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Serum 25-hydroxyvitamin D is associated with major cardiovascular risk factors and cardiac structure and function in patients with coronary artery disease

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Diastolic dysfunction

Abstract *Background and aims:* Vitamin D deficiency has been associated with increased risk for cardiovascular (CV) disease, but the possible effects of Vitamin D on cardiac structure and function are not well characterized.
Methods and results: The correlation between 25-hydroxyvitamin D levels and metabolic and cardiac echocardiographic parameters was studied in ARTEMIS study population including 831diabetic and 659 non-diabetic patients with stable coronary artery disease (CAD). Low levels of Vitamin D were associated with high BMI ($p < 0.001$), high total and LDL cholesterol and triglyceride levels ($p < 0.001$ for all) in both diabetics and non-diabetics. Among non-diabetic patients, low Vitamin D was also associated independently with elevated systolic and diastolic blood pressure ($p < 0.005$). Low Vitamin D levels were independently associated with reduced left ventricular (LV) ejection fraction ($p < 0.005$) and increased left atrial diameter ($p < 0.03$) measured by cardiac ultrasound by 2-dimensional echo. In the non-diabetic group, low Vitamin D levels were associated with impaired LV filling (high E/E′) ($p < 0.03$) and low E/A mitral flow pattern measured by Doppler echocardiography ($p < 0.05$). Among diabetics, low Vitamin D levels were also related to increased LV end-systolic diameter ($p < 0.05$) and right ventricular diameter ($p < 0.005$). The association between LV diastolic filling (E/E′) and Vitamin D levels was significant ($p < 0.01$) after adjustment for the commonly recognized risk factors of diastolic dysfunction in linear regression analysis.
Conclusions: Low Vitamin D is associated with several major cardiovascular risk factors and cardiac structural changes including impaired systolic and diastolic function, which together may explain the association of low Vitamin D to worse cardiovascular outcome.

Introduction

Mortality and cardiovascular events have been demonstrated to be higher in populations with decreased Vitamin D concentration [1–3]. Recent studies also suggest that patients with coronary artery disease (CAD) have generally lower levels of Vitamin D compared to healthy population

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[2] although much heterogeneity in the results connecting Vitamin D to cardiovascular disease exists. Furthermore, both animal [4] and human [5,6] studies have reported associations between Vitamin D levels and cardiac function and morphology. Higher Vitamin D levels have recently been associated with better left ventricular (LV) systolic function and smaller LV end-systolic diameter (LVESD) [7]. In addition, vitamin D analog therapy has been shown to reduce left atrial volume in patients with left ventricular hypertrophy and chronic kidney disease [8]. However, the data on the effects of Vitamin D on diastolic function in patients with CAD are limited. The low Vitamin D levels commonly observed among Type 2 diabetics [9] may be among the factors explaining their increased risk for cardiovascular disease (CVD) [9,10]. Diastolic dysfunction has been reported to be common among diabetic patients [11], and diabetic subjects have higher left ventricular mass index, higher left atrium volume and lower ejection fraction [12] than non-diabetics. The role of Vitamin D in explaining these differences is not known.

The early mechanisms behind poor cardiovascular outcome in patients with low levels of Vitamin D should be clarified. It is therefore worthwhile to investigate more thoroughly the possible effect of Vitamin D on cardiac remodeling, such as left ventricular hypertrophy, impaired systolic and diastolic function, all of which are associated with increased cardiovascular morbidity and mortality in various populations [13]. We therefore studied the correlation between plasma Vitamin D levels, cardiovascular risk factors and echocardiographic measures in a study population consisting of 1490 non-diabetic and diabetic patients with angiographically diagnosed CAD.

Methods

Subjects and study protocol

The ARTEMIS study, conducted in the Division of Cardiology of the Oulu University Hospital (Oulu, Finland), has been registered at ClinicalTrials.gov, Record 1539/31/06. The patients were selected from the angiography registry and were consecutive Type 2 diabetes (T2D) patients referred to coronary angiography at our hospital because of chest pain or acute coronary event. Risk profile analysis was performed 6 months after coronary angiography. Control subjects without known diabetes were selected from the same registry and were also consecutive patients referred to coronary angiography, and matched for major demographic or clinical factors affecting survival. The patients with a novel diagnosis of diabetes were excluded from the control cohort and moved into the diabetic group. Therefore, the number of diabetics is higher than non-diabetics.

