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Association Between Low Blood 25-Hydroxyvitamin D and High C-Reactive Protein Levels in Community-Dwelling Japanese People Aged 40-74 Years

Takuya Abe,[1,2] Yumi Watanabe,[1] Kaori Kitamura,[1] Keiko Kabasawa,[3] Toshiko Saito,[1] Akemi Takahashi,[4] Ribeka Takachi,[5] Ryosaku Kobayashi,[4] Rieko Oshiki,[4] Shoichiro Tsugane,[6] Kei Watanabe[7] and Kazutoshi Nakamura[1]

[1]Division of Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[2]Department of Clinical Engineering and Medical Technology, Niigata University of Health and Welfare, Niigata, Japan
[3]Department of Health Promotion Medicine, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
[4]Department of Rehabilitation, Niigata University of Rehabilitation, Niigata, Japan
[5]Department of Food Science and Nutrition, Nara Women's University Graduate School of Humanities and Sciences, Nara, Japan
[6]International University of Health and Welfare Graduate School of Public Health, Minato-ku, Tokyo, Japan
[7]Division of Orthopaedic Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

Low blood 25-hydroxyvitamin D (25[OH]D) levels at which C-reactive protein (CRP) levels begin to rise vary. This study investigated the association between blood 25(OH)D and elevated CRP levels and determine the cut-off of low 25(OH)D for elevated CRP in middle-aged and older individuals in the Murakami cohort, Japan. This study used a cross-sectional study design with 2,863 subjects aged 40-74 years living in the community. Plasma 25(OH)D levels were determined with the Liaison® 25OH Vitamin D Total Assay, and serum high sensitivity CRP (hs-CRP) levels were determined with a latex nephelometry assay using an automatic analyzer. Multivariate logistic regression analysis was used to calculate odds ratios (ORs) for high hs-CRP (≥ 3 mg/L) with covariates including sex, age, BMI, physical activity, smoking, drinking, and disease history. Median age of subjects was 65 years, and median 25(OH)D level was 47.4 nmol/L. The proportion of subjects with high hs-CRP levels was 4.1%. The adjusted OR of 25(OH)D < 20 nmol/L was higher (OR = 3.22, 95% CI: 1.42-7.31) than that of the reference (25[OH]D 40-49 nmol/L). In subgroup analysis, the adjusted OR of 25(OH)D < 20 nmol/L was significantly higher than the reference in the BMI ≥ 22.8 (median) group (OR = 4.52) but not in the BMI < 22.8 group (OR = 1.61) (P for interaction = 0.0892), and the adjusted OR was significantly higher in the age ≥ 65 group (OR = 8.51) but not in the age < 65 group (OR = 2.22). Low blood 25(OH)D and high CRP levels were associated, with 25(OH)D 20 nmol/L being the cut-off, which was lower than previously reported values.

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Introduction

Low-grade systemic chronic inflammation plays a pivotal role in the pathogenesis of many chronic diseases, such as cardiovascular disease, cancer, and diabetes, which contribute to increased mortality and morbidity rates worldwide (Furman et al. 2019). There are many causes of low-grade systemic chronic inflammation, including chronic

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Correspondence: Kazutoshi Nakamura, MD, Division of Preventive Medicine, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-dori, Niigata 951-8510, Japan.
e-mail: kazun@med.niigata-u.ac.jp

infection, lifestyle, psychological stress, and environmental factors (Furman et al. 2019), with nutrition being one of the most important (Di Giosia et al. 2022).

