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The role of functional vitamin D deficiency and low vitamin D reservoirs in relation to cardiovascular health and mortality

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Abstract

Objectives: The role of vitamin D deficiency in cardiovascular disease (CVD) is controversial. Inherent biological and analytical limitations compromise the specificity of widely used 25-hydroxyvitamin D [25(OH)D] cut-offs. Simultaneous determination of 25(OH)D and 24,25-dihydroxyvitamin D [24,25$(OH)_2$D] permits a functional assessment of vitamin D metabolism. The present study compared the associations of functional vitamin D deficiency and low vitamin D reservoirs with CVD mortality and CVD burden.

Methods: 25(OH)D, 24,25$(OH)_2$D, the degree of coronary obstruction on angiography, high-sensitive cardiac troponin T (hs-cTnT), N-terminal brain natriuretic peptide (NT-proBNP), and 10-year CVD mortality were obtained from 2,456 participants of the LURIC (Ludwigshafen Risk and Cardiovascular Health) study.

Results: Neither low 25(OH)D concentrations nor functional vitamin D deficiency were associated with the number of atherosclerotic coronary arteries or the degree of coronary obstruction. Over a median follow-up of 9.9 years, 454 participants died (23.6 %) due to CVD. CVD mortality was doubled in individuals with 25(OH)D concentrations below the widely used cut-off for deficiency of <50 nmol/L [20 ng/mL] (21.6 vs. 11.5 %). In individuals with and without functional vitamin D deficiency, CVD mortality was 25.0 and 16.7 %, respectively. NT-proBNP and heart failure prevalence were also higher in vitamin D deficient individuals.

Conclusions: Vitamin D deficient individuals have markedly higher CVD mortality, but only marginally higher hs-cTnT concentrations. A higher prevalence of heart failure and higher NT-proBNP concentrations suggest a link between vitamin D deficiency and cardiac function. The traditional and metabolic assessment of vitamin D status showed comparable associations for the different parameters of cardiac health.

Keywords: vitamin D; functional deficiency; cardiovascular mortality

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Introduction

Vitamin D deficiency is a highly prevalent condition in developed countries [1] that compromises the mineralization of bone [2–5]. In addition to its pivotal role in calcium and phosphate homeostasis, vitamin D has pleiotropic functions that affect virtually all organs and tissues. For example, vitamin D modulates cell growth and differentiation, immune function, glucose homeostasis, cognitive function and hormonal actions [4, 6, 7]. Furthermore, observational studies have shown associations of vitamin D deficiency with a broad range of clinical conditions including malignancies, autoimmune diseases, neuropsychiatric diseases and endocrinopathies. There is also evidence that links vitamin D to cardiovascular function and

cardiovascular disease (CVD) [6, 8]. Existing studies suggest that vitamin D deficiency promotes oxidative stress, systemic inflammation, activation of the renin-angiotensin-aldosterone system, endothelial dysfunction, hypertension, cardiac hypertrophy, and myocardial fibrosis. The aforementioned functions of vitamin D are mediated by genomic and non-genomic effects [9]. Genomic effects occur upon binding of vitamin D to the cytoplasmatic vitamin D receptor (VDR) that subsequently translocates into the nucleus. There, it binds to specific response elements in the promotor region of multiple genes involved in cell proliferation, differentiation, DNA repair and apoptosis. Furthermore, activation of the nuclear factor kappa B (NFk-B) pathway down-regulates the expression of inflammatory cytokines and angiotensin. The non-genomic effects of vitamin D, which are mediated by the membrane-associated rapid response steroid receptor ($1,25D_3$-MARRS), regulate the phosphorylation of endothelial nitric oxide synthase (eNOS), which is essential for endothelial function.

In clinical practice, patients' vitamin D status is assessed by measuring serum 25-hydroxyvitamin D [25(OH)D], an inactive prohormone that represents the body's vitamin D reservoir. Existing guidelines unanimously recommend the use of universal 25(OH)D cut-offs for results interpretation [4, 10]. However, these cut-offs are based primarily on risk for adverse bone-related outcomes, such as rickets, osteomalacia, osteoporosis, and fragility fractures [11]. Furthermore, the concept of universal cut-offs that apply to all patients has recently been questioned by observations that people with dark skin have significantly lower 25(OH)D serum concentrations than those with light skin despite comparable bone health [12, 13]. Moreover, only a fraction of individuals with low 25(OH)D concentrations has impaired bone mineralization [5] and accelerated bone metabolism [13], which suggests that a sole 25(OH)D measurement may incompletely reflect biology.

In the field of CVD, observational studies showed inconsistent associations with serum 25(OH)D, which prompted Gholami and colleagues to perform a meta-analysis [14]. This meta-analysis revealed a significant association between low 25(OH)D concentrations and CVD mortality, but not CVD incidence. A recent large prospective cohort study from UK Biobank adds to this finding by showing that low 25(OH)D concentrations are significantly associated with a higher incidence of coronary heart disease (CHD), CVD events and CVD mortality [15]. In contrast, several negative intervention studies question a mechanistic role of vitamin D deficiency in CVD [16–18]. However, a large part of the participants was actually not vitamin D deficient, which limits the scope of beneficial effects of vitamin D supplementation.

Recently, Herrmann and colleagues proposed a new metabolic approach for the diagnosis of functional vitamin D deficiency [13] that appears to be more specific in identifying individuals with functionally relevant vitamin D deficiency. In their study, the presence of functional vitamin D deficiency was associated with a substantially accelerated bone metabolism and increased all-cause mortality. The diagnosis of functional vitamin D deficiency is based on the simultaneous measurement of 25(OH)D and its principal catabolite 24,25-dihydroxyvitamin D [$24,25(OH)_2D$], which are used to calculate the vitamin D metabolite ratio (VMR). This new approach could also be useful to strengthen existing knowledge on the association between vitamin D deficiency and CVD. Therefore, 25(OH)D, $24,25(OH)_2D$, and VMR were determined in a large cohort of cardiovascular patients that underwent cardiac catheterization. CVD related mortality was recorded as primary outcome. In addition, imaging-derived parameters of CVD burden, serum high-sensitive cardiac troponin T (hs-cTnT) and N-terminal-pro-brain-natriuretic peptide (NT-proBNP) were assessed. The present study explored the associations of functional vitamin D deficiency with CVD mortality and CVD burden, and compared them with the traditional definition of vitamin D deficiency.

