Original Article

Ageing affects the association between serum 25-hydroxyvitamin D concentrations and cardiorespiratory fitness in middle-aged and elderly men

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Background and Objectives: The studies investigated the association between serum 25-hydroxyvitamin D (25(OH)D) concentrations and cardiorespiratory fitness (CRF) are few, and the results are controversial. We aim to evaluate the association of serum 25(OH)D concentrations with CRF in adults, and assess whether the associations vary with ageing. **Methods and Study Design:** The study included 78 middle-aged (30-64 years) and 83 elderly (65-79 years) Japanese men. Hand grip strength and leg extension power were measured using hand grip and leg dynamometers, respectively. CRF was measured via a maximal graded exercise test and quantified as the peak oxygen uptake (VO_2peak). Fasting serum 25(OH)D concentrations were determined by enzyme-linked immunosorbant assay. **Results:** Serum 25(OH)D concentrations were positively related to hand grip strength (r=0.331, $p<0.001$), leg extension power (r=0.353, $p<0.001$), CRF (r=0.285, $p<0.001$) in all adults after adjustment for age. Significant interaction between 25(OH)D and age on CRF was observed ($p<0.05$). Age-related reduction of CRF was prevented in high 25(OH)D group ($p<0.05$). In the elderly, subjects in high 25(OH)D group had higher CRF compared with those in low group ($p<0.05$). **Conclusions:** We found that serum 25(OH)D concentrations significantly related with leg extension power, hand grip strength and CRF after adjustment for age. The relationship of vitamin D and CRF was affected by ageing. Higher serum 25(OH)D concentrations are a key predictor for CRF in the elderly, but not in middle-aged men.

Key Words: vitamin D, hand grip strength, leg extension power, cardiorespiratory fitness, advancing age

INTRODUCTION

Over the last few decades, vitamin D receptors (VDRs) have been also observed in more than 30 different types of cells within body, including skeletal muscle, adipose tissue, and cardiomyocytes.[1,2] Therefore, in addition to its role in maintaining bone health, higher circulating 25(OH)D concentrations have been associated with improved cardiovascular outcomes and higher levels of physical fitness.[3]

Poor cardiorespiratory fitness (CRF) is recognized as an independent risk factor for cardiovascular morbidity and mortality.[4] Unlike numerous studies have investigated the association of serum 25(OH)D with muscle strength,[5-12] reports of relationships between serum 25(OH)D and CRF are limited.[13-15] Farrell et al observed that 25(OH)D concentrations were significantly related with CRF.[13] The similar results were also report in healthy adults with lower levels of physical activity, but not in higher group.[14] Ellis et al reported that the positive relationship between 25(OH)D and CRF were found in African Americans, but not in European Americans.[15] The inconsistency in results may be partially explained by difference in ethnic group and physical activity levels. Physical activity was observe to be positively related with 25(OH)D concentrations and CRF.[16,17] However few studies have considered the influence of physical activity and most of them assessed by questionnaires.

Ageing is associated with a decrease in muscle strength and CRF,[18,19] and which further increases the incidence of sarcopenia and cardiometabolic diseases in the elderly. The decreased expression of VDR in ageing muscle tissue and cardiomyocytes could be a contributing factor.[20] Compared with young adults, vitamin D supplementation,

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which may increase muscle VDR expression,[21] may be more effective in those aged ≥65 years.[11,22] However the relationships between serum 25(OH)D concentrations and CRF were unclear in the ageing process. Thus, we aim to evaluate the associations of serum 25(OH)D concentrations with CRF, and assess whether the associations are affected by ageing.

METHODS

Subjects

The participants were recruited through the Internet, posters, and a local newspaper insert in Saitama, Japan (35°N latitude). Approximately 99.6% of data were collected during winter and spring. Seventy-eight middle-aged (30-64 years) and 83 elderly (65-79 years) Japanese men were enrolled, and the health status was confirmed using medical questionnaires; blood pressure was measured by accredited medical personnel. We excluded subjects who were taking vitamin D supplements or medications that could affect bone or mineral metabolism. Diabetes status was defined in accordance with World Health Organization criteria;[23] 8 subjects (5.0%) had type 2 diabetes; 36 (22.4%) received antihypertensive drugs, and 8 (5.0%) received lipid-lowering drugs. The current and/or former smoking status was assessed with a questionnaire. All participants provided written informed consent to participate before enrolling in the study. All procedures performed in the study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the Ethical committee of Waseda University (2011-045).