Vitamin D, primarily recognized for its role in maintaining bone metabolism, has garnered increasing attention due to its potential immunomodulatory effects beyond the skeletal system. Recent studies have demonstrated the involvement of vitamin D in immune response regulation and inflammatory modulation (Yin and Agrawal 2014). In this context, previous studies have explored the relationship between levels of blood 25-hydroxyvitamin D (25[OH]D), a useful marker of vitamin D status, and C-reactive protein (CRP), a sensitive biomarker of systemic inflammation (Amer and Qayyum 2012; Mellenthin et al. 2014; Tepper et al. 2014; Srikanth et al. 2016; de Oliveira et al. 2017; Liu et al. 2017; Rafiq et al. 2020; Twardowski et al. 2021; Laird et al. 2023; Zhou and Hypponen 2023). However, findings of those studies were not consistent; among nine studies reported to date, seven found an inverse association between blood 25(OH)D and CRP levels (Amer and Qayyum 2012; Mellenthin et al. 2014; Tepper et al. 2014; de Oliveira et al. 2017; Rafiq et al. 2020; Laird et al. 2023; Zhou and Hypponen 2023), whereas two found a null association (Srikanth et al. 2016; Twardowski et al. 2021). Moreover, levels of blood 25(OH)D at which CRP levels began to rise varied from 25 to 53 nmol/L among the seven studies reporting the inverse association (Amer and Qayyum 2012; Tepper et al. 2014; de Oliveira et al. 2017; Zhou and Hypponen 2023). Thus, there is a need for further investigation regarding the association between blood 25(OH)D and CRP levels. Furthermore, as previous studies on this topic were all conducted in white populations (Amer and Qayyum 2012; Mellenthin et al. 2014; Tepper et al. 2014; Srikanth et al. 2016; de Oliveira et al. 2017; Rafiq et al. 2020; Twardowski et al. 2021; Laird et al. 2023; Zhou and Hypponen 2023), it will be important to conduct studies in non-white populations because associations between blood 25(OH)D levels and some health outcomes have been reported to vary by ethnicity (van Ballegooijen et al. 2015; Xia et al. 2020).

Low levels of vitamin D have been a topic of interest in clinical medicine, with vitamin D deficiency defined as blood 25(OH)D levels < 50 nmol/L in terms of bone/mineral events in Japan (Okazaki et al. 2017). Nevertheless, cut-off values for blood 25(OH)D levels differ among bone/mineral events (Okazaki et al. 2017), and may also differ by outcomes other than bone/mineral events. Therefore, clarifying the cut-off value for the association between blood 25(OH)D levels and CRP levels is important.

The present study aimed to investigate the association between blood 25(OH)D levels and elevated CRP levels and determine the cut-off of low 25(OH)D for elevated CRP in community-dwelling middle-aged and older individuals in Japan. The findings of this study will provide insights into the potential preventive and therapeutic implications of optimizing vitamin D status for reducing systemic chronic inflammation.

Materials and Methods

Subjects

The present study used a cross-sectional study design. The predictor was plasma 25(OH)D, and the outcome was the presence of elevated serum high-sensitivity CRP (hs-CRP). A total of 8,497 individuals aged 40-74 years participated in the plasma 25(OH)D examination of the Murakami cohort study conducted in 2011-2013 (Nakamura et al. 2015, 2018). Of these, 2,863 individuals who also provided serum samples for examining hs-CRP formed the subjects of the present study. All subjects provided written informed consent. The protocol of this study was approved by the Ethics Committee of Niigata University (Nos. 452, 481, and 1324).

Procedures

Blood samples, including serum and plasma, for 2,863 of the 8,497 participants were collected at annual health checkup examinations held by the local government. Only participants recruited at annual health check examinations were included as subjects of the present study, because serum samples were collected only in that setting. Numbers of subjects with blood samples collected in April, May, June, July, August, September, October, and November were 78, 833, 1517, 339, 16, 13, 66, and 1, respectively. Height and body weight were measured, and body mass index (BMI) was calculated by dividing body weight in kilograms by height in meters squared.

A self-reported questionnaire survey was conducted to obtain information on lifestyles. Total physical activity level was estimated by calculating metabolic equivalent (MET)-hours/day, which was obtained by multiplying the time spent in each activity (/day) by its MET intensity. Nutrient intakes, including the intake of energy, protein, fat, and vitamin D, were assessed with a validated food frequency questionnaire (Yokoyama et al. 2016). Smoking habit was classified as non-smoker, past smoker, 1-20 cigarettes/day, and ≥ 20 cigarettes/day, and alcohol consumption was classified into five categories as non- or rare-drinkers, 1-149, 150-299, 300-449, and ≥ 450 g of ethanol per week. Information on disease histories of myocardial infarction, stroke, and diabetes was obtained by asking participants if they had these histories.