Materials and methods

Study design and participants

The LURIC (Ludwigshafen Risk and Cardiovascular Health) study was a prospective observational study performed at the Heart Centre in Ludwigshafen (Germany), a cardiology unit in a tertiary care medical centre in south-west Germany. Aim of the study was the investigation of environmental and genetic risk factors, and their interactions [19]. Between June 1997 and January 2000, patients were recruited upon hospitalization for elective diagnostic coronary angiography when the following inclusion criteria were met: German ancestry (limitation of genetic heterogeneity), clinical stability (except for acute coronary syndromes) and availability of coronary angiogram. Exclusion criteria were any acute illness other than acute coronary syndromes, any chronic disease where non-cardiac disease predominated and a history of malignancies within the past five years. Written informed consent was obtained from each participant prior to inclusion. The study was in accordance with the Declaration of Helsinki and approved by the Ethics Committee at the Medical Association of Rheinland-Pfalz (Ärztekammer Rheinland-Pfalz).

Follow-up and endpoints

Information about survival was obtained from local community registries. Two experienced physicians who were blinded to any data of patients except for the information from the death certificates independently classified the causes of death. Death certificates were reviewed to classify the deceased into those who died from cardiovascular and non-cardiovascular events. In the case of disagreement about classification, the final decision was made by one of the principal investigators of LURIC after an appropriate review of the data. The primary endpoint in this study was death due to cardiovascular causes and included sudden cardiac death (SCD), fatal myocardial infarction (MI), death due to heart failure (HF), death after intervention to treat coronary artery disease (CAD), stroke and other deaths due to heart disease [20].

Routine measurements

Fasting baseline venous blood was collected on the day of the coronary angiography at the Heart Centre Ludwigshafen, Germany. After immediate centrifugation, plasma was aliquoted and stored at −80 °C for later analysis of several biomarkers.

NT-proBNP was measured by electrochemiluminescence on an Elecsys 2010 analyzer (Roche Diagnostics, Rotkreuz, Switzerland). Inter-assay CVs were 3.2 and 2.0 % at mean values of 157 and 5,125 µg/L. hs-cTnT was determined using the immunoassay for MODULAR E170 series automated analyzers (Roche Diagnostics, Rotkreuz, Switzerland). The lower limit of detection is 5 ng/L, the 99th percentile of a healthy reference population is 14 ng/L. The inter-assay CVs were 9 and 1 % at 13.5 ng/L and above 30 ng/L, respectively.

Determination of vitamin D metabolites

25(OH)D and 24,25$(OH)_2$D were measured simultaneously with an established LC-MS/MS method [21]. Intra- and interday imprecision for all vitamin D metabolites tested was <8.6 % and <11.5 %, respectively. The limit of detection (LOD) for 25(OH)D and 24,25$(OH)_2$D were 0.60 and 0.12 ng/mL (1.5 and 0.3 nmol/L), and limit of quantitation (LOQ, defined as the lowest concentration where the assay gives an inter-assay imprecision of <15 %) were 1.24 and 0.40 ng/mL (3.1 and 1.0 nmol/L), respectively [21]. Recovery varied between 76.1 and 84.3 %. The method performed satisfactorily in the Vitamin D External Quality Assessment Scheme (DEQAS) since 2020. In this program, target values are assigned by the Center's for Disease Control and Prevention (CDC) reference measurement procedure using certified reference material (SRM 2972a) from the National Institute of Standards and Technology [NIST]) [22–24]. The conversion of SI units (nmol/L) to mass units (ng/mL) was performed by division by 2.496.

Participants were classified as having a low vitamin D metabolite profile when 24,25$(OH)_2$D was below 1.2 ng/mL (3 nmol/L) and the vitamin D metabolite ratio (VMR), calculated by multiplying the ratio of 24,25$(OH)_2$D/25(OH)D by 100, was less than 4 %. Both criteria are arbitrary and have been derived earlier [13]. Although these criteria are arbitrary, they are aligned with the Austrian Stroke Prevention Study (ASPS), where the association of vitamin D metabolites with cognitive function has been investigated [25]. Individuals who satisfied only one of the two functional criteria were classified as having a suboptimal vitamin D metabolite profile.

Other variables measured

Obstructive CAD was assessed by coronary angiography using the maximum luminal narrowing estimated by visual analysis. Obstructive CAD was defined as zero-, one-, two-, or three-vessel disease based on the number of luminal narrowing of 50 % in zero, one, two, or three of the major coronary arteries, i.e. left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA). Individuals with stenoses <20 % were considered not to have CAD in this classification.

The severity of CAD was assessed using the Friesinger score: Lesion sizes were quantified in LAD, LCX, and RCA. The lesion sizes in these three regions were graded from 0 to 5 points (0: no disease; 1: lesions <50 % area stenosis; 2: single lesion >50 % but <90 %; 3: multiple lesions >50 % but <90 %; 4: 90 % lesion area; 5: 100 % occlusion present). The Friesinger score was then calculated as the sum of the most severe lesion grade for each of the three regions [26].

In an alternative approach, CAD severity was assessed according to the method described by Gensini [27]. The Gensini score was developed to consider a coronary lesion's severity score, a region multiplying factor and a collateral adjustment factor. The final Gensini score was calculated as the sum of all the adjusted lesion scores of 15 coronary segments, the exact algorithm for the calculation of single lesion scores was described elsewhere [28].

Diagnosis of HF and further classification of heart failure patients with reduced ejection fraction (HFrEF) and preserved ejection fraction (HFpEF) were previously described [29].

Statistics

Descriptive statistics of the baseline characteristics are provided as medians and interquartile ranges (IQRs). Categorical variables are expressed as numbers and percentages.

Subsequently, survival curves were calculated by Kaplan-Meier analyses. Hazard ratios (HRs) were calculated by Cox proportional hazards regression. The proportional hazard assumption was checked by examination of scaled Schoenfeld residuals.

