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Association of Serum 25-Hydroxyvitamin D Levels with Markers for Metabolic Syndrome in the Elderly: A Repeated Measure Analysis

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The purpose of current study was to investigate associations of serum 25-hydroxyvitamin D (OHVD) levels with markers for metabolic syndrome in elderly Koreans. We conducted a panel study on 301 individuals over 60 yr old in Seoul, Korea, and repeatedly measured serum OHVD, glucose, insulin, and lipid levels. Mixed effect model and generalized estimating equations were used to investigate relationships between serum OHVD levels with marker levels for metabolic syndrome and each of its categories. Of all subjects, 76.6% were vitamin D deficient (< 50 nM) and 16.9% were insufficient (< 75 nM). Inverse association was demonstrated between serum OHVD levels and insulin ($P = 0.004$), triglyceride ($P = 0.023$) and blood pressure (systolic blood pressure: $P = 0.002$; diastolic blood pressure: $P < 0.001$). Vitamin D deficiency was found to increase risk of 'hypertriglyceridemia' category of metabolic syndrome (odds ratio: 1.73, 95% confidence interval: 1.13-2.66). In conclusion, we found from our repeated measure analysis that decreasing serum OHVD levels are associated with increasing insulin resistance, increasing serum triglyceride levels and increasing blood pressure in elderly Koreans, and confirmed on the risk of 'hypertriglyceridemia' in vitamin D deficient subjects.

Key Words: Vitamin D; Insulin Resistance; Metabolic Syndrome; Elderly; Mixed Effect Model

INTRODUCTION

Concerns have been raised on hypovitaminosis D due to its high worldwide prevalence, and elderly population has received attention in this matter as they are more prone to develop vitamin D deficiency than younger population. More than 90% of the pigmented populace in the United States (including Asians) are reported to have vitamin D insufficiency (50-75 nM or 20-30 ng/mL), and estimated prevalence of vitamin D deficiency (defined as < 50 nM or < 20 ng/mL) in elderly has been reported to be about 50% worldwide (1, 2).

The role of vitamin D in many biological systems has been actively researched in recent years. At the same time, insulin resistance, a physiological state where insulin becomes less effective in lowering blood sugars, and metabolic syndrome, a group of cardiometabolic disease risk factors, has also received much attention for their increasing prevalence and consequent effects. The issue on the association of serum 25-hydroxyvitamin D (OHVD) levels with insulin resistance (IR) and metabolic syn-drome (MS) has become a subject supported and challenged by increasing reports of evidence as well as controversy. With increasing reports on the role of vitamin D on elements of diabetes and metabolic syndrome such as adiposity, glucose homeostasis, lipid profiles and blood pressure, further investigation for evidence is greatly encouraged (3-9).

While longitudinal studies and intervention trials have shown inconsistent results for the association between vitamin D and IR or MS, numerous cross-sectional studies have demonstrated in favor of the relationship. However, limitation of the study designs has brought about a demand for better approach. Thus we carried out a panel study, as it controls time-invariant individual characteristics by design, therefore is suitable for analyzing of short-term changes in continuous variables.

In a national level prospective cohort study of 35,671 subjects followed in Korea from 2004 to 2006, significant increasing trend for MS was observed with increasing age ($P < 0.001$ for both sex) (10). Along with susceptibility to hypovitaminosis D, the fact that elderly population is more vulnerable to cardiometabolic

disease risks draws attention to further examine the association between low serum OHVD concentration and metabolic syndrome in the elderly. Confirming the association would be more important as supplementing vitamin D is practical and uncomplicated, such as sunbathing and dietary modification, especially for the less-active elderly population.

The purpose of our study was to investigate the relationship of serum OHVD levels with IR and MS in elderly Koreans by repeated measures up to 3 times.

MATERIALS AND METHODS

Subjects

The study is a part of the Korean Elderly Environmental Panel (KEEP) study on Koreans over 60 yr old residing in Seongbuk-gu, Seoul, Republic of Korea. Of all 560 subjects, this research is focused on 301 subjects who had their serum OHVD levels measured more than once out of three opportunities, from August 2008 through August 2010.