To obtain plasma, non-fasting blood specimens were drawn with EDTA-2Na-containing tubes. Serum was obtained by centrifuging the specimens at 3,000 rpm for 10 min. Plasma and serum were immediately stored at 4°C and stored at −80°C until biochemical analysis. Plasma 25(OH)D levels were determined with the Liaison® 25OH Vitamin D Total Assay (DiaSorin Inc.; Stillwater, MN, USA). Intra- and inter-assay coefficient of variation (CV) values were 3.2-8.1 % and 6.9-12.7 %, respectively. Serum hs-CRP levels were determined by a latex nephelometry assay with an automatic analyzer (Behring Nephelometer

II; Siemens Healthcare Diagnostics Inc.; Deerfield, IL, USA; inter-assay CV value, 3.1%). hs-CRP levels greater than 3 mg/L were considered high CRP (Pearson et al. 2003). Non-fasting blood lipid levels were used to determine whether these values affect the association between 25(OH)D and hs-CRP levels. Serum low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were measured in the same health-check settings as above. Details regarding baseline blood collection and examination have been described previously (Nakamura et al. 2015).

Statistical analysis

Median and interquartile ranges were used to characterize continuous variables. Differences in median values and proportions between the two groups in Table 1 were tested by the Wilcoxon rank sum test and chi-square test, respectively. Logistic regression analysis was used to calculate odds ratios (ORs) for high hs-CRP according to 25(OH)D levels for every 10 nmol/L. The mode of the distribution of 25(OH)D levels, i.e., 25(OH)D 40-49 nmol/L, was set as the reference. Multivariate logistic regression analysis was performed with covariates including sex, age, BMI, total physical activity, smoking, alcohol consumption, and disease history. Subgroup multivariate logistic regression analysis was also conducted, stratified by sex, age group (< 65 vs. ≥ 65 [median] years), and BMI (< 22.8 vs. ≥ 22.8 [median] kg/m^2). Finally, for sensitivity analyses, multivariate logistic regression analyses were performed further adjusted for serum lipid levels. The association between 25(OH)D groups and high hs-CRP was analyzed after excluding subjects who reported either a history of myocardial infarction, stroke, or diabetes. Only serum HDL cholesterol levels were associated with high hs-CRP and included in the multivariate analyses. SAS statistical software (release 9.4, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses. $P < 0.05$ was considered statistically significant.

Results

Median age of subjects was 65 years (interquartile range, 59-70 years), and median plasma 25(OH)D level was 47.4 nmol/L (interquartile range, 36.4-59.9 nmol/L). The proportion of subjects with high hs-CRP was 118/2, 863 (4.1%). Characteristics of subjects with normal and high hs-CRP levels are shown in Table 1. The proportion of male was significantly higher in the high hs-CRP group (50.9%) than in the normal hs-CRP group (39.7%). BMI of the high hs-CRP group (23.8 kg/m^2) was significantly higher than that of the normal hs-CRP group (22.8 kg/m^2). Characteristics of subjects according to plasma 25(OH)D levels are shown in Supplementary Table S1. The proportion of male, age, total physical activity level, proportions of current smokers/drinkers, serum lipids, and history of diabetes were significantly associated with plasma 25(OH) D levels.

Table 1. Characteristics of subjects with normal and high hs-CRP levels.

	Normal hs-CRP (≤ 3 mg/L) (N = 2,745)	High hs-CRP (> 3 mg/L) (N = 118)	*P* value
Male (%)	1090 (39.7)	60 (50.9)	0.0157
Age (years)	65 (59,70)	64 (61,69)	0.9184
BMI (kg/m^2)	22.8 (20.9,24.8)	23.7 (21.7,25.9)	0.0026
Total physical activity (MET-h/day)	44.8 (39.2,54.1)	44.7 (40,52.5)	0.9262
Energy intake (kcal/day)	2062 (1649,2585)	1984 (1635,2476)	0.2698
Protein intake (g/day)	71.2 (54.3,92.5)	69.7 (52.9,90.5)	0.6964
Fat intake (g/day)	53.8 (39.0,74.3)	51.9 (37.3,72.7)	0.4284
Vitamin D intake (mg/day)	8.4 (4.9,13.3)	8.2 (4.4,12.3)	0.5895
Current smoker (%)	344 (12.6)	17 (14.4)	0.5522
Current drinker (%)	1,402 (51.1)	60 (50.9)	0.9551
Plasma 25-hydroxyvitamin D (nmol/L)	47.4 (36.4,59.7)	48.9 (36.2,64.4)	0.3399
hs-CRP (mg/L)	0.22 (0.11,0.5)	4.76 (3.82,9.21)	< 0.0001
LDL cholesterol (mg/dl)	112 (95,132)	113 (92,129)	0.3921
HDL cholesterol (mg/dl)	60 (50,70)	51 (44,59)	< 0.0001
Triglyceride (mg/dl)	111 (76,162)	108 (72,173)	0.9756
History of myocardial infarction (%)	17 (0.6)	1 (0.9)	0.7588
History of stroke (%)	53 (1.9)	4 (3.4)	0.2666
History of diabetes (%)	187 (6.8)	7 (5.9)	0.7095

Values are presented as median (interquartile range in parentheses) for continuous variables and number (percent in parentheses) for categorical variables.

