

Original Article

Physical performance and life quality in postmenopausal women supplemented with vitamin D: a two-year prospective study

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Aim: To investigate the effects of calcium and vitamin D supplementation on bone turnover marker levels, muscle strength and quality of life in postmenopausal Chinese women.

Methods: A total of 485 healthy postmenopausal Chinese women (63.44±5.04 years) were enrolled in this open-label, 2-year, prospective, community-based trial. The participants were divided into group A, B, C, which were treated with calcium (600 mg/d) alone, calcium (600 mg/d) and cholecalciferol (800 IU/d) or calcium (600 mg/d) and calcitriol (0.25 µg/d), respectively, for 2 years. Serum levels of 25-hydroxyvitamin D, parathyroid hormone, β-CTX and P1NP were measured, and the muscle strength and quality of life were assessed at baseline and at 12- and 24-month follow-ups.

Results: Four hundred and sixty one participants completed this study. Serum levels of 25-hydroxyvitamin D were significantly increased in group C, but not changed in groups A and B at 24-month follow-up. Serum levels of parathyroid hormone, bone turnover marker β-CTX and bone formation marker P1NP were significantly decreased in group C, while serum levels of β-CTX were increased in group A at 24-month follow-up. The participants in group C maintained the grip strength, while those in groups A and B exhibited decreased grip strength at 24-month follow-up. The quality of life for the participants in groups B and C remained consistent, but that in group A was deteriorated at 24-month follow-up.

Conclusion: Supplementation with calcitriol and calcium modifies the bone turnover marker levels, and maintains muscle strength and quality of life in postmenopausal Chinese women, whereas supplementation with cholecalciferol and calcium prevents aging-mediated deterioration in quality of life.

Keywords: postmenopausal women; cholecalciferol; calcitriol; 25-hydroxyvitamin D; parathyroid hormone; β-CTX; P1NP; bone turnover; muscle strength; quality of life

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Introduction

Aging in elderly people, especially in postmenopausal women, is accompanied by reductions in muscle strength, physical performance and quality of life. Several studies have demonstrated that low serum 25(OH)D levels correlate to poor physical performance and decreased physical activity^[1–5]. Although inconsistently, previous studies have suggested that vitamin D supplementation results in less severe functional limitations, fewer falls and fractures and enhanced mental health^[6–11]. Huang *et al* demonstrated that vitamin D supplementation

[1200 IU daily if serum 25(OH)D levels are insufficient (from 20 to 29 ng/mL) or 50 000 IU weekly if serum 25(OH)D levels are deficient (<20 ng/mL)] improved quality of life, pain and sleep in individuals with chronic pain^[12]. The positive effects of vitamin D supplementation on muscle strength, physical performance and quality of life have been attributed to the influence of vitamin D on myocyte and neuron metabolism and function^[13–16]. To the best of our knowledge, relatively few studies have focused on the effects of vitamin D and calcium supplementation on the physical performance and quality of life in Asian populations, especially in the Chinese population. The primary objective of this 2-year prospective trial was to evaluate the effects of calcium supplementation in combination with either cholecalciferol or calcitriol on bone turnover

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marker levels, muscle strength, physical performance and quality of life in postmenopausal Chinese women in Shanghai.

Materials and methods

Study subjects

The study design was a 2-year, prospective, open-label clinical trial that was aimed at investigating the effect of calcium (600 mg/d) supplementation in combination with either cholecalciferol (800 IU/d) or calcitriol (0.25 µg/d) on bone turnover marker levels, muscle strength, physical performance and quality of life in postmenopausal Chinese women.

Community-dwelling postmenopausal women were recruited from communities in the Xuhui region of Shanghai. Potentially eligible participants were called by telephone and were persuaded to visit the hospitals in their corresponding districts to be screened based on the inclusion and exclusion criteria. Women who met the following primary inclusion criteria were considered: 1) ambulatory, community-dwelling and postmenopausal; 2) capable of independently completing the tests involved in the study; and 3) normal blood counts, normal results on liver and kidney function tests and normal serum levels of calcium, phosphorus, alkaline phosphatase (ALP), and parathyroid hormone (PTH). The exclusion criteria were as follows: 1) the presence of a disease deemed to affect vitamin D metabolism, such as cancer, hyperthyroidism, diabetes mellitus, primary hyperparathyroidism or pituitary, adrenal or rheumatic disease; 2) vitamin D and/or calcium supplementation within the prior 3 months; and 3) administration of medications that increase or decrease bone, muscle or vitamin D metabolism, including bisphosphonate, raloxifene, calcitonin, parathyroid hormone, hormone replacement drugs, thiazolidinedione, antiepileptics, aromatase inhibitors, and glucocorticoids. A total of 485 healthy postmenopausal Chinese females of Han ethnicity with a mean age of 63.44±5.04 years met the inclusion and exclusion criteria and were enrolled in this study. This study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. All of the participants signed informed consent forms prior to entering the study.

