Sex differences in serum 25-hydroxyvitamin D reflect differences in 25-hydroxyvitamin D_3 levels but not in D_2 levels

Short title: Sex differences in serum 25-hydroxyvitamin D

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Abstract

Background & Aims: Serum 25-hydroxyvitamin D (25(OH)D) comprises 25(OH)D_3 and 25(OH)D_2. Although sex differences in 25(OH)D levels have been reported, it remains unclear whether the difference lies in the profiles of 25(OH)D. We determined serum 25(OH)D_3, 25(OH)D_2, 3-*epi*-25-hydroxyvitamin D_3 (3-*epi*-25(OH)D_3), and (24*R*)-24,25-dihydroxyvitamin D_3 (24,25$(OH)_2D_3$) levels measured by LC-MS/MS in healthy adults not consuming supplements and analyzed their profiles.

Methods: The serum 25(OH)D levels of 5,959 participants were measured by CLEIA. Levels of vitamin D metabolites (25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$) of 96 participants with no history of osteoporosis, hypertension, cardiac disease, cerebrovascular disease or diabetes and whose alanine transaminase, serum creatinine, total cholesterol, and hemoglobin A1c levels were within the reference ranges were measured.

Results: Serum 25(OH)D, 25(OH)D_3, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels were significantly higher in men than those in women, but there was no significant difference in the 25(OH)D_2 levels. Strong correlations were observed between 25(OH)D and 25(OH)D_3, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels in both sexes. Serum 25(OH)D and 25(OH)D_2 levels and serum 25(OH)D_3 and 25(OH)D_2 levels were not correlated.

Conclusions: Serum 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ profiles were determined for healthy participants not consuming supplements. Sex differences in 25(OH)D levels reflected differences in 25(OH)D_3, not 25(OH)D_2.

Keywords: 25-hydroxyvitamin D, 25-hydroxyvitamin D_3, 25-hydroxyvitamin D_2, LC-MS/MS, sex difference

Abbreviations

25(OH)D, 25-hydroxyvitamin D; 3-*epi*-25(OH)D, 3-*epi*-25-hydroxyvitamin D; $24,25(OH)_2D$, (24*R*)-24,25-dihydroxyvitamin D; LC-MS/MS, liquid chromatography-tandem mass spectrometry; CLEIA, chemiluminescent enzyme immunoassay; TC, total cholesterol; ALT, alanine transaminase; HbA1c, hemoglobin A1c; BMI, body mass index; Ca, serum calcium; IP, serum inorganic phosphorus; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HPLC, high-performance liquid chromatography

1. Introduction

Vitamin D is an important vitamin with anti-rachitic activity, and it promotes the absorption of calcium in the intestinal tract and its deposition in bones.[1] Vitamin D is transported by the blood to the liver, where it is hydroxylated at position 25 of the side chain to form 25-hydroxyvitamin D (25(OH)D). 25(OH)D is then metabolized to 3-*epi*-25-hydroxyvitamin D (3-*epi*-25(OH)D) or (24*R*)-24,25-dihydroxyvitamin D (24,25$(OH)_2$D). Although 25(OH)D has no biological activity, it has a half-life of approximately 3 weeks in the blood and is used as an indicator of vitamin D excess or deficiency in vivo.[2] In the U.S., vitamin D deficiency is defined as a serum 25(OH)D level of <20 ng/mL and insufficiency as 20–29 ng/mL.[3-9] Assessment criteria for vitamin D deficiency/insufficiency in Japan are consistent with those in the U.S.[10] In recent years, vitamin D deficiency has been reported to be associated with various diseases such as coronavirus disease 2019 (COVID-19), and cardiovascular disease.[11-13] Several countries, including Japan, have reported a deficient state of 25(OH)D, especially in women.[14-16]

However, the body has two different forms of vitamin D: vitamin D_2 or 25$(OH)D_2$, derived from ergosterol (a component of mushroom cell membranes), and vitamin D_3 or 25$(OH)D_3$, a cholesterol-derived cholecalciferol (pre-vitamin D_3) metabolized in the skin.[17-19] Vitamin D_3 and D_2 are thought to have similar bioactivities in the body, but in recent years, differences have been reported in terms of maintenance and improvement of blood vitamin D levels;[20-22] therefore, it is important to distinguish between vitamin D_3 and D_2.

The previous findings on vitamin D have been reported by using immunoassays for 25(OH)D. However, immunoassays measure the total 25(OH)D and are not able to distinguish between 25$(OH)D_3$ and 25$(OH)D_2$. Recently, liquid chromatography-tandem mass spectrometry (LC-MS/MS) has made it possible to distinguish between 25$(OH)D_3$ and 25$(OH)D_2$. Yu et al. reported that the 25(OH)D levels did not differ between men and women,

but the 25(OH)D_3 level was significantly higher and the 25(OH)D_2 level was significantly lower in men.[23] However, the participants in their study were taking supplements, and the measured 25(OH)D level did not exclude the effect of supplementation. In addition, sex differences in 25(OH)D metabolites, 3-*epi*-25(OH)D or 24,25$(OH)_2$D were not studied. This study aimed to determine the profiles of 25(OH)D components by using LC-MS/MS and to examine the sex differences of 25(OH)D components in healthy participants not consuming any dietary supplements.