Missing values: 3 for total physical activity, 4 for smoking, 2 for alcohol consumption, and 10 for serum LDL cholesterol, HDL cholesterol, and triglyceride.

The distribution of plasma 25(OH)D levels by sex are shown in Supplementary Fig. S1. Numbers of male and female in each 25(OH)D group were as follows: 14 and 83 in the < 20 nmol/L group, 57 and 220 in the 20-29 nmol/L group, 145 and 399 in the 30-39 nmol/L group, 226 and 458 in the 40-49 nmol/L group, 259 and 288 in the 50-59 nmol/L group, 223 and 153 in the 60-69 nmol/L group, 140 and 64 in the 70-79 nmol/L group, and 86 and 48 in the ≥ 80 nmol/L group, respectively. The mode of the 25(OH)D level distribution was 50-59 nmol/L for male, 40-49 nmol/L for female, and 40-49 nmol/L for both male and female. Associations of sex, age group, BMI, total physical activity level, smoking, and alcohol consumption with odds of high hs-CRP are shown in Supplementary Fig. S2. Male (P = 0.0164) and higher BMI (P for trend = 0.0005) were significantly associated with odds of high hs-CRP (Supplementary Fig. S2A,C), but age group was not (P for trend = 0.6924) (Supplementary Fig. S2B). Total physical activity level (P for trend = 0.7036), smoking (P for trend = 0.1530), and alcohol consumption (P for trend = 0.5187) were not associated with odds of high hs-CRP (Supplementary Fig. S2D-F). Associations of serum lipids levels with odds of high hs-CRP are shown in Supplementary Fig. S3. HDL cholesterol levels were inversely and significantly associated with odds of high hs-CRP (P for trend < 0.0001, Supplementary Fig. S3B), but LDL cholesterol (P for trend = 0.5298) and triglyceride (P for trend = 0.7720) levels were not (Supplementary Fig. S3A,C).

Unadjusted and adjusted ORs for high hs-CRP according to plasma 25(OH)D levels are shown in Fig. 1 and Supplementary Table S2. Unadjusted and adjusted ORs of the 25(OH)D < 20 nmol/L group were significantly higher than those of the reference group (OR = 2.69, 95% CI: 1.27-5.74 and 3.22, 95% CI: 1.42-7.31, respectively). Overall P for trend values were 0.4560 for the unadjusted model and 0.8129 for the adjusted model. We also calculated ORs for high hs-CRP according to 25(OH)D levels for every 5 nmol/L in subjects with 25(OH)D < 30 nmol, and determined the adjusted ORs of 25(OH)D– < 15 and 15-19-nmol/L groups to be 5.31 (95% CI: 1.60-17.62) and 1.94 (95% CI: 0.64-5.89), respectively, compared to the reference, and that of the 25(OH)D-20-24 nmol/L group to be 0.80 (95% CI: 0.23-2.78) (Supplementary Table S3).

Adjusted ORs for high hs-CRP according to plasma 25(OH)D levels by sex are shown in Fig. 2 and Supplementary Table S4. The adjusted OR of the 25(OH)D < 20 nmol/L group in female was significantly higher than that of the reference group (OR = 3.56, 95% CI: 1.34-9.52, Fig. 2B), but the adjusted OR of the 25(OH)D < 20 nmol/L group in male was not (OR = 2.54, 95% CI: 0.46-13.89, Fig. 2A). There were no other groups in male or female having a significantly higher OR. P for interaction between 25(OH)D < 20 nmol/L and sex on odds of high hs-CRP was 0.9184.

Adjusted ORs for high hs-CRP according to plasma 25(OH)D levels by age group are shown in Fig. 3 and Supplementary Table S5. The adjusted OR of the 25(OH)D < 20 nmol/L group was significantly higher than that of the reference group in those aged ≥ 65 years (OR = 7.27, 95% CI: 1.35-39.18, Fig. 3B) and < 65 years (OR = 2.67, 95% CI: 1.00-7.10, Fig. 3A). There were no other groups in those aged < 65 or ≥ 65 years having a significantly higher OR. P for interaction between 25(OH)D < 20 nmol/L and age group on odds of high hs-CRP was 0.3627.