The patients with and without T2D were matched in terms of age (<40 years, 40–50 years, 50–60 years, 60–70 years, 70–80 years), sex (1:1), history of recent (<3 months) myocardial infarction (1:1) and type of coronary intervention after angiography (1:1 revascularization, CABG or PCI). Patients with age <18 years, or >85 years, Type 1 diabetes (diabetes occurrence in childhood and adolescence, absolute deficiency of insulin secretion), impaired glucose tolerance (n = 314) or impaired fasting glucose (n = 103), NYHA class IV, permanent pacemaker or implantable cardioverter defibrillator (ICD), or significant valvular disease were excluded from this study, as were patients who had life expectancy <1 year. A diagram showing how many subjects were excluded is shown in Fig. 1. The study was performed according to the

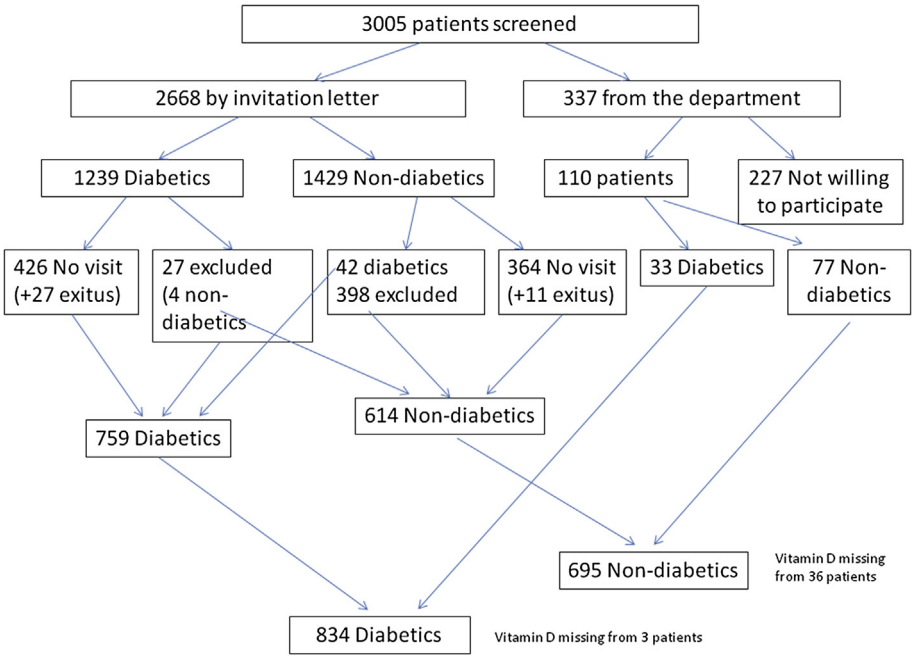

Figure 1 The bivariate correlation between E/E′ and vitamin D concentrations in diabetics, non-diabetics and all the subjects (Figure).

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Declaration of Helsinki, the protocol was approved by the local research ethics committee of the Northern Ostrobothnia Hospital District, and all the subjects gave written informed consent.

After fasting blood samples had been drawn, the subjects without known diabetes were given a 75-g glucose load. Both 1-h plasma and 2-h capillary glucose were determined, except from those previously known to be diabetic. T2D was verified according to current WHO criteria [14].

Blood samples and urine samples were obtained for the analysis of lipids, plasma glucose and glycated hemoglobin (HbA1c) levels. These analyses were performed using the Siemens Advia 1800 clinical chemistry system (Siemens Healthcare Diagnostics, Tarrytown, NY 10591). Hs-CRP, high-sensitivity C-reactive protein, was analyzed with the Siemens BN ProSpec immunonefelometry system (Siemens Healthcare Diagnostics, Tarrytown, NY 10591), insulin was analyzed with the Siemens Centaur XP immunoanalyzer (Siemens Healthcare Diagnostics, Tarrytown, NY 10591) and Vitamin D was assessed using the DiaSorin Liaison immunochemiluminometric assay (Diasorin S.p.A., Saluggia, Italy). Blood pressure was measured in a supine position after a 10-min resting period.

Echocardiographic measurements

Two-dimensional, M-mode and Doppler echocardiography were performed and interpreted according to the American Society of Echocardiography (ASE) guidelines by three cardiologists (O.-P.P., E.S.L. and J.L.) utilizing General Electric Vivid 7 ultrasound machine with its analysis program. Each echo was reviewed by only one out of three cardiologists. The echocardiographic parameters chosen for the present study were left ventricular mass index (LVMI), ejection fraction (EF) (EF (%) 2D-mode = EF measured in 2D-mode, EF (%) M-mode = EF measured in M-mode), E/E′ (ratio of early transmitral flow velocity to early diastolic mitral annulus velocity), E/A (ratio of Vmax of the E and A wave), LA (left atrium diameter), LA volume, RA (right atrium) diameter, Sint (velocity time integral of S wave), Dint (velocity time integral of D wave), LVEDD (LV end diastolic diameter), LVESD (LV end systolic diameter), RVEDD (right ventricular end diastolic diameter), isovolumic relaxation time (IVRT) and septal wall thickness. LVM was calculated using the formula recommended by the American Society of Echocardiography (ASE) (LV mass $= 0.8 \times (1.04\ ((\mathrm{LVIDd} + \mathrm{PWTd} + \mathrm{SWTd})^3 - (\mathrm{LVIDd})^3)) + 0.6$ g). LVMI was calculated by dividing LV mass with body surface area (BSA).

