Development and validation of a novel prediction model for osteoporosis

from serotonin to fat-soluble vitamins

From First Hospital of China Medical University. Shenyang, China

Cite this article:
Bone Joint Res 2025;14(2):
111–123.

DOI: 10.1302/2046-3758.
142.BJR-2023-0409.R2

Correspondence should be sent to Lin Tao taolindr@163.com

J. Wang,[1] L. Shan,[2] J. Hang,[3,4,5,6] H. Li,[1] Y. Meng,[1] W. Cao,[1] C. Gu,[1] J. Dai,[7,8,9] L. Tao[1]

[1]Department of Orthopedics, First Hospital of China Medical University, Shenyang, China
[2]Department of Intelligent Computing, School of Intelligent Medicine, China Medical University, Shenyang, China
[3]Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China
[4]National Clinical Research Center for Obstetrics and Gynecology, Beijing, China
[5]Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China
[6]Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Beijing, China
[7]National Clinical Research Center for Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, China
[8]Mass Spectrometry Precision Medicine Research Center, Department of Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, China
[9]Laboratory Medicine Innovation Unit, Chinese Academy of Medical Sciences, Shenyang, China

Aims

We aimed to develop and validate a novel prediction model for osteoporosis based on serotonin, fat-soluble vitamins, and bone turnover markers to improve prediction accuracy of osteoporosis.

Methods

Postmenopausal women aged 55 to 65 years were recruited and divided into three groups based on DXA (normal, osteopenia, and osteoporosis). A total of 109 participants were included in this study and split into healthy (39/109, 35.8%), osteopenia (35/109, 32.1%), and osteoporosis groups (35/109, 32.1%). Serum concentrations of serotonin, fat-soluble vitamins, and bone turnover markers of participants were measured. Stepwise discriminant analysis was performed to identify efficient predictors for osteoporosis. The prediction model was developed based on Bayes and Fisher's discriminant functions, and validated via leave-one-out cross-validation. Normal and empirical volume under the receiver operating characteristic (ROC) surface (VUS) tests were used to evaluate predictive effects of variables in the prediction model.

Results

Significant variables including oestrogen (E2), total procollagen type 1 amino-terminal propeptide (TP1NP), parathyroid hormone (PTH), BMI, vitamin K, serotonin, osteocalcin (OSTEOC), vitamin A, and vitamin D3 were used for the development of the prediction model. The training accuracy for normal, osteopenia, and osteoporosis is 74.4% (29/39), 80.0% (28/35), and 85.7% (30/35), respectively, while the total training accuracy is 79.8% (87/109). The internal validation showed excellent performance with 72.5% testing accuracy (72/109). Among these variables, serotonin and vitamin K exert important roles in the prediction of osteoporosis.

Conclusion

We successfully developed and validated a novel prediction model for osteoporosis based on serum concentrations of serotonin, fat-soluble vitamins, and bone turnover markers. In addition, interactive communication between serotonin and fat-soluble vitamins was observed to be critical for bone health in this study.

Bone & Joint Research

Article focus

- A novel prediction model for osteoporosis was developed in this study based on serotonin, fat-soluble vitamins, and bone turnover markers.

Key messages

- We successfully developed and validated a novel prediction model for osteoporosis based on serum concentrations of serotonin, fat-soluble vitamins, and bone turnover markers, with a total training accuracy of 79.8% (87/109).
- Serotonin and fat-soluble vitamins were demonstrated in this study to interact with each other, with their combined effects influencing bone health and contributing to the development of postmenopausal osteoporosis.

Strengths and limitations

- Bayesian and Fisher's discriminant analysis were performed in this study to develop the prediction model for osteoporosis, which provides novel approaches to develop prediction models.
- A limitation of this study is that the prediction model was only tested on the training cohort, without validation on an independent validation cohort, which is required in future studies to increase the prediction accuracy of this prediction model.

Introduction

As a degenerative metabolic disease caused by imbalanced skeletal remodelling, osteoporosis has become a global public health problem. Osteoporosis is the main cause of fractures for postmenopausal women.[1] Early diagnosis and interventions in high-risk populations are essential for delaying the development of osteoporosis and thereby improving treatment outcomes.[2,3] However, the capability of early diagnosis is limited due to the lack of sensitive biomarkers. Despite the fact that measurement of bone mineral density (BMD) by dual energy x-ray absorptiometry (DXA) is presently the most recommended tool for osteoporosis risk monitoring, it was restricted by the safety of repeated measurements and the bone status of the detection sites.[4] Therefore, it is important to identify effective predictive biomarkers and develop a novel prediction model for osteoporosis accordingly.

Serotonin is a common neurotransmitter present in the central nervous system and intestine, which is also involved in the regulation of bone remodelling. Serum concentrations of serotonin are positively correlated with BMD of the lumbar spine and femoral neck among postmenopausal women.[5,6] Extremely low levels of E2 are common among postmenopausal women, which is the leading risk factor for osteoporosis.[7] Serotonin is demonstrated to be the messenger of E2 that exerts its physiological and pathological effects.[8] Naturally occurring changes in E2 alter serotonin concentration via two pathways: increasing the production of tryptophan hydroxylase (rate-limiting enzyme for serotonin synthesis), and inhibiting the expression of serotonin reuptake transporter (SERT) thus promoting the action of serotonin.[9,10] Serotonin is also regarded as a positive regulator of bone mass through the serotonin receptors expressed by osteoblasts.[11-13] Meanwhile, serotonin is also strongly implicated in the regulation of the mammalian circadian clock, which is essential for the maintenance of bone health.[14,15]

Additionally, E2 also regulates the metabolism of fat-soluble vitamins.[16,17] Postmenopausal women have shown abnormal levels of fat-soluble vitamins, while the consumption of these vitamins as supplements has been confirmed to reduce bone loss caused by E2-deficient osteoporosis.[18-21] Meanwhile, fat-soluble vitamins interact with serotonin, which is involved in bone metabolism as mentioned above. Vitamin D activates the transcription of the serotonin-synthesizing gene tryptophan hydroxylase 2 (TPH2) through vitamin D response element (VDRE), and inhibits the transcription of TPH1 in tissues outside the blood-brain barrier.[22] Vitamin K is capable of stimulating platelet transportation of serotonin.[23] Additionally, supplement of vitamins A, K, and E alters serotonin levels among different populations.[24-26] Therefore, the interactive communication between serotonin and fat-soluble vitamins appears to be critical for the onset and progression of osteoporosis, providing novel potential for the prediction of osteoporosis.

Numerous prediction models for osteoporosis or related fractures have been developed.[27,28] Among these models, the Fracture Risk Assessment Tool (FRAX), which contains seven dichotomous clinical risk factors, has been applied as a standard to evaluate the individualized ten-year probability of osteoporotic fractures since its development in 2008.[29] However, as no quantitative index is included in the FRAX tool, it is only used to predict the probability of osteoporotic fractures instead of the risk of osteoporosis, let alone the prediction of early phase of osteoporosis.[30] The International Osteoporosis Foundation's (IOF) One-Minute Osteoporosis Risk Test is also commonly used for screening osteoporosis, yet a high false positive rate has been observed.[31,32] Other common prediction models of osteoporosis, such as the Osteoporosis Self-Assessment Tool for Asians (OSTA)[33] and the Beijing Friendship Hospital Osteoporosis Self-Assessment Tool (BFH-OST),[34] are mainly based on indirect indicators including age, height, BMI, and menopausal time. Due to the lack of direct indicators reflecting bone status, the accuracy of osteoporosis prediction is relatively low.

As serotonin and fat-soluble vitamins are critically involved in bone metabolism, we intended to elucidate their relationship with the development of osteoporosis and developed a prediction model based on serum levels of fat-soluble vitamins and serotonin. In addition, serum levels of bone turnover markers including β-cross-linked C-telopeptide of type 1 collagen (CROSSL), oestrogen (E2), osteocalcin (OSTEOC), parathyroid hormone (PTH), and total procollagen type 1 amino-terminal propeptide (TP1NP), which were frequently used to evaluate bone metabolism, have also been included in our prediction model to improve accuracy.[35,36] This study is a forerunner to establish a prediction model for osteoporosis based on the status of fat-soluble vitamins and serotonin among postmenopausal women, which we hope will provide novel potential for the prediction and early diagnosis of osteoporosis.

Methods

Study design, patient recruitment, and data collection

This study was approved by the Ethics Committee of the First Hospital of China Medical University ([2020]-248).

Postmenopausal women aged 55 to 65 years were recruited from the community through open advertisements, which included social media, posters, electronic billboards, and word of mouth, from 1 March to 30 September 2021. The research team of orthopaedists were responsible for recruiting the participants. All authors had full responsibility for data collection, analysis, interpretation, and writing of the report. Potential participants were screened for eligibility and excluded if they had any of the following: 1) shift workers;[37] 2) lower limb joint injury or surgery; 3) cognitive impairment; 4) uncontrolled cardiovascular disease; 5) malignant tumours; 6) receiving x-ray or radiation therapy; and 7) other medical and medication history known to influence BMD and serum concentrations of fat-soluble vitamins in this study (Supplementary Table i). All participants were fully informed of the procedure and potential risks associated with this study by the research team members (JW, HL), and signed informed consent. Once recruited into the study, all participants were allowed to come to the orthopaedic ward for blood collection on any morning from Monday to Friday, while related imaging examination was carried out in the physical examination centre on the same weekdays. The requirements and procedures for blood collection and imaging examinations are detailed in the following sections. A flow diagram of study recruitment is provided in Figure 1.