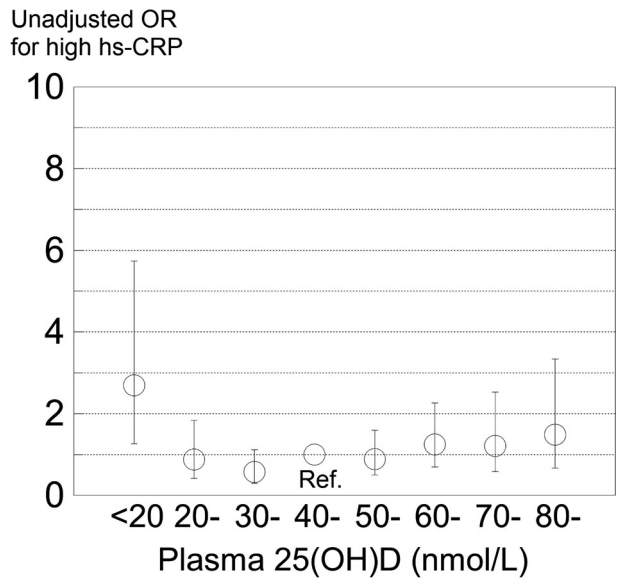
Adjusted ORs for high hs-CRP according to plasma 25(OH)D levels by BMI are shown in Fig. 4 and Supplementary Table S6. The adjusted OR of the 25(OH)D < 20 nmol/L group was significantly higher than that of the reference group (OR = 4.52, 95% CI: 1.65-12.37, Fig. 4B) in those with BMI ≥ 22.8 (median) but not in those with BMI < 22.8 (OR = 1.61, 95% CI: 0.33-7.97, Fig. 4A). There were no other groups in those with BMI < 22.8 or ≥ 22.8 having a significantly higher OR. P for interaction between 25(OH)D < 20 nmol/L and BMI on odds of high hs-CRP was 0.0892 (marginally significant).

Regarding sensitivity analyses, adjusted ORs for high hs-CRP according to plasma 25(OH)D levels after excluding subjects who reported a history of myocardial infarction, stroke, or diabetes (sensitivity analysis) are shown in Supplementary Fig. S4 and Supplementary Table S7. The adjusted OR of the 25(OH)D < 20 nmol/L group was significantly higher than that of the reference group (OR = 3.72, 95% CI: 1.56-8.89). In addition, the strength of associations between 25(OH)D levels and odds of high hs-CRP was similar when HDL cholesterol level was added as a covariate (Supplementary Tables S2, S4-S6).

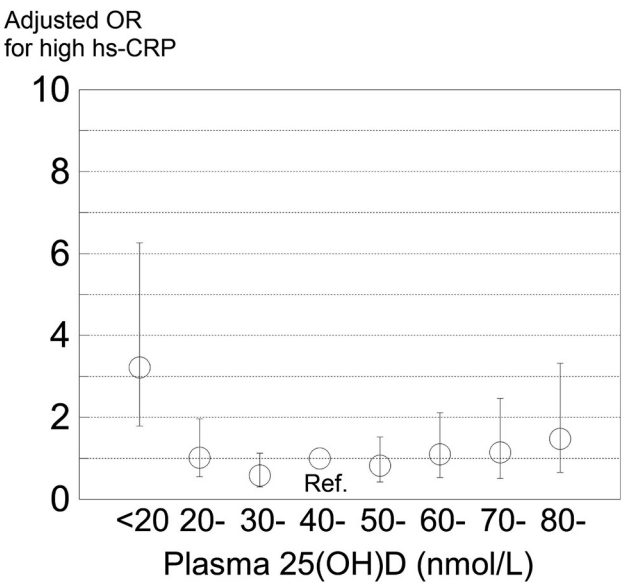
Discussion

The present study examined the association between blood 25(OH)D levels and high hs-CRP in community-dwelling Japanese people, and obtained the following findings: 1) blood 25(OH)D levels < 20 nmol/L were associated with high hs-CRP, 2) the association was stronger in the higher BMI group (BMI ≥ 22.8) than in the lower BMI group (BMI < 22.8), and 3) the association tended to be stronger in older people.

