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ORIGINAL RESEARCH ARTICLE

Plasma calcidiol, calcitriol, and parathyroid hormone and risk of new onset heart failure in a population-based cohort study

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Abstract

Background Heart failure (HF) is a major problem in the Western world, with increasing prevalence and incidence. Because HF cannot be cured, prevention of HF is of utter importance. Calcidiol, calcitriol, and parathyroid hormone (PTH) have been identified as risk factors for cardiovascular disease. However, their association with new onset HF remains to be established. We investigated whether calcidiol, calcitriol, and PTH could be used to identify those subjects at risk for new onset HF, and if they had additive predictive value over established risk predictors like N-terminal-pro Brain-type natriuretic peptide and highly sensitive Troponin-T.

Methods and results We examined 7470 HF-free participants in Prevention of Renal and Vascular End-stage Disease, a community-based cohort study in Groningen, the Netherlands (latitude 53°N, mean age: 49 years, 48% male). During follow-up time of 12.6 years (interquartile range: 12.3–12.9), 281 participants (4%) developed HF: 181 (66%) HF with reduced and 94 (34%) HF with preserved ejection fraction (HFrEF [left ventricular ejection fraction $\leq$40%], and HFpEF [left ventricular ejection fraction $\geq$50%], respectively). Mean (±SD) of calcidiol was 58 (±24) nmol/L, mean calcitriol 145 (±48) pmol/L, and median (interquartile range) PTH was 3.7 (3.0–4.6) pmol/L. Calcidiol levels were univariately associated with new onset HF [hazard ratio (HR) 0.82 (95% CI 0.69–0.96)], but calcitriol levels were not [HR 0.85 (95% CI 0.71–1.03)]. PTH levels kept their predictive value after adjustment for age, sex, and day of blood withdrawal (HR 1.26 [95% CI 1.04–1.53]). However, in our full model this association was lost [HR 1.10 (95% CI 0.92–1.32)]. Calcidiol, calcitriol, and PTH could not differentiate between new onset HFrEF or HFpEF.

Conclusions After adjustment for confounding factors, a single measurement of plasma calcidiol, calcitriol, or PTH was not associated with risk of developing HF. Screening for these markers to identify subjects at risk for new onset HF cannot be advocated.

Keywords Heart failure; Risk factor; Vitamin D; Parathyroid hormone; Population studies

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Introduction

Heart failure (HF) is one of the leading causes of morbidity and mortality in the Western world. Despite optimal treatment, the prognosis of HF remains poor, and approximately 50% of the patients die within 5 years after first diagnosis.[1] The incidence of HF is strongly associated with age, and because of the globally ageing population, HF is expected to become a major burden for society. HF cannot be cured yet, and many efforts are made to prevent individuals from developing HF. Relatively simple and important strategies that contribute to prevention of HF are control of hypertension and prevention of myocardial infarction (MI). However, another approach that may be useful in preventing new onset HF could be the identification of subjects at risk for HF. Although several predication models have been

established,[2,3] their predictive value is modest, and it remains difficult to predict the risk for future HF events in individual subjects. Therefore, a continuous search for new prediction models with novel markers is warranted.

The role of vitamin D biology has been well established in bone homeostasis. In recent decades, however, it has been suggested that vitamin D may as well be of importance in the development of cardiovascular (CV) disease, and in particular HF. Several observational studies reported an association between vitamin D as well as parathyroid hormone (PTH) and the development of CV disease and HF.[4–6] In addition, it was already shown that elevated PTH levels were associated with increased risk of HF in elderly, although calcidiol, the biologically inactive form of vitamin D, was not.[7,8] Mechanistically, vitamin D and PTH exert roles in blood pressure,[9] cardiomyocyte hypertrophy,[10,11] myocardial fibrosis,[12,13] and inflammation.[10] However, it remains to be established whether PTH and vitamin D are either independent predictors in the development of all cases of HF, or HF with reduced ejection fraction (HFrEF) or HF with preserved ejection fraction (HFpEF).

In a large (mostly white) population-based cohort study, we analyse how calcidiol, calcitriol, and PTH are related to each other, as well as to other baseline characteristics and laboratory values. We further investigate whether circulating calcidiol, calcitriol, and PTH are associated with risk for development of new onset HF, and if this association remains after correction for more established predictors like N-terminal-pro-Brain-type natriuretic peptide (NT-proBNP) and highly sensitive Troponin-T (hs-TnT).

