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Coronary Microvascular Dysfunction Induced by Primary Hyperparathyroidism is Restored after Parathyroidectomy

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Abstract:

Background - Symptomatic primary hyperparathyroidism (PHPT) is associated with increased cardiovascular mortality. However, data on the association between asymptomatic PHPT and cardiovascular risk are lacking. We assessed coronary flow reserve (CFR), as a marker of coronary microvascular function, in asymptomatic PHPT of recent onset.

Methods and Results - We studied 100 PHPT patients (pts) (80 F, aged 58±12 years) without cardiovascular disease, and 50 controls matched for age and gender. CFR in the left anterior descending coronary artery was detected by transthoracic Doppler echocardiography, at rest and during adenosine infusion. CFR was the ratio of hyperemic diastolic flow velocity (DFV) to resting DFV. In PHPT, CFR was lower than in controls (3.0±0.8 vs 3.8±0.7, p<0.0001). CFR was abnormal (≤2.5) in 27 (27%) pts compared with controls (4%) (p=0.0008). CFR was inversely related to parathyroid hormone (PTH) levels (r=-0.3; p<0.004). In pts with CFR ≤2.5 PTH was higher (26.4 [16-37] vs18. [13-25] pmol/L, p<0.007) whereas calcium levels were similar (2.9±0.1 vs 2.8±0.3 mmol/L, p=0.2). At multivariable linear regression analysis, PTH, age and heart rate were the only factors associated with CFR (p=0.04, p=0.01 and p=0.006, respectively). At multiple logistic regression analysis only PTH increased the probability of CFR ≤2.5 (p=0.03). In all PHPT pts with CFR ≤2.5, parathyroidectomy normalized CFR (3.3±0.7 vs 2.1±0.5, p<0.0001).

Conclusions - PHPT pts have coronary microvascular dysfunction which is completely restored after parathyroidectomy. PTH independently correlates with the coronary microvascular impairment, suggesting a crucial role of the hormone to explain the increased cardiovascular risk in PHPT.

Key words: coronary flow reserve; microvascular dysfunction; risk factors; PTH; hyperparathyroidism

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Introduction

Parathyroid hormone (PTH) regulates calcium (Ca^{2+}), phosphate and vitamin D homeostasis and plays a crucial role in mineral metabolism and bone turnover. Untreated primary hyperparathyroidism (PHPT) is characterized by chronically elevated PTH and hypercalcemia with bone and renal disorders and is associated with increased cardiovascular morbidity and mortality.[1] In symptomatic PHPT, parathyroidectomy has been shown to reduce cardiovascular mortality and has therefore become mandatory.[2] Nowadays, the features of PHPT have shifted from a clinically manifest condition associated with severe hypercalcemia, kidney stones, bone disorders and neuromuscular involvement, towards an asymptomatic state. Currently, in most patients, PHPT is diagnosed on routine biochemistry, in the absence of any clinical signs of PTH alterations.[3] Data on the cardiovascular involvement in asymptomatic PHPT are limited and it is not known whether calcium or PTH alterations may impair cardiovascular function already in the initial phase of the disease.[2, 4] Recently, mild alterations of circulating PTH, at the upper levels of the normal range[5], have been shown to predict cardiovascular mortality in a large cohort of elderly men. However, there is no evidence that reducing PTH levels will reduce cardiovascular risk in the general population.[5] Furthermore, in asymptomatic PHPT the optimal timing for parathyroidectomy is still controversial and it is unknown whether early surgical intervention results in improved cardiovascular outcome.[6] Traditionally, the association between PHPT and cardiovascular disease has been related to the higher prevalence in these patients of classical cardiovascular risk factors, like diabetes mellitus (DM), dyslipidemia, hypertension[1] and to mineral homeostasis disruption.[7] However, several experimental studies have shown that PTH can selectively target the vascular wall, acting on specific receptors present on endothelial and smooth muscle cells.[8, 9] Therefore, PTH-mediated structural and functional cardiovascular

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alterations might play a causal role in the pathophysiology of atherosclerosis and ultimately heart failure.[7, 10]. Several investigations have documented PHPT-associated endothelial dysfunction in the peripheral circulation[11, 12] as well as increased arterial stiffness.[13] Indeed, the reversal of vascular dysfunction after successful parathyroidectomy further supports the concept of a specific role of PTH in the pathogenesis of cardiovascular disease in PHPT.[11, 12, 14] The aim of the present study was to evaluate the influence of PHPT on coronary microvascular function, assessed by coronary flow reserve (CFR) by transthoracic Doppler echocardiography (TDE), in healthy subjects and in patients with asymptomatic PHPT without evidence for epicardial coronary artery disease (CAD) as assessed by multislice CT (MSCT) coronary angiography. Coronary functional abnormalities represent the first marker of the atherosclerotic process in the coronary circulation and therefore CFR may become of clinical relevance in the management and treatment of PHPT in its asymptomatic initial phase.

Methods

Patients

In this cross-sectional study, we enrolled 100 asymptomatic PHPT patients (80 F, aged 58±12 years), referred to the University Hospital of Padua for parathyroidectomy due to solitary PTH-secreting adenoma. Baseline evaluation included physical examination and collection of clinical and laboratory data (**Table 1**). The median time from PHPT diagnosis was 6 months (range 1-109 months). Patients with history or clinical evidence of cardiopulmonary, renal (serum creatinine >133 μmol/L in men and >120 μmol/L in women) or hepatic disease, and malignant or infectious disease were excluded. Mild PHPT (i.e. total serum calcium <3.0 mmol/L) was equally represented in patients with and without concurrent cardiovascular risk factors. The

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nonrandomized control group consisted of 50 normal volunteers recruited from institutional personnel, matched for age and gender. They did not undergo any cardiovascular conditioning program. None had CAD. All control subjects were asymptomatic, without any history of heart disease. Exclusion criteria for all subjects included any of the following conditions: cerebral vascular disease, carotid artery bruit, peripheral bruit or abnormal pulse, history of angina or a myocardial infarction, hypertension requiring treatment, use of vasodilating drugs and alcohol intake >10 oz per week. All participants had normal ECG at rest and during adenosine-induced hyperemia. Patients and controls came from the same geographic area (North-East of Italy). The absence of CAD was evaluated by clinical history, physical examination and electrocardiogram. Cardiovascular risk factor definition is provided in the online-only Data Supplement. The study was approved by the institutional ethics committee and all patients gave written informed consent. None of the PHPT patients eligible for the study declined to participate in CFR evaluation.