Dietary intake

Daily alcohol consumption and calcium and vitamin D intake were assessed using a brief self-administered diet history questionnaire (BDHQ). The BDHQ is a 4-page questionnaire that yields information on the consumption frequency of selected foods to estimate the dietary intake of 58 food and beverage items.[24] The validity of the nutrient intake data assessed with the BDHQ was confirmed using semi-weighed 16-day dietary records as a reference.[24] On the basis of the dietary macronutrient intake assessed using the BDHQ, vitamin D and calcium intake were calculated.

Body composition

Body weight and percent body fat (assessed by bioelectrical impedance analysis) were measured using an electronic scale (Inner Scan BC-660, Tanita Inc., Tokyo, Japan) without shoes and with light clothes; height was measured with a stadiometer (YL-65, Yagami, Inc., Nagoya, Japan). The body mass index (BMI) was calculated by dividing body weight in kilograms by the square of height in meters (kg/m^2).

Cardiorespiratory fitness

CRF was measured via a maximal graded exercise test using a cycle ergometer (Ergomedic 828E, Monark, Varberg, Sweden or Aerobike 75 XLII, Combi, Tokyo, Japan) and quantified as the peak oxygen uptake (VO_2peak). The graded cycle exercise began at a workload of 45-90W, which was then increased by 15 W/min until the subject could not maintain a pedaling frequency of 60 rpm. The subject's heart rate and rating of perceived exertion (RPE) were monitored every minute throughout the test. The expired gas of subjects was collected and the rates of oxygen consumption and carbon dioxide production were measured and averaged over 30-s intervals using an automated gas analyzer (Aeromonitor AE-280S, Minato Medical Science, Tokyo, Japan), and the highest observed value of VO_2 was defined as the VO_2peak (mL/kg/min).

Muscular fitness

Hand grip strength (kilograms) was measured using a hand grip dynamometer (YX, Yagami Inc., Nagoya, Japan). Subjects were instructed to complete 2 handgrip contraction trials bilaterally, alternating hands between trials. Subjects held the dynamometer with the arm completely extended in the standing position and were verbally encouraged to exert the maximal force possible. The highest values attained using each hand were considered the right-hand and left-hand grip strength scores. The mean value of the two maximal grip strength scores were used. Leg extension power was measured using a dynamometer (Anaero Press 3500, Combi Wellness, Tokyo, Japan) in the sitting position. The subject's waist and ankles were firmly fastened by Velcro straps. The seat was moved to a position at which the knee joint angle was about 90 degrees. The subjects were advised to extend their legs vigorously. The highest recorded power output (Watt) of five measurements (15 s rest between trials) was taken as the definitive measurement.

Physical activity

Physical activity was measured using an activity monitor (Kenz Lifecorder, Suzuken Co. Ltd., Nagoya, Japan) worn continuously for 10 days. The subjects were instructed in the instrument's use, and wore it on their belt or waistband at the right midline of the thigh during waking hours, except while bathing or swimming. On a scale with points 0, 0.5, and 1–9, the Lifecorder system determined the level of physical activity intensity every 4 s. The amount of time spent at intensity levels 4–6 and 7–9 were used as the amount of time spent in MPA and VPA, respectively. The time spent in moderate- and vigorous-intensity physical activity (MVPA) were calculated from MPA and VPA using continuous 7 days of monitored activity. Additional details have been published elsewhere.[25]

Blood sample analysis

Blood samples were collected in Venoject-II AutoSep tubes without anticoagulant (gel, 9mL; Terumo Corporation, Tokyo, Japan), from an antecubital vein of a forearm between 0830 and 1000 h by accredited medical personnel after a 12-h overnight fast, and centrifuged at 3,000×g for 15 min at 4°C and stored at −80°C. Serum 25(OH)D concentrations were measured in duplicate with commercially available enzyme-linked immunosorbent assay kits (25(OH)D; Immundiagnostik AG, Bensheim, Germany) according to the manufacturer's instructions. The control samples provided by the Immundiagnostik AG group were analyzed with each run for quality control. The intra- and inter- kit variations were 8.9% and 10.6%, respectively. We divided subjects into low and high 25(OH)D groups according to the median values of 25(OH)D concentrations (36.5 nmol/L).