Methods

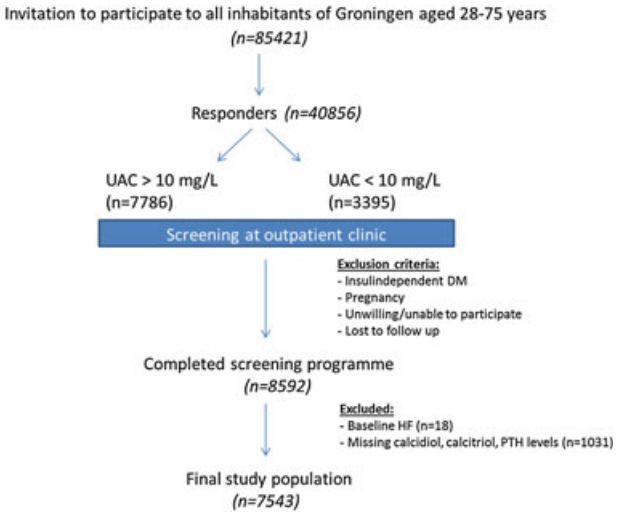
This study was performed using the data of the PREVEND (Prevention of Renal and Vascular End-stage Disease) cohort study in Groningen, the Netherlands (latitude 53°N), and has been described in more detail before.[14,15] The PREVEND study is a large prospective population-based cohort study and was designed in 1997 to investigate albuminuria and (development of) renal and CV diseases. The flowchart for selection and inclusion of the final population is provided in *Figure 1*.

Sample storage, biomarkers, and measurement of vitamin D

At baseline (1997–98), EDTA plasma samples were collected from all participants and stored at −80°C until used for biomarker assessment. NT-proBNP, highly sensitive C-reactive protein, hs-TnT, and urinary albumin concentration were determined as described before.[2]

Baseline plasma calcidiol and calcitriol levels were measured by solid phase extraction isotope dilution that was followed by liquid chromatography–tandem mass spectrometry (Spark-Holland Symbiosis system, Emmen, the Netherlands). The intra-assay and inter-assay coefficients of variation (CoV) for calcidiol were 7.2 and 6.7%, respectively, and for calcitriol 5.0 and 14.1%, respectively. We used an automated two-site immunoassay (Roche, Diagnostics, Indianapolis, IN, USA) to measure baseline plasma intact PTH levels, with an intra-assay CoV of 3.4–5.8% and an inter-assay CoV of <9%.[9] Calcidiol levels were expressed in pmol/L (of note 2.4 pmol/L is equivalent to 1 pg/mL), and calcitriol levels were expressed in nmol/L (2.5 nmol/L is equivalent to 1 ng/mL).

Figure 1 Flowchart of selection and inclusion of final study population. DM, diabetes mellitus; HF, heart failure; PTH, parathyroid hormone; UAC, urinary albumin concentration.



Ascertainment of new onset heart failure

In this study, follow-up time was defined as the time between the baseline visit and the date of new onset HF or the date of the last follow-up (1 January 2010), whatever date came first. Participants of the PREVEND study were known to have a low migration rate. Nevertheless, participants were censored at the day they moved to an unknown destination. The diagnosis of each individual HF case was made using an extensive validation and identification process. Brouwers *et al*. provided a simplified overview of the validation and identification process of this study.[15] Briefly, health care of participants was covered by the two main hospitals in the region. The local Ethics Committee of both hospitals granted access to hospital records of PREVEND participants. Patient files were checked for the presence of HF at baseline or during follow-up for new onset HF. HF was suspected when signs, symptoms, and objective evidence of HF were

reported, according to the criteria of the Heart Failure Guidelines of the European Society of Cardiology. All cases of suspected new onset HF (586 individual cases) were adjudicated by two experts in the field of HF. Anonymized clinical charts, hospitalization, and physician office records were used to ascertain new onset HF. After this review process patients were considered to have 'definite new onset HF', 'definite no new onset HF', or 'definite HF prior to start date PREVEND'. In case consensus was not reached on an individual case, the committee made an joint decision. At that time, European Society of Cardiology guidelines did not provide left ventricular ejection fraction (LVEF) cut-offs for diagnosis of HFrEF or HFpEF; therefore, HF was classified using the following cut-offs: HFrEF LVEF $\leq$ 40% and HFpEF LVEF $\geq$ 50%. To prevent blending of epidemiological profiles, patients with a LVEF between 41 and 49% ($n = 6$) were excluded from final analysis. Aetiology and date of HF onset were also obtained from clinical charts. Data on LVEF were available in 98.4% of cases with new onset HF. In the other six cases, the diagnosis of HF was confirmed through the joint decision of an expert panel (at least two cardiologists) because of insufficient data on LVEF.

Information on dates and causes of death for every participant was obtained from Statistics Netherlands and coded according to the 10th revision of the International Classification of Diseases.