Statistical analyses

The SPSS (versions 19.0 and 21.0; SPSS, Inc., Chicago, IL, USA) statistical package was used in all statistical analyses. Analyses of mean values were performed using one-way analysis of variance (ANOVA), one-way analysis of covariance (ANCOVA) with Bonferroni correction for multiple and pairwise comparisons and with multivariable linear regression analyses. For ANOVA and ANCOVA analyses the subjects were divided into tertiles of Vitamin D levels. Log-transformed values were used as appropriate to normalize the skewed distributions whenever needed (HbA1c, fasting glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride concentration, insulin, ejection fraction measured in 2D mode, E/E′ and septal wall, E/A) $P < 0.05$ was regarded as statistically significant. Correlations were tested with Pearson's correlation. Comparison of frequencies between the groups was performed by the chi-square test.

Results

The current study is a subgroup analysis of the Artemis study. The study population consisted of 831 CAD patients with T2D and 659 stable CAD patients without T2D. More than 70% of the patients had undergone coronary intervention before entering the study. 31% of the subjects had experienced non-ST segment elevation and 18% ST segment elevation myocardial infarction. The prevalence of cerebrovascular disease was 29%. About 9% of the patients had cancer.

Table 1 The use of medications in non-diabetic (n = 659) and diabetic (n = 830) coronary artery disease patients

	n (%)		P-value
	Non-diabetics	Diabetics	
Medication			
Beta blockers	549 (83%)	745 (90.7%)	$p < 0.001$
ACEI/ARB	248 (34%)	361 (43.4%)	0.021
Statins	600 (91%)	754 (90.7%)	0.892
Anticoagulants	647 (98%)	812 (97.7%)	0.601
Calcium antagonists	110 (16.7%)	269 (32.4%)	$p < 0.001$
Nitrates	203 (30.8%)	371 (44.6%)	$p < 0.001$
Diuretics	141 (21.4%)	409 (49.2%)	$p < 0.001$
Liraglutide		3 (0.4%)	
Oral diabetes medication		458 (55.1%)	
Insulin		61 (7.3%)	
Insulin + oral Diabetes medication		152 (18.3%)	

ACEI, angiotensin conversion enzymes inhibitor; ARB, angiotensin II receptor blocker.
ACEI/ARB, patients using either ACEI or ARB medications.

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Table 2 Characteristics and cardiovascular risk factors of the non-diabetic subjects (n = 659) according to the vitamin D tertiles.

Vitamin D tertile (T)	Lowest T		Inter mediate T		Highest T		All subjects		P**	p†
Vitamin D concentration (nmol/l)	44.6	(8.4)	65.0	(4.9)	91.2	(14.9)	66.9	(21.6)		
n	220		220		219		659			
Age (years)	64.8	(9.1)	67.2	(8.5)	67.1	(8.5)	66	(9.0)	0.005	0.011
Gender M (%)	70.0		70.9		67.6		69.5		0.737	
BMI (kg/m^2)	27.6	(3.9)	26.8	(3.6)	25.4	(3.0)	26.6	(3.6)	$p < 0.001$	$p < 0.001^{1), 3)}$
Smokers, %	12		8		7		9		0.442	0.396
Waist	96.9	(10.9)	94.6	(11.3)	90.5	(10.1)	94.0	(11.1)	$p < 0.001$	0.1
Systolic BP (mmHg)	148.7	(22.7)	147.3	(25.7)	143.0	(25.6)	146.0	(24.0)	0.04	$0.004^{1)}$
Diastolic BP (mmHg)	82.5	(10.4)	80.7	(11.4)	78.4	(10.9)	81	(11.0)	$p < 0.001$	$0.001^{1)}$
HbA1c (%)	5.8	(0.4)	5.8	(0.4)	5.7	(0.3)	5.7	(0.4)	0.108	0.218
Fasting glucose (mmol/l)	5.4	(0.5)	5.4	(0.4)	5.3	(0.5)	5.4	(0.5)	0.096	0.35
Total cholesterol (mmol/l)	4.3	(0.9)	4.0	(0.8)	3.9	(0.7)	4.1	(0.8)	$p < 0.001$	$p < 0.001^{1), 2), 3)}$
LDL-cholesterol (mmol/l)	2.5	(0.8)	2.3	(0.8)	2.1	(0.6)	2.3	(0.8)	$p < 0.001$	$p < 0.001^{1), 3)}$
HDL-cholesterol (mmol/l)	1.3	(0.3)	1.35	(0.3)	1.4	(0.3)	1.4	(0.3)	0.005	0.220
Triglyserides (mmol/l)	1.4	(0.6)	1.2	(0.6)	1.0	(0.4)	1.2	(0.6)	$p < 0.001$	$p < 0.001^{1), 2)}$
Insulin	11.6	(8.4)	10.0	(5.6)	8.7	(5.0)	10.1	(6.6)	$p < 0.001$	0.073