Four hundred and eight subjects attended initial series of visits between September and December 2008, and 302 subjects attended second series of visits from April to September 2009. Third series of visits were attended by 173 subjects, between March to August 2010. Of the 302 subjects who made at least two visits, one subject was excluded as she participated from the second series of visits.

Variables

A standard questionnaire was used by trained interviewers to collect information on age, sex, smoking (current/ex-/non-smoker), alcohol drinking (yes/no), regular exercise (yes/no), and self-reported diabetes, hypertension, and dyslipidemia. A food frequency questionnaire on previous one year's dietary intake was also carried out to calculate total (both animal and vegetable) calcium intake. Blood pressure after five minutes-rest, height and weight were measured, and venous blood samples were collected to measure the following: fasting blood sugar (FBS), insulin, triglyceride (TG), high-density lipid (HDL) cholesterol, OHVD.

Data on moving-average amount of sunshine in Seongbuk-gu from the day each subject visited were gained from the Korea Meteorological Administration (http://www.kma.go.kr). The physical examination and blood test were repeated on the second and third series of visits.

Insulin resistance (IR) was measured using the homeostasis model assessment of insulin resistance (HOMA-IR) index, which is calculated as [glucose × insulin]/22.50. For the definition of abnormal levels of the markers, we used criteria for metabolic syndrome according to National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) Criteria for Asian Americans: triglyceride ≥ 1.695 mM (≥ 150 mg/dL), HDL cholesterol

< 1.036 mM (< 40 mg/dL) for men and < 1.295 mM (< 50 mg/dL) for women, blood pressure ≥ 130/85 mmHg or current use of antihypertensive medications, and fasting glucose ≥ 5.55 mM (≥ 100 mg/dL) or previously diagnosed type 2 diabetes or using oral hypoglycemic agents or insulin. With lack of data for waist circumference, we used alternative definition of BMI ≥ 23 kg/m² instead.

Laboratory methods

All blood samples were collected in 8 mL plain tubes around 10 a.m. in the morning after overnight fasting and centrifuged within 30-60 min of collection. It was then serum-separated in screw cap tubes and immediately stored in -70°C freezers until analysis.

Analyses were performed in a central laboratory (Neodin Medical Laboratories, Seoul, Korea). Hexokinase method with Hitachi 7600 II analyzer (Hitachi, Tokyo, Japan) was used for measuring fasting blood glucose levels. Enzymatic caliometry was used for measuring serum lipid levels. Serum insulin levels were measured using competitive RIA method on 1470 WIZARD equipment (PerkinElmer, Turku, Finland), and serum OHVD levels were measured using chemiluminescentimmunoassay on LIAISON equipment (DiaSorin Inc., Stillwater, MN, USA). Quality control for OHVD was maintained using Levey-Jening chart, with mean ± 2SD values set as allowable range.

Statistical analysis

Data analysis was carried out using SAS 9.2 (Cary, NC, USA) and R version 2.12.1 (The Comprehensive R Archive Network, http://cran.rproject.org). Mixed effect model was used for calculating the estimate effect of log-transformed OHVD levels on continuous variables by multiple regression, and generalized estimating equation model was used to calculate the estimate effect of OHVD on abnormal levels of the components of MS (discrete variables). For the graphical analysis of relationship between OHVD and marker levels, we used generalized additive mixed model.

As participants made different number of visits, the non-random loss of follow-up due to different number of repeated measures may lead to selection bias (11). Thus weighting follow-up observations was carried out, by inverse probability of attaining a follow-up response (12). With data from participants who made more than one visit, we performed logistic regression to predict follow-up probability (follow-up = 1, missing = 0), with covariates as previously measured age, sex, BMI, number of years of schooling, blood pressure, season, and temperature. Weight value of 1 was given to first observations of each participant, and more weight was given to more likely missing observations (13).

For multiple regression analysis, age and sex were adjusted in model 1; age, sex, smoking, alcohol, exercise, diabetes, hyper-

tension and dyslipidemia were adjusted in model 2; variables in model 2 plus 7-days moving average for amount of sunshine were adjusted in model 3. In examining the association between OHVD levels and each of MS components, we used the same models.

Ethics statement

The study protocol was approved by the institutional review board of Seoul National University Hospital (IRB No. H-0804-045-241). Written informed consent was received from all participants.

