의학석사 학위논문

Association between Serum 1,25(OH)2 vitamin D3 and Carotid Plaques and Intima-Media Thickness

혈중 1,25(OH)2 vitamin D3농도와 경동맥 경화반 및 내중막 두께와의 연관성

2013년 2월

서울대학교 대학원
의학과 가정의학 전공
김 계 형

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월경 1,25(OH)2 vitamin D3농도와 경동맥 경화반 및 내중막 두께와의 연관성

2012년

김계형

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2012

Kyae Hyung Kim

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Association between Serum 1,25(OH)2 vitamin D3 and Carotid Plaques and Intima-Media Thickness

by
Kyae Hyung Kim

A thesis submitted to the Department of Family Medicine in partial fulfillment of the requirements for the Degree of Master of Science in Family Medicine at Seoul National University College of Medicine

December 2012

Approved by Thesis Committee:

Professor Young Joo Park **Chairman**
Professor Belong Cho **Vice chairman**
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논문제목 : Association between Serum 1,25(OH)2 vitamin D3 and Carotid Plaques and Intima-Media Thickness

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초 록

서론: 많은 연구결과들에 의하여 낮은 농도의 비타민 D가 심혈관 질환과 연관되어 있다고 알려져 있으나 심혈관계 질환 예방을 위한 최적의 비타민 D 농도는 아직 밝혀져 있지 않다. 이에 본 연구는 한국 성인에서 혈중 1,25(OH)2 vitamin D3농도와, 불현성 동맥경화 지표인 경동맥 경화반 및 내중막 두께와의 연관성를 알아보고자 하였다.

방법: 본 연구는 2006년 3월 1일부터 2009년 12월 30일까지 서울대학교 병원 건강증진센터를 내원한 19-85세 사이의 1,815 명의 한국 성인남녀를 대상으로 하였다. 경동맥 초음파를 이용하여 경동맥 내중막 두께, 경동맥 경화반의 유무, 개수, 부피를 측정하여 혈중 1,25(OH)2 vitamin D3 5분위수 농도와 상관관계를 분석하였다.

결과: 여성 참가자들에서 경동맥 경화반의 유무, 개수, 부피는 혈중 1,25(OH)2 vitamin D3 농도와 J 또는 U 커브의 상관관계를 보였다. 다변량 로지스틱 회귀분석 모형에서 혈중 1,25(OH)2 vitamin D3 농도의 제 3분위수 (31.8-38.8 pg/ml) 와 제 4분위수 (31.8-38.8 pg/ml) 농도에서 경동맥 경화반이 유의하게 감소하였으며, 그 오즈비는

각각 0.26 (95% confidence interval [CI], 0.09–0.74) , 0.38 (95% CI, 0.15–0.94) 이었다. 경동맥 경화반의 개수와 부피를 Analysis of covariance로 분석하였을 때도 제 3분위수와 4분위수의 혈중 1,25(OH)2 vitamin D3 농도에서 유의하게 감소하는 결과를 보였다. 남성 참가자들에서는 경동맥 경화반과 혈중 1,25(OH)2 vitamin D3 농도의 유의한 관계를 관찰할 수 없었다. 또한 모든 참가자들에게서 경동맥 내중막 두께와 혈중 1,25(OH)2 vitamin D3 농도의 관계는 통계적으로 유의하지 않았다.

결론: 본 연구결과로 미루어 볼 때 한국 여성에게서 매우 낮거나 높은 농도가 아닌 적절한 혈중 1,25(OH)2 vitamin D3 농도에서 경동맥 경화반의 감소가 관찰되었다. 이와 관련하여 향후 심혈관계 질환 예방을 위한 최적의 비타민 D 농도를 탐구하는 다양한 전향적인 연구가 필요할 것으로 생각된다.

주요어: 경동맥 경화반, 경동맥 내중막 두께, 경동맥 경화, 비타민 D, $1,25(OH)_2$ vitamin D_3

학 번: 2010-21779

Abstract

Introduction

Many epidemiological studies have found that low vitamin D level is associated with cardiovascular diseases. But the optimal level for cardiovascular health remains unclear. The present study aimed to assess the association between $1,25(OH)_2$ vitamin D_3 concentration and subclinical carotid atherosclerosis.

Methods

1,815 Korean participants aged 19-86 who underwent medical checkups were enrolled between March 2006 and December 2009. Cross-sectional relationships between quintiles of serum $1,25(OH)_2$ vitamin D_3 concentration and common carotid artery (CCA) structures using ultrasonography (CCA intima-media thickness [IMT], occurrence, numbers, and volume of plaque) were investigated adjusting for possible confounders.

Results

Among female participants, occurrence of plaque, numbers and volumes of CCA plaques showed the J or U shaped association with $1,25(OH)_2$ vitamin D_3 concentration. In the concentration of 3rd quintile (31.8-38.8 pg/ml) and 4th quintile (38.9-48.5 pg/ml), participants showed decreased adjusted Odds

ratio (aOR) for occurrence of CCA plaque (aOR 0.26, 95% confidence interval [CI], 0.09–0.74, 3rd quintile and aOR 0.38, 95% CI, 0.15–0.94, 4th quintile, respectively) in multivariate logistic regression model. Similarly, deceased number and volume of CCA plaque were demonstrated in of 3rd and 4th quintile (p=0.022 and 0.023 for number of plaque, and p =0.030 and 0.020 for volume of plaque in analysis of covariance [ANCOVA], respectively). Among male participants, we found no association between occurrence, numbers and volumes of CCA plaques and 1,25$(OH)_2$ vitamin D_3 concentration. We found no significant association between CCA IMT and 1,25$(OH)_2$ vitamin D_3 concentration both in ANCOVA and in multivariate logistic regression analysis in the whole participants.

Conclusions

The results of present study indicate that adequate vitamin D concentration, not too low or too high level, is associated with decrease of subclinical carotid atherosclerosis among Korean women, but not among Korean men. Further studies are needed to elucidate optimal levels of vitamin D for cardiovascular health.

Keywords: carotid intima-media thickness, carotid plaque, carotid atherosclerosis, vitamin D, 1,25$(OH)_2$ vitamin D_3

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Contents

Lists of Table and Figures

Introduction

Vitamin D inadequacy is highly prevalent across the world. In United States, the proportions with serum 25(OH)D levels below 30 ng/mL were 73%, 89%, and 95% in the females in age categories of 1–5, 20–49, and 70 years and older. (1) In Asian countries, prevalence rate among postmenopausal women using this cut-off value of 30ng/mL of serum 25(OH)D levels is 92% in Korea, 90% in Japan, and 47% in Thailand respectively (2).

Many epidemiological and biological studies have found that low level of vitamin D is associated with non-skeletal conditions such as obesity (3) hypertension (4) diabetes (5, 6) cardiovascular (7) or cerebrovascular outcomes (8) and mortality (9). The mechanism is not fully elucidated. Vitamin D, the major steroid hormone absorbed from the skin and diet, is metabolized in the liver to 25 (OH) vitamin D (10, 11). Then, 25 (OH) vitamin D, the major circulating and storage form of vitamin D, is metabolized in the kidneys by the enzyme 1-alpha-hydroxylase to its active form, $1,25(OH)_2$ vitamin D_3 (10, 11). Some studies demonstrated that vitamin D receptors and 1-alpha-hydroxylase are known to be present in vascular endothelial cells (12) and known to display anti-hypertrophic activity in the animal heart (13). Vitamin D also upregulates glucose trasporter 4 (GLUT4) translocation and glucose utilization (14), decreases lipogenesis (15), as well as induces anti-atherogenic monocyte/macrophage phenotype (16).

Undoubtedly, vitamin D deficiency is the most common nutrition-responsive medical condition (17). Therefore, it would be an effective strategy to identify

the risk of vitamin D inadequacy in preclinical stage and correct its level to adequacy to prevent further diseases. Guidelines define adequate 25(OH) vitamin D_3 concentration as greater than 30ng/mL (10, 18). And they also define vitamin D insufficiency exists at concentrations between 15 and 30 ng/mL, and deficiency at concentrations less than 15 ng/mL (10, 18). But there is no higher cut-off value of it adequacy. Recently, several studies suggested that both low and high levels of vitamin D linked to increased mortality (19, 20), but there is a lack of evidence for morbid cardiovascular diseases. Moreover, not only in cardiovascular mortality or morbidity, but there is also a need for more evidences in primordial conditions in the general population. Bogalusa Heart study and Pathobiological Determinants of Atherosclerosis in Youth (PDAY) study revealed that young person, even a youth of twenties, could already have subclinical atherosclerosis and the prevention of cardiovascular disease should begin early in life (21, 22). In this context, there is a special need for studies that also evaluate the association of subclinical atherosclerosis and optimal vitamin D concentration.

