Vitamin D, parathyroid hormone and cardiovascular risk: the good, the bad and the ugly
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Background
Vitamin D (VitD) and parathyroid hormone (PTH) represent pillars in the homeostasis of calcium and bone metabolism, through their reciprocal regulation. PTH enhances the tubular reabsorption of calcium and phosphorus by increasing the efficiency of intestinal calcium absorption by about 80%, reducing phosphorus absorption by about 30–40% and phosphorus by increasing the efficiency of intestinal calcium absorption by about 80%, reducing phosphorus absorption by about 30–40%,

25-Hydroxyvitamin D insufficiency and increased cardiovascular risk (CVR) association is still debated. The vitamin D (VitD)-dependent parathyroid hormone (PTH) is considered as the possible actuator of VitD effects on CVR. To investigate the association of CVR, PTH and VitD, we carried out blood pressure measurements and blood samples and collected information on dietary habits, anamnestic, clinical and metabolic data of 451 participants in the Salerno area (Southern Italy) during the World Hypertension Day (17 May). CVR was calculated according to the Framingham CVR charts. The overall population mean age was 51.6 ± 0.7 years, and female sex was slightly prevalent (55%). VitD deficiency (<20 ng/ml) was most frequent (59.7%). In this population, VitD and CVR did not correlate. VitD and PTH inversely correlated ($r = -0.265, P < 0.001$) as expected. PTH was in direct correlation ($r = 0.225, P < 0.001$) with CVR. Elevated PTH (75 percentile; ≥49.5 pg/ml) levels identify a population with higher CVR (11.8 ± 0.5 vs. 8.5 ± 0.3, $P < 0.001$). In a multivariate analysis, both age and PTH correlate to CVR, but not VitD. In conclusion, VitD does not directly affect CVR in the overall population. Rather, increased PTH might be a better predictor of CVR.

Keywords: 25-hydroxyvitamin D cholecalciferol, aging, blood pressure, cardiovascular events, cardiovascular risk

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Methods
Study design and population
This is an observational study conducted on the general population in six Southern Italy villages: Castelnuovo Cilento, Salerno, Polla, Sarno, San Gregorio Magno and Satriano di Lucania. A total of 808 participants (45% men and 55% women, 14–85 years) were recruited during the XI, XII and XIII World Hypertension Day that occurs every year on 17 May. The study was approved by the relevant institutional Ethical Committee of Salerno University. Written informed consent was obtained from all participants. The study is registered in the ClinicalTrials.gov database (NCT03305276).

Data collection
On the occasion of the World Hypertension Day 2015–2017, booths were organized in the major squares of the mentioned villages, and volunteers of the Medical School of Salerno collected through means of questionnaires the anamnesis and dietary habits. They also measured blood pressure (BP) after 5 min in the sitting position, three times with an interval of 2 min using validated, European Society of Hypertension (ESH) approved electronic oscillometers (A100, Microlife, Padua, Italy), according to the European Society of Cardiology/ESH Guidelines. Anamnestic information also included questions on previous cardiovascular conditions or events (coronary heart disease) and cerebrovascular accidents (transient ischemic accidents and stroke). Ongoing therapy and VitD supplementation were also annotated. A venous blood sample was drawn from the antecubital vein, and blood was collected for biochemical analysis at the University Hospital Centralized Service. Data were digitally stored for analysis.

Anamnesis and biochemical data regarding age, sex-specific cholesterol, HDL cholesterol, SBP, cigarette smoking, preexisting conditions and lifestyles were used for the calculation of CVR according to the Framingham Cardiovascular Risk Score. Anamnestic information also included questions on previous cardiovascular conditions or events (coronary heart disease) and cerebrovascular accidents (transient ischemic accidents and stroke). Ongoing therapy and VitD supplementation were also annotated. A venous blood sample was drawn from the antecubital vein, and blood was collected for biochemical analysis at the University Hospital Centralized Service. Data were digitally stored for analysis.

Laboratory assessment of blood samples
A venous blood sample was collected in two tubes of 5.0 ml and centrifuged the same day. The time of the last meal was recorded during the data collection. Blood glucose, insulin, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, VitD and PTH were assessed. Glomerular filtration rate was estimated (eGFR) using the equation of Chronic Kidney Disease Epidemiology Collaboration. Familiarity for cardiovascular disease was defined as cardiovascular events in parents and siblings less than 50 years old.

Grouping and statistical analysis
For statistical analysis, out of 808 volunteers, we selected participants aged 20–80 years old, as Framingham risk score can only be calculated up to 80 years old. Availability and quality data check was performed. Furthermore, we used the statistical identification of outliers, and data above or below 3 SD were excluded for VitD, PTH and eGFR (Fig. 1). In the end, the analysis was performed on 451 participants. Participants were divided into three age groups (20–40, 41–60 and 61–80 years old) for selected statistical purposes, as indicated. We used the lowest quartile of VitD and the highest quartile of PTH as the cutoff for the identification of low-VitD (<9 ng/ml) or high-PTH (>49.5 pg/ml) populations.

