing CV risk factors that explain the discrepant findings in the literature. The Third International Workshop on the Management of Asymptomatic Primary Hyperparathyroidism recently reviewed the evidence for management of mild asymptomatic PHPT and requested prospective cohort studies of parathyroid adenomectomy's (PTX's) impact on CV aspects of PHPT [9].

1.2 DIAGNOSIS AND CLINICAL PRESENTATION OF PHPT

The diagnostic hallmark of PHPT is hypercalcemia in combination with high or non-suppressed levels of PTH. However, the biochemical spectrum of PHPT varies greatly between individuals. Some patients have a PTH level that is within the reference range but still inappropriately high relative to the hypercalcemia, while others have a so-

called normocalcemic PHPT in which an elevated PTH is combined with a consistently normal calcium level. The diagnosis of normocalcemic PHPT requires that other causes of secondary hyperparathyroidism, such as vitamin D deficiency or renal insufficiency, have been excluded [9]. In a large prospective study with 10,000 patients with surgically proven PHPT, PTH levels remained within the normal range (on average) in 16.5% of the patients and normocalcemic PHPT was found in 2.5% [10].

Conditions other than PHPT may also lead to disturbances in PTH and calcium levels. The most frequent other cause of hypercalcemia is malignancy. Measurement of intact PTH has resulted in substantially improved diagnostic discrimination since in malignancy-related hypercalcemia, PTH levels are characteristically suppressed [11]. Less commonly, hypercalcemia is due to granulomatous diseases [12], immobilisation [13] or drugs such as lithium [14] or thiazide diuretics [15]. Another differential diagnostic consideration in PHPT is familial hypocalciuric hypercalcemia (FHH), an autosomal dominant disorder caused by inactivating mutations of the calcium-sensing receptor (CaSR), which makes the parathyroid glands less sensitive to calcium. FHH can be distinguished from PHPT by measuring the calcium/creatinine clearance ratio in urine, which is less than 0.01 in FHH and more than 0.02 in patients with PHPT. When the diagnosis is in doubt, CaSR gene sequence testing can be obtained [16].

In most cases, PHPT is a sporadic disease; a history of cervical irradiation is a recognized risk factor but the exact cause is unknown in the majority of cases. In less than 1%, PHPT is part of a defined hereditary syndrome such as multiple endocrine neoplasia (MEN) type 1 or 2, hyperparathyroidism-jaw tumour syndrome and familial isolated hyperparathyroidism [16].

The disease's clinical profile has changed dramatically in recent decades. Nowadays, at least in the Western world, the disorder is often detected by chance in almost asymptomatic patients. Even though the majority of patients with PHPT have no obvious symptoms or signs, many of them are reported to present non-specific neuropsychological symptoms such as weakness, easy fatigability, depression, cognitive impairment and irritability affecting the quality of life [17]. Overt skeletal and renal diseases are rare but the prevalence of nephrolithiasis and osteoporosis with related fracture is higher in PHPT patients compared to the healthy population [18-21].

1.3 PARATHYROID HORMONE

PTH is an 84 amino acid peptide hormone that is secreted from the chief epithelial cells of parathyroid glands and has a plasma half-life of 2–4 minutes. The N-terminate fragment (1-34) of the PTH molecule acts through type 1 PTH/PTHrP receptors and is the biologically active domain [22]. PTH plays a critical role in maintaining an adequate calcium-phosphorus homeostasis through its impact on bones and kidneys. Calcium is the principal regulator of parathyroid glandular activity, through the CaSR on the surface of parathyroid cells. The rate of secretion of PTH is inversely dependent on the concentration of extracellular ionized calcium (Ca++). The biological actions of PTH, Figure 1, include increased bone turnover and release of calcium and phosphate from bone; stimulation of calcium resorption and decreased phosphate reabsorption from the renal tubules; and increased intestinal absorption of calcium by up-regulation of 1-alphahydroxylase, the enzyme that converts 25-hydroxyvitamin D (25-OH-D) into its active form 1,25 dihydroxyvitamin D (1,25(OH)2D) in proximal renal tubules [23, 24]. A small increase in the extracellular Ca++ level leads to activation of the CaSR, which inhibits PTH secretion; conversely, a decrease in the Ca++ level deactivates the receptor complex and stimulates PTH secretion.

Serum PTH is routinely analyzed with a two-site antibody immunoradiometric assay (IRMA), which recognizes the complete intact PTH (1-84) molecule and incorporates two different antibodies that bind to unique sites on the PTH molecule and have excellent specificity and sensitivity [25].

1.4 CALCIUM

Calcium is an essential ion in the human body; precise control of the extracellular calcium level is necessary to ensure optimum function of essential physiological processes. Most of the calcium in the body is stored in the skeleton, which serves as a reservoir. In plasma, normal calcium concentrations are maintained by calciotropic hormones, in particular PTH and the active form of Vitamin D, 1,25(OH)2D, Figure 1. Normal levels of total calcium range from 2.15 to 2.50 mmol/L. Of the total amount, 50% is free Ca++, 10% is combined with various anions (including bicarbonate, citrate, phosphate, lactate and sulphate) and the remaining 40% is bound to serum proteins, mainly albumin [26]. Free Ca++ is the physiologically important component of total calcium. In plasma, the Ca++ concentration is normally maintained within a narrow range (1.15–1.33 mmol/L).

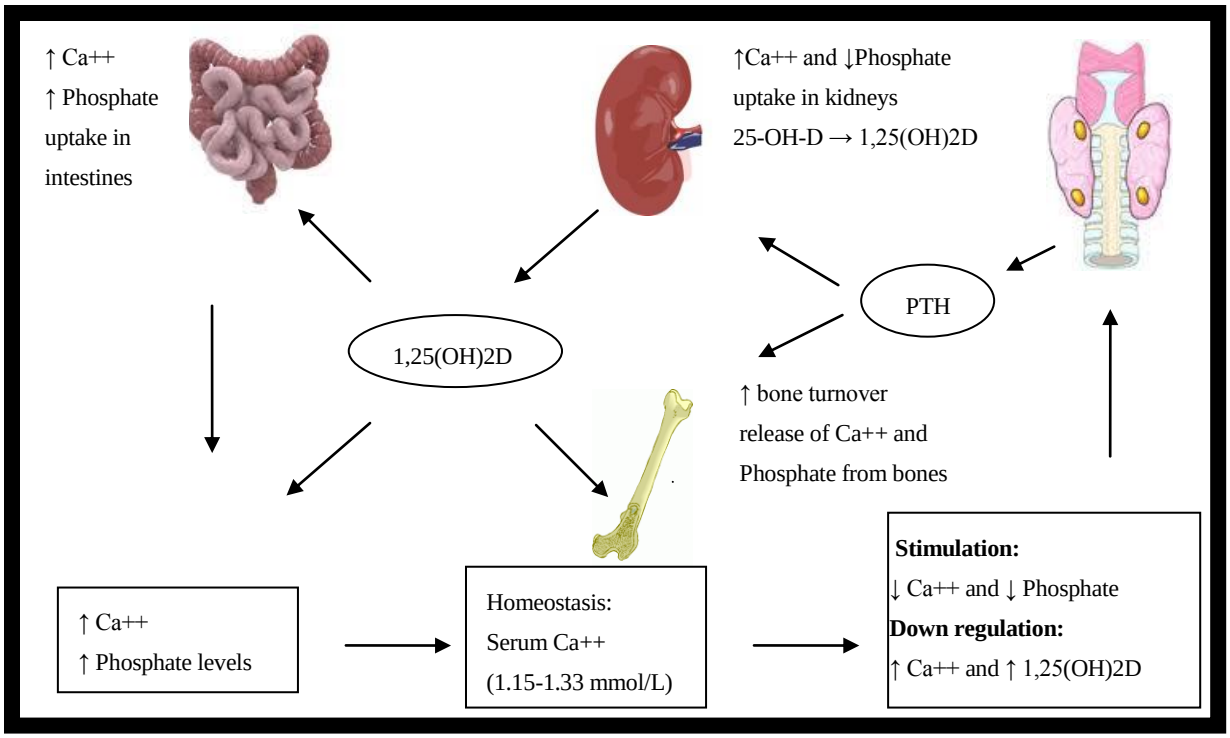


Figure 1. *Calcium homeostasis. PTH increases Ca++ resorption and decreases phosphate reabsorption in kidney, increases activation of vitamin D in kidney and stimulates bone turnover and release of calcium and phosphate from bone. 1,25(OH)2D enhances absorption of calcium and phosphate in intestine and stimulates bone resorption.*

1.5 VITAMIN D

The major supplies of vitamin D3 come from exposure to sunlight and dietary uptake. Vitamin D3 is converted in the skin from its precursor 7-dehydrocholesterol by ultraviolet light. Two sequential hydroxylations of vitamin D3, first in the liver to 25-OH-D and then in the kidney, convert it into its biologically active form 1,25(OH)2D [27]. By binding to vitamin D receptors, 1,25(OH)2D increases the absorption of calcium and phosphate in the intestines, stimulates bone resorption and reduces serum PTH levels by decreasing parathyroid gland activity and indirectly by increasing serum calcium, Figure 1. A low level of 1,25(OH)2D results in a transient decrease in serum calcium and a compensatory rise in PTH levels; it is one of the causes of secondary hyperparathyroidism [28]. The level of 25-OH-D seems to be the best indicator of vitamin D status. 25-OH-D is more stable than the active form; its circulating level in serum is roughly 500–1000 x that of the active form and it correlates well with the biological effects of vitamin D [27].

1.6 PTH AND THE CARDIOVASCULAR SYSTEM

Besides the target organs, bone and kidney, PTH affects a wide variety of other organ systems, including the CV system. PTH has positive inotropic and chronotropic effects on the heart [29, 30]. PTH activates protein kinase C of cardiomyocytes and leads to hypertrophic growth and re-expression of fetal-type protein in cardiomyocytes. This hypertrophic effect of PTH might contribute to left ventricular hypertrophy in patients with hyperparathyroidism [31-33].

The link between PTH and vascular tone is complex. PTH has been associated with both hypo- and hypertensive effects. While short-term infusion of PTH may induce vasodilation and hypotension, long-term infusion results in elevated blood pressure (BP) and hypercalcemia [34, 35]. Several mechanisms have been suggested to explain PTH's vasoactive effects. PTH has a direct vasodilatory effect on the vascular smooth muscle cell: through binding to the PTH receptor, the level of cAMP increases and this reduces the intracellular influx of calcium and leads to vasodilation [36]. On the other hand, PTH might increase the intracellular influx of calcium into the endothelial cells [37]. PTH also has an indirect action on the vasculature: through changes in cytokines and hormones, augmented renin activity and plasma calcium level, which alters the BP [38-40].

PTH has been associated with several parameters involved in the metabolic syndrome, such as insulin resistance, high BP, BMI and dyslipidemia, all of which are important risk factors for CV diseases [41].

1.7 CALCIUM AND THE CARDIOVASCULAR SYSTEM

Calcium is a crucial signal molecule in the CV system and plays an important role in many physiological processes. Ca++ acts as a second messenger via changes in intracellular Ca++ levels mediated by the actions of calcium channels, and as first messenger through a G-protein-coupled CaSR [42, 43]. Calcium appears to interact with the CV system on several levels, by an increase in vascular resistance mediated via a direct effect on vascular smooth muscle as well as indirectly by increments in blood levels of various vasopressive substances [44]. Experimentally induced acute hypercalcemia has increased the BP [40, 45]. In a long-term follow-up study on more than 30,000 individuals, high serum calcium levels were associated with increased mortality, mainly from CV diseases [46]. In population-based studies, even a calcium level that is high up in the normal range and without a diagnosis of PHPT has been recognized as a

prospective risk factor for myocardial infarction and carotid plaque thickness, suggesting a role of extracellular calcium levels in the atherosclerotic process [47, 48].

