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A significant positive association of vitamin D deficiency with coronary artery calcification among middle-aged men: for the ERA JUMP study

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Abstract

Objective—Although a significant positive association of vitamin D deficiency with coronary heart disease has been demonstrated in cross-sectional as well as prospective studies, only a few studies have examined the association of vitamin D deficiency with subclinical atherosclerosis. We examined whether vitamin D deficiency is associated with subclinical atherosclerosis, as measured by coronary artery calcification (CAC) in asymptomatic adults.

Methods—In a population-based cross-sectional study, 195 men aged 40 to 49 years without cardiovascular disease were randomly selected (98 Caucasian and 97 Japanese-American men). A liquid-chromatography-tandem-mass-spectrometry was utilized to measure serum vitamin D. CAC was examined by electron-beam-computed-tomography using standardized protocols and read centrally at the University of Pittsburgh using the Agatston's methods. To investigate an association between vitamin D deficiency (defined as 25(OH)D<20 ng/mL) and CAC (defined as Agatston score ≥10), we utilized multivariable logistic regression models.

Results—Prevalence of CAC and vitamin D deficiency was 27.2% and 10.3%, respectively. Participants with CAC were significantly older, had significantly higher BMI, and had higher rates

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of smoking. Those with CAC were 3.31 times likely to be vitamin D deficient, after adjusting for traditional cardiovascular risk factors (OR=3.31, 95%CI 1.12-9.77).

Conclusions—In this population-based study of healthy middle-aged men, vitamin D deficiency had a significant positive association with the presence of CAC.

Keywords

vitamin D deficiency; atherosclerosis; coronary artery calcification; epidemiology

INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in the U.S.[1] The major underlying cause of CHD is atherosclerosis.[1] Coronary artery calcification (CAC), a non-invasive and highly reliable method to measure coronary atherosclerosis, is positively and independently associated with future CHD.[2-4]

Recent epidemiological evidence showed that vitamin D deficiency is associated with increased risk of CHD [5-10] and CHD risk factors [9-21]. A meta-analysis of twenty-four prospective studies showed an inverse linear association between vitamin D ranged 20-60 nmol/L and CVD risk.[10] The Multi-Ethnic Study of Atherosclerosis (MESA) demonstrated a high risk of developing CHD events associated with vitamin D deficiency.[6] In cross-sectional studies and a meta-analysis of prospective studies, vitamin D deficiency has been demonstrated to increase the risk of hypertension, glucose intolerance, and diabetes mellitus [9-21].

Despite the evidence linking vitamin D deficiency to CHD, the findings on the association between vitamin D deficiency and CAC, remain inconclusive.[5, 22, 23] Michos et al.[22] and Pilz et al.[23] reported no significant association of vitamin D deficiency with CAC, whereas Lim et al. showed significant trends between CAC and vitamin D (<15, 15-29, 30 ng/ml).[5]

Moreover, previous studies between vitamin D deficiency and CAC were conducted in diseased individuals such as diabetes,[24, 25] or elderly individuals.[5, 22] No previous studies have examined the association in healthy middle-aged populations. Therefore, we aimed to examine whether vitamin D deficiency is associated with subclinical atherosclerosis as measured by CAC among 195 healthy men aged 40 to 49 years, in the ‘Electron-Beam Tomography, Risk Factor Assessment among Japanese and U.S. Men in the Post-World War II Birth Cohort’ (ERA-JUMP) study.

MATERIALS AND METHODS

Study population

Detailed descriptions of the ERA-JUMP study population and measurements have been previously published.[26] Briefly, between 2002 and 2006, 613 men aged 40 to 49 years, were randomly selected from two study centers (310 white men from Pennsylvania, U.S. were randomly enrolled from the voter registration list of Allegheny County; 303 Japanese-

Americans men from Honolulu, Hawaii, U.S. were randomly selected among the offspring of the participants of the Honolulu Heart Program. These Japanese Americans were third or fourth generations of Japanese without ethnic admixture.[27]) All participants were without cardiovascular or other severe diseases. For this vitamin D study, 200 men were randomly selected from two centers (100 Caucasians and 100 Japanese Americans). After excluding five participants due to incomplete data, the final sample was 195 men (98 Caucasians and 97 Japanese Americans). All participants signed an informed consent. This study was approved by the Institutional Review Boards of the two institutions (the University of Pittsburgh, Pittsburgh, U.S. and the Kuakini Medical Center, Honolulu, U.S.).

