Changes in Serum 25 Hydroxyvitamin D Concentrations in Male University Long-Distance Runners Following Vitamin D3 Supplementation During Winter: A Randomized Controlled Trial

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Abstract

Background

Vitamin D insufficiency and deficiency remain a global problem, even among athletes. In this study, we evaluated the serum 25 hydroxyvitamin D [25 (OH) D] concentrations of male long-distance runners during winter and the effect of short-term vitamin D_3 supplementation to elucidate their dietary vitamin D requirements.

Methods

Using double-blind randomization, 34 athletes on the "Ekiden" (long-distance road race) team at University A were divided into a vitamin D group (n = 17, 25 μg/day of vitamin D_3 intake) and a placebo group (n = 17, placebo tablet intake). Both groups ate normal meals with either the supplement or placebo included, and all subjects underwent a 31-day dietary survey. The pre- and post-intervention body composition and blood test were measured and compared.

Results

Prior to intervention, the vitamin D intake amount was 16.7 ± 7.2 μg/day, but the serum 25 (OH) D concentration was 28.9 ± 5.7 ng/mL, and 22 (65%) of the 34 subjects had insufficient vitamin D (<30 ng/mL). During the intervention period, the mean amount of vitamin D intake was 40.0 ± 5.5 μg/day in the vitamin D group and 13.4 ± 7.7 μg/day in the placebo group. Serum 25 (OH) D concentrations increased from 30.7 ± 6.7 ng/mL to 35.4 ± 6.6 ng/mL in the vitamin D group and from 27.1 ± 3.9 ng/mL to 28.5 ± 4.4 ng/mL in the placebo group, respectively ($p < 0.001$, $p < 0.01$). After intervention, the serum 25 (OH) D concentration in the vitamin D group was significantly higher ($p < 0.001$). Additionally, the rate of vitamin D sufficiency was 82% in the vitamin D group and 43% in the placebo group, indicating a significant difference ($p < 0.01$).

Conclusions

Our results demonstrate that post-intervention serum 25 (OH) D concentration was related to both mean vitamin D intake amount during the intervention period and pre-intervention serum 25 (OH) D concentration. However, to better quantify the required amount of vitamin D, further study of the effect of vitamin D supplementation on bone health is needed.

Background

Vitamin D is involved in both bone and mineral metabolism. In addition to promoting the absorption of phosphorus and calcium, which are its main physiological actions, it is also involved in immune functions [1] and skeletal muscle functions [2]. Vitamin D is found in foods, such as fish and mushrooms, and is produced in the skin as a result of ultraviolet irradiation. After metabolic conversion in the liver to 25 hydroxyvitamin D [25 (OH) D], it binds to vitamin D binding protein (DBP) and then circulates via the bloodstream. Because of 25 (OH) D's relatively long half-life of approximately 3 weeks, vitamin D nutritional status is used as an index to determine the amount of vitamin D that an individual obtains from their diet and skin [3]. It has been previously reported that low 25 (OH) D levels are related to decreased intake of vitamin D and fish [4], and these decreased

25 (OH) D levels increase the levels of serum parathyroid hormone (PTH), which promotes bone resorption, thereby increasing the risk of reduced bone density and fractures [5, 6].

In recent years, vitamin D insufficiency and deficiency have become a global problem [7], and it has been reported that these conditions also exist among athletes engaged in various sports [8–11]. Low 25 (OH) D concentrations, indicative of vitamin D insufficiency and deficiency, are frequently noted among indoor athletes who have little exposure to ultraviolet light during the winter months [10, 11]. This is related to the risk of fatigue fracture [12–14], and vitamin D and calcium (Ca) intake are important to bone health and the prevention of stress fractures among athletes [15–17]. In recent years, studies have reported that vitamin D and Ca intake promote muscle protein synthesis [18], reduce the occurrence of upper respiratory tract infections [19], and improve aerobic capacity [20].

Dietary Reference Intake guides in the United States and Canada [21] established a serum 25 (OH) D concentration level that does not increase the risk of fracture and recommend a vitamin D intake of 15 μg/day for individuals aged ≤ 70 years. Alternatively, the Dietary Reference Intake for Japanese (2020 edition) [22] has formulated an estimated amount of 8.5 μg/ day for individuals aged 18–29 years. However, this value is based on the median intake of healthy people; the amount required differs per individual and is influenced by individual vitamin D intake, latitude, season, outdoor activity amount, and the presence or absence of sunscreen use, which affects the amount of vitamin D produced by the skin.

Thus, the objective of the present study was to determine winter serum 25 (OH) D concentrations in athletes and to investigate changes in the serum 25 (OH) D concentration that occur as a result of vitamin D_3 intervention to elucidate the amount of vitamin D required in male long-distance runners.