The present study clearly showed that blood 25(OH)D levels < 20 nmol/L were associated with high blood CRP levels. Our results are consistent with several previous studies (Amer and Qayyum 2012; Mellenthin et al. 2014; Tepper et al. 2014; de Oliveira et al. 2017; Rafiq et al. 2020; Laird et al. 2023; Zhou and Hypponen 2023) that reported an inverse association between blood 25(OH)D and CRP levels. However, low blood 25(OH)D levels at which CRP levels begin to rise vary from study to study; previous population-based, cross-sectional studies reported cut-off values of 25-50 nmol/L (Zhou and Hypponen 2023), 30 nmol/L (de Oliveira et al. 2017), 28-35 nmol/L (Tepper et al. 2014), and 53 nmol/L (Amer and Qayyum 2012). Compared with these values, the cut-off value obtained in the present study was lower, at 20 nmol/L. This may partly be explained by the lower prevalence (4.1%) of high blood

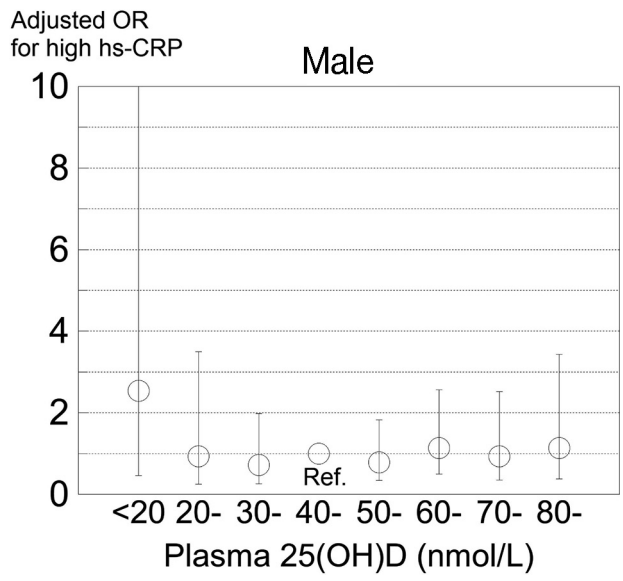


A



B

Fig. 1. Unadjusted and adjusted odds ratios for high hs-CRP according to plasma 25(OH)D levels for the unadjusted model (A) and the adjusted model (B).
Overall P for trend values were 0.4560 for the unadjusted model and 0.9402 for the adjusted model. Covariates of the adjusted model are sex, age, and BMI.



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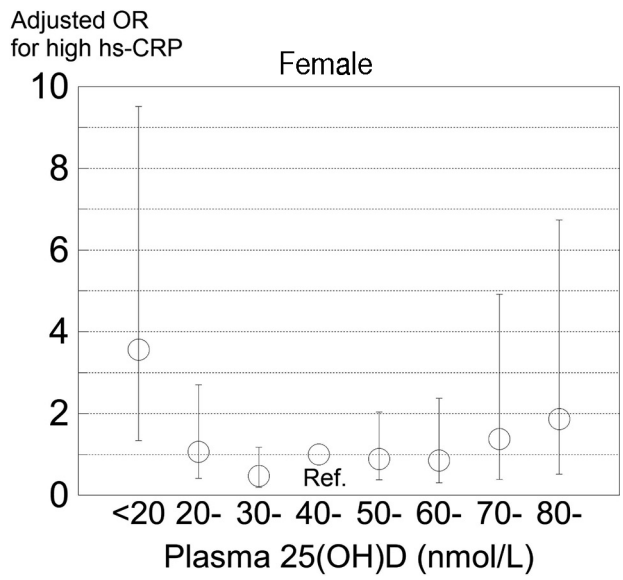


