The Association between Atherosclerotic Disease Risk Factors and Serum 25-Hydroxyvitamin D Concentration in Japanese Subjects

Akane YASUOKA[1], Naoko TSUGAWA[2,3], Chihiro URA[4], Honami OGASAWARA[3], Kiyoshi TANAKA[5], Kei MIZUNO[6,7,8,9], Yasuyoshi WATANABE[6,7,8,10] and Akiko KUWABARA[1,11,*]

[1] *Department of Clinical Nutrition, Graduate School of Comprehensive Rehabilitation, Osaka Prefecture University, 3–7–30 Habikino, Habikino, Osaka, 583–8555, Japan*
[2] *Faculty of Nutrition, Kobe Gakuin University, 518, Ikawadanicho-Arise, Nishi-ku, Kobe, Hyogo 651–2180, Japan*
[3] *Department of Health and Nutrition, Osaka Shoin Women's University, 4–2–26 Hishiyanishi, Higashiosaka, Osaka 577–8550 Japan*
[4] *Department of Packaged Food Engineering, Toyo College of Food Technology, 4–23–2 Minami-Hanayashiki, Kawanishi, Hyogo 666–0026, Japan*
[5] *Research Support Center, Shizuoka General Hospital, 4–27–1, Kita-ando, Aoi, Shizuoka 420–8527, Japan*
[6] *RIKEN Compass to Healthy Life Research Complex Program, 6–7–1 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650–0047, Japan*
[7] *Laboratory for Pathophysiological and Health Science, RIKEN Center for Biosystems Dynamics Research, 6–7–3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650–0047, Japan*
[8] *Center for Health Science Innovation, Osaka Metropolitan University, 3–1 Ofuka-cho, Kita-ku, Osaka, Osaka 530–0011, Japan*
[9] *Department of Healthcare Solution Science, Graduate School of Science, Technology and Innovation, Kobe University, 6–7–3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650–0047, Japan*
[10] *Department of Essential Healthcare Science, Graduate School of Science, Technology and Innovation, Kobe University, 6–7–3 Minatojima-minamimachi, Chuo-ku, Kobe, Hyogo 650–0047, Japan*
[11] *Department of Nutrition, Graduate School of Human Life and Ecology, Osaka Metropolitan University, 3–7–30 Habikino, Habikino, Osaka 583–8555, Japan*

(Received December 21, 2022)

Summary Recent studies have described that vitamin D deficiency/insufficiency is associated with hypertension, insulin resistance, and dyslipidemia, which are major components of metabolic syndrome causing atherosclerosis. Therefore, we investigated the relationship between serum 25-hydroxyvitamin D [25(OH)D] concentration and atherosclerotic disease risk factors in healthy Japanese adults. In the present cross-sectional study, 1,177 subjects (348 males and 829 females) aged 20–72 y living in Japan (34.7–35.0˚N) were evaluated for vitamin D status by measuring serum 25(OH)D concentration. Atherosclerotic disease risk factors were defined as the presence of two or more of the following three risk factors: high blood pressure, dyslipidemia, and hyperglycemia. The percentages of vitamin D deficient and insufficient subjects were 33% and 46% in males and 59% and 32% in females, respectively. Subjects with atherosclerotic disease risk factors were significantly older and had higher BMI than those without it in both sexes. Male subjects with atherosclerotic disease risk factors had significantly lower physical activity and serum 25(OH)D concentration than those without it. In a logistic regression analysis adjusted for confounding factors, serum 25(OH)D concentration showed a significant inverse association with risk factors of atherosclerotic disease in males (OR=0.951, 95%CI: 0.906–0.998), but not in females. A covariance structure analysis also suggested that serum 25(OH)D level has a direct association with risk factors of atherosclerotic disease. In conclusion, we have demonstrated that low serum 25(OH)D level is a significant factor for increased atherosclerotic disease risk factors in males.

Key Words atherosclerotic disease risk factors, 25-hydroxyvitamin D, metabolic syndrome, vitamin D insufficiency, Japanese

Metabolic syndrome (MetS) refers to a state in which accumulated visceral fat causes insulin resistance, clustering of multiple risks such as hypertension, dyslipidemia, and hyperglycemia, thereby enhancing the progression of atherosclerosis and increasing the risk for cerebrovascular diseases and cardiovascular events (*1*). Considering that the above-mentioned diseases are associated with increased mortality and morbidity in many countries including Japan (https://www.mhlw.

*To whom correspondence should be addressed.
E-mail: kuwabara.akiko@omu.ac.jp

go.jp/toukei/saikin/hw/jinkou/geppo/nengai20/dL/gaikyouR2.pdf), and percentage of subjects strongly suspected to have MetS was as high as 28.2% in males and 10.3% in females according to the National Health and Nutrition Examination Survey 2019 (https://www.mhlw.go.jp/content/000711007.pdf), the prevention of MetS and arteriosclerosis is of crucial societal importance.

The most fundamental function of vitamin D is to promote the absorption of calcium from the intestinal tract, and its deficiency increases the risk of rickets and osteomalacia (*2*). Even vitamin D insufficiency, milder than deficiency, is a serious risk for fracture. Recently, extra-skeletal action of vitamin D is increasingly recognized, and vitamin D deficiency/insufficiency has been also reported to affect hypertension, insulin resistance, and dyslipidemia (*3*). Vitamin D deficiency/insufficiency is highly prevalent worldwide and also in Japan (*4*). Therefore, improving vitamin D status was considered likely to be beneficial for prevention of MetS.

