

Diploma thesis

**Cardiovascular Risk in Patients with Primary
Hyperparathyroidism**

submitted by

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Zusammenfassung

Einleitung:

Zunehmende Evidenz deutet auf eine Assoziation zwischen erhöhten Parathormonkonzentrationen und gesteigertem kardiovaskulären Risiko hin. Die zugrundeliegenden Mechanismen der bei PatientInnen mit primärem Hyperparathyreoidismus gehäuft auftretenden kardiovaskulären Beeinträchtigungen sind jedoch noch immer unklar. In dieser Studie werden daher Assoziationen zwischen der Parathormonkonzentration und verschiedenen kardiovaskulären Surrogatparametern bei PatientInnen mit einem primären Hyperparathyreoidismus untersucht.

Methoden und Ergebnisse:

Die vorliegende Arbeit befasst sich mit Baseline-Daten der randomisierten, doppelblinden, Plazebo-kontrollierten “Effect of Eplerenone on Parathyroid Hormone Levels in Patients with Primary Hyperparathyroidism” (EPATH) Studie. Eingeschlossen wurden PatientInnen, die gemäß internationalen Richtlinien mit primärem Hyperparathyreoidismus diagnostiziert wurden und mind. 18 Jahre alt waren. Es erfolgte eine standardisierte Nüchtern-Blutabnahme am Morgen, sowie eine 24h-Blutdruckmessung, welche mit einem validierten Gerät (Mobil O Graph, I.E.M., Stolberg, Deutschland) durchgeführt wurde. Unsere Analyse umfasste 155 TeilnehmerInnen (78,7% weiblich, 21,3% männlich; Gruppe 1) mit einem mittleren Alter von 67,4 (+/- 10,3) Jahren. Die Parathormonkonzentration (Median) lag bei 99 (IQR 82-125) pg/ml. Für eine zweite Analyse wurden PatientInnen, die Cinacalcet einnahmen, ausgeschlossen (Gruppe 2). Der weitere Ausschluss von PatientInnen, die PTH-modifizierende Medikamente (ACE-Hemmer, AT1-Blocker, MR-Antagonisten, Thiazid-, sowie Schleifendiuretika) regelmäßig einnahmen, führte zur Bildung einer dritten Gruppe. In der multivariaten linearen Regressionsanalyse adjustierten wir für Alter, Geschlecht, Body Mass Index, HbA1c, antihypertensive Medikation, Serum-Kalzium, Serum-Cholesterol, die glomeruläre Filtrationsrate, sowie für den Blutdruck. Parathormon zeigte sich in Gruppe 3 als starke Determinante der mittleren 24h-Pulswellengeschwindigkeit, der Nacht-Pulswellengeschwindigkeit, sowie des systolischen Nachtblutdruckes. In den Gruppen 1 und 2 stellte sich keine Assoziation dar.

Diskussion:

In einer ausgewählten Kohorte aus PatientInnen mit primärem Hyperparathyreoidismus zeigte sich ein Zusammenhang zwischen dem Plasmaparathormonspiegel und der Pulswellengeschwindigkeit einerseits, sowie des systolischen Nachtblutdruckes andererseits. Unsere Daten unterstützen die Auffassung, dass die vaskuläre Funktion durch PTH beeinflusst wird. Für die Beurteilung modulatorischer Effekte auf das Gefäßsystem bei Patienten mit PTH-Überschuss sollten weitere klinische und experimentelle Studien durchgeführt werden.

Abstract

Introduction:

Accumulating evidence indicates that high parathyroid hormone (PTH) is associated with increased cardiovascular risk. The underlying causes of cardiovascular impairment frequently seen in patients with primary hyperparathyroidism (pHPT) are however still unclear. This investigation is conducted to add information to the controversially discussed issue of cardiovascular risk associated with PTH excess. Thus, we analyzed the associations between PTH concentration and cardiovascular surrogate parameters in patients with pHPT.

Methods and Results:

We used baseline data of the randomized, double-blind, placebo-controlled “Effect of Eplerenone on Parathyroid Hormone Levels in Patients with Primary Hyperparathyroidism” (EPATH) Trial. Inclusion criteria were age of at least 18 years and a diagnosis of pHPT according to international guidelines. Standardized blood sampling was performed after an overnight fast and 24h ambulatory blood pressure measurement (ABPM) was performed by a validated non-invasive device for ambulatory hemodynamic monitoring (Mobil O Graph, I.E.M., Stolberg, Germany). Our analysis comprised 155 pHPT patients (78.7% females, 21.3% male; Group 1) with a mean age of 67.4 years (+/- 10.3 years) and a median PTH of 99 (IQR 82-125) pg/ml. For a second (subgroup) analysis we excluded patients with a continuous intake of cinacalcet (Group 2). Further exclusion of patients taking ACEI, ARB, MRB, thiazide or loop diuretics on a regular basis led to a third group. In multivariate linear regression analyses adjusted for age, sex, antihypertensive medication, body mass index (BMI), hemoglobin A1c (HbA1c), 25-hydroxyvitamin D, serum calcium, glomerular filtration rate and systolic blood pressure PTH emerged as a strong predictor of mean 24h PWV, nighttime PWV and nighttime SBP in Group 3. In Group 1 and 2 there was no significant association.

Conclusion:

In a selective cohort of patients with pHPT plasma PTH was related to mean 24h PWV, nighttime PWV and nighttime SBP. Our data strengthen the notion that PTH may impact on vascular function. Interventional and mechanistic trials are needed to evaluate modulatory effects on vasculature in patients with high PTH.

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Abbreviations

25(OH)D	25-hydroxy vitamin D
ABPM	ambulatory blood pressure measurement
ACEI	angiotensin converting enzyme inhibitor
Alx	augmentation index
ARB	angiotensin receptor blocker
BMI	body mass index
CaSR	calcium sensing receptor
CVD	cardiovascular disease
DALY	disability-adjusted life years
DBP	diastolic blood pressure
eGFR	estimated glomerular filtration rate
ESC	European Society of Cardiology
FHH	familial hypocalciuric hypercalcemia
HbA1c	hemoglobin A1c
HR	heart rate
IGT	impaired glucose tolerance
IQR	interquartile range
LVH	left ventricular hypertrophy
MAP	mean arterial pressure
MRA	mineralocorticoid receptor antagonist/antagonism
NT-proBNP	N terminal pro brain natriuretic peptide
pHPT	primary hyperparathyroidism
PTH	parathyroid hormone
PTH-rP	parathyroid hormone related peptide
PTX	parathyroidectomy
PWA	pulse wave analysis
PWV	pulse wave velocity
RAAS	renin-angiotensin-aldosterone-system
SBP	systolic blood pressure
SCORE	systematic coronary risk evaluation
SD	standard deviation
WHO	World Health Organization

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1 Introduction

Parathyroid hormone (PTH) is crucial for the maintenance of calcium homeostasis. Balanced calcium levels in turn are essential for several important biological functions, e.g. cell-signaling, neuromuscular performance and bone metabolism. Primary hyperparathyroidism (pHPT) is characterized by excess PTH release in the absence of known or recognized stimuli. It is the third-most common endocrine disorder with only diabetes mellitus and hyperthyroidism being more frequent. (1) The prevalence of pHPT varies between 1 and 21 per 1000 (estimated incidence of 27-30 per 100 000 person-years) depending on laboratory methods and the population studied. (1, 2)

It is well established that pHPT is associated with increased prevalence of cardiovascular diseases (CVD) and with increased cardiovascular mortality. However, underlying mechanisms remain unclear in large parts and previous studies were limited by low sample sizes, insufficient data quality or retrospective character. As a consequence, it remains unclear whether high PTH per se may be the driving force for CVD.

The present work analyzes cross-sectional data from one of the largest pHPT cohorts worldwide, the EPATH Trial, to assess the relationship between PTH and cardiovascular risk factors as well as cardiovascular surrogate parameters.

In the following I will provide a short overview of the physiology of PTH and the mechanism of pHPT. CVD and cardiovascular risk factors will be described briefly before I will focus on CVD and risk in patients with pHPT. Emphasis will be placed on 24-hours ambulatory blood pressure measurement (ABPM).

1.1 Physiology of parathyroid hormone

PTH is produced and secreted by the chief cells of the parathyroid glands. Before PTH evolves into its major storage, secreted and biologically active version, it has to pass several steps. Initially, pre-pro-PTH (a 115-amino acid polypeptide) is cleaved at its N-terminal portion resulting in pro-PTH. In a second step the intact form of PTH (84 amino acids) is generated by cleavage of a 6-amino acid N-terminal extension of the 90-amino acid pro-PTH. (3)

In healthy adults circulating immunoreactive PTH includes intact PTH (5-30%), C-terminal fragments (70-95%) and N-terminal fragments (small percentage). Plasma half-life of intact PTH is 2-4 minutes. The C-terminal fragments are cleared by the kidneys and, thus, plasma half-life is five to ten times higher. (3)

Exocytosis of PTH is regulated via the calcium sensing receptor (CaSR) in the surface of the parathyroid chief cells. (4) These receptors constantly measure plasma calcium concentrations in order to trigger respective changes in PTH liberation. According to variations in ionized calcium levels an increased or diminished PTH release is initiated. CaSR are expressed likewise in bone, kidney and gut – the three major organs involved in calcium homeostasis. (1) PTH gene expression and proliferation of parathyroid cells is inhibited whenever vitamin D receptors on the surface of the parathyroid chief cells are occupied with activated vitamin D (1.25-dihydroxy vitamin D, calcitriol). Hyperphosphatemia contributes to the PTH regulation by enhancing PTH synthesis and secretion and through growth induction of the parathyroid cells.