RESULTS

Baseline characteristics of study subjects are shown in Table 1. Of the 301 subjects, 68 were male and 233 were female, and their average age was 70.4 ± 5.23 (range: 60-84 yr). Percentages for current smoking and alcohol drinking were very low (smoking: 6.0%, alcohol drinking: 17.6%) while percentage of subject population on regular exercise was relatively high (38.4%).

Mean serum OHVD level was 41.66 ± 19.436 nM, ranging from 5.0 to 159.0 nM. Of all the measurements, 76.6% fell under vitamin D deficiency (< 50 nM) while 16.9% and 6.6% fell under insufficiency (50-75 nM) and sufficiency (> 75 nM). Percentages of subjects having hypertension, diabetes and dyslipidemia were derived from questionnaire results, and were found to be 45.5%, 16.0%, and 11.3% respectively.

Serum OHVD levels were calculated in percentiles and divided into quartiles, as Q1 (< 20.754 nM), Q2 (20.754-32.448 nM), Q3 (32.448-48.298 nM), and Q4 (≥ 48.298 nM). Characteristics of study subjects across quartiles of OHVD levels on all three visits are described in Table 2. For mean and standard deviation values for each variable across quartiles of OHVD levels, *P* values were significant for body mass index (BMI), SBP, DBP, triglyceride (TG) levels, HOMA-IR, and amount of sunshine of the study area on the day each subject visited. Significant difference across quartiles of OHVD levels were also observed with subjects' smoking, alcohol drinking and regular exercise.

Numbers and their percentages of subjects for abnormal categories of components for metabolic syndrome across quartiles of serum OHVD levels are shown in Table 3. Mantel-Haenszel chi-square test for trend showed significant difference in hypertriglyceridemia and hypertension across quartiles of OHVD levels.

Effects of serum OHVD levels on continuous variables (BMI, TG, HDL cholesterol, glucose, insulin, HOMA-IR, SBP, and DBP) across differently adjusted models were analyzed by mixed model

Table 1. Baseline characteristics of study subjects by gender

Parameters	All		Male		Female		P value
	No./mean	%/SD	No./mean	%/SD	No./mean	%/SD	
No. (%)	301		68		233		
Age (yr)	70.4	5.23	71.5	4.29	70.1	5.45	0.022
Body mass index (kg/m^2)	24.72	2.950	24.04	3.068	24.91	2.893	0.524
Systolic blood pressure (mmHg)	133.3	17.85	131.7	20.37	133.7	17.06	0.059
Diastolic blood pressure (mmHg)	75.3	10.64	75.1	11.66	75.3	10.35	0.206
Vitamin D (nM)	41.658	19.436	51.218	18.034	38.863	18.975	0.633
Triglyceride (mM/L)	1.558	0.943	1.532	1.038	1.566	0.916	0.184
HDL cholesterol (mM/L)	1.279	0.316	1.204	0.352	1.300	0.302	0.103
Glucose (mM/L)	5.261	1.222	5.356	1.282	5.228	1.204	0.495
Insulin (pM/L)	51.230	38.207	44.342	32.754	53.239	39.498	0.072
HOMA-IR	31.5	27.828	27.54	22.86	32.58	29.07	0.021
Calcium intake (mM/d)	17.92	8.1675	18.865	8.3325	17.6575	8.1225	0.781
Total amount of sunshine (hr)							
lag 0	5.6	3.88	5.9	3.73	5.5	3.92	0.627
lag 0-1	5.8	4.01	5.7	4.38	5.8	3.91	0.221
lag 0-7	6.0	3.77	5.8	4.16	6.0	3.66	0.170
lag 0-14	6.4	3.50	5.9	3.60	6.6	3.47	0.666
Daily mean temperature (°C)							
lag 0	15.3	9.42	16.6	9.69	14.8	9.32	0.665
lag 0-1	15.5	8.87	17.0	8.83	15.1	8.86	1
lag 0-7	15.2	9.63	16.7	8.64	14.7	9.88	0.194
lag 0-14	16.1	9.74	18.2	8.80	15.6	9.94	0.237
Smoking (%)	18	6.0	17	25	1	0.4	< 0.001
Alcohol drinking (%)	53	17.6	32	47.1	21	9.0	< 0.001
Regular exercise (%)	183	61.6	38	55.9	145	63.3	0.269
Hypertension (%)	137	45.5	35	51.5	102	43.8	0.263
Diabetes (%)	48	16.0	12	17.7	36	15.5	0.664
Dyslipidemia (%)	34	11.3	8	11.8	26	11.2	0.890

HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index.