2. Materials and Methods

2.1 Study population

The enrollment process of the study participants is shown in Figure 1. A total of 6,753 individuals underwent health examinations between March 2017 and March 2018 at Hidaka Hospital, Japan. Of these, we excluded 794 individuals who indicated in a questionnaire that they were taking supplements containing vitamin D. The 25(OH)D levels were thus measured by chemiluminescent enzyme immunoassay (CLEIA) in 5,959 participants. Next, in the study of LC-MS/MS, the participants who had replied by self-reported questionnaires and were found to have a history of osteoporosis, hypertension, cardiac disease, cerebrovascular disease, dyslipidemia, or diabetes, were excluded from the group of 5,959. Participants whose alanine transaminase (ALT), serum creatinine, total cholesterol (TC), or hemoglobin A1c (HbA1c) levels deviated from the common reference range established by the Japanese Clinical and Laboratory Standards Conference[24] were also excluded. Only participants with sufficient residual serum were included. As a result, 96 participants remained and these 96 samples were measured by LC-MS/MS. The demographic data of the participants are shown in Table 1.

The study was approved by the Ethics Committees of Hidaka Hospital (No.122) and Gunma University School of Medicine Ethics Review Board for Medical Research Involving Human Subjects (No. HS2020-139) and was performed with written informed consent.

2.2 Measurements

The serum 25(OH)D level was measured by CLEIA using the Unicel DXI 800 (Beckman Coulter, Tokyo, Japan). The intra- and inter-assay coefficients of variation for 25(OH)D were 4.1% and 6.5%, respectively. Vitamin D deficiency was defined as a serum 25(OH)D level <20 ng/mL. Insufficiency was defined as a serum 25(OH)D level <30 ng/mL,

but ≥20 ng/mL. Sufficiency was defined as a serum 25(OH)D level ≥30 ng/mL. Serum TC, ALT, and creatinine levels were measured by enzymatic assays using an automatic analyzer (TBA-c8000; Canon Medical Systems Corporation, Tokyo, Japan). HbA1c level was measured by high-performance liquid chromatography (HPLC) using an automatic analyzer (HLC-723G9; Tosoh, Tokyo, Japan).

Samples for the measurement of 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels were pretreated using JeoQuant™ (JEOL Ltd., Tokyo, Japan). Serum 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels were analyzed using liquid chromatography–tandem mass spectrometry with an HPLC system (Shimadzu, Kyoto, Japan) coupled to a 4000 QTRAP tandem mass spectrometer (AB SCIEX, Tokyo, Japan). The HPLC flow rate was 300 μL/min. The solvent composition of the mobile phase was composed of a 0.1% (v/v) formic acid containing water (solvent A) and acetonitrile (solvent B). The elution program was as follows: 0-0.5 min, 70% A (30% B); 0.5-7.0 min, 42% A (58% B); 7.0-8.0 min, 10% A (90%B); 8.0-9.0 min, 70% A (30% B). An OSAKA SODA core shell column (CAPCELL CORE C18, 2.1×100 mm) at 40 ℃ was used for the vitamin D metabolites separation. Sample were ionized using an electrospray ionization. The capillary voltage and temperature were set at 5,500 V and 500 °C, respectively. The selected reaction monitoring transitions used for each analyte were as follows: 619.3 → 341.1 for 25(OH)D_3 and 3-*epi*-25(OH)D_3, 631.3 → 341.1 for 25(OH)D_2, and 635.3 → 341.1 for 24,25$(OH)_2D_3$. LC-MS/MS data were analyzed by Analyst® (version 1.5.1; AB SCIEX). The chromatograms are shown in Figure 2. The intra-assay coefficients of variation for 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ were 2.2%, 2.4%, 5.3%, and 3.7%, respectively.

2.3 Statistical analysis

Data are presented as median values with 25th and 75th percentiles, rather than as mean values with standard deviations, because almost all the variables were not normally distributed. Accordingly, differences between two groups were tested using the Kruskal-Wallis tests with Dunn-Bonferroni post hoc tests. Spearman rank correlation was used to examine bivariate relationships between parameters. SPSS® (version 27.0.1; IBM®) was used for statistical analysis.

3. Results

As shown in Table 1, a sex difference was observed in the 25(OH)D level among the participants. The 25(OH)D levels were 21.5 (17.5–26.0) ng/mL in the 3,631 male participants and were 17.2 (14.2–20.7) ng/mL in the 2,328 female participants. The 25(OH)D level was significantly lower in women (P<0.001). Of the male participants, 48.5% had insufficiency and 39.7% had deficiency. Of the female participants, 27.5% had insufficiency and 70.2% had deficiency (Table 2). The mean 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels in the 96 participants were 18.3 (14.4–23.6), 0.30 (0.23–0.37), 0.64 (0.47–0.95), and 1.09 (0.66–1.58) ng/mL, respectively. The mean 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels in the 41 male participants were 20.7 (15.8–25.9), 0.30 (0.23–0.38), 0.71 (0.59–1.09), and 1.52 (0.88–1.78) ng/mL, respectively. The mean 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels in the 55 female participants were 17.1 (12.6–20.7), 0.30 (0.24–0.36), 0.59 (0.43–0.79), and 0.82 (0.58–1.21) ng/mL, respectively. The 25(OH)D_3, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels were significantly higher in men than those in women (P = 0.004, 0.001, <0.001, respectively), but there was no significant difference in the 25(OH)D_2 level (Fig. 3).