Body weights and heights were measured in all participants, while they were wearing light clothing without shoes. Body-mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Blood pressure in the right arm of the seated participants was assessed, after participants emptied their bladder and sat quietly for five minutes, using an appropriate-sized cuff, with an automated sphygmomanometer (BP-8800, Colin Medical Technology, Komaki, Japan). Blood pressure was measured twice and the average of the two measurements was used.

Venipuncture was performed after 12-hour fasting. The blood samples were stored at −80°C and then shipped on dry ice to the Heinz Laboratory, University of Pittsburgh, to measure lipids, glucose, insulin, and fatty acids. The protocols standardized by the Centers for Disease Control and Prevention were utilized to measure serum lipids.[28] Gas-capillary-liquid chromatography (PerkinElmer Clarus 500; PerkinElmer, Waltham, MA) was used to measure serum fatty acids in the percentage unit of total fatty acid amount.[29] Marine n-3 fatty acids were defined as the sum of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3).[29] C-reactive protein (C-RP) was measured at the University of Vermont by a colorimetric competitive enzyme-linked immunosorbent assay.[30]

A self-administered questionnaire was utilized to obtain information on demography and other factors. Current smokers were defined as individuals who smoked cigarettes during the prior month. Pack-years of smoking was calculated as the number of years of smoking multiplied by the number of cigarettes and then divided by 20. Ethanol consumption for alcohol drinkers was calculated with alcohol types and amount of drinking (i.e., grams per day). Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or use of anti-hypertensive medications. Diabetes mellitus was defined as fasting glucose level ≥7 mmol/L (126 mg/dL) or use of anti-diabetic medication.

Vitamin D measurement

Serum 25(OH)D levels were measured at the Mayo medical laboratory with liquid chromatography-tandem mass spectrometry (LC-MS/MS) (ThermoFisher Scientific, Franklin, Massachusetts 02038 and Applied Biosystems-MDS Sciex, Foster City, CA 94404), including both 25(OH) $VitD_2$ and 25(OH) $VitD_3$.[31] Circulating serum 25(OH)D, which reflects total levels of vitamin D from both sun exposure as well as dietary intake, is widely used to measure vitamin D levels in the body.[32] The intra-assay coefficients of variation (CV) of 25(OH)D_2 were 4.4%, 3.3%, and 4.2% at 14ng/mL, 41ng/mL, and

124ng/mL, respectively. The inter-assay CVs were 6.1%, 6.2%, and 4.7% at 15ng/mL, 43ng/mL, and 128 ng/mL, respectively.[31] As for 25(OH)D_3, the intra-assay CVs were 3.8%, 2.4%, and 4.7% at 25ng/mL, 54ng/mL, and 140 ng/mL, respectively. The inter-assay C.Vs were 6.4%, 6.8%, and 5.0% at 24ng/mL, 52ng/mL, and 140 ng/mL, respectively.[31] Vitamin D deficiency was defined as less than 20 ng/mL (50 nmol/L) of 25(OH)D.[22, 33-36]

Coronary artery calcification (CAC)

CAC was measured with electron-beam-computed tomography (EBCT) (Imatron C150, GE Medical Systems, South San Francisco, U.S.) as previously reported.[37] Using a standardized protocol, scanners were regularly calibrated. The scanned images were sent to the Cardiovascular Institute, University of Pittsburgh. One trained reader read the image using a Digital-Imaging-and-Communications-in-Medicine workstation and Accu-Image software (AccuImage Diagnostic Corporation, San Francisco, USA) without any information of each participant's characteristics or the study centers. The Agatston scoring method was used to calculate the coronary calcium score.[38] To define the presence of CAC, a coronary calcium score ≥10 was used.[29] The reproducibility of the Agatston calcium scores had an intra-class correlation of 0.98.[39]