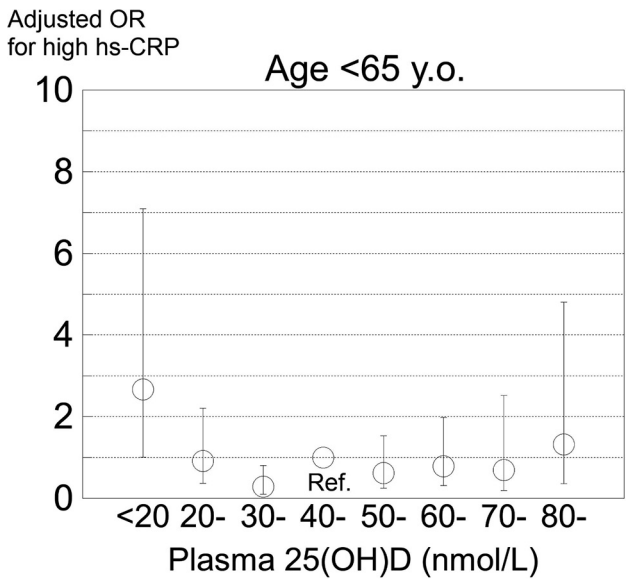
B

Fig. 2. Adjusted odds ratios for high hs-CRP according to plasma 25(OH)D levels by sex for male (A) and female (B). Covariates are age and BMI.

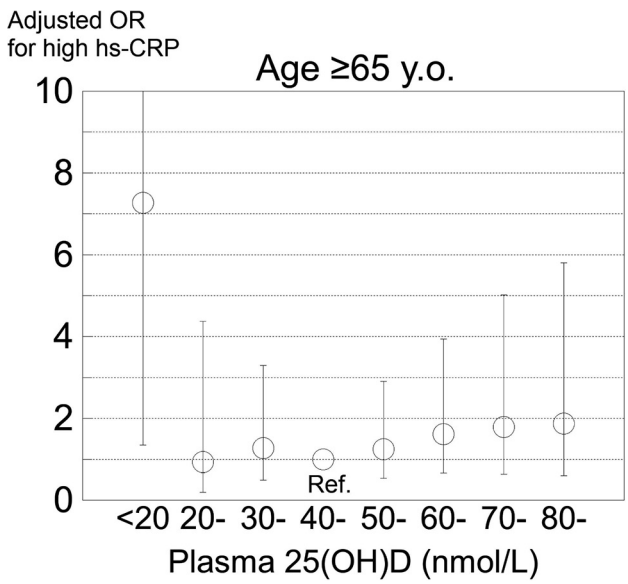
CRP (> 3 mg/L) in the present study cohort, i.e., Japanese people aged 40-74 years, compared with US adult populations of similar ages (37% in their 40s, 44% in their 50s, 49% in their 60s, and 47% in their 70s) (Woloshin and Schwartz 2005). Indeed, Japan is one of the countries with the lowest cardiovascular risk in the world (Global Burden of Metabolic Risk Factors for Chronic Diseases Collaboration 2014).

The low cut-off value of blood 25(OH)D for high CRP may also be explained by ethnicity. Regarding bone metabolism, blood 25(OH)D levels are linearly associated with bone parameters, including bone mineral density and fracture, in white people but not in black or Asian people (Cauley et al. 2011; van Ballegooijen et al. 2015). It is currently unknown whether ethnicity is a factor involved in the association between blood 25(OH)D levels and systemic chronic inflammation, and how this association is affected by ethnic differences will be a topic of future studies.

The observed association between low blood 25(OH)D levels and high CRP levels adds to the growing body of evidence suggesting the role of vitamin D deficiency in systemic inflammation (Furman et al. 2019). Although the exact mechanisms behind this association are not yet fully

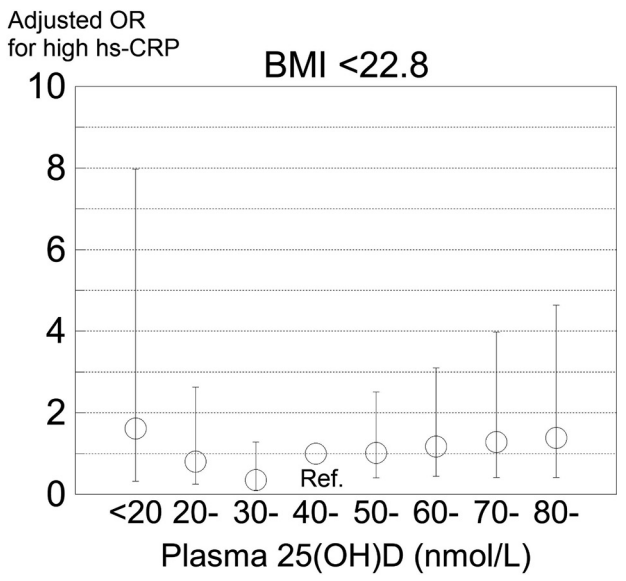


A



B

Fig. 3. Adjusted odds ratios for high hs-CRP according to plasma 25(OH)D levels by age group for subjects aged < 65 years (A) and ≥ 65 years (B).
Covariates are sex, age, and BMI.