1.8 CARDIOVASCULAR MANIFESTATIONS IN PHPT

1.8.1 Mortality

The increased mortality in patients with moderate to severe PHPT is well confirmed in several European studies [6, 49-51]. Malignancy and CV disorders were the major causes of death in PHPT patients [6, 8, 52]. The higher mortality rate persists long after surgical cure but decreases with time after PTX [6, 53]. However, the increased mortality has not been confirmed in North American studies [54, 55]. One explanation can be a milder disease in North American patients, who had a lower serum calcium level and fewer symptoms than the European patients. This hypothesis was confirmed by Nilsson et al., who analyzed mortality over a 30-year period in 10,995 patients who underwent PTX. They observed an increased CV mortality which remained over 15 years after PTX, but the risk of death in CV diseases, except for stroke, normalized in patients enrolled later in the study [7]. Preoperative PTH, calcium levels and adenoma weight have all been reported to correlate to mortality risk [47, 54, 56, 57]. An elevated serum PTH level has been shown to be an independent predictor of impaired long-term survival in an unselected aged population [58, 59]. The decreased mortality shown in the majority of recent studies seems to be connected with milder biochemical disturbances but it could also be due to earlier diagnoses and interventions or recent advances in therapy for CV diseases [9]. A study with a cohort of untreated patients with mild PHPT found an increased mortality and morbidity from several conditions, including CV disease, cancer, diabetes and hypertension [60]. Mortality data in mild PHPT are still limited and future studies are needed to confirm these findings.

1.8.2 Cardiac structure and function

Cardiac structure and function in symptomatic PHPT patients have been extensively studied and numerous abnormalities, such as left ventricular hypertrophy (LVH), diastolic dysfunction and cardiac conduction abnormalities, have been reported [61]. Both calcium and PTH have been implicated in the development of cardiac diseases [47, 62]. A higher prevalence of myocardial and valvular calcification has been reported in PHPT patients with marked hypercalcemia [33, 63].

LVH, a strong predictor of future CV death, has been reported in both hypertensive and normotensive patients with PHPT [32, 33, 64]. It has been suggested that LVH in PHPT is independent of hypertension and is associated with PTH level [32, 65]. The hypertrophic action of PTH on cardiomyocytes, in combination with an increased left ventricular afterload due to increased arterial stiffness and central arterial pressure in PHPT, may explain the mechanism behind LVH in PHPT patients [31, 66, 67]. The reversibility of LVH after PTX in some studies further supports a coupling between disease-related biochemical disturbances and LVH [32, 33].

Diastolic dysfunction is associated with a marked increase in all-cause mortality and is frequently reported in patients with PHPT, with varying reversibility after PTX [65, 68-72]. A decreased E/A ratio, a Doppler-derived (E, early) and (A, atrial contraction) ratio of transmitral peak flow velocity, prolonged isovolumic relaxation time (IVRT) and deceleration time (DT) are all variables which may indicate impaired left ventricular relaxation and diastolic dysfunction. A reduced E/A ratio, an independent indicator of increased all-cause mortality as well as CV mortality, has been shown in PHPT [68, 69, 73, 74]. Increased IVRT, indicating diastolic filling impairment, have also been found in PHPT [68, 70, 71]. The interpretation of some of the data is hampered by the presence of confounding risk factors; further studies with patients with mild PHPT without other CV risk factors are required to confirm these findings.

1.8.3 Vascular structure and function

Increased carotid intima media thickness (IMT), an early predictor of systemic atherosclerosis, is associated with coronary and cerebrovascular events [75]. Increased carotid IMT has been found in patients with severe but not mild PHPT [76-80].

Vascular endothelium plays an important role in the biology of the arterial wall through the release of vasoactive and trophic factors. Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis and plays a key role in the pathophysiology of CV diseases [81]. Endothelial function has been studied in a few PHPT studies with somewhat contradictory results [79, 82-84]. The majority of the studies used flow-mediated vasodilatation of the brachial artery for the evaluation of endothelial function and found an impairment of endothelial dependent vasodilation (EDV) in patients with PHPT compared to healthy controls which normalized after PTX [82, 83]. However, this finding was not confirmed in another study [84]. Nilsson et al. evaluated endothelial function by

the invasive forearm method and showed a lower endothelial function index (EFI) in PHPT patients compared to controls. The EFI correlated inversely to the level of calcium and increased after PTX. The same group also reported a dose-related impairment in endothelial vasodilatory function during acute hypercalcemia in healthy subjects [40].

The augmentation index (AI%), an indirect measure of arterial stiffness and endothelial function, is an independent predictor of CV mortality. An increased AI% has been reported in patients with PHPT [66, 67]. The presence of PHPT was a stronger predictor of elevated AI% than other factors, such as age, gender, smoking, hypertension, hyperlipidemia and diabetes mellitus and the increase in AI% was associated with evidence of more active parathyroid disease [66]. These findings indicate a causal link between endothelial dysfunction and PHPT.

1.8.4 Hypertension

Hypertension, a major risk factor for CV morbidity and mortality, is frequently seen in association with PHPT even among those with mild disease [85, 86]. The link between PTH and hypertension has been confirmed in several studies, both in PHPT and in epidemiological trials [87-89]. Elevated levels of PTH have been demonstrated in patients with essential hypertension [89, 90]. The mechanisms underlying hypertension in sporadic PHPT are not clearly understood. Suggested explanations for the hyperparathyroid hypertension include increased total peripheral resistance due to an exaggerated CV response to vasoactive hormones and/or abnormalities in the renin-angiotensin-aldosteron axis [38] and an abnormal vasodilatory response [79, 82]. The effect of PTX on BP has been studied, with contradictory results. While some studies have reported a decrease in BP after surgical cure [69, 91, 92], others have shown no effect [88, 93]. Adequate BP lowering treatment makes a major contribution to reducing the risk of CV events [94]. Long duration of hypertensive and hyperparathyroid conditions may cause irreversible changes in the vasculature, which may explain why the majority of studies indicate that hypertension is not reversible after surgical cure. For this reason, the presence of hypertension in patients with PHPT is currently not an indication for PTX [9].

1.9 VITAMIN D AND PHPT

Vitamin D deficiency has been identified as a risk factor for cancer mortality, CV diseases and diabetes, together with other components of the metabolic syndrome [95, 96].

Values of 25-OH-D below 50 nmol/L are considered to represent vitamin D deficiency and values between 50–75 nmol/L are considered to represent vitamin D insufficiency [27]. Several studies have shown that vitamin D deficiency occurs more frequently in patients with PHPT compared with the general population, and is usually associated with an aggravated form of the disease [97, 98]. In areas with endemic vitamin D deficiency, coexistent PHPT has been associated with higher preoperative PTH values and larger adenomas [97, 99]. The exact causal link between vitamin D deficiency and PHPT is not clear. One of several possible explanations could be that chronic vitamin D deficiency stimulates parathyroid glands and leads to hyperplasia and adenoma growth [100, 101]. Other possible mechanisms could be that the increased 1,25(OH)2D levels in PHPT patients, due to enhanced renal conversion of 25-OH-D to 1,25(OH)2D in kidneys, may inhibit the dermal production of vitamin D3 and 25-OH-D in the liver. There are also data suggesting that the half-life of 25-OH-D is shortened in PHPT due to increased hepatic inactivation [102, 103].

The guidelines from an expert panel in 2009 state that the level of 25-OH-D should be assessed in all patients suspected of having PHPT, and that vitamin D deficiency should be corrected to achieve a serum level of 25-OH-D above 50 nmol/L [104].

1.10 LIPOPROTEIN DISTURBANCES IN PHPT

The association between increased low density lipoprotein (LDL)-cholesterol, triglycerides and decreased high density lipoprotein (HDL)-cholesterol on the one hand and enhanced risk of CV mortality and morbidity on the other is well established [105]. Apolipoprotein B (apo-B) and apolipoprotein A1 (apo-A1) are thought to be better predictors of acute myocardial infarction than total cholesterol and LDL-cholesterol [106]. PHPT has been associated with dyslipidemia, such as increased cholesterol, triglycerides (TG) and decreased level of HDL-cholesterol [67, 107]. Dyslipidemia in mild PHPT has been reported in a few studies, with an improvement after PTX [5, 108].

1.11 CARDIOVASCULAR RISK MARKERS

Plasma levels of several haemostatic and inflammatory variables have been associated with CV events in numerous prospective epidemiological trials and in a few PHPT cohorts [109-113]. Higher levels of platelet count, D-Dimer and coagulation factors VII and X activity have been observed in PHPT patients compared to controls; the potential

hypercoagulability in PHPT patients may augment the risk of atherosclerotic and atherothrombotic complications [111].

Inflammation plays an important roll in the atherosclerotic process. Inflammatory biomarkers such as high sensitive C-reactive protein (hs-CRP) and interleukin-6 (IL-6) have been identified as predictors of CV events [114, 115]. Both normal and increased levels of IL-6 or/and CRP have been shown in PHPT studies, with varying normalization after PTX [116-118]. The discrepancy between the results may partly be explained by differences between the studies in disease severity and CV risk factors.

The biochemical marker plasminogen activator inhibitor-1 (PAI-1) is a principal inhibitor of fibrinolysis and is associated with CV diseases and diabetes [119]. An elevated PAI-1 level can predict future risk of myocardial infarction and diabetes [120, 121]. A reduced fibrinolytic capacity due to increased plasma levels of PAI-1 has been suggested to play an important role in the pathogenesis of CV diseases, particularly in patients with hypertriglyceridemia [122]. An increased PAI-1 level has been reported in patients with PHPT and may be involved in the pathogenesis of CV diseases in these patients [123, 124].

The large glycoprotein von Willebrand factor (VWF) is mainly produced by vascular endothelial cells and plays an important role in platelet adhesion and aggregation. An increased level of VWF has been associated with the risk of coronary heart disease and acute ischemic stroke [125, 126]. The role of VWF in PHPT patients has been studied in a few studies, none of which found an abnormal VWF level [111, 116].

Insulin-like growth factor 1 (IGF-1) is structurally and functionally very similar to insulin and plays an important role in the regulation of cell proliferation and differentiation. It is synthesized mainly in the liver under the control of growth hormone. IGF-1 is expressed in most tissues and is implicated in the pathogenesis of insulin resistance and CV diseases. Low IGF-1 levels are associated with the presence of insulin resistance, metabolic syndrome and CV diseases [127].

The amino acid homocysteine is emerging as a distinct marker of the risk of CV disease. Elevated levels of homocysteine have been reported as an independent predictor of ischemic heart disease and stroke in healthy populations [128].

1.12 TREATMENT OF PRIMARY HYPERPARATHYROIDISM

The only cure for PHPT is surgical removal of the abnormal parathyroid tissue. The standard operation is a full neck exploration with identification of all glands. Thanks to improved techniques for preoperative localisation of parathyroid adenoma (ultrasound and scintigraphy) and methods for peroperative PTH assays, many patients are now operated with a minimally invasive approach [129]. Both the traditional and the minimal invasive procedures have a success rate of > 95% and very low morbidity when performed by experienced endocrine surgeons [130].

The long-term effects of PTX in symptomatic patients have been evaluated in many epidemiological trials. An improvement in bone mineral density (BMD), a reduced fracture risk, positive effects on cognitive function and kidney stone incidence and decreased CV mortality and morbidity have been demonstrated after PTX [33, 53, 131-133]. While it is generally agreed that PHPT patients with end-organ signs and symptoms should be offered PTX, the indication for surgery in asymptomatic patients is still debated. The current criteria for surgery according to the third international guidelines for asymptomatic PHPT are: age < 50 years, serum calcium levels > 0.25 mmol/L above the upper limit of normal, creatinine clearance < 60 ml/min and BMD detected T-score < −2.5 at any site and/or previous fracture fragility [104]. Recent data show that even asymptomatic mild PHPT patients have subtle neurocognitive symptoms and a declining BMD over time that improve after PTX [9]. Surgery is recommended for asymptomatic PHPT patients who meet any of the guidelines criteria and is always an option in those who do not fulfil the criteria when medical surveillance is not possible.

2 AIMS OF THE THESIS

Our knowledge of CV manifestations in PHPT is mainly based on data from cohorts that include patients with varying disease severity and pre-existing CV risk, which may confound the results and explain the discrepant findings in the literature. The main objective of this thesis was to evaluate the CV function in a selected group of patients with mild PHPT without known CV diseases or risk factors.

- To investigate the acute effect of an elevated PTH level on endothelial vasodilatory function in young healthy subjects (study I).

- To evaluate cardiac structure and function by echocardiography and Doppler tissue imaging (DTI) in patients with mild PHPT and the effect of PTX (study II).