Data are means (standard deviation). P-values obtained from ANCOVA analyses before (**) and after (†) adjustment for age, BMI (except for BMI), sex, and examination month. In post-hoc analyses significance between tertiles 1 and 3 ($^{1)}$), 1 and 2 ($^{2)}$) or 2 and 3 ($^{3)}$).
BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

First, we tested "vitamin D * diabetes" interactions in linear regression. Since this interaction term was significant for several of the echocardiographic parameters (LVMI, EF by M-mode, E/E′, E/A, LA diameter and volume, Sint, Dint, LVESD and septal wall, $p < 0.001$ for all), we considered diabetics and non-diabetics separately in all the analyses. Mean concentration of vitamin D in the group with normal glucose tolerance was 66.9 nmol/L and in the Type 2 diabetes mellitus group 60.4 nmol/L ($p < 0.001$). Vitamin D levels were lower among diabetics even after adjustment for body mass index (BMI), age, examination month and gender ($p = 0.003$). The mean age of the non-diabetic and diabetic groups was 66 and 67 years, respectively. The percentage of male patients was about 69% in both groups. Hypertension was more prevalent among diabetics (80%) than non-diabetics (55%) ($p < 0.001$). The mean duration of diabetes was 8.6 years. Considering medications, beta blockers ($p < 0.001$), angiotensin conversion enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) ($p = 0.002$), calcium antagonists ($p < 0.001$), nitrates ($p < 0.001$) and diuretics ($p < 0.001$) were more commonly used in the diabetic than in the non-diabetic study group (Table 1).

Concentrations of vitamin D and cardiovascular risk factors

Patients with normal glucose tolerance

When the Vitamin D tertiles were compared, patients with the lowest Vitamin D concentration were the youngest after adjustment for BMI, sex and Vitamin D measurement time (month) ($p = 0.011$). BMI was also strongly associated with Vitamin D concentration ($p < 0.001$) (Table 2) with the patients belonging to the highest Vitamin D tertile having the lowest BMI. In addition, patients with higher Vitamin D levels had lower systolic ($p = 0.004$) and diastolic ($p = 0.001$) blood pressure after adjustments. A significant association

Table 3 Characteristics and cardiovascular risk factors of the diabetic subjects (n = 830) according to the vitamin D tertiles.

Vitamin D tertile (T)	Lowest T		Inter mediate T		Highest T		All subjects		P**	p†
Vitamin D concentration (nmol/l)	38.7	(9.0)	58.2	(4.9)	84.4	(14.3)	60.4	(21.3)		
n	277		279		274		830			
Age (years)	66.1	(8.8)	67.1	(8.0)	68.3	(7.8)	67.2	(8.3)	0.006	0.158
Gender M (%)	73.6		68.5		65.7		69.2		0.121	
BMI (kg/m^2)	30.7	(5.2)	30.4	(4.6)	28.8	(4.5)	30.0	(4.9)	$p < 0.001$	$p < 0.001^{1), 3)}$
Smokers, %	11		10		5		9		0.938	0.624
Waist	105.3	(13.6)	105.6	(13.3)	101.1	(12.5)	104.0	(13.3)	$p < 0.001$	0.072
Systolic BP (mmHg)	146.4	(25.8)	149.5	(25.1)	147.2	(23.8)	147.7	(24.9)	0.322	0.388
Diastolic BP (mmHg)	81.3	(13.0)	81.3	(11.7)	79.6	(11.9)	81.0	(12.0)	0.157	0.331
HbA1c (%)	7.2	(1.4)	7.1	(1.2)	6.8	(0.97)	7.0	(1.2)	$p < 0.001$	$0.002^{1), 3)}$
Fasting glucose (mmol/l)	7.9	(2.4)	7.5	(2.1)	7.2	(1.8)	7.5	(2.2)	0.004	$0.015^{1)}$
Total cholesterol (mmol/l)	4.1	(1.0)	3.8	(0.8)	3.7	(0.7)	3.9	(0.9)	$p < 0.001$	$p < 0.001^{1), 2)}$
LDL-cholesterol (mmol/l)	2.4	(0.8)	2.2	(0.7)	2.1	(0.63)	2.2	(0.7)	$p < 0.001$	$p < 0.001^{1), 2)}$
HDL-cholesterol (mmol/l)	1.2	(0.3)	1.2	(0.3)	1.2	(0.3)	1.2	(0.3)	0.062	0.606
Triglyserides (mmol/l)	1.6	(1.1)	1.5	(0.8)	1.3	(0.5)	1.6	(0.9)	$p < 0.001$	$p < 0.001^{1), 2), 3)}$
Insulin	27.6	(30.5)	28.6	(48.4)	48.4	(371)	34.8	(216)	0.44	0.322