The correlations between the parameters are shown in Figure 4. In the male participants, significant correlations were observed between the 25(OH)D and 25(OH)D_3, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels (ρ = 0.762, P<0.001, ρ = 0.587, P<0.001, and ρ = 0.604, P<0.001, respectively), but there was no significant correlation between the 25(OH)D and 25(OH)D_2 levels (ρ = 0.078, P = 0.630). In the female participants, significant correlations were observed between the 25(OH)D and 25(OH)D_3, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ levels (ρ = 0.799, P<0.001, ρ = 0.665, P<0.001, and ρ = 0.746, P<0.001, respectively), but there was no significant correlation between the 25(OH)D and 25(OH)D_2 levels (ρ = 0.254, P = 0.062). Furthermore, there was no significant correlation between the 25(OH)D_3 and

25(OH)D_2 levels in both male or female participants (ρ = -0.008, P = 0.959 and ρ = 0.118, P = 0.393, respectively).

4. Discussion

This study confirmed that there was a significant difference in serum 25(OH)D levels between sexes, with significantly higher levels in men. Vitamin D deficiency has been reported in many countries, including Japan,[14-16] and we confirmed that it was also deficient in the participants of the present study; vitamin D insufficiency/deficiency was observed in 88.2% of the men and 97.8% of the women.

Vitamin D metabolites ($25(OH)D_3$, $25(OH)D_2$, 3-*epi*-$25(OH)D_3$, and $24,25(OH)_2D_3$) were measured, and their profiles were analyzed. The $24,25(OH)_2D$ level was markedly lower in our study, especially in women, than that reported by Kim et al.[25] Previously, Chailurkit et al. reported that the 3-*epi*-25(OH)D level was significantly higher in men.[26] We also found that $25(OH)D_3$ and $24,25(OH)_2D_3$ levels were significantly higher in men. Kobayashi et al. reported a sex difference in the $25(OH)D_3$ levels measured using HPLC, but the sex difference in the $25(OH)D_2$ levels has not been reported.[27] In contrast, Yu et al. reported that the 25(OH)D levels did not differ between sexes and that the $25(OH)D_3$ level was significantly higher in men, and the $25(OH)D_2$ level was lower in men.[23] Furthermore, the $25(OH)D_3$ level was negatively correlated with the $25(OH)D_2$ level.[23] Swanson et al. also reported a negative correlation between the $25(OH)D_3$ and $25(OH)D_2$ levels.[28] Our results showed a sex difference in the 25(OH)D levels but no significant difference in the $25(OH)D_2$ levels. Furthermore, our results differ in that we found no correlation between the $25(OH)D_3$ and $25(OH)D_2$ levels in either sex. Both Yu et al. and Swanson et al. noted the possibility that their study groups may have included vitamin D supplement users; therefore, the influence of supplements may be considered. Their results suggest that supplemental vitamin D_2 or D_3 intake may have led to a compensatory decline in vitamin D_3 or D_2, respectively.

We analyzed the profiles of $25(OH)D_3$, $25(OH)D_2$, 3-*epi*-$25(OH)D_3$, and $24,25(OH)_2D_3$ in healthy adults not taking vitamin D supplements and to clarify sex differences of each

metabolite. Although there are reports of low 25(OH)D levels and those of associations with various diseases, the vitamin 25(OH)D reference values in healthy participants have not been established. Furthermore, there are no reports on 25(OH)D_3 and 25(OH)D_2 levels in healthy subjects not taking supplements. Vitamin D_3 is mainly produced by UV in the skin. Vitamin D_2 is taken from vegetables, such as mushrooms. Both the vitamin D_3 and D_2 are transported to the liver and are metabolized to 25(OH)D_3 and 25(OH)D_2. The physiological effects of vitamin D_3 and D_2 have been thought to be equivalent. However, recent reports have revealed differences between the two vitamin D components.[20-22] These reports have shown that vitamin D_3 stays in the blood longer than vitamin D_2 and is more significant in maintaining serum 25(OH)D levels. Therefore, measuring and distinguishing between vitamin D_3 and vitamin D_2 are important in the management of vitamin D deficiency.

We found a sex difference in the 25(OH)D_3 level but not in the 25(OH)D_2 level. Additionally, we found a strong correlation between the 25(OH)D and 25(OH)D_3 levels, but not between the 25(OH)D and 25(OH)D_2 levels. Furthermore, considering that the serum 25(OH)D_2 level is less than 1/50 of the 25(OH)D_3 level, the difference in the serum 25(OH)D level between the sexes may reflect the difference in the 25(OH)D_3 level. An association between sunlight exposure and 25(OH)D_3 level has been reported.[29] Furthermore, women tend to avoid the sun's ultraviolet light more than men; this practice has been associated with vitamin D deficiency.[30] Our study supports the results of a previous study on the etiology of vitamin D deficiency in women. The limitation of this study was that the presence of the disease was determined by self-reported questionnaires. In further studies, a medical examination by a physician should be considered to exclude the possibility of inclusion of diseased participants who may have affected the results.