- To study the vascular structure, function and intima-media thickness in the carotid and radial arteries and the echogenecity of the intima-media complex in the carotid artery in mild PHPT and the effect of PTX (study III).

- To analyze the prevalence of vitamin D deficiency and other biochemical markers associated with an increased CV risk in mild PHPT and the effect of PTX (study IV).

3 MATERIAL AND METHODS

3.1 SUBJECTS AND STUDY DESIGN

3.1.1 Study I

The study participants were recruited from the responders to a local newspaper advertisement in Uppsala. We started with a pilot study with 3 healthy volunteers (1 man and 2 women), aged 22–25 years, to determine an appropriate rate of intra-arterial infusion of PTH to achieve a PTH level above the upper normal range (6.9 pmol/L). Thereafter, 10 healthy volunteers (5 men and 5 women, aged 21–28 years) were included in the study. Female subjects underwent a pregnancy test before inclusion. None of the subjects were taking any medication, had a history of any disease known to affect the CV system or used nicotine regularly. Each subject gave written consent to participate in the study, which was approved by the Ethics Committee of Uppsala University.

3.1.2 Studies II–IV

The patient population was consecutively recruited from the referrals for parathyroid surgery due to PHPT at the Karolinska University Hospital in Stockholm, Sweden, between January 2006 and November 2008. During the study period, a total of 410 PHPT patients (319 women) were treated with PTX at the clinic. The following inclusion criteria were used: patients with PHPT being considered for PTX, no history of CV diseases or any other disease known to affect the CV system, no medication affecting the CV system, no diabetes mellitus or renal diseases, no arterial hypertension (defined as systolic blood pressure (SBP) > 140 mmHg and diastolic blood pressure (DBP) > 90 mmHg), body mass index (BMI) < 28 and age > 18 and < 70 years. Fifty-three patients met the inclusion criteria and agreed to participate in the study. After inclusion, two patients were dropped from the study: a woman who regretted her decision to undergo PTX and a man who was diagnosed with familial hypocalciuric hypercalcemia by specific mutation analysis after parathyroid exploration with the finding of normal parathyroids. None of the subjects were current smokers at inclusion. A majority of the patients had no overt symptoms or signs associated with PHPT; except for a history of kidney stone disease (n=9) and BMD below −2.5 at any site (n=6). Mild PHPT was defined as plasma calcium less than 2.75 mmol/L and/or the

absence of overt symptoms related to hypercalcemia. The healthy control group (matched by age, gender and geographic area) was randomly selected from the population registry of the city of Stockholm. These subjects received a mailed invitation to participate in the study and were recruited if they fulfilled the inclusion criteria described above except that they had normal P-calcium and normal P-PTH. Two controls were replaced before entering the study, one because of a high P-PTH level, the other because of hypertension. Finally, 51 patients and 51 controls (16 men and 35 women) were included in the study.

Blood samples were collected for biochemical analyses and the CV system was evaluated by echocardiography, DTI, carotid artery ultrasound, high resolution ultrasound of the radial artery and pulse-wave analysis at baseline. Fifty patients were re-examined after a mean follow-up time of 15±4 months (range 7 to 28 months) after PTX. One woman only participated in the blood analyses at follow-up. Two patients were on cholesterol-lowering treatment (atorvastatin) and were excluded from the analyses in studies III–IV along with their matched controls. Another patient and his matched control were excluded in study III due to carotid image storage failure. Each subject gave written consent to participate in the study, which was approved by the Local Ethics Committee of Stockholm.

3.2 METHODS

3.2.1 Forearm blood flow measurement (study I)

Forearm venous occlusion plethysmography is a well-established method for measuring forearm blood flow (FBF) [134, 135]. The method is based on measurement of the increase in forearm volume after the venous return has been occluded by placing an inflated cuff (at a pressure of 30–40 mm Hg for 7 seconds) around the upper arm. At this pressure, venous occlusion occurs without any modification of the arterial inflow. The change in upper arm circumference is recorded by a mercury-in-silastic strain gauge placed around the forearm and connected to a calibrated plethysmograph. FBF is expressed graphically and is calculated (slope of the curve) as the mean of at least five consecutive recordings Figure 2. With this method, cannulation of the brachial artery can be used to infuse vasoactive drugs in one arm without causing any alterations in systemic haemodynamics or in blood flow in the contralateral arm. FBF is then measured in both forearms and the contralateral arm serves as a control. Arterial

infusion of metacholine (MCh), a muscarinic receptor agonist that causes an endothelium-mediated vasodilator response, is used for evaluation of endothelium-dependent vasodilation (EDV). Arterial infusion of sodium nitroprusside (SNP), which directly affects the vascular smooth muscle cells, is used for evaluation of endothelium-independent vasodilation (EIDV). The endothelial function index is calculated as the ratio of FBF during the highest dose of MCh, 4 µg/min (EDV), to the highest dose of SNP, 10 µg/min (EIDV). This index expresses the contribution of endothelial nitric oxide (NO) release to the vasodilatory process. Evaluation of the reproducibility of measurements of EDV and EIDV using this technique has shown a variation of 5–8% in the short- (2 h) and long-term (3 weeks) perspectives [135]. A previous study performed in the same laboratory by one of the authors showed that local intra-arterial infusion of saline did not significantly change either baseline FBF or the responses to MCh and SNP and the endothelial function index in the active arm [136].

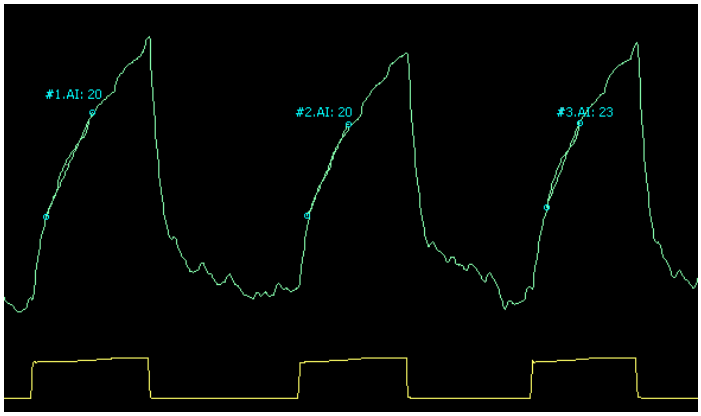


Figure 2. *Measurement of forearm blood flow using venous occlusion plethysmography.*

3.2.2 Experimental procedure (study I)

The subjects were investigated after an overnight fast in the supine position in a room maintained at a constant temperature. An arterial cannula was inserted in the brachial artery (active arm) for administration of the study drugs (PTH, SNP and MCh). The contralateral arm was used as control. The experimental procedure is shown schematically in Figure 3. The levels of PTH and Ca++ were analyzed in the active arm in all subjects and in the control arm as well in 4 subjects (for evaluation of systemic influence) at baseline and after 30 min of PTH infusion. FBF was measured at baseline before drug infusions in both arms. Thereafter, vasodilation in the active arm was achieved by infusion of one of the vasodilatory drugs (SNP or MCh) for 5 min. The

drugs were given in a random order at a maximum rate of 1 ml/min. The infused dosages were 2 and 4 µg/min for MCh and 5 and 10 µg/min for SNP. FBF was recorded in both arms at the end of the infusion. This procedure was repeated with the other vasodilatory drug after 20 min of washout time. The PTH infusion was started after these baseline measurements. After 30 min of PTH infusion, the blood flow measurements were repeated as described above.

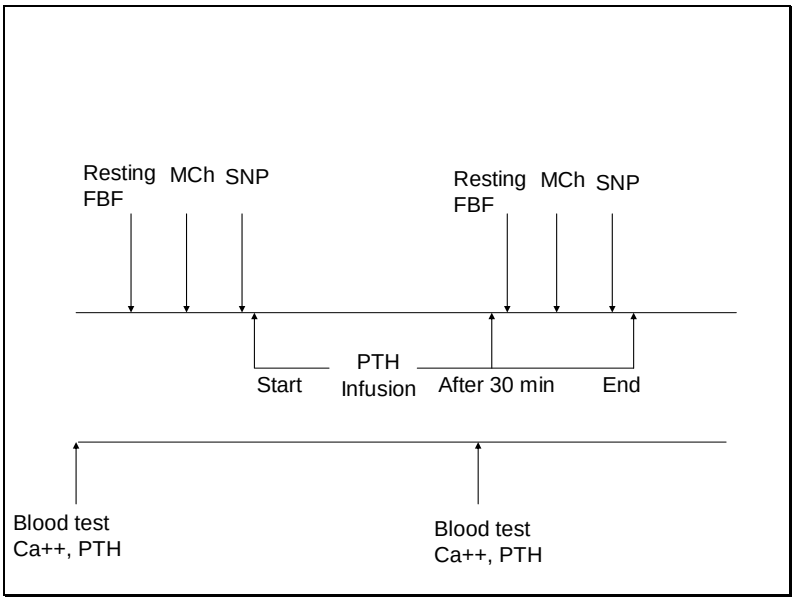


Figure 3. *Experimental procedure, study I.*

3.2.3 Intra-arterial infusion of PTH (study I)

We used Preotact® (Nycomed), a full-length parathyroid hormone (PTH 1-84), diluted with saline in three steps to 0.2µg/ml. In a pilot study with 3 volunteers, we determined the intra-arterial infusion rate that was needed to attain a PTH level above the upper normal range (6.9 pmol/L) in the active arm without any systemic effect. Guided by the results of the pilot study, a dose rate of 70 ml/h during 30 minutes was chosen for the PTH infusion.

3.2.4 Blood pressure, BMI and GFR (studies II–IV)

BP was measured in both arms after at least 30 minutes of rest, using an appropriately sized cuff and automatic monitor (Digital Automatic Blood Pressure Monitor, Omron M7). The mean values of systolic and diastolic BP in the two arms were calculated and heart rate was noted. BMI was calculated by dividing weight (kg) by the square of height (m). Renal function was estimated by calculating the glomerular filtration rate

(GFR) according to Cockroft-Gault's formula: GFR= (140 − age in yr) x (weight in kg/P-creatinine) x (1.23 in men, 1.04 in women).

3.2.5 Body surface area (studies II and III)

Body surface area (BSA) was calculated with the formula: BSA= (0.0001) × (71.84) × $(\text{weight})^{0.425}$ × $(\text{height})^{0.725}$ where weight was measured in kilograms and height in centimetres.

3.2.6 Echocardiography (study II)

All examinations were performed by one experienced echocardiographer, using an ultrasound scanner Vivid 7 (General Electric, Horten, Norway) equipped with DTI capabilities. The patients were placed in the left lateral recumbent position and the two-dimensional, M-mode and Doppler echocardiography was performed in accordance with the guidelines of the American Society of Echocardiography [137]. All images were stored digitally on an ultrasound database Echopac. Standard echocardiographic measurements included right ventricular end-diastolic diameter (RVDd), left atrial end-systolic area, left ventricular (LV) end-diastolic (LVDd) and end-systolic diameter (LVDs), end-diastolic interventricular septum thickness (IVSd) and left ventricular posterior wall thickness (PWTd). The fractional shortening (FS %), ejection fraction (EF %) and atrioventricular plane (AV-Plane) displacement were calculated from M-mode recordings. LV mass was calculated with the formula: LVM (grams) = 0.8 [1.04 $[(\text{LVDd} + \text{IVSd} + \text{PWTd})^3 - (\text{LVDd})^3]] + 0.6$ g. The LV mass was divided by BSA to calculate the LV mass index (LVMI). The cut-off point for LV hypertrophy (LVH) using the LVM/BSA ratio was 150g/m² for males and 120g/m² for females [138]. Transmitral inflow and pulmonary venous flow velocities were obtained with pulsed wave Doppler. The velocities of early transmitral diastolic flow (E) and flow velocity during atrial contraction (A), its ratio (E/A), deceleration time (DT) and isovolumic relaxation time (IVRT) were measured, Figure 4.