Data are means (standard deviation). P-values obtained from ANCOVA analyses before (**) and after (†) adjustment for age, BMI (except for BMI), sex, and examination month. In post-hoc analyses significance between tertiles 1 and 3 ($^{1)}$), 1 and 2 ($^{2)}$) or 2 and 3 ($^{3)}$).

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between Vitamin D and several plasma lipids was found in patients with normal glucose tolerance. The lowest total cholesterol, LDL cholesterol and triglyceride concentrations ($p < 0.001$ for all) were seen among the group with the highest levels of Vitamin D (Table 2). Patients in the highest Vitamin D tertile were more likely to have lipid medication ($p = 0.013$) than others. Otherwise, no differences in medications were observed between Vitamin D tertiles. The above associations between plasma lipids and Vitamin D levels persisted even when the differences in lipid medication were taken into account.

Patients with Type 2 diabetes

Considering patients with Type 2 diabetes, a similar association between high Vitamin D concentration and low BMI ($p < 0.001$) as in non-diabetics was observed after adjustment for covariates (Table 3). Furthermore, Vitamin D concentrations were associated with metabolic control. HbA1c and fasting glucose were the lowest among the patients who belonged to the highest (3rd) Vitamin D tertile. Differences between Vitamin D tertiles and HbA1c were significant before ($p < 0.001$) and after ($p = 0.002$) adjustments. Analysis of covariance for fasting glucose was significant after ($p = 0.015$) adding the same covariates into the model. Total cholesterol, LDL cholesterol and triglycerides associated significantly with Vitamin D concentrations ($p < 0.001$ after adjustments), with the lowest concentrations seen in patients in the highest Vitamin D tertile. Patients in the highest tertile were more likely to have lipid-lowering medication ($p = 0.001$) and anticoagulants ($p = 0.018$). Otherwise, no association between medication and Vitamin D concentration was found. Again, the above associations between plasma lipids and Vitamin D levels persisted even when the differences in lipid medication were taken into account.

Concentrations of vitamin D and echocardiographic parameters

Patients with normal glucose tolerance

Table 4 shows the echocardiographic measurements according to Vitamin D tertiles in patients with normal glucose tolerance. The lowest EF (%) M-mode was seen in the lowest Vitamin D tertile ($p = 0.007$). The latter association persisted after adjustment for age, BMI, sex and Vitamin D measurement month ($p = 0.005$). Also, the E/E′ and E/A ratios (reflecting left ventricular diastolic function) were affected by the changes in Vitamin D levels so that the highest Vitamin D levels were associated with the lowest E/E′ ratio and the highest E/A ratio. In ANCOVA analysis the association was still evident for E/E′ ($p = 0.028$) and E/A ($p = 0.030$) after the covariates (same as above) were taken into account. Left atrium diameter was smaller in the group of patients with the highest as compared to those with the lowest Vitamin D tertile (Table 4). The significance was different on both a univariable ($p = 0.001$) and multivariable ($p = 0.026$) adjusted basis. A similar association was seen in LA volume before ($p = 0.001$) adjustments, but when age, BMI, sex and Vitamin D measurement time were added to the same model, the association was non-significant ($p = 0.088$). In the non-diabetic study group Dint height was the highest among the patients with the highest concentrations of Vitamin D (3rd tertile) ($p = 0.002$).

Table 4 Echocardiographic parameters on the non-diabetic subjects (n = 659) according to the vitamin D tertiles.