Frequencies are reported as a percentage (%), whereas continuous variables are presented as a mean ± standard error. The Student t test was used to compare continuous normally distributed variables, after calculation of the equality of variances with test of Levene. The Bonferroni method, for the analysis of contrasts in average, was used in the analysis of the variance in presence of more than two populations to compare. The bivariate correlation was made to evaluate the association between the variables of interest. Linear regression was used for the estimation of the expected value of a dependent variable (i.e. PTH and CVR) to fixed factors and covariates (age groups, VitD). The interaction between fixed factors and covariates was also assessed. All data were analyzed by G.I. and R.G. using SPSS IBM Corporation, United States of America version 23.0. The significance was set for values of P less than 0.05.

Results
Baseline characteristics of overall population
Overall population features are shown in Table 1. The prevalence of CVR factors is in line with the European...

Fig. 1
Flow chart for data selection

Flowchart describing the selection process leading to the size of population included in the analysis.
estimates: 47.6% of hypertension, 7% of diabetes and 32.8% of smokers. In the general population, SBP and age have a positive correlation (Pearson correlation \( r = 0.528, P < 0.001 \)), as expected. A significant share of the population shows low levels of VitD, and the 59.7% of participants have serum levels of VitD below 20.0 ng/ml. In particular, 19.1% has deficiency (below 10.0 ng/ml) and 40.6% has insufficiency (between 10.1 and 20.0 ng/ml). Within the deficient group, women are predominant with a percentage of 66.3%.

**Vitamin D and cardiovascular risk**

As expected,\(^2\) VitD and PTH inversely correlate (Fig. 2). No effect of aging was observed on VitD (data not shown), whereas PTH increases along groups of age (\( R^2 = 0.061, P < 0.001 \)). The relationship between VitD and PTH, though, remains significant at each age group (20–40, 40–60 and 60–80 years, Fig. 2). No effects of age on the relationship between VitD and PTH were observed in a multivariate analysis that included PTH as the dependent variable, and groups of age as a fixed factor and VitD as a covariate (VitD: \( F = 35.931, P < 0.001 \); age groups: \( F = 17.517, P < 0.001 \); VitD \& age groups: \( F = 1.232, P = 0.293 \)).

We then assessed the potential relationship between VitD and CVR. In our population, no linear regression was described between the two variables (\( B = 0.043; F = 0.818 \), n.s.). Similarly, low-VitD participants presented the same Framingham Risk Score as the control group (5.5 ± 0.38 vs. 4.9 ± 0.6, respectively, n.s.).

**Parathyroid hormone and cardiovascular risk**

We then assessed the effect of PTH on CVR. Given the association between age and PTH, it is possible to speculate that PTH serum levels might be a better predictor of CVR. Indeed, in the Framingham CVR calculation, age represents an important determinant. First, we divided the population according to high and low PTH levels in serum, using the above described 75 percentile of serum PTH as a cutoff (49.5 ng/ml). High-PTH participants show higher CVR (7.47 \( \pm 0.7 \) vs. 4.6 \( \pm 0.35 \); \( P < 0.001 \)). Furthermore, PTH and CVR are in linear regression (\( R = 0.034; \) beta = 0.184; \( F = 15.553; P < 0.001 \)). Given the interaction between PTH and age, to identify the independent role of PTH, we tested the PTH effect on CVR in a model including also age groups (multivariate analysis). Although, as expected, CVR increases with age (\( F = 124.85, P < 0.001 \)), also PTH effect on CVR is statistically significant (\( F = 24.231, P < 0.001 \)) and independent from age (PTH \& age: \( F = 0.169, \) n.s.).

**Focus on people with age between 41 and 60 years**

We hypothesized that if the driving mechanism of PTH on CVR is older age, this would limit the significance of our results, given the obvious impact of age on CVR. Therefore, to attenuate the effect of aging on the predictive role of PTH on CVR, we decided to compare participants with normal and elevated PTH in an age range that does not affect significantly CVR, that is in the group ranging from 41 to 60 years. At this age, high PTH
levels identify participants with double CVR (6.35 ± 1.27 vs. 3.75 ± 0.37, P < 0.001). Apparently, the only determinant of the increased risk is the SBP, all the other parameters being similar between the two groups, including eGFR (Table 2). Confirming what observed in Fig. 2, people with higher PTH also show significantly lower VitD values (Table 2).