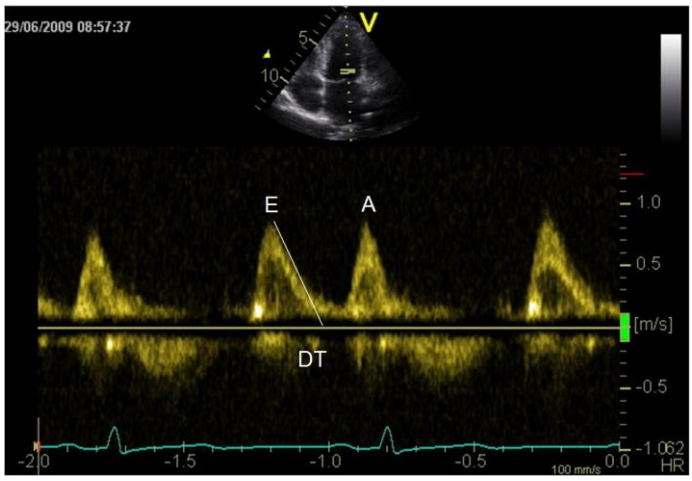


Figure 4. *Measurement of transmitral diastolic flow velocities by pulsed-Doppler echocardiography. E= early transmitral diastolic flow, A= flow velocity during atrial contraction and DT= deceleration time.*

3.2.7 Doppler tissue imaging (study II)

Pulsed DTI was recorded in the apical four-chamber view at three sites: the tricuspid annulus for the right ventricle and in the septal, Figure 5, and lateral parts of the mitral annulus for the left ventricle. A 3-mm sampling volume was used. Peak systolic (S´), early diastolic (E´) and late diastolic (A´) velocities recorded by DTI were measured. E´/A´ and E/ E´ for the septal and the lateral part of the mitral annulus were calculated.

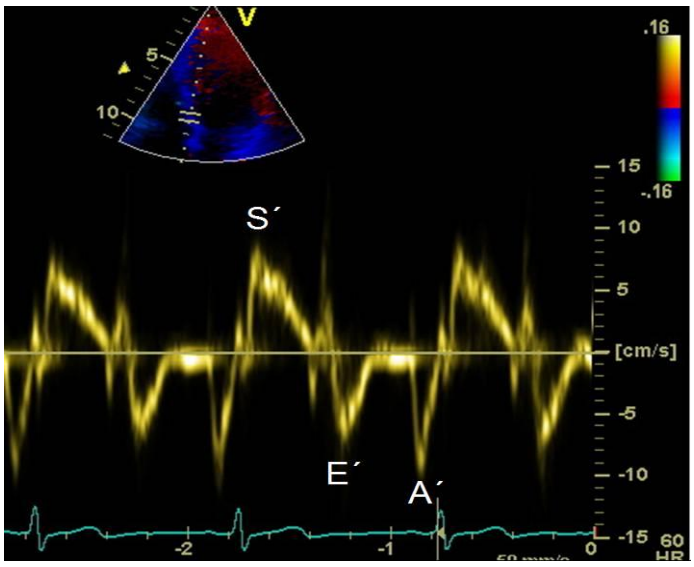


Figure 5. *Measurement of peak myocardial velocities in the septal part of the mitral annulus by DTI. S´- peak systolic, E´- early diastolic and A´- late diastolic velocities.*

3.2.8 Carotid artery ultrasound (study III)

Two dimensional images of the common carotid artery (CCA) were obtained with an 8 MHz transducer, 7L (Vivid 7, General Electric, Horten, Norway). All subjects were examined in supine position by one experienced sonographer. The CCA was evaluated 1–2 cm proximal to the bulb. Diastolic images at the time of the electrocardiographic R-wave were stored digitally on EchoPAC, Image Vault 5.0 system. For detection of intima media thickness (IMT_{cca}) and intima media grey scale median (IM-GSM), three digitized images were imported to the automated software, Artery Measurement Software (AMS) [139, 140].

AMS was developed in collaboration between the Department of Signals and Systems at Chalmers University of Technology and the Physiology Group at the Wallenberg Laboratory, Gothenburg University, Gothenburg, Sweden. A region of interest (ROI) was manually placed at the beginning of the bulb, and IMT_{cca} and lumen diameter (LD_{cca}) were calculated from around 100 discrete measurements through a 10 mm long segment, Figure 6. The borders of the IMT_{cca} and LD_{cca} were identified automatically by the programme and could be manually corrected if necessary. LD_{cca} was measured from the intima-lumen interface of the near wall to the lumen-intima interface of the far wall. Intima media thickness was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia of the far wall [141], Figure 6. IM-GSM was calculated from image analyses of pixels in the range of 0 (black) and 255 (white). The adventitia was used as the reference for white and the blood as the reference for black.

IMT_{cca}, IM-GSM and LD_{cca} are presented as a mean value from six images, three images from left respectively right CCA. Lumen diameter and IMT_{cca} values are shown in mm, while IM-GSM is shown as median grey. The image analyses and calculations were performed in random order by the same investigator.

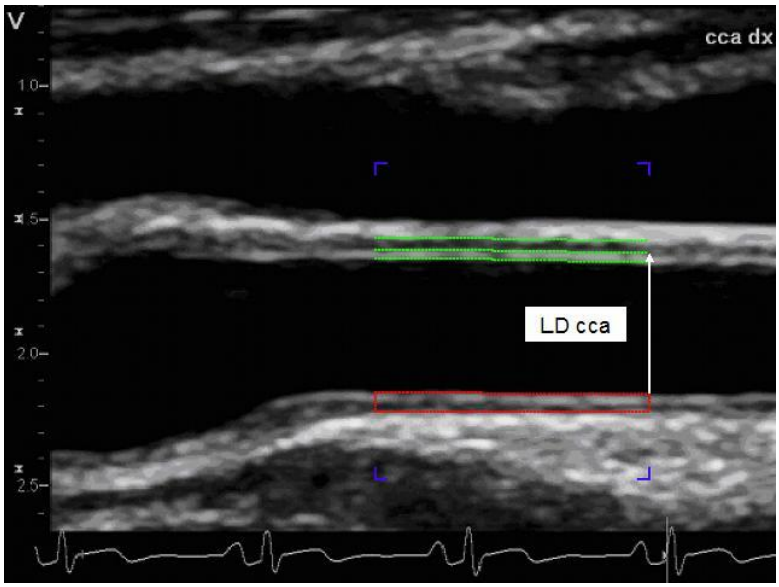


Figure 6. *The IMT_{cca} and IM-GSM are measured in the far wall (red box). The diastolic LD_{cca} is measured from the intima-lumen interface of the near wall to the lumen-intima interface of the far wall (white arrow).*

3.2.9 High resolution ultrasound of the radial artery (study III)

A high resolution ultrasound investigation of the radial artery was performed in 43 patients. The images of the intima media of the radial artery were obtained by high resolution ultrasound, using a 55 MHz transducer (Vevo 770, VisualSonics, Toronto, Canada). The subjects were examined in supine position. B-mode images were recorded from the right radial artery in a longitudinal projection. All cine loops were stored on an external disk and measured off-line using VisualSonics software. The thickness of the radial artery intima media (IMT_{rad}) and lumen diameter (LD_{rad}) were measured according to the leading edge principle [141]. Three sets of measurements were performed from each of three representative images. The IMT_{rad} of the far wall is presented as a mean value from 9 measurements corresponding to end-diastole, the smallest lumen diameter. The IMT_{rad} was defined as the distance from the lumen-intima interface to the media-adventitia interface, Figure 7. Diastolic LD_{rad} was measured from the M-mode image, defined by the distance between the intima-lumen interface of the near wall and the lumen-intima interface of the far wall. LD_{rad} is presented as a mean value taken from three representative beats.

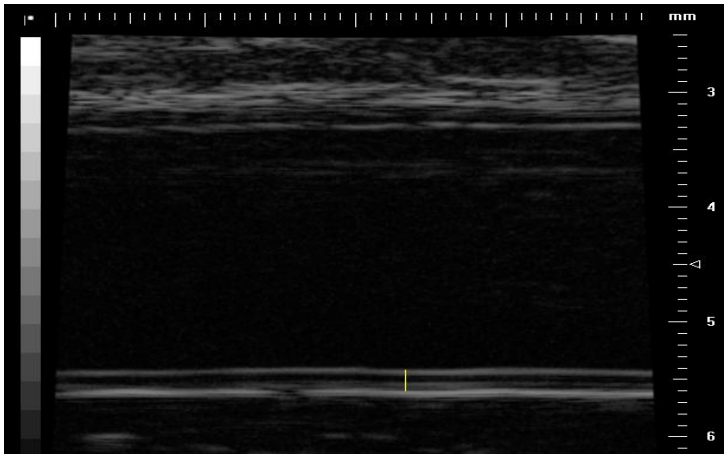


Figure 7. *The IMT_{rad} in the right radial artery was measured from the lumen-intima interface to the media-adventitia interface.*

3.2.10 Pulse-wave analysis (study III)

The subjects were studied in supine position after resting for 60 minutes. Pulse-wave analysis (PWA) was performed noninvasively using SphygmoCor equipment connected to a computer with SphygmoCor 2000 software (version 7.01, AtCor Medical, Sydney, Australia). PWA was registered with a single high-fidelity tonometer gently pressed against the artery (SPT-301B, Millar Instruments, Houston, Texas, USA). The recorded artery waves were collected and processed by the system software. To assess AI%, the radial artery wave was recorded. The corresponding aortic pressure waveform was generated from an averaged radial artery waveform using a validated transfer factor [142, 143], Figure 8. AI% is defined as the difference between the first and the second peaks of the central aortic waveform, expressed as a percentage of the pulse pressure [144]. AI% is presented as a mean value taken from two or more optimal recordings. The radial wave was calibrated to the brachial systolic and diastolic BP, using an automatic monitor (Digital Automatic Blood Pressure Monitor, Omron M7). All measurements were performed by the same investigator.

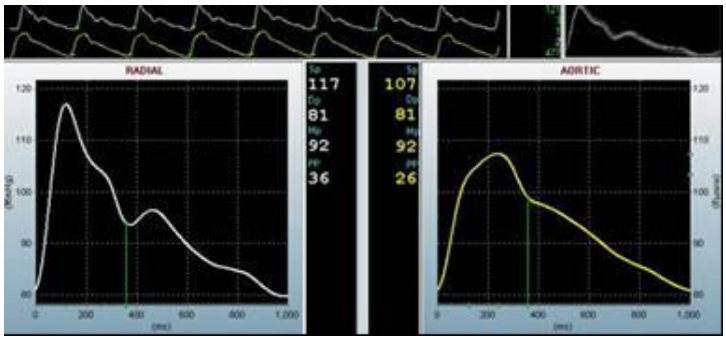


Figure 8. *Pulse- wave analysis - the recorded radial waveform and the derived aortic pressure waveform.*

3.3 IMAGE ANALYSES

3.3.1 Study II

All variables describing cardiac dimensions and structure were analyzed off-line by one observer unaware of the subject's status. The remaining Doppler-derived variables were analyzed by another observer. All measurements were averaged from at least 3 heart beats. To assess the measurement variability, the two observers re-measured 20 randomly selected echocardiographic and DTI examinations. The coefficient of variation was calculated for variables for determination of inter- and intra-observer variability. Coefficient of variation was calculated as the mean percent error, defined as the standard deviation of the absolute differences between the two sets of measurements, divided by the mean of two measurements.

The coefficient of variation for DTI assessed myocardial velocities was 3–4% for inter-observer and 2–3% for intra-observer analyses. The variability for transmitral flow velocity was: inter-observer coefficient of variation 2–4 %, intra-observer coefficient of variation 1–2%. The coefficient of variation for M-mode atrioventricular plane displacement was 2–4% for inter-observer and 2–3% for intra-observer analyses.

3.3.2 Study III

The image analyses and calculations were performed in random order by the same sonographer. To assess the intra- and inter-observer variability of IMT_{cca} and IM-GSM measurements, two observers re-measured 30 randomly selected subjects. Six images, three from each side of CCA, were measured and a mean value was calculated. For evaluation of observer variability, the coefficient of variation was calculated as described above. Intra- and inter-observer variability in terms of coefficients of variation were for IMT_{cca} 2.90% and 3.80%, for LD_{cca} 0.40% and 0.70%, for IM-GSM 2.74% and 3.81%, for IMT_{rad} 5.78% and 10.94% and for LD_{rad} 3.16 and 3.56%.