Patient demographic and clinical characteristics

Postmenopausal osteoporosis (PMOP) is the main type of primary osteoporosis. Therefore, postmenopausal women aged 55 to 65 years were included in this study to develop the prediction model for PMOP. Of note, postmenopausal women aged over 65 years were not included in this study because female osteoporosis patients aged over 65 years or male patients aged over 70 years are generally considered to have senile osteoporosis (SOP).[38] Among 180 samples included in the entire dataset, 36 were excluded due to missing data. In addition, six participants were excluded due to long-term night-shift work, five were excluded due to recent surgery history, eight were excluded due to use of glucocorticoids, 14 were excluded due to use of anti-osteoporosis medications, and two were excluded due to use of hypolipidaemic agents. The total recruitment process and results are shown in Figure 1. All participants were examined in this study by DXA and were divided into three groups based on T value: healthy (39/109, 35.8%), osteopenia (35/109, 32.1%), and osteoporosis (35/109, 32.1%). Characteristics of the model development cohort are summarized in Table I, Figure 2, and Supplementary Figure a. There were no significant differences in age and sex among different groups. Significant differences including serotonin, vitamin A, vitamin K, vitamin D3, weight, BMI, E2, PTH, and TP1NP were observed among subgroups. None of the other clinical parameters were statistically significant (Table I). Of note, 2.7% (101/109) and 55.9% (60/109) of all participantshad vitamin D insufficiency and deficiency. It is noted that there is a linear association between serum concentrations of vitamin K1 and triglyceride, indicating that vitamin K1 population reference intervals should be expressed as a ratio of the triglyceride concentration.[39] In order to determine whether the vitamin K results in this study were influenced by serum triglyceride concentrations, we examined the lipid levels of the participants included in this study. As shown in Table I, mean serum concentrations of triglyceride among participants in different groups were 0.98 (SD 0.11), 0.96 (SD 0.14), and 0.96 mmol/L (SD 0.12), which exhibited no statistical difference (Table I). Therefore, the serum concentrations of vitamin K1 could be further used to establish the prediction model in this study.

Bone measures and clinical assessments

BMD of the left femoral neck and lumbar spine (L1-L4) were measured by DXA. The mean value of Chinese healthy women was used to calculate the T-score. According to WHO criteria, osteoporosis is considered when BMD is 2.5 SDs below the young mean value ($T < -2.5$), while osteopenia is considered when $-2.5 < T < -1.0$.

Serum fat-soluble vitamins, serotonin, and bone turnover marker levels

Blood was collected by venipuncture at 7:00 am in the fasting state (≥ 10 hrs). Serum was collected and aliquoted for storage at -80°C until use. For the measurement of serum fat-soluble vitamins and serotonin levels, serum samples were analyzed using liquid chromatography–tandem mass spectrometry (LC-MS/MS, SCIEX 6500 QTRAP; SCIEX, USA). The fat-soluble vitamins assay kit for the high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) (License No. LXZZ 20202400103; Shandong Yingsheng Biotechnology Co., China) that we used was approved by the National Medical Products Administration, which can be used to accurately quantify vitamin A, vitamin E, 25-OHD2, 25-OHD3, and vitamin K in human serum. This method has also been used for routine clinical specimen testing. Notably, among these fat-soluble vitamins, vitamin K is a class of menaquinone derivatives, and we detected K1 (phylloquinone) in our research, which is light-sensitive. Therefore, the centrifugation and separation of serum were completed within two hours after blood collection, which was then treated with protective agent (containing citric acid, β-mercaptoethanol aqueous solution) and stored at -80°C. Serum concentrations of bone turnover markers were measured via chemiluminescence. Standard curves were generated according to the manufacturer's instructions, and the corresponding concentrations were calculated.

Development and validation of the prediction model

Model development and validation was performed according to transparent reporting for individual prognosis or diagnosis (TRIPOD) guidance of multivariable prediction models.[40] Raw data obtained from 145 participants, including age, height, weight, BMI, serum levels of fat-soluble vitamins (VA, VD2, VD3, VE, and VK), bone turnover markers (CROSSL, E2, OSTEOC, PTH, and TP1NP), and serotonin, were filtered first via stepwise discriminant analysis (SDA) to identify efficient predictors for osteoporosis. Selected variables were then used for the development and validation of the prediction model. Cases with missing data were removed from the model in this study.

Development of the prediction model was based on Bayes and Fisher's discriminant functions through SDA. With the original complete data of individual cases without missing values and the prior probability of each group being one-third, Bayesian and Fisher's discriminant functions were generated through SDA to determine the cluster centroids and individual classifications. Briefly outlining the modelling approach,

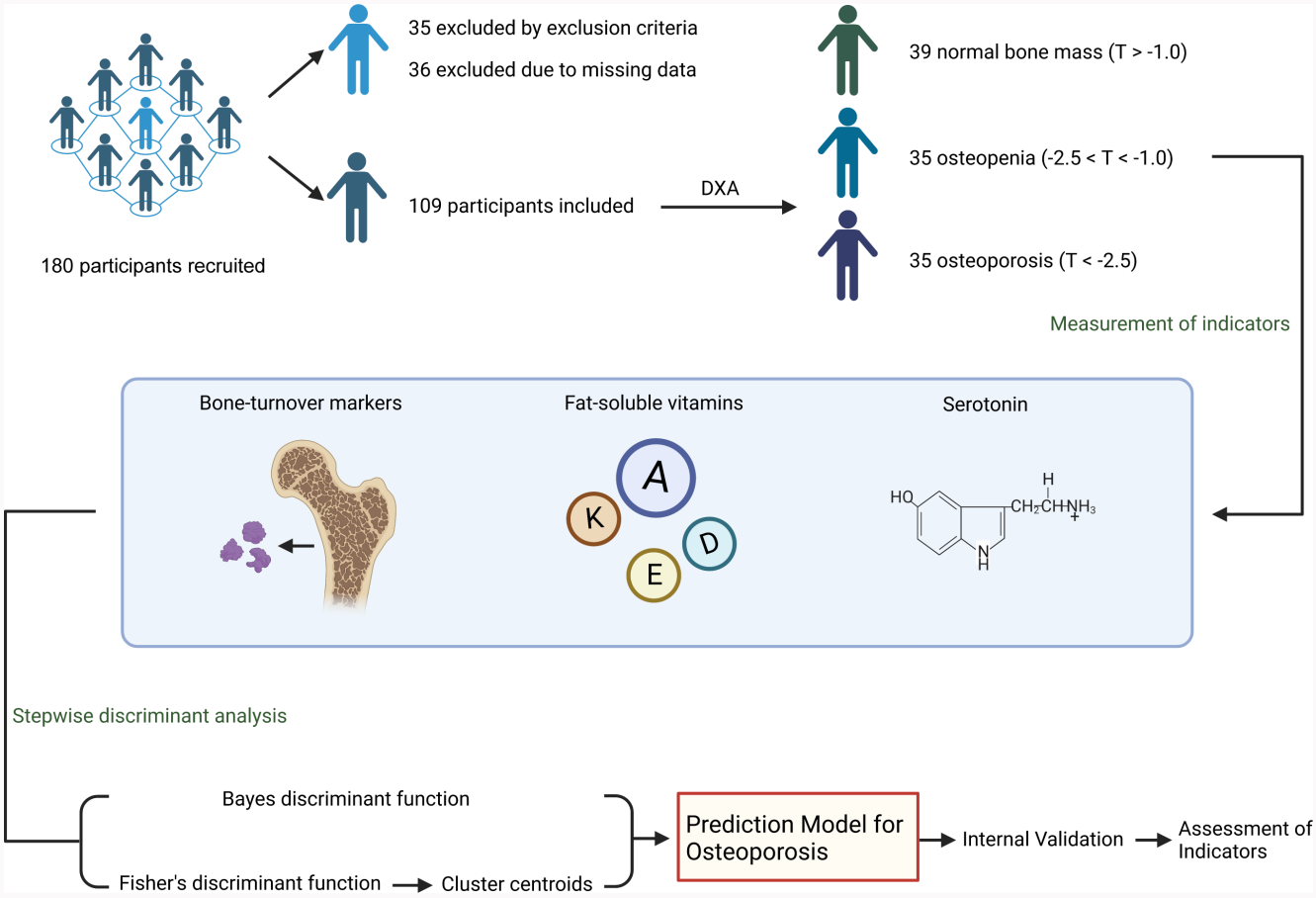

Fig. 1
Flow diagram of participant recruitment and data analysis. A total of 180 participants were recruited, with 71 excluded due to missing data and exclusion criteria. Participants included in this study were divided into three groups according to bone mineral density (BMD). Serum was collected for the measurement of serotonin, fat-soluble vitamins, and bone turnover markers. Methods for model development and validation are displayed in the following step. DXA, dual energy x-ray absorptiometry.

three discriminant equations for osteoporosis, osteopenia, and normal bone mass were obtained through Bayes discriminant analysis. Substituting the variable values of each individual into the three discriminant equations, the classification with the largest function value is the classification to which the individual belongs. Meanwhile, three cluster centroids of osteoporosis, osteopenia, and normal bone mass and two discriminating equations were determined by Fisher's discriminant analysis. Substituting the variable values of each individual into the two discriminant equations and calculating the distance from the cluster centroids, the classification of the individual is also obtained according to the distance. Validation of the prediction model was performed by leave-one-out cross-validation (LOOCV). All data were analyzed using SPSS v. 24.0 (IBM, USA).

Assessment of prediction model

Receiver operating characteristic (ROC) analysis is normally used to assess the training and test accuracy of diagnostic markers and classification procedures in general. The ROC surface was considered to be the generalization of the ROC curve, and the summarizing index of the volume under the ROC surface (VUS) was normally used to evaluate the diagnostic accuracy of biomarkers in a three-class classification model.[41,42] The trinROC R package[43] was used for the VUS test in this study to assess the discriminatory power of the prediction model.

Statistical analysis

In this study, efficient predictors for osteoporosis were filtered via SDA. Development of the prediction model was performed according to Bayes and Fisher's discriminant functions, while validation of the prediction model was performed via leave-one-out cross-validation (LOOCV). The one-sample Kolmogorov-Smirnov test was used to assess the normality of data before performing statistical tests. Spearman's rank correlation was applied to test for correlations. One-way analysis of variance (one-way ANOVA) was used to calculate p-values displayed in the baseline characteristics of participants, stepwise optimization, and three-class trinROC analyses. F-test was performed to calculate exact F-values in the stepwise optimization of the prediction model. Z-statistic was used to determine the statistical significance level of three-class trinROC analyses. All data were analyzed using SPSS version 24.0 (IBM, USA).