Table 2. Characteristics of study subjects across quartiles of serum OHVD levels, over all visit periods combined

Parameters	Q1: < 20.754 nM		Q2: 20.754-32.448 nM		Q3: 32.448-48.298 nM		Q4: ≥ 48.298 nM		P value
	No./mean	%/SD	No./mean	%/SD	No./mean	%/SD	No./mean	%/SD	
No. (%)	194		193		195		194		
Age (yr)	72.0	5.63	71.8	5.77	70.2	5.01	71.3	4.77	0.004
Female sex (%)	171	87.69	158	81.87	144	73.85	134	69.07	< 0.001
Body mass index (kg/m^2)	24.83	3.104	25.28	3.094	24.85	2.869	24.07	2.706	0.001
Systolic blood pressure (mmHg)	132.6	16.92	131.1	17.01	130.8	17.14	126.1	17.68	0.002
Diastolic blood pressure (mmHg)	74.8	10.99	74.2	10.40	73.8	10.25	71.0	9.90	0.002
Triglyceride (mM/L)	1.64	0.950	1.57	0.944	1.43	0.748	1.43	0.812	0.031
HDL cholesterol (mM/L)	1.34	0.354	1.33	0.326	1.32	0.333	1.33	0.359	0.934
Glucose (mM/L)	5.59	1.267	5.60	1.564	5.44	1.377	5.37	1.063	0.246
Insulin (pM/L)	60.84	79.470	62.42	64.310	52.09	37.489	48.79	40.675	0.055
HOMA-IR	40.68	62.514	43.2	61.956	32.94	28.962	31.14	34.092	0.044
Calcium intake (mM/d)	15.763	6.345	19.3	8.371	16.795	8.634	18.828	8.005	0.091
Total amount of sunshine (hr)									
lag 0	5.7	4.24	5.6	4.22	5.1	4.10	4.8	4.00	0.167
lag 0-1	5.5	4.20	5.3	4.03	5.6	4.35	5.3	4.10	0.869
lag 0-7	6.2	4.31	5.7	4.14	5.5	4.08	5.8	3.90	0.372
lag 0-14	5.9	4.09	5.9	4.24	5.9	3.81	5.9	3.84	0.999
Daily mean temperature (°C)									
lag 0	12.7	7.62	15.8	8.05	17.9	8.65	18.5	8.35	< 0.001
lag 0-1	12.9	7.21	15.8	8.14	18.1	8.17	18.8	7.90	< 0.001
lag 0-7	11.8	7.52	15.3	8.33	17.9	8.48	18.3	9.18	< 0.001
lag 0-14	10.6	7.82	14.4	9.38	17.8	8.50	18.3	8.82	
Current smoking (%)	5	2.6	10	5.2	8	4.1	23	11.9	0.004
Current drinking (%)	23	11.8	34	17.6	37	19.0	44	22.7	0.006
No regular exercise (%)	135	67.0	112	59.3	114	59.7	108	55.7	0.007
Hypertension (%)	94	48.2	87	45.1	94	48.2	78	40.2	0.192
Diabetes (%)	25	12.8	33	17.1	26	13.3	39	20.1	0.123
Dyslipidemia (%)	19	9.7	18	9.3	19	9.7	34	17.5	0.021

HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index.