Sabanayagam et al found vitamin D deficiency is associated with prehypertension in a representative sample of US adults (23). Gonzalez-Molero and Huwemoen respectively reported that subjects with vitamin D deficiency showed high incidence of diabetes in a prospective study (24, 25). Kojima et al found Low dietary vitamin D predicts 34-year incident stroke (8). Those studies focused on low levels of vitamin D with subclinical vascular conditions. However, higher level of vitamin D, below toxicity level, has not

been an object of concern.

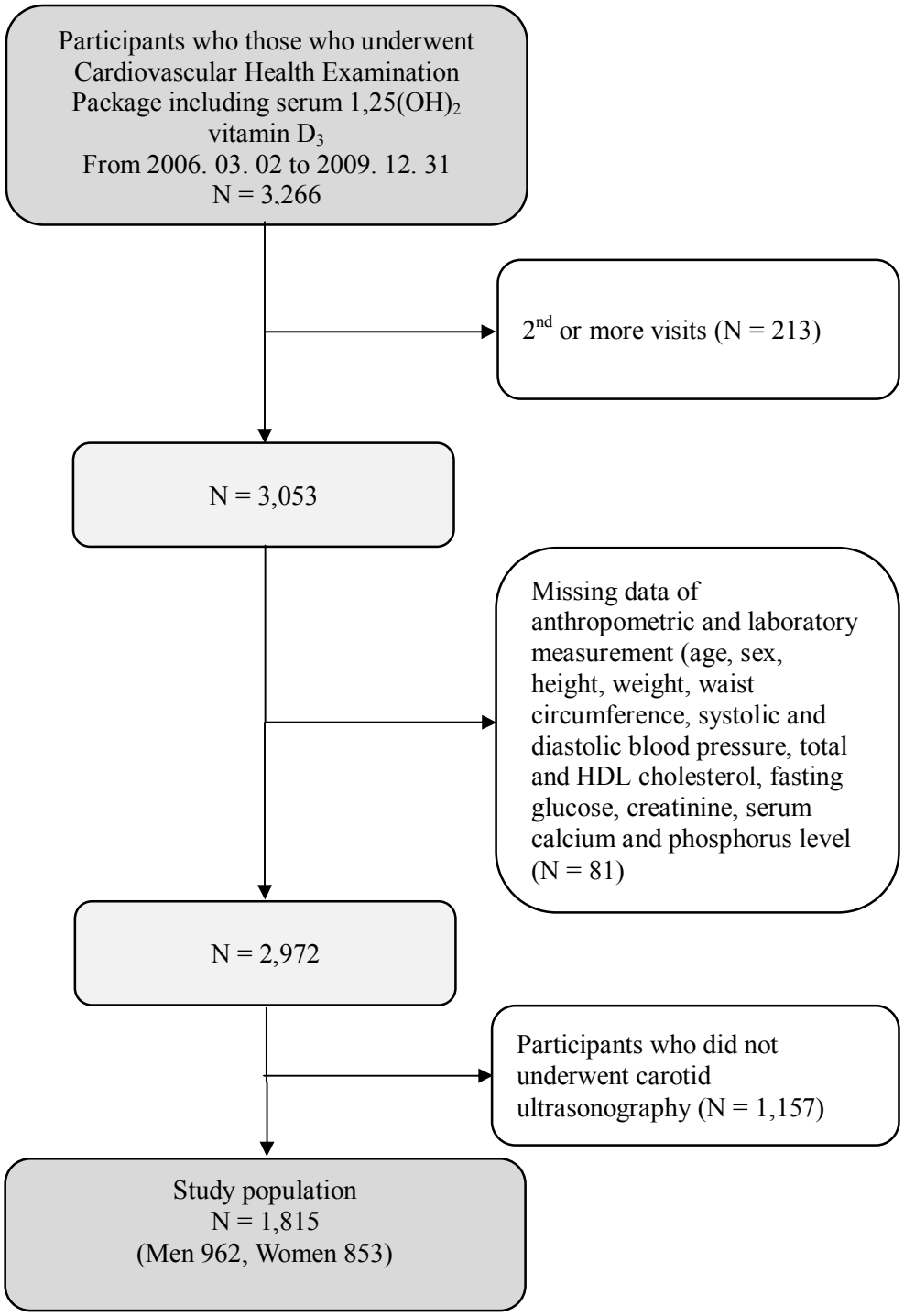
Carotid Intima-media thickness (IMT) is a surrogate marker for subclinical atherosclerosis and predicts future cardiovascular and cerebrovascular outcomes (26). High-resolution B-mode Ultrasonography is known to detect changes in the arterial wall structure, including intima-media thickness (IMT), atherosclerotic plaque, and plaque volume (27). Recently, carotid plaque are also known to be an important predictor of cardiovascular disease events as well (28, 29). We evaluated both CCA IMT and plaque. In regards to assess vitamin D status, we used $1,25(OH)_2$ vitamin D_3 concentration in the present study. Although 25-OH vitamin D is recommended to determine a patient's vitamin D status (10), however, previous studies showed that both 25 (OH) vitamin D_3 and $1,25(OH)_2$ vitamin D_3 levels may show independent associations for cardiovascular outcomes (30). In the present study, our aim was to study in a cross-sectional analysis whether serum serum $1,25(OH)_2$ vitamin D_3 concentration are associated with carotid IMT, number of plaque, or plaque volumes among 962 men and 853 women in Korea.

Materials and Methods

Study population

The participants in this study were screened as detailed in Figure 1. Among the persons who visited the Health Promotion Center, Seoul National University Hospital, for health check-ups from March 1st 2006 to December 30th 2009, only those who underwent Cardiovascular Health Examination Package including serum $1,25(OH)_2$ vitamin D_3 were selected. Participants who had underwent anthropometric measurements, health questionnaire survey and laboratory tests including measurement of serum $1,25(OH)_2$ vitamin D_3, and carotid ultrasonography were included in the study. The health questionnaire survey was double-checked through health interviews by health screening doctors. In total, 1,815 participants were included in the study. All the participants provided informed consent. The study procedures followed the tenets of the Declaration of Helsinki and were approved by the Institutional Review Board of Seoul National University Hospital.

Figure 1. Flow diagram of inclusion or exclusion of study participants



Question survey, anthropometric and laboratory assessments

All the participants completed a questionnaire about previous medical history, current medication, smoking habits, regular exercise, education level, and income. With regard to smoking, the participants were grouped as nonsmokers, past smokers, or current smokers.

From 2006 to 2007, the participants answered a questionnaire on the average quantity and frequency of alcohol consumption per week and type of alcohol during the previous 1−month period (31, 32) to minimize recall bias of the participants, our center chose the most recent previous 1−month period. The Quantity-Frequency (QF) questions on alcohol consumption within a given time frame are known to produce higher estimates than global QF questions (e.g., consumption during the entire year). From 2008 to 2009, participants chose the most recent period either previous 1-month or 1-week period. Synthetically, we produced weekly alcohol consumption by standard drink (approximately 12 grams of pure alcohol) of each participant.

With regard to previous medical history of hypertension, from 2006 to 2007 the participants was asked if they had current medication for hypertension, while from 2008 to 2009 the participants was asked if they had both diagnosed and taken medication. For the purpose of analysis participants were classified as either the normal or hypertension patients. Regarding diabetes, from 2006 to 2007 the participants was asked if they had been diagnosed or

not and if diabetes was well controlled or not while from 2008 to 2009 the participants was asked if they had been both diagnosed and taking medication. We classified the participants into simple two groups: the normal group or diabetes group.

Through 2006-2009, regular exercisers were defined if the participant answered if he or she regularly exercises, or if he or she had light, moderate, or vigorous activities for more than 30 minutes at least 3 times a week.

Height, weight, and weight circumference (WC) were measured, with the participants wearing light, single-thickness clothing, and standing erect with the feet together. WC was measured at the smallest circumference of the torso. BMI was calculated as the ratio of weight to the square of height (kg/m2). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the left arm, with the participant in the sitting position after 5 minutes of rest.