Statistical analysis
All statistical analyses were performed by using the SPSS version 22.0 software (SPSS, Inc., Chicago, IL, USA). Data were assessed for normality using a Kolmogorov-Smirnov test prior to all statistical analyses and presented as follows: normally distributed variables, mean ± SD; skewed variables, medians (interquartile ratio; IQR); and categorical variables, percentages (unless otherwise indicated). Student t-test (for normally distributed variables), Mann-Whitney U test (for non-normally distributed variables), or chi-square test (for categorical variables) was used to evaluate differences between groups. Non-normally distributed variables were log or square root-transformed to obtain a normal distribution before the correlation analysis. Associations among variables were determined using the Pearson correlation coefficient, and a partial correlation analysis adjusted for age was performed. The influence of 25(OH)D concentrations and age on physical fitness was evaluated by two-way analysis of covariance adjusted for age, season, percent body fat, MVPA, vitamin D intake, smoking status, and lipid-lowering, antidiabetic drug use and antihypertension drug use. A post hoc test with Bonferroni correction was used to identify significant differences if a significant main effect or interaction was identified. Statistical significance was set at $p<0.05$.

RESULTS
The characteristics of the subjects are presented in Table 1. The median 25(OH)D concentration was 36.5 (IQR:27.3-49.0) nmol/L; 77.5% of the subjects were vitamin D-deficient (<50 nmol/L) and 15.6% were vitamin D-insufficient (50-75 nmol/L). The elderly group showed lower mean height, weight, BMI, hand grip strength, leg extension power and CRF, and higher 25(OH)D values than did the middle-aged group ($p<0.05$).

Table 2 presents the correlations of serum 25(OH)D concentrations with other variables. Pearson correlation coefficient analysis showed that serum 25(OH)D correlated positively with age (r=0.215, $p=0.006$), hand grip strength (r=0.204, $p=0.009$), calcium intake (r=0.244, $p=0.002$), and vitamin D intake (r=0.326, $p<0.001$). Partial correlation analysis revealed that serum 25(OH)D concentrations correlated positively with hand grip strength (r=0.331, $p<0.001$), leg extension power (r=0.353, $p<0.001$), CRF (r=0.285, $p<0.001$), calcium intake (r=0.206, $p=0.010$) and vitamin D intake (r=0.296, $p<0.001$) after adjustment for age.

To evaluate interaction effects between 25(OH)D and age on the indicators of physical fitness, two-way ANOVA was performed after adjustment for several confounding factors including age, season, percent body fat, MVPA, vitamin D intake (Table 3 and Figure 1). Significant interaction between 25(OH)D and age on CRF was observed ($p<0.05$), but non-significant interactions were found on hand grip strength, leg extension power and MVPA. In the elderly, subjects in high 25(OH)D group had higher CRF compared with those in low group (32.7 vs 28.6 mL/kg/min, $p<0.05$), which was not observed in middle-aged group. Age-related decrease of CRF was observed in low 25(OH)D group (33.6 vs 28.6 mL/kg/min, $p<0.05$), while the reduction was prevented in high 25(OH)D group. The association between 25(OH)D and CRF remained after further adjustment for muscle strength ($p_{\text{interaction}}=0.030$).