Echocardiography

Transthoracic Doppler echocardiography was performed using a commercially available ultrasound system (Vivid 7, GE Medical System, Inc., Hortem, Norway). All images were analyzed offline by a single investigator, blinded to all clinical data. Echocardiographic methods are detailed in the online-only **Data Supplement.**

Coronary Flow Velocity Reserve Assessment

Images were obtained in the distal part of the LAD using a 7-MHz transducer. The coronary blood flow was obtained by Color Doppler flow-mapping guidance, and a sample volume was positioned within the color signal in the left anterior descending coronary artery (LAD) by the pulse wave Doppler. After baseline recordings of flow velocity, adenosine was administered by

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intravenous infusion (140 μg/kg/min) for 3 minutes, obtaining hyperemic Doppler flow profiles. The CFR was estimated to be the ratio of hyperemic to baseline peak diastolic coronary flow velocities[15]. A CFR ≤2.5 was considered abnormal[16], and the population was dichotomized according to this cut-off. All patients abstained from caffeine-containing drinks for at least 24 h before testing.

All CFR measurements were stored digitally for future offline analysis blinded for all clinical variables. The intra-observer and inter-observer variability of CFR measurements was 4.3 % and 5.8 %, respectively.

MSCT Coronary Angiography Protocol and Interpretation

Patients with abnormal CFR underwent MSCT coronary angiography to exclude epicardial CAD. Coronary MSCT was performed using a 64-slice dual-source scanner (Definition, Siemens Medical System, Forchheim, Germany). MSCT protocol is detailed in the online-only **Data Supplement.**

Laboratory Methods

Blood samples for biochemical and metabolic laboratory measurements were obtained at 08:00 a.m., after overnight fasting. Serum PTH was measured using DiaSorin LIAISON® N-tact™ PTH kit (Saluggia, Italy), with the CLIA method, normal range 1.8-7.7 pmol/L (17-73 ng/L). Intra- and inter-assay coefficients of variation were less than 10%. All other biochemical variables were assayed in plasma or serum using standard methods.

Statistical Analyses

Continuous variables with no/mild skew were presented as mean ± SD; skewed measures as median with first and third quartiles (Q1, Q3). Discrete variables were summarized as frequencies and percentages. The distribution of the data was analyzed with one-sample

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Kolmogorov-Smirnov test. Logarithmic transformation was performed to achieve normal distribution for skewed variables. Categorical variables were compared with χ^2 test or Fisher exact test, as appropriate. Continuous data were compared with 2-tailed paired or unpaired *t* test (for normally distributed datasets) or with Mann-Whitney *U* test or Wilcoxon signed rank test (for skewed variables). Bivariate correlations were assessed by Pearson's coefficient (r). Unadjusted and multiple linear regression analysis were performed between CFR and risk factors or clinical conditions. Stepwise logistic regression analysis was used to model normal versus abnormal CFR as a function of PTH and other coronary risk factors or clinical conditions. Baseline characteristics were chosen for entry into multivariable models on the basis of their discrimination between low and high CFR as well as on unadjusted association with CFR≤2.5 of p≤0.1. A combination of forward and backward selection procedures was used to aid in determining the best model of factors independently associated with CFR. This was followed by forcing potential confounders into the models and determining their effect on the relationship of interest. Non-significant risk factors were removed if they did not significantly add to the model. Summary statistics for the regression models included the *c*-statistic (a measure of association of predicted probabilities and observed prevalence of a binary outcome) and R^2 (re-scaled for use in logistic regression by Cox & Snell method). Intra-observer and inter-observer reproducibility of CFR was evaluated by linear regression analysis and expressed as correlation of coefficients (r) and standard error of estimates (SEE), and by the intra-class correlation coefficient (ICC). Reproducibility is considered satisfactory if the ICC is between 0.81 and 1.0. Intra-observer and inter-observer reproducibility measurements were calculated in all 100 pts. All tests were 2-sided and statistical significance was accepted if the null hypothesis could be rejected at $p<0.05$. Data were analyzed with SPSS software version 18.0 (Chicago, SPSS, Inc., Chicago, Illinois). The

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authors had full access to the data and take responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Results

Baseline Clinical Features and Coronary Flow Reserve Evaluation

Patient characteristics are presented in **Table 1**. CFR in patients was lower than in control subjects (3.0±0.8 vs 3.8±0.7, p<0.0001) (See **Supplemental Table**). The prevalence of CFR≤2.5 was higher in patients compared to controls (27% vs 4%; OR 8.9, p=0.0008). Severe CFR (<2) impairment was found in 8 (8%) patients and in none of controls. CFR studies were always well tolerated. Overall, during adenosine infusion heart rate increased compared to baseline (74±12 beats/min vs 94±16 beats/min, increase 20.27±12.38, p<0.0001), systolic blood pressure decreased (132±17 vs 114±18 mmHg, decrease 15 mmHg (10, 25), p<0.0001), as well as diastolic blood pressure (79±14 vs 67±11 mmHg, decrease 10 mmHg (0, 20), p<0.0001), whereas peak diastolic velocity in the LAD increased (23±6 vs 69±20 cm/s, increase 46.18±18.38, p<0.0001). There were no significant electrocardiographic changes or LV wall motion abnormalities during adenosine infusion in any of our patients. 14 patients were treated with statins, 10 with aspirin, 2 with nitrates, 14 with ACE-inhibitors, 8 with calcium-antagonists and 18 received β-blockers.