Discussion

Our data propose two major advancements of knowledge. First, within the general population, VitD levels fail to associate with CVR. Second, PTH, a VitD dependent hormone, is a more reliable predictor of CVR and affects in particular SBP.

Although increasing evidence accredits PTH as a relevant player in CVR, the underlying mechanism is still unclear: a possible explanation relates to the interference with other classical CVR factors. In our population, indeed, we show that PTH increases with age, weight, BMI, SBP, LDL and BUN. In particular, our paper shows for the first time the close relationship between age and PTH. Consequently, also the normality ranges for PTH should consider aging. In our study, we identify 49.5 pg/ml as the upper limit. Using this cutoff, we can draw two major results. First, the group with at least 49.5 pg/ml of PTH presents higher CVR. The inverse correlation between PTH and VitD appears preserved, being the VitD values significantly lower in the group with elevated PTH. In this population, though, the increased CVR can be attributed to aging, which can also be considered as the cause of increased PTH. In particular, age-dependent contraction of the kidney function expressed as the reduction of eGFR and increasing of BUN values can cause altered homeostasis of calcium/phosphate and consequent secondary hyperparathyroidism. To exclude an effect of per se on the increased CRV, we limited our analysis to the population comprised between 41 and 60 years old. In this group, PTH is not influenced by kidney function, which remains similar between high and low PTH groups (Table 2). Moreover, the relationship with VitD is preserved. PTH still predicts the CVR in this subgroup. To finally identify the independent effect of PTH on CVR, we run a multivariable linear modeling, including parameters of aging and PTH levels to correlate significantly and independently with CVR.

The second result is that in a younger and homogenous population, the association between elevated PTH and doubled CVR can be explained by the increased BP, as all other parameters (age, sex, smoking, diabetes, total cholesterol and HDL cholesterol) remain similar between high and low PTH groups. In this group, lower VitD is the only determinant of PTH levels, and therefore its deficiency can lead to increased BP values by increasing PTH.

The use of 49.5 pg/ml as a cutoff for PTH confirms the need to have reference values for each age group for this parameter. We propose that different ranges should be considered for different age groups. Indeed, excluding the elderly and kidney failure, at the age 41–60 years, participants with elevated PTH show significantly higher values of BP, glucose and LDL.

The molecular mechanism underlying the increased BP has not been elucidated. A number of pieces of evidence suggest that PTH has vascular effects. Endothelial dysfunction is one mechanism thought to link PTH to vascular changes: PTH, indeed, may increase serum levels of endothelin-1 and IL-6. Furthermore, PTH may stimulate the vascular smooth muscle cells to produce factors including collagen and beta-1 integrin which could, in turn, remodel the peripheral vasculature. PTH may increase renin release and activate the renin–angiotensin system, a complex process mediated by serum calcium, renal 1-alpha hydroxylase and resultant changes in 1,25(OH)2D.

Our study does not allow the identification of the mechanisms underlying the increased BP associated with increased PTH; hence this issue deserves further specific investigation.

Perspectives

Although in the general population VitD fails to predict CVR, PTH represents a more reliable and promising
biomarker. Although PTH increases with aging, also in younger populations, the cutoff of 49.5 pg/ml can predict increased CVR. The major determinant is the increase of SBP values. Being the variation of PTH values in this age group almost exclusively linked to VitD deficiency, the supplementation of VitD may become an important stronghold to reduce the CVR through the decrease of serum PTH values.

Limitations

Our study, although performed in a significantly large population, has clear geographical limitations, and these results need to be replicated in larger studies including different geographical areas in Italy and in the world. Moreover, a prospective design is not included in our observation, and therefore, we cannot draw outcome implications from our results.

Novelty and significance

What is New: PTH is a better biomarker than VitD to predict CVR.