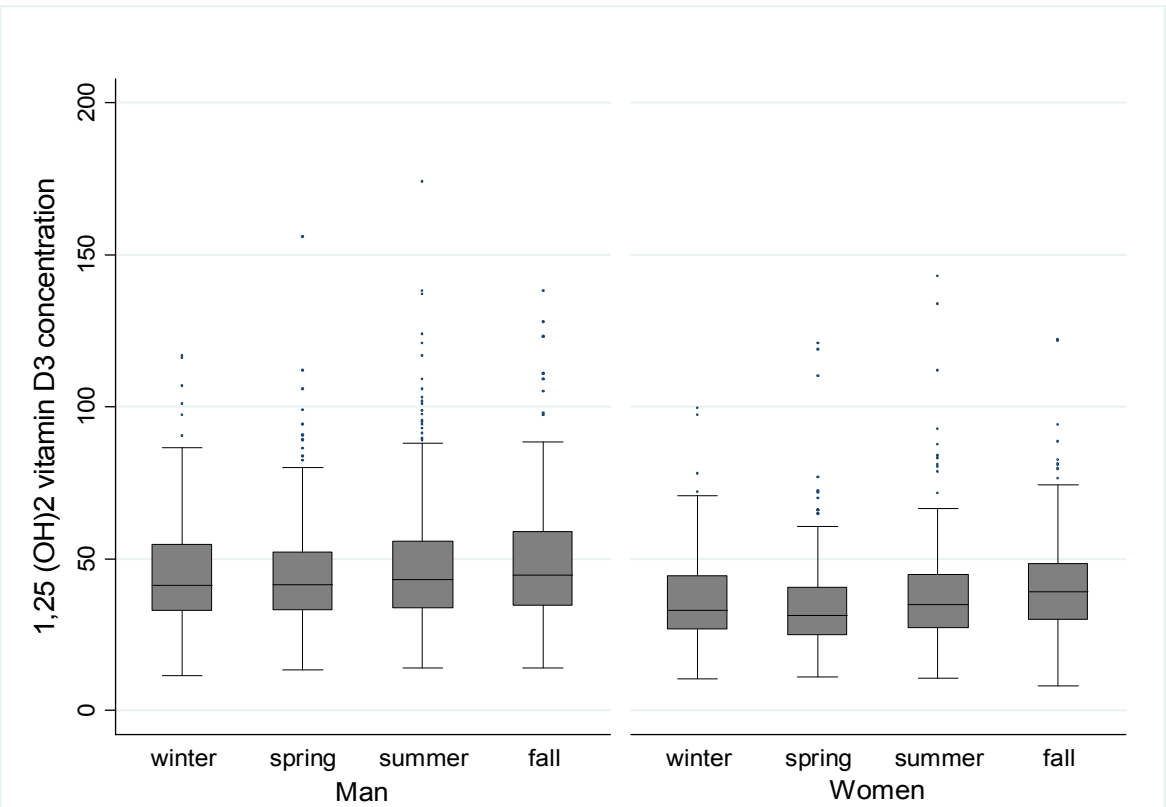
Laboratory tests included serum calcium and phosphorus concentration, fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol, serum creatinine, high sensitivity C-reactive protein (hs-CRP), and homocysteine after . The level of Non-HDL cholesterol was calculated: [TC (mmol/L) − HDL cholesterol (mmol/L)]. Serum HbA1c and insulin were also measured and recorded.

Serum 1,25(OH)$_2$ vitamin D$_3$ measurement

Serum was collected in serum separator tubes and the plasma was separated

by centrifugation and frozen at -70℃. $1,25(OH)_2$ vitamin D_3 was measured in thawed samples using a radioimmunoassay kit (Diasorin, Stillwater, MN, USA) which has a threshold of detection of 4 pg/mL. The Intra- and inter-assay coefficients of variation were 7.9% and 10.5%, respectively. We divided the participants into quintiles on the basis of total $1,25(OH)_2$ vitamin D_3 concentration and the lowest concentration group defaulted to the reference group. The seasonal distribution of $1,25(OH)_2$ vitamin by sex was described in Figure 2.

Figure 2. Seasonal distribution of 1,25 $(OH)_2$ vitamin D_3 concentration of study participants



Measurement of carotid artery structure

The ultrasonography of carotid arteries was performed while the subject was lying in the supine position with the head slightly extended using high-resolution B-mode ultrasound (IU22 Ultrasound System, Phillips, Eindhoven, the Netherlands) equipped with a 7.5MHz linear array transducer. Intima-media thickness was determined as the distance from the media-adventia interface to the intima-lumen interface on the posterior (far) wall (27). Qualified two radiologist evaluated three points of each common carotid artery free from advanced atherosclerotic lesion or plaque. The mean value of six measurements was determined as ‘mean Common carotid artery (CCA)-IMT' diameter (33). Further, the radiologists also reported the presence and number of carotid plaques and carotid artery stenosis, and described the volume of plaques as three dimensional measure. The presence of plaques was defined as localized echo structures encroaching into the vessel lumen for which the wall thickening that was at least 50% greater than that of the surrounding vessel wall or greater than 1.5mm.

Statistical analysis

All analyses were performed using STATA statistical software version 12.0 (Stata Corp., College Station, TX, USA). Figure 3 and 4 shows that CCA IMT and ,25$(OH)_2$ vitamin D_3 distribution were both left skewed according to the Shapiro−Wilks test (all $p<0.001$). The distributions of serum triglycerides

were left skewed. The clinical characteristics according to serum $1,25(OH)_2$ vitamin D_3 status were evaluated with ANOVA to determine statistical differences in the continuous variables, and with χ^2 tests or Fisher's exact test for differences in the discrete variables. Pearson's correlation analysis was used in assessing the univariate associations between various risk factors and CCA-IMT.

We constructed an analysis of covariance (ANCOVA) model and linear models from the multivariate-adjusted mean areas of mean CCA-IMT, mean numbers and volumes of CCA plaque within the $1,25(OH)_2$ vitamin D_3 categories. The model 1 was adjusted for age, BMI, regular exercise, cigarette smoking and weekly alcohol consumption, season of blood draw. The model 2 was further adjusted for the model 1 covariables and diabetes, hypertension, fasting glucose level, systolic blood pressure, serum total and Non-HDL cholesterol level. Model 3 was adjusted the aforementioned model 2 covariables and serum creatinine, calcium phosphorus level.

Furthermore, The association between $1,25(OH)_2$ vitamin D_3 level and increased CCA IMT and occurrence of CCA plaques were assessed using multivariate logistic regression analyses. The adjusted models were constructed as three models as above.

Figure 3. Histogram of 1,25 $(OH)_2$ vitamin D_3 concentration of study participants