Hemodynamic Parameters Based On Coronary Flow Reserve Value

Heart rate at rest and during adenosine infusion was higher in patients with CFR≤2.5 (80±13 vs 72±11 beats/min, p=0.005 and 100±16 vs 92±15 beats/min, p=0.04, respectively). Among patients, systolic blood pressure at rest was higher in those patients with CFR≤2.5 (138±17 vs 130±16 mmHg, p=0.03, respectively). Baseline peak diastolic flow velocity was higher in

patients with CFR≤2.5 (26±9 vs 22±4 cm/s, p<0.002). Hyperemic peak diastolic flow velocity as well as CFR were significantly lower in patients with CFR≤2.5 compared with patients with normal CFR (57±15 vs 74±16 cm/s, p<0.002 and 2.0±0.4 vs 3.4±0.6, p<0.0001, respectively). Intra- and inter-observer reproducibility of CFR measurements are shown in the online-only **Data Supplement.**

Characteristics of Patients with Coronary Microvascular Dysfunction (CFR≤2.5)

The clinical characteristics of patients with CFR≤2.5 and patients with CFR>2.5 are given in **Table 2**. PTH levels were higher in patients with CFR≤2.5 (**Figure 1**). Patients with coronary microvascular dysfunction (CFR≤2.5) were older and with a higher prevalence of hypertension. Diastolic dysfunction and plasma calcium levels were comparable in the two groups as well as echocardiographic features and LV mass. Therapy was not different between groups (data not shown). 26 patients with CFR≤2.5 had normal coronary arteries at MSCT. Only 1 patient had a mild right coronary stenosis (<50%). Calcium score was <10 in all patients with CFR≤2.5.

Factors Associated With Coronary Flow Reserve

At unadjusted linear regression analysis PTH (p=0.004), age (p=0.01), heart rate (p=0.007) and systolic blood pressure (p=0.02) were significantly associated with CFR. By multivariable analysis only PTH (p=0.01), age (p=0.04) and heart rate (p=0.006) were independently associated with CFR (**Table 3**). PTH also independently correlated with DPV during adenosine (p=0.01). If hypertensive and diabetic patients were excluded from the analysis, PTH (p=0.01), age (p=0.02) and heart rate (p=0.01) remained the only factors associated with CFR.

Factors Associated With Coronary Microvascular Dysfunction (CFR≤2.5)

At unadjusted logistic regression analysis the significant or marginally significant (p<0.1) risk factors were: PTH (p<0.01), age (p<0.03), BMI (p=0.08), history of hypertension (p=0.03),

systolic blood pressure (p=0.03), heart rate (p<0.004), history of dyslipidemia (p=0.08) and diastolic dysfunction (p=0.06). Factors independently associated with abnormal CFR are summarized in **Table 4**. When modeled using a stepwise regression and adjusting for baseline differences, factors independently associated with CFR≤2.5 were: PTH (odds ratio [OR] 3.03, 95% confidence interval [CI] 1.56 to 5.9; p=0.01) and heart rate (OR 1.08, 95% CI 1.02 to 1.13; p=0.02) (**Table 4**). Substituting the basal heart rate did not greatly affect the model and no other clinical characteristic entered as a significant covariate. To exclude the modulating effect of other variables, we added also variables marginally significant at unadjusted logistic regression analysis (model 3). These were not independently associated with CFR≤2.5, and their addition to the model did not affect the robust relationship between PTH and CFR≤2.5. When other conditions (gender, time from diagnosis, current smoking, history of diabetes, use of statins or nitrates) were forced into the model (potentially overfitting it), PTH and heart rate remained significantly associated with CFR≤2.5 (p=0.01 and p=0.03, respectively). The C statistic for model 1 was 0.746 (95% CI 0.633-0.859) without PTH and 0.794 (0.693-0.895) with PTH.

Coronary Flow Reserve after Parathyroidectomy

All 27 PHPT patients with pre-operative CFR ≤2.5 showed a complete CFR normalization (CFR 2.1±0.5 vs 3.3±0.7, increase 1.21±0.6, p<0.0001) 6 months after parathyroidectomy (**Figure 2**). After parathyroidectomy, PTH and serum calcium levels decreased (26.4 [16-37] vs 5.7 [4.4-7.4] pmol/L, p<0.0001 for PTH and 2.9 ± 0.1 vs 2.1 ± 0.1 mmol/L, p<0.001, for calcium). Four previously hypertensive PHPT patients, did not longer required antihypertensive drugs after parathyroidectomy. No other significant variations (new medications, evidence of cardiac, renal or cerebrovascular damage, change in diet, life-style, smoking, physical exercise or body weight) occurred in PHPT patients after parathyroidectomy.

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Discussion

Our study demonstrates that asymptomatic PHPT of recent onset is associated with coronary microvascular dysfunction in patients without CAD. Indeed, early after short term successful parathyroidectomy, we observed a complete normalization of this impairment, suggesting a novel role of PTH in the pathophysiology of cardiovascular disease.

Chronically increased PTH levels have long been associated with higher morbidity, all-cause and cardiovascular mortality in PHPT[2], but also in secondary hyperparathyroidism (SHPT)[7, 17], in which PTH is secreted by parathyroid glands, seeking to restore the dyshomeostasis of essential cations (i.e. hypocalcemia and hypomagnesemia), in conditions like chronic renal failure[18], low renin hypertension[19]_ENREF_32, primary aldosteronism (PA)[20], heart failure[10, 21, 22] and vitamin D deficiency[7, 17, 23]. The latter is particularly common among the female and the elderly, where lactose intolerance and reduced dietary Ca^{2+} may be also contributory[23]. Patients having symptomatic PHPT or SHPT are at increased cardiovascular risk for long time after successful parathyroidectomy. This evidence implicates the presence of an irreversible cardiovascular damage[2], which was initially attributed to an "uremic-like" toxicity exerted by chronic PTH elevations intertwining with multiple cardiovascular risk factors, reported at higher prevalence in PHPT and SHPT patients.[1, 17]. Nowadays in western countries, PTH alterations are diagnosed in an early phase when overt bone, renal and cardiovascular complications have not developed yet. Nevertheless, as recently shown, mild alterations of circulating PTH, at the upper levels of the normal range, are already associated with a higher incidence of cardiovascular events in individuals without any other sign of disturbed mineral metabolism.[5] Moreover, in patients referred to coronary angiography[24], PTH levels have been associated with all-cause and cardiovascular mortality, after adjustments for common

cardiovascular risk factors and parameters of mineral metabolism. In patients with stable CAD followed over 8 years, baseline PTH levels were an independent prognostic factor for secondary cardiovascular event incidence and all-cause mortality.[25]

As a consequence, the hypothesis of specific cardiovascular actions of PTH, extending beyond the control of bone and mineral metabolism, has evolved over time and is supported by multiple lines of evidence[1, 5, 17, 22]. Interestingly, in different tissues PTH may exert an ionophoric effect causing intracellular Ca^{2+} overload and reactive oxygen species (ROS) generation[18, 22, 26]. Indeed, impaired antioxidant defenses due to low intracellular zinc (Zn^{2+}) are often coupled to intracellular Ca^{2+} overload and further worsen the redox imbalance leading to severe cell injury and death[26].