Results

Stepwise optimization of the prediction model

SDA was performed to identify effective predictors for osteoporosis. The probability of F was used as the criterion for

Table I. Baseline characteristics of participants. All data are shown as mean (SD).

Characteristic	Normal bone mass	N	Osteopenia	N	Osteoporosis	N	p-value*
Serotonin (pg/ml)	154.85 (68.84)	39	124.45 (58.87)	35	121.26 (59.43)	35	0.042
Vitamin A (ng/ml)	458.53 (131.51)	39	495.16 (138.77)	35	485.3 (150.88)	35	0.507
Vitamin E (μg/ml)	17.22 (6.61)	39	15.2 (6.94)	35	14.39 (4.36)	35	0.122
Vitamin K (ng/ml)	0.48 (0.52)	39	0.27 (0.22)	35	0.24 (0.18)	35	0.006
Vitamin D3 (ng/ml)	21.48 (7.52)	39	19.88 (8.85)	35	16.66 (7.28)	35	0.033
Vitamin D2 (ng/ml)	2.25 (1.6)	39	2.5 (3.18)	35	1.65 (1.02)	35	0.226
Age (yrs)	60.18 (6.87)	39	60.26 (7.83)	35	61.86 (5.74)	35	0.509
Height (cm)	161.08 (4.23)	39	160.43 (5.24)	35	158.74 (4.83)	35	0.103
Weight (kg)	66.93 (8.82)	39	63.2 (7.64)	35	58.29 (8.26)	35	< 0.001
BMI (kg/m^2)	25.8 (3.29)	39	24.59 (3.07)	35	23.09 (2.84)	35	0.001
CROSSL (ng/ml)	525.71 (259.31)	39	695.02 (301.58)	35	590.76 (284.29)	35	0.038
E2 (ng/ml)	59.1 (30.02)	39	69.51 (32.62)	35	31.5 (15.89)	35	< 0.001
OSTEOC (ng/ml)	23.12 (9.74)	39	22.58 (6.65)	35	19.32 (7.63)	35	0.108
PTH (ng/ml)	40.69 (16.48)	39	29.84 (9.5)	35	42.24 (19.7)	35	0.002
TP1NP (ng/ml)	58.63 (22.74)	39	73.21 (20.33)	35	48.36 (25.97)	35	< 0.001

*One-way analysis of variance.
CROSSL, β-cross-linked C-telopeptide of type 1 collagen; OSTEOC, osteocalcin; PTH, parathyroid hormone; TP1NP, total procollagen type 1 amino-terminal propeptide.

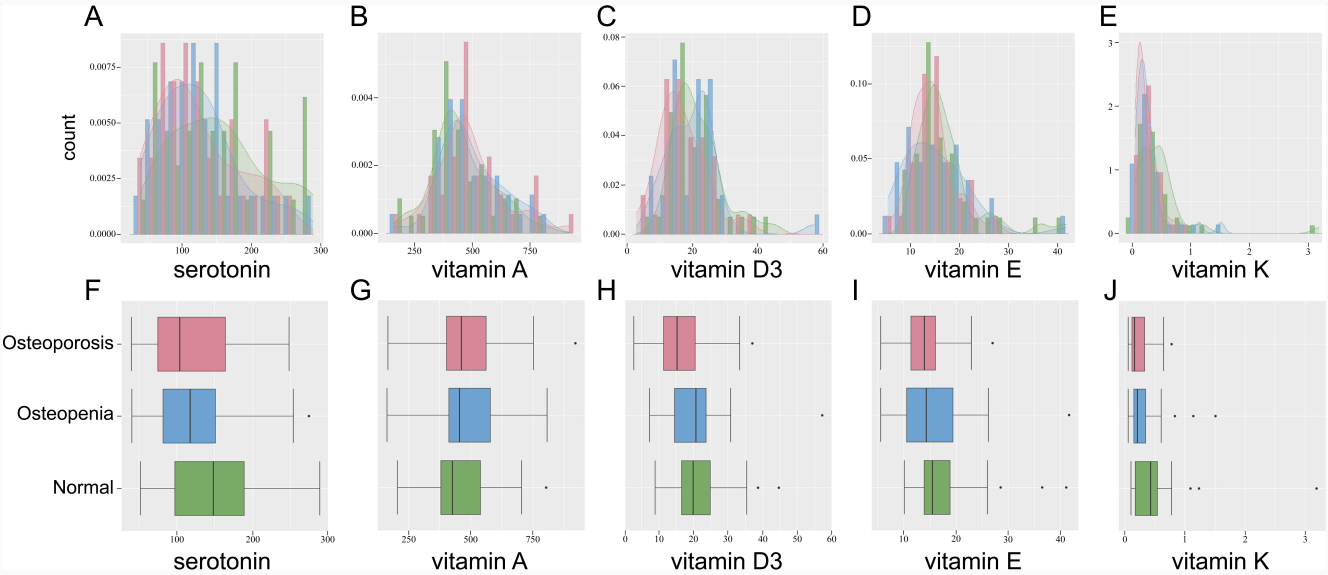

Fig. 2
Distribution of serotonin and fat-soluble vitamins among the three groups. Red indicates osteoporosis, blue indicates osteopenia, and green indicates normal bone mass. a) to e) Histograms of serotonin and fat-soluble vitamins (vitamin A, vitamin D3, vitamin E, and vitamin K). f) to j) Box plots of serotonin and fat-soluble vitamins (vitamin A, vitamin D3, vitamin E, and vitamin K).

entering and removing variables; a variable was entered into the model if the significance level of its F-value was < 0.10, and was removed if it was > 0.20. Significant variables including E2, TP1NP, PTH, BMI, vitamin K, serotonin, OSTEOC, vitamin A, and vitamin D3, sequenced according to statistical values, were used for further development of the prediction model (Table II). Detailed steps for the SDA are available in Supplementary Table ii. However, vitamin E was excluded by SDA. In order to further understand the status of fat-soluble vitamins among postmenopausal women and their association with osteoporosis progression, vitamin E was included artificially.

Table II. Wilks' lambda and significance test of stepwise input variables.*†

		Wilks' lambda							
						Exact F-values			
Step	Input	Statistical value	Freedom 1	Freedom 2	Freedom 3	Statistical value	Freedom 1	Freedom 2	p-value
1	E2	0.744	1	2	106.000	18.202	2	106.000	< 0.001
2	TP1NP	0.631	2	2	106.000	13.608	4	210.000	< 0.001
3	PTH	0.579	3	2	106.000	10.907	6	208.000	< 0.001
4	BMI	0.535	4	2	106.000	9.442	8	206.000	< 0.001
5	Vitamin K	0.504	5	2	106.000	8.330	10	204.000	< 0.001
6	Serotonin	0.476	6	2	106.000	7.577	12	202.000	< 0.001
7	OSTEOC	0.452	7	2	106.000	6.956	14	210.000	< 0.001
8	Vitamin A	0.429	8	2	106.000	6.525	16	198.000	< 0.001
9	Vitamin D3	0.392	9	2	106.000	6.505	18	196.000	< 0.001

At each step, the variables that minimize the overall Wilks' lambda are entered in a stepwise fashion.
Statistical test: F-value and F-test. F-values refer to statistical value in column G, with freedom 1 and freedom 2 (columns H and I). F-test was performed to calculate exact F-values (columns G to J).
*The largest significance level of F-value to enter into the model is 0.10.
†The minimal significance level of F-value to be removed from the model is 0.20.
E2, oestrogen; OSTEOC, osteocalcin; PTH, parathyroid hormone; TP1NP, total procollagen type 1 amino-terminal propeptide.

Development and validation of the prediction model

Bayes discriminant function and Fisher's discrimination function were generated simultaneously to develop the prediction model for osteoporosis based on the ten variables mentioned above.

The Bayes discriminant functions are expressed as below (Y1, Y2, and Y3 refer to normal bone mass, osteopenia, and osteoporosis, respectively):

Y1=0.044**serotonin*+0.004***VA*+0.242***VE*+2.583***VK*+0.217***VD*3 +2.560***BMI*+0.020***E2*+0.154***OSTEOC*+0.120***PTH*+0.106***TP*1*NP*-51.441

Y2=0.034**serotonin*+0.012***VA*+0.196***VE*+0.728***VK*+0.156***VD*3+2.366***BMI*+0.053***E*+0.052***OSTEOC*+0.088***PTH*+0.152***TP*1*NP*-47.653

Y3=0.033**serotonin*+0.012***VA*+0.183***VE*+0.795***VK*+0.077***VD*3+2.291***BMI*-0.004***E*+0.120***OSTEOC*+0.135***PTH*+0.086***TP*1*NP*-40.537

The Fisher's discrimination functions are expressed as below:

Y1=0.002**serotonin*+0.001***VA*+0.14***VE*+2.45***VK*+0.050***VD*3+0.068***BMI*+0.025***E2*-0.020***OSTEOC*-0.020***PTH*+0.027***TP*1*NP*-4.374

Y2=0.007**serotonin*+0.005***VA*+0.034***VE*+1.271***VK*+0.057***VD*3+0.148***BMI*-0.012**E2+0.058***OSTEOC*-0.014***PTH*-0.020***TP*1*NP*-4.083

Centroids of the three clusters are obtained based on Fisher's discrimination functions. The open circle indicates the participants, while squares in green, blue, and red represent centroids in the corresponding group (Figures 3a to 3c). The overall distribution is shown in Figure 3d, with open circles representing the normal bone mass group, squares representing the osteopenia group, and crosses representing the osteoporosis group. The distribution showed that the prediction model could clearly separate the participants into three clusters, indicating the good discriminatory ability of this prediction model (Figure 3d). Based on the above Bayes and Fisher's discrimination functions, we could calculate the training accuracy of the prediction model for osteoporosis. As shown in Table III, the training accuracy for the model is 79.8% (87/109). Meanwhile, the internal validation showed excellent performance with 72.5% test accuracy (72/109). Predictive results of the discriminant analysis of 108 cases are shown in Supplementary Table iii. The field diagram of prediction model based on Fisher's discrimination function is also shown in Supplementary Figure b. In the field diagram, the lines composed of the numbers 1, 2, and 3 divide the diagram into three parts, with areas composed of two "1/2/3" lines and a border representing the normal bone mass, osteopenia, and osteoporosis groups, respectively (Supplementary Figure b).