Methods

Subjects and study period

From an initial candidate pool of 43 male runners on the “Ekiden” (long-distance road race) team at University A, 34 athletes, who consented to participate in the study and for whom all relevant data was available, were included in this study. The subjects were athletes who engaged in training mainly in long-distance running outdoors six days per week. Using a random number table, double-blind randomization was performed, and the subjects were categorized into a group that ingested vitamin D_3 supplement tablets (25 μg/day: vitamin D group) and a group that ingested placebo tablets (placebo group). Both groups ate normal meals with either the supplement or the placebo included. Those who did not participate in training owing to injury or physical deconditioning and those who were taking calcium and vitamin D supplements were excluded from the study. The intake rate of the test foods distributed to the subjects was confirmed using a daily survey, which showed that the intake rate during the survey period was 100%.

The intervention period was a 31-day period between January 25 and February 23, 2019. This study was approved by the Institutional Review Board of Teikyo University (no. 18-155), and all subjects provided informed written consent.

Measured items and measurement methods

Bone mineral content (BMC) and bone mineral density (BMD) were measured using dual-energy X-ray absorptiometry (Horizon, TOYO MEDIC, Tokyo). Weight, body fat percentage, and minerals content were measured both prior to and following the intervention using 8-electrode multi-frequency impedance (InBody770, InBody Japan, Tokyo). Body mass index (BMI) was calculated based on body height and weight. Body height, BMC, and BMD were measured prior to intervention only.

Pre- and post-intervention fasting blood tests were obtained to measure serum 25 (OH) D concentration (CLEIA method), serum intact PTH (ECLIA method), serum calcium concentration (Ca, Arsenazo III method), serum inorganic phosphorus concentration (P, direct Molybdate method), and serum interleukin-6 (IL-6, CLEIA method). Blood tests were performed under contract by SRL Tokyo Medical (Tokyo). Serum 25 (OH) D levels of ≥30 ng/mL were considered to indicate sufficient vitamin D nutritional status [23], whereas those <30 ng/mL indicated insufficient vitamin D.

During the 31-day intervention period of this study, food and drink intake were monitored using a diet record and photographs of the foods and drinks consumed. Excel Eiyo-kun software (Ver.8.0, Kenpakusha, Tokyo) was used to calculate the caloric intake as well as the mean protein, fat, carbohydrate, vitamin D, and calcium intake amounts.

Statistical analysis

All measured values are shown as the mean ± standard deviation. The Shapiro–Wilk test was used to test the normality of all the variables. Pre- and post-intervention comparisons were performed using the paired *t*-test. Comparisons of the vitamin D and placebo groups were performed under the assumption of post-F-test homogeneity of variance and using the student's *t*-test. Comparisons of the vitamin D nutritional status between groups were performed using the chi-squared test. Simple regression analysis with post-intervention serum 25 (OH) D concentration as the dependent variable and all factors as the independent variables was performed to analyze the relationship between post-intervention serum 25 (OH) D concentration and the amounts of both energy intake and nutrient intake during the intervention period. Investigation of the factors that contribute to post-intervention serum 25 (OH) D concentration was performed using multiple regression analysis (stepwise) with post-intervention serum 25 (OH) D concentration as the dependent variable and pre-intervention serum 25 (OH) D concentration, vitamin D intake amount, and calcium intake amount as the independent variables. Analysis of variance (ANOVA) was used to test whether the multiple regression was significantly established.

The relationship between the rate of change in the pre- and post-intervention serum 25 (OH) D concentrations and all other factors was analyzed using Pearson's product moment correlation coefficient. All statistical analyses were performed using SPSS Statistic ver.23.0 (IBM, New York, US). All tests were two-sided tests, and the standard of statistical significance was set at 5%.

Results

Pre-intervention subject characteristics

The subjects' mean age was 19.8 ± 0.9 years, the whole body BMC was 2303 ± 211 g, the whole body bone density was 1.126 ± 0.058 g/cm^2, and the young adult mean (YAM) of the whole body bone density was 96.6 ± 5.5%. The pre-intervention vitamin D intake amount was 16.7 ± 7.2 µg/day, and 31 subjects (91%) had intake amounts that exceeded the target amount. The serum 25 (OH) D concentration was 28.9 ± 5.7 ng/mL, with 12 subjects (35%) showing sufficient vitamin D (≥ 30 ng/mL) and 22 (65%) showing insufficient vitamin D (< 30 ng/mL). There were no significant differences between the vitamin D group and the placebo group regarding age, body height, weight, body fat percentage, BMC, BMD, YAM value, blood indices, energy, or nutritional intake amounts (Table 1).