Circulating calcium is crucial for several important biological functions, e.g. cell-signaling, neuromuscular performance and bone metabolism. Therefore, calcium levels are physiologically maintained within narrow ranges.

PTH regulates the calcium balance in three different ways by affecting bone, the kidneys and the intestines. PTH secretion and activity result in increased plasma calcium concentration which in turn lowers PTH secretion from the parathyroid glands. In the bone tissue, PTH leads to indirect stimulation of osteoclasts which ensues in bone destruction with liberation of calcium. In the distal tubules and the thick ascending limb of the kidneys PTH enhances active reabsorption of calcium. An increase in ionized calcium concentration is additionally achieved through reduction of phosphate reabsorption and, thus, higher calcium:phosphate ratio. In

order to augment calcium absorption in the intestines PTH functions as a coupler between kidneys and intestine in the production of activated vitamin D. PTH up-regulates the key-enzyme responsible for converting vitamin D into its active form 1.25-dihydroxy vitamin D. While the activation occurs in the kidneys, activated vitamin D increases calcium absorption in the intestine. Calcitriol then again inhibits proliferation of parathyroid cells and lowers PTH excretion by binding to the vitamin D receptor.

Serum phosphate regulation through PTH takes place in the same above mentioned tissues. Bone breakdown leads to phosphate release, although slightly less than calcium liberation. In the intestines, activated vitamin D results in enhanced absorption of calcium and phosphate with phosphate being less dependent on the presence of activated vitamin D. While in the kidneys PTH increases calcium reabsorption, phosphate elimination is initiated. In summary, PTH results in a small net drop of serum phosphate concentration.

Normal values of PTH are 1.5-6.5 pmol/l (15-65 ng/l). Reasons for increased values of PTH are elevated serum phosphate concentrations, especially due to chronic kidney disease, vitamin D deficiency, lack of calcium, autonomic production of PTH in the parathyroid glands (pHPT) through swelling or adenoma or pseudohypoparathyroidism which describes the inability of the body to respond to PTH. (1) On the contrary, anything resulting in hypercalcemia, such as hyperthyreosis, overdosage of vitamin D, bone breakdown caused by a tumor or sarcoidosis, can lead to lower-than-normal values of PTH. Other reasons for diminished PTH levels are hypomagnesemia, accidental removal of the parathyroid glands in the course of neck surgery or dysfunction of the parathyroid glands, e.g. due to radiation.

1.2 Primary hyperparathyroidism

1.2.1 Epidemiological facts and etiology

The term primary hyperparathyroidism is characterized by excess PTH release out of one or more parathyroid glands in the absence of known or recognized stimuli. The uncontrolled overproduction of PTH leads to inappropriate elevation of the serum calcium concentration. It is the third-most common endocrine disorder with only diabetes mellitus and hyperthyroidism being more frequent. (1)

While a single gland adenoma occurs in up to 85%, multigland adenomas exist in 15-20% of the patients with pHPT. In approximately half of the patients with multiple adenoma genetic disorders (e.g. multiple endocrine dysplasia syndrome 1 or 2a) may be found. (5) Parathyroid carcinomas or ectopic glands (e.g. around the thyroid gland or in the mediastinum) are rare causes for pHPT. (6) In a small number of patients ionizing radiation or genetic abnormalities are likely to be seen as reason for pHPT. Familial hypocalciuric hypercalcemia (FHH) can be found in some patients with familial hyperparathyroidism. In these patients, mutations in the CaSR gene, in GNA1 or in AP2S1 result in the inability of monitoring and consecutively reacting adequately to changes in circulating ionized calcium. (7) Since surgery would not be curable, those affected by FHH have to be differentiated from patients with pHPT.

The prevalence of pHPT varies between 1 and 21 per 1000 (estimated incidence of 27-30 per 100 000 person-years) depending on laboratory methods and the population studied. (1, 2) Since the technical capabilities enabled serum calcium determination in routine biomedical screening in the early 1970s, the incidence of pHPT increased rapidly. (8) PHPT occurs two to three times more often in women than in men. (1, 9) This ratio increases with age up to a five-fold excess in people older than 75 years. (1) The peak incidence is suggested to be between the 5th and 6th decade of life. (5)

1.2.2 Diagnosis

Hypercalcemia or high calcium levels accompanied by inappropriately high PTH concentrations can be seen as hallmark for pHPT. According to calcium levels, pHPT may be subdivided into normocalcemic and hypercalcemic pHPT. Due to variations of albumin, the major calcium-binding protein, the total serum calcium concentration has to be albumin-adjusted. The formula should be applied if serum albumin is less than 4mg/dL: corrected calcium = measured total serum calcium in mg/dL + 0.8 x (4.0 - patient's serum albumin concentration in g/dL). (10) When assessing patients with potential pHPT, there might be mild or moderate hypercalcemia. In patients with normal total serum calcium concentration ionized serum calcium may still be elevated. PTH (1-84) may be in the reference range, but is inappropriate for the respective hypercalcemia. (1) Serum phosphate concentrations are diminished or in low-normal ranges. A mild hyperchloraemic (chloride > 103mmol/l) metabolic acidosis is an ordinary finding. PHPT features a couple of other biochemical attributes, such as elevated concentrations of calcitriol and reduced phosphate reabsorption in the kidneys. (5)

Since pHPT is the most common cause of hypercalcemia, the workup of a patient suspected with pHPT has to include all differential diagnoses of increased calcium levels. In the general population, malignant disorders are responsible for a large proportion of hypercalcemia. In order to distinguish pHPT and malignancy (together accounting for over 90% of hypercalcemia), immunoassays for intact PTH and PTH-related protein (PTH-rP), the humoral mediator of malignancy-associated hypercalcemia, are needed. (5) High PTH is indicative of pHPT. On the contrary, PTH-related protein is high in most cases of malignancy-associated hypercalcemia while PTH is low or undetectable in these patients. Other causes of hypercalcemia include sarcoidosis, lithium intake, diuretic use, tamoxifen, milk alkali syndrome, vitamin D intoxication, increased calcium intake, adrenal insufficiency, and thyrotoxicosis. (11) Usually, in non-parathyroid causes hypercalcemia is adjunct to PTH in low or low/normal ranges.

The diagnosis of pHPT requires comprehensive assessment of potential causes for secondary or tertiary hyperparathyroidism. Common causes of secondary hyperparathyroidism are chronic kidney disease, drug intake (e.g. bisphosphonates, anticonvulsants or furosemide) or pseudohypoparathyroidism. (12) In the course of

excluding secondary reasons for PTH elevation, FHH is an important differential diagnosis. FHH is a consequence of a hereditary mutation of the CaSR gene rendering the receptor insensitive for circulating calcium. Those affected present with hypercalcemia accompanied by PTH concentration that is modestly increased or within normal ranges. Usually, patients are healthy despite a long history of hypercalcemia. Davies et al. could show that the increased renal tubular reabsorption of calcium remained enhanced after parathyroidectomy (PTX). (13) Since surgery is not curative, it is important to distinguish between classic pHPT and FHH. **Table 1** shows several factors that can facilitate differentiation.

Table 1: Comparison of clinical and biochemical findings in FHH and pHPT (1)

	FHH	pHPT
AGE (YEARS)	<40	>50
SEX	Equal men and women	Mainly women
SYMPTOMS	Unrelated to calcium	Related to calcium
PLASMA ALBUMIN-ADJUSTED CALCIUM (MMOL/L)	2.55–3.5	2.55–4.5
INTACT PARATHYROID HORMONE (PMOL/L)	Most within reference range (0.9–11.0; median 3.0)	Most above reference range (2.5–84.5; median 8.2)
PLASMA MAGNESIUM (MMOL/L)	Trend higher (0.78–1.18; median 0.94)	Trend lower (0.34–1.03; median 0.84)
PLASMA 1,25-DIHYDROXYVITAMIN D (PMOL/L)	Within reference range (54–134; median 87)	Often raised (62–212; median 105)
CaCl/CrCl	Most <0.01 (0.001–0.018; median 0.005)	Most >0.015 (0.001–0.060; median 0.019)

CaCl/CrCl=calcium creatinine clearance ratio

A calcium creatinine clearance ratio lower than 0.01 is suggested as the cut-off separating FHH from pHPT. Thus, a 24-hour urine collection for measurement of calcium and creatinine (and estimated glomerular filtration rate (eGFR)) should be obtained.

The clinician must also take a careful history to ensure that the patient is not taking lithium or thiazide diuretics as these can confound biochemical testing. Since low 25-hydroxy vitamin D (25(OH)D) levels stimulate PTH secretion, patients diagnosed with vitamin D deficiency have to undergo supplement therapy before PTH concentration is reevaluated. Secondary hyperparathyroidism due to lack of vitamin D can be suspected, if increasing vitamin D concentrations are accompanied by a significant decrease of PTH. (14) Especially in normocalcemic patients suspected of pHPT, vitamin D insufficiency/deficiency has to be excluded prior to diagnostic work-up for pHPT.