In addition, we also randomly divided the cohort into two separate groups in a ratio of approximately 2:1 in order to assess prediction ability of the prediction model in a separate test cohort. The training cohort consisted of 71 samples, while the validation cohort comprised 38 samples. The development and validation of the new prediction model were therefore based on two independent cohorts. The prediction accuracy of the new model was 74.6%, while the validation accuracy calculated by LOOCV in the training cohort was 66.2%. The external predictive accuracy of the model in the validation cohort was 57.9% (Supplementary Table iv). We also randomly divided the cohort into two separate groups in a ratio of approximately 4:1, with 88 in the training cohort and 21 in the validation cohort. The prediction accuracy of the new model was 70.5%, while the validation accuracy calculated by LOOCV in the training cohort was 62.5%. The external predictive accuracy of the model in the validation cohort was 66.7% (Supplementary Table iv). It can be seen that although

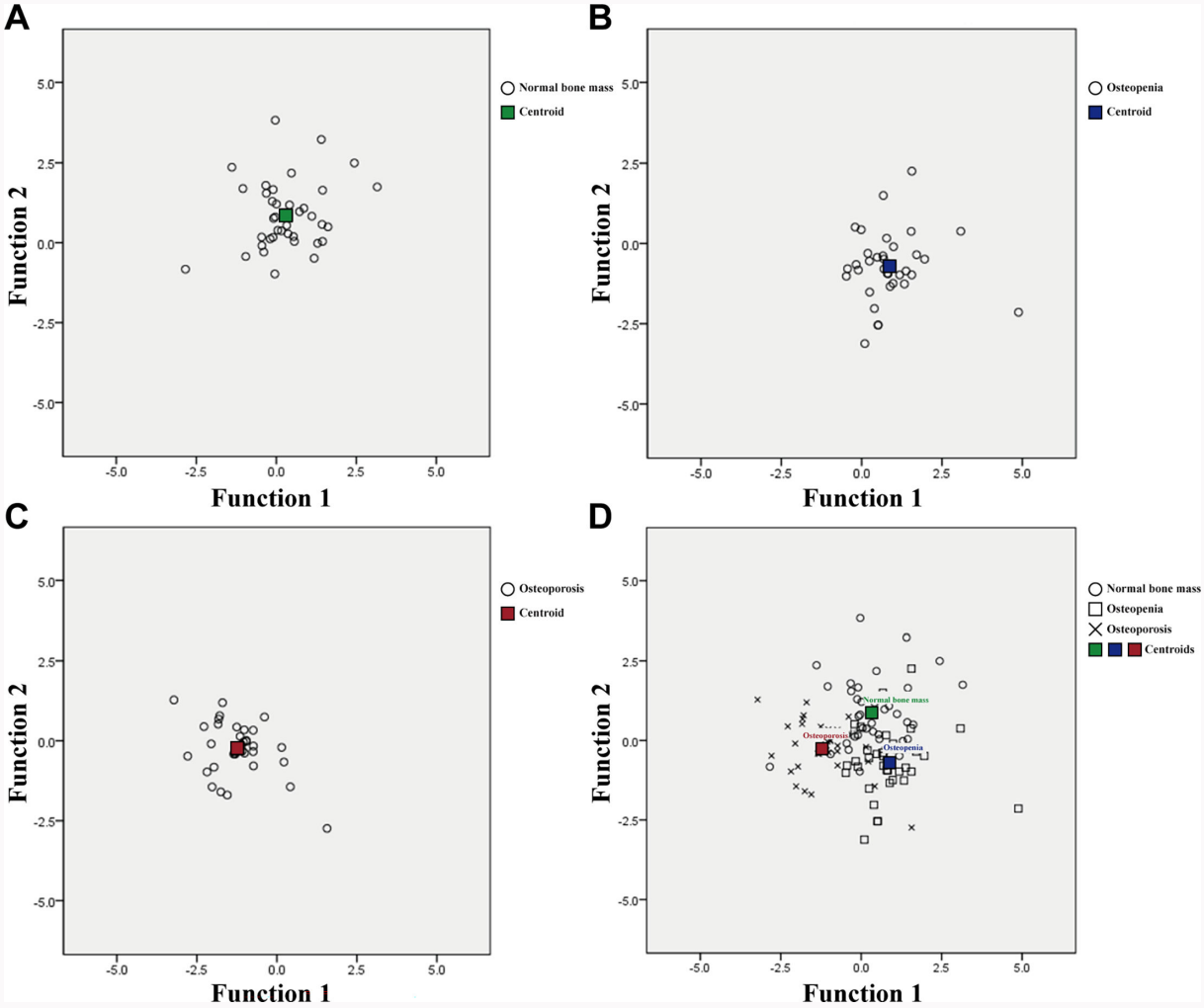

Fig. 3

Cluster centroids of three groups based on Fisher's discrimination function. Functions 1 and 2 were obtained based on Fisher's discrimination functions. Abscissa and ordinate were obtained by substituting the variables into equations. a) to c) Centroids of three groups. Open circles represent individuals in each group. Centroids of three groups (normal bone mass, osteopenia, and osteoporosis) are indicated in coloured squares (green, blue, and red, respectively). d) All individuals are indicated as open circles, squares, and crosses, representing normal bone mass, osteopenia, and osteoporosis, respectively. Centroids of three groups are coloured in green, blue, and red.

dividing the samples into independent training and validation cohorts allowed us to externally validate the model, the reduction in sample size led to a small decrease in prediction and validation accuracy. Therefore, sufficient samples may increase the accuracy and stability of the models.

Assessment of predictive effects of serotonin and fat-soluble vitamins

We first focused on the role of serotonin and fat-soluble vitamins in the prediction of osteoporosis. The variables were sorted according to Wilks' lambda, which emphasized the sequence of variables to improve the accuracy of the whole model while retaining other variables such as BMI and TP1NP (Supplementary Table ii). The rank order in this principle was vitamin K > serotonin > vitamin A > vitamin D3. We also adopted Bayes discriminant analysis for each variable including serotonin, vitamin K, vitamin A, and Vitamin D3 to determine their prediction ability. The order of training accuracy was: serotonin (44.4%) > vitamin K (42.8%) > vitamin D3 (37.2%) > vitamin A (32.4%) (Supplementary Table v). In addition, VUS test was applied in this study to measure and estimate the test accuracy for the prediction model with three ordinal predictive groups. The order of the variables based on normal VUS was vitamin K > serotonin > vitamin D3> vitamin A when variables were assumed to follow normal distribution, while the order based on empirical VUS was vitamin D3> serotonin > vitamin K > vitamin A (Table IV, Figure 4). Three-class trinROC analyses of other variables (BMI, E2, OSTEOC, PTH, and TP1NP) are displayed in Supplementary Figure c.

Analysis of co-activity between parameters

Although the prediction model in this study exhibited good training accuracy, the prediction model based on univariate analysis exhibited poor training accuracy with the training accuracy of only three variables over one-third (serotonin, vitamin K, and vitamin D3). This finding indicates that there might be interactions among these variables, which play important roles in the early prediction of osteoporosis. In order to account for co-activity between parameters, we first conducted a normality test on the variables and found that none of them conformed to a normal distribution.

Table III. Aggregated results of discriminant analysis.*†

Data type	Display format	Classification	Predicted results			
			Normal bone mass	Osteopenia	Osteoporosis	Total
Original data	Count	Normal bone mass	29	5	5	39
		Osteopenia	5	28	2	35
		Osteoporosis	1	4	30	35
	%	Normal bone mass	74.4	12.8	12.8	100.0
		Osteopenia	14.3	80.0	5.7	100.0
		Osteoporosis	2.9	11.4	85.7	100.0
Cross-validation	Count	Normal bone mass	23	9	7	39
		Osteopenia	6	26	3	35
		Osteoporosis	1	4	30	35
	%	Normal bone mass	59.0	23.1	17.9	100.0
		Osteopenia	17.1	74.3	8.6	100.0
		Osteoporosis	2.9	11.4	85.7	100.0

*79.8% (87/109) of original cases were correctly classified.
†72.5% (72/109) of cross-validation cases were correctly classified.

