

Arterial Structure and Function in Mild Primary Hyperparathyroidism Is Not Directly Related to Parathyroid Hormone, Calcium, or Vitamin D

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Abstract

Objective: Elevated levels of calcium and parathyroid hormone (PTH), characteristics of primary hyperparathyroidism (PHPT), may be associated with cardiovascular morbidity and mortality in the general population. We evaluated the possible vascular effects of these risk factors in patients with mild PHPT by using standard methods and new imaging techniques.

Design: A prospective case-control study.

Subjects and Methods: Forty-eight patients with mild PHPT without any known cardiovascular risk factors were studied at baseline and at one year after parathyroidectomy (PTX) in comparison with 48 healthy age- and gender-matched controls. We measured biochemical variables, augmentation index (AIx), aortic pulse wave velocity (PWV_{ao}), radial (IMT_{rad}) and common carotid artery (IMT_{cca}) intima media thicknesses, and the grayscale median (IM-GSM) of the latter.

Results: No significant differences were observed between PHPT patients and controls at baseline for AIx (28.6±12.2 vs. 27.7±12.8%), IMT_{rad} (0.271±0.060 vs. 0.255±0.053 mm), IMT_{cca} (0.688±0.113 vs. 0.680±0.135 mm), or IM-GSM (82.3±17.2 vs. 86.5±15.3), while PWV_{ao} was slightly higher in patients (8.68±1.50 vs. 8.13±1.55, p<0.05). Systolic blood pressure (SBP), calcium, and PTH were higher in patients compared with controls, and decreased after PTX, while vitamin D was lower in patients and increased after PTX. While AIx, PWV_{ao}, IMT_{rad}, and IMT_{cca} were related to SBP, neither correlated to vitamin D levels. Only PWV_{ao} correlated weakly to plasma PTH (r=0.29, p<0.01) and ionized calcium (r=0.22, p<0.05) but showed no relation when age and SBP were adjusted for.

Conclusion: We found normal arterial function despite high calcium, PTH, and low vitamin D levels, in patients with mild PHPT without cardiovascular risk factors. The cardiovascular risk associated with low vitamin D and/or high PTH and calcium levels may be explained by their coupling to blood pressure and other risk factors rather than direct effects on arterial structure.

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Introduction

Associations between high calcium and parathyroid hormone (PTH) as well as low 25-OH-D levels and cardiovascular risk have been reported in the general population [1,2,3,4]. Recently, elevated PTH levels have been found to predict cardiovascular mortality in patients with coronary disease [5], as well as in the general population [6]. However, other recent data indicate no association between low 25-OH-D levels and all-cause mortality in older men [7].

Primary hyperparathyroidism (PHPT) is characterized by elevated calcium levels, inappropriately increased PTH levels, and is often associated with vitamin D deficiency [8].

Thus, PHPT is a good model to determine the influence of these alterations on the vasculature.

An increased mortality rate has been noted in both unselected PHPT [9], as well as in mild PHPT [10]. However, the majority of previous studies on PHPT include patients with varying disease severity in combination with pre-existing cardiovascular risk factors, which may confound relationships. Nevertheless, it is still unclear whether vascular structure and function are affected in mild PHPT without cardiovascular risk factors [11].

Endothelial function and vasodilatation regulated by nitric oxide (NO) release is of importance in the early atherosclerotic process. Endothelial vasodilatory dysfunction has been described in patients with PHPT [12]. In studies of mild PHPT, the

augmentation index (AIx), an indirect measure of the endothelial function and arterial stiffness, has been reported to be increased [13,14]. Increased pulse wave velocity (PWV_{ao}) has been found in PHPT patients with hypertension [15]; however, conflicting results are reported [16]. Carotid intima-media thickness (IMT_{cca}) measured by ultrasound is associated with a risk of myocardial infarction and stroke [17,18]. Alterations in carotid IMT_{cca} have been reported in patients with mild PHPT [19,20]; however the results are somewhat conflicting [16,21]. Among PHPT patients with or without cardiovascular risk factors, IMT_{cca} was found to be increased only in the group with risk factors [22]. Recently, echogenicity of the carotid intima media complex has been demonstrated to be related to cardiovascular risk factors [23], and the intima thickness of the radial artery (IMT_{rad}) has been shown to be increased in hypertension [24], but none of these indices have yet been studied in PHPT.

With mild PHPT without concomitant disease or known cardiovascular risk factors as a model, our aim was to determine the potential effects of PTH, calcium, and vitamin D on arterial structure and function. Thus, applying the as mentioned novel diagnostic techniques, we compared patients with age- and gender matched healthy controls, and evaluated the vascular effects of normalizing PTH, calcium, and vitamin D by parathyroidectomy (PTX).

Materials and Methods

Subjects

In a prospective, previously described [25,26], case-control study at the Karolinska University Hospital in Stockholm, 410 PHPT patients (319 women) accepted for PTX between January 2006 and November 2008 were consecutively evaluated for possible participation. Patients accepted for PTX were included in the current study if they fulfilled the following criteria: calcium <3.0 mmol/L; no diagnosed hypertension, diabetes mellitus, or renal diseases; no medication affecting the cardiovascular system; no current smoking; BMI <28; and age >18 and <70 years. The majority of our patients had plasma calcium levels below 2.75 mmol/L, and only five patients had plasma calcium levels between 2.76–2.97 mmol/L at the time of inclusion. Fifty-three patients with mild PHPT were consecutively included in the study. Five of the 53 patients were excluded; one man due to later findings of familial hypocalciuric hypercalcaemia, one woman regretted her decision to undergo PTX, one man was excluded because of carotid image storage failure and finally, two men were excluded because of lipid lowering medication. The final population consisted of 48 patients (13 men and 35 women). Of these, 13 PHPT patients had a history of kidney stones (n = 9) and/or osteoporosis (n = 6); while the remaining 35 patients had no classical symptoms coupled to PHPT, 22 of whom were diagnosed with PHPT at a routine health check-up, and the others sought medical attention because of fatigue or diffuse symptoms.

A healthy control group, age- and gender-matched, was randomly selected from the population registry of the city of Stockholm. They were informed by mail and asked to participate in the study. If they fulfilled the inclusion criteria, described above, they were included in the study. If anyone declined, a new randomly chosen control from the population registry was contacted. Two controls were replaced before entering the study: one because of hypertension and the other because of a high PTH level. The median (25, 75 percentiles) time from baseline to follow-up was 1.06 and 1.48 years. One woman did not participate in the vascular examinations at follow-up. The blood pressure and all examinations were performed the morning after an over-night fast,

with a resting period of 30–60 minutes in supine position. The same investigator performed the ultrasound examinations and pulse wave analysis. Each participant provided written consent to participate in the study, which was approved by the Local Ethics Committee, Regional Ethical Review Board, EPN, of Stockholm, Sweden.

Blood Pressure, Body Mass Index

Blood pressure (BP) was measured in both arms using a digital automatic blood pressure monitor Omron M7 (Omron Healthcare Co., LTD, Kyoto, Japan). The mean values of systolic and diastolic blood pressure (SBP and DBP) in both arms were calculated. Mean arterial blood pressure (MAP) was calculated as $DBP + (SBP - DBP) / 3$. Body mass index (BMI) was calculated by dividing weight (kg) by the square of height (m²). Body surface area (BSA) was calculated by the formula: $BSA (m^2) = 0.007184 \times (\text{weight, kg})^{0.425} \times (\text{height, cm})^{0.725}$.