Statistical analyses

In order to compare general characteristics of risk factors between CAC ≥10 and CAC <10 and between vitamin D deficient and sufficient groups, t-test for continuous variables and Chi-square tests for categorical variables were used. After confirming no interaction according to centers (p=0.8677), we pooled the data of Pittsburgh and Honolulu. Taking into account seasonal variations of sun exposure that might have affected to vitamin D exposure, [40] we included the collection date of serum samples for 25(OH)D measurements in our models. To test the association between vitamin D deficiency and CAC, multivariable logistic regression models were established as followings: the crude model was adjusted for center and collection date of 25(OH)D; the Model 1 was further adjusted for age (continuous), BMI (continuous, kg/m^2), smoking (pack-years), drinking (ethanol consumption), C-RP (continuous), and triglycerides (continuous); the Model 2 was additionally adjusted for hypertension (yes/no) and diabetes (yes/no); the Model 3 was further adjusted for marine n-3 fatty acids (continuous). We adjusted for marine n-3 fatty acids, because fish is the major dietary source of vitamin D.[35] All reported *P*-values were based on two-sided tests with the significance of less than 0.05. All statistical analyses were performed using SAS 9.2 for Windows (SAS Institute, Inc., Cary, North Carolina, U.S.).

RESULTS

Comparisons of the general characteristics among the study participants

Table 1 shows prevalence of CAC and the general characteristics of study participants. The prevalence of CAC ≥10 was 27.2% (n=53). As compared to participants with CAC <10, those with CAC ≥10 were significantly older, had higher BMI, and higher pack-years of smoking. Those with CAC ≥ 10 tended to have higher levels of systolic and diastolic blood

pressure, triglycerides, total cholesterol, and higher prevalence of alcohol drinkers, though not statistically significant.

Comparisons of risk factors between vitamin D deficient and sufficient groups

Table 2 presents the comparisons of risk factors between the vitamin D deficient and sufficient groups of the study participants. Prevalence of vitamin D deficiency was 10.3% (n=20). Participants with vitamin D deficiency were significantly older compared to those with vitamin D sufficiency. Additionally, those with vitamin D deficiency were more likely to have higher systolic and diastolic blood pressure, high levels of alcohol consumption, than those with vitamin D sufficiency.

Odds ratios of coronary artery calcification associated with vitamin D deficiency

Table 3 lists the estimated odds ratios and 95% confidence intervals of CAC associated with vitamin D deficiency from multivariable logistic models. After adjusting for center, collection date of 25(OH)D, age, BMI, pack-years of smoking, ethanol consumption, C-RP, and triglycerides in the model I, participants with CAC ≥10 were 3.01 times more likely to be vitamin D deficient, as compared to those with CAC <10 (OR=3.01, 1.03-8.75). After further adjusted for hypertension and diabetes, the estimated association remained significant (OR=3.13, 1.07-9.15). In the final model, after further adjusting for serum marine n-3 fatty acids, compared to participants with CAC <10, those with CAC ≥10 were 3.31 times more likely to be vitamin D deficient, even after adjusting for traditional cardiovascular and other risk factors (OR=3.31, 1.12-9.77).

DISCUSSION

In this population-based study of healthy middle-aged men, vitamin D deficiency had a significant positive association with coronary atherosclerosis as measured with CAC. This association remained significant after adjusting for traditional cardiovascular risk and other factors, including age, BMI, smoking (pack-year), drinking (ethanol consumption), C-RP, triglycerides, hypertension, diabetes, and marine n-3 fatty acids. To our knowledge, this is the first study to examine the association between vitamin D deficiency and CAC in healthy middle-aged population.