Definitions

Blood pressure was measured during two visits, using an automatic Dinamap XL Model 9300 series device. Hypertension was defined as a systolic blood pressure $>$ 140 mm Hg, a diastolic blood pressure $>$ 90 mm Hg, or when an individual reported to use antihypertensive medication. Body mass index was calculated as the ratio of weight to height squared (kg/m^2), and individuals with a body mass index $>$ 30 kg/m^2 were considered obese. Hypercholesterolemia was either present if lipid-lowering medication was used, or total serum cholesterol exceeded 6.5 mmol/L (251 mg/dL) in participants without history of MI, or 5.0 mmol/L (193 mg/dL) in participants with a history of MI. A history of MI was present in those individuals who reported that they had been hospitalized for at least 3 days as a result of this condition. Individuals were diagnosed to have type 2 diabetes when the use of antidiabetic drugs was reported, and/or a fasting plasma glucose of $>$126 mg/dL was measured, or a non-fasting plasma glucose of $>$200 mg/dL was measured. We calculated urinary albumin excretion (UAE) as the average of two consecutive 24 h urine collections. The simplified modification of diet in renal disease formula was used to calculate the estimated glomerular filtration rate. Smokers were those individuals who reported that they had used nicotine within the previous year. We used the Modular ECG Analysis System[16] to record standard 12-lead electrocardiograms. Presence of atrial fibrillation (AF) was defined using Minnesota codes 8.3.1 and 8.3.3.

Statistical analysis

Continuous data were represented as means ± standard deviation (SD) for normally distributed data and as medians with interquartile ranges (IQR) for skewed distributions. Baseline differences were tested using Student's *t*-test or Kruskal–Wallis test, as appropriate. Discrete and categorical data were presented as frequencies (%), and differences between groups were tested using a standard χ^2 test. A *P*-value of $<$0.05 was designated as significant.

Linear variables were included as linear covariates in our model. Discrete and non-linear variables were included as categorical variables. Calcidiol and calcitriol levels were normally distributed and included as continuous variables. PTH levels were distributed in a skewed manner and transformed to a log-scale. Because of the overselection of subjects with elevated UAE, we corrected for UAE in all models by using a statistical weighting method. This method enabled us to extrapolate and generalize our conclusions as if we were studying a general population.[17]

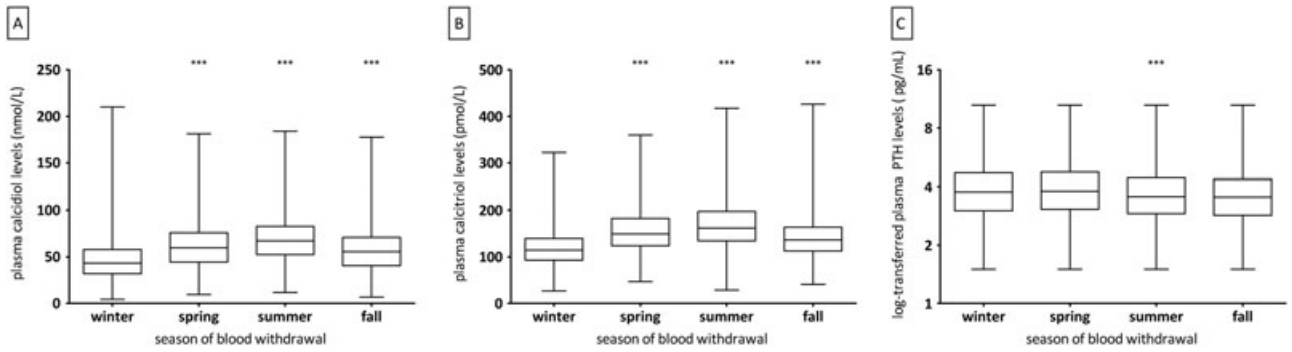
Vitamin D levels are known to significantly fluctuate depending on the time of year of blood sampling. We tested the relationship between PTH, vitamin D, and day of blood withdrawal in our subjects (one-way ANOVA, post hoc testing with Bonferroni). In addition, we included two variables $R = \cos[2\pi/365.25]$*day and $S = \sin([2\pi/365.25]$*day,[18] to correct for the time of the year of blood sampling in all subsequent analyses unless stated otherwise.[18]

Cross-sectional univariable regression analyses were performed to study the univariate associations between calcidiol, calcitriol, PTH, and baseline characteristics. Results were standardized. To compare relative strength of the various outcomes, we presented outcomes as beta coefficients.

Linearity of the relationship between calcidiol, calcitriol, PTH, and new onset HF was tested and not violated. For the longitudinal analyses we built Cox proportional hazard regression models to study the associations between plasma vitamin D and PTH with risk of new onset HF. Hazard ratios (HRs) are reported with respective 95% confidence interval [95% CI]. Cause-specific hazard analyses were performed to study the associations of calcidiol, calcitriol, and PTH with risk of HFpEF (LVEF $\geq$ 50%) and HFrEF (LVEF $\leq$ 40%). A *P*-value for competing risk (P_{cr}) $<$ 0.10 between HFrEF and HFpEF was considered statistically significant.[19,20] Proportional hazard assumptions were tested and satisfied.