Augmentation Index and Pulse Wave Velocity

Augmentation index (AIx) and aortic pulse wave velocity (PWV_{ao}) were noninvasively measured using SphygmoCor equipment connected to a computer with SphygmoCor 2000 software (version 7.01, AtCor Medical, Sydney, Australia). Recordings were performed using a single high-fidelity tonometer gently pressed to the radial, carotid, and femoral arteries (SPT-301B, Millar Instruments, Houston, Texas, USA). To measure AIx, the radial artery waves were recorded and processed by the system software. The corresponding aortic pressure waveform was generated from an averaged radial artery waveform using a validated transfer factor [27,28]. AIx 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 [29,30]. To obtain PWV_{ao}, the pulse waveform was collected sequentially from femoral and carotid artery sites, and by using the R-wave (of a simultaneously recorded electrocardiography, ECG) as a reference, the mean difference in time (ΔT) between sites A (carotid) and B (femoral) was calculated. The surface distance ($x_{\text{subtracted}}$) was measured as (carotid-jugulum distance) subtracted from (jugulum-femoral distance). The direct distance over the body surface (x_{direct}) was obtained by a standardized formula: $x_{\text{direct}} = 0.45x_{\text{subtracted}} + 0.21 \times \text{height} + 0.08$ (m) [31]. The use of x_{direct} leads to an overestimation of the real PWV_{ao}, and a scaling factor was used to convert PWV_{ao} distance to “real” PWV_{ao}. The real PWV_{ao} was calculated as: $0.8 \times x_{\text{direct}} / \Delta T$, m/s, [32,33,34]. The brachial artery blood pressure was measured in connection with the examination, as a mean value of two measurements, by using an automatic monitor Omron M7 (Omron Healthcare Co., LTD., Kyoto, Japan). The radial and aortic blood pressure was calibrated against the brachial artery blood pressure. The aortic AIx and PWV_{ao} are presented as mean values from two recordings.

Carotid Artery Ultrasound

Two-dimensional images of the common carotid artery (CCA) were acquired using an 8 MHz transducer, 7L, Vivid 7 (General Electric Company, Horten, Norway). The CCA was evaluated 1–2 cm proximal to the carotid bulb. Diastolic images at the time of the electrocardiographic R-wave were stored digitally on EchoPAC (Image Vault 5.0 system, General Electric Company, Horten, Norway). For detection of IMT_{cca} and IM-GSM, six digitized images were imported to validated automated software, Artery Measurement Software (AMS) [35,36]. 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 (www.wlab.gu.se),

Gothenburg University, Gothenburg, Sweden. A region of interest (ROI) of a 10 mm long segment was manually placed proximal to the carotid bulb, **Figure 1, upper panel**. The borders of the IMT_{cca} of the far wall and the inner lumen diameter (LD_{cca}) were identified automatically by the program and the analysis could be manually corrected if necessary. IMT_{cca} 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. LD_{cca} was defined as the distance from the leading edge of the intima-lumen interface of the near wall and the leading edge of the lumen-intima interface of the far wall [37].

The IM-GSM was calculated in the same intima-media segment of the far wall that was evaluated for IMT_{cca} , and from analysis of pixels on a scale from 0 (black) to 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 mean values of six images from both the right and left CCA. Lumen diameter and IMT_{cca} values are shown in mm, while IM-GSM is shown as median gray. At the end of the study, the same investigator performed the image analyses and calculations in random order. Intra- and interobserver variability was measured in 30 randomly selected participants, and the calculations in terms of coefficients of variation (CV) were: 2.90% and 3.80% for IMT_{cca} , 0.40% and 0.70% for LD_{cca} , and 2.74% and 3.81% for IM-GSM. Bland-Altman plots for IMT_{cca} and IM-GSM are shown in **Figure 2**.

High-resolution Ultrasound

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). B-mode images were recorded from the right radial artery in a longitudinal projection, 1–2 cm proximal to the fold separating the palm of the hand from the forearm. All cine-loops were stored on an external disk and measured offline 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 [37]. Three sets of measurements were performed from each of the three representative images. The IMT_{rad} of the far wall is presented as a mean value from nine measurements corresponding to the 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 1, lower panel**. 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. Intra- and interobserver variability in terms of CV were 5.78 and 10.94% for IMT_{rad} , and 3.16 and 3.56% for LD_{rad} .

Biochemical Variables

Plasma concentrations of total calcium, creatinine, glucose, high sensitivity C-reactive protein (hsCRP), phosphate, serum concentration of total cholesterol, triglycerides (TG), apolipoprotein A-1 (Apo-A1), and apolipoprotein B (Apo-B) were estimated using Synchron LX[®] 20 system (Beckman Coulter Inc., Brea, CA, USA). Serum-ionized calcium (Ca^{++}) was analyzed on ABL 800 (Radiometer, Copenhagen, Denmark), and plasma concentration of intact parathyroid hormone (PTH) was measured with electrochemiluminescence immunoassay on the Modular E system (Roche Diagnostics GmbH, Mannheim, Germany). Total serum IGF-1 was analyzed by chemiluminescence immunoassay on DPC Immulite 2000 (Siemens Healthcare Diagnostics, Munich, Ger-

many). Serum concentrations of 25-OH-D were measured by chemiluminescence on Liasor[®] (DiaSorin, S.p.A, Italy). The plasma concentrations of VWF antigen were determined with latex immunoassay on the BCS XP system (Siemens Healthcare Diagnostics, Munich, Germany). The plasma activity of PAI-1 was measured by a functional enzymatic ELISA assay (Chromolyze PAI-1) from Medinor (Axis-Shield, Dundee, U.K.). The samples of 25 hydroxyvitamin D (25-OH-D), von Willebrand factor (VWF) antigen, and PAI-1 were frozen at $-70^{\circ}C$ and analyzed in the same series in order to minimize inter-assay variation. Renal function was estimated by calculating the glomerular filtration rate (GFR) according to Cockcroft-Gault's formula: $GFR = (140 - \text{age in yr}) \times (\text{weight in kg/creatinine}) \times (1.23 \text{ in men or } 1.04 \text{ in women})$.

Vitamin D deficiency was defined as 25-OH-D below 50 nmol/L.

Statistics

Statistical analyses were performed with PASW Statistics v18 (PASW Inc., Chicago, Illinois, U.S.A.). Data are expressed as mean \pm SD. Comparisons between the patients at baseline and the control group were performed with the Mann-Whitney U test for unpaired data, and for intra-individual analyses, Wilcoxon signed rank sum test was used. Spearman's rank correlation coefficients were computed to assess relationships between variables. Univariate and stepwise multiple linear regression analyses were used to evaluate relationships and adjust for confounders. Results from the multiple regression analyses were presented as standardized beta and adjusted R^2 .