Vitamin D tertile (T)	Lowest T		Inter- mediate T		Highest T		All subjects		p**	p†
n	220		220		219		659			
LVMI (g/m^2)	105.1	(25.4)	108.5	(25.5)	103.8	(23.9)	106.0	(25.0)	0.126	0.219
HR mean	66.8	(8.8)	65.5	(8.5)	65.1	(7.3)	65.8	(8.2)	0.092	0.39
EF (%) 2D-mode	64.5	(8.5)	64.4	(8.3)	64.5	(7.2)	64.5	(8.0)	0.97	0.73
EF (%) M-mode	64.4	(10.2)	66.5	(9.1)	67.0	(8.5)	65.9	(9.3)	0.007	0.005[1),2)]
E/E′	10.3	(3.8)	10.0	(3.5)	9.5	(3.2)	9.9	(3.5)	0.073	0.028[1)]
E/A	1.6	(0.59)	1.6	(0.6)	1.7	(0.9)	1.6	(0.7)	0.037	0.030[1)]
LA (mm)	41.6	(6.1)	40.7	(5.8)	39.4	(5.9)	40.6	(6.0)	0.001	0.026[1)]
LA volume (cm^3)	21.1	(4.2)	20.5	(4.8)	19.5	(4.5)	20.3	(4.6)	0.001	0.088
RA (mm)	16.1	(3.9)	16.3	(4.0)	16.3	(4.5)	16.2	(4.1)	0.9	0.46
Sint (mm)	18.9	(4.1)	19.5	(4.8)	19.3	(4.6)	19.2	(4.5)	0.33	0.35
Dint (mm)	11.6	(3.1)	12.5	(3.4)	12.6	(3.4)	12.2	(3.3)	0.004	0.002[1), 2)]
LVEDD (mm)	50.7	(5.9)	50.9	(5.7)	50.1	(5.5)	50.6	(5.7)	0.281	0.65
LVESD (mm)	32.6	(6.4)	32.0	(6.0)	31.2	(5.5)	32.0	(6.0)	0.038	0.22
RVEDD (mm)	27.1	(5.2)	27.7	(4.9)	26.9	(4.8)	27.2	(5.0)	0.21	0.45
IVRT (ms)	101.4	(19.2)	100.0	(21.7)	98.5	(21.6)	99.9	(20.9)	0.36	0.163
Septal wall (mm)	10.8	(1.8)	10.9	(1.8)	10.6	(1.9)	10.8	(1.8)	0.214	0.732

Data are means (standard deviation *). P-values obtained from ANCOVA analyses before (**) and after (†) adjustment for age, BMI (except for BMI), sex, and examination month. In post-hoc analyses significance between tertiles 1 and 3 ([1)]), 1 and 2 ([2)]) or 2 and 3 ([3)]).
EF (%) 2D-mode = ejection fraction measured in 2D-mode, EF (%) M-mode = ejection fraction measured in M-mode, E/A = Ratio where E is E wave in mitral inflow pattern and A is A wave in mitral inflow pattern, both measured by pulse wave Doppler, LA = left atrium diameter, LA volume = left atrium volume, RA = right atrium diameter, Sint = S wave in pulmonary venous flow pattern, measured by pulse wave Doppler, Dint = D wave in pulmonary venous flow pattern, measured by pulse wave Doppler, LVEDD = left ventricular end diastolic diameter, LVESD = left ventricular end systolic diameter, RVEDD = right ventricular end diastolic diameter, IVRT = isovolumic relaxation time AMI, acute myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST-elevation myocardial infarction; PCI, percutaneous coronary intervention CABG, coronary artery by-pass grafting; E/E′, ratio of early transmitral flow velocity to early diastolic mitral annulus velocity.

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Table 5 Echocardiographic parameters on the diabetic subjects (n = 830) according to the vitamin D tertiles.