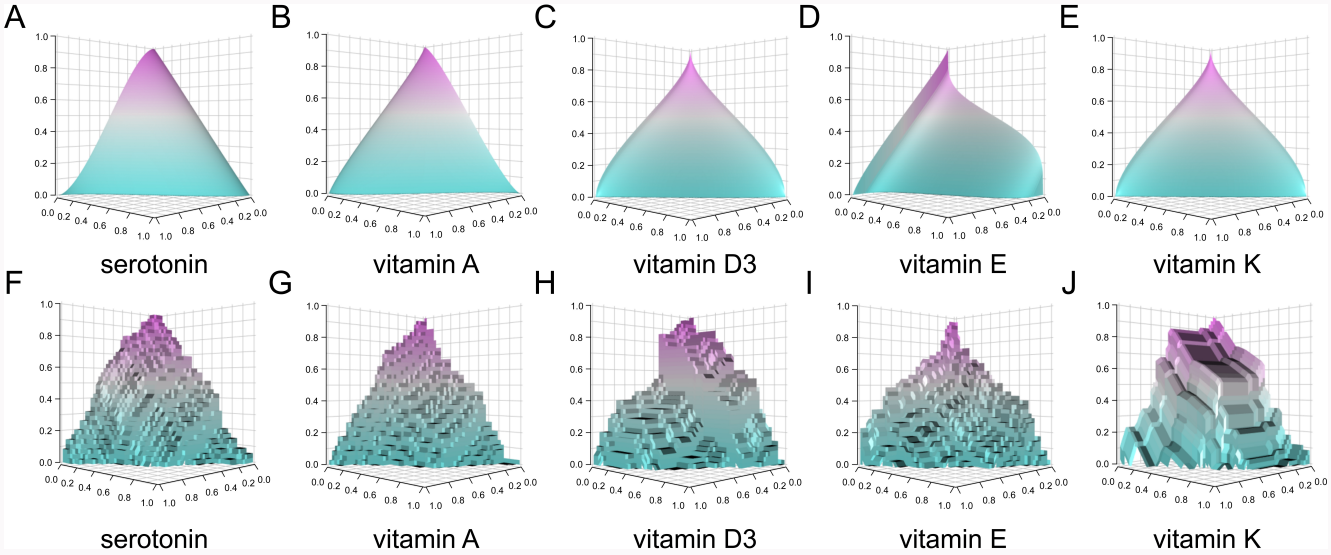

Fig. 4
Three-class trinROC analyses of serotonin and fat-soluble vitamins. Three-class trinROC analyses were used to evaluate diagnostic accuracy of biomarkers in this model. Volume under surface of each variable represents corresponding prediction accuracy. a) to e) Normal-VUS tests of serotonin, vitamin A, vitamin D3, vitamin E, and vitamin K. f) to j) Empirical-VUS tests of serotonin, vitamin A, vitamin D3, vitamin E, and vitamin K. ROC, receiver operating characteristic; VUS, volume under the ROC surface.

Therefore, we used Spearman's rank correlation to verify the correlation between the variables. The results showed that the coefficient of rank correlation between vitamin A and vitamin D3 was 0.465 ($p < 0.001$), while the coefficient of rank correlation between vitamin E and vitamin K was 0.481 ($p < 0.001$), demonstrating that there were moderate correlations in vitamin A-vitamin D3 and vitamin E-vitamin K. Related contents were added in the manuscript (Supplementary Tables vi and vii).

Discussion

Main findings

In the present study, we established a novel prediction model based on serotonin, fat-soluble vitamins, and bone turnover markers according to Bayes discrimination analysis and Fisher's discrimination analysis. The prediction model performed well with a total training accuracy of 79.8% (87/109) and LOOCV of 72.5% (72/109). We observed that low BMD was associated with low serum concentrations of vitamin K, serotonin, and vitamin D3, as well as high levels of vitamin A. No significant difference was found for vitamin E. Of all

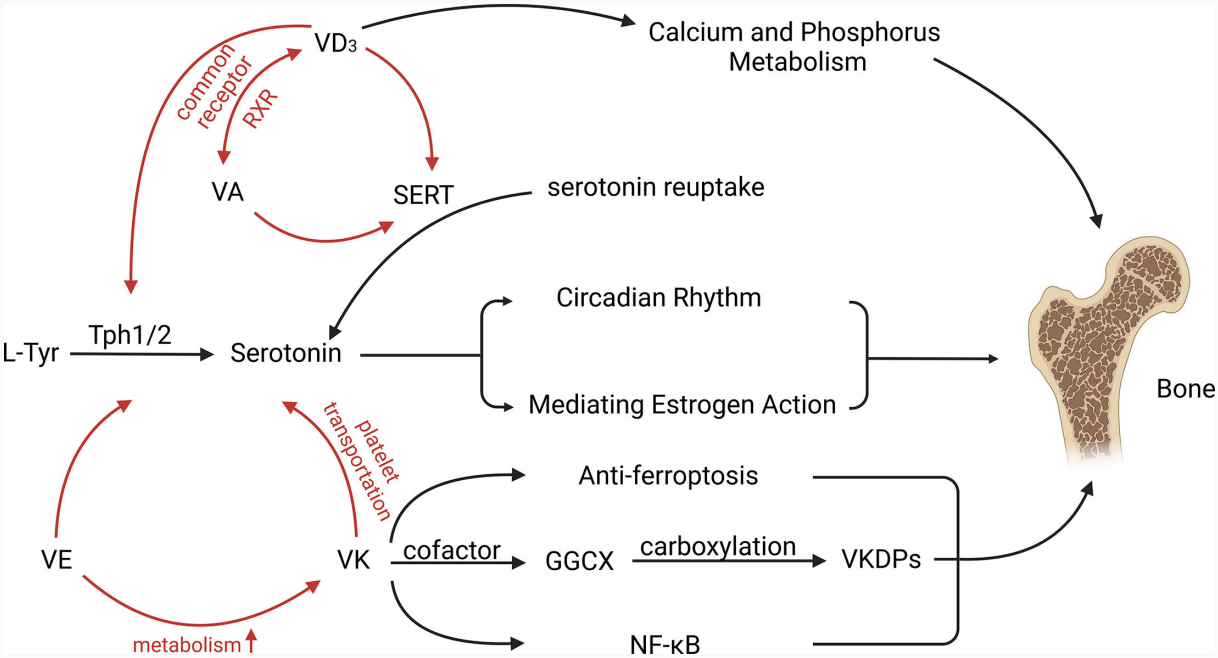

Fig. 5
Effects of serotonin and fat-soluble vitamins on the pathogenesis of osteoporosis. Interactive communication between serotonin and fat-soluble vitamins (vitamin A, vitamin D3, vitamin E, and vitamin K) is critical for bone health. Red arrows represent interactions between serotonin and fat-soluble vitamins. Serotonin, vitamin K, and vitamin D3 exert the major function to regulate bone metabolism. Serotonin modulates bone status through circadian rhythm and mediating oestrogen actions. Vitamin K is involved in bone remodelling via three pathways: anti-ferroptosis effects, nuclear factor kappa B (NF-κB) pathway, and participating carboxylation of vitamin K-dependent proteins (VKDPs). Vitamin D3 could regulate calcium and phosphorus metabolism to affect bone health. Vitamin A and vitamin E have indirect roles in bone remodelling via regulating the effects of serotonin, vitamin D3, or vitamin K. GGCX, gamma-glutamyl carboxylase; RXR, retinoid X receptor; SERT, serotonin reuptake transporter.

Table IV. Three-class trinROC analyses of the variables.

Variable	Normal VUS	p-value	Order	Empirical VUS	p-value	Order
Serotonin	0.252	0.05315	5	0.260	0.05028	5
Vitamin A	0.196	0.45334	9	0.203	0.40344	8
Vitamin E	0.212	0.24705	7	0.183	0.67132	9
Vitamin K	0.282	0.01158	2	0.307	0.0088	3
Vitamin D3	0.243	0.07129	6	0.275	0.02823	4
BMI	0.310	0.00258	1	0.320	0.0051	2
E2	0.280	0.01432	3	0.331	0.00549	1
OSTEOC	0.265	0.03643	4	0.257	0.06625	6
PTH	0.179	0.76394	10	0.178	0.79323	10
TP1NP	0.197	0.44933	8	0.205	0.4088	7

Statistical test: If a single classifier is investigated, the null hypothesis is V US1 = 1/6 with the Z-statistic: $Z = \frac{\widehat{VUS_1} - 1/6}{\sqrt{\widehat{Var(VUS_1)}}}$

E2, oestrogen; OSTEOC, osteocalcin; PTH, parathyroid hormone; ROC, receiver operating characteristic; TP1NP, total procollagen type 1 amino-terminal propeptide; VUS, volume under the ROC surface.

these metrics, serotonin and vitamin K are the most significant predictors. Surprisingly, however, vitamin D3 achieved poor prediction effects. Notably, the prediction model based on univariate analysis exhibited poor training accuracy, and only three variables achieved training accuracy over one-third. Therefore, the interaction among these variables, rather than a single variable alone, plays an important role in the early prediction of osteoporosis, which could be explained by the variables' molecular mechanisms (Figure 5).

Bayesian and Fisher's discriminant rules are used to develop the prediction model for osteoporosis. Bayesian and Fisher's discriminant analyses are two statistical analyses for modelling that are essentially the same, yielding identical predictive accuracy and validation accuracy. However, their presentation format varies, catering to different users' needs for model use. Bayes discriminant function could clearly obtain the predictive probabilities of which disease status the patient has, which is more suitable for presentation to patients, while Fisher's discriminant function visually shows the degree of clustering of different groups through class centroids, with larger distances between class centroids indicating better differentiation between each population to some extent, which is more suitable for professionals to evaluate predictive models and the effectiveness of differentiating patient subtypes in clinical settings. Therefore, the two discriminant methods are essentially the same, which can be selected based on the users and purposes of the model.

Interpretations

Serotonin is a neurotransmitter present in the central nervous system and intestine. Both centrally and peripherally produced serotonin was demonstrated to affect bone status.[44] The

relationship between peripheral serotonin and bone remains controversial. Many studies have shown that serum serotonin concentrations are lower among postmenopausal women and positively associated with BMD, indicating its positive effects on bone metabolism.[45–47] However, other studies have demonstrated that serotonin levels are inversely associated with BMD,[11,44,48,49] and in vitro experiments also show conflicting results.[50–52] Several explanations would probably account for this paradoxical effect: serum serotonin level, which is largely affected by tryptophan intake, may obviously differ between the populations, or different sampling times in distinct studies could impact its accuracy as serum serotonin concentration exhibits a 12-hour circadian rhythm and seasonal variation.[53,54] Additionally, it was further explained that the association between serum serotonin and non-vertebral fracture risk seems to be U-shaped, with higher fracture risk at low and high serum levels than at median levels.[55]

Vitamin K is involved in blood coagulation and bone metabolism. Similar to our study, numerous studies have indicated that low serum levels of vitamin K are associated with low BMD and an increased risk for osteoporotic fractures.[56–59] Indeed, vitamin K mainly participates in bone metabolism as a cofactor of gamma-glutamyl carboxylase (GGCX), which catalyzes the carboxylation of vitamin K-dependent proteins (VKDPs).[60–62] In addition, vitamin K regulates the proliferation and differentiation of osteoblasts and osteoclasts through the signal transduction pathway of nuclear factor kappa B (NF-κB), which is crucial for osteoclast development and resorption.[63] It is also noted that vitamin K is a potent anti-ferroptotic compound that could protect cells against ferroptosis.[64] Targeting ferroptosis has been confirmed in many studies to alleviate and prevent osteoporosis.[65,66] Consequently, vitamin K might be a novel biomarker for the prediction of osteoporosis.