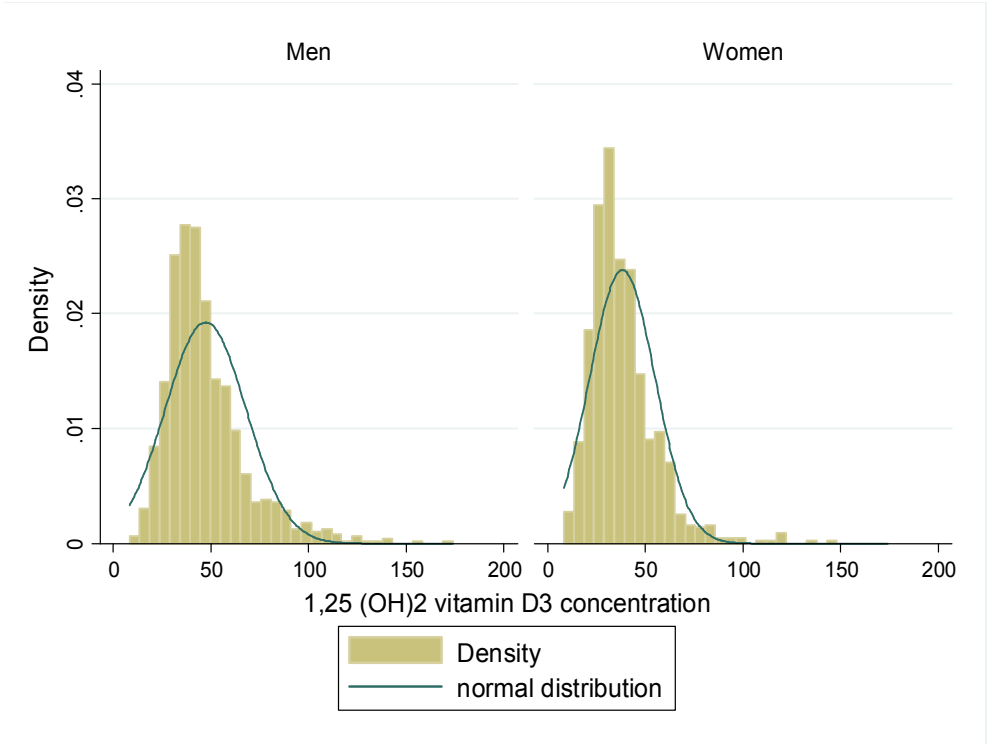
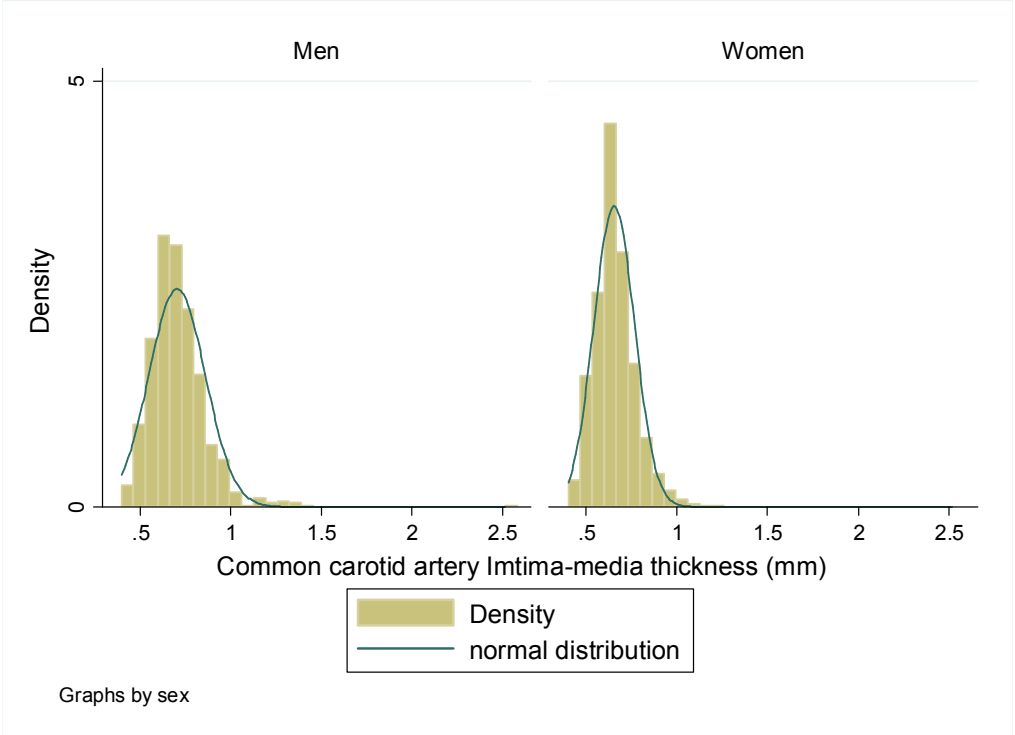


Figure 4. Distribution of mean CCA IMT of study participants



Results

Baseline characteristics

The characteristics of the male participants are presented in Table 1. The mean age, height, weight, WC were not significantly different across five quintile groups (all $p>0.05$) while BMI decreased as as $1,25(OH)_2$ vitamin D_3 concentration increased ($p = 0.045$). The amount of weekly alcohol consumption increased as $1,25(OH)_2$ vitamin D_3 concentration increased ($p<0.001$). Regular exercise, cigarette smoking, past history of diabetes and hypertension were not associated with $1,25(OH)_2$ vitamin D_3 concentration (all $p>0.05$). Serum calcium, total cholesterol and homocysteine level were shown to increase as with $1,25(OH)_2$ vitamin D_3 increased ($p = 0.007$, 0.002 and 0.014, respectively). Other laboratory tests results showed no statistical significance.

Table 2 showed that the characteristics of female participants. In female participants, $1,25(OH)_2$ vitamin D_3 concentration is more closely related to various factors than in male participants. The age of female participants tends to increase with increasing $1,25(OH)_2$ vitamin D_3 concentration ($p<0.001$). The proportion of regular exercisers increased with $1,25(OH)_2$ vitamin D_3 increased ($p<0.001$). Serum calcium and total cholesterol level increased according to $1,25(OH)_2$ vitamin D_3 concentration ($p = 0.012$ and 0.001, respectively) while serum non-HDL cholesterol showed marginal significance ($p = 0.051$). Among female participants, CCA IMT tended to increase as

$1,25(OH)_2$ vitamin D_3 concentration increase, while occurrence of plaque decreased ($p = 0.025$).

Season of blood draw showed statistically different according to $1,25(OH)_2$ vitamin D_3 concentration in female participants ($p = 0.007$). Figure 2 showed histograms of $1,25(OH)_2$ vitamin D_3 by sex. Mean $1,25(OH)_2$ vitamin D_3 concentration was 47.18 $\pm$ 20.73 pg/dl in male and 38.22 $\pm$ 16.79 pg/dl in female participants ($p < 0.001$ from T-test between two groups, Data not shown).

Table 1. Characteristics of study participants according to 1,25(OH)2 vitamin D3 concentration in Men (n = 962)

Variables		Quintiles of 1,25(OH)2 vitamin D3 (median)						p [a]	p [b]
		Total	<32.0 (27.2) pg/ml	32.0-38.9 (35.1) pg/ml	39.0-46.8 (42.7) pg/ml	46.9-59.8 (51.7) pg/ml	>59.9 (73.6) pg/ml		
N	962		*n* = 193	*n* = 192	*n* = 193	*n* = 193	*n* = 191		
Age, yr	962	55.1 ± 10.3	54.7 ± 10.5	54.0 ± 11.5	55.4 ± 9.8	56.0 ± 10.0	55.3 ± 9.5	0.367	0.158
Height, cm	962	169.1 ± 6.0	168.3 ± 5.5	169.9 ± 6.4	169.2 ± 5.4	168.9 ± 5.9	169.1 ± 6.3	0.122	0.620
Weight, kg	962	72.0 ± 11.3	72.2 ± 11.5	73.5 ± 11.6	71.1 ± 10.4	72.1 ± 11.7	71.1 ± 11.3	0.212	0.170
BMI, kg/m^2	962	25.1 ± 3.2	25.4 ± 3.5	25.3 ± 3.1	24.7 ± 3.1	25.2 ± 3.3	24.7 ± 3.1	0.110	0.045
WC, cm	962	89.4 ± 8.9	89.4 ± 9.0	90.4 ± 9.2	88.6 ± 8.4	89.6 ± 9.6	89.0 ± 8.4	0.361	0.456
Weekly alcohol intake (standard drink)	888	10.6 ± 15.1	6.9 ± 8.7	6.9 ± 9.5	10.2 ± 13.2	13.1 ± 17.8	16.1 ± 20.4	<0.001	<0.001
Regular exercise (n,%)	953	479 (50.2)	93 (48.6)	91 (48.1)	97 (50.5)	102 (53.6)	96 (50.2)	0.840	
Cigarette smoking (n,%)	935							0.081	
Never		213 (22.7)	52 (27.6)	39 (21.3)	44 (23.2)	45 (23.9)	33 (17.6)		
Past		420 (44.9)	80 (42.5)	86 (46.9)	97 (51.3)	74 (39.3)	83 (44.3)		
Current		302 (32.3)	56 (29.7)	58 (31.6)	48 (25.4)	69 (36.7)	71 (37.9)		
DM (n,%)	925	121 (13.1)	26 (14.0)	23 (12.5)	27 (14.4)	24 (12.8)	21 (11.5)	0.924	
HTN (n,%)	934	268 (28.6)	50 (26.7)	47 (25.5)	59 (30.8)	59 (30.8)	53 (28.8)	0.670	
SBP (mmHg)	962	131.7 ± 15.6	131.8 ± 18.0	130.1 ± 16.0	131.3 ± 14.9	131.9 ± 14.2	133.6 ± 14.6	0.278	0.120
DBP (mmHg)	962	80.1 ± 11.0	80.1 ± 12.7	78.9 ± 10.4	79.3 ± 10.5	80.5 ± 10.5	81.7 ± 10.4	0.095	0.056
FBS (mg/dL)	962	98.1 ± 23.3	97.0 ± 20.7	96.7 ± 24.4	99.0 ± 22.9	98.9 ± 23.8	99.0 ± 24.6	0.754	0.245
Calcium	962	9.22 ± 0.34	9.15 ± 0.35	9.23 ± 0.34	9.21 ± 0.33	9.24 ± 0.35	9.28 ± 0.31	0.007	0.001
Phosphorus	962	3.26 ± 0.46	3.26 ± 0.51	3.26 ± 0.41	3.26 ± 0.43	3.25 ± 0.46	3.29 ± 0.47	0.903	0.503
HbA1c (%)	962	5.93 ± 0.75	5.92 ± 0.78	5.93 ± 0.79	5.94 ± 0.70	5.95 ± 0.75	5.91 ± 0.75	0.990	0.988
Insulin (μIU/ml)	945	10.22 ± 4.94	9.96 ± 4.79	10.19 ± 4.54	10.19 ± 4.76	10.64 ± 6.00	10.11 ± 4.48	0.741	0.512
Total Cholesterol (mg/dL)	962	200.5 ± 34.0	193.0 ± 33.1	199.8 ± 32.2	199.5 ± 36.4	205.2 ± 28.7	205.0 ± 37.6	0.002	<0.001
Non-HDL cholesterol (mg/dL)	962	150.3 ± 34.4	145.4 ± 34.0	151.3 ± 32.6	149.3 ± 36.5	153.7 ± 28.5	151.6 ± 39.1	0.173	0.059