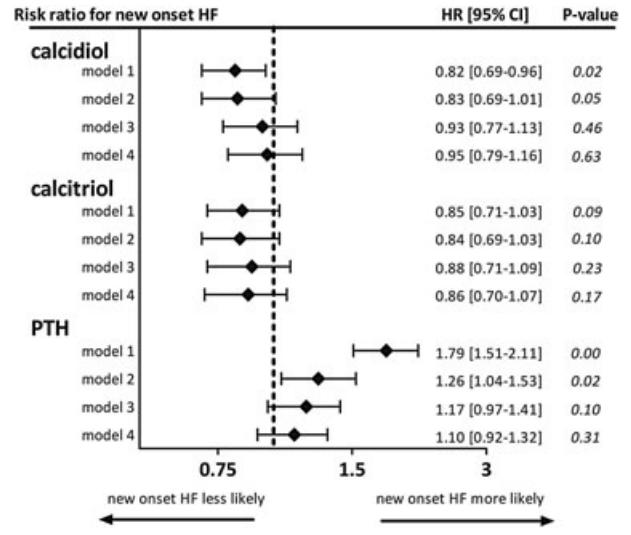
To describe the independent associations of calcidiol, calcitriol, and PTH with new onset HF, we built several models adjusting for possible confounders. In the first model,

Figure 2 (A) Correlation of plasma calcidiol and season of blood withdrawal. (B) Correlation of plasma calcitriol and season of blood withdrawal. (C) Correlation of plasma PTH levels and season of blood withdrawal. Data are presented in Tukey boxplot [median (interquartile range)]. * $P < 0.05$ and *** $P < 0.001$ as compared with previous season. Data are analysed using one-way ANOVA (post hoc testing with Bonferroni). Outliers are not presented.



we examined the univariate association between calcidiol, calcitriol, and PTH with risk of new onset HF. In model 2, we adjusted for age, sex, and season of blood withdrawal. In the third model, we added other covariates that are associated with new onset HF (smoking, hypertension, hypercholesterolemia, history of MI, obesity, AF, serum cystatin C, UAE, highly sensitive C-reactive protein, and estimated glomerular filtration rate[15]). Finally, we created a fourth model that additionally adjusted for NT-proBNP and hs-TnT.[2]

Figure 3 Risk ratio for new onset HF [HR (95% CI)] per 1 SD increment in plasma calcidiol and calcitriol, or per log-transformed increase of PTH. Model 1: univariate association. Model 2: age, sex, and time of year of blood withdrawal (using the cosinor model). Model 3: model 2 + active smoking, history of myocardial infarction or stroke, hypertension, hypercholesterolemia, obesity (body mass index > 30 kg/m^2), atrial fibrillation, urinary albumin excretion, highly sensitive C-reactive protein, Cystatin C, and estimated glomerular filtration rate. Model 4: model 3 + N-terminal pro-Brain-type Natriuretic peptide and highly sensitive troponin-T.



All analyses were performed using StataIC version 11.0 (StataCorp, Texas, USA).

Results

A total of 7470 participants were evaluated in this study. Baseline characteristics are presented in *Table 1*. Mean (±SD) of calcidiol was 58 (±24) nmol/L, mean calcitriol 145 (±48) pmol/L, and median (IQR) PTH was 3.7 (3.0–4.6) pmol/L. Mean age of study participants was 49 ± 12 years. Of all participants, 96% were white, and 52% were females. Half of the subjects had their blood drawn in the winter.

Cross-sectional associations of calcidiol, calcitriol, and PTH with demographics, laboratory values, and co-morbidities

Univariate associations of changes in calcidiol, calcitriol, and PTH levels with baseline variables are presented in *Table 2*. Levels of calcidiol and calcitriol correlated significantly with season of blood withdrawal (beta coefficient for calcidiol: 0.19, $P < 0.001$; beta coefficient calcitriol: 0.20, $P < 0.001$), but this was less pronounced for PTH (log-transformed coefficient for PTH: -0.08, $P < 0.001$; *Figure 2*).

We further observed that the levels of calcidiol and calcitriol were strongly associated with each other, whilst the association between PTH and calcidiol was less pronounced. Furthermore, we found no association between PTH and calcitriol levels. For PTH, the strongest association per 1 log-transformed increase was with age (*Table 2*).