The coefficient of variation (CV), defined as the standard deviation of the absolute differences between the measurements, divided by the mean of two measurements was used to evaluate the intra- and interobserver variability. The reproducibility was illustrated according to Bland-Altman [38]. All tests were two-tailed and $p < 0.05$ was considered to be statistically significant.

Results

Clinical Data

Clinical data of healthy controls as well as PHPT patients at baseline and follow-up are presented in **Table 1**. The patients mean age at baseline was 54 ± 8.9 years (range 33 to 68 years). Fifteen patients were below 50 years of age. Forty-five patients had a single parathyroid adenoma, and three had multiglandular disease; median weight of removed tissue 409 mg. Heart rate and diastolic blood pressure did not differ, while systolic blood pressure was slightly higher in patients ($p < 0.05$).

Biochemical Data

Biochemical data are presented in **Table 2**. Calcium and PTH levels were significantly higher and phosphate levels were lower in patients compared with controls and normalized after PTX. The vitamin D levels increased after surgery, but 19 out of 48 patients (40%) had a 25-OH-D level below 50 nmol/l at follow-up, 15 ± 4 months after PTX. There were no significant differences between the subgroups with 25-OH-D levels < 50 nmol/L, and > 50 nmol/L, regarding SBP, AIx, PWV_{ao} , IMT_{cca} , IMT_{rad} , IM-GSM, S-Ca, ionized Ca, or PTH levels. Further, these measures did not differ between those with 25-OH-D levels below or above the median (39 nmol/L).

Vascular Data

Vascular structural and functional results are presented in **Table 3**. We found no differences between patients and controls regarding AIx, estimated aortic blood pressure, IMT_{cca} , IM-GSM

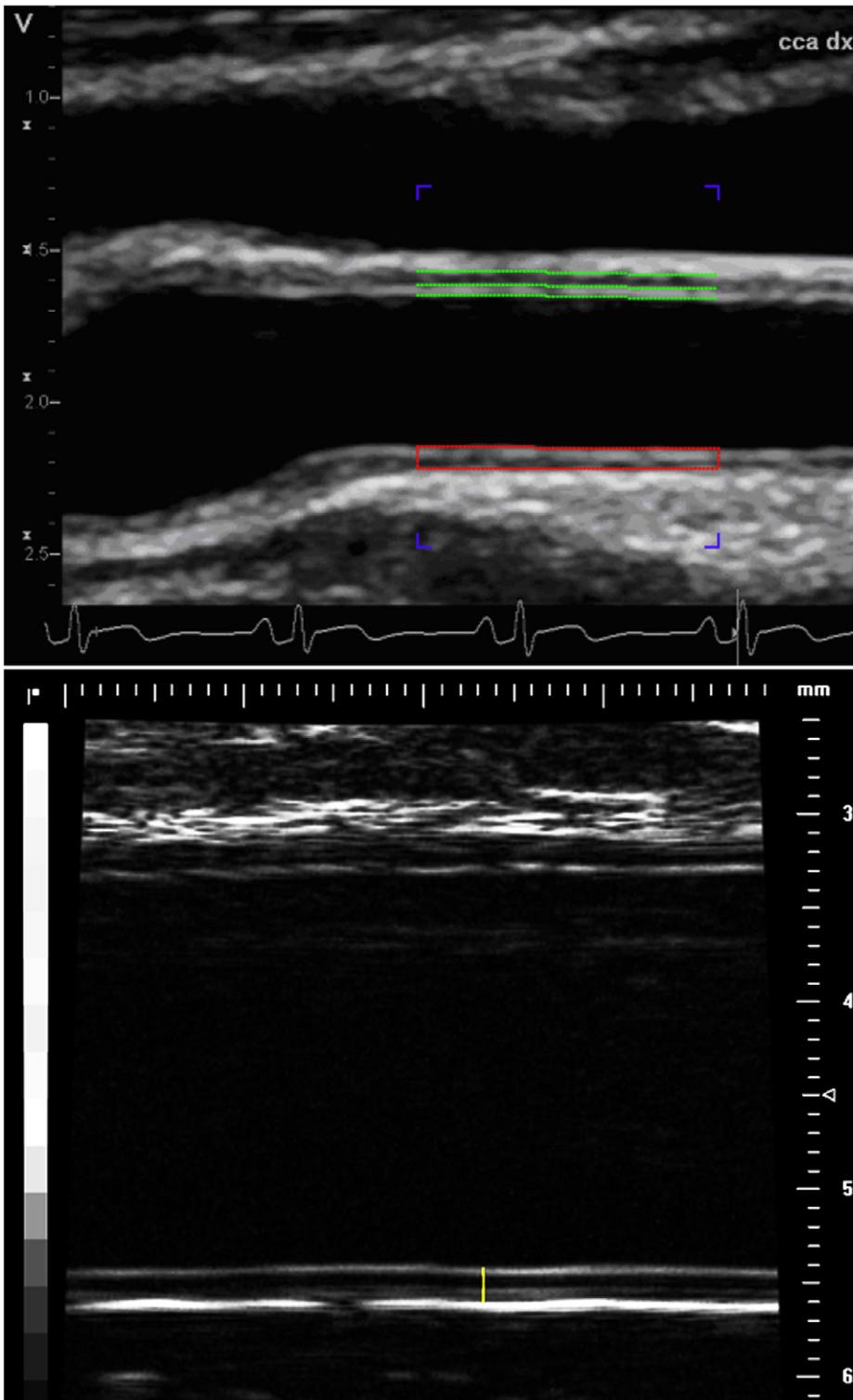


Figure 1. Carotid and radial artery ultrasound images. The red rectangular outline in the upper panel delineates the measurement of the right common carotid artery far wall intima media thickness (IMT_{cca}) and grayscale median (IM-GSM). The vertical yellow line in the lower panel indicates the intima media thickness of the radial artery (IMT_{rad}).
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values, or lumen of the CCA and no changes were observed during follow-up. PWV_{ao} was slightly higher in patients 8.68 ± 1.50 (mean \pm SD) compared to controls 8.13 ± 1.55 (mean \pm SD), $p < 0.05$, (range 6.6–13.2 for patients and 5.3–13.3 for controls),

and did not change during follow-up. The IMT_{rad} did not differ between the groups and remained unchanged at follow-up, while the LD_{rad} was similar to that of controls; however, a marginal increase was noted after one year ($p < 0.05$).