The mechanisms underlying the above-mentioned MetS prevention by vitamin D have been postulated as below; 1α,25-dihydroxy-vitamin D [1,25$(OH)_2$D] which is the active form of vitamin D negatively regulates renin transcription through a vitamin D receptor (VDR)-mediated mechanism (5). Lack of VDR was associated with impaired glucose tolerance and insulin secretion (*6*), since 1,25$(OH)_2D_3$ may play important roles in insulin receptor levels and insulin-stimulated glucose transport (*7*). The effect of vitamin D on lipid metabolism might be exerted through the effect of 1,25$(OH)_2D_3$ and 25-hydroxyvitamin D [25(OH)D] on sterol regulatory element binding protein-1c (SREBP-1c), which regulates lipogenesis, and peroxisome proliferator-activated receptor α (PPARα), and promotion of lipid β-oxidation (*8*, *9*). Other possible mechanisms include vitamin D affecting serum lipids through increased calcium levels, which may reduce the formation and/or secretion of triglyceride in the liver, and influence both insulin secretion and sensitivity, thereby indirectly affecting lipid metabolism (*10*). These mechanisms, however, have not been fully established. In a meta-analysis of cross-sectional studies, serum 25(OH)D, which is the best indicator of vitamin D nutritional status, was negatively associated with the prevalence of MetS (*11*). Although various meta-analyses have been reported, most of the studies were conducted on Western subjects, suggesting the need for studies in Japanese. A more recent cross-sectional study of 1,790 Japanese workers has also described that higher serum 25(OH)D level was associated with lower odds for MetS (*12*). However, this paper is not free from methodological problems, e.g., analysis of males and females as a whole despite marked gender differences in MetS prevalence. In addition, physical activity had a positive correlation with serum 25(OH)D concentration (*13*–*15*) and were also associated with the risk of atherosclerotic disease (*16*, *17*). Thus, deciding whether vitamin D affects the atherosclerotic disease risks directly or indirectly through physical activity would greatly enhance our understanding of vitamin D action.

Based on these considerations, we have investigated the relationship between 25(OH)D concentrations and the risk factors of atherosclerotic disease, evaluated the predictive value of serum 25(OH)D for the risk, and determined the possible involvement of physical activity in mediating the vitamin D action in healthy Japanese adults in males and females.

MATERIALS AND METHODS

Subjects. All subjects were recruited at RIKEN; Institute of Physical and Chemical Research (Japan) in Kobe (34.7˚N) between September 2018 and February 2019, including 1,193 subjects aged 20–72 y living in Kinki Area, Japan (34.7–35.0˚N). Sixteen subjects were excluded because of the lack of 25(OH)D measurements and/or atherosclerotic disease risk factors, and data from 1,177 (348 males and 829 females) subjects were used for analyses. Detailed information was given, and written consent was obtained from the subject. The study protocol was approved by the ethical committee in RIKEN (Approval number: Kobe2 2017-04(6)).

Measurement of serum 25-hydroxyvitamin D concentration. No specification was made regarding the time of blood collection. After centrifugation, serum was kept frozen at −80˚C until analysis. Serum vitamin D metabolites were measured by liquid chromatography-tandem mass spectrometry (LC-APCI-MS/MS) (*18*), with the following modification; derivation of extracted vitamin D metabolites by 4-[2-(6,7-dimethoxy-4-methyl-3-oxo-3,4-dihydroquinoxalyl)ethyl]-1,2,4-triazoline-3,5-dione (DMEQ-TAD) to acquire higher sensitivity by increased ionization efficiency (*19*). The subjects were divided into three groups based on their serum 25(OH)D concentrations: deficiency (<20 ng/mL), insufficiency (≥20 and <30 ng/mL), and sufficiency (≥30 ng/mL) (*2*, *20*).

Assessment of covariates. Information on demographic characteristics, e.g. age and sex, and medical histories were obtained. Collection of basic information, including smoking history, drinking habits, use of vitamin D or medicine, physical activity, and questionnaire on diet were completed online. BMI was calculated from the individual height and weight. The season of blood draw was divided into summer (July–September), fall (October–December), and winter (January–March). Serum concentrations of the following parameters were measured; serum concentration of total cholesterol (Tch), HDL-cholesterol (HDL-C), diacron reactive oxygen metabolites (d-ROMs), and blood hemoglobin A1c (HbA1c) level.