Table 1
Pre-intervention subject characteristics

		All (n = 34)			Vitamin D group (n = 17)			Placebo group (n = 17)			*P* value
Age	(yrs)	19.8	±	0.9	20.0	±	0.8	19.5	±	0.9	n.s.
Body height	(cm)	171.0	±	5.0	171.0	±	5.0	170.9	±	5.1	n.s.
Weight	(kg)	55.7	±	4.8	54.5	±	5.4	56.8	±	4.0	n.s.
Body fat percentage	(%)	11.5	±	2.1	11.2	±	1.5	11.9	±	2.6	n.s.
Minerals contents	(g)	2,888	±	298	2,840	±	347	2,935	±	240	n.s.
Whole body bone mineral content	(g)	2,303	±	211	2,257	±	235	2,348	±	179	n.s.
Whole body bone density	(g/cm^2)	1.126	±	0.058	1.122	±	0.067	1.130	±	0.050	n.s.
YAM	(%)	96.6	±	5.6	96.8	±	6.0	96.4	±	5.4	n.s.
Serum 25(OH)D concentration	(ng/mL)	28.9	±	5.7	30.7	±	6.7	27.1	±	3.9	n.s.
Serum intact PTH concentration	(pg/mL)	26	±	15	24	±	8	28	±	6	n.s.
Serum calcium concentration	(mg/dL)	9.6	±	0.3	9.8	±	0.3	9.6	±	0.2	n.s.
Serum inorganic Phosphorus concentration	(mg/dL)	3.6	±	0.3	3.6	±	0.3	3.6	±	0.3	n.s.
Serum interleukin-6 concentration	(pg/mL)	0.76	±	0.68	0.68	±	0.29	0.83	±	0.93	n.s.
Energy intake	(kcal/day)	3,075	±	479	3,152	±	569.0	3,012	±	396	n.s.
Protein intake	(g/day)	119.5	±	25.6	124.2	±	29.4	115.6	±	22.1	n.s.
Fat intake	(g/day)	83.0	±	23.8	85.7	±	26.3	80.8	±	22.0	n.s.
Carbohydrate intake	(g/day)	449.4	±	79.0	458.1	±	88.7	442.3	±	71.6	n.s.
Vitamin D intake	(μg/day)	16.7	±	7.2	18.6	±	8.6	15.1	±	5.6	n.s.

		All (n = 34)			Vitamin D group (n = 17)			Placebo group (n = 17)			*P* value
Calcium intake	(mg/day)	859	±	478	964	±	586	773	±	363	n.s.
mean ± SD											
YAM: Young Adult Mean											
Serum 25(OH)D: Serum 25 hydroxyvitamin D											
Serum intact PTH: Serum parathyroid hormone											
Pre- vs. post-intervention comparisons: Paired t-test											
Vitamin D group and placebo group comparisons: Student’s t-test after F-test ($p > 0.05$)											
n.s. : not significant											

The results of the chi-squared test showed that there were no significant differences between the two groups in terms of the percentages of vitamin D sufficiency and insufficiency ($p = 0.14$). The percentages of vitamin D sufficiency were 47% in the vitamin D group and 53% in the placebo group.

Mean energy intake amount and nutritional intake amount during the intervention period

During the intervention period, the mean vitamin D intake amount in the vitamin D group was 40.0 ± 5.5 μg/day, of which 15.0 ± 5.5 μg/day was derived from the diet, whereas the mean vitamin D intake amount in the placebo group was 13.4 ± 7.7 μg/day. No significant difference was found in the mean vitamin D intake amounts between the two groups (Table 2). There were also no significant intergroup differences in the mean energy, protein, fat, carbohydrate, and calcium intake amounts.

Table 2
Mean energy intake and nutrient intake amounts during the intervention period

		Vitamin D group (n = 17)			**Placebo group (n = 17)**			***P* value**
Energy	(kcal/day)	3,076	±	342	2,987	±	228	n.s.
Protein	(g/day)	121.1	±	13.9	115.3	±	11.7	n.s.
Fat	(g/day)	91.9	±	11.6	89.4	±	9.4	n.s.
Carbohydrates	(g/day)	427.0	±	47.7	419.9	±	32.2	n.s.
Vitamin D (meals only)	(μg/day)	15.0	±	5.5	13.4	±	7.7	n.s.
Total vitamin D (meals+vitamin D_3 supplement)	(μg/day)	40.0	±	5.5	13.4	±	7.7	< 0.001
Calcium	(mg/day)	1,035	±	284	898	±	142	n.s.
mean ± SD								
Vitamin D group and placebo group comparisons: Student's t-test after F-test ($p > 0.05$)								
n.s., not significant								

Relationship between post-intervention serum 25 (OH) D concentration and the mean energy and nutrient intake amounts during the intervention period

The simple regression analysis' results showed that the post-intervention serum 25 (OH) D concentration exhibited a significant correlation with the mean intake amounts of vitamin D ($r = 0.54$, $p = 0.001$) and calcium ($r = 0.34$, $p < 0.05$).