Descriptive statistics and Mann–Whitney U-tests were performed using SAS University edition (2024 SAS Institute Inc., Cary, NC). Violin plots, bar charts, dot-and-whisker plots, Kaplan–Meier analyses, and Cox proportional hazards regression analyses were carried out using R version 4.1.2 and the ggstatsplot package version 0.12.2 (http://www.r-project.org).

Results

Vitamin D status and CVD

Anthropometric data and general medical characteristics of the participants are summarized in Table 1. Plasma samples for the measurement of vitamin D metabolites were available from 2,456 participants (Supplementary Material, Supplementary Figure). 1,701 (69.3 %) out of 2,456 participants had a 25(OH)D concentration below 50 nmol/L, but only 521 (21.2 %) fulfilled the criterion for functional vitamin D deficiency. The prevalence of vitamin D deficiency was not significantly associated with the presence and severity of coronary artery disease (CAD), regardless of the approach used (Figure 1). Although low 25(OH)D concentrations occurred more frequently in participants with advanced coronary artery disease (two or three-vessel disease) than in those with no or only one affected coronary artery, this difference did not reach statistical significance. Likewise, functional vitamin D deficiency was equally prevalent amongst participants with no or mild CAD and those with advanced CAD. The Friesinger score, which classifies the severity of coronary obstruction for each of the three main vessels in three categories, was comparable in individuals with low and normal concentrations of 25(OH)D (Figure 2) as well as the Gensini Score (Figure 3), which also takes into account collaterals as well as increased flow with diameter reduction [28]. Similarly, this score did not differ between individuals with and without functional vitamin D deficiency. Also, hs-cTnT, a marker of myocardial ischemia, was similar in participants with and without vitamin D deficiency, independently of the diagnostic approach used (Figure 4).

Table 1: General characteristics of with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile.

	Functional assessment			Assessment by fixed 25(OH)D cutoff		
Parameter	**Low vitamin D metabolite profile**	**Unremarkable vitamin D metabolite profile**	**p-Value**	**<50 nmol/L**	**≥50 nmol/L**	**p-Value**
n	521	925		1,701	755	
Age, median (range), years	67 (21–92)	62 (19–87)	<0.001	65 (21–92)	61 (19–87)	<0.001
Gender, n (%), female	229 (44)	213 (23)	<0.0001	595 (35)	196 (26)	<0.001
BMI, median (IQR), kg/m^2	27 (25–30)	27 (25–29)	0.220	27 (25–30)	27 (25–29)	0.246
Nt pro-brain natriuretic peptide (IQR), pg/mL	366 (152–1,081)[a]	238 (92–659)[b]	<0.001	333 (129–1,049)[c]	206 (84–600)[d]	<0.001
High-sensitivity troponin T, median (range), ng/L	12.61 (0.00–5,860.00)[e]	9.00 (0.00–6,210.00)[f]	<0.001	11.27 (0.00–6,740.00)[g]	8.00 (0.00–5,040.00)[h]	<0.001
25(OH)D, median (IQR), nmol/L [ng/mL]	23.7 [9.5] (16.7–32.6)	60.9 [24.4] (50.4–74.1)	<0.001	30.4 [12.2] (20.8–39.9)	65.4 [26.2] (57.6–77.1)	<0.001
25(OH)D3, median (IQR), nmol/L	22.5 (15.6–30.5)	59.2 (49.1–72.9)	<0.001	28.7 (19.6–38.4)	64.1 (56.3–75.9)	<0.001
25(OH)D2, median (IQR), nmol/L	1.04 (0.70–1.51)	1.10 (0.76–1.61)	<0.001	1.08 (0.70–1.56)	1.12 (0.77–1.65)	<0.001
24,25(OH)$_2$D3, median (IQR), ng/mL	0.56 (0.30–0.95)	4.70 (3.72–6.18)	<0.001	1.43 (0.78–2.35)	4.97 (3.80–6.60)	<0.001
VMR median (IQR), %	2.8 (1.9–3.4)	8.1 (6.94–9.7)	<0.001	5.3 (3.7–7.0)	7.6 (6.2–9.3)	<0.001
ACS, %	31.7 %	31.8 %	0.964	31.7 %	31.0 %	0.711
Diabetes mellitus (ADA), %	38.4 %	25.3 %	<0.001	35.2 %	25.0 %	<0.001
Cancer, %	7.9 %	6.0 %	0.177	7.2 %	6.6 %	0.454
Menopause, % of women	89.2 %	87.3 %	0.546	89.1 %	86.6 %	0.360

BMI, body mass index; VMR, vitamin D metabolite ratio; 25(OH)D, 25-hydroxyvitamin D; 25-hydroxyvitamin D; 24,25(OH)$_2$D$_3$, 24,25-dihydroxyvitamin D$_3$; ADA: diabetes as classified according to diagnostic criteria of the American diabetes association [30]. Nt pro-brain natriuretic peptide measurements available for [a]514, [b]910, [c]1673, [d]744 patients; high-sensitivity troponin T measurements available for [e]520, [f]922, [g]1,698, [h]754 patients.