Creatinine (mg/dL)	962	1.09 ± 0.17	1.09 ± 0.27	1.08 ± 0.13	1.09 ± 0.15	1.09 ± 0.14	1.09 ± 0.15	0.984	0.851
hs-CRP (mg/L)	917	0.19 ± 0.52	0.29 ± 0.85	0.14 ± 0.28	0.18 ± 0.50	0.17 ± 0.30	0.18 ± 0.41	0.086	0.113
Homocysteine (umol/L)	934	11.0 ± 3.1	11.1 ± 2.7	11.0 ± 2.4	11.3 ± 2.4	11.3 ± 3.2	12.0 ± 3.5	0.014	0.003
IMT, mm	962	0.70 ± 0.15	0.70 ± 0.14	0.71 ± 0.21	0.69 ± 0.12	0.71 ± 0.14	0.69 ± 0.14	0.377	0.480
IMT ≥ 0.75 mm (n,%)	962	329 (34.2)	73 (37.8)	64 (33.3)	66 (34.2)	63 (32.6)	63 (32.9)	0.822	
IMT ≥ 0.90 mm (n,%)	962	85 (8.8)	17 (8.8)	22 (10.9)	12 (6.2)	19 (9.8)	16 (8.3)	0.560	
Presence of plaque (n,%)	951	152 (15.9)	29 (15.0)	27 (14.2)	32 (16.7)	28 (14.8)	36 (19.0)	0.711	
No of plaque	951	0.25 ± 0.69	0.22 ± 0.61	0.20 ± 0.55	0.26 ± 0.74	0.26 ± 0.78	0.30 ± 0.77	0.377	0.159
Volume of plaque, mm^3	915	20.9 ± 199.1	16.5 ± 87.4	6.1 ± 31.2	17.3 ± 123.3	15.4 ± 79.2	50.0 ± 415.3	0.271	0.106
Season of measurement (n,%)	962							0.418	
Spring (mar to may)		225 (23.3)	52 (26.9)	44 (22.9)	52 (26.9)	35 (24.8)	41 (21.4)		
Summer (Jun to Aug)		224 (23.2)	50 (25.9)	50 (26.0)	51 (26.4)	55 (28.5)	54 (28.2)		
Fall (Sep to Nov)		260 (27.0)	39 (20.2)	51 (26.5)	50 (25.9)	55 (28.5)	58 (30.3)		
Winter (Dec to Feb)		253 (26.3)	52 (26.9)	47 (24.4)	40 (20.7)	48 (24.8)	38 (19.9)		

Data are presented as the means and standard deviation [SD], or number and percentages

[a] *p* value from ANOVA for continuous variables or chi-squared test for categorical variables.

[b] p value from linear regression analysis

[C] p value from Fisher's exact test

Abbreviations: BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, higi-density lipoprotein; CRP, C-reactive protein; IMT, intima-media thickness

Table 2. Characteristics of study participants according to 1,25(OH)2 vitamin D3 concentration in Women (n = 853)

			Quintiles of 1,25(OH)2 vitamin D3 (median)					p [a]	p [b]
	n	Total	<25.5 (21.4) pg/ml	25.5-31.7 (28.7) pg/ml	31.8-38.8 (35.0) pg/ml	38.9-48.5 (51.7) pg/ml	>48.6 (59.1) pg/ml		
N	853		*n* = 171	*n* = 173	*n* = 169	*n* = 170	*n* = 170		
Age, yr	853	53.9 ± 10.2	53.9 ± 10.2	54.9 ± 10.2	55.4 ± 8.2	57.2 ± 8.5	58.9 ± 8.5	<0.001	<0.001
Height, cm	853	156.3 ± 5.3	156.5 ± 4.9	156.3 ± 5.7	156.2 ± 5.3	156.9 ± 5.1	155.4 ± 5.3	0.143	0.240
Weight, kg	853	58.9 ± 9.1	58.5 ± 10.0	59.6 ± 9.8	59.8 ± 9.1	59.4 ± 8.3	57.1 ± 7.7	0.035	0.178
BMI, kg/m^2	853	24.1 ± 3.4	23.8 ± 3.8	24.4 ± 3.8	24.5 ± 3.5	24.1 ± 3.2	23.6 ± 2.8	0.102	0.366
WC, cm	853	85.7 ± 9.2	84.5 ± 9.3	86.2 ± 9.3	86.4 ± 9.5	86.3 ± 9.1	85.3 ± 8.8	0.225	0.424
Weekly alcohol intake (standard drink)	750	1.2 ± 5.6	1.0 ± 4.4	1.0 ± 3.7	0.8 ± 2.4	0.7 ± 2.3	2.3 ± 10.5	0.098	0.118
Regular exercise (n,%)	838	378 (45.1)	58 (34.5)	73 (42.4)	73 (43.7)	80 (48.4)	94 (56.6)	<0.001	
Cigarette smoking (n,%)	796							0.401	
Never		740 (93.0)	136 (88.8)	151 (94.9)	143 (92.2)	158 (95.1)	152 (93.2)		
Past		27 (3.4)	10 (6.5)	3 (1.8)	6 (3.8)	4 (2.4)	4 (2.4)		
Current		29 (3.6)	7 (4.5)	5 (3.1)	6 (3.8)	4 (2.4)	7 (4.2)		
DM (n,%)	804	52 (6.4)	9 (5.7)	7 (4.3)	10 (6.2)	12 (7.3)	14 (8.5)	0.594	
HTN (n,%)	823	196 (23.8)	35 (21.4)	35 (21.4)	38 (23.3)	37 (22.2)	51 (30.7)	0.222	
SBP (mmHg)	853	128.2 ± 17.3	126.2 ± 15.9	129.8 ± 17.7	126.5 ± 16.1	128.4 ± 18.8	130.1 ± 17.6	0.124	0.134
DBP (mmHg)	853	76.7 ± 10.6	76.1 ± 10.3	77.3 ± 10.6	76.1 ± 10.7	76.9 ± 11.2	77.1 ± 10.5	0.777	0.562
FBS (mg/dL)	853	92.5 ± 17.2	91.2 ± 15.8	92.9 ± 18.1	91.9 ± 14.9	92.7 ± 18.3	93.7 ± 18.4	0.732	0.269
Calcium	853	9.20 ± 0.38	9.12 ± 0.39	9.18 ± 0.35	9.23 ± 0.37	9.24 ± 0.38	9.20 ± 0.38	0.026	0.012
Phosphorus	853	3.72 ± 0.55	3.70 ± 0.66	3.69 ± 0.57	3.74 ± 0.49	3.77 ± 0.51	3.69 ± 0.52	0.631	0.713
HbA1c (%)	853	5.85 ± 0.60	5.84 ± 0.60	5.83 ± 0.53	5.83 ± 0.60	5.85 ± 0.70	5.90 ± 0.56	0.778	0.312
Insulin (μIU/ml)	825	9.91 ± 4.05	9.63 ± 4.43	10.32 ± 4.59	9.85 ± 3.81	10.20 ± 3.84	9.53 ± 3.47	0.309	0.741
Total Cholesterol (mg/dL)	853	206.8 ± 37.3	198.0 ± 31.2	205.0 ± 40.3	212.6 ± 38.2	206.1 ± 37.0	212.3 ± 37.5	0.001	0.001
Non-HDL cholesterol (mg/dL)	853	148.3 ± 36.7	142.2 ± 32.0	148.0 ± 39.1	153.9 ± 37.3	147.1 ± 36.0	150.4 ± 38.1	0.051	0.084