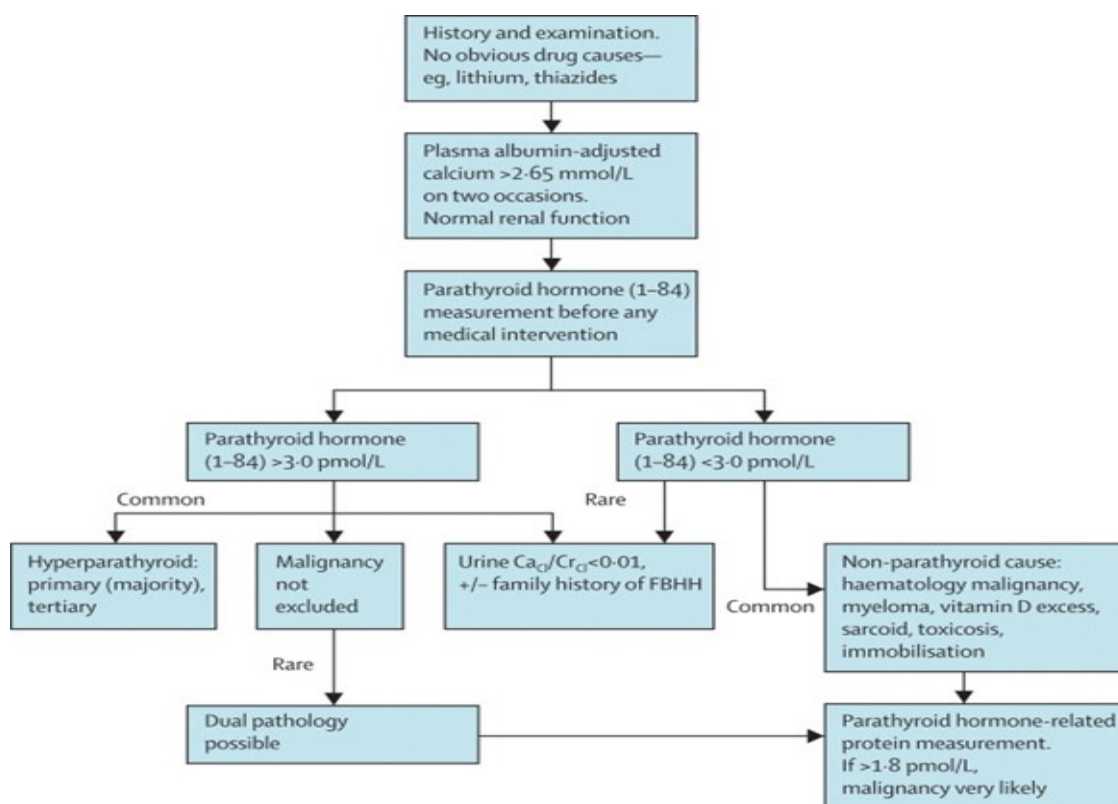


Figure 1: Initial investigation of hypercalcaemia

Biochemical investigation of hypercalcaemia should result in measurement of PTH (1–84) on a sample taken before any medical intervention, with the initial classification into parathyroid or non-parathyroid causes on the basis of the combination of concentrations of PTH and calcium. Further complex tests might be needed to establish the precise cause of hypercalcaemia in some patients. Intact PTH (1–84) measured with Nichols Institute diagnostic method. Ca_C/Cr_C=calcium creatinine clearance ratio. FBHH=familial benign hypocalciuric hypercalcaemia. (1)

1.2.3 Clinical presentation

In most cases pHPT is detected by an incidental finding of hypercalcemia and 70-80% of patients present without signs or symptoms of disease. (1) How clinical features of pHPT changed over the last decade is shown in **Table 2**.

	1930–1970	1970–2000
Nephrolithiasis	51–57%	17–37%
Hypercalciuria	36%	40%
Overt skeletal disease	10–23%	1.4–14%
Asymptomatic	0.6–18%	22–80%

Table 2: Changing clinical presentation of pHPT (15)

Nephrolithiasis accounts for the most frequent clinical manifestation in symptomatic patients. While overt skeletal disease is a rare finding, osteoporosis with related fractures is increasing. (1)

Several studies examined the effect of PTX on bone mineral density (BMD) and bone turnover in patients with pHPT. Having tested the effect of surgery against observation in patients diagnosed with mild pHPT, Bollerslev et al. found normalized levels of calcium and PTH after PTX. Biochemical markers of bone turnover decreased by 35% and, consecutively, BMD in the spine (2.7%; $P < 0.01$) and femoral neck (1.1%; $P < 0.02$) increased significantly whereas BMD and bone markers were stable in the observation group. (16)

There is growing evidence that PTH is associated with increased risk for CVD and mortality. Hagström et al., Pilz et al and Tomaschitz et al. found elevated plasma PTH to be associated with significantly higher risk of cardiovascular mortality (17-19).

Arterial hypertension is frequently seen in patients with pHPT. Broulik et al. documented a prevalence of hypertension in 69.8% of pHPT-patients which was significantly higher in comparison to the age-, sex-, BMI- and smoking status-matched control group. (20) Other studies documented that markers of arterial stiffness are increasingly prevalent among patients with pHPT. (21, 22)

Other clinical manifestations of PTH excess are acute pancreatitis or neuromuscular symptoms. Diabetes, gastrointestinal ulcers, gout, hyperlipidemia and increased

weight are known to be associated with pHPT. Psychiatric complaints including depression, fatigue, lethargy and an overall feeling of dullness can be revealed in so-called asymptomatic patients. (14) Acute hypercalcemic crisis is a life-threatening, but seldom complication.

1.2.4 Asymptomatic primary hyperparathyroidism

The term asymptomatic primary hyperparathyroidism refers to a condition defined as hyperparathyroidism that lacks specific symptoms or signs traditionally associated with hypercalcemia or PTH excess. According to the current guidelines for the management of asymptomatic pHPT there is growing consensus that PTX may be appropriate in the majority of patients with asymptomatic pHPT. (10) This agreement is based upon several cohort studies demonstrating signs of disease progression in up to 30% of long-term monitored patients and BMD increases in those who underwent surgery. It is suggested that PTX lowers the lifetime fracture risk in patients with asymptomatic pHPT. Since cardiovascular manifestations and reversible neurocognitive elements are not yet definite, corresponding algorithms cannot be established so far. However, it is pointed out that these topics are of interest for future investigations. (10)

1.2.5 Management

PTX is the only potential cure for patients with pHPT and, thus, surgical treatment is recommended for most symptomatic patients. Since today many patients are incidentally diagnosed with pHPT, surgery is not required as long as signs and symptoms of disease are lacking.

The “2013 Guidelines” recommend PTX for patients with so called “asymptomatic” pHPT if certain criteria for surgical treatment are met. The Guidelines for surgery are listed in **Table 3**.

Serum calcium	>1,0 mg/dl (>0.25 mm/liter)
Renal	Creatinine clearance < 60 cc/min 24-h urine for calcium > 400 mg/d (> 10 mmol/d) and increased stone risk by biochemical stone risk analysis ^c Presence of nephrolithiasis or nephrocalcinosis by x-ray, ultrasound, or CT
Skeletal	BMD by DXA: T-score < -2,5 at lumbar spine, total hip, femoral neck, or distal 1/3 radius ^d and/or previous vertebral fracture by x-ray, CT, MRI or VFA
Age	< 50 yrs

Table 3: Guidelines for parathyroid surgery in asymptomatic pHPT^a

^a Surgery is also indicated in patients for whom medical surveillance is neither desired nor possible.

^b Consistent with the position established by the International Society for Clinical Densitometry, the use of Z-scores instead of T-scores is recommended in evaluating BMD in premenopausal women and men younger than 50 yr.

^c Most clinicians will first obtain a 24-hour urine for calcium excretion. If marked hypercalciuria is present (> 400 mg/d [> 10 mmol/d]), further evidence of calcium-containing stone risk should be sought by a urinary biochemical stone risk profile, available through most commercial laboratories. In the presence of abnormal findings indicating increased calcium-containing stone risk and marked hypercalciuria, a guideline for surgery is met.

^d The history of a fragility fracture at any site would define someone as having a complication of pHPT, and thus the individual would be automatically considered to be a surgical candidate.

Modified from reference 10.

Because pHPT may present with a plethora of symptoms, differentiating between symptomatic and asymptomatic pHPT can be intricate. Unspecific symptoms that are common in general society, e.g. fatigue, weakness, exhaustion, polydipsia, polyuria, nocturia, constipation, back pain, joint pain, bone pain or depression, can reduce the quality of life distinctively but quantification of impairment or even attribution to pHPT is difficult. Patients often do not realize the severity of their symptoms until they undergo PTX and experience symptomatic relief. Therefore, the workup of patients with pHPT should be performed thoroughly with the patient's history being extremely relevant. (23)

Medical management can be appropriate (1) in asymptomatic pHPT without overt disease progression, (2) if surgery is contraindicated or the patient is unwilling to undergo PTX or (3) if the patient's clinical status is deteriorating. (10)

With subject to the outcome of further research on cardiovascular manifestations, neurocognitive changes, and long-term stability of BMD in this disease, patients with asymptomatic pHPT, who do not meet the criteria for surgery, can be followed safely at regular intervals. Serum calcium, eGFR and serum creatinine should be checked annually. Reevaluation of bone density is recommended after 1-2 years depending on country-specific advisories or the need for closer monitoring if the clinical situation is appropriate. (10)

Since long-term data for drug management are lacking, none of the four classes of potential medical approaches (bisphosphonates, estrogen, selective estrogen receptor modulators, or calcimimetics) are currently recommended as alternative for surgery but pharmacological intervention may be beneficial and needs further investigation. (10)

1.3 Cardiovascular disease and risk factors

1.3.1 Cardiovascular disease – a short definition

Cardiovascular disease is a chronic disorder developing unnoticed for a long time. (23) By the time symptoms occur, CVD has usually progressed to an advanced stage. (23) CVDs include diseases of the heart, vascular diseases of the brain and diseases of blood vessels. Eventually, CVD causes mass disability accompanied by an increasing loss in disability-adjusted life years (DALY) and is a major cause of premature death. (23) In Europe 47% of all deaths can be attributed to CVD. Estimated costs for the European Union economy are amounting to almost €196 billion a year. (24) The World Health Organization (WHO) currently attributes one-third of all global deaths to CVD, with developing countries, low-income and middle-income countries accounting for over 80% of the DALYs lost due to CVD. Since CVD is strongly related to lifestyle features, such as the use of tobacco, unhealthy dietary habits, physical inactivity, and psychosocial stress, the WHO has further stated that over three-quarters of all CVD mortality may be prevented with adequate changes in lifestyle. (25) In consequence, aiming at improving disease prevention and controlling risk factors is substantial.