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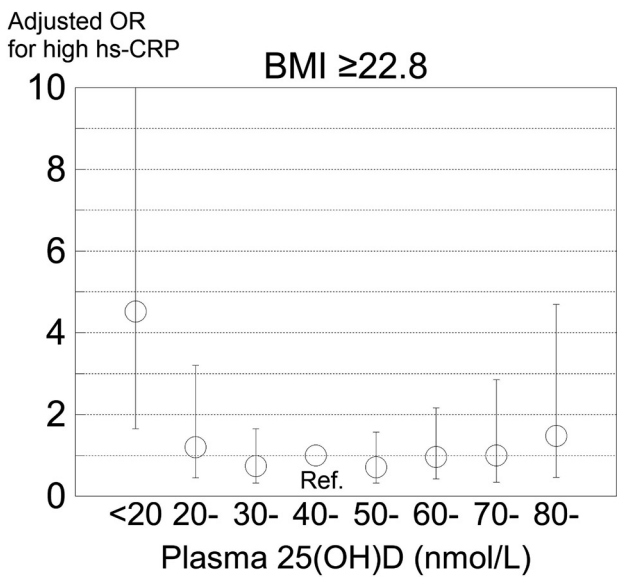

B

Fig. 4. Adjusted odds ratios for high hs-CRP according to plasma 25(OH)D levels by BMI group for subjects with BMI < 22.8 (median) (A) and BMI ≥ 22.8 kg/m^2 (B).
Covariates are sex, age, and BMI.

understood, several biological pathways have been proposed based on the anti-inflammatory and anti-atherogenic effects of vitamin D, including protection against endothelial dysfunction, alteration of macrophage function and gene expression, and inhibition of smooth-muscle cell proliferation (Yin and Agrawal 2014).

The association between low blood 25(OH)D levels < 20 nmol/L and high CRP levels was stronger in the higher BMI group (BMI ≥ 22.8) than in the lower BMI group (BMI < 22.8). This finding may partly be explained by the fact that individuals with higher BMI have higher CRP levels (Rocha and Libby 2009), which was also demonstrated in the present study (Supplementary Fig. S2C), and that vitamin D deficiency is associated with unfavorable metabolic phenotypes such as overweight and obesity via various mechanisms (Pourshahidi 2015). One hypothesis is that the underlying mechanism is related to adiposity. Low blood 25(OH)D levels have been reported to be correlated with low blood adiponectin concentrations in obese individuals (Stokić et al. 2015). Therefore, vitamin D deficiency may adversely affect adipose tissue-derived adipocytokines, such as adiponectin, which may cause lower grade systemic inflammation in overweight individuals.

The association between low blood 25(OH)D levels < 20 nmol/L and high CRP levels tended to be more robust and possibly higher in older subjects than in younger sub-

jects. Because vitamin D has anti-inflammatory and anti-atherogenic effects, vitamin D deficiency affects older individuals more adversely than younger individuals. This finding clearly indicates that vitamin D deficiency in older people is detrimental in terms of CRP-related diseases, including cardiovascular disease, hypertension, diabetes mellitus, and kidney disease (Tang et al. 2017).

This study has some limitations. First, due to the cross-sectional study design, a causal relationship between blood 25(OH)D and CRP levels could not be established. However, a recently published Mendelian randomization study suggested that elevated CRP levels are caused by low blood 25(OH)D levels (Zhou and Hypponen 2023). Second, since we did not evaluate other inflammatory biomarkers, a full understanding of the association between vitamin D deficiency and low-grade systemic inflammation has yet to be gained. Third, although we considered several potential confounders of the association between 25(OH)D and high hs-CRP, we did not control other unknown confounders. Finally, because relatively few individuals had low 25(OH)D levels < 20 nmol/L, the 95% CI for the OR of 25(OH)D < 20 nmol/L was large. A larger sample size will be needed in future studies.

In conclusion, the present study found an association between low blood 25(OH)D levels and high CRP levels, with a cut-off 25(OH)D of 20 nmol/L, in middle-aged and older Japanese people. This cut-off value appeared to be lower than previously reported values. Further studies from different regions and ethnic groups are warranted.

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Conflict of Interest

The authors declare no conflict of interest.

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Supplementary Files

Please find supplementary file(s);
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