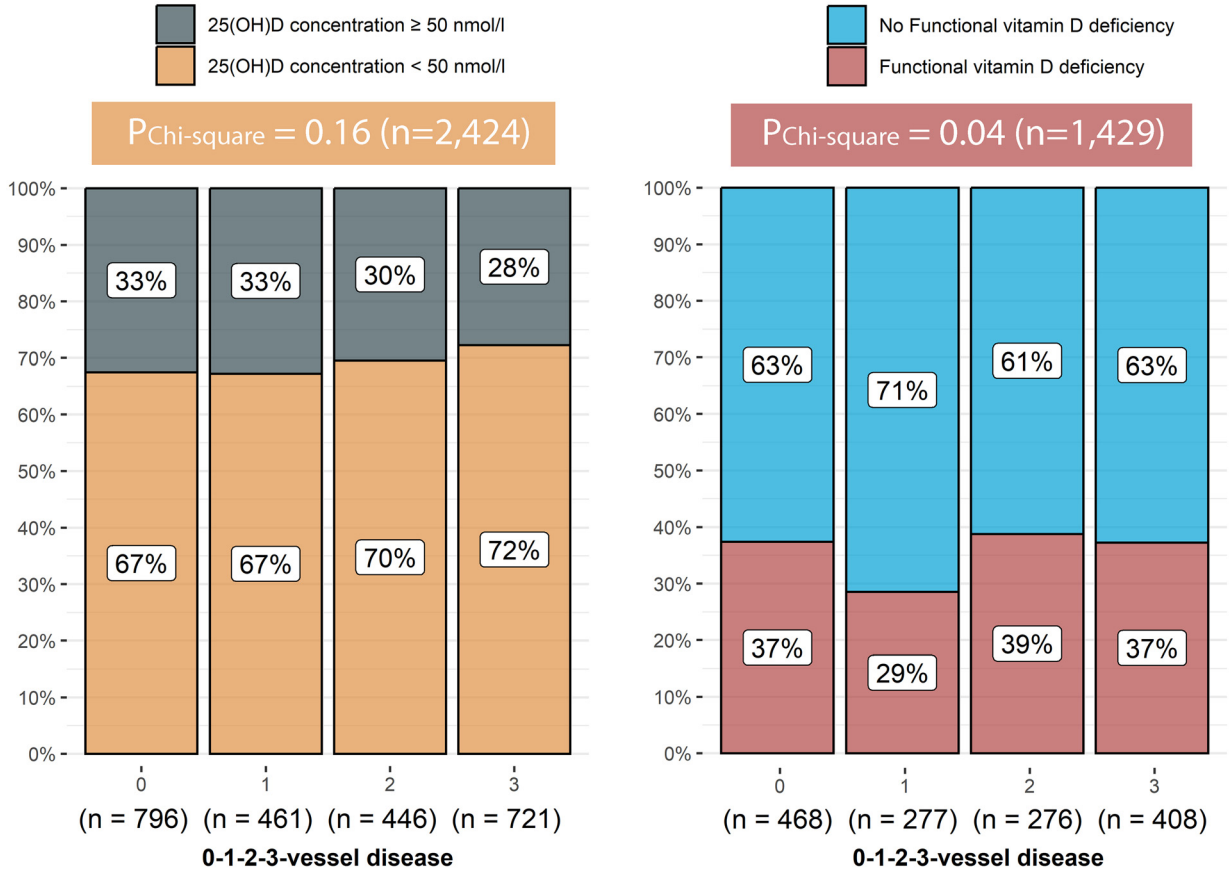

Figure 1: Degree of coronary obstruction on angiography in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. Coronary artery disease (CAD) was assessed by coronary angiography using the maximum luminal narrowing estimated by visual analysis. The angiographic severity of disease was defined as zero-, one-, two-, or three-vessel disease based on the number of luminal narrowings of 50 % in zero-, one-, two-, or three of the major coronary arteries.

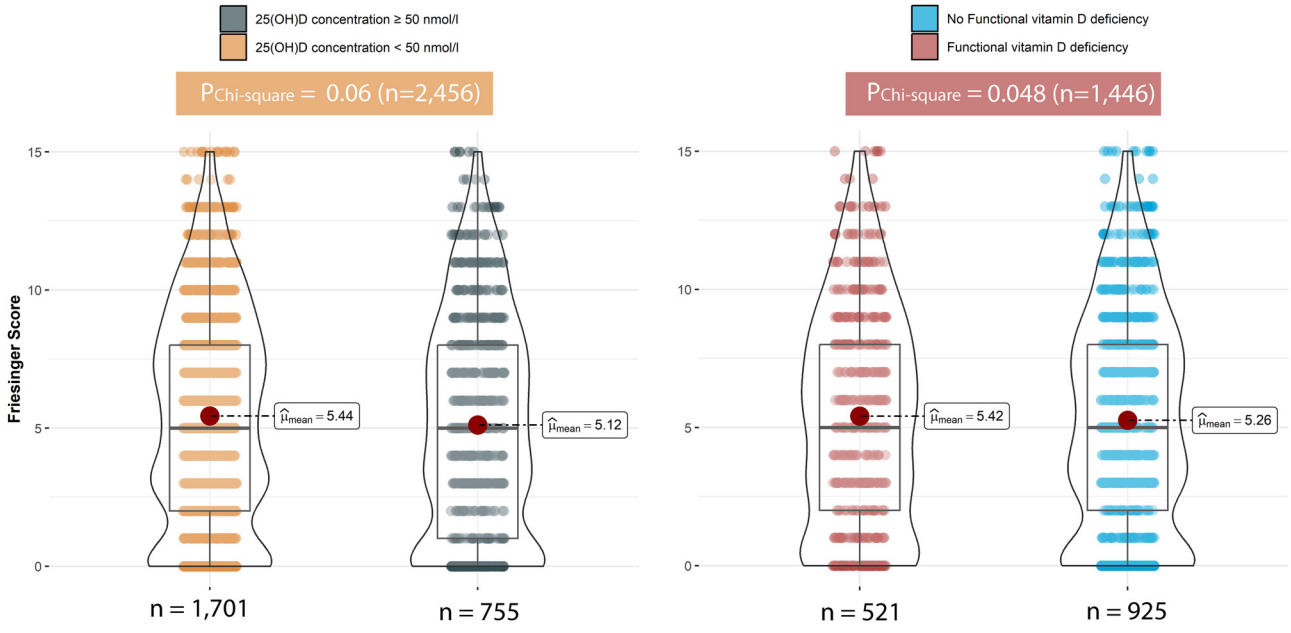

Figure 2: Friesinger scores in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. Lesion sizes were quantified in three regions (left anterior descending artery [LAD], left circumflex artery [LCX] and right coronary artery [RCA]) of the coronary circulation in coronary angiography. The lesion sizes in these three regions were graded from 0 to 5 (0: no disease; 1: lesions <50 % area stenosis; 2: single lesion >50 % but <90 %; 3: multiple lesions >50 % but <90 %; 4: 90 % lesion area; 5: 100 %). The Friesinger score was then calculated as the sum of most severe lesion grade for each of three regions [26].