1.3.2 Risk factors for cardiovascular disease at a glance

Cardiovascular risk is defined as the likelihood of a person developing a cardiovascular event over a certain period of time. According to the WHO, risk factors of CVD can be grouped as follows:

Behavioural risk factors:

1. tobacco use
2. physical inactivity
3. unhealthy diet (rich in salt, fat and calories)
4. harmful use of alcohol.

Metabolic risk factors:

5. raised blood pressure (hypertension)
6. raised blood sugar (diabetes)

7. raised blood lipids (e.g. cholesterol)
8. overweight and obesity

Other risk factors:

9. poverty and low educational status
10. advancing age
11. gender
12. inherited (genetic) disposition
13. psychological factors (e.g. stress, depression)
14. other risk factors (e.g. excess homocysteine)

Raised blood pressure can be seen as the worldwide leading risk factor of CVD. When ranking risk factors in terms of attributable deaths, hypertension is followed by tobacco use, raised blood glucose, physical inactivity and overweight and obesity. (26) It is understood that long-term exposure to behavioral risk factors leads to increased prevalence of metabolic disorders. Manifestations of cardiovascular risk factors, e.g. hypertension and diabetes, link CVD to renal impairment. (26)

As some risk factors are non-modifiable, respective patients should receive regular check-ups. Generally speaking, the prevalence of CVD increases with age. Women are at lower risk until they pass the menopause, after which their risk for heart disease is similar to that in men. CVD is more likely to occur in patients with a family history of a first-degree blood relative who has had coronary heart disease or stroke before the age of 55 years (for a male relative) or 65 years (for a female relative).(26) Rapid and accurate assessment of a patient's cardiovascular risk is essential for a clinician in order to make precise management decisions. This requirement led to the implementation of a risk estimation system, the so-called SCORE (Systematic Coronary Risk Evaluation). The SCORE chart helps to estimate the 10-year risk of fatal CVD considering age, gender, smoking status, systolic blood pressure (SBP) and total cholesterol as risk factors. (23)

1.4 Parathyroid hormone and cardiovascular risk

Higher PTH concentrations have been associated with fatal CVD in most studies but the mechanisms underlying PTH related cardiovascular damage as well as the cardiovascular risk profile of patients with pHPT are still obscure.

1.4.1 Parathyroid hormone and mortality

Current evidence points to PTH as a relevant marker that predisposes to higher all-cause and cardiovascular mortality. In a population-based cohort comprising 633 elderly white men and women studied by van Ballegooijen et al. this finding could be validated. The study showed a significantly reduced survival for the highest PTH quartile. (27) Another study goes in line with these results by exposing an estimated 30% greater risk for heart failure in patients with PTH \geq 65pg/ml. (28) Pilz et al. showed that PTH is associated with increased mortality and fatal cardiovascular events in a well-selected cohort of patients undergoing coronary angiography. In this study Kaplan – Meier curves followed by a log-rank test revealed that all-cause and cardiovascular mortality significantly increased in the highest two PTH quartiles ($P < 0.001$ for both). This led to the conclusion that PTH is an independent risk factor for sudden cardiac death. Interestingly, PTH levels were independently related to incident cardiovascular mortality exclusively in patients with high aldosterone concentrations. (18, 19) This finding supports the growing notion that cardiovascular damage related to PTH might be modified by aldosterone. A prospective study among a community-based cohort of 958 elderly men indicated that higher plasma PTH concentrations were linked to increased risk for cardiovascular mortality. Since these results remained stable in participants with PTH within normal ranges, Hagström et al. suggested that even in asymptomatic patients PTH levels could provide important prognostic information. In this cohort elevated plasma levels of PTH accounted for 20% of the population-attributable risk proportion for cardiovascular mortality which might be of interest for public health. (17) However, whether the mentioned findings apply to both genders remains unknown.

1.4.2 Cardiovascular disease and risk in patients with primary hyperparathyroidism

The cardiovascular risk profile of patients with pHPT has been discussed controversially over the last years. Whether or not autonomously elevated levels of PTH causes damage has been debated in several studies. According to Andersson et al. pHPT is associated with hypertension, cardiac arrhythmias, alteration in the vascular wall and disturbances in the renin-angiotensin-aldosterone-system (RAAS). (29) Presumed mechanisms of the observed cardiovascular modifications are illustrated in **Figure 2**.

In general, it is well approved today that symptomatic pHPT-patients have an increased risk of all-cause and cardiovascular mortality before and after treatment with PTX. (30) However, studies indicate a better postsurgical outcome when operated after a shorter duration of disease with a more favourable biochemical profile. (29)

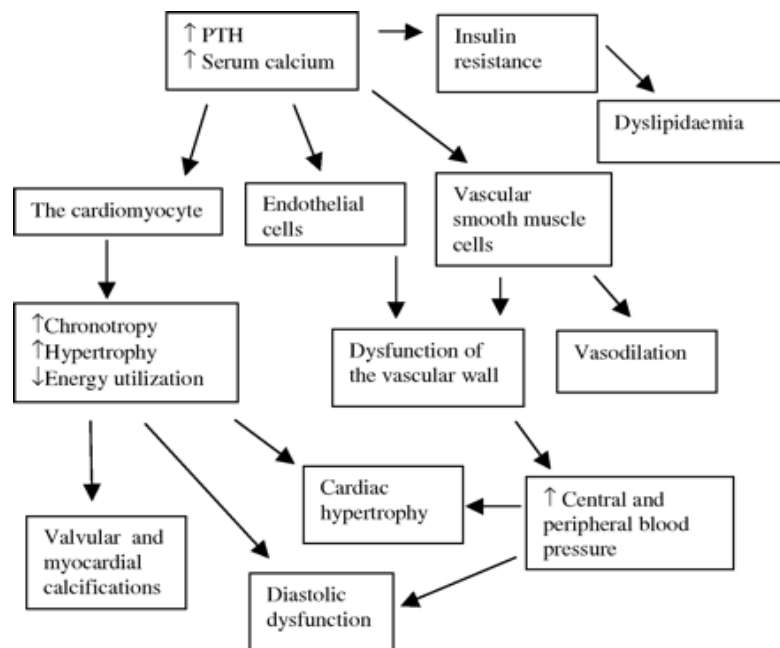


Figure 2: Presumed mechanisms behind the observed increase in cardiac disease associated with pHPT (18)

1.4.2.1 Primary hyperparathyroidism and vascular stiffening

A few studies found an association between pHPT and markers of arterial stiffness. Aortic pulse wave velocity (PWV) was found to be significantly higher among patients with pHPT and improved after PTX. (21) In another study pHPT presented as a stronger predictor of arterial stiffness than traditional risk factors, e.g. age or smoking status. The increased risk was further associated with evidence of more active parathyroid disease. (22)

Further research is needed to clarify whether stiffening of the arteries leads to augmented pressure and, in consequence, to an increased afterload in patients with pHPT. In comparison with healthy controls (matched for brachial blood pressures) Smith et al. have indeed found higher aortic blood pressure in patients with mild pHPT but the study is limited by analyzing only a small cohort comprising 21 participants with pHPT. (31)

1.4.2.2 Impact on cardiac function

Left ventricular hypertrophy (LVH) is known to be a powerful predictor of cardiovascular mortality in the general population and regression of LVH is related to improved outcomes. (32) Recent studies indicate that pHPT might be associated with LVH but further trials are needed to add proof to this suggestion. PTH receptors can be found on cardiomyocytes, but unlike in smooth muscle cells, where PTH decreases the Ca²⁺ influx, PTH induces a rise of intracellular calcium levels when binding to the PTH/PTHrP receptor on cardiomyocytes. (33) In consequence, the activated protein kinase C pathway leads to hypertrophic and metabolic effects. (34) Among a group of patients with pHPT Stefenelli et al. found hypertrophy of the interventricular septum in 81.6%. Interestingly, only 55% of these patients had a history of hypertension which is known to be a major cause of LVH. A follow-up after PTX revealed a significant reduction of left ventricular wall thickness in non-hypertensive patients. This led to the conclusion that PTH plays an important role in the maintenance or progression of LVH. (35) Although there is no definitive conclusion that PTH mediated LVH, Andersson et al. favor the suggestion that pHPT is associated with LVH, independent of blood pressure. Hypertrophy is declining

after PTX but the regression process takes years to be completed. (29) Another potential cause of increased prevalence of LVH among patients with pHPT might be augmented central blood pressure but since others could not support this finding, further evidence is needed. (31, 36)