Furthermore, the multiple regression analysis demonstrated that both the mean vitamin D intake amount ($p < 0.001$) and pre-intervention serum 25 (OH) D concentration ($p < 0.001$) significantly contributed to the post-intervention serum 25 (OH) D concentration (Table 3).

Table 3
Factors that contribute to the post-intervention serum 25 (OH) D concentration

		Partial regression coefficient	Standard partial regression coefficient	Standard coefficient β	*P* value	95%CI	
						MIN	MAX
Constant		5.919	2.653		0.03	0.501	11.337
Mean vitamin D intake amount	(μg/day)	0.146	0.037	0.332	< 0.001	0.071	0.221
Pre-intervention serum 25 (OH) D concentration	(ng/mL)	0.989	.097	0.857	< 0.001	0.790	1.187

n = 34

R^2 = 0.844, ANOVA < 0.001

Multiple regression (stepwise) analysis was performed with the post-intervention 25 (OH) D concentration as the dependent variable and the pre-intervention serum 25 (OH) D concentration, mean vitamin D intake amount and mean calcium intake amount as the independent variables.

Serum 25(OH)D: Serum 25 hydroxyvitamin D

Changes in pre- vs. post-intervention in the vitamin D group and the placebo group

There was a significant decrease in body fat percentage in both groups; the value in the vitamin D group decreased from 11.2 ± 1.5% to 10.5 ± 1.6% and that in the placebo group decreased from 11.9 ± 2.6% to 10.5 ± 2.5% ($p < 0.01$ and $p < 0.001$, respectively). In contrast, the mineral contents showed a significant increase in both groups; the value in the vitamin D group increased from 2840 ± 347 g to 2891 ± 338 g and that in the placebo group increased from 2935 ± 240 g to 3000 ± 222 g ($p < 0.05$ and $p < 0.01$, respectively). The serum 25 (OH) D concentration showed a significant increase in both groups, with the value in the vitamin D group increasing from 30.7 ± 6.7 ng/mL to 35.4 ± 6.6 ng/mL and that in the placebo group increasing from 27.1 ± 3.9 ng/mL to 28.5 ± 4.4 ng/mL ($p < 0.001$ and $p < 0.01$, respectively). In the vitamin D group, the amount of increase in serum 25 (OH) D per 1 μg vitamin D_3 was 0.19 ± 0.15 μg. The serum Ca concentration showed a significant decline only in the placebo group, from 9.6 ± 0.2 mg/dL to 9.3 ± 0.2 mg/dL ($p < 0.001$), whereas the serum IL-6 concentrations showed a significant decline in the vitamin D group only, from 0.68 ± 0.29 pg/mL ($p < 0.01$). Weight, serum intact PTH concentration, and serum P concentration exhibited no pre- vs. post-intervention intergroup differences.

The analysis of variance results showed that the serum 25 (OH) D and serum Ca concentrations were significantly higher ($p < 0.001$ and $p < 0.05$, respectively,) and the serum intact PTH concentration was significantly lower ($p < 0.05$, Fig. 1) in the vitamin D group than in the placebo group. The percentage of sufficient vitamin D was 82% in the vitamin D group and 43% in the placebo group, showing a significant intergroup difference ($p < 0.01$).

Relationship between the rate of pre- vs. post-intervention change in serum 25 (OH) D concentration

The rate of change in the pre- vs. post-intervention serum 25 (OH) D concentrations was found to be positively correlated to the mean vitamin D intake amounts ($r = 0.423$, $p < 0.05$; Fig. 2). No significant relation was found with mean energy and other nutrients' intake amounts. Furthermore, investigation of the rate of change between the pre- vs. post-intervention serum 25 (OH) D concentrations in the vitamin D group exhibited a negative correlation with the pre-intervention serum 25 (OH) D concentration ($r = -0.485$, $p < 0.05$; Fig. 3).

Discussion

In this study, we investigated changes in the winter-time serum 25 (OH) D concentrations of university long-distance runners following vitamin D_3 supplementation. The subjects were athletes who engaged in training mainly in long-distance running outdoors six days per week.