Definition of atherosclerotic disease risk factors. MetS is characterized by increased visceral adiposity, which is evaluated using computed tomography, waist circumference (WC) being the surrogate for the screening purpose (*21*). WC measurement, however, carries various diagnostic problems such as no internationally acknowledged cut-off value with marked country-to-country variation. Also, WC measurement is optional, or not included in the diagnosis of MetS outside Japan (*1*). Although the Japanese diagnostic criteria for MetS

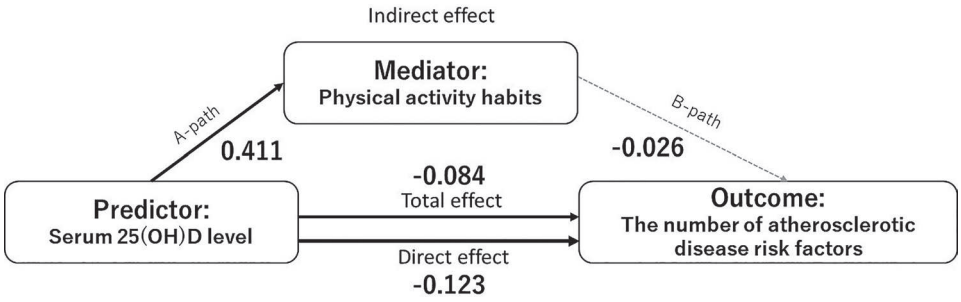

Fig. 1. A model for conceptualizing physical activity habits as a mediator of the relationship between serum 25(OH)D concentrations and the number of atherosclerotic disease risk factors in males. In the figure, the numbers of A-path and B-path indicate the value of the standardized total effect. Solid and double lines indicate significant effects. The model was adjusted by age and BMI. The direct effect of serum 25(OH)D concentration on the number of atherosclerotic disease risk factors was greater than the indirect effect. Chi-square test=4.654, p= 0.098.

include increased WC plus at least two of the following three indices: high blood pressure, dyslipidemia, and hyperglycemia, WC measurements were not included in this study based on the above consideration. Since the subjects in the present study included those with non-fasting blood samples, we have adopted an assessment of possible metabolic syndrome employed in the National Health and Nutrition Survey Japan (https://www.mhlw.go.jp/content/000711004.pdf), which had similar blood sampling conditions. In brief, participants were diagnosed as having atherosclerotic disease risk factors if they have two or more of the following three factors: (1) high blood pressure: systolic blood pressure (SBP)≥130 mmHg, diastolic blood pressure (DBP)≥85 mmHg, or under-treatment of hypertension; (2) dyslipidemia: HDL-C<40 mg/dL or under-treatment of dyslipidemia; (3) HbA1c≥6.0% or under-treatment of diabetes.

Statistical analysis. Statistical analyses were performed using IBM SPSS Statistics version 27.0 J (IBM, Armonk, NY, USA). Median (1st quartile, 3rd quartile) were calculated for all continuous variables, while absolute number and percentages were calculated for categorical variables. Comparison between the two independent groups was made by Mann-Whitney test. Contingency tables were analyzed by chi-square test in complex sample analysis. Binomial logistic regression analyses were performed to identify factors contributing to risk factors of atherosclerotic disease. Model 1 was adjusted by age, and sex (adopted for the analysis of total only), and model 2 was additionally adjusted by BMI, smoking history, drinking habits, and physical activity. The same adjustment factors were used in the analysis with categorical data on vitamin D nutritional status. A receiver operating characteristic curve (ROC) was used to calculate the detective ability and cut-off value of serum 25(OH)D level for the presence of risk factors of atherosclerotic disease.

Two parameters, quite likely to be associated with risk factors of atherosclerotic disease; serum 25(OH)D concentration and physical activity, were significantly associated in men (Spearman's correlation r=0.381, p<0.001). Then, vitamin D status was considered likely to affect the atherosclerotic disease risk factors both directly and indirectly through physical activity, since muscle strength is significantly influenced by vitamin D status. Therefore, we performed covariance structure analysis in males to determine the potential mediation effect of physical activity, which consisted of serum 25(OH)D level (cause), physical activity (mediator), and the number of atherosclerotic disease risk factors (outcome) (Fig. 1). The mediation models the maximum likelihood method was used to verify the mediation effects, the SE (standard error) for direct and indirect effects (*22*). This analysis was performed using IBM SPSS Amos version 27.0 J (IBM).

The statistical significance assumed for all analyses was defined as a two-tailed p<0.05.

RESULTS

Study subjects consisting of 1,177 subjects (348 males and 829 females) were analyzed. The percentage of subjects with atherosclerotic disease risk factors was 14.4% in males and 3.3% in females. The percentages of subjects with vitamin D deficiency and insufficiency were 33% and 46% in males and 59% and 32% in females. Table 1 describes the characteristics of subjects with or without atherosclerotic disease risk factors. In both genders, subjects with atherosclerotic disease risk factors were significantly older and had higher BMI than those without it. Blood pressure and HbA1c level were higher, and HDL-C was lower in both subjects with atherosclerotic disease risk factors. Male subjects with atherosclerotic disease risk factors had a higher percentage of rarely performing physical activity than those without it, while in females, the prevalence of elevated d-ROMs was significantly higher in subjects with atherosclerotic disease risk factors than the counterpart. Male subjects with atherosclerotic disease risk factors had significantly lower serum 25(OH)D concentration and lower prevalence of vitamin D sufficiency than those without it.

Table 2 shows that serum 25(OH)D concentration was significantly inversely associated with the presence of risk factors of atherosclerotic disease in males, but not in females in model 1. After additional adjustment

Table 1. Characteristics of the study participants with and without atherosclerotic disease risk factors.