In particular, PTH-mediated intracellular Ca^{2+} accumulation coupled to induction of excessive oxidative stress in cardiomyocytes and their mithochondria, has been linked to cells death and subsequent myocardial tissue repair. In the long term, progressive parenchymal loss and reparative fibrosis compromise cardiac function and cause heart failure. Moreover, Ca^{2+} overload has been related to the electrical remodeling and arrhythmogenic potential characterizing cardiomyocytes in heart failure[22]

The complexity of PTH functions is further highlighted by data indicating the existence of a bidirectional link between PTH and renin-angiotensin-aldosterone-system (RAAS), which may be relevant for the regulation of calcium metabolism and in the pathogenesis of cardiovascular disease[22, 27]. In particular, PTH can stimulate directly aldosterone secretion from the adrenals[27] as well as indirectly by modulating angiotensin-II signaling [28]. Increased aldosterone levels are a well-known mediator in the pathogenesis of cardiovascular disease (i.e. higher risk of left ventricular hypertrophy and sudden cardiac death) and atherosclerosis, due to

pro-inflammatory, pro-thrombotic, and pro-fibrotic effects. [29]

On the other hand, in primary or secondary aldosteronism[22] the appearance of ionized hypocalcemia and hypomagnesemia, secondary to cations excretory loss causes SHPT and bone resorption. Adrenalectomy and blockade of the mineralocorticoid receptor normalize PTH profile in PA patients[20]. Interestingly, in a model of secondary aldosteronism developing SHPT[30], PTH-mediated intracellular Ca^{2+} overload and oxidative stress have been reported to promote an inflammatory phenotype in immune cells infiltrating coronary vessels, suggesting that PTH-mediated Ca^{2+} overload and oxidative stress have a permissive role in the cardiovascular remodeling mediated by aldosterone. Also in patients with PHPT aldosterone levels are elevated and correlate positively with PTH values[31], likely explaining the development of arterial hypertension and cardiovascular damage in patients with PHPT.

In line with other reports[1, 17, 22], suggesting that PTH may influence the vascular system, namely endothelial and vascular smooth muscle cells (VSMC)[8] and may cause arterial stiffening[14] and endothelial dysfunction[11, 12], our findings points to the coronary vasculature as a novel putative PTH target. Data available on PTH effects on the vessel wall are controversial. Whereas acute PTH administration seems to have a vasorelaxant effect[8, 32], its chronic elevation accompanied by excessive ROS generation might contribute to VSMC contraction[33] and to the impairment of the endothelial vasoprotective properties, thus promoting atherosclerosis [34, 35]. Along this line, in PHPT patients[12] reduced nitric oxide (NO) bioavailability has been associated to peripheral endothelial dysfunction reversible after parathyroidectomy. Although molecular mechanisms regulating coronary function in PHPT are unknown, it is conceivable to hypothesize that the Ca^{2+}-overloading paradigm could play an important role to explain our findings in the coronary microvasculature. Accordingly, PTH-mediated intracellular Ca^{2+} overloading occurring

in the coronary endothelial cells would result in the induction of oxidative stress which is known to impair NO bioavailability. NO is not only a major vasodilatory molecule, but also the principal physiological antiatherosclerotic mediator, due to its anti-inflammatory, anti-proliferative, immune-modulatory properties[36].

Thus, compromised NO bioavailability might be a major determinant of the impaired coronary microvascular dysfunction we observed and, on a broader perspective, a crucial factor explaining the higher risk for cardiovascular morbidity associated to PTH disturbances. In particular, PTH controls expression and activity of endothelial nitric oxide synthase (e-NOS) through PKC and PKA pathways[9]. SHPT due to chronic renal insufficiency has been associated to reduced e-NOS expression and NO generation. Impaired NO availability was reversed by parathyroidectomy as well as by calcium channel blockade, suggesting that calcium dyshomeostasis might play a role in the phenomenon[37].

In endothelial cell PTH induces the appearance of calcium channels and modulates the Ca^{2+} signaling pathways, resulting in activation of protein kinase C[38]. Some PKC isoforms, like the PKC-beta family, are known to be activated by oxidative stress and to promote further ROS production with detrimental effect on the vascular wall[39]. Recently, *in vitro,* the mitochondrial Ca^{2+} homeostasis, a determinant of cell survival, has been shown to become dysfunctional in condition of intracellular oxidative stress involving PKC beta and prolyl isomerase 1 (Pin1)[40]. Moreover, in the presence of SHPT due to renal failure, increased PTH expression has been linked to decreased Pin1 activity[41]. Moreover Pin1 pharmacological inhibition as well as genetic deletion has been shown to cause endothelial dysfunction and hypertension due to decreased e-NOS activity and NO production[42]. Thus, it is conceivable to speculate that in a condition of elevated PTH, PKC isoforms activation and/or decreased Pin1 activity might contribute to

endothelial dysfunction due to decreased NO production and increased oxidative stress. To date, however, the involvement of PKC isoforms as well as that of Pin1-signaling has not been investigated in the coronary microvasculature in the presence of PTH disturbances and further studies are awaited.

From a pathophysiological point of view, reduced CFR can result from different alterations combined, such as impaired vasodilation, enhanced vasoconstrictor responsiveness, and/or structural remodeling of the coronary microvasculature. In PHPT an involvement of both endothelial-dependent [9, 43] and independent pathways[44] has been reported. Baseline coronary flow was higher in patients with CFR ≤ 2.5 compared with patients with CFR > 2.5, which is consistent with a resting microvascular vasorelaxation and could alone account for the lower CFR. This result could be in correlation with a PTH mediated endothelium-independent vasorelaxing effects, on VSMC[8, 44]. Interestingly, in some patients we observed a reduced CFR due to abnormal flow velocity increase during adenosine. Although CFR assessment by adenosine does not allow us to dissect between endothelial-dependent and/or independent abnormalities[45], this functional alteration can be recognized as the earliest detectable impairment in the process leading to the coronary microvasculopathy. We could not exclude the presence of structural abnormalities affecting the microcirculation (e.g. arteriolar remodeling and calcification) by means of currently available imaging techniques. However, the CFR normalization after parathyroidectomy seems to rule out this possibility, at least in our patient cohort in which vascular calcifications are unlikely, because the disease was mild (serum total calcium was >3 mmol/L in only 12% of patients) and in the early stage (median time from diagnosis 6 months).