Long-distance running places a repeated weight load on the bones, and it is associated with a high prevalence of fatigue fracture caused by local decreases in bone mass, resulting from the bone's inability to sufficiently repair itself [24]. Previous studies have reported on the control of decreases in bone mass and density, which are associated with long-distance running, high bone turnover, and inhibited bone formation owing to increased PTH [25–27]. This indicates that a nutritional approach designed to ensure preventative bone health is required. Warden et al. [28] reported that calcium and vitamin D are required to prevent stress fractures in long-distance runners, and a study by Lappe et al. [15] that focused on new military recruits found that calcium and vitamin D supplements had a suppressive effect on fatigue fracture onset. Of these two nutrients, vitamin D insufficiency and deficiency are particularly problematic in Japan.

The bone density of the subjects in the present study was 100% below YAM, which––as Fredericson et al. [29] reported––indicates low bone density. This result is indicative of athletes involved in long-distance running. The pre-intervention vitamin D intake amount was 16.7 ± 7.2 μg/day, which exceeded the target amount, but the vitamin D nutritional status index––serum 25 (OH) D concentration––was 28.9 ± 5.7 ng/mL, and the high percentage of subjects with vitamin D insufficiency (65%) suggests that the target amount currently in use is insufficient. Serum 25 (OH) D concentration is affected by ultraviolet light irradiation; it displays seasonal variations, with lower levels during the winter than in summer [10, 11]. This suggests a need to review vitamin D intake amounts in individuals who engage in outdoor activities to ensure that they obtain sufficient amounts of vitamin D.

Holick et al. [30] reported that individuals with vitamin D insufficiency or deficiency require 37.5–50 μg of vitamin D supplementation to maintain a serum 25 (OH) D concentration of at least 30 ng/mL. In their vitamin D interventional study of underwater patrol staff (divers) receiving limited amounts of ultraviolet light, Gasier et al. [31] reported that their serum 25 (OH) D concentration improved following supplementation with 50 μg/day of vitamin D over a 12-week period, and significant effectiveness was not obtained through the use of < 50 μg/day. In the present study, the subjects in the vitamin D group obtained an average of 40 μg/day of vitamin D during the intervention period from their normal diet and 25 μg/day of vitamin D supplement, and the percentage of vitamin D sufficiency in that group increased considerably from 47–82%. In contrast, investigation of the mean vitamin D intake during the intervention period in the placebo group showed that the percentage of vitamin D sufficiency declined from 53–43% over the course of the study. Our post-intervention comparison of the two groups showed that the vitamin D group had a lower serum intact PTH concentration and a higher serum Ca concentration than the placebo group. Comparison of the vitamin D and placebo groups also suggested that although vitamin D supplementation resulted in improved vitamin D nutritional status in the vitamin D group, improvement in the vitamin D nutritional status in the placebo group did not lead to sufficient improvement in vitamin D nutritional status.

There was no significant difference in the mineral contents between the two groups; however, the concentration of serum IL-6 showed a significant decline in the vitamin D group only. Vitamin D is also known to play a role in the regulation of inflammatory cytokines, such as down-regulating the expression of TNF-alpha and IL-6 in the general population [32]. Although few studies have described this relationship in athletes, training load can increase inflammatory cytokines (TNF-alpha and IL-6) and inhibit bone formation. Thus, further investigation is needed regarding the effects of sufficient vitamin D supplementation on reduced risk of disability.

Vitamin D and calcium supplements resulted in an increased serum calcium concentration and control of PTH activation, which supports bone health [33]. Calcium and vitamin D intake is effective in the prevention of fatigue fracture in athletes [15]. In their randomized controlled trial, Gaffney-Stomberg et al. [34] also found that these supplements contributed to the improvement in bone status under conditions wherein the risk of fatigue fracture is elevated owing to increased serum PTH levels during training with repeated mechanical loads. In the current study, the mean calcium intake amounts in both groups during the intervention period exceeded the recommended amount. Thus, the significant difference between the groups at the post-intervention time point was likely owing to the effects of the amounts of vitamin D intake.

Vitamin D supplementation is believed to be effective on serum 25 (OH) D concentration during the winter months, when the baseline serum 25 (OH) D level is low and ultraviolet light is less plentiful [35]. In the present study, the mean vitamin D intake amount was positively correlated to the amount of change in the serum 25 (OH) D concentration, and it was dose responsive. In addition, in the vitamin D group, the pre-intervention serum 25 (OH) D concentration was found to be negatively correlated to the rate of change in the serum 25 (OH) D concentration. Multiple regression analysis showed that the post-intervention serum 25 (OH) D concentration was related to the mean vitamin D intake amount during the intervention period and the pre-intervention serum 25 (OH) D concentration. These results suggest that the effectiveness of vitamin D supplementation using pre-intervention serum 25 (OH) D concentrations also needs to be investigated in the subjects of this study.