Table 1. Characteristics of the subjects[†]

Variable	Total (n=161)	Middle-aged (n=78)	Elderly (n=83)	p[‡]
Age (yr)	65.0 (58.0-69.0)	57.5 (44.0-63.0)	69.0 (67.0-73.0)	0.000[*]
Height (cm)	169.1±6.4	170.6±5.7	167.8±6.9	0.007[*]
Weight (kg)	68.3±9.1	70.7±9.1	66.1±8.5	0.001[*]
BMI (kg/m^2)	23.4 (22.3-25.3)	24.0 (22.5-25.6)	23.1 (22.1-25.0)	0.051
Body fat (%)	21.1±4.2	21.2±4.1	21.1±4.4	0.943
Hand grip strength (kg)	37.6±7.4	40.9±6.5	34.6±6.9	0.000[*]
Leg extension power (W/kg)	17.0±5.1	19.6±5.1	14.6±3.7	0.000[*]
MVPA (min/day)	38.8±23.8	39.2±20.3	38.6±26.7	0.874
CRF (mL/kg/min)[§]	32.4±7.6	36.6±6.9	28.4±5.9	0.000[*]
25(OH)D (nmol/L)	36.5 (27.3-49.0)	32.4 (23.6-46.5)	37.8 (28.5-58.4)	0.026[*]
Alcohol consumption (g/day)[¶]	18.1 (4.5-41.9)	17.7 (5.7-40.3)	21.2 (2.5-46.0)	0.600
Calcium intake (mg/day)[¶]	620.5 (437.9-782.9)	591.1 (392.1-768.0)	649.8 (474.2-802.9	0.070
Vitamin D intake (μg/day)[¶]	13.1 (9.1-20.8)	12.6 (9.0-17.0)	14.5 (9.4-24.5)	0.103
Antihypertensive drug use (%)	22.4	16.7	27.7	0.093
Lipid-lowing drug use (%)	5.0	6.4	3.6	0.485
Diabetes (%)	5.0	2.6	7.2	0.279
Smoking status (%)	47.8	43.6	51.8	0.297

BMI: body mass index; MVPA: moderate- to vigorous-intensity physical activity; CRF: cardiorespiratory fitness; 25(OH)D: 25-hydroxyvitamin D.
[†]Data are mean±SD, median (interquartile range), or percentages. Data were analyzed using Student's test (for normally distributed variables), Mann-Whitney U test (for non-normally distributed variables), or Chi-square test (for categorical variables).
[‡]Middle -aged vs elderly.
[§]Middle-aged: n=76, elderly: n=80.
[¶]Middle-aged: n= 78, elderly: n=82.
[*]Indicates significance ($p<0.05$).

Table 2. Correlations between serum 25(OH)D concentrations and subject characteristics †‡

	25(OH)D		25(OH)D (age adjusted)	
	r	*p*	r	*p*
Age (year)	0.215	0.006		
BMI (kg/m²)	-0.054	0.500	-0.053	0.513
Body fat (%)	-0.032	0.690	-0.050	0.533
Hand grip strength (kg)	0.204	0.009	0.331	0.000
Leg extension power (W/kg)	0.110	0.164	0.353	0.000
MVPA (min/day)	-0.005	0.952	-0.017	0.830
CRF (mL/kg/min)§	0.086	0.287	0.285	0.000
Alcohol consumption (g/day)¶	0.071	0.371	0.052	0.517
Calcium intake (mg/day)¶	0.244	0.002	0.206	0.010
Vitamin D intake (μg/day)¶	0.326	0.000	0.296	0.000

BMI: body mass index; 25(OH)D: 25-hydroxyvitamin D; MVPA: moderate- to vigorous-intensity physical activity; CRF: cardiorespiratory fitness.
†Data are mean±SD, median (interquartile range), or percentages. Data were analyzed using Student's test (for normally distributed variables.
‡25(OH)D, calcium intake and alcohol consumption were square root transformed; vitamin D intake were log-transformed.
§Middle-aged: n=76, elderly: n=80.
¶Middle-aged: n= 78, elderly: n=82.