A recent evaluation of myocardial perfusion conducted by G-SPECT has demonstrated

that CFR is significantly reduced in PHPT non-CAD patients compared with control subjects[46], depending on disease duration. On the contrary, in our population PTH, age and heart rate were independently associated with CFR and not with disease duration. This difference might be explained by the very short median time of PHPT duration of our patients, reflecting an early phase in the development of PHPT-cardiovascular abnormalities. However, since the relation between CFR and disease duration has been described[46], we adjusted the multivariable models for this variable too. Furthermore, no follow-up data were available in Marini et al. study. On the contrary, CFR evaluation by means of a non-invasive technique such as TDE provided us a simple, objective, rapid diagnostic tool to detect coronary microvascular dysfunction and to follow it over time. Interestingly, CFR evaluation, performed 6 months after parathyroidectomy, allowed us to observe a complete recovery of coronary microvascular function.

Study Limitations

Firstly, our study is cross-sectional and conclusions about causal and temporal order between PTH and CFR cannot be drawn. Secondly, sample variability in PTH and serum calcium assays may increase random error. However, all assays used in this study have acceptable levels of precision. Nevertheless, it is possible that the lack of significant results for serum calcium is in part due to random error. Thirdly, it cannot be also excluded that disturbances in the RAAS, which are known to be associated with PHPT, may contribute to structural and functional alterations of the microvascular wall.[31, 47] Fourth, despite the relatively large cohort of patients, the low number of patients with abnormal CFR limits statistical power and creates the risk of over-fitting the models when adding covariates. However, in this study, the addition of covariates, in any case, has influenced the relationship between PTH and CFR. Finally, none of the control subjects underwent MSCT or coronary angiography. We think that it would be

unethical to submit asymptomatic subjects with normal CFR to invasive diagnostic examinations. Similarly, patients with normal CFR did not undergo MSCT for ethical reasons.

Conclusion

Our findings unmask a specific role of PTH excess in early coronary microvascular dysfunction and show that parathyroidectomy performed shortly after PHPT diagnosis is able to completely restore this impairment. In our study, PTH, *per se*, correlates with CFR, independently from established cardiovascular risk factors and other determinants of mineral metabolism. Our data can help to explain why PHPT is associated to increased cardiovascular mortality and risk although molecular mechanisms involved need to be further investigated.

Conflict of Interest Disclosures: None.

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Table 1. Characteristics of Study Population (n=150)

	Controls (n=50)	PHPT (n=100)	p
Age, years	59 ± 12	58 ± 12	0.6
Male gender, n (%)	10 (20)	20 (20)	0.8
PTH, pmol/L	2.0 (0.8-2.4)	20 (15-29)	<0.0001
Serum total calcium, mmol/L	2.3 ± 0.1	2.95 ± 0.1	<0.0001
Cardiovascular risk factors			
Current smokers, n (%)	6 (12)	12 (12)	0.8
Hypertension, n (%)	22 (44)	45 (45)	0.7
Diabetes, n (%)	5 (10)	10 (10)	0.8
Obesity, n (%)	8 (16)	12 (12)	0.5
Hyperlipidemia, n (%)	11 (22)	23 (23)	0.7
Echocardiographic characteristics			
IVS_d, mm	9.0 ± 2.2	8.4 ± 1.5	0.2
PWT_d, mm	8.4 ± 1.4	8.1 ± 1.4	0.5
$LVID_d$, mm	53 ± 4.9	51.6 ± 5.9	0.4
$LVID_s$, mm	32.6 ± 6.4	32.4 ± 7	0.9
LV mass, g	170 ± 53	158 ± 49	0.2
LV mass index, g/m^2	102 ± 26	100 ± 26	0.4
LV mass/$height^{2.7}$, $g/m^{2.7}$	46 ± 13	44 ± 13	0.3
Relative WT	0.32 ± 0.05	0.32 ± 0.09	0.9
LV hypertrophy, LVMI, n (%)	17 (34)	32 (32)	0.8
LV hypertrophy, LVMH, n (%)	22 (44)	40 (40)	0.9
LVEF, %	65 ± 4	66 ± 3	0.7
E-wave, cm/s	80.1 ± 14.1	78.1 ± 12.2	0.7
A-wave, cm/s	60.2 ± 10.2	59.1 ± 14.1	0.8
E/A	1.38 + 0.21	1.35 + 0.42	0.8
DT, ms	192 ± 15	197 ± 17	0.5
IVRT, ms	70 ± 5	73 ± 4	0.6
PV_s/PV_d	1.20 ± 0.18	1.23 ± 0.21	0.6
E/E' Septal	7.41 ± 1.42	7.61 ± 1.71	0.7
E/E' Lateral	6.51 ± 1.34	6.38 ± 1.72	0.5
Grade of diastolic dysfunction			0.8
None, n (%)	23 (46)	43 (43)	
Mild, n (%)	20 (40)	43 (43)	
Moderate, n (%)	7 (14)	13 (13)	
Severe, n (%)	0 (0)	1 (1)	

Unless specified otherwise, the values are means ± SD or median (Q1, Q3).
A-wave = flow velocity during atrial contraction; BMI=Body mass index; CFR= coronary flow reserve; DT = deceleration time; E-wave = early transmitral diastolic flow velocity; E/E' = a ratio of early transmitral diastolic flow velocity (E) and early diastolic velocity recorded by Doppler tissue imaging (E') in the mitral annulus; IVRT = isovolumetric relaxation time; IVSd = diastolic interventricular septal thickness; LV = left ventricular; LVEF = left ventricular ejection fraction; $LVID_d$ = left ventricular internal diameter in diastole; $LVID_s$ = left ventricular internal diameter in systole; LVMI = left ventricular mass index; LVMH = left ventricular mass/ $height^{2.7}$; PTH=parathyroid hormone; PV_d = diastolic pulmonary vein velocity; PV_s = systolic pulmonary vein velocity; PWTd = diastolic posterior wall thickness; WT = wall thickness.