3.4 LABORATORY METHODS

3.4.1 Study I

Anaerobic venous samples from the active arm were analyzed at baseline and after PTH infusion, for Ca++ (i- STAT 1- Abbott, normal range 1.1–1.3 mmol/L) and intact plasma PTH (Immunolite 2500, Diagnostics Product Company LA, CA, USA; intact P-

PTH, normal range 1.1–6.9 pmol/L). Plasma PTH and Ca++ levels were also analyzed in the contralateral arm in 4 subjects to exclude a systemic effect during the infusions.

3.4.2 Studies II–IV

Blood samples in patients and controls were drawn in the fasting state in the morning. Plasma concentrations of total calcium, alkaline phosphatase (ALP), creatinine, glucose, phosphate, hs-CRP and homocysteine, and serum concentrations of total cholesterol, triglycerides (TG), apolipoprotein A1 (apo-A1) and apolipoprotein B (apo-B) were measured using the Synchron LX ® 20 system (Beckman Coulter inc., Brea, CA, USA). Serum Ca++ concentrations were analyzed on ABL 800 (Radiometer, Copenhagen, Denmark) and plasma concentrations of intact PTH were determined with electrochemiluminescence immunoassay on the Modular E system (Roche Diagnostics GmbH, Mannheim, Germany). Total serum insulin-like growth factor 1 (IGF-1) was analyzed by chemiluminescence immunoassay on DPC Immulite 2000 (Siemens Heathcare Diagnostics, Munich, Germany). Serum concentrations of 25-OH-D were measured by chemiluminescence on Liason ® (DiaSorin, S.p.A, Italy); values below 50 nmol/L were considered to represent vitamin D deficiency and values between 50 and 75 nmol/L, vitamin D insufficiency [27]. The plasma concentrations of VWF antigen were determined with a latex immunoassay on the BCS XP system (Siemens Healthcare Diagnostics, Munich, Germany), and the plasma activity of PAI-1 by a functional enzymatic ELISA assay (Chromolyze PAI-1) from Medinor (Axis-Shield, Dundee, U.K.). In order to minimize inter-assay variation, the pre- and postoperative samples of 25-OH-D, VWF antigen and PAI-1, together with the sample from the randomized controls, were frozen at –70°C and then analyzed in the same series. All the analyses could not be completed in some cases because of technical problems or missed blood sample collection.

3.5 STATISTICS

The sample size for study I was based on earlier similar experimental studies [40]. The sample size for studies II–IV was based on differences and standard deviation (SD) of diastolic function from earlier studies to guarantee a power level of 80% at a confidence level of 95% [71, 145]. Statistical analysis was performed with the PASW for Windows statistical package 18.0 (PASW Inc; Chicago, IL, USA). Data are expressed as mean ± SD. Comparisons between the group of patients at baseline and the control group were performed with the Mann-Whitney *U* test for unpaired data. The Wilcoxon signed rank sum test was used for intra-individual analyses. Relationships between

variables were assessed with Spearman's ρ correlation test. All tests were done two-tailed and $P < 0.05$ was considered to be statistically significant. Multivariate linear regression and stepwise analyses were used in study III to evaluate relationships and adjust for confounders.

4 RESULTS

4.1 FBF MEASUREMENT AND BIOCHEMICAL DATA (STUDY I)

Biochemical data, resting FBF, EDV and EIDV values in the active arm for all study subjects are presented in Table 1. Ca++ and PTH values were normal in all subjects at baseline. After 30 minutes of PTH infusion, the PTH level increased significantly in the active arm, while Ca++ remained unchanged, Table 1. Resting FBF, EDV and EIDV did not change during PTH infusion, Table 1. EDV (MCh 4 µg/min) correlated inversely to the Ca++ level in the active arm (baseline r= –0.75; p< 0.05, after PTH infusion, r= –0.68; p<0.05).

Table 1. Forearm blood flow and biochemical data in the active arm for all subjects at baseline and after parathyroid hormone infusion.

Variables	**Baseline** (n=10)	**PTH-Infusion** (n=10)	**P**
Ca++ (1.1–1.3 mmol/L)	1.26±0.02	1.25±0.04	ns
PTH (1.1–6.9 pmol/L)	3.6±1.2	13.8±4.0	p< 0.01
Resting FBF (ml/min/100 ml tissue)	4.5±1.5	5.0±2.3	ns
EDV (MCh, 2 µg/min)	20.0±13.4	21.5±13.0	ns
EDV (MCh 4 µg/min)	24.7±13.1	29.1±15.9	ns
EIDV (SNP 5 µg/min)	16.2±3.5	18.3±8.2	ns
EIDV (SNP 10 µg/min)	22.2±7.9	24.0±9.7	ns
Endothelial function index	1.09±0.41	1.25±0.57	ns

Values are shown as mean ± SD. Ca++= ionized calcium, PTH= parathyroid hormone, FBF= forearm blood flow, EDV= endothelium-dependent vasodilation, MCh= metacholine, EIDV= endothelium-independent vasodilation, SNP= sodium nitroprusside.

FBF, Ca++ and PTH measured in the control arm remained mainly unchanged during the whole experiment, Table 2.

Table 2. Forearm blood flow and biochemical data for all subjects before and after parathyroid hormone infusion in the control arm.

Variables	**Baseline** (n=10)	**PTH-Infusion** (n=10)	**P**
Ca++ (1.1–1.3 mmol/L)	1.26±0.02 [a]	1.28±0.02 [a]	ns
PTH (1.1–6.9 pmol/L)	3.2±0.86 [a]	3.5±0.39 [a]	ns
Resting FBF (ml/min/100 ml tissue)	3.9±1.7 [b]	3.5±1.8	p<0.05
EDV (MCh, 2 µg/min)	3.7±1.1	3.1±1.4	ns
EDV (MCh 4 µg/min)	3.9±1.7	3.5±1.3	ns
EIDV (SNP 5 µg/min)	4.3±2.8	3.5±1.8	ns
EIDV (SNP 10 µg/min)	3.9±2.2	3.6±1.6	ns

[a] n =4, [b]n=9. Values are shown as mean ± SD. Ca++= ionized calcium, PTH= parathyroid hormone, FBF= forearm blood flow, EDV= endothelium-dependent vasodilation, MCh= metacholine, EIDV= endothelium-independent vasodilation, SNP= sodium nitroprusside.

4.2 CLINICAL AND BIOCHEMICAL DATA (STUDIES II–IV)

Clinical and biochemical data of patients and controls are given at baseline and after PTX in Table 3. Forty-eight patients had a single parathyroid adenoma and 3 patients had multiglandular disease. The majority of our patients had no overt symptoms or signs associated with PHPT; except for a history of kidney stone disease (n=9) and BMD below −2.5 at any site (n=6). The plasma calcium levels were less than 2.75 mmol/L, in the majority of cases, only 5 patients had plasma calcium levels between 2.76–2.97 mmol/L at the time of inclusion. Normocalcemia was achieved in 49 of 51 patients after the first PTX; the other two became normocalcemic after a second operation, when a second parathyroid adenoma and an intrathyroidal adenoma, respectively, were removed. All patients were normocalcemic at the postoperative follow-up one month after PTX (not shown). There were no PTX-related complications. The mean total wet weight of the excised abnormal parathyroid tissue was 619±837 mg (median 409 mg). The patients were re-examined a mean of 15±4 months after successful PTX. Plasma intact PTH, plasma calcium and Ca^{++} were

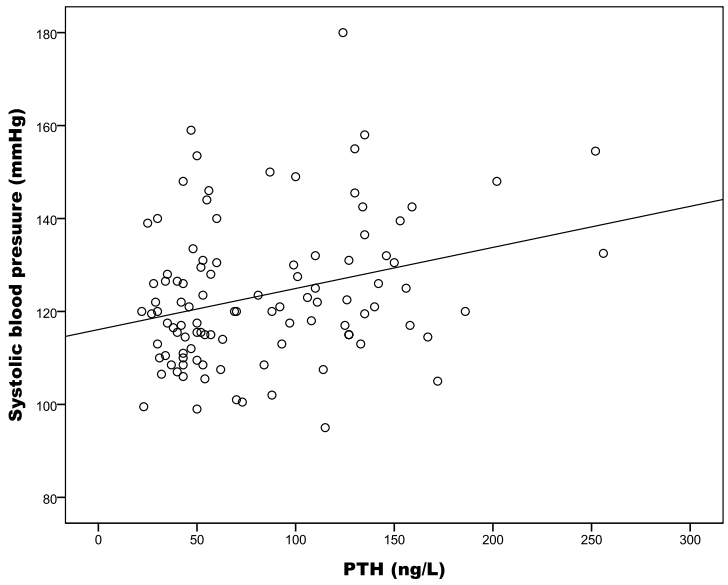
significantly higher in patients at baseline compared to healthy controls, while serum phosphate levels were significantly lower, Table 3. PTH, calcium and phosphate concentrations normalized after PTX and did not differ from controls at the follow-up visit, Table 3. Systolic blood pressure (SBP) was significantly higher in the PHPT group compared to controls and decreased after PTX, Table 3. SBP correlated to the levels of PTH ($r=0.26$, $P<0.01$, Figure 9A) and Ca^{++} ($r=0.25$, $P<0.05$, Figure 9B). Diastolic blood pressure (DBP) decreased after PTX, Table 3. BMI increased after PTX only in female PHPT (23.5±2.7 vs. 24.3±3.3, kg/m^2, $P<0.01$), not in men (25.3±3.0 vs. 25.6±3.7, kg/m^2, ns).

Table 3. Clinical and biochemical data for healthy controls and for patients with primary hyperparathyroidism before and 15±4 months after parathyroidectomy.

Variable	**Controls**	**PHPT cases**		**P**	
	n=51	**Baseline** n=51(35♀)	**Follow-up** n=51(35♀)	**PHPT baseline vs. controls**	**PHPT baseline vs. follow-up**
Age (yr.)	55.2±8.9	54.3±8.8	55.6±8.7		
BMI (kg/m^2)	23.4±2.1	24.1±2.9	24.7±3.2	ns	<0.05
BSA	1.78±0.17	1.83±0.18	1.85±0.18	ns	<0.05
SBP at rest, (mmHg)	119.6±12.6	127.6±17.1	124.6±16.6[a]	<0.05	<0.05
DBP at rest, (mmHg)	76.1±7.7	80.3±9.6	78.4±8.6[a]	ns	<0.05
S-Ca++ (mmol/L)	1.25±0.04	1.46±0.07	1.25±0.05	<0.001	<0.001
P-Calcium (mmol/L)	2.29±0.08	2.62±0.13	2.28±0.08	<0.001	<0.001
P-Albumin (g/L)	38.4±2.6	39.4±2.5	38.5±2.9	<0.05	<0.05
P-PTH (ng/L)	44.1±12.4	122.6±43.6	48.8±15.2	<0.001	<0.001
P-Phosphate (mmol/L)	1.1±0.2	0.84±0.2	1.0±0.2	<0.001	<0.001
P-Creatinine (µmol/L)	69.7±13.7	67.3±12.6[a]	69.1±16.6[b]	ns	ns
P-Glucose (mmol/L)	5.0±0.35	5.0±0.44[b]	5.0±0.51[a]	ns	ns

[a] n=50, [b]n=47–49. Values are shown as mean ±SD. BMI= body mass index, BSA= body surface area, SBP= systolic blood pressure, DBP= diastolic blood pressure, Ca++= ionized serum calcium, PTH= parathyroid hormone.

A.