While left ventricular systolic function does not seem to be potentially affected by pHPT, literature is diverging regarding the effects of pHPT on diastolic function. Given an elevated prevalence of LVH in pHPT-patients, one could expect an increased filling impairment leading to diastolic dysfunction. (26) In fact, two studies showed a prolonged isovolumetric relaxation time in patients with pHPT. (37, 38) These findings add support to the notion that pHPT might influence diastolic function but since the results are limited by small cohorts, conclusions must be drawn cautiously. In addition, so far there has been no consensus whether PTX leads to improvement of diastolic function. (29)

Among patients with pHPT valvular and myocardial calcifications are increasingly prevalent. (35)

1.4.2.3 Primary hyperparathyroidism and Hypertension

Patients with pHPT frequently present with arterial hypertension, even those with mild disease. (39) Broulik et al. could demonstrate a reduction of blood pressure in hypertensive pHPT patients after PTX. They observed decreased blood pressure levels in individuals never receiving antihypertensive therapy as well as in individuals on medication for hypertension, the latter suggesting pathogenetic participation of pHPT on blood pressure elevation. (20) The results of Heyliger et al. are in line with the above mentioned results as blood pressure decreased significantly in hypertensive pHPT patients (n=147) six months after PTX. The reduction occurred in all hypertensive patients, regardless of antihypertensive therapy which was not modified during the post-surgery period. (40) In another study, 81% of patients with pHPT presented with hypertension, 57% showed a “non-dipping” pattern, 35% of essential hypertensives and 15% of normal subjects, respectively. (41) Several studies have demonstrated that hypertensive individuals with a “non-dipping” blood pressure pattern show an increased frequency of target organ damage. (42) However, the presence of hypertension in patients with pHPT

is not currently defined as an indication for PTX, since the majority of studies indicate that hypertension is not permanently reversible with surgical cure. (39)

1.4.2.4 Primary hyperparathyroidism and Glucose Metabolism

As shown by different studies, pHPT presents with an increased frequency of altered glucose metabolism. Reduction of both basal and whole-body insulin sensitivity, in spite of increased insulin secretion, result in impaired glucose tolerance (IGT). Hypercalcemia was found to be significantly related to insulin sensitivity, suggesting a link between severity of the disease and occurrence of IGT. (43, 44)

1.4.2.5 Primary hyperparathyroidism and Dyslipidemia

In postmenopausal women with pHPT Hagström et al. found unfavorable alterations in the lipoprotein fraction, associated with phenotype IV dyslipidemia and increased risk of cardiovascular complications. PTX induced effective normalization. (45) Another study in patients with normocalcemic pHPT revealed proatherosclerotic dyslipidemia, increased fasting glucose and urate. Again, PTX had a positive effect on these cardiovascular surrogate parameters. (46)

1.4.2.6 Primary hyperparathyroidism and Obesity

According to a meta-analysis by Bolland et al. increased body weight is consistently present in cohorts of subjects with pHPT. (47) Whether PTX leads to modifications in body composition has not been shown so far.

1.4.2.7 Gender Differences

Han et al. found that male pHPT patients had larger adenomas at the time of surgery and obesity, type 2 diabetes and coronary artery disease was more prevalent compared to female pHPT patients. (48) With serum calcium and phosphate levels being similar in either sex, women presented with significantly higher concentrations of intact parathyroid hormone, thus, probably reflecting more severe disease. (49)

1.4.3 Aim of the study

To analyze cross-sectional relationships between PTH and cardiovascular risk factors in a large cohort of patients with pHPT.

1.5 The EPATH Trial

The “Effects of eplerenone on parathyroid hormone levels in patients with primary hyperparathyroidism” (EPATH) Trial is a single-center, randomized, double-blind, placebo-controlled trial. The aim of the study is to investigate whether treating patients with pHPT with eplerenone (a mineralocorticoid receptor antagonist (MRA)) leads to changes of PTH levels, influences markers of bone metabolism and results in beneficial effects on cardiovascular and renal parameters. (50) Since the Workgroup of the “2008 Guidelines” stated that further trials are needed in those patients with pHPT in whom surgery is not recommended, one objective of the EPATH study is to clarify the impact of PTH on cardiovascular morbidity and mortality. Another important goal of the EPATH trial is to document potential benefits of mineralocorticoid receptor antagonism on the cardiovascular system, bone metabolism and kidney function. For the present cross-sectional investigation baseline data of participants with pHPT were used. The EPATH cohort comprises patients with normocalcemic and with hypercalcemic pHPT.

The primary endpoint is defined as the mean change of PTH level. Secondary endpoints are mean change of 24-hours systolic and diastolic ambulatory blood pressure levels from baseline to the last visit, mean change of further biomarkers of cardiovascular disease from baseline to the last visit as assessable by 24-hours ABPM.

The EPATH trial is based on the notion of a potential interrelationship between aldosterone and PTH derived from previous animal studies and observational studies in humans. In these studies, mineralocorticoid receptor blockade resulted in decreased levels of PTH, normalized bone metabolism, increased bone density and decreased blood pressure and cardiovascular pathology.

Taking the interrelation between elevated aldosterone and PTH levels in patients with pHPT into consideration, it is hypothesized that mineralocorticoid antagonism (MRA) leads to improvements in cardiovascular and bone health. Thus, it is expected that in patients with pHPT, inhibitory effects mediated by MRA eplerenone results in diminished PTH levels, improved bone metabolism, increased bone density and decreased blood pressure and cardiovascular pathology.

2 Methods

2.1 Study population

For the present cross-sectional analyses we used data from the EPATH Trial. We included subjects who took part in the screening for trial participation and had biochemically confirmed hypercalcemic or normocalcemic pHPT. Hypercalcemic pHPT was defined as 1) albumin adjusted plasma calcium > 2.55 mmol/L or 2) ionized plasma calcium >1.35 mmol/L or 3) regular use of cinacalcet for hypercalcemia and inappropriately high PTH (PTH > 46 pg/mL). Normocalcemic pHPT was defined as PTH > 65 pg/mL and normal albumin adjusted plasma calcium above the median of the reference range (>2.35 mmol/L) and plasma ionized calcium within normal ranges in the absence of chronic kidney disease (eGFR > 40 mL/min/m²) and 25(OH)D deficiency (25(OH)D ≥ 20 ng/dL) as potential causes for secondary hyperparathyroidism and no regular use of cinacalcet. Study participants were recruited from the outpatient clinic of the Department of Internal Medicine, Department of Cardiology and Division of Endocrinology and Metabolism, Medical University of Graz, Austria. (50)

2.2 Study design

The EPATH trial was designed as an 8-weeks treatment period interventional trial. Participants were randomized to a double-blind treatment with either eplerenone or placebo. Eplerenone was commenced with 25mg once daily and titrated to 50mg once daily after 4 weeks. The study comprised 5 visits (at baseline, after 1, 4, 5 and 8 weeks) in order to monitor changes in biochemical parameters such as serum potassium closely. (50)

After 8 weeks primary and secondary outcomes were evaluated. The primary endpoint was defined as the mean change of PTH levels. Secondary endpoints were mean change of 24-hours systolic and diastolic ambulatory blood pressure levels from baseline to the last visit, mean change of N terminal pro brain natriuretic peptide (NT-pro-BNP), mean change of biomarkers of bone metabolism from baseline to the fifth visit, 24-hours urinary protein and albumin excretion from baseline to the fifth visit, echocardiographic parameters of left ventricular (LV) structure and function. (50)

For the present cross-sectional investigation we analyzed baseline data of the EPATH trial.

The study was performed in accordance with the Good Clinical Practice guidelines and the declaration of Helsinki.

2.3 Laboratory assessment

Blood sampling was performed in the morning (7 to 11 a.m.) after an overnight fast. Patients rested ten minutes in the sitting position before blood collection. After sampling all blood samples were centrifuged within one hour. At latest four hours after blood collection all samples were analyzed. Blood samples for the determination of intact PTH (1-84) were kept at 4°C before analysis. Measurement of iPTH (1–84) (pg/mL) was performed by electrochemoilluminescence immunoassay “ECLIA” (Elecsys immunoassay analyzer; CobasW; Roche Diagnostics GmbH, Mannheim, Germany; reference range, 15–65 pg/mL, interassay coefficient of variation 3.0%–6.5% for PTH 26.7–261 pg/mL). HbA1c and total cholesterol were determined using standard laboratory kits. (50)

2.4 ABPM assessment

ABPM was performed using an automated non-invasive, oscillometric device (Mobil O Graph, I.E.M., Stolberg, Germany). The device was validated according to the criteria of the British Hypertension Society. (51) In order to allow correct choice of cuff size, arm circumferences were measured and recorded. Measurements of blood pressure and heart rate were taken for 24 hours every 20 minutes between 06:00 and 10:00h and every 30 minutes between 10:00 and 06:00h. (50) For later statistical analyses measurements were divided into daytime measurements (09:00-21:00h), evening (21:00–01:00h) and nighttime measurements (01:00-06:00h) according to the European Society of Cardiology (ESC) position paper 2013 on ABPM. (52) If the ambulatory recordings were longer than 24 hours, only the first 24 hours were used for analysis starting the 24-hours timeframe from the first valid recorded measurement. (53)

2.5 Statistical analyses

Continuous variables are expressed as mean +/- standard deviation (SD) or (those with skewed distribution) as medians and interquartile ranges (IQR) and categorical variables as numbers and percentages.