	Total (n=1,177)			Males (n=348)			Females (n=829)		
	No	Yes	p-value	No	Yes	p-value	No	Yes	p-value
n	1,100	77		298	50		802	27	
Age, y	45 (35, 53)	53 (47, 58)	<0.001	46.5 (34.0, 54.0)	53.0 (46.0, 58.0)	<0.001	45 (36, 52)	54 (50, 58.5)	<0.001
BMI, kg/m^2	21.2 (19.5, 23.6)	25.8 (23.0, 28.1)	<0.001	23.35 (21.4, 25.2)	25.9 (23.7, 28.1)	<0.001	20.6 (19, 22.3)	25.6 (21.15, 28.1)	<0.001
≥BMI 25 kg/m^2, n (%)	157 (14.3)	45 (58.4)	<0.001	85 (28.5)	30 (60.0)	<0.001	72 (9.0)	15 (55.6)	<0.001
Years of education, y	16 (14, 16)	16 (14, 16)	0.497	16 (16, 18)	16 (16, 16)	0.064	16 (14, 16)	14 (14, 16)	0.238
Smoking status, n (%)			<0.001			0.155			0.282
Never smoked	804 (73.1)	40 (51.9)		121 (40.6)	15 (30.0)		683 (85.2)	25 (92.6)	
Ever smoked	296 (26.9)	37 (48.1)		177 (59.4)	35 (70.0)		119 (14.8)	2 (7.4)	
Alcohol consumption, n (%)			0.403			0.384			0.149
Never/Less than ones per month	413 (37.5)	31 (40.3)		66 (22.1)	14 (28.0)		347 (43.3)	17 (63.0)	
2 to 3 times per month/Less than ones per week	315 (28.6)	20 (26.0)		82 (27.5)	13 (26.0)		233 (29.1)	7 (25.9)	
2–3 times per week	145 (13.2)	6 (7.8)		50 (16.8)	4 (8.0)		95 (11.8)	2 (7.4)	
More than 4 times per week	227 (20.6)	20 (26.0)		100 (33.6)	19 (38.0)		127 (15.8)	1 (3.7)	
Physical activity habits, n (%)[1]			0.382			0.007			0.784
Rarely	504 (45.8)	40 (51.9)		92 (30.9)	27 (54.0)		412 (51.4)	13 (48.1)	
1 to 2 times per month	216 (19.6)	15 (19.5)		89 (29.9)	9 (18.0)		127 (15.8)	6 (22.2)	
Ones per week	192 (17.5)	14 (18.2)		59 (19.8)	9 (18.0)		133 (16.6)	5 (18.5)	
More than twice per week	181 (16.5)	7 (9.1)		58 (19.5)	4 (8.0)		123 (15.3)	3 (11.1)	
The season of blood draw, n (%)			0.122			0.396			0.317
Summer	166 (15.1)	13 (16.9)		61 (20.5)	10 (20.0)		105 (13.1)	3 (11.1)	
Fall	530 (48.2)	28 (36.4)		134 (45.0)	18 (36.0)		396 (49.4)	10 (37.0)	
Winter	404 (36.7)	36 (46.8)		103 (34.6)	22 (44.0)		301 (37.5)	14 (51.9)	
SBP, mmHg	112 (103, 124)	135 (127, 144)	<0.001	122 (114, 132)	137 (130, 144)	<0.001	109 (101, 119)	132 (123, 140)	<0.001
DBP, mmHg	75 (68, 83)	89 (83, 97)	<0.001	81 (74, 89)	91 (84, 103)	<0.001	73 (66, 79)	87 (82, 92)	<0.001
Tch, mg/dL	203 (181, 230)	212 (191, 241)	0.061	200 (181, 221)	205 (187, 229)	0.172	205 (181, 235)	220 (194, 256)	0.037
LDL-C, mg/dL	116 (99, 140)	129 (108, 154)	0.005	119 (101, 139)	121 (105, 149)	0.286	115 (98, 140)	133 (123, 158)	0.005
HDL-C, mg/dL	71 (59, 83)	55 (46, 66)	<0.001	58 (49, 72)	51 (45, 63)	<0.001	75 (65, 86)	60 (53, 71)	<0.001
HbA1c, %	5.3 (5.1, 5.4)	5.8 (5.4, 6.3)	<0.001	5.3 (5.1, 5.4)	6.0 (5.4, 6.4)	<0.001	5.3 (5.1, 5.4)	5.7 (5.4, 6.1)	<0.001
d-ROMs, U.CARR	355 (309, 406)	364 (308, 424)	0.377	321 (280, 363)	337 (293, 368)	0.173	368 (320, 418)	416 (385, 484)	<0.001
High d-ROMs, n (%)	880 (80.0)	61 (79.2)	0.869	188 (63.1)	34 (68.0)	0.504	692 (86.3)	27 (100.0)	0.039
25(OH)D, ng/mL	19.7 (15.6, 24.2)	20.3 (16.1, 24.6)	0.971	24.0 (19.0, 29.3)	20.8 (16.8, 25.8)	0.007	18.4 (14.9, 23.5)	18.5 (14.1, 23.5)[1]	0.889
Categories of vitamin 25(OH)D, n (%)			0.292			0.020			0.849
Deficiency	564 (51.3)	38 (49.4)		91 (30.5)	23 (46.0)		473 (59.0)	15 (55.6)	
Insufficiency	395 (35.9)	33 (42.9)		138 (46.3)	23 (46.0)		257 (32.0)	10 (37.0)	
Sufficiency	141 (12.8)	6 (7.8)		69 (23.2)	4 (8.0)		72 (9.0)	2 (7.4)	

Data are Median (Q1, Q3) for continuous variables, while absolute number and percentages for categorical various.