Conclusion

The serum levels of 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ measured by LC-MS/MS in healthy participants not taking supplements were determined. The sex differences in 25(OH)D level reflected the differences in the 25(OH)D_3 level but not in the 25(OH)D_2 level.

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Conflict of interest: Reagents for the serum 25(OH)D assay were provided by Beckman Coulter.

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Ethical approval: The study was conducted following informed consent and was approved by the Ethical Committees of Hidaka Hospital (No.122) and Gunma University School of Medicine Ethical Review Board for Medical Research Involving Human Subjects (No. HS2020-139).

Guarantor: Dr. Masami Murakami

Contributorship

Hiroki Machida contributed to the conceptualization, methodology, data collection and analysis, and writing and revision of the original draft.

Katsuhiko Tsunekawa contributed to data analysis, reviewing, and editing of the manuscript.

Koji Sakamaki contributed to data collection.

Takao Kimura contributed to reviewing and editing of the manuscript.

Yumiko Abe contributed to data analysis, reviewing, and editing of the manuscript.

Masami Murakami contributed to the conceptualization, methodology, supervision, review, and editing of the manuscript.

All authors read and approved the final manuscript.

Provenance and peer review

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Research data (data sharing and collaboration)

There were no linked research datasets for this study. The data will be made available upon request.

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Figure legends

Figure 1. Enrollment of the study participants

CLEIA, chemiluminescent enzyme immunoassay; LC-MS/MS, liquid chromatography-tandem mass spectrometry

Figure 2. Chromatograms of 25(OH)D_3, 3-*epi*-25(OH)D_3, 25(OH)D_2, and 24,25$(OH)_2D_3$; The selected reaction monitoring transitions used for each analyte were as follows: (A) 619.3 → 341.1 for 25(OH)D_3 and 3-*epi*-25(OH)D_3. (B) 631.3 → 341.1 for 25(OH)D_2. (C) 635.3 → 341.1 for 24,25$(OH)_2D_3$.

Figure 3. The mean levels of serum vitamin D metabolites in men and women. (A) 25(OH)D_3. (B) 25(OH)D_2. (C) 3-*epi*-25(OH)D_3. (D) 24,25$(OH)_2D_3$. *P* values were calculated using the Kruskal-Wallis tests with Dunn-Bonferroni post hoc tests.

Figure 4. The relationship of the 25(OH)D level with 25(OH)D_3, 25(OH)D_2, 3-epi-25(OH)D_3, and 24,25$(OH)_2D_3$ levels. (A) Association between 25(OH)D and 25(OH)D_3 levels in men. (B) Association between 25(OH)D and 25(OH)D_2 levels in men. (C) Association between 25(OH)D and 3-*epi*-25(OH)D_3 levels in men. (D) Association between 25(OH)D and 24,25$(OH)_2D_3$ levels in men. (E) Association between 25(OH)D_3 and 25(OH)D_2 levels in men. (F) Association between 25(OH)D and 25(OH)D_3 levels in women. (G) Association between 25(OH)D and 25(OH)D_2 levels in women. (H) Association between 25(OH)D and 3-*epi*-25(OH)D_3 levels in women. (I) Association between 25(OH)D and 24,25$(OH)_2D_3$ levels in women. (J) Association between 25(OH)D_3 and 25(OH)D_2 in women. Correlation coefficients were calculated using Spearman rank correlation.

Table 1. Demographic data of the participants

	5,959 participants for measured of 25(OH)D			96 participants for measured of detailed components of 25(OH)D by LC-MS/MS		
	Men (n = 3,631)	Women (n = 2,328)	*P* value	Men (n = 41)	Women (n = 55)	*P* value
Age (year)	50 (43–58)	50 (43–57)	0.050	48 (44–55)	50 (44–58)	0.543
Height (m)	1.71 (1.67–1.75)	1.58 (1.55–1.62)	<0.001	1.73 (1.69–1.74)	1.59 (1.55–1.62)	<0.001
Weight (kg)	68.6 (62.4–75.5)	53.6 (48.8–59.9)	<0.001	66.1 (61.5–72.7)	52.0 (47.1–57.4)	<0.001
BMI (kg/m^2)	23.5 (21.6–25.6)	21.4 (19.7–23.8)	<0.001	22.3 (20.9–24.5)	20.8 (18.8–22.7)	0.003
ALT (U/L)	23 (17–32)	16 (12–20)	<0.001	22 (18–25)	13 (12–18)	<0.001
Creatinine (mg/dL)	0.85 (0.77–0.93)	0.63 (0.57–0.69)	<0.001	0.85 (0.80–0.94)	0.63 (0.60–0.69)	<0.001
Ca (mg/dL)	9.4 (9.2–9.6)	9.4 (9.2–9.6)	<0.001	9.4 (9.2–9.6)	9.2 (9.1–9.4)	0.013
IP (mg/dL)	3.2 (2.9–3.5)	3.6 (3.3–3.9)	<0.001	3.3 (2.9–3.6)	3.6 (3.2–3.8)	0.006
TC (mg/dL)	205 (184–228)	210 (188–233)	<0.001	216 (195–225)	204 (182–231)	0.756