All analyses were performed in the entire cohort (Group 1) and in those participants who were not regularly taking the PTH-modifying drug cinacalcet (Group 2). As a final step we additionally excluded patients taking angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blocker (ARB), MRA, thiazide diuretics or loop diuretics on a regular basis (Group 3) as these drugs may affect PTH concentrations as well.

In order to evaluate potential correlations between plasma levels of intact PTH and parameters of the 24-hours APBM, Pearson's correlation test was performed. We assessed the correlation between PTH and:

- Mean 24-hours systolic blood pressure (SBP)
- Mean 24-hours diastolic blood pressure (DBP)
- Mean nighttime SBP
- Mean nighttime DBP
- Mean 24-hours PWV
- Mean nighttime PWV
- HbA1c
- Total cholesterol
- Smoking status

In case of a significant bivariate correlation between PTH and the respective dependent variable, multivariate linear regression analyses were performed with the respective cardiovascular risk factor as dependent variable. We adjusted for age, sex, antihypertensive medication, body mass index (BMI), HbA1c, 25(OH)D, serum calcium, eGFR and mean SBP were appropriate.

3 Results

3.1 Group 1 (entire cohort)

The entire pHPT cohort comprised 155 participants of the EPATH study screening visit and consisted of 33 (21.3%) males and 122 (78.7%) females, with a mean age of 67.4 years (+/- 10.3 years). Median plasma levels of intact PTH were 99 pg/ml (IQR 82-125 pg/ml). Mean 24-hours SBP was 124.9 mmHg +/- 11.6 mmHg and mean 24-hours PWV was 9.6 m/s +/- 1.7 m/s. Median HbA1c was 37 (35 – 40) mmol/mol, mean total cholesterol was 202 +/- 42 mg/dL and mean BMI was 28 +/- 5 kg/m². Baseline characteristics are provided in **Table 4**.

Baseline characteristics (n=155)	
Plasma levels of intact PTH in pg/ml	99 (IQR 82-125)
Age in years	67.4 +/- 10.3
Female in %	78.7
BMI in kg/m ³	28 +/- 5
24-hours SBP in mmHg	124.9 +/- 11.6
24-hours DBP in mmHg	75.9 +/- 8.3
Daytime SBP in mmHg	128.1 +/- 11.5
Daytime DBP in mmHg	77.5 +/- 8.9
Nighttime SBP in mmHg	117.4 +/- 16.8
Nighttime DBP in mmHg	68.8 +/- 10.1
24-hours PWV in m/s	9.6 +/- 1.7
Daytime PWV in m/s	9.9 +/- 1.7
Nighttime PWV in m/s	9.4 +/- 1.8
24-hours HR in beats/min	70.4 +/- 9.3
Daytime HR in beats/min	75.1 +/- 11.2
Nighttime HR in beats/min	61.4 +/- 8.8
HbA1c in mmol/mol	37 (35- 40)
Total cholesterol in mg/dL	202 +/- 42
Active smokers % / packyears	13.5 / 31.3 +/- 16.4

Table 4: Baseline characteristics

Abbreviations: PTH parathyroid hormone, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure PWV pulse wave velocity, HR heart rate, HbA1c hemoglobin A1c

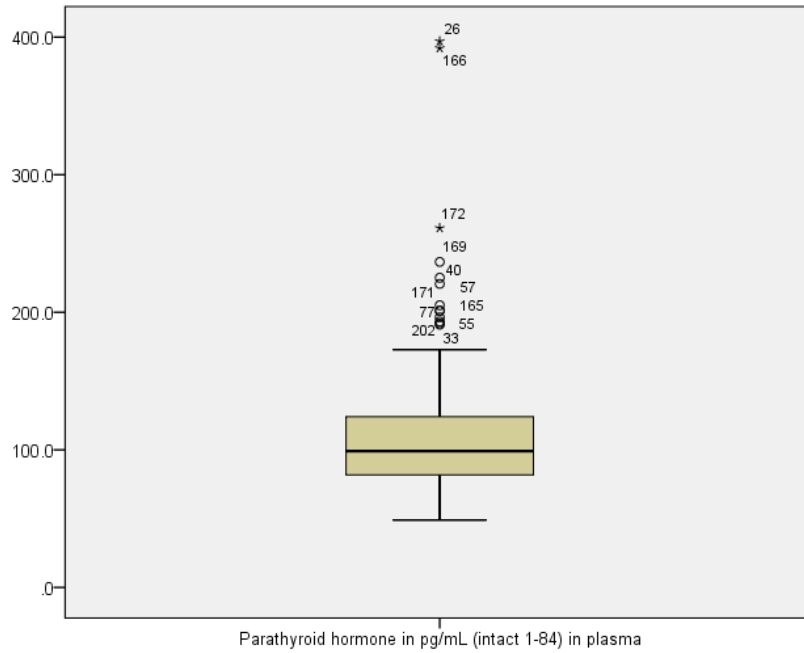


Figure 3: Distribution of PTH levels

		PTH	P-Value
Sex	<i>Male</i>	107 (IQR 74-160)	0.27
	<i>Female</i>	99 (IQR 82-116)	
Diabetes	<i>Yes</i>	93 (IQR 72-148)	0.98
	<i>No</i>	99 (IQR 82-123)	
Smokers	<i>Yes</i>	78 (IQR 70-104)	<0.01
	<i>No</i>	106 (IQR 86-131)	

Table 5: Plasma levels of PTH in subgroups of the entire cohort stratified according to sex, diabetes and smoking. P for between group differences (Student's t-test)

In bivariate correlation analyses PTH was correlated with mean 24-hours PWV ($r=0.244$, $P<0.01$) and with nighttime PWV ($r=0.228$, $P<0.01$) (**Table 6**).

n=155	Intact PTH	
	r	P-Value
Mean 24-hours PWV	0.244	< 0.01
Nighttime PWV	0.228	< 0.01
Mean 24-hours SBP	0.062	0.46
Mean 24-hours DBP	0.011	0.90
Nighttime SBP	0.070	0.41
Nighttime DBP	0.079	0.36
Total Cholesterol	-0.053	0.52
HbA1c	0.006	0.94

Table 6: Bivariate correlation analysis (Group 1)
Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c hemoglobin A1c

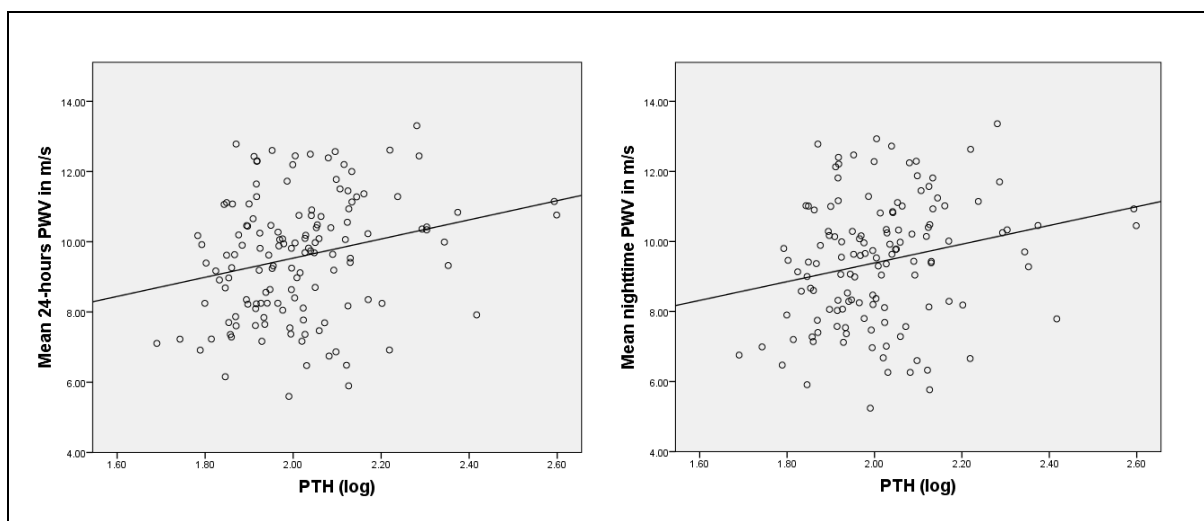


Figure 4: Bivariate correlation between mean 24-hours PWV and nighttime PWV and intact PTH
Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity

In multivariate linear regression analyses we found no association between PTH and cardiovascular risk factors.