Table 3. Joint association of age and 25(OH)D groups with parameters related to physical fitness†

Age (years)	Middle-aged		Elderly		Age	25(OH)D	Interaction
25(OH)D	Low 25(OH)D	High 25(OH)D	Low 25(OH)D	High 25(OH)D	*p*	*p*	*p*
N	46	32	35	47			
Hand grip strength (kg)	38.5±1.1	39.8±1.2	34.2±1.2	37.7±1.1	0.028*	0.022*	0.255
Leg extension power (W/kg)	16.3±0.6	18.3±0.7	15.7±0.7	17.8±0.6	0.548	0.001*	0.921
MVPA (min/day)‡	40.4±4.4	40.4±4.6	38.4±4.6	37.0±4.1	0.625	0.857	0.867
CRF (mL/kg/min)§	33.6±0.9	34.2±1.0	28.6±1.0¶	32.7±0.9††	0.007*	0.006*	0.044*

25(OH)D: 25-hydroxyvitamin D; MVPA: moderate- to vigorous-intensity physical activity; CRF: cardiorespiratory fitness.
†Data are presented as the adjusted mean ± SE. Data were analyzed using two-way analysis of covariance adjusted for age, season, percent body fat, MVPA, vitamin D intake, smoking status, and lipid-lowering, antidiabetic drug use and antihypertension drug use.
‡Data was also adjusted by the above confounding factors except MVPA.
§Middle-aged: n=76, Elderly: n=80. 25(OH)D groups were divided according to the median serum 25(OH)D concentrations (36.5nmol/L).
¶$p<0.05$ vs. Middle-aged group within the same 25(OH)D group.
††$p<0.05$ vs. low 25(OH)D in elderly group.
*Indicates significance ($p<0.05$).

DISCUSSION

In the present study, we found that serum 25(OH)D concentrations positively related with leg extension power, hand grip strength and CRF after adjustment for age. Furthermore, significant age-related reduction of CRF was observed in low 25(OH)D group, while the reduction was prevented in high 25(OH)D group; in the elderly, increased serum 25(OH) concentrations were closely related with improve CRF. Our results indicate that increased serum 25(OH)D concentrations could play an important role in maintaining and promoting higher levels of CRF in the ageing process.

The prevalence of vitamin D deficiency was high in the present study, which may related with the determination season. More than 99% subjects' blood were obtained during winter and spring, when vitamin D synthesis from sunlight is very limited.[26] Kobayashi et al found that in Japan the highest 25(OH)D concentrations were observed in August,[27] an gradually decreased until February. The results suggest that subjects, especially the elderly are encouraged to exercise outside, consume fatty fish, and take appropriate supplements for adequate vitamin D intake, especially in winter and early spring.

The present study indicated that serum 25(OH)D was significantly related with hand grip strength and leg extension power after adjustment for age. These results are consistent with those of several previous studies.[5-9] Grimaldi et al found that age is an important predictor of

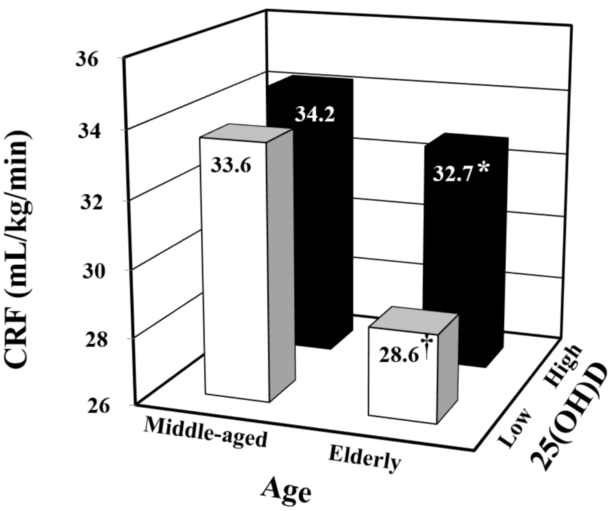

Figure 1. Joint association of age and 25(OH)D groups with CRF.

several muscle strength indicators,[6] and the non-significant relationship between serum 25(OH)D and physical function score became significant after age-adjustment.[11] Interventional studies of vitamin D supplementation on muscle function have also suggested that the elderly may benefit more from vitamin D supplementation than would younger adults,[22] and that serum 25(OH)D concentrations may have a stronger influence on physical fitness in the elderly.