Our finding of a significant positive association between vitamin D deficiency and CAC is consistent with some [5, 7] but not all previous results [22, 41]. In accordance with our study, a population-based cohort study among 921 elderly aged 65 and over demonstrated significant trends in the frequency of CAC according to vitamin D groups (<15.0, 15.0-29.9, 30.0 ng/ml).[5] Similarly, another study showed that $1,25(OH)_2D$ had an inverse association with CAC among 173 patients with high risk for cardiovascular disease.[42] Furthermore, the MESA reported that incident CAC was significantly associated with low 25(OH)D in 397 participants with chronic kidney disease (23% risk increase per 10 ng/mL of 25(OH)D decrease),[7] supporting our result that vitamin D deficiency is associated with CAC. In contrast, Michos et al. found no significant association between serum 25(OH)D levels and CAC in 650 Amish adults.[22] The inconsistent results between vitamin D deficiency and

CAC are, in part, because the participants of the Michos' study were relatively old and 86% of the participants were insufficient vitamin D.[22]

A Women's Health Initiative (WHI) calcium/vitamin D supplemental trial demonstrated that vitamin D supplementation for seven years had no significant effect on CAC progression among 754 post-menopausal women aged 50 to 59 (p=0.74).[41] No significant effect of vitamin D supplementation in this trial may be attributable to relatively older women with hysterectomy and low compliance where some women stopped their assigned medication before the trial was completed. Also, additional reasons of not finding a significant effect could be due to not only very low prevalence of CAC but also a secondary analysis to examine the effect of vitamin D supplementation on CAC where the primary aim was to examine the effect of conjugated equine estrogens (CEE) group on CAC [41]. The possible underlying mechanisms associating vitamin D deficiency with development and progression of CAC may be through pro-atherosclerotic processes such as increased inflammatory or immune process [43], endothelial dysfunction [44], and vascular smooth muscle cell (VSMC) proliferation and migration[45]. Particularly, vascular calcification can progress through VSMC apoptosis into vascular osteoblast cells, leading to mineralization and bone matrix within vascular well [46, 47]. Vitamin D inhibits VSMC differentiation and matrix mineralization [46]. Also, Vitamin D reduces inflammatory response [46, 47], which is a crucial causative process of atherosclerosis.

Our study is unique in a sense that we adjusted for omega-3 fatty acids, an important potential but not commonly adjusted confounder. This is because oily fish is known as one of main resources of vitamin D[48] along with other dietary sources (eggs and fortified food products such as milk, orange juice).[17, 32, 35] However, after adjusting for omega-3 fatty acids, the association was not attenuated and remained significant.

This study has several strengths. First, the study population came from population-based samples, which are representative population of healthy adults. Second, the measurements of serum 25(OH)D by LC-MS/MS have been demonstrated as accurate and precise with a lower CV as compared to immunoassay method. However, this study also has several limitations. First, the study participants were all men aged 40-49 years, which limits the generalizability to other populations (e.g. older or women). Second, the sample size is relatively small. Nonetheless, we found the significant positive association of vitamin D deficiency with CAC. Third, the cross-sectional study design cannot establish causality, which limits the inference of the study results.

In conclusion, we observed that vitamin D deficiency had a significant positive association with coronary atherosclerosis. Future studies with prospective design are warranted to examine the causal relation between vitamin D deficiency and subclinical atherosclerosis in the general population.

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Abbreviations

CAC	coronary artery calcification
CHD	coronary heart disease
EBCT	Electron beam computed tomography

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Table 1

Characteristics of the study participants (n=195)