What is Relevant: Although PTH increases with aging, also in younger populations, the cutoff of 49.5 pg/ml can predict increased CVR. In this younger population, the major determinant of Framingham CVR is the increased SBP. A descending hypothesis is that VitD supplementation might reduce PTH levels and BP values, normalizing cardiovascular risk.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80 (6 Suppl):1698S–1696S.
2. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357:266–281.
3. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. J Am Coll Nutr 2003; 22:142–146.
4. Zerwelh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr 2008; 87:1097S–1091S.
5. Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006; 81:353–373.
6. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet 1998; 351:805–806.
7. Thomas MK, Lloyd-Jones DM, Thadhani RL, et al. Hypovitaminosis D in medical inpatients. N Engl J Med 1998; 338:777–783.
8. Chapuy MC, Preziosi P, Maamer M, Amadou S, Galan P, Hercberg S, Meunier PJ. Prevalence of vitamin D insufficiency in an adult normal population. Osteoporos Int 1997; 7:439–443.
9. Holick MF, Sinis ES, Binkley N, et al. Prevalence of vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab 2005; 90:3215–3224.
10. Michos ED, Melamed ML. Vitamin D and cardiovascular disease risk. Curr Opin Clin Nutr Metab Care 2008; 11:7–12.
11. Li YC, Qiao G, Uskokovic M, Xiang W, Zheng W, Kong J. Vitamin D: a negative endocrine regulator of the renin–angiotensin system and blood pressure. J Steroid Biochem Mol Biol 2004; 89:90–387–392.
12. Ramsos G, Tsepe P, Zialka S. Vitamin D, the renin-angiotensin system, and insulin resistance. Int Urol Nephrol 2008; 40:419–426.
13. Norman AW, Frankel JB, Heldt AM, Grodsky GM. Vitamin D deficiency inhibits pancreatic secretion of insulin. Science 1980; 209:823–825.
14. Carthy EP, Yamashita W, Hsu A, Qiu BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension 1989; 13 (6 Pt 2):954–959.
15. Deluca HF, Cantorna MT. Vitamin D: its role and uses in immunology. FASEB J 2001; 15:2579–2585.
16. Cerit L. Bermuda triangle; heart failure, atrial fibrillation, and vitamin D deficiency. J Cardiovasc Med 2017; 18:121.
17. Cerit L, Kemal H, Gulisen K, Ozcem B, Cerit Z. Duygu H. Relationship between vitamin D and the development of atrial fibrillation after on-pump coronary artery bypass graft surgery. Cardiovasc J Afr 2017; 28:104–107.
18. Gruber KR, Marx W, Plz S, et al. Vitamin-D concentrations, cardiovascular risk and events—a review of epidemiological evidence. Rev Endocr Metab Disord 2017; 18:259–272.
19. Al Mheid I, Gugiyama AA. Vitamin D and cardiovascular disease: controversy unresolved. J Am Coll Cardiol 2017; 70:89–100.
20. Rostand SG, Drueke TB. Parathyroid hormone, vitamin D, and cardiovascular disease in chronic renal failure. Kidney Int 1999; 56:383–392.
21. Andersson P, Rydberg E, Willenheimer R. Primary hyperparathyroidism and heart disease—a review. Eur J Heart 2004; 25:1776–1787.
22. Usdin TB, Bonnir TI, Harta G, Mezev E. Distribution of parathyroid hormone-2 receptor messenger ribonucleic acid in rat. Endocrinology 1996; 137:4285–4297.
23. Mancia G, Fogard R, Barkiewiez K, et al. 2013ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2013; 31:1281–1357.
24. D’Agostino RB Sr, Grundy S, Sullivan LM, Wilson P, CHD Risk Prediction Group. Validation of the Framingham coronary heart disease prediction scores: results of a multiple ethnic groups investigation. JAMA 2001; 286:180–187.
25. Izzo R, de Simone G, Giudici R, Chinati M, Trimarco V, De Luca N, Trimarco B. Effects of nutraceuticals on prevalence of metabolic syndrome and on calculated Framingham Risk Score in individuals with dyslipidemia. J Hypertens 2010; 28:1482–1487.
26. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009; 150:604–612.
27. Hsu CY. CKD-EPI eGFR categories were better than MDRD categories for predicting mortality in a range of populations. Ann Intern Med 2012; 157:JC5–JC12.
28. Gruson D, Perracin B, Atna SA, et al. 1,25-Dihydroxyvitamin D to PTH(1-84) ratios strongly predict cardiovascular death in heart failure. PLoS One 2015; 10:e0135427.
29. Anderson JL, Vanwoerom RC, Horne BD, et al. Parathyroid hormone, vitamin D, renal dysfunction, and cardiovascular disease: dependent or independent risk factors? Am Heart J 2011; 162:331–339 e2.
30. Garcia de la Torre N, Wass JA, Turner HE. Parathyroid hormone has a prosclerotic effect on vascular smooth muscle. J Clin Endocrinol Metab 2013; 98(12 suppl):F1218.
31. Pilz S, Tomaschitz A. Role of vitamin D in arterial hypertension. Expert Rev Cardiovasc Ther 2010; 8:1599–1608.
32. Grant FD, Mandel SJ, Brown EM, Williams GH, Seely EW. Interrelationships between the renin-angiotensin-aldosterone and calcium homeostatic systems. J Clin Endocrinol Metab 1992; 75:988–992.
33. Fitzpatrick LA, Bilezikian JP, Silverberg SJ. Parathyroid hormone and the cardiovascular system. Curr Osteoporos Rep 2008; 6:77–83.
34. Bennerwalde WH. The role of calcium in the regulation of renin secretion. Am J Physiol Renal Physiol 2010; 298:F1–F11.