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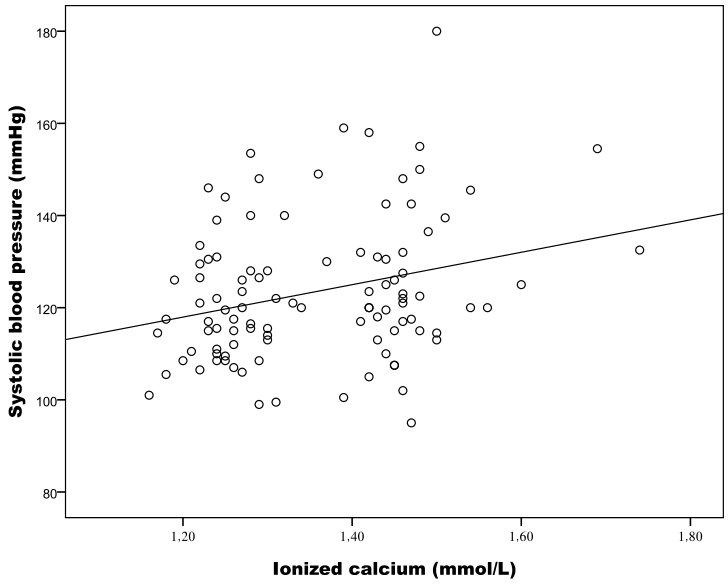

Figure 9 A and B. *Systolic blood pressure for the whole group of PHPT patients and the healthy control subjects was correlated to the PTH level (r=0.26; P<0.01, A) and the ionized calcium level (r=0.25; p<0.05, B).*

4.3 ECHOCARDIOGRAPHY (STUDY II)

There were no differences between the groups in the variables describing cardiac dimensions and structure, Table 4. The systolic and diastolic left ventricular functions evaluated by Doppler echocardiography were within normal limits and there were no differences between the groups at baseline, Table 5. The regional peak systolic myocardial velocities (S´) measured with DTI in the base of the right and left ventricles

decreased after PTX, Table 5. For variables with a significant change after PTX we also tested post-PTX results for possible differences compared to the control group. Compared to our age- and gender-matched healthy controls, the PHPT group had numerically lower peak systolic velocities after PTX but the difference reached statistical significance only in the lateral part of the mitral annulus, Table 5. For comparison, previously published normal values for DTI myocardial velocities for right and left ventricles are shown in Table 5 [146, 147]. The values are in the same range as in our healthy control group.

The peak systolic velocity of the septal part of the mitral annulus correlated significantly and positively to the Ca^{++} level ($r=0.35$, $p<0.05$) in the PHPT group at baseline. IVRT was increased in the male PHPT group compared to the female PHPT group and the healthy controls (PHPT males; 97±20, PHPT females; 82±15, controls males; 84±13, controls females; 82±17 $P<0.05$). The E/A ratio was inversely correlated to the PTH level ($r= -0.33$, $p<0.01$), SBP ($r= -0.40$, $p<0.01$) and DBP ($r= -0.46$, $p<0.01$).

Table 4. Echocardiographic variables for healthy controls and patients with primary hyperparathyroidism before and 15±4 months after parathyroidectomy.

Variable	**Controls**	**PHPT cases**		**P**	
		Baseline	**Follow-up**	**PHPT baseline vs. controls**	**PHPT baseline vs. follow-up**
	n=51	n=51(35♀)	n=50(34♀)		
HR, at rest (per min)	61±9	62±10	60±9	ns	ns
RVDd (cm)	2.77±0.41	2.70±0.36	2.77±0.36	ns	ns
LVDd (cm)	4.89±0.41	4.94±0.40	4.94±0.40	ns	ns
LVDs (cm)	3.12±0.40	3.12±0.37	3.15±0.33	ns	ns
Left atrial area (cm^2)	19.20±2.80	19.91±3.08	19.94±2.55	ns	ns
IVS (cm)	0.91±0.13	0.94±0.16	0.96±0.15	ns	ns
PWT (cm)	0.86±0.16	0.88±0.11	0.86±0.13	ns	ns
LVMI (g/m^2) Males	93.7±11.7	99.3±18.4	98.4±16.0	ns	ns
Females	80.6±20.1	81.8±12.3	80.4±14.8	ns	ns

Values are shown as mean ±SD. HR= heart rate, RVDd= right ventricular end-diastolic diameter, LVDd= left ventricular end-diastolic diameter, LVDs= left ventricular end-systolic diameter, IVS= Interventricular septum thickness, PWT= left ventricular posterior wall thickness, LVMI= left ventricular mass index.

Table 5. Systolic and diastolic function variables by echocardiography and Doppler tissue imaging for healthy controls and patients with primary hyperparathyroidism before and 15±4 months after parathyroidectomy.

Variable	Controls	PHPT cases		P	
		Baseline	Follow-up	PHPT baseline vs. controls	PHPT baseline vs. follow-up
	n=51	n=51(35♀)	n=50(34♀)		
Systolic Variable					
S´-RV (cm/s) (13.5±1.7)[1]	13.85±1.77	14.23±1.85	13.48±1.79	ns	<0.05
S´-IVS (cm/s) (7.7±1.7)[2]	8.23±1.00	8.48±0.96	7.97±0.85	ns	<0.05
S´-LVL (cm/s) (8.5±2.2)[2]	9.80±1.89	9.61±2.05	8.87±1.63*	ns	<0.05
EF (%)	65.29±6.17 [a]	66.18±5.78 [a]	65.75±4.84 [a]	ns	ns
FS (%)	36.29±5.14 [a]	37.02±4.33 [a]	36.46±3,81 [a]	ns	ns
AV- plane RV (cm)	2.64±0,32	2.58±0.37	2.50±0.39	ns	ns
AV-plane IVS (cm)	1.44±0.20	1.42±0.18	1.39±0.19	ns	ns
AV-plane LV (cm)	1.57±0.21	1.55±0.18	1.55±0.18	ns	ns
Diastolic Variable					
E-wave (cm/s)	80.18±14.44	75.76±15.09	75.77±13.47	ns	ns
A-wave (cm/s)	59.59±12.48	60.58±15.27	61.41±12.70	ns	ns
E/A	1.39±0.33	1.30±0.34	1.27±0.28	ns	ns
DT (ms)	197.9±22.6	199.7±27.9	200.8±29.3	ns	ns
IVRT (ms)	82.36±16.20	86.67±18.42	86.32±19.42	ns	ns
PVs / PVd	1.16±0.27	1.22±0.34	1.21±0.32	ns	ns
E/ E´ Septal	7.81±1.73	7.69±1.74	7.54±1.58	ns	ns
E/ E´ Lateral	6.38±1.43	6.35±1.84	6.50±1.61	ns	ns

*[a] n=49–50. Values are shown as mean ±SD. S´= the regional peak systolic myocardial velocities measured by DTI, RV= right ventricular wall, IVS= interventricular septum, LVL= left ventricular lateral wall, EF= ejection fraction, FS= fractional shortening. AV-Plane= atrioventricular plane displacement, E-wave= early transmitral diastolic flow velocity, A-wave= flow velocity during atrial contraction, DT= deceleration time, PVs= systolic pulmonary vein velocity, PVd= diastolic pulmonary vein velocity. E/ E´ = a ratio of early transmitral diastolic flow velocity (E) and early diastolic velocity recorded by DTI (E´) in the mitral annulus. [1] The reference for published normal values for DTI myocardial velocities for RV [146]. [2] The reference for published normal values for DTI myocardial velocities for LV [147]. * The peak systolic velocity in the lateral part of the mitral annulus was lower in patients after PTX compared to controls, P<0.05.*

4.4 VASCULAR ULTRASOUND AND PULSE-WAVE ANALYSIS (STUDY III)

The results of vascular ultrasound and pulse-wave variables are summarized in Table 6. AI% did not differ between patients and controls and did not change after PTX, neither did the estimated aortic blood pressure. We found no differences between patients and controls regarding IMT_{cca} or lumen of the CCA and no changes were seen during follow-up. IM-GSM values did not differ and there was no significant change after PTX. The IMT_{rad} of the right radial artery did not differ between the groups and was unchanged at follow-up. While the radial artery lumen diameter was similar to that of controls, a marginal increase in LD_{rad} was noted after one year ($p<0.05$). Univariate and multivariate analyses of vascular variables to baseline data showed that AI% was related to age (beta 0.53, $p<0.001$) and inversely to weight (beta –0.41, $p<0.001$), while IMT_{cca} was related to age (beta 0.55, $p<0.001$) and IM-GSM to weight (beta –0.33, $p=0.02$). Another multivariate analysis, adjusted for age and weight, of vascular variables (AI%, IMT_{cca} and IM-GSM) in relation to biochemical data specifically abnormal in PHPT (PTH, Ca++, phosphate) showed no relation between vascular variables and PTH and Ca++; only a relation between AI% and phosphate (beta –0.22, $P=0.004$) was found.

Table 6. Augmentation index and ultrasound measurements for healthy controls and patients with mild primary hyperparathyroidism before and 15±4 months after parathyroidectomy

Variable	Controls	PHPT cases		P	
		Baseline	Follow-up	PHPT baseline vs. controls	PHPT baseline vs. follow-up
	n=48 (35♀)	n=48 (35♀)	n=48 (35♀)		
AI%	27.7±12.8	28.6±12.2	29.8±11.9	ns	ns
Ao SBP (mmHg)	109.8±15.3	114.4±17.3	114.8±15.8	ns	ns
Ao DBP (mmHg)	75.0±9.7	78.1±11.3	78.0±8.7	ns	ns
IMT_{rad} (mm)	0.255±0.053[a]	0.271±0.060[c]	0.271±0.046[b]	ns	ns
LD_{rad} (mm)	1.87±0.35[a]	1.79±0.30[c]	1.90±0.38[b]	ns	<0.05
IMT_{cca} (mm)	0.680±0.135	0.688±0.113	0.702±0.119	ns	ns
LD_{cca} (mm)	5.94±0.62	6.03±0.45	6.03±0.47	ns	ns
IM-GSM	86.5±15.3	82.3±17.2	81.8±15.9	ns	ns

[a]47, [b]43, [c]40–41. Values are shown as mean ±SD. AI= augmentation index, Ao SBP= aortic systolic blood pressure, Ao DBP= aortic diastolic blood pressure, IMT_{rad} = intima media thickness in right radial artery, LD_{rad}= lumen diameter in right radial artery, IMT_{cca}= intima media thickness in both left end right common carotid artery, LD_{cca}= lumen diameter in common carotid artery, IM-GSM= intima media grey scale median in both left end right common carotid artery.

4.5 VITAMIN D AND CARDIOVASCULAR RISK MARKERS (STUDY IV)

The serum 25-OH-D level was significantly lower in patients compared to controls at baseline and increased postoperatively, Table 7. Preoperative vitamin D deficiency (25-OH-D < 50 nmol/L) was found in 77% of the patients (n=37/48) compared to 20% of the controls, (n=9/46). Another 21 % of the patients (n=10/48) and 59% of the controls (n=27/46) had insufficient vitamin D levels (50 > 25-OH-D < 75 nmol/L). At follow-up 15±4 months after PTX, 40% (n=19/48) of the patients were still vitamin D deficient and 33% (n=16/48) were vitamin D insufficient. Except for a higher postoperative PTH level, patients with 25-OH-D levels below 50 nmol/L did not differ from those above this level as regards BMI, age, Ca++ and GFR in relation to vitamin D status, Table 8.

The distributions of PTH and calcium in relation to vitamin D are illustrated in Figure 10 and Figure 11. Postoperatively, 25-OH-D was inversely correlated to the PTH level ($r = -0.34$; $P<0.05$). Seven patients had elevated PTH levels at follow-up (range 68–92 ng/L) in combination with normal Ca++ (range 1.20–1.31 mmol/L). The mean 25-OH-D level was significantly lower in patients with a persistently elevated PTH level compared to those with normal PTH level at follow-up (25-OH-D; 45±19 vs. 61±19 nmol/L; $p<0.001$); 5 of 7 patients had a 25-OH-D level below 50 nmol/L.

The level of ALP was higher in PHPT patients compared to the healthy controls and correlated to the PTH and the Ca++ levels ($r=0.53$, $p<0.001$) as well as inversely to 25-OH-D ($r = -0.33$, $p<0.01$) and decreased significantly after PTX, Table 7.

The TG level was slightly higher in patients compared to controls; otherwise there were no differences between the groups regarding age, BMI, coagulation, inflammatory, metabolic and lipid markers, Table 7. After PTX, TG level decreased slightly, while BMI, apo-B and hs-CRP increased in patients. The level of calcium correlated to VWF:Ag ($r=0.33$, $p<0.05$) and the TG level correlated to PAI-1 activity ($r=0.44$, $p<0.01$).

Table 7. Vitamin D status and cardiovascular risk markers for healthy controls and patients with primary hyperparathyroidism before and 15±4 months after parathyroidectomy.