Chi-square test.

Mann-Whitney U test.

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D.

[1] One missing value for males, 7 missing value for females.

Table 2. Multivariable-adjusted ORs (95%CI) for risk factors of atherosclerotic disease according to continuous of serum 25(OH)D.

25(OH)D	Number of cases, *n* (%)	ORs (95%CI)	
		Model 1	Model 2
Total	77/1,177 (6.5)	0.946 (0.914–0.979)	0.962 (0.928–0.996)
p-value		0.001	0.031
Males	50/348 (14.4)	0.933 (0.895–0.974)	0.951 (0.906–0.998)
p-value		0.001	0.042
Females	27/829 (3.3)	0.970 (0.916–1.026)	0.987 (0.930–1.048)
p-value		0.284	0.668

Backward Elimination (Likelihood Ratio).
Model 1 adjusted covariates for age (continuous) and sex (total only, males or females).
Model 2 adjusted covariates for model 1 plus BMI (<25 or ≥25), smoking history (never or ever), drinking habits (never/less than ones a month, 2–3 times a month/less than ones a week, 2–3 times a week or 4 times a week or more), and physical activity (not at all, 1–2 times a month, ones a week or 2 times a week or more).
25(OH)D, 25-hydroxyvitamin D.

Table 3. Multivariable-adjusted ORs (95%CI) for risk factors of atherosclerotic disease according to categorical type of serum 25(OH)D.

25(OH)D	Number of cases, *n* (%)	ORs (95%CI)	
		Model 1	Model 2
Total			
Categorical type of 25(OH)D			
Deficiency	38 (6.3)	1.00 (reference)	1.00 (reference)
Insufficiency	33 (7.7)	0.744 (0.439–1.262)	0.929 (0.531–1.623)
Sufficiency	6 (4.1)	0.234 (0.092–0.598)	0.330 (0.125–0.868)
Males			
Categorical type of 25(OH)D			
Deficiency	23 (20.2)	1.00 (reference)	1.00 (reference)
Insufficiency	23 (14.3)	0.596 (0.307–1.157)	0.694 (0.341–1.412)
Sufficiency	4 (5.5)	0.164 (0.052–0.511)	0.258 (0.076–0.874)
Females			
Categorical type of 25(OH)D			
Deficiency	15 (3.1)	1.00 (reference)	1.00 (reference)
Insufficiency	10 (3.7)	0.954 (0.411–2.215)	1.275 (0.505–3.221)
Sufficiency	2 (2.7)	0.564 (0.123–2.587)	0.708 (0.131–3.833)

Backward Elimination (Likelihood Ratio).
Model 1 adjusted covariates for age (continuous) and sex (total only, males or females).
Model 2 adjusted covariates for model 1 plus BMI (<25 or ≥25), smoking history (never or ever), drinking habits (never/less than ones a month, 2–3 times a month/less than ones a week, 2–3 times a week or 4 times a week or more), and physical activity (not at all, 1–2 times a month, ones a week or 2 times a week or more).
25(OH)D, 25-hydroxyvitamin D.

(model 2), the association remained statistically significant only in males with the odds ratio (95%CI) of 0.951 (0.906–0.998). Furthermore, the odds ratio was significantly lower only in the vitamin D sufficiency group compared to the vitamin D deficiency group (Table 3).

The relationship between 25(OH)D level and the presence of atherosclerotic disease risk factors was analyzed by the ROC analysis. The area under the curve (AUC) was 0.62 (95%CI: 0.54–0.70) in males (Fig. 2). Although serum 25(OH)D concentration significantly predicted atherosclerotic disease risk factors, we have decided not to calculate the cutoff values, since the AUC was not considered high enough to yield a cutoff value with high reliability.

As shown in Fig. 1, serum 25(OH)D level directly exerted a negative effect on the atherosclerotic disease risk factors. Although serum 25(OH)D exerted a strong positive effect on physical activity habit, the contribution of physical activity habit on the risk was limited. Then, it was considered that the overall indirect effect of vitamin D status was small, and serum 25(OH)D level mostly directly decreased the risk factors of atherosclerotic disease.

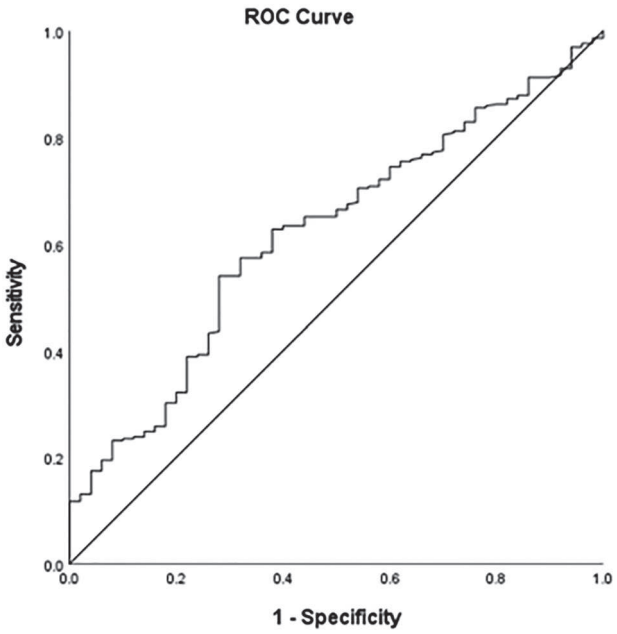

Fig. 2. ROC curve of serum 25(OH)D concentration reflecting the presence of risk factors of atherosclerotic disease. The detective ability and cut-off value of serum 25(OH)D level for atherosclerotic disease risk factors were evaluated by ROC analysis. For the presence of atherosclerotic disease risk factors, AUC was 0.62 (95%CI: 0.54–0.70), and the cut-off value was determined to be 23 ng/mL with the specificity and sensitivity being 54.0% and 63.5%, respectively.