Table 7: Multivariate linear regression analysis between mean 24-hours PWV and nighttime PWV and intact PTH			
Group 1	Intact PTH		
	R ²	β-coefficient	P-value
Mean 24-hours PWV	0.913	0.044	0.12
Nighttime PWV	0.892	0.037	0.26
Adjusted for age, sex, antihypertensive medication, BMI, HbA1c, 25(OH)D, serum calcium, eGFR and mean 24-hours SBP			

Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, BMI body mass index, 25(OH)D 25-hydroxy vitamin D, Hba1c hemoglobin A1c, eGFR estimated glomerular filtration rate, SBP systolic blood pressure

3.2 Group 2 (exclusion of subjects taking cinacalcet)

Excluding those subjects with a regular intake of cinacalcet led to a sample size of 136 subjects (mean age 66.8+/-10.2 years, 109 (80.1%) females). In bivariate correlation analysis PTH was correlated with mean 24-hours PWV ($r=0.280$, $P<0.01$), mean nighttime PWV ($r=0.280$, $P<0.01$) and nighttime SBP ($r=0.203$, $P=0.03$) (**Table 8**).

n=136	Intact PTH	
	r	P-Value
Mean 24-hours PWV	0.280	< 0.01
Nighttime PWV	0.280	< 0.01
Mean 24-hours SBP	0.149	0.10
Mean 24-hours DBP	0.002	0.98
Nighttime SBP	0.203	0.03
Nighttime DBP	0.150	0.10
Total Cholesterol	-0.066	0.45
HbA1c	0.068	0.43

Table 8: Bivariate correlation analysis (Group 2)

Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c hemoglobin A1c

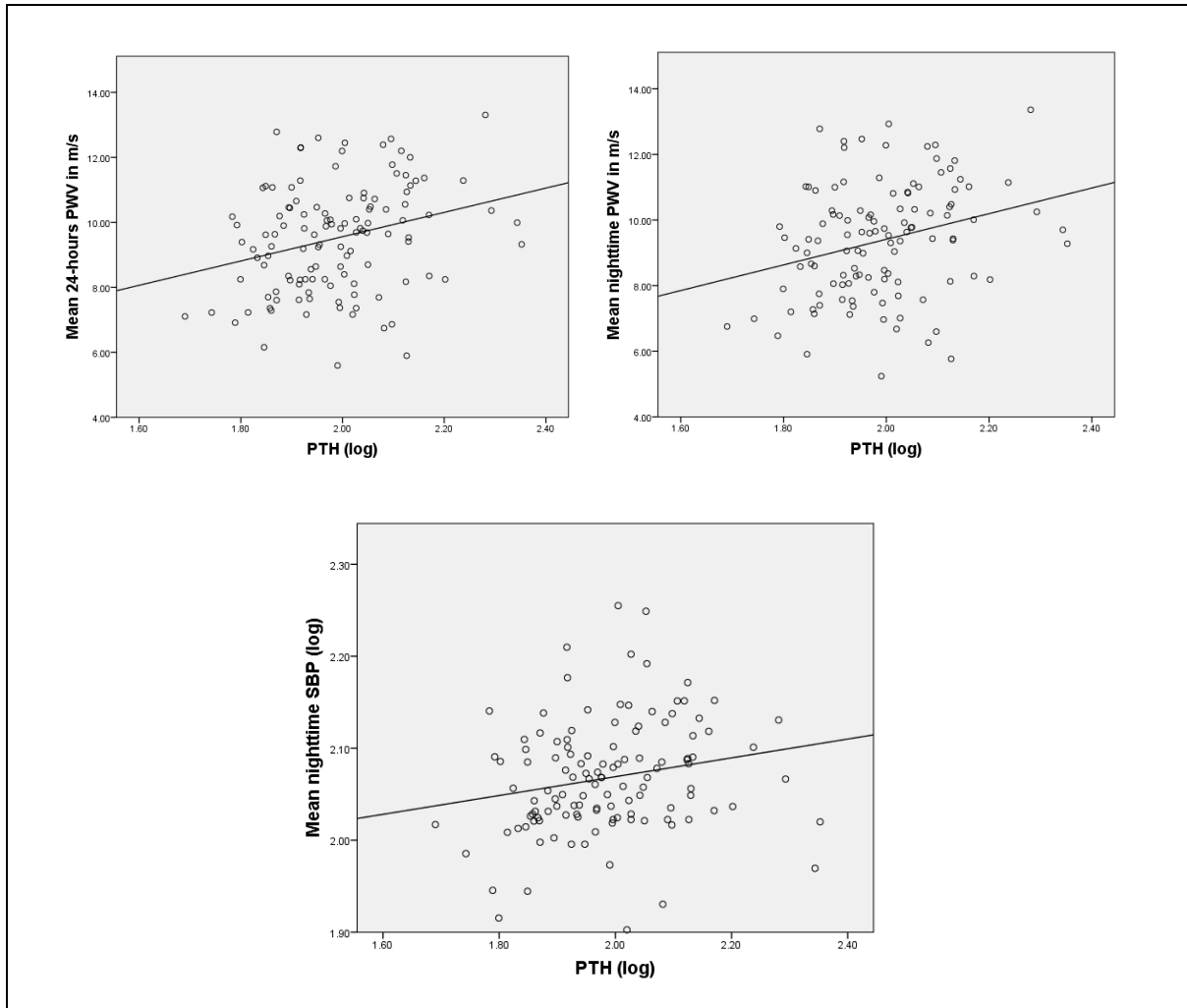


Figure 5: Bivariate correlation between mean 24-hours PWV, nighttime PWV and nighttime SBP and intact PTH
Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, SBP systolic blood pressure

		PTH	P-Value
Sex	<i>Male</i>	88 (IQR 70-134)	0.78
	<i>Female</i>	97 (IQR 81-112)	
Diabetes	<i>Yes</i>	91 (IQR 70-141)	0.86
	<i>No</i>	98 (IQR 79-113)	
Smokers	<i>Yes</i>	77 (IQR 70-103)	<0.01
	<i>No</i>	99 (IQR 84-120)	

Table 9: Plasma levels of PTH in subgroups of Group 2 stratified according to sex, diabetes and smoking. P for between group differences (Student's t-test)

In multivariate linear regression analyses we found no association between PTH and cardiovascular risk factors.

Table 10: Multivariate correlations between mean 24-hours PWV, nighttime PWV and nighttime SBP and intact PTH			
Group 2	Intact PTH		
	R ²	β-coefficient	P-value
Mean 24-hours PWV	0.952	0.060	0.08
Nighttime PWV	0.885	0.061	0.12
Nighttime SBP	0.234	0.141	0.14
adjusted for age, sex, antihypertensive medication, BMI, HbA1c, 25(OH)D, serum calcium, eGFR and mean 24-hours SBP			

Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, BMI body mass index, 25(OH)D 25-hydroxy vitamin D, Hba1c hemoglobin A1c, eGFR estimated glomerular filtration rate, SBP systolic blood pressure

3.4 Group 3 (after further exclusion of subjects taking ACEI, ARB, MRA, thiazide diuretics or loop diuretics)

The additional exclusion of subjects taking ACEI, ARB, MRA, thiazide diuretics or loop diuretics on a regular basis led to a sample size of 75 subjects (mean age 65.1 +/- 11.3 years, 63 (84.0%) females). In bivariate correlation analysis PTH was correlated with mean 24-hours PWV ($r=0.328$, $P<0.01$) and mean nighttime PWV ($r=0.305$, $P=0.02$), mean 24-hours SBP ($r=0.255$, $P=0.03$) and mean nighttime SBP ($r=0.370$, $P<0.01$) (**Table 11**).

n=75	Intact PTH	
	r	P-Value
Mean PWV	0.328	< 0.01
Nighttime PWV	0.305	0.02
Mean SBP	0.255	0.03
Mean DBP	0.008	0.95
Nighttime SBP	0.370	< 0.01
Nighttime DBP	0.201	0.11
Total Cholesterol	-0.183	0.12
HbA1c	0.152	0.20

Table 11: Bivariate correlation analysis (Group 3)
Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, SBP systolic blood pressure, DBP diastolic blood pressure, HbA1c hemoglobin A1c