HDL-C (mg/dL)	56 (47–66)	69 (59–81)	<0.001	60 (52–70)	70 (60–78)	0.008
LDL-C (mg/dL)	122 (101–143)	115 (95–138)	<0.001	124 (107–138)	113 (87–134)	0.120
HbA1c (%)	5.7 (5.5–6.0)	5.7 (5.5–5.9)	0.001	5.6 (5.4–5.8)	5.6 (5.4–5.8)	0.908
25(OH)D (ng/mL)	21.5 (17.5–26.0)	17.2 (14.2–20.7)	<0.001	22.3 (19.2–26.6)	17.9 (13.8–22.4)	<0.001

Data are expressed as medians (25th–75th percentile).

LC-MS/MS, liquid chromatography-tandem mass spectrometry; BMI, body mass index; ALT, alanine transaminase; Ca, serum calcium; IP, serum inorganic phosphorus; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, hemoglobin A1c; 25(OH)D, 25-hydroxyvitamin D

Table 2. Percentile values of 25(OH)D deficiency, insufficiency, and sufficiency defined by serum 25(OH)D level in men and women

	Serum 25(OH)D levels (ng/mL)		
	<20.0 (deficiency)	20.0≤25(OH)D<30.0 (insufficiency)	≥30.0 (sufficiency)
Men (n = 3,631)	1441 (39.7%)	1762 (48.5%)	428 (11.8%)
Women (n = 2,328)	1635 (70.2%)	641 (27.5%)	52 (2.2%)

Deficiency was defined as a serum 25(OH)D level <20 ng/mL, insufficiency was defined as a serum 25(OH)D level ≥20 ng/mL and <30 ng/mL, sufficiency was defined as a serum 25(OH)D level ≥30 ng/mL according to Assessment criteria for vitamin D deficiency/insufficiency in Japan.[10]

25(OH)D, 25-hydroxyvitamin D

Figure 1.