Anthropometry

Both at baseline and after 12 and 24 months of treatment, the body weight and height of each participant were measured while they were wearing a light gown and no shoes using a SK-CK Ultrasonic Body Scale. Body mass index (BMI) was calculated as body weight in kg divided by height in m².

Groups and treatment

The participants were separated into 3 groups: A) supplementation with calcium only, B) supplementation with calcium and cholecalciferol, and C) supplementation with calcium and calcitriol. For 2 years, cholecalciferol (Cholecalciferol, Xingsha Pharmaceuticals Co, Xiamen, China) was administered at 800 IU/d, and calcitriol (Calcitriol Soft Capsules, CP Haier Pharmaceuticals Co, Qingdao, China) was administered at 0.25 µg/d. Each of the subjects received 1 pill/day of Caltrate

D (containing 600 mg of calcium and 125 IU of vitamin D₃, Wyeth Pharmaceuticals, Suzhou, China) for elemental calcium supplementation. Because Caltrate D contained 125 IU of vitamin D₃, group B received 925 IU/d of cholecalciferol in total. All of the subjects were instructed to take the drugs and to perform physical training daily during the 24-month study period.

Serum collection and measurement

Fasting blood samples were collected at baseline and at the 12-month and 24-month follow-ups to measure serum levels of calcium, phosphate, albumin, cholesterol, triglycerides (TG), blood urea nitrogen (BUN), creatinine (Cr), alanine aminotransferase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (γ-GT), ALP, intact PTH (iPTH), beta C-terminal cross-linked telopeptides of type I collagen (β-CTX), amino-terminal propeptide of type I collagen (P1NP), osteocalcin (OC) and 25(OH)D. The serum levels of 25(OH)D, PTH, β-CTX, OC and P1NP were measured using an automated Roche electro-chemiluminescence system (E170, Roche Diagnostic GmbH, Mannheim, Germany). All of the biochemical markers were measured in one batch at the same time point using a single lot of reagents according to both the manufacturer's instructions and specialized laboratory-quality assay procedures. The intra-assay coefficients of variation (CVs) for 25(OH)D were 5.7% at a level of 25.2 ng/mL, 5.7% at a level of 39.9 ng/mL and 5.4% at a level of 65.6 ng/mL. The inter-assay CVs for 25(OH)D were 9.9% at a level of 25.2 ng/mL, 7.3% at a level of 39.9 ng/mL and 6.9% at a level of 65.6 ng/mL. The lower limit of detection of 25(OH)D was 4 ng/mL (10 mmol/L)^[17]. The intra- and inter-assay CVs were 1.4% and 2.9% for PTH, 2.5% and 3.5% for β-CTX, 2.9% and 4.0% for OC, and 2.3% and 2.8% for P1NP, respectively^[18].

Muscle strength and physical performance measurements

Muscle strength and physical performance were assessed at baseline and at the 12-month and 24-month follow-ups. Handgrip strength was calculated using a Jamar Hand dynamometer (5030 J1, Sammons Preston, Inc, Bolingbrook, IL, USA). Three measurements were recorded from each hand, and the average values of the right and left hands were used for analysis. A chair-rising test was performed as follows: the participants were asked to stand up from a chair with folded arms five times as quickly as possible, and the time required to stand was recorded.

Quality of life assessment

To assess changes in health-related quality of life, a Short Form 36 Health Survey (SF-36) questionnaire was used after being translated into Chinese^[19]. All of the participants were asked to respond to 36 questions, 35 of which are compressed into eight multi-item scales: physical functioning (PF), role-physical (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role-emotional (RE) and mental health (MH). The study participants completed the questionnaires both at baseline and after 12 and 24 months of intervention.

Statistical analysis

Statistical analyses were performed using the statistical package SPSS version 19.0 (SPSS, Inc, Chicago, IL, USA). The data were assessed for normality, and data transformation was applied whenever appropriate. Continuous variables are presented as the mean±SD. Data that were not normally distributed are presented as medians and inter-quartile ranges. ANOVA, chi-square tests and appropriate nonparametric tests were used to detect differences in demographic and lifestyle characteristics, health conditions, physical performance, and biochemical marker levels between the groups. These tests were also used to examine differences in absolute changes in physical performance and in biochemical marker levels between baseline and the 12- or 24-month follow-ups. $P < 0.05$ was considered significant.

Results

Figure 1 presents a flowchart describing the study. A total of 485 postmenopausal women who presented normal indicators of liver and kidney function and blood calcium and phosphorus levels were included in the study and were separated into three groups. Nine subjects withdrew from the study to receive anti-osteoporosis treatment. Two subjects were lost to follow-up because they changed their addresses. Twelve subjects failed to complete the study for unknown reasons. Ultimately, 461 participants completed the study. At the

12-month and 24-month follow-ups, the serum concentrations of calcium, phosphorous, and PTH and the results of liver and kidney function in the three groups all remained within normal ranges. No obvious adverse events were reported over the course of the study.