Another fat-soluble vitamin, vitamin D, is responsible for calcium and phosphorus homeostasis. Vitamin D insufficiency and deficiency were defined as serum 25-hydroxyvitamin D3 values < 30 ng/ml and 20 ng/ml, respectively.[67] Consistent with our study, increased vitamin D concentration has also been demonstrated to be related to high BMD, while low levels of vitamin D3 might increase the risk of fractures.[68–70] However, it is worth noting that serum concentrations of vitamin D are not associated with the risk of fractures.[71] It was also observed among healthy populations that a supplement of vitamin D3 does not provide demonstrable health benefits.[72] These findings indicate that the relationship between vitamin D3 and bone health is not a simple direct linear relationship based on concentrations, but rather it functions in conjunction with other indicators (such as other types of fat-soluble vitamins). In addition, no significant differences were observed in serum concentrations of vitamin A and vitamin E among the three subgroups. The relationships between serum concentrations of vitamin A, vitamin E, and BMD among different populations remain controversial. Elevated serum concentration of vitamin A is associated with an increased risk of low BMD,[73] while in a large, nationally representative sample in the USA population, no significant associations are observed between fasting serum retinyl esters and any measure of bone mineral status.[74] A similar situation also occurs for vitamin E.[75–77]

It is interesting to note that serotonin and fat-soluble vitamins could interact with and regulate each other. Among fat-soluble vitamins, vitamin A and vitamin D share a common nuclear receptor: retinoid X receptor. Therefore, high levels of vitamin A could reduce vitamin D function.[78] Vitamin E could interfere with the activity of vitamin K, which might be explained by the same metabolic pathways. Vitamin E may strengthen the xenobiotic pathways of vitamin K and accelerate the excretion of all vitamin K forms.[79] In addition, the status of optimal fat-soluble vitamins may contribute to maintaining serotonin concentrations. Daily rhythms and supplement of vitamin D could also regulate the production of serotonin via regulating the expression of TPH1, TPH2, and SERT.[80,81] Serum serotonin level is reported to be directly regulated by vitamin A supplements, which is also regulated by SERT expression,[24] and supplement of vitamin E could result in obvious upregulation of serotonin level in rats.[25] Moreover, serum concentration of serotonin is observed to be decreased among osteotomy rats when supplemented with vitamin K.[26] As a result, the evaluation of only a single or several fat-soluble vitamins is not enough to reflect their exact effects on bone metabolism, while their net interactions might play a determining role.

Strengths and limitations

This study has several strengths. As discussed above, recent studies about the relationship between fat-soluble vitamins and bone metabolism have yielded different, even contradictory conclusions.[70,71] It is not enough to evaluate the effect of single fat-soluble vitamins due to their tight interactions. Therefore, serum concentrations of all fat-soluble vitamins were measured in this study, and VA, VD3, VE, and VK were selected for the prediction model of osteoporosis. According to these results, it is efficient to evaluate the global status of fat-soluble vitamins and serotonin. In addition, all blood samples were collected at around 7:00 am when the plasma concentration of serotonin reaches its peak to minimize the effects of serotonin rhythms, and uniform detection by LC-MS/MS was applied to rule out the errors caused by different detection methods.[82] All these strengths guarantee the reliability of our data. However, this study might also be limited due to the insufficient sample size and single-centre participants' recruitment. Thus, LOOCV was performed in this study for estimating the predictive accuracy.

Implications

We successfully developed and validated a clinical model to predict the risk of osteoporosis based on several biomarkers, including serum concentrations of serotonin, fat-soluble vitamins, and bone turnover markers. As precision medicine is currently being emphasized,[83,84] application of a clinical prediction model and risk calculator can be a useful tool for an individualized approach. Our studies provide a novel potential for the early prediction that serum levels of fat-soluble vitamins and serotonin could reflect bone status. In addition, due to the various advantages of prediction models for various diseases, such as facilitating early diagnosis and prevention of diseases, and personalized healthcare, various kinds of disease prediction models have been constructed.[85–87] We proposed a novel method to develop the prediction model based on Bayes and Fisher's discriminant analyses. Meanwhile, machine

learning has also frequently been used to construct various prediction models.[88,89] Therefore, apart from the application presented in this study, we could also calculate the critical values for different indicators within different subgroups and assign values to variable within different ranges based on their coefficients in the Bayes and Fisher functions. That is to say, we could categorize the probabilities of the three classifications not as precise numerical values, but within probability intervals, which means that the probability of being classified as Category A falls within a certain range, followed by the probability of Category B within the next range, and then the probability of Category C in the subsequent range. Stacking is an ensemble learning technique that combines multiple different models to enhance prediction accuracy. In stacking, each model makes predictions independently, and then these predictions are used as inputs and passed onto another model, typically referred to as the meta-model or secondary learner, which makes the final prediction. Subsequently, using computational methods (such as artificial intelligence or machine learning) to combine different models, together with the idea of stacking, provides a means of updating the existing prediction models.

In summary, a clinical prediction model to predict the risk of osteoporosis was developed in this study based on serum concentrations of serotonin, fat-soluble vitamins, and bone turnover markers. The prediction model showed excellent performance with 79.8% (87/109) training accuracy. Serotonin and fat-soluble vitamins interact and regulate each other, while the combined effects impact bone status and contribute to the pathogenesis of osteoporosis. We developed a novel prediction method for osteoporosis in this study, which provides new avenues of investigation for the debates about the effects of serotonin and fat-soluble vitamins on bone status.

Supplementary material

Figures showing distribution of other variables (BMI, E2, OSTEOC, PTH, and TP1NP) among the three groups (osteoporosis, osteopenia, and normal bone mass), field diagram of the prediction model based on Fisher's discrimination function, and three-class trinROC analyses of other variables. Tables showing: inclusion and exclusion criteria; stepwise tolerance, F significance, and Wilks' lambda of variables; predictive results of discriminant analysis of 108 cases; performance of various prediction models with different classification principles; Bayes discriminant analysis for serotonin, vitamin K, vitamin D3, and vitamin A; and normality test and correlation test among serotonin and fat-soluble vitamins, using one-sample Kolmogorov-Smirnov test and Spearman's rank correlation.

References

1. **Rizzoli R**, **Biver E**, **Brennan-Speranza TC**. Nutritional intake and bone health. *Lancet Diabetes Endocrinol*. 2021;9(9):606–621.
2. **Radominski SC**, **Bernardo W**, **Paula AP**, **et al**. Brazilian guidelines for the diagnosis and treatment of postmenopausal osteoporosis. *Rev Bras Reumatol Engl Ed*. 2017;57 Suppl 2:452–466.
3. **Parveen B**, **Parveen A**, **Vohora D**. Biomarkers of osteoporosis: an update. *Endocr Metab Immune Disord Drug Targets*. 2019;19(7):895–912.
4. **Marra M**, **Sammarco R**, **De Lorenzo A**, **et al**. Assessment of body composition in health and disease using bioelectrical impedance analysis (BIA) and dual energy x-ray absorptiometry (DXA): a critical overview. *Contrast Media Mol Imaging*. 2019;2019:3548284.
5. **Quevedo I**, **Zuniga AM**. Low bone mineral density in rotating-shift workers. *J Clin Densitom*. 2010;13(4):467–469.
6. **Bukowska-Damska A**, **Skowronska-Jozwiak E**, **Peplonska B**. Night shift work and osteoporosis: evidence and hypothesis. *Chronobiol Int*. 2019;36(2):171–180.
7. **Song S**, **Guo Y**, **Yang Y**, **Fu D**. Advances in pathogenesis and therapeutic strategies for osteoporosis. *Pharmacol Ther*. 2022;237:108168.
8. **Rybaczyk LA**, **Bashaw MJ**, **Pathak DR**, **Moody SM**, **Gilders RM**, **Holzschu DL**. An overlooked connection: serotonergic mediation of estrogen-related physiology and pathology. *BMC Womens Health*. 2005; 5:12.
9. **Hiroi R**, **McDevitt RA**, **Neumaier JF**. Estrogen selectively increases tryptophan hydroxylase-2 mRNA expression in distinct subregions of rat midbrain raphe nucleus: association between gene expression and anxiety behavior in the open field. *Biol Psychiatry*. 2006;60(3):288–295.
10. **Benmansour S**, **Arroyo LD**, **Frazer A**. Comparison of the antidepressant-like effects of estradiol and that of selective serotonin reuptake inhibitors in middle-aged ovariectomized rats. *Front Aging Neurosci*. 2016;8:311.
11. **Kristjansdottir HL**, **Lewerin C**, **Lerner UH**, **et al**. High serum serotonin predicts increased risk for hip fracture and nonvertebral osteoporotic fractures: the MrOS Sweden Study. *J Bone Miner Res*. 2018;33(9):1560–1567.
12. **Tanaka K**, **Hirai T**, **Ishibashi Y**, **Izumo N**, **Togari A**. Modulation of osteoblast differentiation and bone mass by 5-HT2A receptor signaling in mice. *Eur J Pharmacol*. 2015;762:150–157.
13. **Collet C**, **Schiltz C**, **Geoffroy V**, **Maroteaux L**, **Launay JM**, **de Vernejoul MC**. The serotonin 5-HT2B receptor controls bone mass via osteoblast recruitment and proliferation. *FASEB J*. 2008;22(2):418–427.
14. **Daut RA**, **Fonken LK**. Circadian regulation of depression: a role for serotonin. *Front Neuroendocrinol*. 2019;54:100746.
15. **Tian Y**, **Ming J**. The role of circadian rhythm in osteoporosis; a review. *Front Cell Dev Biol*. 2022;10:960456.
16. **Everts HB**, **Silva KA**, **Schmidt AN**, **et al**. Estrogen regulates the expression of retinoic acid synthesis enzymes and binding proteins in mouse skin. *Nutr Res*. 2021;94:10–24.
17. **Saki F**, **Sadeghian F**, **Kasaee SR**, **Koohpeyma F**, **Ranjbar Omrani GH**. Effect of prolactin and estrogen on the serum level of 1,25-dihydroxy vitamin D and FGF23 in female rats. *Arch Gynecol Obstet*. 2020;302(1): 265–271.
18. **Muhammad N**, **Luke DA**, **Shuid AN**, **Mohamed N**, **Soelaiman IN**. Two different isomers of vitamin e prevent bone loss in postmenopausal osteoporosis rat model. *Evid Based Complement Alternat Med*. 2012;2012: 161527.
19. **Ilesanmi-Oyelere BL**, **Brough L**, **Coad J**, **Roy N**, **Kruger MC**. The relationship between nutrient patterns and bone mineral density in postmenopausal women. *Nutrients*. 2019;11(6):1262.
20. **Somekawa Y**, **Chigughi M**, **Harada M**, **Ishibashi T**. Use of vitamin K2 (menatetrenone) and 1,25-dihydroxyvitamin D3 in the prevention of bone loss induced by leuprolide. *J Clin Endocrinol Metab*. 1999;84(8): 2700–2704.
21. **Prynne CJ**, **Mishra GD**, **O'Connell MA**, **et al**. Fruit and vegetable intakes and bone mineral status: a cross sectional study in 5 age and sex cohorts. *Am J Clin Nutr*. 2006;83(6):1420–1428.
22. **Patrick RP**, **Ames BN**. Vitamin D hormone regulates serotonin synthesis. Part 1: Relevance for autism. *FASEB J*. 2014;28(6):2398–2413.
23. **Bosin TR**, **Schaltenbrand SL**. Stimulation of platelet serotonin transport by substituted 1,4-naphthoquinone-induced oxidant stress. *Biochem Pharmacol*. 1991;41(6–7):967–974.
24. **Guo M**, **Zhu J**, **Yang T**, **et al**. Vitamin A improves the symptoms of autism spectrum disorders and decreases 5-hydroxytryptamine (5-HT): a pilot study. *Brain Res Bull*. 2018;137:35–40.
25. **Cordero K**, **Coronel GG**, **Serrano-Illán M**, **Cruz-Bracero J**, **Figueroa JD**, **De León M**. Effects of dietary vitamin E supplementation in bladder function and spasticity during spinal cord injury. *Brain Sci*. 2018;8(3):38.
26. **Tasatargil A**, **Cadir B**, **Dalaklioglu S**, **Yurdakonar E**, **Caglar S**, **Turkay C**. Effects of vitamin K1 supplementation on vascular responsiveness and oxidative stress in a rat femoral osteotomy model. *Cell Biochem Funct*. 2007;25(5):485–490.