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Table 2. Comparison Between Patients with Microvascular Dysfunction (CFR≤2.5) and Patients with Normal Coronary Flow Reserve (n=100)

	CFR≤2.5 (n=27)	CFR>2.5 (n=73)	p
Age, years	63 ± 12	57 ± 12	0.03
Male gender, n (%)	5 (18)	15 (20)	0.8
Time from diagnosis, months	8 (4-25)	8 (4-21)	0.7
PTH, pmol/L	26.4 (16-37)	18 (13-25)	<0.007
Serum total calcium, mmol/L	2.9 ± 0.1	2.8 ± 0.3	0.1
Cardiovascular risk factors			
Current smokers, n (%)	3 (11)	9 (12)	0.8
Hypertension, n (%)	17 (63)	28 (38)	0.02
Diabetes, n (%)	3 (11)	7 (9.5)	0.8
Obesity, n (%)	4 (15)	8 (11)	0.6
Hyperlipidemia, n (%)	9 (33)	14 (19)	0.1
Echocardiographic characteristics			
IVS_d, mm	8.6 ± 2	8.3 ± 1.2	0.5
PWT_d, mm	8.3 ± 1.3	8.1 ± 1.1	0.6
$LVID_d$, mm	52 ± 4.1	51 ± 5	0.7
$LVID_s$, mm	33.4 ± 6	31.3 ± 5	0.4
LV mass, g	160 ± 51	156 ± 47	0.3
LV mass index, g/m^2	100 ± 22	99 ± 19	0.4
LV mass/$height^{2.7}$, $g/m^{2.7}$	46 ± 11	44 ± 12	0.4
Relative WT	0.32 ± 0.04	0.31 ± 0.07	0.8
LV hypertrophy, LVMI, n (%)	9 (33)	23 (31)	0.3
LV hypertrophy, LVMH, n (%)	13 (48)	27 (37)	0.5
LVEF, %	63 ± 2	65 ± 5	0.8
E-wave, cm/s	77.2 ± 13.1	79.1 ± 11.2	0.6
A-wave, cm/s	62.1 ± 10.1	58.1 ± 11.1	0.3
E/A	1.32 ± 0.21	1.37 ± 0.32	0.3
DT, ms	199 ± 13	195 ± 16	0.4
IVRT, ms	72 ± 5	75 ± 5	0.4
PV_s/PV_d	1.25 ± 0.16	1.22 ± 0.18	0.5
E/E' Septal	7.81 ± 1.32	7.53 ± 1.61	0.5
E/E' Lateral	6.41 ± 1.21	6.39 ± 1.51	0.4
Grade of diastolic dysfunction			0.8
None, n (%)	12 (44)	31 (42)	
Mild, n (%)	12 (44)	31 (42)	
Moderate, n (%)	3 (12)	10 (14)	
Severe, n (%)	0	1 (2)	

Unless specified otherwise, the values are means ± SD or median (Q1, Q3). Abbreviations as in Table 1

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Table 3. Unadjusted and Multivariable Linear Regression Analysis Between Coronary Flow Reserve (Dependent Variable) and Risk Factors or Clinical Conditions (Independent Variables) (n=100)

	Unadjusted Analysis	Multivariable Analysis	
	p	β	p
Age	0.01	-0.232	0.04
Male gender	0.5	-0.127	0.2
Time from diagnosis	0.8	0.046	0.6
PTH	0.004	-0.242	0.01
Serum total calcium	0.2	0.034	0.7
Current smokers	0.1	-0.174	0.07
Hypertension	0.09	-0.049	0.6
Diabetes	0.6	0.167	0.1
Obesity	0.4	0.02	0.8
Hyperlipidemia	0.1	-0.126	0.2
HR at time of the CFR measurement	0.007	-0.265	0.006
SBP at time of the CFR measurement	0.02	-0.052	0.6
LVEF	0.3	-0.078	0.4
Diastolic dysfunction	0.7	0.077	0.4

R^2 of the multivariable model = 0.545

CFR = coronary flow reserve; HR = heart rate; SBP = systolic blood pressure; other abbreviations as in Table 1

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Table 4. Factors Independently Associated With Reduced Coronary Flow Reserve