We also assessed the associations between levels of vitamin D metabolites, PTH, and prevalence of several morbidities. Lower levels of calcidiol were associated with

Table 1 Baseline characteristics of all subjects participating in PREVEND without HF during follow up and of subjects with new onset HF during follow-up

	All subjects ($n = 7470$)	No heart failure ($n = 7189$)	Heart failure ($n = 281$)	*P*-value	HFrEF ($n = 181$)	HFpEF ($n = 94$)	*P*-value
Age (years)	\49 ± 12	48 ± 12	62 ± 9	<0.001	62 ± 10	62 ± 9	0.73
Males (%)	48	47	61	<0.001	70	44	<0.001
Race (%)							
Caucasian	96	96	98		98	98	
Negroid	1	1	—		—	—	
Asian	2	2	1		1	1	
Other	1	1	1		1	1	
BMI (kg/m^2)	26 ± 4	26 ± 4	28 ± 5	<0.001	28 ± 4	29 ± 6	0.04
SBP (mm Hg)	128 ± 20	127 ± 19	145 ± 22	<0.001	143 ± 20	148 ± 26	0.08
DBP (mm Hg)	74 ± 10	73 ± 10	80 ± 10	<0.001	80 ± 10	79 ± 10	0.50
Blood withdrawal in winter (%)	50	50	48	0.43	48	49	0.89
Smoking or quit <1 year (%)	38	38	38	0.91	44	28	0.01
Myocardial infarction (%)	6	5	25	<0.001	29	17	0.03
Hypertension (%)	30	28	68	<0.001	66	72	0.29
Hypercholesterolemia (%)	26	25	46	<0.001	48	39	0.15
Type 2 diabetes (%)	1	1	4	<0.001	4	5	0.58
Atrial fibrillation (%)	1	1	4	<0.001	4	4	0.85
Glucose (mmol/L)	4.8 ± 1.1	4.8 ± 1.0	5.4 ± 1.7	<0.001	5.4 ± 1.6	5.6 ± 1.8	0.41
Total cholesterol (mmol/L)	5.6 ± 1.1	5.6 ± 1.1	6.0 ± 1.1	<0.001	6.0 ± 1.1	6.0 ± 1.0	0.87
HDL cholesterol (mmol/L)	1.3 ± 0.4	1.3 ± 0.4	1.2 ± 0.4	<0.001	1.2 ± 0.4	1.3 ± 0.4	0.29
Triglycerides (mmol/L)	1.4 ± 1.0	1.4 ± 1.0	1.6 ± 0.8	0.003	1.6 ± 0.8	1.5 ± 0.8	0.69
Serum creatinin (μmol/L)	83 ± 16	83 ± 16	87 ± 17	<0.001	90 ± 16	82 ± 16	<0.001
eGFR (mL/min/1.73 m^2)	81 ± 14	81 ± 14	76 ± 16	<0.001	75 ± 14	78 ± 18	0.15
Cystatin C (mg/dL)	0.8 [0.7–0.9]	0.8 [0.7–0.9]	0.9 [0.8–1.0]	<0.001	0.91 ± 0.2	0.9 [0.8–1.0]	0.29
hs-CRP (mg/L)	1.2 [0.5–2.8]	1.2 [0.5–2.8]	2.5 [1.1–4.8]	<0.001	2.5 [1.2–4.5]	2.0 [0.8–4.5]	0.12
hs-TnT (ng/L)	2.5 [2.5–4.0]	2.5 [2.5–4.0]	6.0 [3.0–10.0]	<0.001	7.0 [4.0–11.0]	5.0 [2.5–8.0]	0.001
NT-proBNP (ng/L)	37 [17–72]	36 [16–69]	102 [43–285]	<0.001	140 [50–351]	80 [36–172]	0.01
Renin concentration (μIU/mL)	18 [11–29]	18 [11–29]	18 [10–31]	0.40	18 [10–34]	17 [8–26]	0.20
UAE (mg/24 h)	9 [6–15]	9 [6–15]	15 [8–33]	<0.001	15 [8–33]	15 [8–35]	0.29
Calcidiol (nmol/L)	58 ± 24	58 ± 24	54 ± 21	0.003	56 ± 22	52 ± 20	0.19
Calcitriol (pmol/L)	145 ± 48	145 ± 48	141 ± 45	0.10	143 ± 46	136 ± 41	0.24
PTH (pmol/L)	3.7 [3.0–4.6]	3.6 [3.0–4.5]	4.3 [3.5–5.4]	<0.001	4.4 [3.4–5.4]	4.2 [3.7–5.4]	1.00

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, highly sensitive C-reactive protein; hs-TnT, highly sensitive troponin T; NT-proBNP, N-terminal pro-Brain-type natriuretic peptide; PTH, parathyroid hormone; SBP, systolic blood pressure; UAE, urinary albumin excretion. Continuous normally distributed data are presented as mean ± SD and compared using Student's *t*-test. Continuous non-normally distributed data are presented as median [interquartile range] and compared using the Kruskall–Wallis test. Categorical variables are presented as frequencies (%) and compared using standard χ^2 test.