Creatinine (mg/dL)	853	0.86 ± 0.12	0.84 ± 0.14	0.87 ± 0.10	0.86 ± 0.10	0.88 ± 0.13	0.86 ± 0.09	0.012	0.089
hs-CRP (mg/L)	811	0.14 ± 0.44	0.16 ± 0.42	0.12 ± 0.27	0.09 ± 0.14	0.13 ± 0.22	0.19 ± 0.82	0.298	0.529
Homocysteine (umol/L)	820	8.51 ± 2.66	8.53 ± 3.79	8.35 ± 2.02	8.39 ± 2.10	8.79 ± 3.06	8.49 ± 1.79	0.595	0.591
IMT, mm	853	0.65 ± 0.11	0.62 ± 0.11	0.65 ± 0.10	0.65 ± 0.11	0.66 ± 0.12	0.67 ± 0.10	0.005	<0.001
IMT ≥ 0.75 mm (n,%)	853	157 (18.4)	24 (14.0)	26 (15.0)	33 (19.5)	34 (20.0)	40 (23.5)	0.142	
IMT ≥ 0.90 mm (n,%)	853	32 (3.7)	5 (2.9)	6 (3.4)	5 (2.8)	8 (4.7)	8 (4.7)	0.848[C]	
Presence of plaque (n,%)	839	86 (10.2)	23 (13.8)	15 (8.7)	12 (7.1)	11 (6.5)	25 (15.0)	0.025[c]	
No of plaque	839	0.14 ± 0.47	0.17 ± 0.47	0.12 ± 0.44	0.10 ± 0.38	0.07 ± 0.28	0.24 ± 0.67	0.010	0.515
Volume of plaque, mm^3	839	5.1 ± 53.3	13.2 ± 112.7	3.4 ± 17.4	0.9 ± 6.3	0.9 ± 4.6	7.5 ± 36.4	0.180	0.297
Season of measurement (n,%)	853							0.007	
Spring (mar to may)		225 (23.3)	50 (29.2)	48 (27.7)	37 (21.8)	29 (17.0)	27 (15.8)		
Summer (Jun to Aug)		224 (23.2)	50 (29.2)	45 (26.0)	51 (30.1)	48 (28.2)	48 (28.2)		
Fall (Sep to Nov)		260 (27.0)	34 (19.8)	39 (22.5)	48 (28.4)	63 (37.0)	60 (35.2)		
Winter (Dec to Feb)		253 (26.3)	37 (21.6)	41 (23.7)	33 (19.5)	30 (17.6)	35 (20.5)		

Data are presented as the means and standard deviation [SD], or number and percentages

[a] *p* value from ANOVA for continuous variables or chi-squared test for categorical variables.

[b] p value from linear regression analysis

[C] p value from Fisher's exact test

Abbreviations: BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, higi-density lipoprotein; CRP, C-reactive protein; IMT, intima-media thickness

Univariate Correlations of various cardiovascular risk factors and mean CCA-IMT

As shown in Table 3, mean CCA-IMT was correlated with variouss variables of cardiovascular risk factors particularly among women. In man, mean CCA-IMT showed significant positive correlations with age, SBP, FBS, serum creatinine, and homocysteine while negatively correlated with weekly alcohol intake and serum calcium level. In women, mean CCA-IMT showed significant positive correlations with age, BMI, WC, SBP, DBP, FBS, HbA1c, total cholesterol, serum phosphorus, homocysteine and serum $1,25(OH)_2$ vitamin D_3 concentration.

Table 3. Pearson's correlation coefficients for univariate associations between various risk factors and mean CCA IMT.

	Men		Women	
	r	P value	*r*	P value
Age	0.439*		0.460*	<0.001
BMI	-0.033		0.164*	<0.001
WC (cm)	0.001	0.952	<0.001	<0.001
Weekly alcohol intake (standard drink)	-0.074*	0.026	0.293	0.7068
SBP (mmHg)	0.078*	0.014	0.214*	<0.001
DBP (mmHg)	-0.020	0.534	0.105*	0.002
FBS (mg/dL)	0.070*	0.029	0.174*	<0.001
HbA1c (%)	0.116	<0.001	0.191*	<0.001
Insulin (μIU/ml)	-0.028	0.376	0.061	0.077
Total Cholesterol (mg/dL)	-0.037	0.250	0.168*	<0.001
Non-HDL cholesterol (mg/dL)	-0.011	0.717	0.195*	<0.001
Calcium	-0.071*	0.027	-0.010	0.750
Phosphorus	-0.001	0.970	0.107*	0.001
Creatinine (mg/dl)	0.110*	<0.001	0.039	0.250
hs-CRP (mg/l)	0.023	0.479	0.046	0.188
Homocysteine (umol/L)	0.089*	0.006	0.072*	0.037
$1,25(OH)_2$ vitamin D_3 (pg/mL)	-0.043	0.179	0.099*	0.003

Abbreviations: BMI, body mass index; WC, waist circumference; DM, diabetes mellitus; HTN, hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL, higi-density lipoprotein; CRP, C-reactive protein; IMT, intima-media thickness

The associations between 1,25(OH)2 vitamin D3 concentration and Carotid atherosclerosis indexes

In ANCOVA, CCA IMT was not associated with $1,25(OH)_2$ vitamin D_3 concentration both in male and in female participants (Table 4 and 5). The R square values were 0.23 in Model 1, 0.24 in model 2, 0.26 in model 3 in men (Table 4) while the value were 0.24 in Model 1, 0.25 in model 2, 0.26 in model 3 in women (Table 5) analyzing CCA IMT with $1,25(OH)_2$ vitamin D_3 concentration.

In women, numbers and volume of CCA plaques were significantly associated with $1,25(OH)_2$ vitamin D_3 concentration (Table 5). The association showed the J or U shape. In the concentration of 3rd quintile (31.8-38.8 pg/ml) and 4th quintile (38.9-48.5 pg/ml), deceased number and volume of CCA plaque were demonstrated (p=0.022 and 0.023 for number of CCA plaque, and p =0.030 and 0.020 for volume of CCA plaque in Model 3, respectively). In the concentration of 5th quintile, no significant difference compared with the reference group of 1st quintile (p=0.571 for number of CCA plaque, and p =0.063 for volume of CCA plaque in Model 3, respectively).

In men, numbers and volume of CCA plaques were not significantly associated with $1,25(OH)_2$ vitamin D_3 concentration, although volume

of CCA plaque showed slightly J shaped association with $1,25(OH)_2$ vitamin D_3 concentration (Table 4).

In multivariate logistic regression analyses for binary outcomes, male participants showed no significant results in increased CCA IMT and CCA plaques (Table 6). But in female participants in Table 7, a reverse J-shaped relationship was reported. There was significantly decreased occurrence of CCA plaque was reported in 3rd quintile (p=0.011 in Model 3) 4th quintile (p=0.036 in Model 3). We found marginally significant decrease in 2nd quintile (p=0.059 in Model 3), however, no significant decrease or increase in 5th quintile (p=0.206 in Model 3).

Table 4. Mean values of carotid indexes of participants according to of $1,25(OH)_2$ vitamin D_3 concentration in men (n = 962).