Vitamin D tertile (T)	Lowest T		Inter- mediate T		Highest T		All subjects		p**	p†
n	277		279		274		830			
LVMI (g/m^2)	107.8	(29.0)	111.4	(27.5)	109.5	(26.9)	110.0	(28.0)	0.322	0.301
HR mean	70.6	(9.8)	69.1	(9.4)	68.6	(9.5)	69.4	(9.6)	0.036	0.16
EF (%) 2D-mode	62.9	(10.7)	63.7	(10.2)	63.9	(9.8)	63.5	(10.3)	0.47	0.511
EF (%) M-mode	60.9	(11.3)	63.2	(11.8)	64.9	(9.9)	63.0	(11.1)	$p < 0.001$	0.003[1)]
E/E′	11.5	(4.1)	11.4	(4.4)	11.2	(4.1)	11.4	(4.1)	0.72	0.241
E/A	1.4	(0.8)	1.5	(0.7)	1.5	(0.5)	1.50	(0.7)	0.715	0.57
LA (mm)	44.0	(6.1)	43.4	(6.3)	41.9	(6.3)	43.1	(6.3)	$p < 0.001$	0.008[1)]
LA volume (cm^3)	22.7	(5.5)	22.0	(5.5)	20.6	(5.1)	21.7	(5.4)	$p < 0.001$	0.015[1)]
RA (mm)	16.7	(4.8)	16.7	(5.0)	16.6	(4.7)	16.7	(4.9)	0.98	0.32
Sint (mm)	17.6	(5.0)	17.6	(5.2)	18.6	(5.3)	17.9	(5.2)	0.035	0.10
Dint (mm)	11.1	(3.6)	11.8	(3.6)	11.9	(3.5)	11.9	(3.6)	0.019	0.015[1),2)]
LVEDD (mm)	50.8	(7.3)	50.5	(6.7)	50.0	(6.3)	50.3	(6.8)	0.11	0.83
LVESD (mm)	34.2	(7.9)	33.1	(7.6)	31.8	(6.5)	33.0	(7.4)	0.001	0.048[1)]
RVEDD (mm)	26.5	(4.9)	27.7	(5.5)	27.3	(5.2)	27.2	(5.2)	0.029	0.002[1), 2)]
IVRT (ms)	101.2	(24.5)	99.4	(24.0)	97.7	(22.0)	99.4	(23.5)	0.243	0.062
Septal wall (mm)	11.4	(2.1)	11.8	(2.3)	11.5	(2.1)	11.6	(2.2)	0.098	0.101

See Table 4 for abbreviations.
Data are means (standard deviation *). P-values obtained from ANCOVA analyses before (**) and after (†) adjustment for age, BMI (except for BMI), sex, and examination month. In post-hoc analyses significance between tertiles 1 and 3 ([1)]), 1 and 2 ([2)]) or 2 and 3 ([3)]).

Patients with Type 2 diabetes

As in the case of patients with normal glucose tolerance, association of EF (%) M-mode with Vitamin D concentrations was also observed in the Type 2 diabetic group (Table 5). The variation of ejection fraction between tertiles was significant ($p < 0.001$). The inclusion of BMI, age, gender and examination time as covariates to the analysis did not change the association to non-significant level ($p = 0.003$). Left atrium diameter was the largest in patients who had the lowest levels of Vitamin D ($p = 0.008$) after the covariates were considered (Table 5). Identically, left atrium volume varied in the same direction ($p = 0.015$). The highest D-wave (Dint) of pulmonary venous flow pattern was related to the highest Vitamin D level before ($p = 0.019$) and after ($p = 0.015$) covariates were taken into account. The highest S-wave (Sint) of pulmonary venous flow pattern showed significant association with the highest Vitamin D tertile before ($p = 0.035$), but not after ($p = 0.10$) adjustment for covariates. LVESD was the largest ($p = 0.048$) and RVEDD the smallest (0.002) among the patients who had the lowest concentrations of Vitamin D.

The possible role of different treatments (specifically beta blockers, ACE inhibitors, statins) on cardiovascular risk factors, cardiac structural changes, vitamin D status and glucose control in diabetics subgroup was also evaluated. The results on these analyses were negative.

Linear regression model for the variation of diastolic dysfunction

We also performed multivariable linear regression model (Table 6) for E/E′ by combining diabetic and non-diabetic population. We included study group (diabetics vs. non-diabetics), age, sex, BMI, mean sitting systolic blood pressure together with Vitamin D levels and examination month of Vitamin D in the model. As expected, study group, age, BMI, sex and systolic blood pressure were associated with E/E′. Vitamin D levels were independently associated with E/E′ ($p < 0.01$) when all the above covariates were included in the model.

E/E′ correlated significantly with vitamin D concentrations in all the patients ($p = 0.004$) (Fig. 2). The effect was seen in non-diabetics ($p = 0.032$) but not in diabetics.

Discussion

The results of the present study show that in both groups, low Vitamin D levels were independently associated with lower ejection fraction measured in M-mode. In the non-diabetic group, low Vitamin D levels were associated with markers of

Table 6 Linear regression model explaining the variation of E/E′.

Variables	Unstandardized regression coefficient	Standardized regression coefficient	R-square change	T-value	*P*
Study group	−1.066	−0.136	0.034	−5.220	<0.001
Age, years	0.114	0.246	0.080	9.911	<0.001
BMI, kg/m^2	0.059	0.069	0.011	2.616	0.009
Sex, 1 = male, 2 = female	1.041	0.123	0.036	4.973	<0.001
Systolic BP, mmHg	0.025	0.155	0.051	6.311	<0.001
Vitamin D examination month	−0.009	−0.009	0.000	−0.360	0.719
Vitamin D level, nmol/l	−0.012	−0.068	0.005	−2.733	0.006