Table 2 Cross-sectional analysis on the association between calcidiol, calcitriol, and PTH with demographics, baseline medical history, and laboratory values

	PTH Log-transformed coefficient	*P*-value	Calcidiol Beta coefficient	*P*-value	Calcitriol Beta coefficient	*P*-value
Age (years)	0.30	<0.001	-0.02	0.09	-0.01	0.55
Males (%)	-0.05	<0.001	0.01	0.36	0.08	<0.001
Race (%)						
Caucasian	1.00	*ref*	1.00	*ref*	1.00	*ref*
Negroid	0.05	<0.001	-0.11	<0.001	-0.01	0.52
Asian	0.05	<0.001	-0.13	<0.001	-0.03	0.02
Other	0.06	<0.001	-0.10	<0.001	-0.02	0.04
BMI (kg/m^2)	0.24	<0.001	-0.08	<0.001	-0.02	0.04
SBP (mm Hg)	0.25	<0.001	-0.04	0.001	0.02	0.05
DBP (mm Hg)	0.21	<0.001	-0.02	0.15	0.02	0.03
Blood withdrawal in winter	-0.04	0.72	0.33	<0.001	0.38	<0.001
Smoking or quit <1 year	-0.22	<0.001	-0.04	<0.001	-0.09	<0.001
Myocardial infarction (%)	0.06	<0.001	-0.02	0.05	-0.02	0.12
Hypertension (%)	0.23	<0.001	-0.03	0.01	0.01	0.21
Hypercholesterolemia (%)	0.07	<0.001	0.00	0.69	0.04	<0.001
Type 2 diabetes (%)	0.04	0.001	-0.03	0.01	-0.03	0.01
Atrial fibrillation (%)	0.04	0.002	-0.02	0.13	0.00	0.93
Glucose (mmol/L)	0.12	<0.001	-0.01	0.30	0.03	0.004
Total cholesterol (mmol/L)	0.07	<0.001	0.00	0.72	0.03	0.01
HDL cholesterol (mmol/L)	-0.06	<0.001	0.09	<0.001	0.11	<0.001
Triglicerides (mmol/L)	0.08	<0.001	-0.04	0.001	-0.02	0.07
Serum creatinin (μmol/L)	0.09	<0.001	0.13	<0.001	-0.09	<0.001
eGFR (mL/min/1.73 m^2)	-0.12	<0.001	-0.17	<0.001	0.02	0.15
Cystatin-C (mg/dL)	0.09	<0.001	0.03	0.03	-0.09	<0.001
hs-CRP (mg/L)	0.06	<0.001	0.01	0.25	0.03	0.01
hs-TnT (ng/L)	0.18	<0.001	-0.02	0.05	-0.05	<0.001
NT-proBNP (ng/L)	0.14	<0.001	0.00	0.69	0.01	0.21
Renin concentration (μIU/mL)	-0.08	<0.001	-0.05	<0.001	-0.12	<0.001
UAE (mg/24 h)	0.12	<0.001	-0.03	0.01	-0.02	0.05
Calcidiol (nmol/L)	-0.24	<0.001			0.54	<0.001
Calcitriol (pmol/L)	0.01	0.96	0.54	<0.001		
PTH (pmol/L)			-0.24	<0.001	0.00	0.96

Data are presented per each SD-increment of plasma calcidiol and calcitriol, and per SD log-transformed increase of PTH. DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hs-CRP, highly sensitive C-reactive protein; hs-TnT, highly sensitive troponin T; NT-proBNP, N-terminal pro-brain-type natriuretic peptide; PTH, parathyroid hormone; SBP, systolic blood pressure; UAE, urinary albumin excretion.

increased prevalence of type 2 diabetes, hypertension and a history of MI, while lower levels of calcitriol were associated with a higher frequency of type 2 diabetes, but a lower frequency of hypercholesterolemia (*Table 1*). Higher PTH levels were associated with higher prevalence of multiple morbidities (hypertension, hypercholesterolemia, type 2 diabetes, AF, and MI), although we observed an inverse association with active smokers (*Table 1*).