27. **Uemura K**, **Otake Y**, **Takashima K**, **et al**. Development and validation of an open-source tool for opportunistic screening of osteoporosis from hip CT images. *Bone Joint Res*. 2023;12(9):590–597.
28. **Sainio H**, **Rämö L**, **Reito A**, **Silvasti-Lundell M**, **Lindahl J**. Prediction of fracture nonunion leading to secondary surgery in patients with distal femur fractures. *Bone Jt Open*. 2023;4(8):584–593.
29. **Kanis JA**, **McCloskey E**, **Johansson H**, **Oden A**, **Leslie WD**. FRAX(®) with and without bone mineral density. *Calcif Tissue Int*. 2012;90(1):1–13.
30. **Compston J**. FRAX--Where are we now? *Maturitas*. 2015;82(3):284–287.
31. **Lin L-P**, **Lai W-J**, **Hsu S-W**, **Lin J-D**. Early osteoporosis risks and associated factors among caregivers working in disability institutions: IOF one-minute osteoporosis risk check. *Int J Environ Res Public Health*. 2020;17(9):3319.
32. **Kharroubi A**, **Saba E**, **Ghannam I**, **Darwish H**. Evaluation of the validity of osteoporosis and fracture risk assessment tools (IOF One Minute Test, SCORE, and FRAX) in postmenopausal Palestinian women. *Arch Osteoporos*. 2017;12(1):6.
33. **Subramaniam S**, **Chan CY**, **Soelaiman IN**, **et al**. The performance of osteoporosis self-assessment tool for Asians (OSTA) in identifying the risk of osteoporosis among Malaysian population aged 40 years and above. *Arch Osteoporos*. 2019;14(1):117.
34. **Ma Z**, **Yang Y**, **Lin J**, **et al**. BFH-OST, a new predictive screening tool for identifying osteoporosis in postmenopausal Han Chinese women. *Clin Interv Aging*. 2016;11:1051–1059.
35. **Watanabe N**, **Miyatake K**, **Takada R**, **et al**. The prevalence and treatment of osteoporosis in patients undergoing total hip arthroplasty and the levels of biochemical markers of bone turnover. *Bone Joint Res*. 2022;11(12):873–880.
36. **Liu YD**, **Liu JF**, **Liu B**. N,N-Dimethylformamide inhibits high glucose-induced osteoporosis via attenuating MAPK and NF-κB signalling. *Bone Joint Res*. 2022;11(4):200–209.
37. **Wang X**, **Jiang W**, **Pan K**, **Tao L**, **Zhu Y**. Melatonin induces RAW264.7 cell apoptosis via the BMAL1/ROS/MAPK-p38 pathway to improve postmenopausal osteoporosis. *Bone Joint Res*. 2023;12(11):677–690.
38. **Zhang D-M**, **Cui D-X**, **Xu R-S**, **et al**. Phenotypic research on senile osteoporosis caused by SIRT6 deficiency. *Int J Oral Sci*. 2016;8(2):84–92.
39. **Azharuddin MK**, **O'Reilly DSJ**, **Gray A**, **Talwar D**. HPLC method for plasma vitamin K1: Effect of plasma triglyceride and acute-phase response on circulating concentrations. *Clin Chem*. 2007;53(9):1706–1713.
40. **Moons KGM**, **Altman DG**, **Reitsma JB**, **et al**. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): explanation and elaboration. *Ann Intern Med*. 2015;162(1):W1–73.
41. **Scurfield BK**. Multiple-event forced-choice tasks in the theory of signal detectability. *J Math Psychol*. 1996;40(3):253–269.
42. **Noll S**, **Furrer R**, **Reiser B**, **Nakas CT**. Inference in receiver operating characteristic surface analysis via a trinormal model-based testing approach. *Stat*. 2019;8(1):e249.
43. **Noll S**, **FurrerR**. trinROC: Statistical Tests for Assessing Trinormal ROC Data. R package version 0.5.2. 2022. https://www.math.uzh.ch/pages/trinROC/ (date last accessed 23 January 2025).
44. **Karsenty G**, **Yadav VK**. Regulation of bone mass by serotonin: molecular biology and therapeutic implications. *Annu Rev Med*. 2011;62:323–331.
45. **Wang Q**, **Chen D**, **Nicholson P**, **et al**. The associations of serum serotonin with bone traits are age- and gender-specific. *PLoS One*. 2014;9(10):e109028.
46. **Karege F**, **Widmer J**, **Bovier P**, **Gaillard JM**. Platelet serotonin and plasma tryptophan in depressed patients: effect of drug treatment and clinical outcome. *Neuropsychopharmacol*. 1994;10(3):207–214.
47. **Wei Q-S**, **Chen Z-Q**, **Tan X**, **et al**. Serum serotonin concentration associated with bone mineral density in Chinese postmenopausal women. *Scand J Clin Lab Invest*. 2017;77(1):40–44.
48. **Mödder UI**, **Achenbach SJ**, **Amin S**, **Riggs BL**, **Melton LJ 3rd**, **Khosla S**. Relation of serum serotonin levels to bone density and structural parameters in women. *J Bone Miner Res*. 2010;25(2):415–422.
49. **Yadav VK**, **Balaji S**, **Suresh PS**, **et al**. Pharmacological inhibition of gut-derived serotonin synthesis is a potential bone anabolic treatment for osteoporosis. *Nat Med*. 2010;16(3):308–312.
50. **Bliziotes M**, **Eshleman A**, **Burt-Pichat B**, **et al**. Serotonin transporter and receptor expression in osteocytic MLO-Y4 cells. *Bone*. 2006;39(6):1313–1321.
51. **Gustafsson BI**, **Thommesen L**, **Stunes AK**, **et al**. Serotonin and fluoxetine modulate bone cell function in vitro. *J Cell Biochem*. 2006;98(1):139–151.
52. **Yadav VK**, **Ryu J-H**, **Suda N**, **et al**. Lrp5 controls bone formation by inhibiting serotonin synthesis in the duodenum. *Cell*. 2008;135(5):825–837.
53. **Opper C**, **Weiner N**, **Xü F**, **Adam W**, **Fruhstorfer H**, **Wesemann W**. Daily variations of functional parameters and density distribution in human blood platelets. *Chronobiol Int*. 1994;11(5):309–319.
54. **Wirz-Justice A**, **Lichtsteiner M**, **Feer H**. Diurnal and seasonal variations in human platelet serotonin in man. *J Neural Transm*. 1977;41(1):7–15.
55. **Vestergaard P**. Serotonin: a new potential risk factor for falls, low BMD, and fracture. *J Bone Miner Res*. 2018;33(9):1558–1559.
56. **Binkley NC**, **Krueger DC**, **Engelke JA**, **Foley AL**, **Suttie JW**. Vitamin K supplementation reduces serum concentrations of under-gamma-carboxylated osteocalcin in healthy young and elderly adults. *Am J Clin Nutr*. 2000;72(6):1523–1528.
57. **Torbergsen AC**, **Watne LO**, **Wyller TB**, **et al**. Vitamin K1 and 25(OH)D are independently and synergistically associated with a risk for hip fracture in an elderly population: a case control study. *Clin Nutr*. 2015;34(1):101–106.
58. **Finnes TE**, **Lofthus CM**, **Meyer HE**, **et al**. A combination of low serum concentrations of vitamins K1 and D is associated with increased risk of hip fractures in elderly Norwegians: a NOREPOS study. *Osteoporos Int*. 2016;27(4):1645–1652.
59. **Nakano T**, **Tsugawa N**, **Kuwabara A**, **Kamao M**, **Tanaka K**, **Okano T**. High prevalence of hypovitaminosis D and K in patients with hip fracture. *Asia Pac J Clin Nutr*. 2011;20(1):56–61.
60. **Booth SL**, **Centi A**, **Smith SR**, **Gundberg C**. The role of osteocalcin in human glucose metabolism: marker or mediator? *Nat Rev Endocrinol*. 2013;9(1):43–55.
61. **Palermo A**, **Tuccinardi D**, **D'Onofrio L**, **et al**. Vitamin K and osteoporosis: myth or reality? *Metabolism*. 2017;70:57–71.
62. **Booth SL**. Roles for vitamin K beyond coagulation. *Annu Rev Nutr*. 2009;29:89–110.
63. **Cosso R**, **Falchetti A**. Vitamin K and bone metabolism: the myth and the truth. *Expert Rev Precis Med Drug Dev*. 2016;1(3):301–317.
64. **Mishima E**, **Ito J**, **Wu Z**, **et al**. A non-canonical vitamin K cycle is a potent ferroptosis suppressor. *Nature*. 2022;608(7924):778–783.
65. **Zhang Z**, **Ji C**, **Wang YN**, **et al**. Maresin1 suppresses high-glucose-induced ferroptosis in osteoblasts via NRF2 activation in type 2 diabetic osteoporosis. *Cells*. 2022;11(16):2560.
66. **Li M**, **Yang N**, **Hao L**, **et al**. Melatonin inhibits the ferroptosis pathway in rat bone marrow mesenchymal stem cells by activating the PI3K/AKT/mTOR signaling axis to attenuate steroid-induced osteoporosis. *Oxid Med Cell Longev*. 2022;2022:8223737.
67. **Hagaman JT**, **Panos RJ**, **McCormack FX**, **et al**. Vitamin D deficiency and reduced lung function in connective tissue-associated interstitial lung diseases. *Chest*. 2011;139(2):353–360.
68. **Thompson M**, **Aitken D**, **Balogun S**, **Cicuttini F**, **Jones G**. Population vitamin D stores are increasing in Tasmania, and this is associated with less BMD loss over 10 years. *J Clin Endocrinol Metab*. 2021;106(8):e2995–e3004.
69. **Abe S**, **Kashii M**, **Shimada T**, **et al**. Relationship between distal radius fracture severity and 25-hydroxyvitamin-D level among perimenopausal and postmenopausal women. *Bone Jt Open*. 2022;3(3):261–267.
70. **Dong W**, **Postlethwaite BC**, **Wheller PA**, **et al**. Beta-caryophyllene prevents the defects in trabecular bone caused by Vitamin D deficiency through pathways instated by increased expression of klotho. *Bone Joint Res*. 2022;11(8):528–540.
71. **Trajanoska K**, **Morris JA**, **Oei L**, **et al**. Assessment of the genetic and clinical determinants of fracture risk: genome wide association and mendelian randomisation study. *BMJ*. 2018;362:k3225.
72. **Bouillon R**, **Manousaki D**, **Rosen C**, **Trajanoska K**, **Rivadeneira F**, **Richards JB**. The health effects of vitamin D supplementation: evidence from human studies. *Nat Rev Endocrinol*. 2022;18(2):96–110.
73. **Navarro-Valverde C**, **Caballero-Villarraso J**, **Mata-Granados JM**, **et al**. High serum retinol as a relevant contributor to low bone mineral density in postmenopausal osteoporotic women. *Calcif Tissue Int*. 2018;102(6):651–656.
74. **Ballew C**, **Galuska D**, **Gillespie C**. High serum retinyl esters are not associated with reduced bone mineral density in the third national