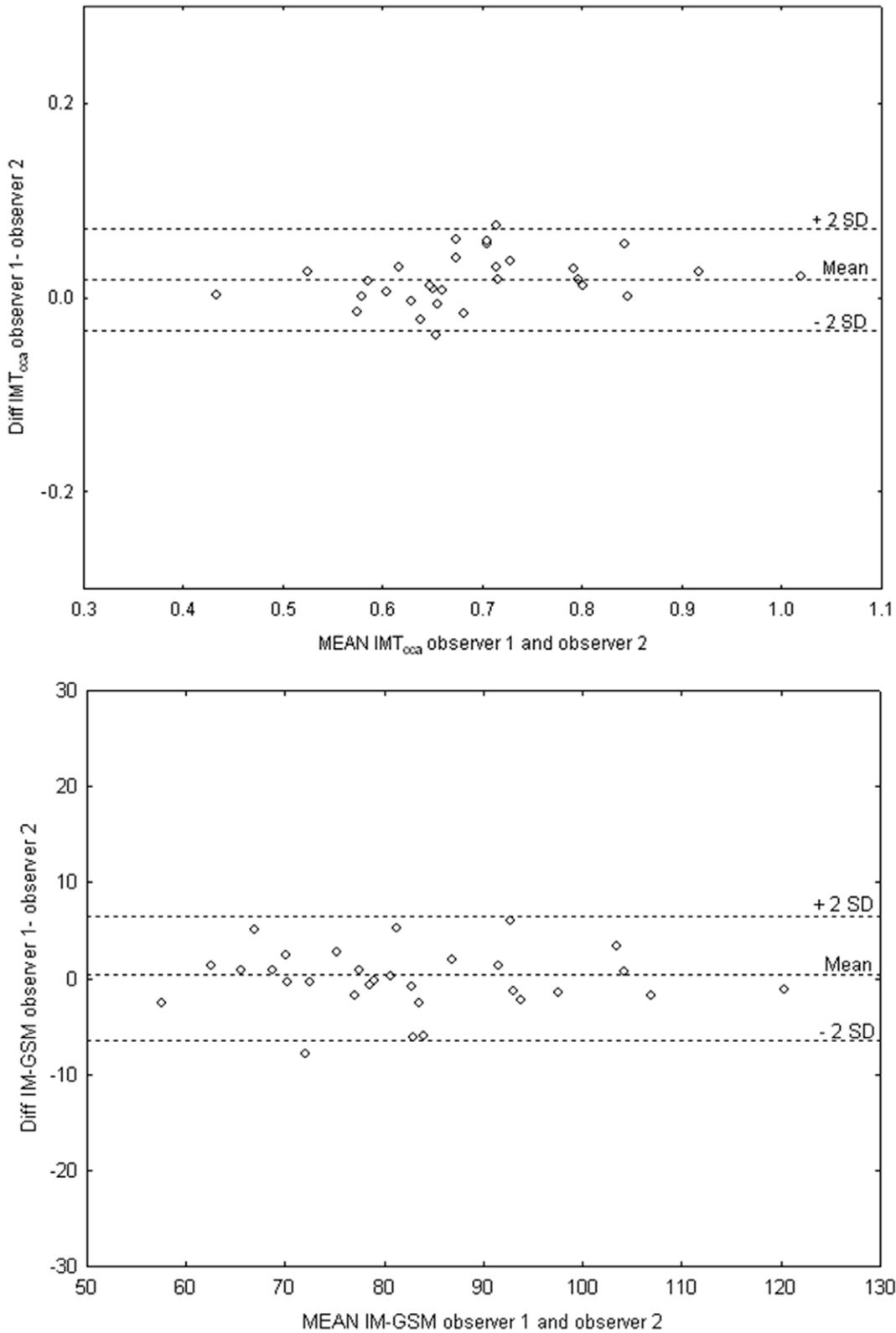


Figure 2. Bland-Altman plots of inter-observer variability. Measurements of the common carotid artery intima media thickness (IMT_{cca}) are illustrated in the upper panel, and of the grayscale median (IM-GSM) in the lower panel. doi:10.1371/journal.pone.0039519.g002

Table 1. Clinical data for healthy controls and patients with mild primary hyperparathyroidism before and after parathyroidectomy.

Variable	Controls n = 48 (35 F)	PHPT		PHPT Baseline	
		Baseline	Follow-up	vs. Controls	vs. Follow-up
		n = 48 (35 F)	n = 48 (35 F)	p-value	p-value
Age, year	54.8±9.0	54.0±8.9	55.3±8.8	0.608	<0.001
HR, bpm	61±9	63±10	60±9 ^a	0.324	0.060
SBP, mmHg	119.1±12.6	126.1±15.8	123.3±15.2 ^a	0.018	0.010
DBP, mmHg	75.9±7.8	79.8±8.8	78.0±8.2 ^a	0.127	0.028
MAP, mmHg	90.3±8.7	95.2±10.2	93.1±9.9 ^a	0.025	0.021
Height, cm	169.1±9.3	171.3±9.0	-	0.173	-
Weight, kg	67.5±10.1	70.6±11.4	72.5±12.0 ^a	0.155	0.003
BSA, m ²	1.77±0.18	1.82±0.18	1.84±0.18 ^a	0.116	0.004
BMI, kg/m ²	23.5±2.1	24.0±3.0	24.7±3.3 ^a	0.381	0.003

^an = 47. Values are shown as means±SD. F = female, HR = heart rate, bpm = beats per minute, SBP = systolic blood pressure, DBP = diastolic blood pressure, MAP = mean arterial pressure, BSA = body surface area, BMI = body mass index.

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Table 2. Biochemical data for healthy controls and patients with mild primary hyperparathyroidism before and after parathyroidectomy.

Variable	Controls n = 48 (35 F)	PHPT		PHPT Baseline	
		Baseline	Follow-up	vs. Controls	vs. Follow-up
		n = 48 (35 F)	n = 48 (35 F)	p-value	p-value
P-PTH (10–65 ng/L)	44.0±12.5	120.0±40.4	49.6±15.7	<0.001	<0.001
P-Calcium (2.15–2.50 mmol/L)	2.29±0.08	2.61±0.12	2.27±0.08	<0.001	<0.001
S-Ca ⁺⁺ (1.15–1.33 mmol/L)	1.25±0.04	1.46±0.06	1.25±0.05	<0.001	<0.001
P-Phosphate (0.80–1.5 mmol/L)	1.06±0.17	0.85±0.16	1.01±0.17 ^a	<0.001	<0.001
Ca*Phosphate	2.43±0.41	2.20±0.39	2.32±0.40	<0.01	0.050
S-25-OH-D (75–250 nmol/L)	64.1±20.6 ^b	40.6±17.1 ^a	58.2±19.5	<0.001	<0.001
P-Creatinine (<90 μmol/L)	69.1±13.6	66.3±12.1 ^a	68.1±15.9 ^b	0.434	0.386
GFR, mL/min (>90 mL/min)	93.0±22.0	100.9±22.2 ^a	103.1±27.7 ^b	0.049	0.861
P-Glucose (4.0–6.0 mmol/L)	5.0±0.34 ^a	5.0±0.46 ^b	4.9±0.52 ^a	0.523	0.471
P-hs-CRP (<3 mg/L)	1.14±1.22 ^b	0.92±0.90 ^a	1.95±5.05 ^c	0.553	0.004
S-Cholesterol (3.9–7.8 mmol/L)	5.71±1.03 ^b	5.74±0.80 ^a	5.85±0.89	0.857	0.163
S-TG (0.45–2.6 mmol/L)	0.86±0.43 ^b	1.00±0.54 ^a	0.93±0.51	0.073	0.045
S-Apo-A1 (1.10–2.10 g/L)	1.73±0.32 ^b	1.72±0.32 ^a	1.69±0.28	0.786	0.760
S-Apo-B (0.50–1.70 g/L)	1.02±0.21	1.01±0.18 ^a	1.06±0.17	0.954	0.003
Apo B/Apo-A1 (<1)	0.60±0.14 ^b	0.61±0.17 ^a	0.64±0.13	0.997	0.117
P-VWF (0.60–1.60 kIE/L)	1.11±0.40 ^b	1.05±0.36 ^b	1.07±0.40 ^b	0.467	0.647
P-PAI-1 (<15 kIE/L)	10.07±6.90 ^b	9.51±7.73 ^b	8.69±7.49 ^b	0.320	0.661
P-Homocysteine (5.0–15.0 μg/L)	9.30±3.00 ^b	9.87±2.75 ^a	9.59±2.29 ^a	0.295	0.266
S-IGF-1 (110–270 μg/L)	137.5±52.9 ^b	146.2±48.3 ^a	143.6±68.2	0.323	0.086

^an = 47,

^bn = 44–46.