		Quintiles of 1,25(OH)2 vitamin D3 (Median)					
		<32.0 (27.2) pg/ml *n* = 193	32.0-38.9 (35.1) pg/ml *n* = 192	39.0-46.8 (42.7) pg/ml *n* = 193	46.9-59.8 (51.7) pg/ml *n* = 193	>59.9 (73.6) pg/ml *n* = 191	*p* value[a]
CCA IMT	Model 1	0.703 (0.009)	0.718 (0.009)	0.696 (0.009)	0.706 (0.009)	0.702 (0.009)	0.630
	Model 2	0.700 (0.009)	0.711 (0.009)	0.696 (0.009)	0.704 (0.009)	0.706 (0.009)	0.831
	Model 3	0.700 (0.009)	0.712 (0.009)	0.696 (0.009)	0.704 (0.009)	0.706 (0.009)	0.815
Number of CCA plaques	Model 1	0.220 (0.052)	0.246 (0.053)	0.260 (0.052)	0.263 (0.051)	0.303 (0.053)	0.868
	Model 2	0.209 (0.053)	0.222 (0.053)	0.258 (0.052)	0.264 (0.051)	0.275 (0.051)	0.898
	Model 3	0.207 (0.053)	0.222 (0.054)	0.258 (0.052)	0.264 (0.051)	0.275 (0.051)	0.893
Volume of CCA plaques	Model 1	20.057 (16.325)	10.164 (16.682)	17.568 (16.515)	12.120 (15.977)	52.158 (16.848)	0.385
	Model 2	21.321 (17.077)	9.059 (17.210)	18.373 (17.069)	11.575 (16.393)	53.543 (17.608)	0.379
	Model 3	22.277 (17.147)	9.191 (17.243)	18.455 (17.103)	11.157 (16.423)	52.789 (17.703)	0.400

Note: Values are mean (s.e.) adjusted for multiple variables

Model 1: Values are odds ratios adjusted for age, BMI, regular exercise, cigarette smoking and weekly alcohol consumption, season of blood draw.

Model 2: Values are odds ratios adjusted for covariables of Model 1 + diabetes, hypertension, serum total and Non-HDL cholesterol level, fasting glucose level and systolic blood pressure.

Model 3: Values are odds ratios adjusted for covariables of Model 2 + serum creatinine level, calcium level and phosphorus level.

[a]*P* value from ANCOVA according to $1,25(OH)_2$ vitamin D_3 concentration

Table 5. Mean values of carotid indexes of participants according to of $1,25(OH)_2$ vitamin D_3 concentration in women (n = 853).

N		Quintiles of 1,25(OH)2 vitamin D3 (median)					*p* value[b]
		<25.5 (21.4) pg/ml *n* = 171	25.5-31.7 (28.7) pg/ml *n* = 173	31.8-38.8 (35.0) pg/ml *n* = 169	38.9-48.5 (51.7) pg/ml *n* = 170	>48.6 (59.1) pg/ml *n* = 170	
CCA IMT	Model 1	0.644 (0.008)	0.656 (0.008)	0.656 (0.008)	0.661 (0.008)	0.659 (0.008)	0.620
	Model 2	0.644 (0.008)	0.655 (0.008)	0.654 (0.009)	0.662 (0.008)	0.660 (0.008)	0.620
	Model 3	0.642 (0.008)	0.655 (0.008)	0.655 (0.008)	0.661 (0.008)	0.660 (0.008)	0.526
Number of CCA plaques	Model 1	0.201 (0.038)	0.117 (0.037)	0.078 (0.037)*	0.066 (0.037)*	0.186 (0.037)	0.031
	Model 2	0.200 (0.038)	0.118 (0.037)	0.072 (0.037)*	0.070 (0.037)*	0.169 (0.037)	0.052
	Model 3	0.198 (0.038)	0.118 (0.037)	0.073 (0.037)*	0.073 (0.037)*	0.166 (0.038)	0.075
Volume of CCA plaques (mm^3)	Model 1	16.863 (4.964)	3.242 (4.815)	1.128 (4.862)*	0.579 (4.807)*	3.476 (4.921)	0.119
	Model 2	17.149 (5.178)	3.336 (4.963)	1.273 (5.024)*	0.499 (4.998)*	3.182 (5.100)	0.137
	Model 3	17.225 (5.207)	3.243 (4.966)	1.453 (5.030)*	0.126 (5.021)*	3.397 (5.109)	0.135

Note: Values are mean (s.e.) adjusted for multiple variables
Model 1: Values are odds ratios adjusted for age, BMI, regular exercise, cigarette smoking and weekly alcohol consumption, season of blood draw.
Model 2: Values are odds ratios adjusted for covariables of Model 1 + diabetes, hypertension, serum total and Non-HDL cholesterol level, fasting glucose level and systolic blood pressure.
Model 3: Values are odds ratios adjusted for covariables of Model 2 + serum creatinine level, calcium level and phosphorus level.
[a]*P* value from ANCOVA according to $1,25(OH)_2$ vitamin D_3 concentration
**P* value <0.05 compared with the reference group

Table 6. Association of 1,25(OH)$_2$ vitamin D$_3$ concentration and increased carotid atherosclerosis in men (n = 962).

		Quintiles of 1,25(OH)2 vitamin D3 (Median)					
		<32.0 (27.2) pg/ml *n* = 193	32.0-38.9 (35.1) pg/ml *n* = 192	39.0-46.8 (42.7) pg/ml *n* = 193	46.9-59.8 (51.7) pg/ml *n* = 193	>59.9 (73.6) pg/ml *n* = 191	*p* value[b]
CCA IMT ≥ 0.75 mm	Model 1	1	0.87 (0.53−1.44)	0.97 (0.59−1.58)	0.68 (0.42−1.13)	0.91 (0.55−1.51)	0.463
	Model 2	1	0.95 (0.56−1.77)	1.06 (0.64−1.77)	0.76 (0.45−1.28)	1.10 (0.65−1.86)	0.949
	Model 3	1	0.96 (0.57−1.63)	1.07 (0.64−1.78)	0.77 (0.46−1.30)	1.11 (0.65−1.89)	0.979
CCA IMT ≥ 0.90 mm	Model 1	1	1.33 (0.61−2.90)	0.77 (0.32−1.81)	1.05 (0.48−2.29)	1.01 (0.45−2.29)	0.810
	Model 2	1	1.28 (0.56−2.92)	0.83 (0.35−2.00)	1.13 (0.50−2.58)	1.17 (0.50−2.73)	0.839
	Model 3	1	1.27 (0.55−2.91)	0.83 (0.34−1.99)	1.09 (0.47−2.51)	1.15 (0.49−2.71)	0.899
Occurrence of CCA plaque	Model 1	1	1.05 (0.55−1.99)	1.12 (0.60−2.08)	0.91 (0.49−1.71)	1.22 (0.66−2.26)	0.688
	Model 2	1	0.92 (0.47−1.82)	1.16 (0.61−2.19)	0.91 (0.47−1.76)	1.10 (0.57−2.11)	0.807
	Model 3	1	0.95 (0.48−2.23)	1.17 (0.61−2.23)	0.94 (0.48−1.82)	1.12 (0.58−2.16)	0.778

Model 1: Values are odds ratios adjusted for age, BMI, regular exercise, cigarette smoking and weekly alcohol consumption, season of blood draw.
Model 2: Values are odds ratios adjusted for covariables of Model 1 + diabetes, hypertension, serum total and Non-HDL cholesterol level, fasting glucose level and systolic blood pressure.
Model 3: Values are odds ratios adjusted for covariables of Model 2 + serum creatinine level, calcium level and phosphorus level.

Table 7. Association of 1,25(OH)$_2$ vitamin D$_3$ concentration and increased carotid atherosclerosis in women (n = 853).