In the vitamin D group, the amount of increase in serum 25 (OH) D concentration per 1 μg of vitamin D_3 was less than has been reported previously [36]. This is likely owing to the fact that the subjects of the present study underwent nutritional education regarding vitamin D intake for the purpose of maintaining daily bone health and were provided a diet fortified with vitamin D, which implies that their pre-intervention serum 25 (OH) D concentration was higher than that of previous studies.

We did not assess bone status or the risk of fatigue fracture resulting from vitamin D_3 intervention, which was one of the limitations of the present study. Although we measured BMC and bone density prior to intervention, because the intervention period was short, at only 31 days, it was impossible to measure post-intervention values; therefore, we were unable to investigate their relation with vitamin D supplement intake. Thus, to identify the required amount of vitamin D intake in individuals with a high rate of fatigue fracture, such as the long-distance runners in the present study, further study of the effectiveness of vitamin D supplementation on bone health is required.

Another limitation of this study was the fact that we were unable to identify the effect of the subjects’ low body fat percentage on serum 25 (OH) D concentration. Because serum 25 (OH) D concentration is negatively correlated to body fat amount [37], it is likely that the low body fat percentage of the subjects in this study increased the response to the blood concentration based on the amount of vitamin D intake. Therefore, there is a need to investigate the body compositions of athletes engaged in different sports to verify that changes in serum 25 (OH) D concentration were the result of vitamin D supplement intake.

Conclusions

Male university long-distance runners who suffered from vitamin D insufficiency showed a significant increase in serum 25 (OH) D concentration and improvement in their vitamin D nutritional status after receiving 25 μg/day vitamin D_3 supplement for a 31-day period during winter. Our results demonstrate that post-intervention serum 25 (OH) D concentration was related to both mean vitamin D intake amount during the intervention period and pre-intervention serum 25 (OH) D concentration. However, to better quantify the required amount of vitamin D, further study of the effect of vitamin D supplementation on bone health is needed.

Abbreviations

25 (OH) D 25 hydroxyvitamin D

DBP Vitamin D binding protein

Ca Calcium

BMC Bone mineral content

BMD Bone mineral density

BMI Body Mass Index

YAM Young adult mean

P Phosphorus

PTH Parathyroid hormone

IL-6 Interleukin-6

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Review Board of Teikyo University (no. 18-155), and all subjects provided informed written consent.

Consent for publication

All participants provided written informed consent for publication of the study.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

MT, and MK collected the data. MT, MK conceived the study and analyzed the results. TN, and JS supervised the study and prepared the manuscript. All authors read and approved the final manuscript.