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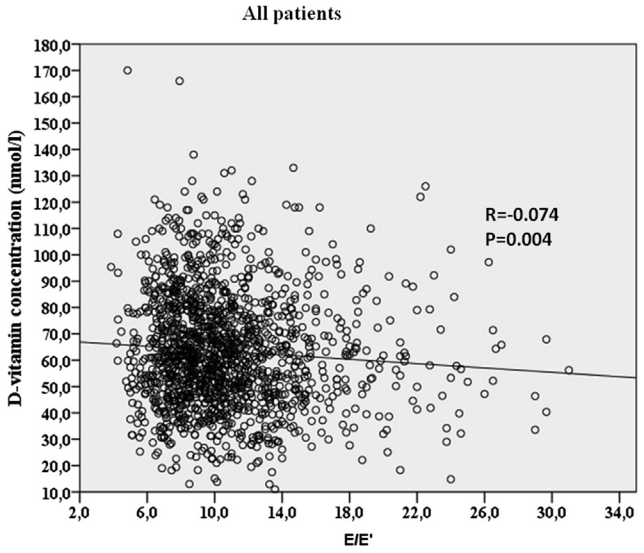

Figure 2 The bivariate correlation between E/E0 and vitamin D concentrations in diabetics, non-diabetics and all the subjects (Figure).

impaired diastolic filling (high E/E′, high E/A). Diabetics in our study were more obese and had lower levels of Vitamin D than non-diabetics as reported earlier [15]. An inverse association between circulating Vitamin D levels and the risk of Type 2 diabetes is a common finding in earlier studies [9,16,17]. Previous reports have suggested Vitamin D levels to be positively associated with a more favorable lipoprotein profile [18], as in our study. In the non-diabetic group, an association between high blood pressure and low Vitamin D was seen, as in earlier studies [19,20].

Cardiac muscle is found to have vitamin D receptors (VDR) and it has been hypothesized that activation of VDR might have beneficial effects on cardiac function in animals [4,21]. We did not see any association between Vitamin D levels and cardiac hypertrophy, which has been seen in some [22–24] but not all [25] studies. However, in the present study, low Vitamin D levels were independently associated with systolic dysfunction in both study groups, but with diastolic dysfunction in the whole study group and among non-diabetics. In animal studies, a direct role for Vitamin D in regulating cardiac contractility has been suggested [26]. In human data, vitamin D deficiency has been linked to the presence of heart failure [27]. However, only few studies have addressed the influence of Vitamin D levels on diastolic dysfunction. The results of the PIVUS and our study showed that higher circulating vitamin D concentrations were associated with better LV systolic function and smaller LVESD [7]. The Hoorn study [28] showed that serum levels of 25(OH) D were not significantly associated with LV structure and function. In the Tromsø Study increased serum 25(OH) D was not associated with better LV systolic function [25]. Recently, like in our non-diabetics, serum levels of 25(OH) D were significantly associated with LV diastolic dysfunction [29] in 281 patients referred to coronary angiography for stable angina pectoris. The causal relationship of Vitamin D with cardiac dysfunction and cardiovascular disease remains highly controversial. The heterogeneity of studies may be caused by different definitions of vitamin D status, age structures, definition and determination of cardiovascular endpoints and other confounding factors. Support from randomized controlled trials for a beneficial effect of Vitamin D on CVD risk is still lacking.

A recent cross-sectional study by Pandit et al. [30] did not find any association between Vitamin D levels and LV diastolic dysfunction. In the latter study patients were unselected and the specific reasons for obtaining Vitamin D levels and echocardiographic examination were not evaluated. Our study population differs from that in the study by Pandit since we had patients with CAD. Our profiling of cardiovascular risk factors was also comprehensive. Thus, our work is an original contribution in the field. Our study patients had relatively low prevalence of absolute vitamin D deficiency. This may be explained by the fact that CAD patients have met medical experts more often than general population and have therefore received lifestyle instructions more often. The Vitamin D status of Finnish adult population has improved in recent years although about one third of Finns are still Vitamin D deficient [31]. The positive association of vitamin D concentrations with age, observed also earlier [31], was still evident after adjustment for BMI in non-diabetic subjects but not in diabetics. It is possible that older Finns get more vitamin D from the diet and also from the sun by spending more time outdoors and by traveling. Therefore, lifestyle factors may play a role in non-diabetic population in explaining the positive relation between vitamin D and age. Unfortunately information on lifestyle habits, calcium intake or vitamin D supplementation is not available in our study being a limitation of the present investigation.

In conclusion, in CAD patients, low Vitamin D may be an indicator of increased cardiovascular risk and systolic and diastolic dysfunction. In future studies, it is an important issue to clarify whether CAD patients would benefit from Vitamin D supplementation.

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