^cOne patient had CRP 35.40 mg/L at follow-up, after excluding the patient, mean hs-CRP was 1.26±1.09 mg/L, p<0.01. Values are shown as means±SD. F = female, P = plasma, S = serum, PTH = parathyroid hormone, S-Ca⁺⁺ = ionised calcium, S-25-OH-D = 25 hydroxyvitamin D, GFR = glomerular filtration rate. P-hsCRP = high sensitivity C-reactive protein, TG = triglycerides, Apo = apolipoprotein, VWF = von Willebrand factor antigen, PAI-1 = plasminogen activator inhibitor-1 activity, IGF-1 = insulin like growth factor.

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Table 3. Augmentation index and ultrasound measurements for healthy controls and patients with mild primary hyperparathyroidism before and after parathyroidectomy.

Variable	Controls n = 48 (35 F)	PHPT		PHPT Baseline	
		Baseline n = 48 (35 F)	Follow-up n = 48 (35 F)	vs. Controls p-value	vs. Follow-up p-value
Alx, %	27.7±12.8	28.6±12.2	29.8±11.9 ^a	0.881	0.333
PWV _{ao} , m/s	8.13±1.55 ^b	8.68±1.50 ^c	8.61±1.37 ^c	0.027	0.095
Ao SBP, mmHg	109.8±15.3	114.4±17.3	114.8±15.8 ^a	0.205	0.473
Ao DBP, mmHg	75.0±9.7	78.1±11.3	78.0±8.7 ^a	0.097	0.181
IMT _{rad} , mean, mm	0.255±0.053 ^a	0.271±0.060 ^c	0.271±0.046 ^b	0.340	0.623
LD _{rad} , mean, mm	1.87±0.35 ^a	1.79±0.30 ^c	1.90±0.38 ^b	0.199	0.020
IMT _{cca} , mean, mm	0.680±0.135	0.688±0.113	0.702±0.119 ^a	0.354	0.172
LD _{cca} , mean, mm	5.94±0.62	6.03±0.45	6.03±0.47 ^a	0.229	0.197
IM-GSM, mean	86.5±15.3	82.3±17.2	81.8±15.9 ^a	0.091	0.941

^an = 47,^bn = 43–45,^cn = 36–41. Values are shown as means ± SD. F = female, Alx = augmentation index, PWV_{ao} = aortic pulse wave velocity, 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 and right common carotid artery, LD_{cca} = lumen diameter in both left and right common carotid artery, IM-GSM = intima media grayscale median in both left and right common carotid artery.

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Correlations

We evaluated the univariate relations of vascular measures in terms of Alx, PWV_{ao}, IMT_{rad}, IMT_{cca}, and IM-GSM to baseline data in the complete study cohort of patients and controls in order to verify expected patterns, **Table 4**. As noted, all vascular data except IM-GSM were related to age and systolic blood pressure. Further, correlations of Alx, PWV_{ao}, IMT_{cca}, IMT_{rad}, and IM-GSM with biochemical variables are also presented in *Table 4*. Vascular function was not related to vitamin D. Alx and PWV_{ao} were weakly related to cholesterol, PWV_{ao}, and IM-GSM also to PTH, and PWV_{ao} to ionized Ca and triglycerides. None of Alx, PWV_{ao}, IMT_{rad}, IMT_{cca}, or IM-GSM correlated to P- Phosphate, hsCRP, Apo-B/Apo-A1, Ca*P product, or homocysteine levels.

Stepwise Regression Analyses

Multiple stepwise regression analyses with indices of vascular function (Alx, PWV_{ao}, IMT_{cca}, IMT_{rad} and IM-GSM, one at a time) as dependent variables and demographic data (age, gender, SBP, height, and weight) as independent variables are presented in **Table 5**. More than 30% of the variation in Alx, PWV_{ao}, and IMT_{cca}, were explained by age and SBP, in Alx also by gender.

We also analyzed the possible influence of the disease-specific biochemical disturbances (PTH, ionized Ca, phosphate, 25-OH-D) on the five indices of vascular function, one at a time, considering also demographic data, **Table 6**. Apart from a weak relation between Alx and phosphate, no vascular measure was related to the biochemical variables.

Discussion

In this prospective case-control study of mild PHPT without any known cardiovascular risk factors or medications affecting the cardiovascular system, we found no indication that circulating levels of vitamin D influenced arterial structure or function. Wall composition (IM-GSM) and aortic stiffness (PWV_{ao}) were weakly related to PTH and the latter also to ionized calcium levels in univariate analysis, but not when adjusting for confounders like

blood pressure. Furthermore, the PTX did not cause any change in indices of vascular function or arterial wall thickness. Our conclusions were substantiated by adding new variables, such as echogenicity of the carotid artery and intima media thickness of the radial artery to the established measures Alx, PWV_{ao}, and IMT_{cca}.

Thus, although vitamin D deficiency is common in patients with PHPT [8], and is associated with cardiovascular disease in the general population [39], we did not observe any relations between 25-OH-D and Alx, PWV_{ao}, IMT_{rad}, IMT_{cca}, or IM-GSM in our study. Neither were these measures related to other biochemical data specifically abnormal in PHPT, such as Ca⁺⁺ and phosphate, apart from a correlation between PWV_{ao} and PTH and ionized Ca and an inverse correlation between IM-GSM and PTH. Interestingly, Reis *et al.* found no association between vitamin D and carotid IMT apart from a weak association of 25-OH-D levels to internal carotid IMT when adjusting for, but not excluding, hypertension. Further, they found no evidence of an association between 1,25(OH)₂ D and common carotid IMT or internal carotid IMT, apart from a weak association to internal carotid IMT among patients with hypertension in one of several subgroup analyses [40]. The higher blood pressure among PHPT patients is not clearly understood. Despite the fact that we excluded patients with hypertension, we found significantly higher SBP, although within normal range, in patients compared with controls. SBP decreased after PTX in our patients, while it did not in another series of mild PHPT patients [41]. We have previously reported a weak but significant correlation between SBP and Ca⁺⁺ as well as PTH levels when including both patients and controls [26]. It has been reported that vitamin D may influence blood pressure through the renin-angiotensin system [42], and a number of possible pathophysiological links to atherosclerosis have been ascribed to vitamin D deficiency, including activation of the renin angiotensin system [43]. Our data add strength to the hypothesis that associations between the atherosclerotic process and circulating levels of vitamin D, ionized Ca, and PTH are mediated through blood pressure and other risk factors. With regard to the

Table 4. Spearman rank order correlations between vascular variables and clinical and biochemical data in the whole group of PHPT patients and controls.