Inconsistent with previous evidence,[28] we were unable to observe a correlation between serum 25(OH)D and BMI. Accumulating evidence shows that compared with Europeans, Asians are more prone to obesity-related morbidity and mortality at a lower BMI, but at larger VFA levels.[29] Furthermore, in our previous studies conducted in Japanese, we found that serum 25(OH)D concentration was more closely related with abdominal fat, such as VFA, rather than with BMI.[30,31]

Circulating 25(OH)D was found to be positively related with CRF after adjusting for age, which is consistent with results in adults across a wide age range,[13-15] though the relationship with ageing was still unclear. In the present study, we evaluated the influence of serum 25(OH)D concentrations on CRF according to age groups. Serum 25(OH)D was an important predictor of CRF in the elderly, but not in the middle-aged group; maintain higher 25(OH)D concentrations could prevent the age-related reduction of CRF. The results were also remained after adjustment for MVPA objectively measured. It is indicated that CRF is simply a surrogate for individuals' MVPA,[17] which could also be related to sun exposure. However, most of previous studies have not considered MVPA's influence. Ardestani et al found that the relationship between 25(OH)D and CRF was significantly affected the levels of MVPA,[14] which was determined by self-report questionnaire and subject to recall bias.

CRF declines with age, and is associated with risk of diseases and the ability to function independently.[32] CRF depends on the oxygen transport by the respiratory and circulatory systems from environmental air to the active muscle and the utilization of oxygen by the mitochondria. Exercise training is indicated to increase CRF levels, which results from more effective cardiac output distribution to active muscles combined with enhanced capacity of trained muscle fibers and oxygen utilization. Unlike young adults, exercise training tends to have greater effect on oxygen utilization in muscle rather than oxygen transport in the elderly, because they usually have diminished capacity to improve cardiac out.[33] VDRs have been observed in cardiac and skeletal muscle tissue, and also in the renin-angiotensin systems.[3] In addition, 1, 25(OH)$_2$D have been implicated in the regulation of mitochondrial function and enzyme function,[34,35] which are likely to influence CRF. Although the clear mechanism was unclear, the above evidence may give an explanation that serum 25(OH)D was a significant predictor in improving CRF in the elderly.

Our study focused on the age-specific relationship of serum 25(OH)D concentrations with CRF, and found that the relationship of vitamin D and CRF was affected by ageing. However, there are several limitations to our study. First, the sun exposure time was not recorded, although season and MVPA as proxy parameters to sun exposure were assessed in the present study. Second, because this was a cross-sectional study, it is difficult to make causal inferences. Third, though the subjects in the present study showed a daily vitamin D intake (13.1 μg/day) higher than the Dietary Reference Intake (5.5 μg/day) for Japanese adults, the prevalence of 25(OH)D deficiency (77.0%) was still high; therefore, whether the findings can be extrapolated to subjects with higher serum 25(OH)D concentrations and other seasons need to be investigated. Finally, because we only examined men in the present study, our results should be interpreted with caution and confirmed in cohorts with women.

In conclusion, we found that serum 25(OH)D concentrations were significantly and positively related with hand grip strength, leg extension power, and CRF in adults with consideration of age influence; additionally, serum 25(OH)D concentrations play a crucial role in maintaining or improving CRF in the elderly, but not in middle-aged men. These results suggest that increasing serum 25(OH)D concentrations related with improved muscle strength, and was a significant predictor of CRF in the elderly.

Positive findings

- Serum 25(OH)D concentrations significantly related with leg extension power, hand grip strength and CRF.
- Higher serum 25(OH)D concentrations are a key predictor for CRF in the elderly, but not in middle-aged men.

Specific recommendations

- Higher levels of vitamin D was beneficial for physical fitness.
- The effect of ageing should be considered when assess the associations among serum 25(OH)D concentrations and physical fitness. The elder appear to be benefit more compared with the younger adults.

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AUTHOR DISCLOSURES

The authors have no conflicts of interest to declare.

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