	Coronary Artery Calcification		
	CAC < 10 n=142	**CAC ≥ 10 n=53**	***P*-value**
Age (year)	45.59±2.81	47.00±2.24	0.0012
BMI (kg/m^2)	27.36±3.99	29.24±4.95	0.0066
Systolic BP (mmHg)	125.38±11.57	127.93±15.88	0.2877
Diastolic BP (mmHg)	75.60±9.09	77.48±11.05	0.2284
Hypertension (%)	26.06	32.08	0.4033
LDL-C (mg/dL)	125.30±34.15	125.07±27.30	0.9645
Triglycerides (mg/dL) *	121.50 (88.00, 202.00)	153.00 (99.00, 231.00)	0.1214
HDL-C (mg/dL)	48.80±12.77	50.85±15.56	0.3513
Total cholesterol (mg/dL)	205.75±36.46	211.28±28.25	0.2654
C-Reactive Protein (mg/L) *	0.81 (0.39, 1.43)	0.93 (0.59, 1.34)	0.7324
Diabetes (%)	9.86	11.32	0.7647
Current smoker (%)	7.04	18.87	0.0155
Pack-years *	0 (0, 0)	0 (0.00, 15.00)	0.0029
Alcohol drinker (%)	38.73	41.51	0.7241
Ethanol intake (g/day) *	3.29 (0, 16.46)	2.78 (0, 28.39)	0.7083
Marine n-3 fatty acids	4.21±1.80	4.45±1.84	0.4095
25(OH)D deficiency (%)	7.75	16.98	0.0586

Mean ± SD with t-test;

* Median (Interquartile range) with wilcoxon test for p-value; BMI=body mass index; LDL-C=low density lipoprotein cholesterol; BP=blood pressure; HDL-C=high density lipoprotein cholesterol; Marine n-3 fatty acids include eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3), and docosahexaenoic acid (22:6n-3); CAC is defined as those with coronary calcium score ≥10;

Table 2

Characteristics between vitamin D deficiency and sufficient groups (n=195)

	Vitamin D Deficiency		
	<20 ng/mL n=20	**≥20 ng/mL n=175**	***P*-value**
Age (year)	47.10±2.59	45.85±2.73	0.0516
BMI (kg/m^2)	27.68±4.63	27.89±4.32	0.8410
Systolic BP (mmHg)	130.33±12.86	125.59±12.84	0.1196
Diastolic BP (mmHg)	77.58±7.83	75.95±9.86	0.4768
Hypertension (%)	35.00	26.86	0.4407
LDL-C (mg/dL)	123.19±29.64	125.47±32.73	0.7656
Teiglycerides (mg/dL)*	124.00 (91.00, 210.50)	131.00 (95.00, 214.00)	0.8591
HDL-C (mg/dL)	50.09±14.29	49.28±13.53	0.8003
Total-C (mg/dL)	206.25±25.04	207.37±35.41	0.8907
C-Reactive Protein (mg/L)*	0.69 (0.38, 1.81)	0.85 (0.40, 1.37)	0.4830
Diabetes (%)	10.00	10.29	0.9682
Current smoker (%)	10.00	10.29	0.9682
Pack-years*	0 (0, 0)	0 (0, 7.50)	0.3915
Alcohol drinker (%)	50.00	38.29	0.3100
Ethanol intake (g/day)*	10.29 (0, 34.97)	3.09 (0, 16.46)	0.5100
Marine n-3 fatty acids	4.21±2.42	4.28±1.73	0.8890
CAC ≥ 10 (%)	45.00	25.14	0.0586

Mean ± SD; BMI=body mass index; LDL-C=low density lipoprotein cholesterol; BP=blood pressure; HDL-C=high density lipoprotein cholesterol; Marine n-3 fatty acids include eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3)

Table 3

Odds ratios on coronary artery calcification (CAC) associated with vitamin D deficiency (n=195)

	Coronary Artery Calcification Odds Ratio (95% Confidence Intervals)
Univariate	2.54 (0.98, 6.61)
Model I	3.01 (1.03, 8.75)
Model II	3.13 (1.07, 9.15)
Model III	3.31 (1.12, 9.77)

Odds Ratios were estimated on the outcome of coronary artery calcification (CAC) associated with vitamin D deficiency; Vitamin D deficiency was defined as 25(OH)D<20ng/mL; Reference groups are 25(OH)D ≥20ng/mL; CAC is defined as those with coronary calcium score ≥10; In the crude model, center and collection date of 25(OH)D were adjusted; in the model I, age, body mass index, smoking (pack-year), drinking (ethanol consumption), C-Reactive Protein, and triglycerides were additionally adjusted; in the model II, hypertension and diabetes were further adjusted; in the model III, marine n-3 fatty acids were further adjusted.