	AIx	PWV _{ao}	IMT _{cca}	IMT _{rad}	IM-GSM
Age, years	0.72***	0.49***	0.51***	0.27*	-0.02
Gender	0.46***	0.04	0.20	-0.02	0.12
SBP, mmHg	0.39***	0.50***	0.44***	0.26*	-0.06
Height, cm	-0.49***	-0.04	-0.19	-0.11	-0.05
Weight, kg	-0.44***	-0.04	0.04	0.07	-0.31**
HR, bpm	-0.13	0.20	0.05	0.06	-0.06
S-Ca ⁺⁺	0.02	0.22*	0.07	0.01	-0.14
P-PTH	0.09	0.29**	0.12	0.13	-0.24*
S-25-OH-D	-0.10	-0.15	-0.05	0.003	0.002
S-Cholesterol	0.27*	0.26*	0.19	0.06	0.12
S-TG	-0.12	0.30**	0.05	0.11	-0.13
P-Phosphate	-0.01	-0.14	-0.003	0.07	0.14
P-Creatinine	-0.31**	-0.22*	-0.16	-0.18	-0.02
PAI-1	0.002	0.24*	-0.07	0.17	-0.16
P-Glucose	-0.09	0.23*	-0.04	0.13	-0.13
GFR	-0.50***	-0.05	-0.16	0.12	-0.28**
IGF-1	-0.46***	-0.09	-0.18	-0.19	-0.13

Statistically significant correlations; * = p<0.05, ** = p<0.01, *** = p<0.001. AIx = augmentation index, PWV_{ao} = aortic pulse wave velocity, IMT_{rad} = intima media thickness in right radial artery, IMT_{cca} = intima media thickness in both left and right common carotid artery, IM-GSM = intima media grayscale median in both left and right common carotid artery, SBP = systolic blood pressure, bpm = beats per minutes, P = plasma, S = serum, S-Ca⁺⁺ = ionized calcium, PTH = parathyroid hormone, S-25-OH-D = 25 hydroxyvitamin D, TG = triglycerides, Apo = apolipoprotein.
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relation between vitamin D status and cardiovascular risk, many issues still remain to be resolved. Recent comprehensive reviews do not support a strong link between vitamin D status or supplementation and cardiometabolic outcome or cardiovascular events [44,45], and another systematic review and meta-analysis, based on 51 trials, found no significant correlation between vitamin D status, hypertension, or cardiovascular mortality [46].

Interestingly, it was recently demonstrated that PHPT causes up-regulation of the matrix metalloproteinase 9 (MMP9) gene and

a number of other inflammatory and metabolic genes in fat tissue, with possible impacts on blood pressure and atherosclerosis [47]. In our study of normotensive PHPT patients and controls, we found slightly higher blood pressure and PWV_{ao} in patients. However, data are not unanimous. While *Rosa et al.* noted PHPT patients to have increased arterial stiffness with and without hypertension [48], *Kösch et al.* showed no difference in PWV between PHPT patients and controls, and found no correlations between PWV and increased PTH levels [16].

Our PHPT patients and healthy controls showed no difference in AIx. This is in contrast to the results of *Smith et al.* [13] and *Rubin et al.* [14], who also studied patients with mild PHPT. *Smith et al.* [13] reported higher AIx in patients with mild PHPT compared to controls, and *Rubin et al.* [14] asserted that PHPT was independently associated with increased AIx. In contrast to *Rubin et al.* [14], we did not find any correlation between AIx and elevated PTH. A plausible explanation for the discrepancy is that *Rubin et al.* [14] studied groups containing PHPT patients with hypertension and patients on cardiovascular medication. The association between the severity of PHPT and cardiovascular risk factors, however, is not universal. Similar amount of risk factors was recently reported in hypercalcemic and normocalcemic PHPT patients although cardiovascular and cerebrovascular diseases were more common in hypercalcemic PHPT [49]. In the same study, however, vascular stiffness was not higher in this group than in normocalcemic PHPT or controls, and in congruence with our material unrelated to calcium and PTH.

Probably, cardiovascular diseases combined with PHPT are more likely to affect the arterial wall and IMT than PHPT alone. This is supported by *Fallo et al.* [22], who included PHPT patients with and without cardiovascular risk factors. When compared to healthy controls, only PHPT patients with concomitant cardiovascular risk factors were found to have an increased IMT of the carotid artery wall. Similarly *Walker et al.* [20] reported increased carotid IMT in PHPT patients, including those with cardiovascular diseases, compared to controls. *Nuzzo et al.* [19] excluded subjects with high blood pressure or clinical cardiovascular disease and still found significantly higher carotid IMT values in 20 selected patients compared with controls. However, the authors did not indicate the lipid levels in their study group. *Kösch et al.* [16] found no disturbance in brachial and carotid IMT or aortic PWV in PHPT patients compared to controls, and *Lumachi et al.* [21] reports no improvement in carotid IMT after 18 months of PTX follow-up; further, no correlations between serum calcium and IMT was found. In our study, with careful exclusion of

Table 5. Stepwise regression analyses with indices of vascular function as dependent variables, and demographic data as independent variables in the whole group of PHPT patients and controls.

Variable	Model for AIx		Model for PWV _{ao}		Model for IMT _{cca}		Model for IMT _{rad}		Model for IM-GSM	
	R ² = 0.57, p<0.001		R ² = 0.37, p<0.001		R ² = 0.32, p<0.001		R ² = 0.08, p<0.01		R ² = 0.14, p<0.001	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Age, years	0.54	<0.001	0.28	<0.01	0.41	<0.001	-	-	-	-
Gender	0.29	<0.001	-	-	-	-	-	-	-	-
SBP, mmHg	0.17	<0.05	0.43	<0.001	0.26	<0.01	0.30	<0.01	-	-
Height, cm	-	-	-	-	-	-	-	-	0.40	<0.01
Weight, kg	-	-	-	-	-	-	-	-	-0.58	<0.001

AIx = augmentation index, PWV_{ao} = aortic pulse wave velocity, IMT_{cca} = intima media thickness in both left and right common carotid artery, IMT_{rad} = intima media thickness in right radial artery, IM-GSM = intima media grayscale median in both left and right common carotid artery, SBP = systolic blood pressure.
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Table 6. Stepwise regression analyses with indices of vascular function as dependent variables, and demographic and biochemical data as independent variables in the whole group of PHPT patients and controls.