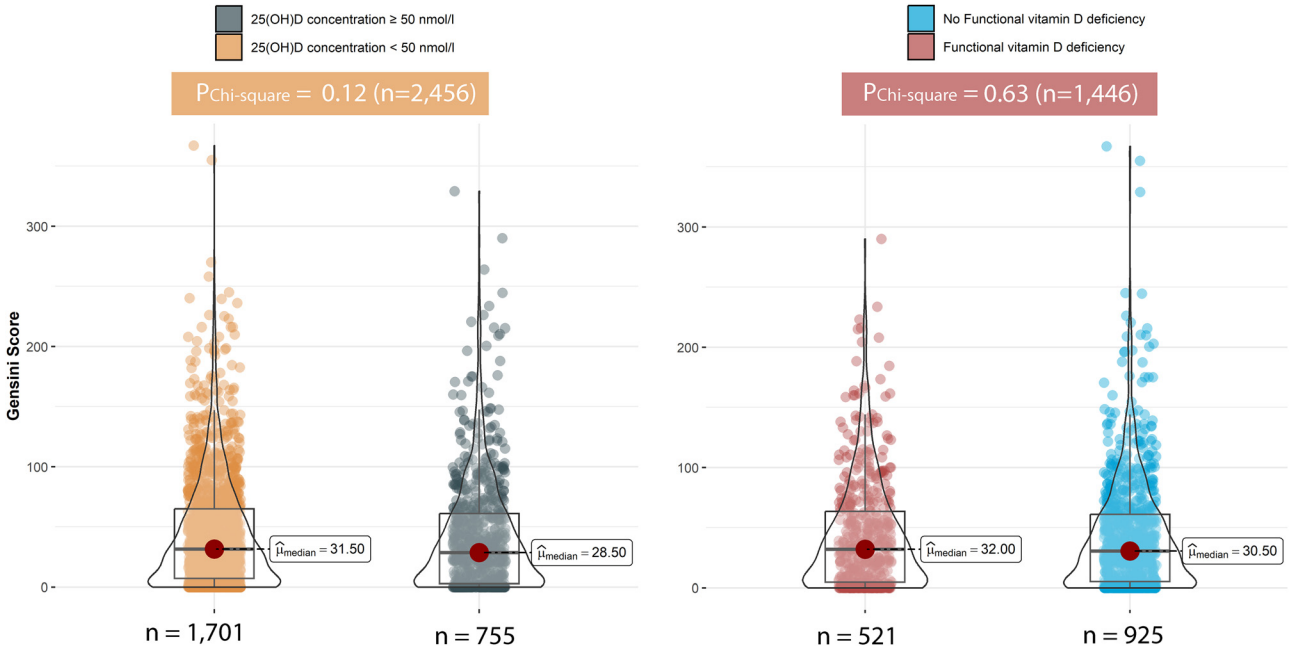

Figure 3: Gensini scores in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. The Gensini scores were calculated as the sum of all the adjusted coronary artery lesion scores of 15 coronary segments from coronary angiography, the exact algorithm for the calculation of single lesion scores was described elsewhere [28].

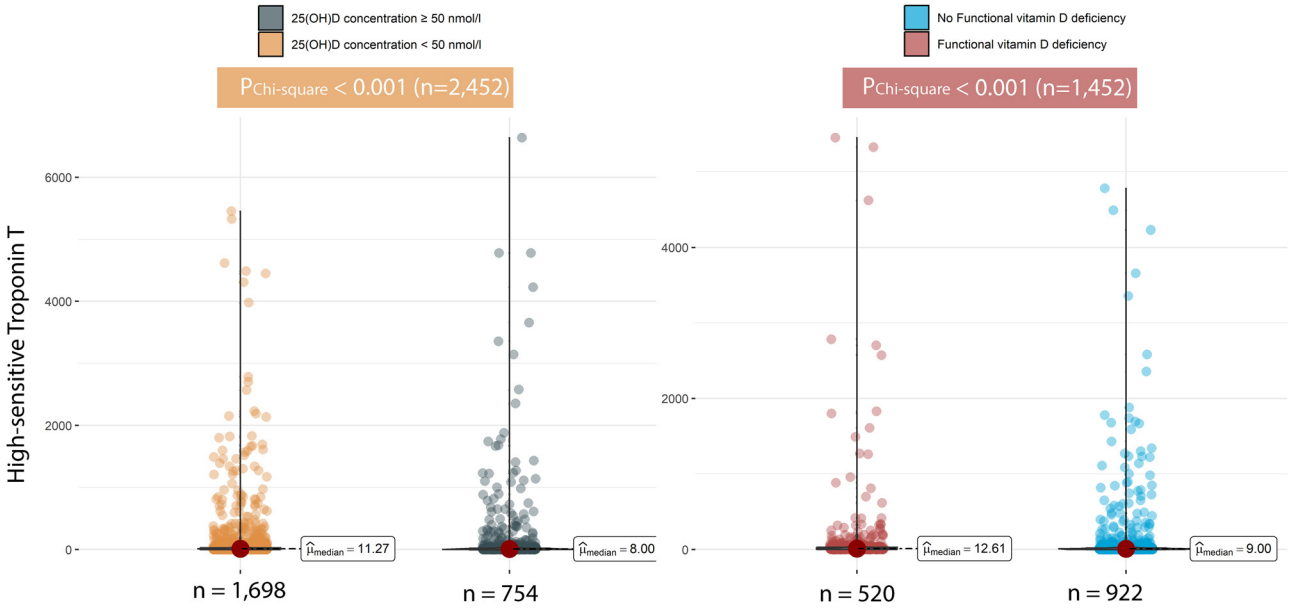

Figure 4: High-sensitive troponin T concentrations in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. High-sensitive troponin T concentrations in ng/L.

Vitamin D status and heart failure (HF)

With both diagnostic approaches, vitamin D deficiency was significantly more prevalent in participants with HF (Figure 5). The highest prevalence of vitamin D deficiency was observed in HF patients with reduced ejection fraction (HF_{rEF}). Likewise, NT-proBNP was significantly higher in participants with low 25(OH)D concentrations or functional vitamin D deficiency (Figure 6). The difference in NT-proBNP between vitamin D deficient and sufficient individuals was comparable with both diagnostic approaches.