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Figures

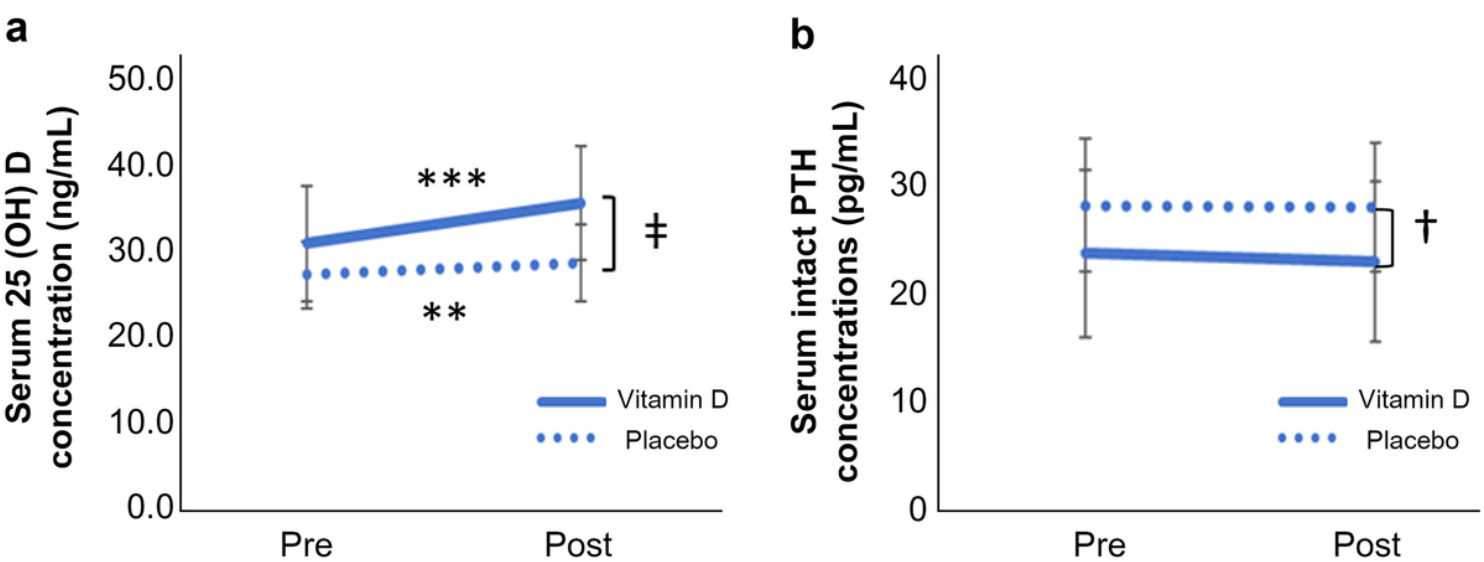

Figure 1

Changes in serum 25 (OH) D and serum intact PTH concentrations a indicates the pre- vs. post-intervention serum 25 (OH) D concentration, and b indicates the pre- vs. post-intervention serum intact PTH concentration.

Comparison of the post-intervention serum 25 (OH) D and serum intact PTH concentrations in the vitamin D and placebo groups to the pre-intervention values was performed using analysis of variance with covariates. †: $p < 0.05$, ‡: $p < 0.001$. Comparisons of pre- vs. post-intervention values in both groups were performed using the paired t-test. **: $p < 0.01$, ***: $p < 0.001$

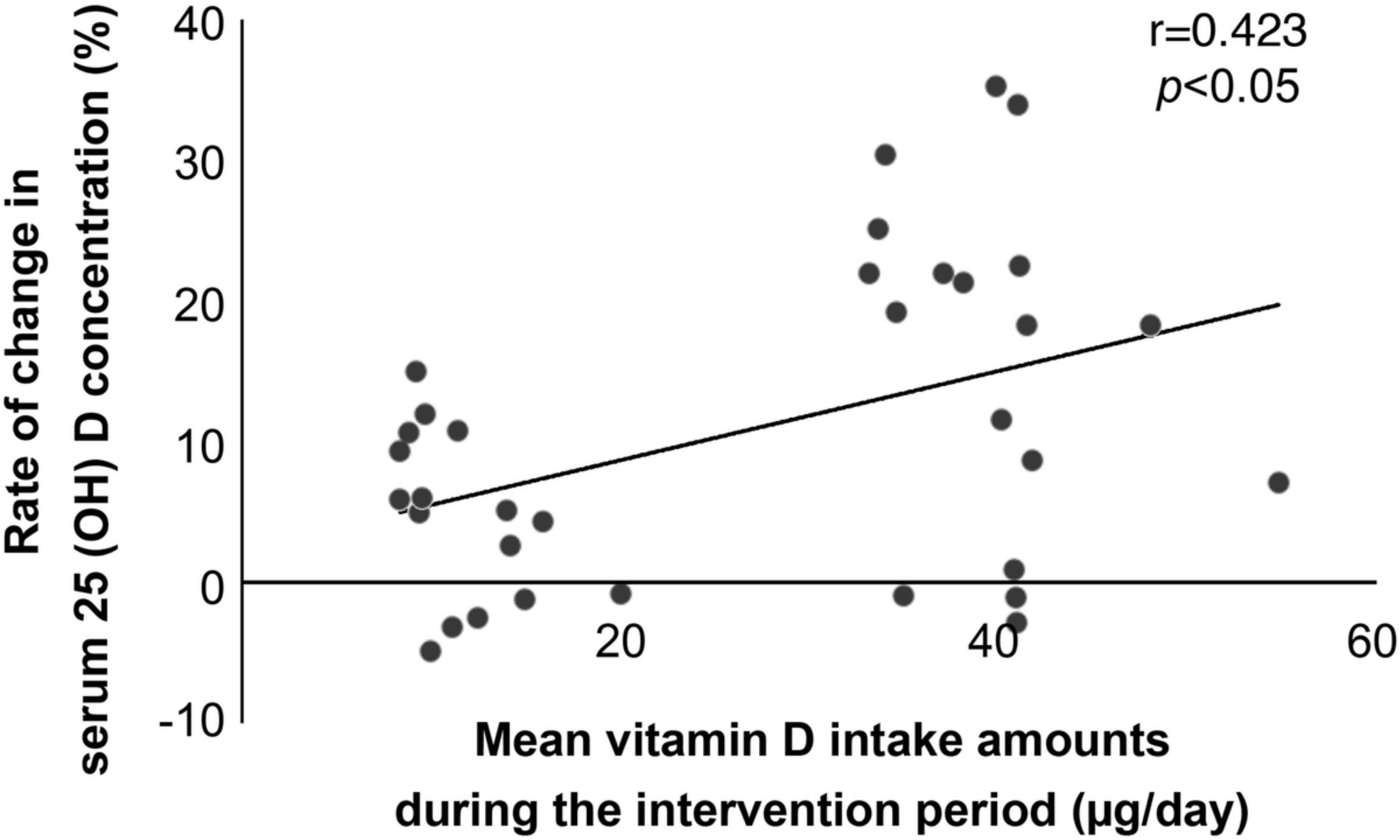

Figure 2

Relationship between serum 25 (OH) D concentration changes and vitamin D intake amounts Analysis performed using Pearson's product moment correlation coefficient.