Variable	Model for Alx		Model for PWV _{ao}		Model for IMT _{cca}		Model for IMT _{rad}		Model for IM-GSM	
	R ² = 0.58, p<0.001		R ² = 0.37, p<0.001		R ² = 0.29, p<0.001		R ² = 0.07, p<0.05		R ² = 0.18, p<0.001	
	β	p-value	β	p-value	β	p-value	β	p-value	β	p-value
Age, years	0.65	<0.001	0.27	<0.05	0.39	<0.001	–	–	–	–
Gender	0.29	<0.001	–	–	–	–	–	–	–	–
SBP, mmHg	–	–	0.44	<0.001	0.26	<0.05	0.28	<0.05	–	–
Height, cm	–	–	–	–	–	–	–	–	0.37	<0.01
Weight, kg	–	–	–	–	–	–	–	–	–0.63	<0.001
P-PTH	–	–	–	–	–	–	–	–	–	–
S-Ca ⁺⁺	–	–	–	–	–	–	–	–	–	–
P-Phosphate	–0.21	<0.01	–	–	–	–	–	–	–	–
S-25-OH-D	–	–	–	–	–	–	–	–	–	–

Alx = augmentation index, PWV_{ao} = aortic pulse wave velocity, IMT_{cca} = intima media thickness in both left and right common carotid artery, IMT_{rad} = intima media thickness in right radial artery, IM-GSM = intima media grayscale median in both left and right common carotid artery, SBP = systolic blood pressure, PTH = parathyroid hormone, P = plasma, S = serum, S-Ca⁺⁺ = ionized calcium, PTH = parathyroid hormone, S-25-OH-D = 25 hydroxyvitamin D.

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patients with cardiovascular risk factors, we did not observe any differences between patients and controls at baseline, apart from slightly higher SBP and PWV_{ao} in patients with a significant reduction in blood pressure one year after PTX. All our vascular measurements, apart from IM-GSM, were related to age and blood pressure. Adjusting for age and blood pressure, PWV_{ao} was not related to circulating levels of PTH, Ca⁺⁺, phosphate, or 25-OH-D.

In order to investigate the vascular structure more thoroughly, we have used new validated techniques in our study, such as grayscale median for the evaluation of echogenicity of the carotid intima media, and a high-resolution ultrasound for measurement of IMT in the radial artery. We have demonstrated that high-resolution ultrasound allows measurement of very thin structures such as IMT of the rat carotid artery wall [50], and it has also been shown that measurement of the human radial artery IMT is feasible by this technique [24]. In the current study, we measured the intima media thickness in the radial artery and found no significant increase in IMT_{rad} in the PHPT patients compared with controls. By measurement of the grayscale in the intima media images of carotid artery, we tried to obtain information regarding the wall composition, with the assumption that fibrotic infiltration should be more echogenic and lipidous transformation should be more echolucent. Earlier studies have shown that IM-GSM in CCA is closely related to the echogenicity in overt carotid plaques; moreover, the same group further reported a relationship between echolucency in the intima-media complex and cardiovascular risk factors in elderly subjects [23,51]. However, our study did not indicate abnormal composition of the carotid artery wall as evaluated by this technique.

Strengths and Limitations

To our knowledge, this is the first study to describe IMT_{rad} and the echogenicity in the intima media of the CCA in patients with

mild PHPT. The strict design, excluding cardiovascular risk factors, is the strength of our study, which should favorably influence the reliability of our results. The material is comparatively large for this type of study; however, it is too small for any prognostic evaluations.

We measured the IMT of the common carotid artery in the far wall only, as we assumed relatively symmetrical wall thickness in our patients and controls. Since most patients with PHPT today do not express typical signs and since calcium- and PTH-levels are not examined routinely, it is rarely possible to estimate the duration of the disease.

Conclusion

We found normal arterial function, despite high PTH and Ca as well as low vitamin D levels, in patients with mild PHPT without cardiovascular risk factors. The cardiovascular risk associated with low vitamin D and/or high PTH and Ca levels may be explained by their coupling to blood pressure and other risk factors rather than direct effects on the arterial structure. Our findings support the importance of adequate blood pressure control in PHPT if PTX is not performed, but do not indicate vascular abnormalities motivating extended follow-up after PTX.

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Author Contributions

Conceived and designed the experiments: ILN ME KC. Performed the experiments: MR PF ILN. Analyzed the data: MR PF KC MJE ILN. Contributed reagents/materials/analysis tools: TG. Wrote the paper: MR KC MJE ILN PF TG.

References

- Fraser A, Williams D, Lawlor DA (2010) Associations of serum 25-hydroxyvitamin D, parathyroid hormone and calcium with cardiovascular risk factors: analysis of 3 NHANES cycles (2001–2006). *PLoS One* 5: e13882.
- Williams DM, Fraser A, Lawlor DA (2011) Associations of vitamin D, parathyroid hormone and calcium with cardiovascular risk factors in US adolescents. *Heart* 97: 315–320.