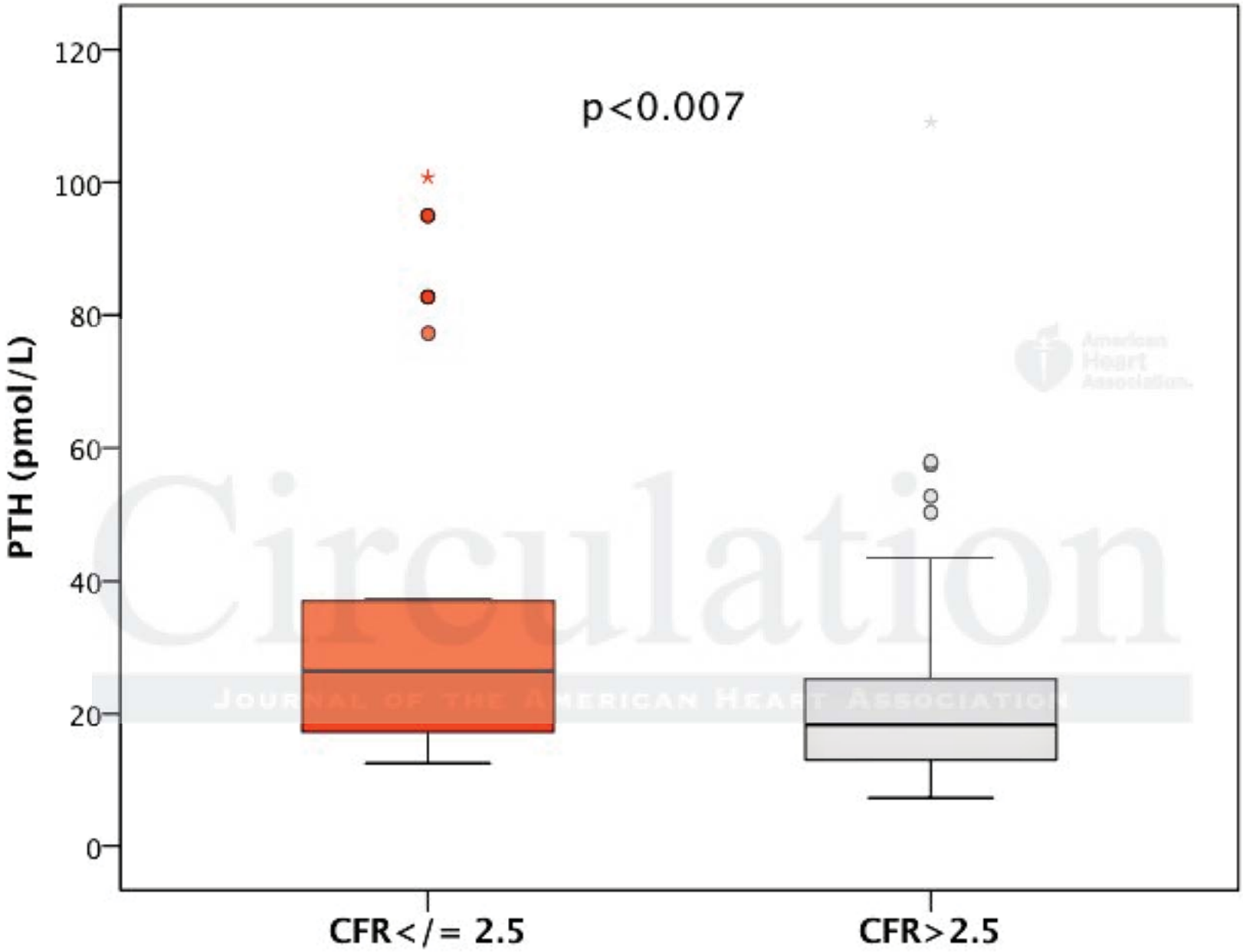
	OR	95% CI	p
1. Model obtained by stepwise regression (c-statistic = 0.79; R^2 = 0.31)			
PTH	3.03	1.56-5.9	0.01
Basal heart rate	1.08	1.02-1.13	0.02
2. Model obtained when not entering basal heart rate (c-statistic = 0.75; R^2 = 0.28)			
PTH	2.71	1.50-5.10	0.03
3. Model obtained including also variables marginally significant (c-statistic = 0.80; R^2 = 0.34)			
PTH	3.01	1.51-6.04	0.01
Basal heart rate	1.08	1.02-1.14	0.02
4. Model obtained forcing other variables (c-statistic = 0.81; R^2 = 0.37)			
PTH	3.00	1.51-5.90	0.01
Basal heart rate	1.08	1.02-1.15	0.03

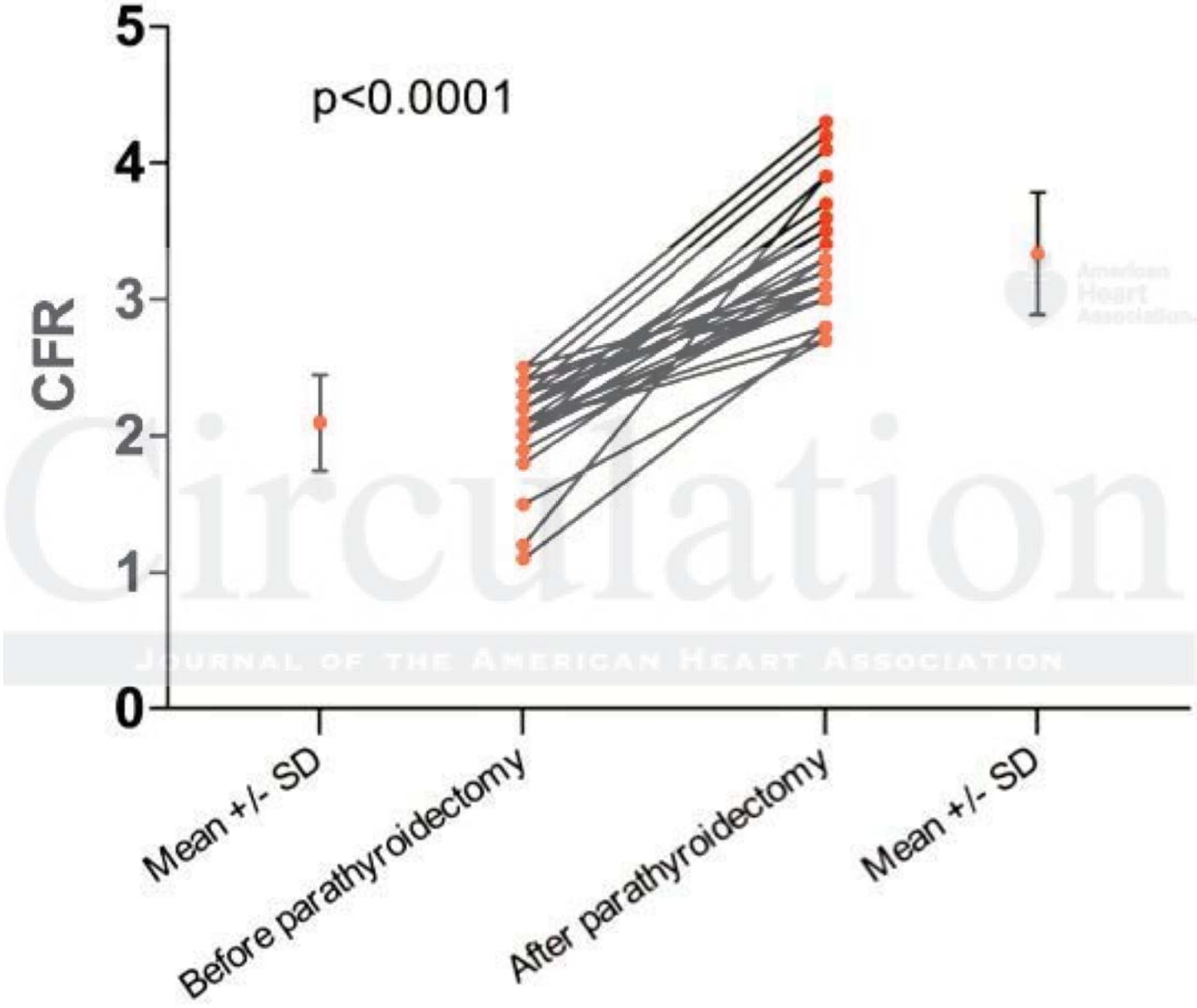
The following variables were considered by the stepwise procedure, but not included in the final models because they did not meet the criteria to enter or to stay in the model: age, history of hypertension or hyperlipidemia, systolic blood pressure, obesity, diastolic dysfunction. The following variables were forced into the model one at a time, but their inclusion did not affect the relationship between CFR≤2.5 and PTH: gender, time from diagnosis, current smoking, history of diabetes, use of statins or nitrates. Abbreviations as in Tables 1 and 2.