Table 3. "Positive" or "negative" proportions in five categories of metabolic syndrome; in total number of measurements, across quartiles of serum OHVD levels

Categories of metabolic syndrome	Total number of measurements		Number of subjects distributed over quartiles of OHVD levels								P value
			Q1: < 20.754 nM		Q2: 20.754-32.448 nM		Q3: 32.448-48.298 nM		Q4: ≥ 48.298 nM		
	No.	%	No.	%	No.	%	No.	%	No.	%	
Body mass index (kg/m^2)											
< 23	203	26.13	50	25.64	47	24.35	43	22.05	63	32.47	0.198
≥ 23	574	73.87	145	74.36	146	75.65	152	77.95	131	67.53	
Triglyceride (mM/L)											
< 1.695	532	68.47	123	63.08	131	67.88	131	67.18	147	75.77	0.012
≥ 1.695	245	31.53	72	36.92	62	32.12	64	32.82	47	24.23	
HDL cholesterol											
≥ 1.036 mM/L (men) or ≥ 1.295 mM/L (women)	448	57.66	106	54.36	112	58.03	116	59.49	114	58.76	0.354
< 1.036 mM/L (men) or < 1.295 mM/L (women)	329	42.34	89	45.64	81	41.97	79	40.51	80	41.24	
Hypertensive											
< 130/85 mmHg	226	29.09	48	24.62	53	27.46	49	25.13	76	39.18	0.005
≥ 130/85 mmHg or currently in medication	551	70.91	147	75.38	140	72.54	146	74.87	118	60.82	
Diabetic											
FBS < 5.55 mM/L	512	65.89	118	60.51	124	64.25	139	71.28	131	67.53	0.065
FBS ≥ 5.55 mM/L or using oral medication/insulin or previously diagnosed T2DM	265	34.11	77	39.49	69	35.75	56	28.72	63	32.47	

HDL cholesterol, high-density lipoprotein cholesterol.

Table 4. Multiple regression of serum OHVD levels on measured outcome variables and HOMA-IR

Variables	Model 1*			Model 2†			Model 3‡		
	Estimate (β)	SE	P value	Estimate (β)	SE	P value	Estimate (β)	SE	P value
Body mass index	-0.09605	0.08722	0.271	-0.1004	0.0875	0.252	-0.09148	0.0886	0.302
Triglyceride	-6.9665	3.4221	0.042	-7.1769	3.4575	0.036	-7.9583	3.493	0.023
HDL cholesterol	0.2381	0.5905	0.687	0.2028	0.5949	0.733	0.2429	0.6019	0.687
Glucose	0.1045	1.1383	0.927	-0.5828	1.0936	0.594	-0.6291	1.1062	0.570
Insulin	-1.1679	0.456	0.011	-1.3329	0.4565	0.004	-1.3507	0.4614	0.004
HOMA-IR	-0.3361	0.1574	0.033	-0.4179	0.1553	0.007	-0.4238	0.1569	0.007
Systolic blood pressure	-2.6426	0.8234	0.001	-2.4871	0.8257	0.003	-2.5667	0.8347	0.002
Diastolic blood pressure	-2.2071	0.5092	< 0.001	-2.1305	0.5124	< 0.001	-2.1463	0.5184	< 0.001