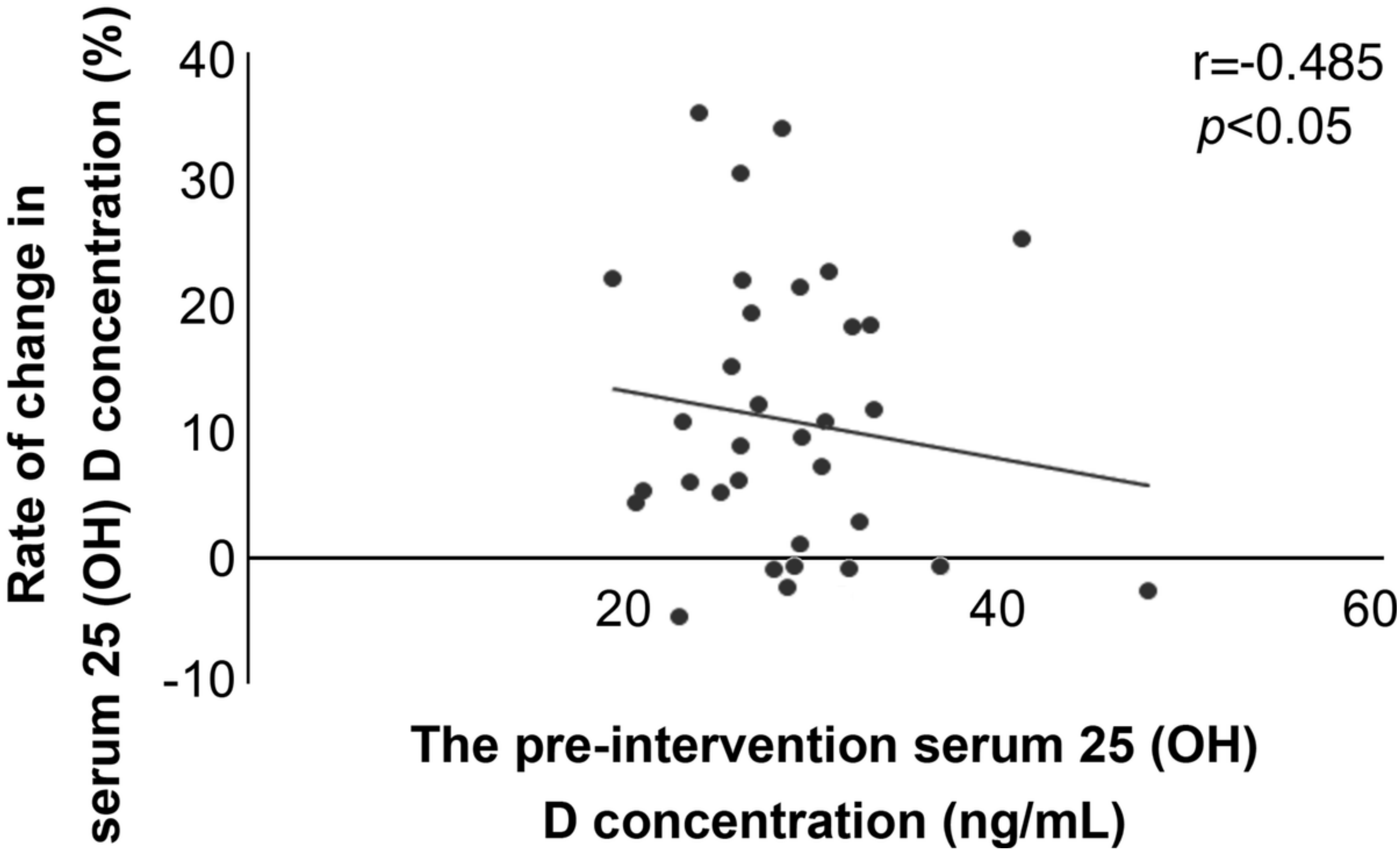

Figure 3

Relationship between the change in and pre-intervention levels of serum 25 (OH) D concentration Analysis performed using Pearson's product moment correlation coefficient.