Variable	Controls	PHPT cases		P value	
		Baseline	Follow-up	PHPT baseline vs. controls	PHPT baseline vs. follow-up
	n=49(35♀)	n=49(35♀)	n=49(35♀)		
P-ALP (< 1.9 µkat/L)	0.91±0.24[c]	1.34±0.42[c]	1.08±0.85[a]	<0.001	<0.001
S-25-OH-D (75–250 nmol/L)	64.6±20.8[a]	40.1±16.5[a]	58.9±19.5[a]	<0.001	<0.001
S-Cholesterol (3.9–7.8 mmol/L)	5.71±1.02[a]	5.76±0.80[a]	5.83±0.89	ns	ns
S-TG (0.45–2.6 mmol/L)	0.86±0.43[a]	1.04±0.60[a]	0.94±0.50	<0.05	<0.05
S-Apo-A1 (1.10–2.10 g/L)	1.73±0.31[a]	1.71±0.33[a]	1.68±0.28	ns	ns
S-Apo-B (0.50–1.70 g/L)	1.02±0.21[a]	1.01±0.18[a]	1.06±0.16	ns	<0.01
Apo-B/Apo-A1 (<1)	0.61±0.14[a]	0.62±0.18[a]	0.65±0.13	ns	ns
P-VWF (0.60–1.60 kIE/L)	1.12±0.41[b]	1.06±0.36[a]	1.08±0.40[a]	ns	ns.
P-PAI-1 (<15 kIE/L)	9.34±6.32[b]	9.97±8.23[a]	8.69±7.41[a]	ns	ns.
P-hs-CRP (<3 mg/L)	1.13±1.21[a]	0.92±0.89[a]	2.00±5.00	ns	<0.01
P-Homocysteine (5.0–15 µg/L)	9.25±3.00[a]	9.96±2.78[a]	9.78±2.64[a]	ns	ns
S-IGF-1 (110–270 µg/L)	136.3±52.9[a]	146.7±47.9[a]	142.7±67.7	ns	ns

[a]n=45–48, [b]n=42–-43, [c]n=34–35, Values are shown as mean ±SD. ALP= alkaline phosphatase, 25-OH-D= 25-hydroxyvitamin D, TG= triglyceride, Apo= apolipoprotein, VWF:Ag= von Willebrand factor, PAI-1= plasminogen activator inhibitor-1 activity, hs-CRP = high sensitive C-reactive protein, IGF-1= Insulin-like growth factor 1.

Table 8. Clinical and biochemical data (mean±SD) for patients with primary hyperparathyroidism, before and 15±4 months after parathyroidectomy in relation to vitamin D status.

Variable	PHPT baseline		P	PHPT follow-up		P
	25-0H-D <50 nmol/L n=37 (26♀)	25-0H-D >50 nmol/L n=11 (9♀)		25-0H-D <50 nmol/L n=19 (16♀)	25-0H-D >50 nmol/L n=29 (18♀)	
Age (yr.)	53.9±9.4	55.5±6.8	ns	56.6±8.4	54.6±9.1	ns
BMI (kg/m^2)	24.0±2.8	23.9±3.4	ns	25.3±2.9	24.2±3.5 [a]	ns
GFR (mL/min)	104.0±23.2	91.6±15.0	ns	105.7±27.2	102.5±28.2[a]	ns
S-Ca++(mmol/L)	1.46±0.07	1.45±0.03	ns	1.26±0.04	1.25±0.05	ns
P-PTH (ng/L)	125.7±39.5	104.3±39.5	ns	57.2±15.3	44.8±14.0	P<0.01
S-25-OH-D (nmol/L)	33.4±10.1	62.5±14.0	P<0.001	41.0±6.7	70.6±15.8	P<0.001

[a] *n=27–28. BMI= body mass index, GFR= glomerular filtration rate according to Cockroft-Gault's formula: GFR=(140 − age in yr) x (weight in kg/P-creatinine) x (1.23 in men, 1.04 in women), Ca++= ionized calcium, PTH= parathyroid hormone, 25-OH-D= 25-hydroxyvitamin D. Vitamin D deficiency defined as 25-OH-D < 50 nmol/L.*

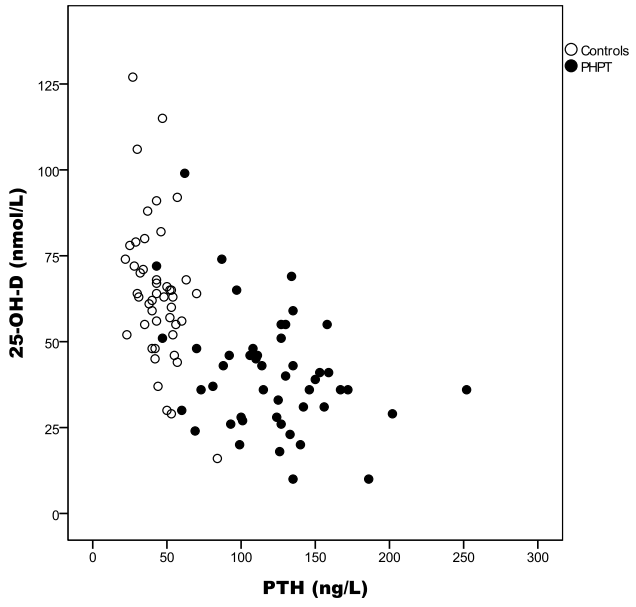

Figure 10. *The distribution of 25-OH-D and parathyroid hormone (PTH) levels for the whole group of PHPT patients and the healthy control subjects*

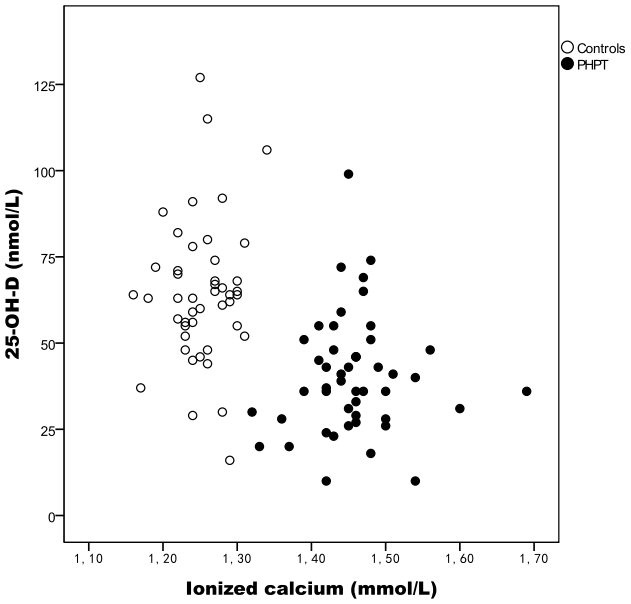

Figure 11. *The distribution of 25-OH-D and the ionized calcium levels for the whole group of PHPT patients and the healthy control subjects.*

5 DISCUSSION

The major goal of this thesis was to analyze the extent and nature of CV involvement in mild PHPT. We performed a prospective case-control study with a strict selection procedure. To avoid the influence of confounding factors, only patients and control subjects without known CV risk factors were included. The group of patients included in our study made up about one-tenth of all the PHPT patients treated with PTX at our clinic during the time period. We used advanced methods to evaluate CV structure and function. Vitamin D status and biochemical risk markers correlated to CV events were analyzed. We also performed an experimental study to analyze the acute effect of elevated PTH on vasodilatory function.

5.1 STUDY I

Measurement of the blood flow response to intra-arterial infusion of vasoactive agents using the invasive forearm model is the golden standard for assessing endothelial function in resistance arteries. The main advantage of this method is that it allows the evaluation of local effects on vasodilatory function since the risk of confounding by systemic effects is negligible. Experimental data suggest that PTH has vasodilatory properties, mediated directly through cAMP-dependent inhibition of L-type calcium channel currents in smooth muscle cells and/or through activation of endothelium-derived nitride oxide production [148, 149]. In the present study, PTH infusion into the brachial artery resulted in a locally elevated PTH and unchanged Ca++ level in the forearm but did not affect the vasodilatory reaction induced by metacholine (EDV) or nitroprusside (EIDV). We observed a numerical but not significant increase in both EDV and EIDV during PTH infusion.

The systemic haemodynamic effects of PTH in humans are complex and have been studied in a few trials with contradictory results. While acute systemic PTH infusion caused hypotension [35], chronic infusion resulted in hypertension [34]. In a study with systemic and local infusion of calcium in healthy subjects, Nilsson et al. observed a dose-related impairment in endothelial vasodilatory function, an increased SBP and a significant drop in PTH level during systemic hypercalcemia but not during local hypercalcemia [40].

The results from these studies support the hypothesis that the main hemodynamic actions of PTH are mediated indirectly through changes in the plasma calcium level and/or an impact on vasoactive hormones such as cortisol and the renin-aldosterone axis, [35, 40, 150]. It should be emphasized that the number of subjects in our study was small and only major effects of PTH could be detected.

5.2 STUDY II

This study was conducted to evaluate BP, cardiac structure, function and the effect of PTX in patients with mild PHPT in comparison to healthy controls. We observed no differences in global systolic or diastolic function, or cardiac morphology in PHPT patients compared to controls. The BP and regional systolic myocardial velocities corresponded to normal values both at baseline and one year after PTX in all subjects. However, the patients had a slightly higher SBP compared to controls and both BP and regional systolic myocardial velocities decreased significantly after PTX. Our results indicate that PHPT patients had a higher systolic myocardial performance at baseline, which seems to be associated with PTH and Ca++ levels. The clinical significance of these findings is not clear. Hypothetically, a disease-related inotropic effect may result in increased vascular resistance and left ventricular workload which, if they persist, can partly explain some of the CV abnormalities seen in PHPT patients. Our hypothesis of a suprasystolic performance is supported by Almqvist et al., who observed a transient decrease in systolic and diastolic function variables in PHPT patients after PTX when the PTH and calcium levels had normalized [65]. The authors interpreted this phenomenon as a result of the withdrawal inotropic effect of PTH.

DTI is a sensitive echocardiographic method which allows early detection of even minor cardiac dysfunction [151]. Mitral annulus velocity determined by DTI is a relatively preload-independent variable and is superior to conventional mitral Doppler indexes [152]. To our knowledge, only two studies have used this method for evaluation of cardiac function in mild PHPT [68, 153]. Similar to Walker et al., we did not find any abnormalities in LVMI or diastolic function in PHPT. Baykan et al. evaluated cardiac function in PHPT patients without CV risk factors and found signs of diastolic disturbances such as lower E, E/A ratio and prolonged IVRT in the PHPT group. We could not confirm these findings. A subgroup analysis revealed a prolonged IVRT in male cases but since the number of cases is only 16, the finding does not allow us to draw any conclusions.

The effect of PTX on BP has not been consistent in literature. The majority of previous data show no effect on BP in PHPT patients after PTX [79, 88, 154]. However, other studies have shown decreased BP after PTX [69, 91, 92]. We observed a minor decrease in both SBP and DBP in our patients after PTX.

Data from observational studies involving more than 1 million individuals without previous vascular disease indicate that mortality from both ischemic heart disease and stroke increases from BP levels as low as 115/75 mmHg. For every 20 mmHg systolic or 10 mmHg diastolic increase in BP, there is a doubling of mortality from both ischemic heart disease and stroke [155]. Early adequate BP control is essential for prevention of future CV events before irreversible CV alterations accrue. BP-lowering treatment provides a proportional reduction in CV risk in hypertensive patients and also in normotensive high-risk patients [156].

5.3 STUDY III

In this ultrasound study of arterial function and structure in the carotid and radial arteries and endothelial function evaluated by PWA, we found no indications of vascular abnormalities in patients with mild PHPT without any known CV risk factors. Neither did PTX cause any differences in either our vascular indices of arterial wall thickness or vascular function. In addition to patients yielding much the same vascular findings as controls, our results were further strengthened by the absence of an effect on arterial measurements from total and Ca++ and PTH.

Increased AI% has been reported in mild PHPT [66, 67]. In contrast to previous findings, AI% was normal in our patients and did not differ from controls.

Data regarding carotid vascular abnormalities in PHPT are conflicting; while some have reported increased IMT_{cca} in PHPT patients [76, 80], others have not found any disturbances [77, 78]. Both severity of PHPT and coexisting CV diseases and risk factors play an important role in the development of cardiovascular complications and explain the conflicting findings in the literature. In our study with mild PHPT and careful exclusion of CV risk we did not observe an increased IMT or any other vascular abnormalities compared to controls. Our results are supported by Fallo et al., who compared PHPT patients with and without CV risk factors to healthy controls and found increased IMT_{cca} only in patients with concomitant CV risk factors.