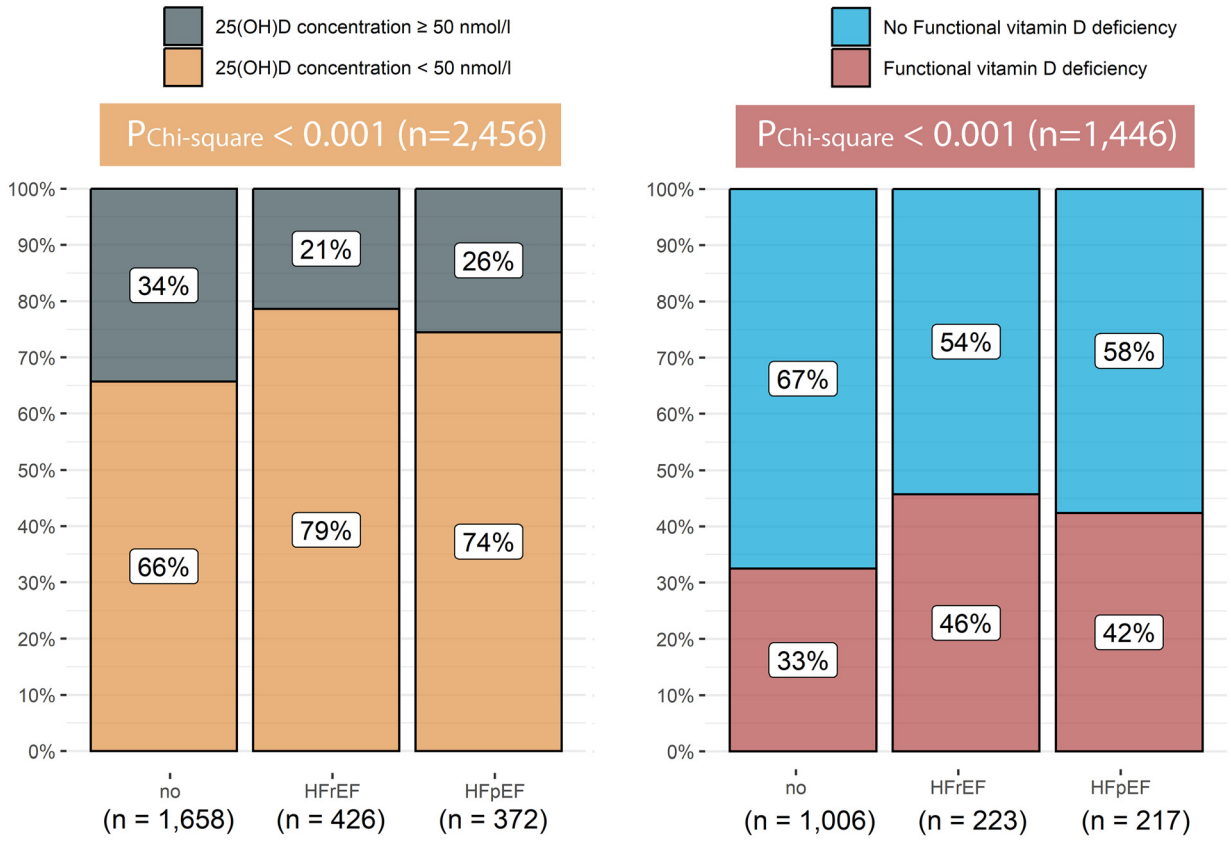

Figure 5: Prevalence of heart failure in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. HF: heart failure; HFrEF: heart failure with reduced ejection fraction; HFpEF: Heart failure with preserved ejection fraction.

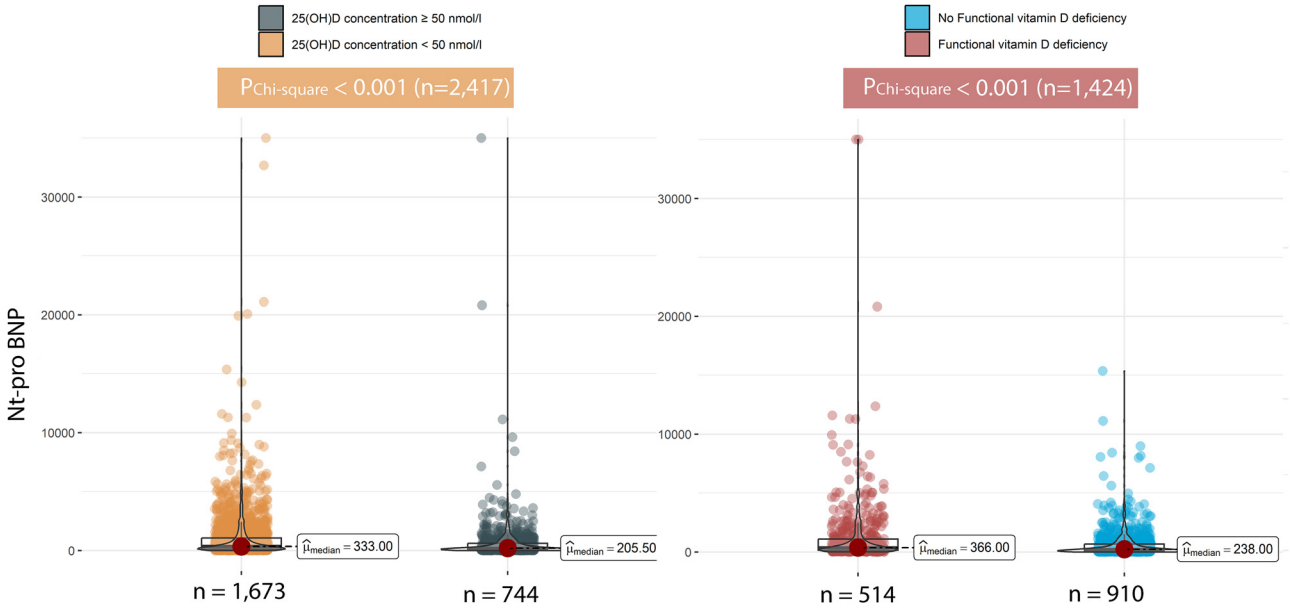

Figure 6: NT-proBNP concentrations in participants with and without 25(OH)D deficiency, and in individuals with and without low vitamin D metabolite profile. NT-proBNP: N-terminal pro B-type natriuretic peptide. NT-proBNP concentrations in pg/mL.