3. Lind L, Hanni A, Lithell H, Hvarfner A, Sorensen OH, et al. (1995) Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. *Am J Hypertens* 8: 894–901.
4. Burgaz A, Byberg L, Rautiainen S, Orsini N, Hakansson N, et al. (2011) Confirmed hypertension and plasma 25(OH)D concentrations amongst elderly men. *J Intern Med* 269: 211–218.
5. Pilz S, Tomaschitz A, Drechsler C, Ritz E, Boehm BO, et al. (2010) Parathyroid hormone level is associated with mortality and cardiovascular events in patients undergoing coronary angiography. *Eur Heart J* 31: 1591–1598.
6. Hagstrom E, Ingelsson E, Sundstrom J, Hellman P, Larsson TE, et al. (2010) Plasma parathyroid hormone and risk of congestive heart failure in the community. *Eur J Heart Fail* 12: 1186–1192.
7. Cawthon PM, Parimi N, Barrett-Connor E, Laughlin GA, Ensrud KE, et al. (2010) Serum 25-hydroxyvitamin D, parathyroid hormone, and mortality in older men. *J Clin Endocrinol Metab* 95: 4625–4634.
8. Silverberg SJ, Shane E, Dempster DW, Bilezikian JP (1999) The effects of vitamin D insufficiency in patients with primary hyperparathyroidism. *Am J Med* 107: 561–567.
9. Hedback G, Oden A (1998) Increased risk of death from primary hyperparathyroidism—an update. *Eur J Clin Invest* 28: 271–276.
10. Yu N, Donnan PT, Flynn RW, Murphy MJ, Smith D, et al. (2010) Increased mortality and morbidity in mild primary hyperparathyroid patients. The Parathyroid Epidemiology and Audit Research Study (PEARS). *Clin Endocrinol (Oxf)* 73: 30–34.
11. Silverberg SJ, Lewiecki EM, Mosekilde L, Peacock M, Rubin MR (2009) Presentation of asymptomatic primary hyperparathyroidism: proceedings of the third international workshop. *J Clin Endocrinol Metab* 94: 351–365.
12. Nilsson IL, Aberg J, Rastad J, Lind L (1999) Endothelial vasodilatory dysfunction in primary hyperparathyroidism is reversed after parathyroidectomy. *Surgery* 126: 1049–1055.
13. Smith JC, Page MD, John R, Wheeler MH, Cockcroft JR, et al. (2000) Augmentation of central arterial pressure in mild primary hyperparathyroidism. *J Clin Endocrinol Metab* 85: 3515–3519.
14. Rubin MR, Maurer MS, McMahon DJ, Bilezikian JP, Silverberg SJ (2005) Arterial stiffness in mild primary hyperparathyroidism. *J Clin Endocrinol Metab* 90: 3326–3330.
15. Rosa J, Raska I Jr, Wichterle D, Petrak O, Strauch B, et al. (2011) Pulse wave velocity in primary hyperparathyroidism and effect of surgical therapy. *Hypertens Res* 34(3): 296–300.
16. Kosch M, Hausberg M, Barenbrock M, Posadzy-Malaczynska A, Kisters K, et al. (2001) Arterial distensibility and pulse wave velocity in patients with primary hyperparathyroidism before and after parathyroidectomy. *Clin Nephrol* 55: 303–308.
17. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, et al. (1999) Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 340: 14–22.
18. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE (1997) Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 96: 1432–1437.
19. Nuzzo V, Tauchmanova L, Fonderico F, Trotta R, Fittipaldi MR, et al. (2002) Increased intima-media thickness of the carotid artery wall, normal blood pressure profile and normal left ventricular mass in subjects with primary hyperparathyroidism. *Eur J Endocrinol* 147: 453–459.
20. Walker MD, Fleischer J, Rundek T, McMahon DJ, Homma S, et al. (2009) Carotid vascular abnormalities in primary hyperparathyroidism. *J Clin Endocrinol Metab* 94: 3849–3856.
21. Lumachi F, Ermani M, Frego M, Pilon F, Filosa T, et al. (2006) Intima-media thickness measurement of the carotid artery in patients with primary hyperparathyroidism. A prospective case-control study and long-term follow-up. *In Vivo* 20: 887–890.
22. Fallo F, Camporese G, Capitelli E, Andreozzi GM, Mantero F, et al. (2003) Ultrasound evaluation of carotid artery in primary hyperparathyroidism. *J Clin Endocrinol Metab* 88: 2096–2099.
23. Andersson J, Sundstrom J, Gustavsson T, Hulthe J, Elmgren A, et al. (2009) Echogenicity of the carotid intima-media complex is related to cardiovascular risk factors, dyslipidemia, oxidative stress and inflammation: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Atherosclerosis* 204: 612–618.
24. Myrdal A, Gan LM, Osika W, Friberg P, Johansson M (2010) Increased intima thickness of the radial artery in individuals with prehypertension and hypertension. *Atherosclerosis* 209: 147–151.
25. Farahnak P, Larfars G, Sten-Linder M, Nilsson IL (2011) Mild primary hyperparathyroidism: vitamin D deficiency and cardiovascular risk markers. *J Clin Endocrinol Metab* 96: 2112–2118.
26. Farahnak P, Ring M, Caidahl K, Farnebo LO, Eriksson MJ, et al. (2010) Cardiac function in mild primary hyperparathyroidism and the outcome after parathyroidectomy. *Eur J Endocrinol* 163: 461–467.
27. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP (1993) An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J* 14: 160–167.
28. Pauca AL, O'Rourke MF, Kon ND (2001) Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. *Hypertension* 38: 932–937.
29. O'Rourke MF (1971) The arterial pulse in health and disease. *Am Heart J* 82: 687–702.
30. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, et al. (2006) Expert consensus document on arterial stiffness: methodological issues and clinical applications. *Eur Heart J* 27: 2588–2605.
31. Vermeersch SJ, Rietzschel ER, De Buyzere ML, Van Bortel LM, Gillebert TC, et al. (2009) Distance measurements for the assessment of carotid to femoral pulse wave velocity. *J Hypertens* 27: 2377–2385.
32. Weber T, Ammer M, Rammer M, Adji A, O'Rourke MF, et al. (2009) Noninvasive determination of carotid-femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. *J Hypertens* 27: 1624–1630.
33. Sugawara J, Hayashi K, Yokoi T, Tanaka H (2008) Age-associated elongation of the ascending aorta in adults. *JACC Cardiovasc Imaging* 1: 739–748.
34. Boutouyrie P, Vermeersch SJ, Mattace-Raso FUS, Wilkinson I, Willum Hansen T, et al. (2010) Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'. *Eur Heart J* 31: 2338–2350.
35. Wendelhag I, Liang Q, Gustavsson T, Wikstrand J (1997) A new automated computerized analyzing system simplifies readings and reduces the variability in ultrasound measurement of intima-media thickness. *Stroke* 28: 2195–2200.
36. Liang Q, Wendelhag I, Wikstrand J, Gustavsson T (2000) A multiscale dynamic programming procedure for boundary detection in ultrasonic artery images. *IEEE Trans Med Imaging* 19: 127–142.
37. Wendelhag I, Gustavsson T, Suurkula M, Berglund G, Wikstrand J (1991) Ultrasound measurement of wall thickness in the carotid artery: fundamental principles and description of a computerized analysing system. *Clin Physiol* 11: 565–577.
38. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1: 307–310.
39. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, et al. (2008) Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 117: 503–511.
40. Reis JP, von Muhlen D, Michos ED, Miller ER 3rd, Appel LJ, et al. (2009) Serum vitamin D, parathyroid hormone levels, and carotid atherosclerosis. *Atherosclerosis* 207: 585–590.
41. Persson A, Bollerslev J, Rosen T, Mollerup CL, Franco C, et al. (2011) Effect of surgery on cardiac structure and function in mild primary hyperparathyroidism. *Clin Endocrinol (Oxf)* 74: 174–180.
42. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, et al. (2004) Vitamin D: a negative endocrine regulator of the renin-angiotensin system and blood pressure. *The Journal of steroid biochemistry and molecular biology* 89–90: 387–392.
43. Brewer LC, Michos ED, Reis JP (2011) Vitamin D in atherosclerosis, vascular disease, and endothelial function. *Curr Drug Targets* 12: 54–60.
44. Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, et al. (2010) Systematic review: Vitamin D and cardiometabolic outcomes. *Ann Intern Med* 152: 307–314.
45. Wang L, Manson JE, Song Y, Sesso HD (2010) Systematic review: Vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 152: 315–323.
46. Elamin MB, Abu Elnour NO, Elamin KB, Fatourehchi MM, Alkatib AA, et al. (2011) Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 96: 1931–1942.
47. Christensen MH, Dankel SN, Nordbo Y, Varhaug JE, Almas B, et al. (2011) Primary hyperparathyroidism influences the expression of inflammatory and metabolic genes in adipose tissue. *PLoS One* 6: e20481.
48. Rosa J, Raska I Jr, Wichterle D, Petrak O, Strauch B, et al. (2011) Pulse wave velocity in primary hyperparathyroidism and effect of surgical therapy. *Hypertens Res* 34: 296–300.
49. Tordjman KM, Yaron M, Izkhakov E, Osher E, Sherkerman G, et al. (2010) Cardiovascular risk factors and arterial rigidity are similar in asymptomatic normocalcemic and hypercalcemic primary hyperparathyroidism. *Eur J Endocrinol* 162: 925–933.
50. Razuvaev A, Lund K, Roy J, Hedin U, Caidahl K (2008) Noninvasive real-time imaging of intima thickness after rat carotid artery balloon injury using ultrasound biomicroscopy. *Atherosclerosis* 199: 310–316.
51. Lind L, Andersson J, Ronn M, Gustavsson T (2007) The echogenicity of the intima-media complex in the common carotid artery is closely related to the echogenicity in plaques. *Atherosclerosis* 195: 411–414.