*Model 1, adjusted for age and sex. †Model 2, adjusted for age, sex, smoking, alcohol, exercise, diabetes, hypertension and dyslipidemia. ‡Model 3, adjusted for all variables in model 2 plus 7-day moving average for amount of sunshine of Seongbuk-gu from the day each subject visited. HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index.
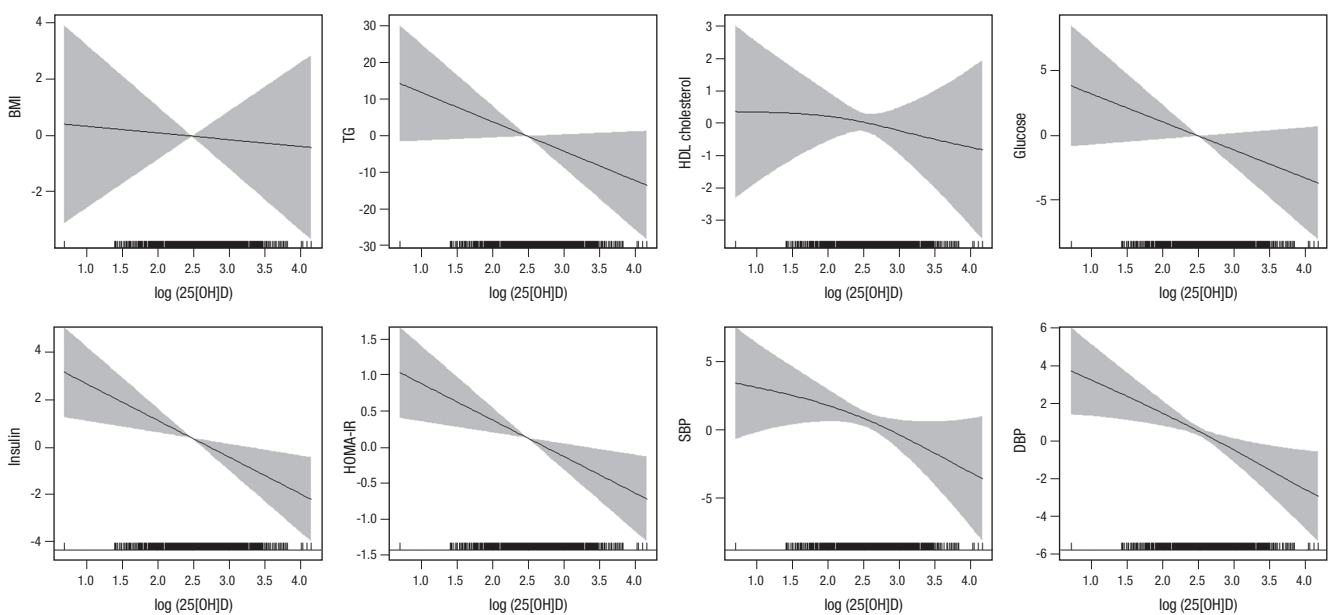

Fig. 1. Association between log-transformed OHVD levels and various outcome variables by generalized additive mixed models adjusted for age, sex, smoking, drinking, regular exercise, diabetes, hypertension, dyslipidemia, 7-day moving average for sunshine amount of Seongbuk-gu from the day each subject visited. BMI, body mass index; TG, triglyceride; HDL cholesterol, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance index; SBP, systolic blood pressure; DBP, diastolic blood pressure; log(25(OH)D), log-transformed 25-hydroxyvitamin D.

analysis, and are shown in Table 4. Significant inverse relationship with serum OHVD levels were found with TG, insulin, HOMA-IR, SBP and DBP across all three models, but no significance was found with BMI, HDL cholesterol and glucose in any of the models. Results were consistent in direction and significance across all the selected moving average for amount of sunshine, and the effect size was greatest at lag 0-7. To confirm for the linear association between log-transformed OHVD levels and various outcome variables in the mixed model analysis, we implemented generalized additive mixed models, as shown in Fig. 1 (14).

Effects of OHVD levels on categorical variables of MS components across differently adjusted models were analyzed by generalized estimating equations. From analysis of outcome variables across all quartiles of OHVD levels, highest odds ratios

for significant variables were estimated at the 75th percentile level of 48.298 nM (data not shown). As 50 nM is the definition level for vitamin D deficiency and as odds ratios at 48.298 nM and 50 nM did not largely differ, we evaluated the estimate effect of vitamin D at 50 nM on abnormal levels of markers. Table 5 shows odds ratios of OHVD deficiency level on abnormal levels of markers across adjusted models. At serum OHVD level of 50 nM, odds ratios and their 95% confidence intervals were significant with only hypertriglyceridemia category of MS, with similar effect size across all models (odds ratio [OR]: 1.74 [95% confidence interval: 1.13-2.70] in model 3).

DISCUSSION

Our results showed statistically significant inverse association of

Table 5. Effect of serum OHVD levels on five categories of MS at definition level for vitamin D deficiency (50nM)