health and nutrition examination survey, 1988-1994. *J Bone Miner Res*. 2001;16(12):2306–2312.
75. **Zhang J**, **Hu X**, **Zhang J**. Associations between serum vitamin E concentration and bone mineral density in the US elderly population. *Osteoporos Int*. 2017;28(4):1245–1253.
76. **Hampson G**, **Edwards S**, **Sankaralingam A**, **et al**. Circulating concentrations of vitamin E isomers: association with bone turnover and arterial stiffness in post-menopausal women. *Bone*. 2015;81:407–412.
77. **Holvik K**, **Gjesdal CG**, **Tell GS**, **et al**. Low serum concentrations of alpha-tocopherol are associated with increased risk of hip fracture. A NOREPOS study. *Osteoporos Int*. 2014;25(11):2545–2554.
78. **Johansson S**, **Melhus H**. Vitamin A antagonizes calcium response to vitamin D in man. *J Bone Miner Res*. 2001;16(10):1899–1905.
79. **Traber MG**. Vitamin E and K interactions--a 50-year-old problem. *Nutr Rev*. 2008;66(11):624–629.
80. **Huiberts LM**, **Smolders KCHJ**. Effects of vitamin D on mood and sleep in the healthy population: interpretations from the serotonergic pathway. *Sleep Med Rev*. 2021;55:101379.
81. **Sabir MS**, **Haussler MR**, **Mallick S**, **et al**. Optimal vitamin D spurs serotonin: 1,25-dihydroxyvitamin D represses serotonin reuptake transport (*SERT*) and degradation (*MAO-A*) gene expression in cultured rat serotonergic neuronal cell lines. *Genes Nutr*. 2018;13:19.
82. **Sauerbier I**, **von Mayersbach H**. Circadian variation of serotonin levels in human blood. *Horm Metab Res*. 1976;8(2):157–158.
83. **Collins FS**, **Varmus H**. A new initiative on precision medicine. *N Engl J Med*. 2015;372(9):793–795.
84. **König IR**, **Fuchs O**, **Hansen G**, **von Mutius E**, **Kopp MV**. What is precision medicine? *Eur Respir J*. 2017;50(4):1700391.
85. **Yeramosu T**, **Ahmad W**, **Bashir A**, **Wait J**, **Bassett J**, **Domson G**. Predicting five-year mortality in soft-tissue sarcoma patients. *Bone Joint J*. 2023;105-B(6):702–710.
86. **Sun Z**, **Liu W**, **Liu H**, **et al**. A new prognostic nomogram for heterotopic ossification formation after elbow trauma: the Shanghai post-Traumatic Elbow Heterotopic Ossification Prediction (STEHOP) model. *Bone Joint J*. 2022;104-B(8):963–971.
87. **Hijikata Y**, **Kamitani T**, **Nakahara M**, **et al**. Development and internal validation of a clinical prediction model for acute adjacent vertebral fracture after vertebral augmentation: the AVA score. *Bone Joint J*. 2022;104-B(1):97–102.
88. **Dijkstra H**, **Oosterhoff JHF**, **van de Kuit A**, **et al**. Development of machine-learning algorithms for 90-day and one-year mortality prediction in the elderly with femoral neck fractures based on the HEALTH and FAITH trials. *Bone Jt Open*. 2023;4(3):168–181.
89. **Yeramosu T**, **Ahmad W**, **Satpathy J**, **Farrar JM**, **Golladay GJ**, **Patel NK**. Prediction of suitable outpatient candidates following revision total knee arthroplasty using machine learning. *Bone Jt Open*. 2023;4(6):399–407.

Author information

J. Wang, PhD, Orthopaedic Surgeon
H. Li, PhD, Orthopaedic Surgeon
Y. Meng, PhD, Orthopaedic Surgeon
W. Cao, PhD, Orthopaedic Surgeon
C. Gu, PhD, Orthopaedic Surgeon
L. Tao, PhD, Orthopaedic Surgeon
Department of Orthopedics, First Hospital of China Medical University, Shenyang, China.

L. Shan, PhD, Researcher, Department of Intelligent Computing, School of Intelligent Medicine, China Medical University, Shenyang, China.

J. Hang, PhD, Researcher, Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Beijing, China; National Clinical Research Center for Obstetrics and Gynecology, Beijing, China; Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China; Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Beijing, China.

J. Dai, PhD, Researcher, National Clinical Research Center for Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, China; Mass Spectrometry Precision Medicine Research Center, Department of Laboratory Medicine, The First Affiliated Hospital of China Medical University, Shenyang, China; Laboratory Medicine Innovation Unit, Chinese Academy of Medical Sciences, Shenyang, China.

Author contributions

J. Wang: Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Data curation.
L. Shan: Conceptualization, Supervision, Visualization, Writing – review & editing.
J. Hang: Funding acquisition, Writing – review & editing.
H. Li: Visualization, Writing – review & editing, Data curation.
Y. Meng: Visualization, Writing – review & editing.
W. Cao: Visualization, Writing – review & editing.
C. Gu: Visualization, Writing – review & editing.
J. Dai: Conceptualization, Writing – review & editing, Supervision.
L. Tao: Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing.

J. Wang, L. Shan, and J. Hang contributed equally to this work.

Funding statement

The authors disclose receipt of the following financial or material support for the research, authorship, and/or publication of this article: funding from the Beijing Nova Program (20220484160), as reported by J. Hang.

ICMJE COI statement

J. Hang reports funding from the Beijing Nova Program (20220484160), related to this study.

Data sharing

The data that support the findings for this study are available to other researchers from the corresponding author upon reasonable request.

Acknowledgements

The authors wish to thank all the participants for their invaluable contribution to the study.

Ethical review statement

This study was approved by the Ethics Committee of the First Hospital of China Medical University ([2020]-248).

Open access funding

The authors report that the open access funding for their manuscript was self-funded.