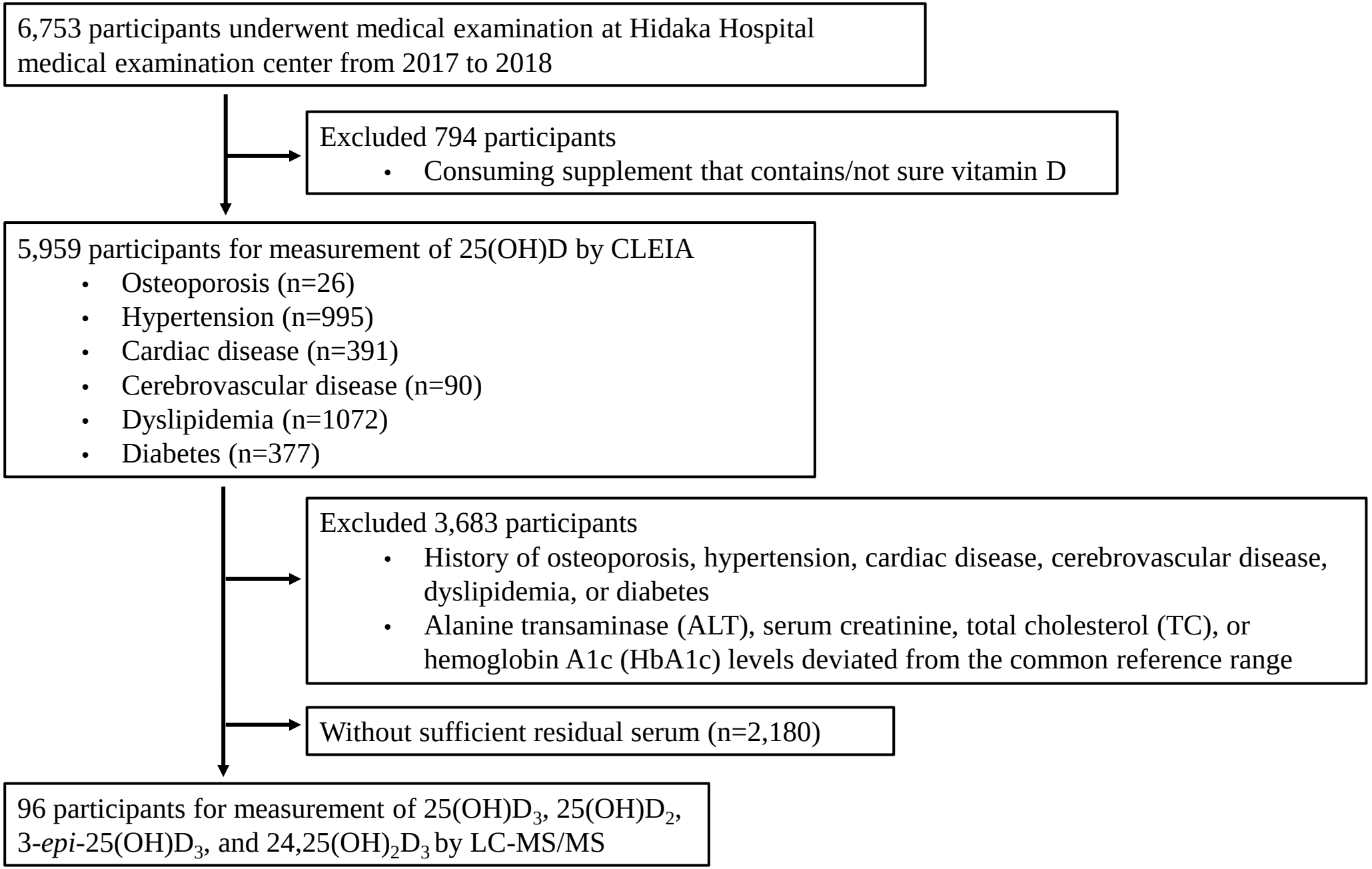

6,753 participants underwent medical examination at Hidaka Hospital medical examination center from 2017 to 2018
Excluded 794 participants
• Consuming supplement that contains/not sure vitamin D
5,959 participants for measurement of 25(OH)D by CLEIA
• Osteoporosis (n=26)
• Hypertension (n=995)
• Cardiac disease (n=391)
• Cerebrovascular disease (n=90)
• Dyslipidemia (n=1072)
• Diabetes (n=377)
Excluded 3,683 participants
• History of osteoporosis, hypertension, cardiac disease, cerebrovascular disease, dyslipidemia, or diabetes
• Alanine transaminase (ALT), serum creatinine, total cholesterol (TC), or hemoglobin A1c (HbA1c) levels deviated from the common reference range
Without sufficient residual serum (n=2,180)
96 participants for measurement of 25(OH)D_3, 25(OH)D_2, 3-*epi*-25(OH)D_3, and 24,25$(OH)_2D_3$ by LC-MS/MS

Figure 2.