Model/variables	Estimate (β)	SE	95% CI		P value	OR	95% CI	
Model 1*								
Body mass index	-0.0482	0.188	-0.4166	0.3202	0.797	0.95	0.66	1.38
Triglyceride	0.5498	0.2193	0.1201	0.9796	0.012	1.73	1.13	2.66
HDL cholesterol	-0.2177	0.1709	-0.5528	0.1173	0.203	0.80	0.58	1.12
Hypertensive	0.2617	0.1887	-0.1083	0.6316	0.166	1.30	0.90	1.88
Diabetic	-0.0207	0.155	-0.3245	0.283	0.894	0.98	0.72	1.33
Model 2†								
Body mass index	-0.0669	0.192	-0.4432	0.3093	0.727	0.94	0.64	1.36
Triglyceride	0.5565	0.2209	0.1235	0.9895	0.012	1.74	1.13	2.69
HDL cholesterol	-0.2065	0.1734	-0.5462	0.1333	0.234	0.81	0.58	1.14
Hypertensive	0.2821	0.1959	-0.1019	0.6661	0.150	1.33	0.90	1.95
Diabetic	0.0245	0.1582	-0.2856	0.3347	0.877	1.02	0.75	1.40
Model 3‡								
Body mass index	-0.0652	0.1928	-0.4431	0.3126	0.735	0.94	0.64	1.37
Triglyceride	0.5488	0.2196	0.1184	0.9791	0.012	1.73	1.13	2.66
HDL cholesterol	-0.2126	0.1757	-0.5569	0.1318	0.226	0.81	0.57	1.14
Hypertensive	0.2857	0.1952	-0.0969	0.6682	0.143	1.33	0.91	1.95
Diabetic	0.0236	0.1584	-0.2869	0.334	0.882	1.02	0.75	1.40

*Model 1, adjusted for age and sex. †Model 2, adjusted for age, sex, smoking, alcohol and exercise. ‡Model 3, adjusted for all variables in model 2 plus 7-day moving average for sunshine amount of Seongbuk-gu from the day each subject visited. HDL cholesterol, high-density lipoprotein cholesterol.

OHVD levels with IR, TG and blood pressure. Subjects with serum OHVD level less than 50 nM showed increased odds ratio of 1.74 for hypertriglyceridemia when we used criteria for MS.

Average serum OHVD level found in our study was 41.66 nM and proportion of vitamin D deficiency (< 50 nM) was 76.6%. These results are comparable with a population-based cross-sectional study on middle-aged and elderly Chinese population, which showed that their average OHVD level was 16.19 ng/mL (40.41 nM) with deficiency proportion of 69.2% (6). Another cross-sectional study on elderly Spanish found average as 17.21 ng/mL (42.96 nM) and proportion of OHVD level below 25 ng/mL (62 nM) as 86% (15). A cohort study on population over 30 yr old (mean age 49.6) with high risk for type 2 diabetes and/or MS found the average level as 22.36 ng/mL (55.81 nM) (16); the slightly higher average level of OHVD in the study could be explained by younger age-distribution of the study population. The lower serum OHVD levels in the elderly is possibly due to specific physiological and lifestyle factors linked to advanced age, such as impaired production of 7-dehydrocholesterol in the skin, insufficient exposure to sunlight, poor dietary intake of vitamin D, as well as chronic diseases, pharmacological treatments and disability (17).

Associations of serum OHVD levels and serum lipids have been reported. The Tromsø study in Norway showed significant increase in total cholesterol, HDL cholesterol and LDL cholesterol levels and decrease in serum TG levels across increasing serum OHVD quartiles in cross-sectional study, but significant association was found with serum TG levels only in longitudinal study (18). Other cross-sectional studies also supported the association of OHVD levels and lipid levels, but significance was confirmed with TG levels only (19, 20).

Our results on OHVD levels and insulin or HOMA-IR showed

clear inverse relationship, and conforms with previous studies that used HOMA-IR as a marker to indicate IR. Numerous cross-sectional studies, with subjects in varying age group, in non-diabetic status or in risk of diabetes and/or MS and in various confounder-adjusted models, reported significant association with low serum OHVD levels and insulin resistance (16, 21-26). In a prospective study on non-diabetic subjects aged 40-69 yr, baseline OHVD level was inversely associated with 10-yr risk of 2-hr glucose, insulin, and HOMA-IR (22).

Recently reported studies favor our finding on the relationship of OHVD levels with blood pressure. In a meta-analysis including 4 prospective studies and 14 cross-sectional studies, the odds ratio for a 16 ng/mL or 39.94 nM (2 SD) increment in serum OHVD level was 0.84 (95% CI 0.78-0.90) (27). The same study group also performed a cross-sectional study where they measured supine and 24-hr blood pressure measurements to lessen classification bias. Their results showed that men with OHVD levels < 15.02 ng/mL or < 37.49 nM had a 3-fold higher prevalence of hypertension compared to those with ≥ 15.02 ng/mL or ≥ 37.49 nM (28). In another recent study on initially normotensive, middle-aged Koreans with median OHVD level of 46.8 nM, odds ratio was substantially higher for new hypertension after 4 yr in subjects with OHVD levels below the median value compared with those above median (OR 2.74; 95% CI 1.40-5.34) (29).