DISCUSSION

The present cross-sectional study has revealed that higher serum 25(OH)D level is associated with a lower risk factors of atherosclerotic disease in males. There have been several reports on the association between serum 25(OH)D level and atherosclerotic disease risk, some showing significant negative correlation, which is consistent with our data (*9*, *23–25*), whereas others reporting no association (*26*, *27*).

One possible reason for such discrepancy would be the different circulating 25(OH)D levels of the subjects. For example, the 25(OH)D levels in the American study subjects were higher than those in our study subjects, with small-subject variance (*26*), whereas in the Indian study, most subjects had deficiency levels of 25(OH)D$<$ 20 ng/mL (*27*). A meta-analysis of cross-sectional studies, however, suggested that circulating levels of 25(OH)D had a significant negative contribution to the risk of atherosclerotic disease (*11*, *28*).

In the present study, a negative association between serum 25(OH)D concentration and the presence of risk factors of atherosclerotic disease was observed only in males, which is consistent with several previous studies (*29–32*). For example, in a study including 43,837 participants aged 18–96 y, an inverse association between 25(OH)D and the risk of MetS was observed, which was more marked in males than in females by sex-stratified analysis (*29*). Involvement of menstrual status has also been reported. For example, Chun et al. have also reported a significantly higher odds ratio of MetS in subjects with vitamin D deficiency (25(OH)D$<$20 ng/mL) in postmenopausal females than those without it, which, however, was not observed in premenopausal females (*33*). Taken together, one of the possible explanations for the observed sex differences would be the effect of estrogen on metabolic disorders (*34*).

We have not shown the cutoff value, since the AUC value was considered not high enough. On the other hand, the presence of atherosclerotic disease risk factors was significantly decreased only at the vitamin D sufficiency level ($\geq$30 ng/mL), not at the vitamin D insufficiency level ($\geq$20 and $<$30 ng/mL). However, the percentage of subjects with vitamin D sufficiency is small, and the data are not sufficiently well evidenced. Of the few studies calculating the cut-off value for the MetS risk, Lu et al. have reported that the serum 25(OH)D cutoff value for MetS risk in Chinese adults was 15.655 ng/mL (*35*). There also have been reports on the cut-off value of 25(OH)D for cardiovascular diseases (CVD); the outcome of atherosclerosis. A dose-response meta-analysis of prospective studies has demonstrated a low risk of CVD events at serum 25(OH)D above 25 ng/mL (*36*) and an increased risk of CVD below 24 ng/mL (*37*). These apparent discrepancies may be due to the older age of Lu's study subjects and lower 25(OH)D values than in the CVD studies. Although no significant relationship was found between blood 25(OH)D levels and MetS in the meta-analyses of longitudinal studies (*11*, *28*), favorable effects have been reported on the MetS components by vitamin D supplementation in the previous RCTs (*3*, *38–40*). Therefore, further studies are needed to clarify the causal relationship and determine the optimal range of circulating levels of 25(OH)D for decreasing the risk of atherosclerotic disease.

We have also shown that serum 25(OH)D negatively affects atherosclerotic disease risk factors mostly through direct action with little contribution by indirect effects through increased physical activity, which is consistent with the report by Su et al., who have described that serum 25(OH)D is a significant predictor of cardiovascular events and death even after adjustment by physical activity (*41*). Thus, the preventive effects of vitamin D on atherosclerosis were considered to be mainly a direct one such as improvement of insulin resistance.

The present study has several strengths. First, sex differences are considered in our analysis. Second, serum 25(OH)D concentrations were measured using LC-MS/MS methods, which is a gold-standard procedure. Third, we have revealed a direct association of serum 25(OH)D level with risk factors of atherosclerotic disease. Our study also has several limitations. Due to the cross-sectional study design, the causal effect of serum 25(OH)D on the risk of atherosclerotic disease cannot be proven. Second, the number of subjects remains modesty. Third, data on vitamin D intake have not been collected.

In conclusion, the present study suggested that serum 25(OH)D concentration is negatively correlated with risk factors of atherosclerotic disease in males.

Authorship

Analysis and interpretation of data, writing the article: AY; carrying out the study, and analysis of data: NT, CU, and HO; review & editing manuscript: KT; designing and carrying out the study: KM and YW; formulating the research question(s), designing the study, analysis, interpretation of data, and writing the article; AK.

Disclosure of state of COI

No conflicts of interest to be declared.

Acknowledgments

We thank Takahito Kitayoshi for helpful support; Kyoko Ebisu, Satoshi Kume, Yusuke Morito, Miho Iwasaki, Kyosuke Watanabe, Kanako Tajima, Yuka Nakatsu and Shoko Tojo for their contribution for data collection.