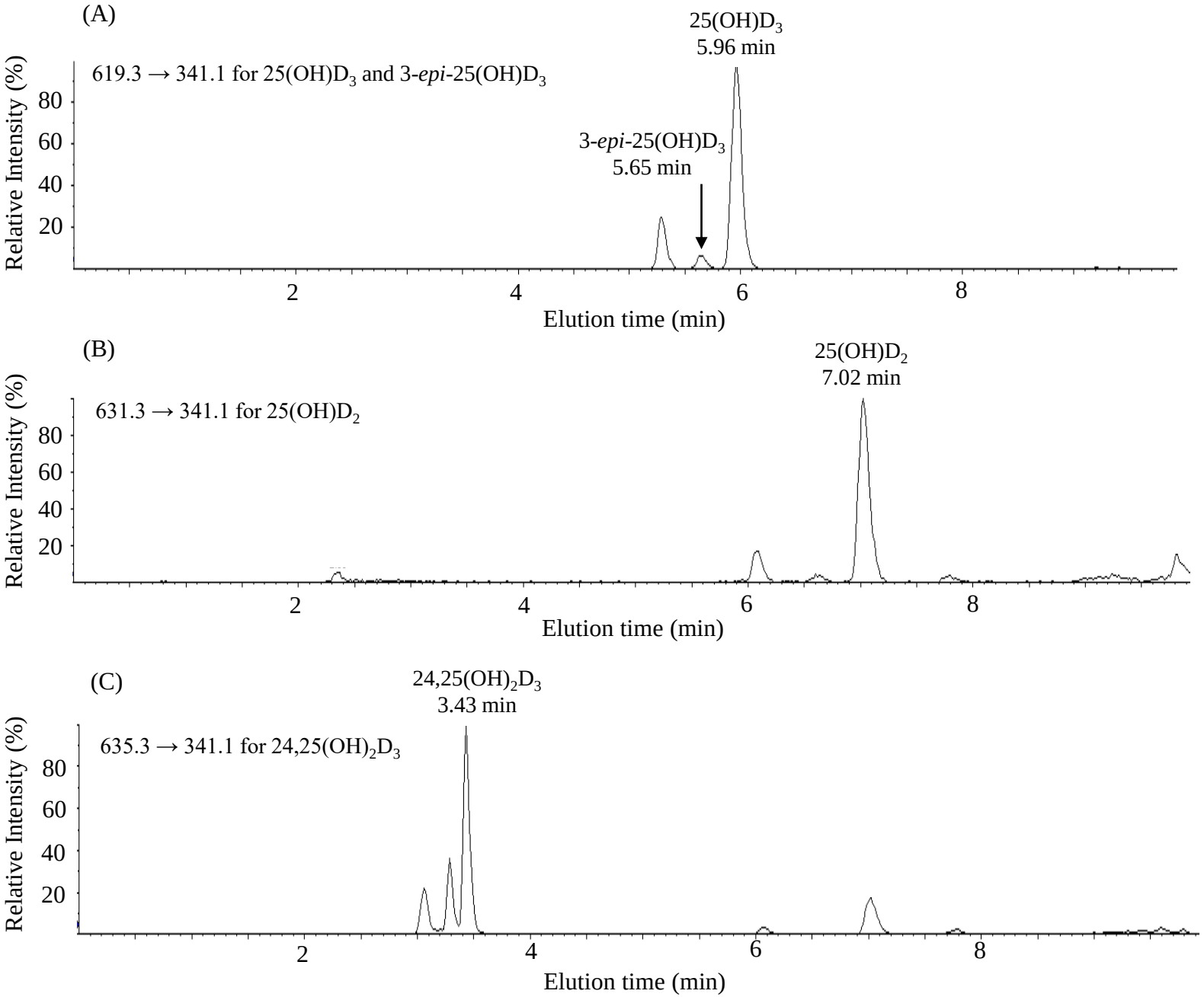

(A)
25(OH)D3
5.96 min
619.3 → 341.1 for 25(OH)D3 and 3-epi-25(OH)D3
3-epi-25(OH)D3
5.65 min
Relative Intensity (%)
Elution time (min)
(B)
25(OH)D2
7.02 min
631.3 → 341.1 for 25(OH)D2
Relative Intensity (%)
Elution time (min)
(C)
24,25(OH)2D3
3.43 min
635.3 → 341.1 for 24,25(OH)2D3
Relative Intensity (%)
Elution time (min)

Figure 3.

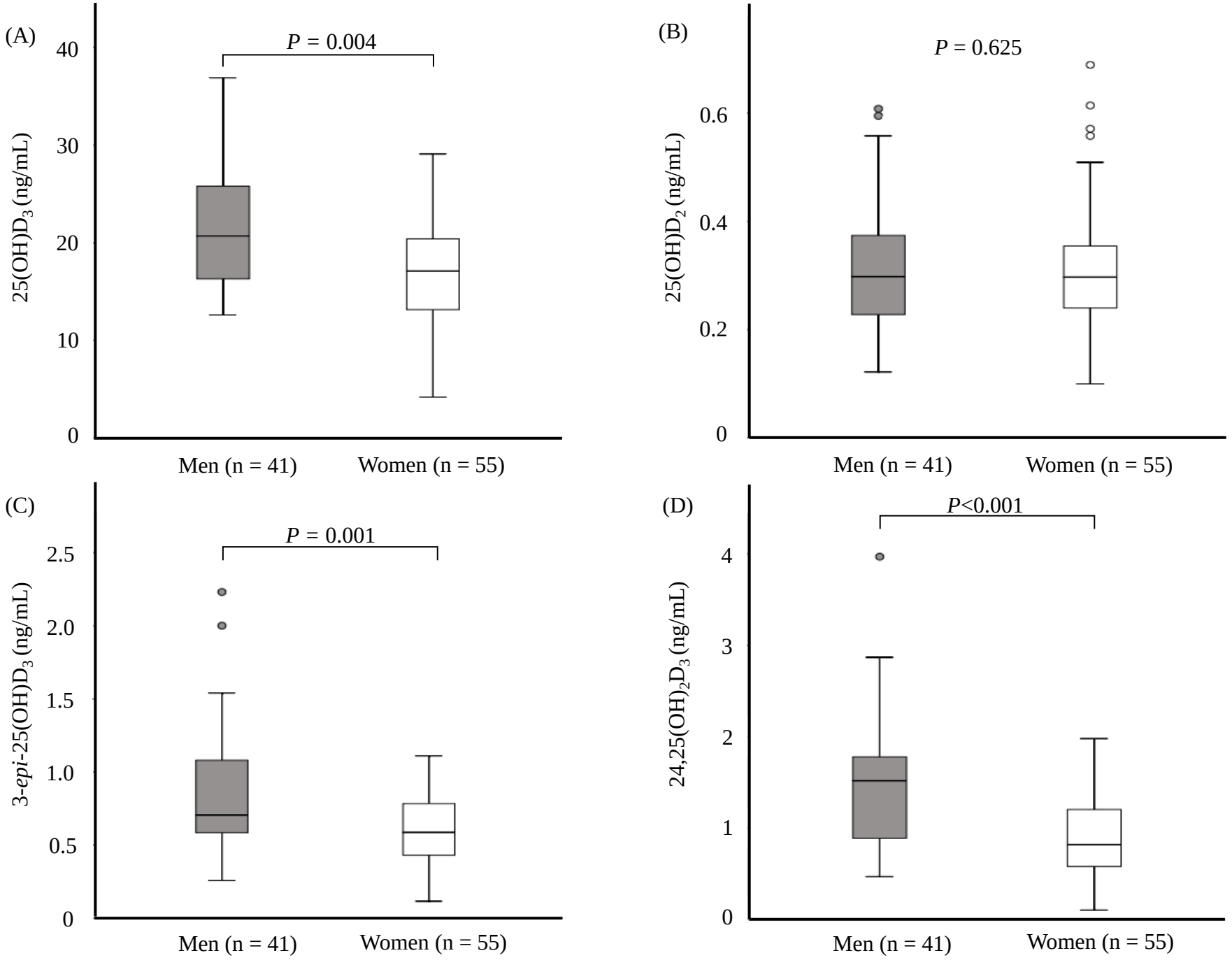
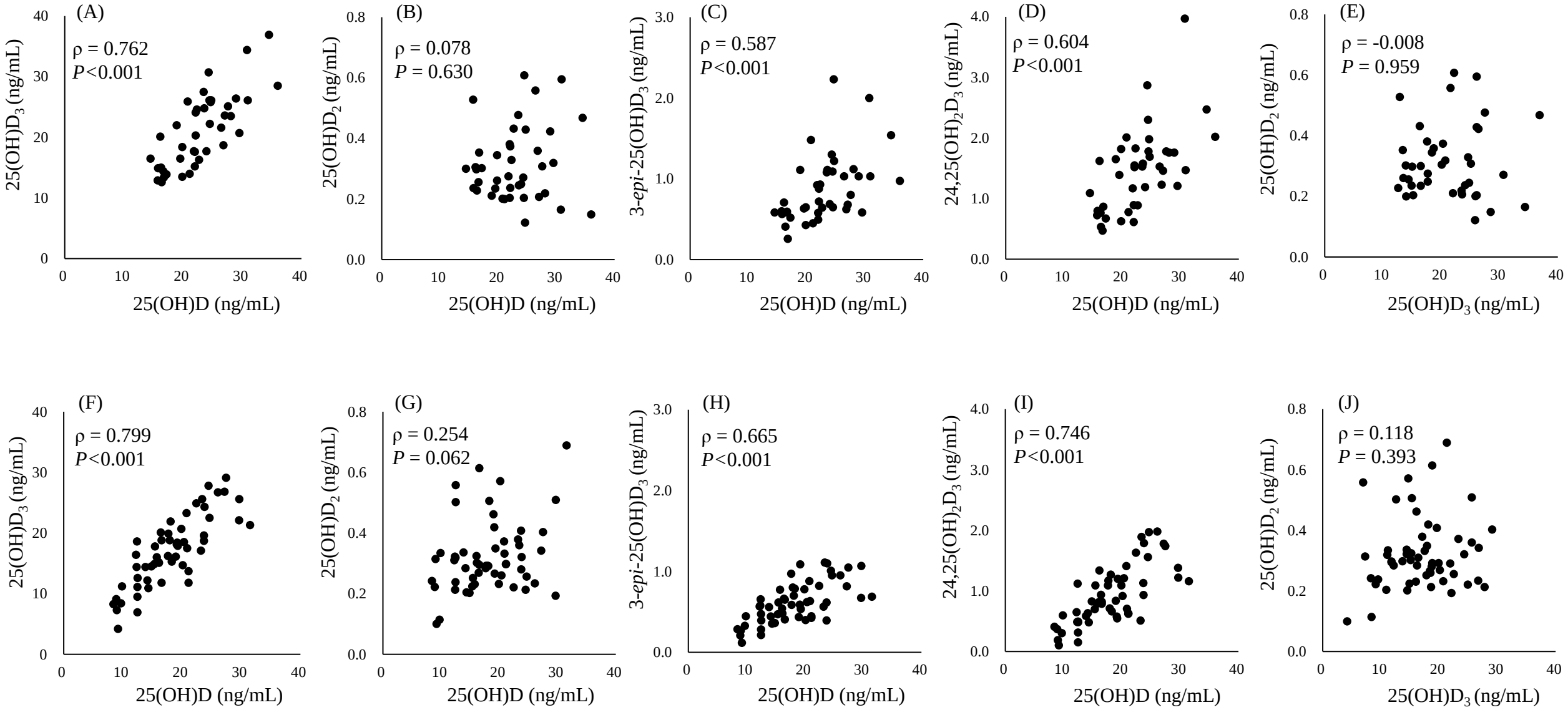