Relationship between OHVD levels and insulin, HOMA-IR, SBP, DBP as continuous variables in our study showed statistical significance, but relationship between OHVD levels and 'hypertensive', 'diabetic' categories of MS did not. The latter insignificant results could be explained by the different value characteristics: serum OHVD, FBS levels and blood pressure reflect changes on daily basis while the 'hypertensive' and 'diabetic'

categories reflect changes on longer term basis, as diabetes and hypertension are disease states attained possibly through years (i.e. not easily changed over time). Another possible explanation for the disappeared significance is that the effect of hypovitaminosis D may not be as influential as to reach body's "threshold" at which diabetes or hypertension can be developed. Although insignificant, increased odds ratios of 'hypertensive' and 'diabetic' categories at vitamin D deficiency level support the assumption ('hypertensive': OR 1.33, 95% CI: 0.91-1.95; 'diabetic': OR 1.02, 95% CI: 0.75-1.40).

Because another Korean study suggested that the relationship between vitamin D and the risk of hypertension might be associated with a low habitual calcium intake and a high prevalence of vitamin D deficiency in Korea (29), we also analyzed for significance of total calcium intake across quartiles of OHVD levels by ANOVA (Table 2), and found no significant association. However, we could not further investigate this association in the mixed effects or generalized estimating equation model analyses, as calcium intake measurement was taken only once.

To our knowledge, this is the first study that has performed a repeated measure analysis in investigating the effect of OHVD levels on IR and MS. The advantage of this approach over existing cross-sectional analyses is to provide increased power to the outcome association. In mixed models, intra-individual variation as well as inter-individual variation is taken into account, thus providing increased validity for the association. However, it must also be noted that repeated measure analysis is limited in deducing causality.

Our study has several limitations. We did not account for parathyroid hormone (PTH) levels. With the role of OHVD/PTH axis as maintaining extracellular calcium homeostasis, PTH levels may play a role for the effect of OHVD. However, effects of PTH levels on IR or MS still remains a controversy as the previous results have been inconsistent. In one study, researchers found MS positively related to PTH levels in older men but not in women (9), while other found no evidence of independent association between PTH levels and MS (5). Another study reported results favoring PTH and not OHVD levels as an independent predictor of MS, but results were limited to morbidly obese Caucasians (30). A more recent study showed inconsistent associations of PTH levels with IR and MS across different OHVD levels (26). In the study on middle-aged Koreans, serum PTH and calcium levels were significantly higher in subjects with MS compared with those without, but the association of serum vitamin D levels and MS/hypertension were unchanged after adjustment for PTH and serum calcium levels, indicating that the associations were independent of these factors (29).

Although we adjusted for regular exercise and moving average of sunshine amount on days each subject visited, it would have been more accurate if we adjusted for actual outdoor physical activity and exposure amount to sunshine. Also, although oral vitamin D supplements are not available in Korea, we did not account for additional measures of vitamin D-rich food intake.

To allow for ethnicity, we used updated NCEP-ATP III criteria for Asian Americans for definition of MS. However, we used calculated BMI instead of waist circumference. Threshold level of 23 kg/m^2 was used as this is the level classified as "overweight" in Asian populations (31). Waist circumference measurements in elderly could be inaccurate; nonetheless, BMI is less reflective of central obesity than waist circumference. Each subjects' visit dates were on different intervals and seasons. To compensate for possible bias effect due to different seasons, we adjusted 7-days moving average for total amount of sunshine on each visit date in the final model.

In conclusion, we have found from our repeated measure analysis that decreasing OHVD levels are associated with increasing insulin resistance, increasing serum triglyceride levels and increasing blood pressure in elderly Koreans, and confirmed on the risk of 'hypertriglyceridemia' in vitamin D deficient subjects.

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