Financial support

This work was supported by JSPS KAKENHI Grant Number 19K11747 and JST Compass to Healthy Life Research Complex Program.

REFERENCES

1) Alberti KGMM, Zimmet P, Shaw J. 2006. Metabolic syndrome—A new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabet Med* **23**: 469–480.
2) Holick MF, Chen TC. 2008. Vitamin D deficiency: A worldwide problem with health consequences. *Am J Clin Nutr* **87**: 1080–1086.
3) Prasad P, Kochhar A. 2016. Interplay of vitamin D and metabolic syndrome: A review. *Diabetes Metab Syndr Clin Res Rev* **10**: 105–112.
4) Asakura K, Etoh N, Imamura H, Michikawa T, Nakamura T, Takeda Y, Mori S, Nishiwaki Y. 2020. Vitamin D status in Japanese adults: Relationship of serum 25-hydroxyvitamin D with simultaneously measured dietary vitamin D intake and ultraviolet ray exposure. *Nutrients* **12**: 743.
5) Li YC, Kong J, Wei M, Chen Z-F, Liu SQ, Cao L-P. 2002. 1,25-Dihydroxyvitamin D3 is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest* **110**: 229–238.
6) Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. 2003. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J* **17**: 509–511.
7) Maestro B, Campion J, Davila N, Calle C. 2000. Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. *Endocr J* **47**: 383–391.
8) Yin Y, Yu Z, Xia M, Luo X, Lu X, Ling W. 2012. Vitamin D attenuates high fat diet-induced hepatic steatosis in rats by modulating lipid metabolism. *Eur J Clin Invest* **42**: 1189–1196.
9) Asano L, Watanabe M, Ryoden Y, Usuda K, Yamaguchi T, Khambu B, Takashima M, Sato S, Sakai J, Nagasawa K, Uesugi M. 2017. Vitamin D metabolite, 25-hydroxyvitamin D, regulates lipid metabolism by inducing degradation of SREBP/SCAP. *Cell Chem Biol* **24**: 207–217.
10) Jorde R, Grimnes G. 2011. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res* **50**: 303–312.
11) Ju SY, Jeong HS, Kim DH. 2014. Blood vitamin D status and metabolic syndrome in the general adult population: A dose-response meta-analysis. *J Clin Endocrinol Metab* **99**: 1053–1063.
12) Akter S, Eguchi M, Kurotani K, Kochi T, Kashino I, Ito R, Kuwahara K, Tsuruoka H, Kabe I, Mizoue T. 2017. Serum 25-hydroxyvitamin D and metabolic syndrome in a Japanese working population: The Furukawa Nutrition and Health Study. *Nutrition* **36**: 26–32.
13) Fernandes MR, Barreto WDRJ. 2017. Association between physical activity and vitamin D: A narrative literature review. *Rev Assoc Med Bras* **63**: 550–556.
14) Chin K, Zhao D, Tibuakuu M, Martin SS, Ndumele CE, Florido R, Windham BG, Guallar E, Lutsey PL, Michos ED. 2017. Physical activity, vitamin D, and incident atherosclerotic cardiovascular disease in whites and blacks: The ARIC Study. *J Clin Endocrinol Metab* **102**: 1227–1236.
15) Zittermann A, Borgermann J, Gummert JF, Pilz S. 2012. Future directions in vitamin D and cardiovascular research. *Nutr Metab Cardiovasc Dis* **22**: 541–546.
16) Martinez-Gomez D, Guallar-Castillon P, Higueras-Fresnillo S, Garcia-Esquinas E, Lopez-Garcia E, Bandinelli S, Rodríguez-Artalejo F. 2018. Physical activity attenuates total and cardiovascular mortality associated with physical disability: A national cohort of older adults. *J Gerontol A Biol Sci Med Sci* **73**: 240–247.
17) Barengo NC, Antikainen R, Borodulin K, Harald K, Jousilahti P. 2017. Leisure-time physical activity reduces total and cardiovascular mortality and cardiovascular disease incidence in older adults. *J Am Geriatr Soc* **65**: 504–510.
18) Tsugawa N, Suhara Y, Kamao M, Okano T. 2005. Determination of 25-hydroxyvitamin D in human plasma using high-performance liquid chromatography-tandem mass spectrometry. *Anal Chem* **77**: 3001–3007.
19) Higashi T, Awada D, Shimada K. 2001. Simultaneous determination of 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 in human plasma by liquid chromatography-tandem mass spectrometry employing derivatization with a Cookson-type reagent. *Biol Pharm Bull* **24**: 738–743.
20) The Japan Endocrine Society, The Japanese Society for Bone and Mineral Research, Ministry of Health, Labour and Welfare, Research Project for Overcoming Intractable Diseases Research Team on Abnormalities in Hormone Receptor Mechanisms. 2017. Guidelines for determining vitamin D deficiency and insufficiency. *Japanese Journal of Endocrinology* **93**: 1–10 (in Japanese).
21) The Examination Committee of Criteria for Metabolic Syndrome. 2005. Definition and criteria of metabolic syndrome. *Nihon Naika Gakkai Zasshi* **94**: 794–809 (in Japanese).
22) Baron RM, Kenny DA. 1986. The moderator–mediator variable distinction in social psychological research: Conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* **51**: 1173–1182.
23) Lee SJ, Lee EY, Lee JH, Kim JE, Kim KJ, Rhee Y, Kim HC, Youm Y, Kim CO. 2019. Associations of serum 25-

hydroxyvitamin D with metabolic syndrome and its components in elderly men and women: The Korean Urban Rural Elderly cohort study. *BMC Geriatr* **19**: 102.

24) Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, Li X, Yang X, Chen Y, Lin X. 2009. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. *Diabetes Care* **32**: 1278–1283.

25) Barchetta I, De Bernardinis M, Capoccia D, Baroni MG, Fontana M, Fraioli A, Morini S, Leonetti F, Cavallo MG. 2013. Hypovitaminosis D is independently associated with metabolic syndrome in obese patients. *PLoS One* **8**: e68689.

26) Reis JP, Von Mühlen D, Kritz-Silverstein D, Wingard DL, Barrett-Connor E. 2007. Vitamin D, parathyroid hormone levels, and the prevalence of metabolic syndrome in community-dwelling older adults. *Diabetes Care* **30**: 1549–1555.

27) Majumdar V, Nagaraja D, Christopher R. 2011. Vitamin D status and metabolic syndrome in Asian Indians. *Int J Obes* **35**: 1131–1134.

28) Hajhashemy Z, Shahdadian F, Moslemi E, Mirenayat FS, Saneei P. 2021. Serum vitamin D levels in relation to metabolic syndrome: A systematic review and dose-response meta-analysis of epidemiologic studies. *Obes Rev* **22**: e13223.

29) Yu S, Song L, Wei Q, Lv Y, Wan Z. 2021. Dose-response relationship between serum 25-hydroxyvitamin D and the risk of metabolic syndrome. *Clin Nutr* **40**: 1530–1536.

30) Kim MH, Lee J, Ha J, Jo K, Lim DJ, Lee JM, Chang SA, Kang MI, Cha BY. 2018. Gender specific association of parathyroid hormone and vitamin D with metabolic syndrome in population with preserved renal function. *Sci Rep* **8**: 1149.

31) Bea JW, Jurutka PW, Hibler EA, Lance P, Martínez ME, Roe DJ, Sardo Molmenti CL, Thompson PA, Jacobs ET. 2015. Concentrations of the vitamin D metabolite 1,25(OH)2D and odds of metabolic syndrome and its components. *Metabolism* **64**: 447–459.

32) Weldegiorgis TZ, Hidru TH, Yang XL, Xia YL, Ma L, Li HH. 2020. Association between serum 25-hydroxyvitamin D concentrations and metabolic syndrome in the middle-aged and elderly Chinese population in Dalian, northeast China: A cross-sectional study. *J Diabetes Investig* **11**: 184–191.

33) Chun H, Kim GD, Doo M. 2020. Differences in the association among the vitamin D concentration, dietary macronutrient consumption, and metabolic syndrome depending on pre- and postmenopausal status in Korean women: A cross-sectional study. *Diabetes Metab Syndr Obes Targets Ther* **13**: 3601–3609.

34) Mauvais-Jarvis F, Clegg DJ, Hevener AL. 2013. The role of estrogens in control of energy balance and glucose homeostasis. *Endocr Rev* **34**: 309–338.

35) Lu Y, Liu M, Pei Y, Li J, Tian H, Cheng X, Fang F, Sun B, Xiao H, Li N, Miao X, Li C. 2015. Low levels of serum 25-hydroxyvitamin D and risk of metabolic syndrome in China. *Int J Clin Exp Med* **8**: 13790–13796.

36) Zhang R, Li B, Gao X, Tian R, Pan Y, Jiang Y, Gu H, Wang Y, Wang Y, Liu G. 2017. Serum 25-hydroxyvitamin D and the risk of cardiovascular disease: Dose-response meta-analysis of prospective studies. *Am J Clin Nutr* **105**: 810–819.

37) Wang L, Song Y, Manson JAE, Pilz S, März W, Michaëlsson K, Lundqvist A, Jassal SK, Barrett-Connor E, Zhang C, Eaton CB, May HT, Anderson JL, Sesso HD. 2012. Circulating 25-hydroxy-vitamin D and risk of cardiovascular disease: A meta-analysis of prospective studies. *Circ Cardiovasc Qual Outcomes* **5**: 819–829.

38) Marquina C, Mousa A, Scragg R, de Courten B. 2019. Vitamin D and cardiometabolic disorders: a review of current evidence, genetic determinants and pathomechanisms. *Obes Rev* **20**: 262–277.

39) Bahrami LS, Ranjbar G, Norouzy A, Arabi SM. 2020. Vitamin D supplementation effects on the clinical outcomes of patients with coronary artery disease: a systematic review and meta-analysis. *Sci Rep* **10**: 12923.

40) Theik NWY, Raji OE, Shenwai P, Shah R, Kalluri SR, Bhutta TH, Hannoodee H, Al Khalili M, Khan S. 2021. Relationship and effects of vitamin D on metabolic syndrome: A systematic review. *Cureus* **13**: e17419.

41) Su Y, Leung J, Lee J, Ho KF, Kwok T. 2020. The effect of physical activity on dose-relationship between serum 25-hydroxyvitamin D and cardiovascular health events in older adults. *Nutr Metab Cardiovasc Dis* **30**: 656–665.