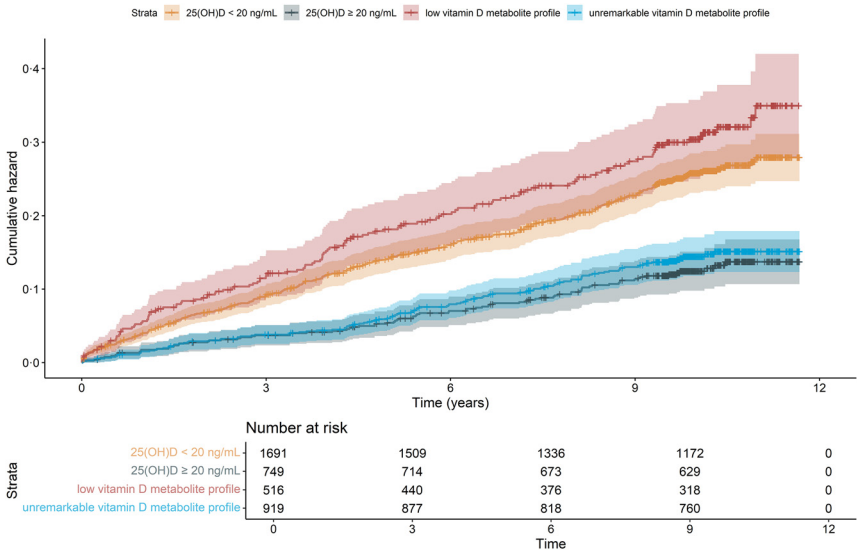

Figure 7: Cumulative hazard for cardiovascular mortality in the LURIC study. Cardiovascular mortality was 25.0 % (130/521) in individuals with a low vitamin D metabolite profile and 22.1 % (204/925) in individuals with an unremarkable vitamin D metabolite profile; 25(OH)D concentrations of <20 ng/mL (<50 nmol/L) were associated with a mortality of 21.6 % (367/1,701); 25(OH)D concentrations ≥20 ng/mL (>50 nmol/L) were associated with a mortality of 11.5 % (87/755). The x-axis displays the time in years elapsed since the baseline visit. A low vitamin D metabolite profile was defined as $24,25(OH)_2D$ <1.2 ng/mL (<3 nmol/L) and a VMR <4 % as calculated by multiplying the ratio of $24,25(OH)_2D/25(OH)D$ by 100.

Vitamin D status and CVD mortality

Over a median follow up-period of 9.9 years, a total of 720 participants died (23.6 %). The relationship between vitamin D status and all-cause mortality has been analysed previously [13]. 454 out of 720 (69.5 %) death cases were due to cardiovascular causes. In vitamin D deficient participants, CVD mortality was significantly higher than in those with vitamin D sufficiency (Figure 7). Individuals with replenished vitamin D reservoirs (25[OH]D ≥50 nmol/L) had a CVD mortality of 11.5 % (87/755), whereas functional vitamin D adequacy was associated with a CVD mortality of 16.7 % (324/1,935). In contrast, low vitamin D reservoirs (25[OH]D <50 nmol/L) were associated with a significantly higher CVD mortality of 21.6 % (367/1,701). With 25.0 % (130/521), CVD mortality was highest in individuals with functional vitamin D deficiency. However, the rather small difference in CVD mortality between individuals with a low 25(OH)D concentration and functional vitamin D deficiency was not significant (p=0.097).

Discussion

In LURIC, CVD mortality was nearly doubled in vitamin D deficient individuals. This increase in CVD mortality was observed with both, the traditional and the functional approach to establish vitamin D deficiency. Although CVD mortality was somewhat higher in individuals with functional vitamin D deficiency, the difference compared to individuals with low 25(OH)D concentrations was not significant. Despite the higher CVD mortality in vitamin D deficient individuals, the present results do not show a link between vitamin D status and CAD burden. For example, vitamin D deficiency was unrelated to the number of atherosclerotic coronary vessels and the degree of obstruction. In line with these observations, the median hs-cTnT concentration was only marginally higher in vitamin D deficient individuals compared to vitamin D sufficient individuals. The functional assessment of vitamin D status used here did not change this. Considering that functional vitamin D deficiency is associated with markedly increased all-cause mortality and alterations of bone metabolism [13], the present results argue against a mechanistic involvement of vitamin D in CAD. Nevertheless, it should be kept in mind that even below the 99th percentile of healthy individuals, small differences in cardiac troponin levels are related to CVD risk [31, 32]. Considering the rather small difference of hs-cTnT between vitamin D deficient and sufficient individuals in LURIC, a significantly larger cohort would be needed to substantiate potential effects of vitamin D in the development and progression of CVD.

The present results are in line with large cohort studies, such as the Atherosclerosis Risk in Communities (ARIC) study (n=11,311) that did not find significantly different hs-cTnT concentrations in participants with and without 25(OH)D deficiency [33]. Also, the number of individuals with elevated hs-cTnT was unrelated to 25(OH)D. Interestingly, in the same cohort, the change of hs-cTnT over 6 years of follow-up was associated to baseline 25(OH)D. However, this association was only significant in women and individuals younger than 56 years. The comparison of our results with those from ARIC is limited by the fundamentally different study cohorts. In ARIC, the participants are significantly younger, free from prevalent cardiovascular disease and include more females than LURIC. Further support for our findings in LURIC shown here comes from the prospective

community-based Cardiovascular Health Study (CHS, n=2,312), where 25(OH)D was neither related to hs-cTnT nor to electrocardiographic indices associated with CVD [34]. While large cohort studies, such as LURIC, ARIC and CHS do not support an association between 25(OH)D and the degree of CAD, some smaller studies reported significant relationships with the number of atherosclerotic coronary vessels [35], indices of coronary obstruction, such as the Gensini score [36], and slow coronary flow [37]. In LURIC, neither the Friesinger score nor the Gensini score were significantly associated with low 25(OH)D concentrations or functional vitamin D deficiency. However, the comparison with previous studies is hampered by their rather small sample size, different ethnicities of the participants and substantially different 25(OH)D concentrations. Furthermore, cardiac troponin or mortality were not reported. In contrast, the rather large cohort size of LURIC with 1701 25(OH)D deficient participants and 521 participants with functional vitamin D deficiency confers substantial strength to the present results.