		Quintiles of 1,25(OH)2 vitamin D3 (median)					p value[b]
		<25.5 (21.4) pg/ml n = 171	25.5-31.7 (28.7) pg/ml n = 173	31.8-38.8 (35.0) pg/ml n = 169	38.9-48.5 (51.7) pg/ml n = 170	>48.6 (59.1) pg/ml n = 170	
CCA IMT ≥ 0.75 mm	Model 1	1	0.85 (0.42−1.74)	1.18 (0.60−2.33)	0.97 (0.49−1.91)	1.20 (0.62−2.33)	0.483
	Model 2	1	0.81 (0.39−1.69)	1.16 (0.58−2.35)	0.97 (0.47−1.96)	1.21 (0.61−2.40)	0.433
	Model 3	1	0.92 (0.44−1.33)	1.33 (0.65−2.72)	1.07 (0.51−2.22)	1.37 (0.68−2.76)	0.298
CCA IMT ≥ 0.90 mm	Model 1	1	0.98 (0.24−3.99)	0.90 (0.21−3.84)	1.40 (0.38−5.12)	1.36 (0.37−4.88)	0.474
	Model 2	1	1.13 (0.27−4.77)	0.66 (0.13−3.30)	1.70 (0.43−6.67)	1.49 (0.39−5.67)	0.402
	Model 3	1	1.67 (0.36−7.66)	1.03 (0.19−5.59)	2.61 (0.60−11.41)	2.09 (0.50−8.74)	0.239
Occurrence of CCA plaque	Model 1	1	0.44 (0.19−1.03)	0.30 (0.11−0.78)*	0.37 (0.16−0.88)*	0.65 (0.31−1.38)	0.320
	Model 2	1	0.41 (0.17−1.00)	0.25 (0.09−0.71)*	0.37 (0.15−0.90)*	0.59 (0.26−1.30)	0.268
	Model 3	1	0.42 (0.17−1.03)	0.26 (0.09−0.74)*	0.38 (0.15−0.94)*	0.59 (0.26−1.32)	0.299

Model 1: Values are odds ratios adjusted for age, BMI, regular exercise, cigarette smoking and weekly alcohol consumption, season of blood draw.
Model 2: Values are odds ratios adjusted for covariables of Model 1 + diabetes, hypertension, serum total and Non-HDL cholesterol level, fasting glucose level and systolic blood pressure.
Model 3: Values are odds ratios adjusted for covariables of Model 2 + serum creatinine level, calcium level and phosphorus level.
*P value <0.05

Discussion

In the present study, male participants showed mean CCA-IMT level of 0.70 ± 0.15mm and female participants showed the level of 0.65 ± 0.11mm, which correspond to previous study of healthy Koreans' IMT with the value of 0.66 ± 0.11 mm (34). Mean 1,25$(OH)_2$ vitamin D_3 concentration, 47.18 ± 20.73 pg/dl in male and 38.22 ± 16.79 pg/dl, does also correspond to the level of previous population−based studies (30, 35). We found significant associations between serum 1,25(OH)2 vitamin D3 concentration and plaque of CCA only in women. Adequate serum 1,25(OH)2 vitamin D3 concentration is associated with decreased occurrence of carotid plaques, numbers and volumes of carotid plaques. And these associations seem to be J-shape or U shape suggesting that there could be a threshold of vitamin D concentration adequate for decreasing subclinical cardiovascular diseases. The lowest risk of plaque seemed to be with the levels between 31.8 and 48.5 pg/ml of 1,25(OH)2 vitamin D3 independently of other factors.

In the present study, we had used serum 1,25$(OH)_2$ vitamin D_3 concentration instead of 25(OH) vitamin D_3. 25 (OH) vitamin D_3 requires additional hydroxylation by 1-alpha-hydroxylase to become active 1,25$(OH)_2$ vitamin D_3. The half-life of 25(OH) vitamin D_3 in the circulation is 2 weeks, whereas the half-life of its active metabolite is less than 4 hours. The concentration of 1,25$(OH)_2$ vitamin D_3 is 1000-fold lower than that of 25 (OH) vitamin D_3.

Therefore, guideline does not recommend $1,25(OH)_2$ vitamin D_3 as a screening tool for vitamin D deficiency (18). However, previous studies showed low 1,25(OH)2 vitamin D3 is also independently associated with cardiovascular outcomes (30, 36), and 25(OH) vitamin D_3 is a predictor of serum $1,25(OH)_2$ vitamin D_3 (37). One study suggest 25 (OH) vitamin D_3 and $1,25(OH)_2$ vitamin D_3 levels may yield similar but independent biologic effects for cardiovascular outcomes (30). Another study found $1,25(OH)_2$ vitamin D_3 showed strong association with renal outcome more than 25 (OH) vitamin D_3 did (36).

Up to now, optimal levels of vitamin D have not been fully established. Regarding cardiovascular mortality, Durup et al. (20) and Hutchinson et al. (19) suggest that a U-shaped or J-shaped association between 25 (OH) vitamin D_3 and all-cause mortality with the optimal 25 (OH) vitamin D_3 level of approximately 50 − 75 mmol/Liter (=24 − 32 ng/mL, to convert to nanogram per milliliter, divide by 2.496). This level seem to correspond to the level of 31 − 50 ng/mL of 1,25(OH)2 vitamin D3 according to the previous study (30), which is similar to the level of decreasing carotid plaque in the present study.

Koreans usually consumes vitamin D far lesser, as low as 6 ug (38), than Recommended Daily Allowance (RDA). RDA for vitamin D intake is the level of 15ng (600IU) in young and middle-aged subjects, and recommendations for diseased patients at risk for vitamin D deficiency is

widely-ranged from 37.5ng (1500IU) to 50ng (2000IU) (18). There should be more evidences for adequate intake of vitamin D preventing cardiovascular diseases.

We found the lowest vitamin D level is associated with subclinical atherosclerosis only in female participants. Allegedly, vitamin D insufficiency is more prevalent in women across the world (39-42). In Korea, vitamin D insufficiency (25(OH) vitamin D_3 <20 mg/dL) was found in 47.3% of males and 64.5% of females of general population (43). It is Not only in its epidemiology, but also in its effect, that some studies suggest there is a gender difference of vitamin D. Correale et al. suggest that there is estrogen-promoted differences in vitamin D3 metabolism and there be a greater protective effect of vitamin D of immunomodulation in women (44). Estrogen stimulates 1,25(OH)2 vitamin D3 accumulation during ovulation and pregnancy, when its levels are high, and active vitamin D3 levels increase in postmenopausal women receiving estrogen therapy (45-47). Treating osteoporosis, a synergistic effect between vitamin D3 and estrogen on increasing bone mineral density has been reported (48). There should be more evidences about gender difference of effect of vitamin D in cardiovascular health.

Carotid ultrasonography is an early and effective marker of onset of cardiovascular diseases which is being real-time, economic, reliable, safe, and now seems to become a standard in vascular assessment methodology (26, 27).

Measurement of carotid plaque is more strongly predictive of cardiovascular events than is measurement of carotid intima-media thickness (IMT) (28, 49). In the present study, we found significant association only in CCA plaque. Recently, Inaba et al. suggested that Diffuse adaptive thickening of carotid wall (measured by CCA-IMT) as a response to ageing and hypertension rather reflects arteriosclerosis than plaque formation, which is a focal process and carotid plaque is more related to atherosclerotic processes such as inflammation, oxidation and endothelial dysfunction (28). Also, the genetic determinants of carotid IMT and carotid plaques may differ (50). Vitamin D mechanism is more related to atherosclerotic processes such as inflammation, oxidation and endothelial dysfunction rather than aging and hypertension (12, 16), therefore, our present study is comparable to those previous results.

Our study has several limitations. First, this study was cross-sectional. Thus, we could not establish a cause-and-effect relationship between serum $1,25(OH)_2$ vitamin D_3 concentration and carotid atherosclerosis. Future longitudinal studies of vitamin D and carotid atherosclerosis are needed. Second, through 2006-2007, health questionnaire survey did not include questions about current medication for hyperlipidemia. Although we controlled total cholesterol and Non-HDL cholesterol level, which could introduce bias. Third, we were unable to evaluate sun exposure exactly, because we do not include survey about weather condition of the survey day and sunscreens the participants might use. Fourth, as aforementioned, We

used serum $1,25(OH)_2$ vitamin D_3 concentration instead of 25(OH) vitamin D_3, which has short half-life. As mentioned above, serum $1,25(OH)_2$ vitamin D_3 has a short half-life and hard to measure.

Conclusions

In the present study, low 1,25$(OH)_2$ vitamin D_3 concentration showed a significant association with increased risk of carotid plaque independent of other factors, implying that low vitamin D alters vascular metabolism. Moreover, the results of present study indicate that adequate vitamin D concentration, not too low or too high level, is associated with decrease of subclinical carotid plaques among Korean women, but not among Korean men. Interventions targeting healthy individuals with lower or higher level of vitamin D may be needed. Our results will be a useful reference for further assessments of vitamin D status.

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