Our conclusions were substantiated by data from innovative techniques, such as grey scale median for the evaluation of echogenicity of the carotid intima media, and high resolution ultrasound for measurement of intima thickness in the radial artery. To our knowledge, this is the first time these kinds of technique have been used for this purpose in patients with mild PHPT. Previous data suggest that high resolution ultrasound allows measurement of very thin structures, such as intima of the carotid artery wall in rats, showing a significant relationship between intima thickness and the histopathology of intima of the carotid artery wall [157]. Information regarding the composition and characteristics of the carotid artery wall can be obtained by using IM-GSM technique. Earlier data have shown that IM-GSM in CCA is closely related to the echogenecity of the carotid plaques and that the echolucency of the carotid intima-media is related to several CV risk factors [158, 159]. Our results did not show any abnormalities in the structural properties of the carotid and radial arteries.

5.4 STUDY IV

The main objective of this study was to evaluate vitamin D status and biochemical risk markers correlated to CV events in patients with mild PHPT without other known CV risk factors. Except for a higher prevalence of vitamin D deficiency and slightly higher TG levels, biomarkers predicting CV diseases did not differ between healthy controls and patients. PTX had an overall positive effect on TG level and vitamin D status.

In line with previous findings [10, 160, 161], vitamin D deficiency (serum 25-OH-D <50 nmol/L) was more common in our patients with PHPT (77%) compared to their well-matched controls (20%) from the same geographic area. The level of 25-OH-D was significantly increased at follow-up 15±4 months after PTX, though 40% of the patients were still vitamin D deficient. Vitamin D deficiency has been suggested to account for a significant number of patients who exhibit normocalcemic PTH elevation after successful PTX [162, 163]. This may explain the persisting postoperative normocalcemic PTH elevation in combination with lower 25-OH-D levels in a small group of our patients.

One weakness of our study could be that we did not analyze 1,25(OH)2D levels. However, the level of 25-OH-D, but not 1,25(OH)2D, has been reported to be correlated to PTH secretion in PHPT and seems to be the best indicator for evaluation of vitamin D status [164]. Furthermore, 25-OH-D is more stable than the active form, its

circulating level in serum is roughly 500-1000 x that of 1,25(OH)2D and it correlates well with the biological effects of vitamin D [27].

The preoperative elevation of serum ALP in our PHPT patients, which correlated with the PTH and Ca++ levels, could be an indication of an accelerated bone turnover, which has been shown in other studies [165].

Few trials have evaluated the level of CV risk markers such as VWF, PAI-1 and hs-CRP in PHPT patients; in keeping with these, we did not observe any abnormalities [111, 116]. An unexpected finding in our study was the increased hs-CRP level after PTX. The clinical significance of this finding is unclear. Plasma hs-CRP levels are influenced by many factors, such as trauma and infection [166]. To avoid confounding factors, evaluation of hs-CRP on two separate occasions a few weeks apart is recommended; failure to do this was a weakness of our study.

Dyslipidemia in mild PHPT has been observed in a few trials [5, 108, 111]. The TG levels were slightly higher in our patients compared to controls but within the reference range and they decreased after PTX. Otherwise there were no inter-group differences in lipid status. The discrepant results could partly be explained by the presence of CV risk factors in previous studies.

5.5 GENERAL DISCUSSION

PHPT is a common endocrine disorder. The clinical profile of the disease has undergone a striking change in developed countries, from a severe symptomatic disease with typical target organ involvement (bone and kidneys) to an almost asymptomatic disorder with no obvious symptoms or signs traditionally associated with the disease. There is a broad consensus that patients with biochemically confirmed PHPT and classical symptoms from bone and kidneys should be offered parathyroid surgery. However, the indication for parathyroid surgery in so-called “asymptomatic” patients without any traditional PHPT symptoms is still debated. One reason for this controversy is the difficulty in predicting which patients will have disease progression or would benefit from early surgery to avoid future complications. Although patients with PHPT often complain of neurocognitive and psychiatric symptoms like memory disturbances, fatigue, anxiety and depression, neuropsychiatric symptoms are still not generally accepted as indications for parathyroid surgery. Neither are the presence of

CV diseases or prevention of future CV complications commonly accepted as indications for parathyroid surgery.

The long-term effects of undiagnosed or conservatively treated PHPT are still largely unknown. Long-term observational data show evidence of biochemical and densitometric stability in the majority of the patients in the first 8–12 years. However, approximately 25 % of the patients show evidence of disease progression, worsening of the biochemical profile after 12 years, and densitometric progression after 8 years [18]. Furthermore, data regarding the extent of CV complications and reversibility after surgical cure in mild asymptomatic PHPT are limited. The latest international workshop for asymptomatic PHPT emphasized that further investigations regarding CV aspects in mild PHPT are needed [9].

The term mild PHPT is frequently used in the literature but there is no generally approved definition. We defined mild hyperparathyroidism as plasma calcium less than 2.75 mmol/L and/or the absence of overt symptoms related to hypercalcemia. The majority of our patients had plasma calcium levels below 2.75 mmol/L; only 5 patients had levels between 2.76–2.97 mmol/L at the time of inclusion. The main objective of this thesis was to study how mild hyperparathyroidism affects the CV function. We applied a strict selection to exclude patients with known CV disease and risk factors or medication that could affect CV function. Only about one-tenth of all the PHPT patients treated at our clinic met the inclusion criteria for the study. The strict selection is the main strength of our study and furthermore adds to the accuracy of our findings since other confounding factors are excluded. However, it might also be considered as a weakness, since our results may not be extended to the large PHPT population in general where comorbidity is common. It is most likely that PHPT may potentiate the negative effects of co-existing risk factors [167].

Despite usage of sensitive methods for evaluation of the CV system, we found no obvious signs of CV involvement in our patients. However, we did observe subtle differences such as slightly higher SPB and TG levels in the PHPT patients compared to controls. Our patients had also a “supersystolic performance”, with higher systolic myocardial velocities, and higher systolic and diastolic BP. Surgical cure had an overall positive effect on all these factors. The clinical significance of these subtle findings is not clear. Hypothetically, if the disease-related “supersystolic” condition persists for a

long time, it might result in an increased vascular resistance and cardiac workload that may contribute to future CV complications. Population studies support that even a small reduction in BP could have a major public health impact on the risk of coronary heart disease and stroke events [168].

A very large proportion of our patients (77%) had vitamin D deficiency. PTX had a positive effect on vitamin D status. However, 40% of our patients remained vitamin D deficient after successful cure. The clinical consequence of this finding is not clear. Vitamin D deficiency represents an important new CV risk factor and has been associated with increased CV morbidity [96, 169]. However, the coupling between vitamin D deficiency and CV morbidity is not fully understood and there is no available evidence that vitamin D supplementation might prevent future CV events.

It is reasonable to believe that the adverse effect of the hyperparathyroid condition may be amplified in the presence of other CV risk factors [167, 170, 171]. It is also reasonable to assume that adequate treatment of PHPT and the coexisting risk factors may improve the CV outcome. However, based on our results, we have no evidence that CV complications can be prevented by parathyroid surgery in PHPT patients without known CV diseases and risk factors. If just the CV aspect is taken into consideration, the indication for surgery in this subgroup of PHPT patients is therefore not clear-cut.

5.6 FUTURE PERSPECTIVES

The natural history of PHPT in conservatively treated patients with asymptomatic mild disease is poorly investigated. To identify risk patients who may benefit from early surgical treatment, future randomized studies, comparing the long-term effects of conservative follow-up and parathyroid surgery respectively, on wide health aspects are required. Furthermore, the influence of vitamin D deficiency and CV aspects in PHPT need further investigation, as do how and when to optimize vitamin D status in PHPT patients with concomitant vitamin D deficiency.

6 CONCLUSIONS

- Experimental acute elevation of the PTH level alone had no effect on endothelial vasodilatory function.

Patients with mild PHPT without known CV risk factors had:

- Normal global systolic and diastolic function and cardiac morphology but a higher systolic myocardial performance and blood pressure at baseline, which seems to be associated with PTH and Ca++ levels and decreased after PTX.

- Normal arterial function and structure in the carotid and radial arteries. No differences were observed between cases and controls. Neither did PTX entail any differences in either our vascular indices of arterial wall thickness or vascular function.

- Higher prevalence of vitamin D deficiency and a slightly higher TG level compared to controls. Otherwise there were no inter-group differences in coagulation, inflammatory metabolic and lipid status. PTX had an overall positive effect on TG level and vitamin D status.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING (SWEDISH SUMMARY)

Primär hyperparatyreoidism (bisköldkörtelöverfunktion, PHPT) är en vanlig sjukdom där ökad utsöndring av parathormon (bisköldkörtelhormon) medför förhöjda kalciumnivåer i blodet. PHPT orsakas oftast av en godartad tumör i en av bisköldkörtlarna. Cirka 1 % av den vuxna befolkningen och 3-4 % av kvinnorna efter klimakteriet drabbas. Idag upptäcks sjukdomen oftast av en slump i samband med rutinmässig blodprovstagning. Många patienter har nästan inga eller väldigt diffusa symptom som trötthet, irritabilitet minskad livsglädje och/eller minnesstörning. Den enda botbara behandlingen är kirurgiskt avlägsnande av onormal bisköldkörtelvävnad.

Tidigare studier har visat ökad sjuklighet och dödlighet i hjärtkärlsjukdomar vid PHPT. Däremot vet vi väldigt lite om riskerna för hjärtkärlsjukdom i samband med mild PHPT. Denna osäkerhet beror delvis på att tidigare studier av PHPT har omfattat individer med olika svårighetsgrader av sjukdomen och som i stor utsträckning belastats även av andra riskfaktorer som kan ha påverkat resultaten.

Huvudsyftet med våra studier i denna avhandling var att undersöka hjärtkärlfunktionen hos patienter med mild PHPT utan kända riskfaktorer för hjärtkärlsjukdom och analysera effekten av operation. Vi valde ut en grupp av patienter utan kända riskfaktorer som högt blodtryck, fetma, diabetes eller medicinering som påverkade hjärtkärlfunktionen. Endast någon tiondel av våra patienter med PHPT som genomgick operation under studieperioden uppfyllde dessa kriterier. Vi startade med att undersöka om kärlfunktionen i underarmen påverkades vid akut förhöjning av parathormonnivån hos friska individer.

Studie I: Kärlfunktionen utvärderades med venös pletysmografi före och under infusion av parathormon i underarmsartär som medförde lokalt förhöjning av parathormonnivån i underarmen hos 10 friska individer. Vi kunde inte påvisa någon direkt inverkan av parathormon på kärlfunktionen.

Studie II-IV: 51 patienter med mild PHPT och 51 slumpmässigt utvalda kontrollpersoner, matchade för ålder och kön deltog i studierna. Hjärtkärlfunktionen

undersöktes med känslig ultraljudsteknik. Vi analyserade nivåer av riskmarkörer för hjärtkärlsjukdomar (blodfetter, inflammatoriska och tromboemboliska markörer) samt D-vitamin i blodet.

Våra studier visade att patienter med mild PHPT hade normal hjärtkärlfunktion jämfört med friska kontroller. Det fanns dock små skillnader mellan grupperna. Patienterna hade något högre systoliskt blodtryck och högre blodfetter jämfört med friska kontroller. D-vitaminbrist var mycket vanligare hos patienterna (77%) jämfört med kontrollerna (20%). Operation hade en positiv inverkan på blodtrycket, blodfetterna och D-vitaminnivåerna. Vi kan inte uttala oss om den kliniska betydelsen av dessa små skillnader mellan grupperna.

Sammanfattningsvis fann vi inte evidens för att patienter med mild PHPT utan kända riskfaktorer för hjärtkärlsjukdom löper ökad risk för hjärtkärlkomplikationer. Man måste vara klar över att den studerade gruppen utgör en höggradigt selekterad grupp av patienter med ”låg riskprofil”. Vår studiedesign tillåter oss inte att uttala oss om PHPT kan potentiera risken för hjärtkärlkomplikationer hos patienter med PHPT och samtidiga riskfaktorer för hjärtkärlsjukdom.

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