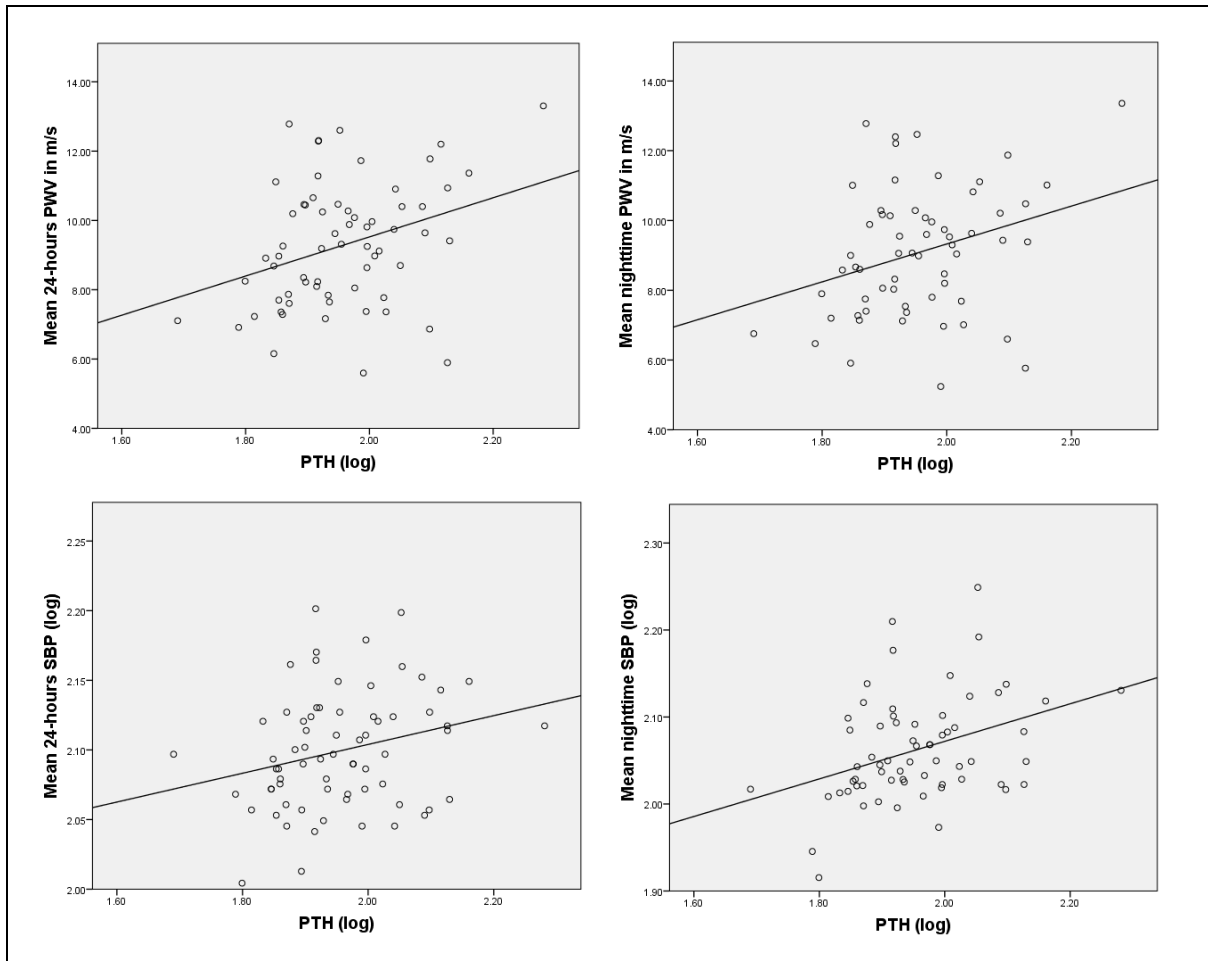


Figure 6: Bivariate correlation between mean 24-hours PWV, nighttime PWV, nighttime SBP and mean SBP and intact PTH
Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, SBP systolic blood pressure

		PTH	P-Value
Sex	<i>Male</i>	82 (IQR 65-119)	0.58
	<i>Female</i>	90 (IQR 78-104)	
Diabetes	<i>Yes</i>	79 (IQR 71-105)	0.40
	<i>No</i>	89 (IQR 77-104)	
Smokers	<i>Yes</i>	75 (IQR 70-101)	0.02
	<i>No</i>	93 (IQR 80-109)	

Table 12: Plasma levels of PTH in subgroups of Group 3 stratified according to sex, diabetes and smoking. P for between group differences (Student's t-test)

In multivariate linear regression analyses PTH was found to be associated with mean 24-hours PWV (beta-coefficient=0.132, P<0.01), nighttime PWV (beta-coefficient=0.135, P=0.01) and nighttime SBP (beta-coefficient=0.175, P=0.02). Further adjustment for smoking did not materially alter our results.

Table 13: Multivariate correlations between mean 24-hours PWV, nighttime PWV, nighttime SBP and mean SBP and Intact PTH			
Group 3	Intact PTH		
	R ²	β-coefficient	P-value
Mean 24-hours PWV	0.927	0.132	<0.01
Nighttime PWV	0.906	0.135	0.01
Nighttime SBP	0.275	0.292	0.03
Mean 24-hours SBP	0.203	0.154	0.24
adjusted for age, sex, antihypertensive medication, BMI, HbA1c, 25(OH)D, serum calcium, eGFR and mean 24-hours SBP			

Abbreviations: PTH parathyroid hormone, PWV pulse wave velocity, BMI body mass index, 25(OH)D 25-hydroxy vitamin D, Hba1c hemoglobin A1c, eGFR estimated glomerular filtration rate, SBP systolic blood pressure

4 Discussion

In the present analysis we found an independent and direct relationship between PTH and mean 24-hours PWV, nighttime PWV and nighttime SBP, respectively, in a well selected cohort of 75 pHPT-patients without a regular intake of several PTH-modifying drugs.

PHPT is associated with increased cardiovascular risk but the underlying pathophysiological mechanisms are poorly understood. (17, 30) The present analyses on patients with pHPT indicate an interplay between PTH and PWV as a marker for arterial stiffness. It could be hypothesized that PTH contributes to vascular diseases by modulating stiffness of blood vessels. The fact that PTH has direct effects on vascular smooth muscle cells and endothelial cells adds support to that notion. (34)

Some studies reported that higher aortic stiffness assessed by PWV is associated with increased cardiovascular risk and all-cause mortality. (54-57) In terms of predicting cardiovascular events, PWV exceeds traditional cardiovascular risk factors, including 24-hours MAP. (55) Measuring PWV is generally accepted as the simplest, non-invasive, robust, and reproducible method to determine arterial stiffness. (56) The rigidity of the vessel walls influences the velocity at which the pulse wave travels through the vasculature - as the vessels get stiffer, pulse wave velocity rises. Ventricular contraction produces a forward pressure wave that is reflected from the periphery. In a physiological condition the reflection of the pulse wave reaches the aortic root during diastole. This leads to an augmentation of diastolic pressure, hence, improving coronary artery perfusion. As vessels stiffen and, consequently, PWV increases, the reflected wave arrives back earlier. As a result systolic pressure gets augmented while diastolic pressure decreases. With rising central pressure cardiac afterload increases, thus, accelerating the development of LVH. Given a further increase of arterial stiffness, the elevated amplitude of the reflected wave further augments central systolic pressure, while reducing coronary artery perfusion. (58) When performing PWA, central pressure, the augmentation index (AIx) and PWV should not be used as interchangeable terms since PWV represents the only direct measure of arterial stiffness. (56) Increased arterial stiffness may lead to enhanced cardiac afterload and, accordingly, to the development of LVH which is likely to contribute to cardiovascular morbidity

and mortality. (31, 32) Given the solely marginal effect of stiffened arteries on brachial blood pressure, the true extent of cardiac afterload is likely to be underestimated by routine brachial sphygmomanometry.

Vascular abnormalities may occur on the basis of structural and functional changes mediated by altered calcium-PTH homeostasis. Metabolic and lipid disorders which are commonly seen in patients with pHPT may contribute to these alterations. (44, 46) However, in the present investigation no associations were found between PTH and parameters of glucose- and lipid-metabolism, respectively. This finding, supports direct PTH mediated effects on the vasculature.

Evidence that echocardiographic LVH is more prevalent among both hypertensive and normotensive patients with pHPT compared to matched controls is consistent with our observations. Reversibility of LVH after PTX further supports this notion. (35, 59) However, since basic studies showed that PTH itself exerts hypertrophic effects on cardiomyocytes the main reason for the documented cardiac abnormalities is still unclear. (34) A possible contribution of various effects of PTH might explain the cardiovascular alterations and performing PWA presumably adds important information to the medical attendance of a patient with pHPT.

We were able to demonstrate a positive correlation between PTH and mean PWV and nighttime PWV. Our data extend former studies that observed higher PWV among both hypertensive and non-hypertensive patients with pHPT compared to corresponding controls. In these studies PTX led to a significant decrease of PWV but a linkage to PTH was not documented. (21, 60) Rubin et al. reported that pHPT emerged as a stronger predictor of arterial stiffness than traditional risk factors, e.g. smoking status, which is congruent with the present analysis. (22) In another study, patients diagnosed with pHPT presented with a significantly higher Alx than a control group that was matched for age, sex and brachial systolic blood pressure. (31) These results strengthen the notion that PTH may impact on vascular function. Contrary to the above mentioned literature Kosch et al. and Ring et al. documented normal arterial function despite high levels of PTH and no changes after PTX, but their studies were limited by a small sample size and only mild pHPT. (36, 61)

Arterial hypertension may be another potential mechanism linking PTH to increased cardiovascular risk. PTH infusions led to increased blood pressure in healthy volunteers implicating that hypertension associated with pHPT results from either

direct or indirect effects of PTH excess per se. (62) In patients with pHPT, even in those with mild disease, hypertension is a common finding. (39) Our data are consistent with this observation showing a direct, albeit modest, correlation between PTH and nighttime SBP.

As previous literature reports alterations in glucose metabolism, lipid profile and increased prevalence of obesity in patients with pHPT, we analyzed the cross-sectional relationship between PTH and HbA1c and total cholesterol levels, respectively. (43-47) Our results did not reveal a correlation between PTH and either of the above mentioned CVD risk factors implicating that PTH, at least in patients with pHPT, does not strongly interfere with glucose or lipid metabolism.

4.1 Strengths and limitations

Our study is strengthened by the comparatively large cohort of patients with pHPT which enabled multivariate analyses. This is to the best of our knowledge the first study which evaluated both 24-hours PWV and (2) its relationship to PTH levels in patients with pHPT, respectively. Therefore, our study adds important information about (1) potential vascular impairment in patients with pHPT and (2) the association between PWV and severity of pHPT disease.

By now, the brachial device we used for pulse wave analysis (PWA) is not as well-established as the widely approved applanation tonometry device SphygmoCor but there is evidence that the Mobil-O-Graph showed equally effective. (63)

Given the cross-sectional study design conclusions regarding causality are not possible. Further longitudinal and interventional studies are warranted to evaluate modulatory effects of PTH on vasculature in patients with PTH excess.

4.2 Conclusion and perspectives

We cross-sectionally analyzed the association of plasma levels of PTH and several risk factors for CVD in patients with pHPT. The present analysis showed a direct correlation of PTH and mean 24-hours PWV, nighttime PWV and nighttime SBP, respectively. These findings indicate potential effects of PTH on the vasculature, thus, possibly compromising cardiovascular health in patients with pHPT. Further studies are needed to evaluate possible modulatory effects of PTH on vasculature in patients with pHPT and its underlying mechanisms.

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