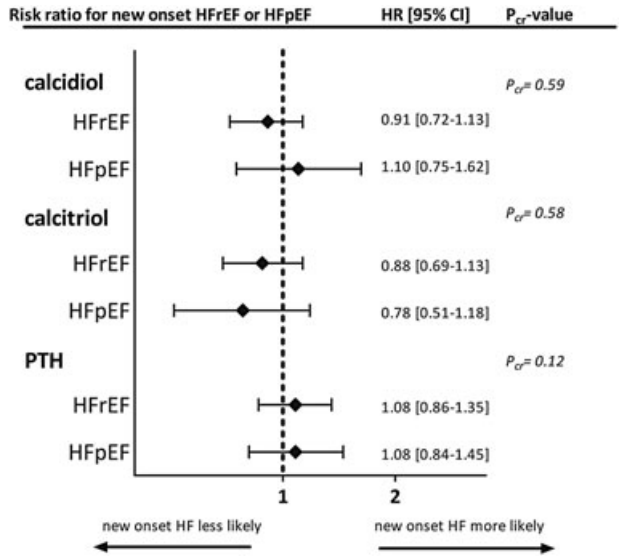
Longitudinal associations of calcidiol, calcitriol, and parathyroid hormone with risk of new onset heart failure

During a median follow-up time of 12.6 years (IQR: 12.3–12.9), 281 participants (4%) developed HF, of whom 181 (66%) were classified with HFrEF and 94 (34%) with HFpEF. While levels of calcitriol were not different between groups, subjects that were diagnosed with HF were more likely to have significantly lower calcidiol levels and higher PTH levels. The levels of calcidiol, calcitriol, and PTH were not significantly different in subjects with HFpEF or HFrEF (*Table 1*).

Univariately, calcidiol levels were per 1 SD increase associated with new onset HF. However, the association was no longer significant after adjustment for age, sex, and season of blood withdrawal, and after further adjustment for other covariates (*Figure 3*). PTH levels were, per log-transformed increment, univariately associated with new onset HF [HR 1.79 (95% CI 1.51–2.11)]. This association remained present after adjustment for age, sex, and season of blood withdrawal (*Figure 3*). However, after addition of other confounders that are associated with risk of new onset HF this association was no longer significant [HR 1.17 (95% CI 0.97–1.41)]. Addition of the biomarkers NT-proBNP and hs-TnT to this model further attenuated this association (*Figure 3*).

We performed cause-specific analyses to analyse if calcidiol, calcitriol, and PTH could be of importance in differentiating between new onset HFrEF or HFpEF. None of these markers could differentiate between new onset HFrEF or new onset HFpEF (*Figure 4*).

Figure 4 Risk ratio for new onset HFrEF or HFpEF (HR (95% CI)] per 1 SD increment in plasma calcidiol, calcitriol, or per log-transformed increase in PTH levels, and their incremental power between HFrEF and HFpEF (P_{cr}-value). In our full model, we adjusted for age, sex, active smoking, history of myocardial infarction or stroke, hypertension, hypercholesterolemia, obesity (body mass index $> 30\ kg/m^2$), atrial fibrillation, urinary albumin excretion, highly sensitive C-reactive protein, N-terminal pro-brain-type natriuretic peptide, highly sensitive troponin T, Cystatin C, estimated glomerular filtration rate, and the cosinor model for time of the year of blood withdrawal.



Discussion

In this population-based study, neither plasma calcidiol, calcitriol, nor PTH were independently associated with risk of new onset HF. Plasma calcidiol and PTH levels were univariately associated with risk of new onset HF. However, after adjustment for age, sex, and time of the year of blood withdrawal calcidiol was no longer associated, whilst plasma PTH lost its predictive value after addition of other covariates to our model. In addition, we observed that calcidiol and calcitriol levels strongly depended on season of blood withdrawal and that PTH levels were more consistent during the year. Levels of calcidiol and calcitriol were strongly associated with each other, and although PTH is a well-known key regulator in vitamin D biology, we did not observe a cross-sectional association with plasma calcitriol and PTH. The association between plasma calcitriol and PTH was only moderate in this cohort of healthy subjects.

The observation that PTH is not associated with risk of new onset HF is in line with results from The Atherosclerosis Risk in Communities (ARIC) study,[21] but contrasts with data from the Multi-Ethnic Study of Atherosclerosis (MESA), suggesting that PTH is a predictor for new onset HF.[22] These discrepancies might be (indirectly) driven by differences in study population. The average age of participants in the MESA (62 years) was markedly higher than in ARICs (range: 56–57 years) and PREVENDs (49 years). In general, kidney function declines with age and changes in PTH levels are predominantly present in patients with advanced stages of chronic kidney disease.[23] Independent from kidney function, increasing age itself is associated with increased plasma PTH concentrations,[24,25] and it has even been suggested to take this effect into account when assessing calcium disorders in elderly individuals.[26] We therefore hypothesize that, despite proper adjustment of age as confounding variable in ARIC, MESA, and our study, differences in (baseline) age have been a major factor in the reported discrepancies. We hypothesize that the (indirect) effect of differences in age has driven the reported discrepancies. Even though not directly within the scope of our study, we observed a univariate significant interaction between age, PTH, and risk of new onset HF that was lost after addition of sex, and time of year of blood withdrawal (data not shown). We suggest that future research should use accurately pre-set age categories to reveal if an interaction between age and PTH is of importance when assessing the association with new onset HF. Based on data from this and ARIC study, however, it appears that screening for PTH to identify subjects at risk for new onset HF has limited value.