While the degree of CAD was not associated with vitamin D deficiency in LURIC, both diagnostic approaches showed a significantly higher prevalence of vitamin D deficiency amongst HF patients. The highest number of vitamin D deficient individuals was seen in HF_{rEF}. A link between vitamin D deficiency and HF is further supported by higher NT-proBNP concentrations in vitamin D deficient individuals. The difference in NT-proBNP between vitamin D deficient and sufficient individuals was approximately 130 pg/mL with both approaches. Higher NT-proBNP concentrations in 25(OH)D deficient individuals have also been reported by others, but there, the association lost significance after adjustment for established confounders including age, sex, physical activity, smoking, hypertension and others [34]. Furthermore, van Ballegooijen and colleagues did not find significant associations of 25(OH)D with structural and functional parameters of HF, such as left ventricular mass, fractional shortening or the E/A-ratio as a surrogate of left ventricular outflow characteristics. Also in ARIC, 25(OH)D was associated with NT-proBNP in men, but not women. Per 1 SD higher 25(OH)D (21.2 nmol/L), the odds ratio of having an elevated NT-proBNP of >100 pg/mL increased by 17 %. This association remained significant after correction for a broad range of confounders. In 787 outpatients with diastolic dysfunction and HF, Nolte et al. found lower 25(OH)D levels to be associated with reduced functional capacity and an increased rate of cardiovascular hospitalizations [38]. Also, in a cross-sectional analysis of 870 elderly individuals (=>70 years) without prior myocardial infarctions, HF, or prevalent valvular disease, 25(OH)D was significantly associated with left ventricular endsystolic diameter, fractional shortening, and ejection fraction [39]. However, a longitudinal analysis in the same cohort failed to show significant relations between 25(OH)D and change in left ventricular geometry and function after 5 years. A retrospective analysis of 1,011 patients by Pandit et al. further adds to the inconclusive results of previous studies [40]. In their study, serum 25(OH)D was not associated with left ventricular diastolic dysfunction, an early manifestation of cardiac disease. In LURIC, structured echocardiographic information on cardiac morphology and function are not available. The inconclusive results of existing studies are further fuelled by intervention studies, such as VITAL. Two years of vitamin D3 supplementation (2,000 IU per day) did not result in significant changes in cardiac structure and function [41]. In line with this result, also the administration of active calcitriol for 48 weeks had no effect on left ventricular mass index [42]. Also NT-proBNP appears to be unaffected by the administration of vitamin D as shown by Schroten et al. [43] Administering 2,000 IU of vitamin D3 for six weeks did not change circulating NT-proBNP and fibrosis markers. On the other hand, various rodent models showed beneficial effects of vitamin D supplementation on cardiac remodeling and function, as well as the expression of atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and myocardial fibrosis in rats with chronic renal failure [44, 45]. In a meta-analysis of seven randomized controlled trials that investigated the effects of vitamin D on cardiovascular outcomes in patients with CHF, Jiang et al. found no significant effects on left ventricular ejection fraction, NT-proBNP, and performance in the 6 min walking test [46]. A more recent review of the literature by Busa et al. also concluded that existing studies failed to show beneficial effects of vitamin D supplementation on clinical outcomes in HF patients [47]. However, available studies differ vastly in their design, which limits comparability. Supplementation regimens from 2,000 IU daily up to 100,000 IU monthly have been used, and study duration varied from 6 weeks to 3.3 years.

An important indirect argument against a significant role of vitamin D in CVD and HF is the fact that the metabolic approach to diagnose vitamin D deficiency confirmed the results obtained with the traditional approach based on a low 25(OH)D concentration. In contrast, in the area of bone metabolism and all-cause mortality, the metabolic approach has shown superior specificity in identifying individuals with increased all-cause mortality risk and accelerated bone metabolism without compromising on sensitivity [13]. This leaves room for speculations that vitamin D is more a lifestyle marker that reflects other CVD risk factors rather than having a direct functional role in cardiovascular health. In a previous study, Thomas et al. reported lower HDL-cholesterol and higher

C-reactive protein and cystatin C concentrations in the 25(OH)D deficient participants of LURIC [48]. Moreover, vitamin D deficient participants had a significantly higher prevalence of CVD, peripheral vascular disease and diabetes. While all these differences could have contributed to a higher CVD mortality in vitamin D deficient individuals, they do not imply causality. Further support for this hypothesis comes from randomized, placebo controlled supplementation studies that also failed to show a reduction of CVD events and CVD death [16–18].

When interpreting our results in the context of the existing literature, it is important to consider the strength and weaknesses of LURIC. A main strength is the rather long follow-up period of 9.9 years and the high number of death cases, where the causes of death were classified by two physicians blinded to baseline characteristics of the participants by reviewing hospital records and death certificates. In addition, two different approaches to establish vitamin D deficiency were used that are based on the measurement of 25(OH)D and 24,25$(OH)_2$D by a validated LC-MS/MS method. The comprehensive phenotyping of LURIC participants through a thorough clinical examination and a coronary angiogram is another strength of this study. As LURIC included a mixed population of Caucasians without any acute or chronic illness except coronary artery disease, the results cannot easily be translated to the general population or other ethnicities, such as Africans or Asians. Furthermore, more specific information on HF-related structural and functional parameters are not available. Finally, follow-up visits for a re-evaluation of cardiovascular health have not been performed.

Conclusions

In summary, the present results show a link between vitamin D deficiency and CVD mortality, but not CVD burden. Moreover, vitamin D deficiency is more prevalent in HF patients and is associated with higher NT-proBNP concentrations. However, the functional approach to diagnose vitamin D deficiency does not discriminate better than the traditional approach between individuals with an increased cardiovascular risk.

Acknowledgments: No Artificial Intelligence (AI) or Machine Learning Tools were employed.

Research ethics: The LURIC study was in accordance with the Declaration of Helsinki and approved by the Ethics Committee at the Medical Association of Rheinland-Pfalz (Ärztekammer Rheinland-Pfalz).

Informed consent: Written informed consent was obtained from each study participant prior to inclusion.

Author contributions: All authors contributed substantially to the interpretation of data for the work, reviewed it critically, have accepted responsibility for the entire content of this manuscript and approved its submission.

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