(A)
P = 0.004
25(OH)D3 (ng/mL)
40
30
20
10
0
Men (n = 41)
Women (n = 55)
(B)
P = 0.625
25(OH)D2 (ng/mL)
0.6
0.4
0.2
0
Men (n = 41)
Women (n = 55)
(C)
P = 0.001
3-epi-25(OH)D3 (ng/mL)
2.5
2.0
1.5
1.0
0.5
0
Men (n = 41)
Women (n = 55)
(D)
P<0.001
24,25(OH)2D3 (ng/mL)
4
3
2
1
0
Men (n = 41)
Women (n = 55)

Figure 4.


(A)
ρ = 0.762
P<0.001
25(OH)D3 (ng/mL)
25(OH)D (ng/mL)
(B)
ρ = 0.078
P = 0.630
25(OH)D2 (ng/mL)
25(OH)D (ng/mL)
(C)
ρ = 0.587
P<0.001
3-epi-25(OH)D3 (ng/mL)
25(OH)D (ng/mL)
(D)
ρ = 0.604
P<0.001
24,25(OH)2D3 (ng/mL)
25(OH)D (ng/mL)
(E)
ρ = -0.008
P = 0.959
25(OH)D2 (ng/mL)
25(OH)D3 (ng/mL)
(F)
ρ = 0.799
P<0.001
25(OH)D3 (ng/mL)
25(OH)D (ng/mL)
(G)
ρ = 0.254
P = 0.062
25(OH)D2 (ng/mL)
25(OH)D (ng/mL)
(H)
ρ = 0.665
P<0.001
3-epi-25(OH)D3 (ng/mL)
25(OH)D (ng/mL)
(I)
ρ = 0.746
P<0.001
24,25(OH)2D3 (ng/mL)
25(OH)D (ng/mL)
(J)
ρ = 0.118
P = 0.393
25(OH)D2 (ng/mL)
25(OH)D3 (ng/mL)