PTH is part of a complex endocrine system that regulates calcium and bone metabolism and initiates integration of many factors, including the vitamin D metabolites calcidiol and calcitriol. Calcidiol is the storage form of vitamin D in the human body, and levels of this metabolite are used to determine vitamin D status. However, biological activity of vitamin D does not only rely on calcidiol, and instead, calcitriol is considered to be the primary and biologically most active metabolite in vitamin D biology.[27] Previous studies have mainly focused on the role of calcidiol as risk marker in HF, and the value of measuring circulating calcitriol to predict new onset HF has not been studied before.

Experimental studies in mice lacking the vitamin D receptor ($VDR^{-/-}$ mice) demonstrated that deficiency of the VDR results in cardiac hypertrophy and hypertension. So mechanistically, vitamin D is thought to directly influence HF pathology by regulating myocyte contractility, cardiac remodelling (regulation of inflammation and cytokines), secretion of natriuretic hormones, and activity of the renin–angiotensin–aldosteron system.[28] However, disappointing outcomes from experimental and epidemiological studies in humans have tempered the enthusiasm that this can be translated easily.[29–31] In line with this, we now show that both plasma calcidiol and calcitriol have no predictive value in identifying subjects at risk for new onset HF in the general population. Therefore, we consider the role for vitamin D as direct modifier in HF pathology a limited one.

Although vitamin D may thus not have added value in predicting new onset HF in the general population, it may still be useful in reducing morbidity and mortality prevalence in patients with HF. The number of morbidities increases with age,[32,33] and it is known that patients with HF often have

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multiple co-morbidities.[34] Unfortunately, patients with co-morbid conditions are more likely to have an advanced stage of HF,[34,35] with a concomitant increased risk for both HF hospitalization and overall mortality.[35] It is, therefore, of relevance to establish screening tools that help identifying those patients at risk for increased co-morbidity prevalence.[36] Interestingly, low levels of vitamin D are associated with abnormalities in laboratory values (e.g. increased serum LDL cholesterol, triglycerides, and decreased serum HDL cholesterol) as well as increased prevalence of frequently reported co-morbidities in patients with HF, such as hypertension and diabetes mellitus.[37] Moreover, vitamin D deficiency is also associated with increased morbidity prevalence in a general population.[33] We, therefore, hypothesize that vitamin D reflects an individual's global health status. Possibly, low levels of vitamin D may be used to identify subjects with overall poor general health who are at risk for increased morbidity prevalence. Further studies are needed to address if screening for vitamin D deficiency could be of added value in the reduction of morbidity and mortality burden in patients with HF.

Strengths and limitations

PREVEND is a large, well-phenotyped, community-based cohort study with a long follow-up. Within this study, extensive information is available on several covariates of study subjects, including sensitive biomarkers that minimizes residual confounding. The validation of incident heart diagnosis has been thorough, and we used a sensitive method, liquid chromatography–tandem mass spectrometry to measure both calcidiol and calcitriol.

This study had several limitations. First, we used a retrospective approach to identify new onset HF. Although all cases were evaluated by seven independent experts in the field of HF, this could have caused detection bias, especially resulting in the underdetection of subjects with HFpEF. Second, we defined prevalent HF at baseline by self-reported HF, and therefore cannot exclude that subjects may have had an episode of HF or suffered from prevalent HF without reporting this.

Third, PREVEND study subjects were predominantly white. External validity of our findings may be limited to white adults, and results may therefore not be readily extrapolated to other ethnicities. Finally, because of the design of the study, there was an enrichment of subjects with mildly elevated urinary albumin concentrations. However, we corrected for this in all analyses. Although we did not find an interaction between vitamin D and urinary albumin concentrations, we cannot fully ensure that this study design has not affected our results.

Conclusion

In this well-characterized, community-based cohort, we have shown that a single measurement of plasma calcidiol, calcitriol, or PTH does not predict new onset HF. Furthermore, we found that plasma calcidiol, calcitriol, and PTH were unable to distinguish between new onset of HFrEF and HFpEF. Based on these data, screening for these markers to identify subjects at risk for new onset HF cannot be advocated.

Conflicts of interest

H.J. Lambers-Heerspink has consultancy agreements with AbbVie, Astellas, Astra Zeneca, Johnson&Johnson, Reata, and Vitae. All honoraria are paid to his employer, the University Medical Center Groningen, the Netherlands. The other authors report no conflicts.

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