Figure Legends:

Figure 1: Box plot of PTH in patients with normal and abnormal CFR. Each box plot displays the smallest value (lowest point on vertical whisker), 25th percentile (bottom of box), median (horizontal line in box), 75th percentile (top of box) and largest values (highest point on vertical wisker).

Figure 2: Plot of individual changes in CFR before and after parathyroidectomy. All 27 PHPT patients with pre-operative CFR ≤2.5, evaluated 6 months after parathyroidectomy showed a complete CFR normalization. Error bars reflect standard deviation.





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SUPPLEMENTAL METHODS

Risk factor definition

Diabetes mellitus was diagnosed when patients were taking hypoglycemic medications or with fasting glycemia >126 mg/dl (7 mmol/L) in two consecutive determinations. Hyperlipidemia was defined as fasting total serum cholesterol >220 mg/dl (5.7 mmol/L) and/or serum triglycerides >1.56 mg/dl (1.8 mmol/L), or when patients were taking an oral lipid-lowering agent. We defined as hypertensive those patients taking antihypertensive drugs or showing a systolic blood pressure ≥ 140 mmHg and/or a diastolic blood pressure ≥ 90 mmHg (average of two or more readings taken in the sitting position in different days). Secondary hypertension was excluded on the basis of standard biochemical, hormonal, and instrumental tests. A BMI ≥30 Kg/m^2 was considered as an index of obesity. Patients smoking at least one cigarette daily for 1 year within the last 5 years were considered smokers.

Echocardiography

From two-dimensional guided M-mode echocardiograms, left ventricular (LV) dimensions were measured by American Society of Echocardiography (ASE) convention; LV mass was calculated by the adjusted ASE method[1] and indexed for body surface area or height. LV mass/body surface area ≤116 g/m^2 in men and ≤104 g/m^2 in women was considered normal. LV mass/ $height^{2.7}$ was considered normal if ≤49.2 $g/m^{2.7}$ in men and ≤46.7 $g/m^{2.7}$ in women. Valvular disease was classified significant if moderate-severe stenosis and/or insufficiency was detected by TDE. None of the patients suffered from significant valvular disease. In each subject, ejection fraction was measured and diastolic dysfunction was defined according to the ASE criteria[2]. These criteria integrate Doppler measurements of the mitral inflow and Doppler tissue imaging of the mitral annulus. This approach is the standard practice in our laboratory and enabled us to classify diastolic function in 4 categories: normal diastolic function, mild diastolic dysfunction (impaired relaxation without evidence of increased filling pressures), moderate diastolic dysfunction (impaired

relaxation or pseudo-normal with moderate elevation of filling pressures), and severe diastolic dysfunction (advanced reduction in compliance).[3]

MSCT protocol

To optimize imaging, participants without contraindications received β-blockade with oral and/or intravenous metoprolol to slow the heart rate to 60-70 beats/min. An optimized dose modulation approach using helical acquisition and reduced voltage was used to decrease radiation exposure. Coronary MSCT was read by consensus of 2 cardiologists and radiologists. Coronary MSCT data sets were evaluated for the presence of significant coronary artery stenosis within the left main coronary artery; proximal, mid, and distal segments of the LAD coronary artery; first and second diagonal branches; proximal, mid, and distal segments of the left circumflex coronary artery; first and second marginal branches; proximal, mid, and distal segments of the right coronary artery; and the posterior descending artery according to the 15-segment American Heart Association classification[4]. The coronary artery calcium score was assessed with dedicated software (Syngo Ca Score-Siemens Medical Solution). The total calcium burden in the coronary arteries was quantified as previously described[4].

SUPPLEMENTAL RESULTS

Correlation between CFR, Clinical and Biochemical Characteristics

Bivariate correlation analysis revealed significant and inverse correlations between CFR and log PTH ($r=-0.3$, $p<0.004$), age ($r=-0.24$, $p=0.01$), heart rate ($r=-0.26$, $p<0.007$) and systolic blood pressure ($r=-0.21$, $p=0.02$), whereas no correlation with serum calcium levels has been observed ($p=0.3$).

Intra- and Inter-Observer Reproducibility of CFR by Transthoracic Echocardiography

Intra-observer and inter-observer reproducibility of CFR measurements were assessed by repeating CFR evaluation twice, 1 h apart, by the same operator (F.T.) in all patients and by another operator (E.O.) in all patients as well. The intra-observer reproducibility was high ($r=0.92$, SEE=0.10); ICC was 0.969. The inter-observer reproducibility was also high ($r=0.90$, SEE=0.11); ICC was 0.961.

Supplemental Table: Hemodynamic Parameters during CFR Evaluation in the Study Population

	Controls (n=50)	PHPT (n=100)	p
Basal heart rate, beats/min	75 (67-82)	74 (65-82)	0.8
Adenosine heart rate, beats/min	96 (86-110)	95 (83-106)	0.7
Basal systolic blood pressure, mmHg	120 (110-127)	130 (120-140)	0.2
Adenosine systolic blood pressure, mmHg	100 (95-115)	110 (100-123)	0.2
Basal diastolic blood pressure, mmHg	80 (67-83)	80 (70-83)	0.8
Adenosine diastolic blood pressure, mmHg	60 (60-70)	70 (60-80)	0.4
Basal peak diastolic velocity, cm/s	22 (18-28)	23 (19-26)	0.4
Adenosine peak diastolic velocity, cm/s	84 (64-97)	71 (55-81)	<0.0001
Coronary flow velocity reserve	3.8 ± 0.7	3.0 ± 0.8	<0.0001

Values are reported as mean ± standard deviation or median (Q1, Q3)

SUPPLEMENTAL REFERENCES

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