Baseline characteristics

The baseline characteristics of the subjects in the three groups are summarized in Table 1. No significant differences were detected in the mean ages of the three groups (63.22±4.90 years, 64.34±4.93 years and 63.35±5.99 years in groups A, B and C, respectively). No significant differences in any of the baseline characteristics were detected between the three groups, except for serum concentrations of calcium, β -CTX, and 25(OH)D, handgrip strength, time to complete the chair rising test and SF-36 questionnaire scores (Table 1). The subjects in group A exhibited higher handgrip strength and SF-36 scores than those in the other two groups. The median serum 25(OH)D level in group C was 20.90 (17.83–27.88) and was higher than that of the other groups, which was 20.03 (16.01–24.15) in group A and 20.63 (16.39–24.45) in group B.

Changes in serum vitamin D and bone turnover marker levels following the different interventions

Serum levels of β -CTX significantly increased in group A, from 370.00 ng/mL (254.00–491.75) at baseline to 417.00 ng/mL

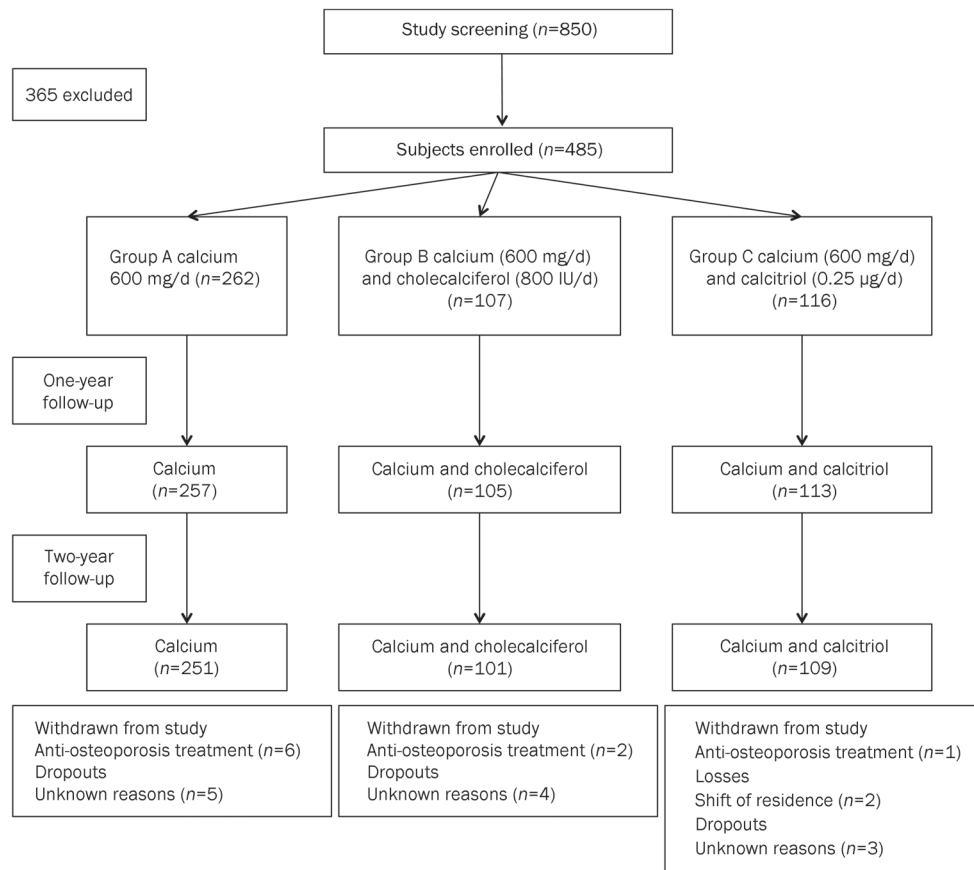


Figure 1. Flowchart describing the study.

Table 1. Characteristics of subjects at baseline for each group. Continuous variables are presented as the means±SD. Non-normally distributed data are presented as the medians and inter-quartile ranges (in parentheses). ^b*P*<0.05 vs group A. ^e*P*<0.05 vs group B.

Characteristic	Group A (n=262) calcium (600 mg/d)	Group B (n=107) calcium (600 mg/d) and cholecalciferol (800 IU/d)	Group C (n=116) calcium (600 mg/d) and calcitriol (0.25 µg/d)
Age (year)	63.22±4.90	64.34±4.93	63.35±5.99
Height (m)	1.54±0.05	1.54±0.05	1.55±0.05
Weight (kg)	59.08±9.25	58.20±9.34	59.50±8.46
BMI (kg/m ²)	24.75±3.36	24.54±3.52	24.69±3.32
Age at menarche (year)	15.80±1.71	15.40±1.82	15.27±1.90
Age at menopause (year)	50.53±3.29	50.22±3.61	49.82±3.25
Calcium (mmol/L)	2.43±0.10	2.38±0.08 ^b	2.40±0.09 ^b
Phosphorous (mmol/L)	1.08±0.11	1.11±0.12	1.10±1.12
ALP (U/L)	81.49±20.14	82.21±15.11	80.18±18.54
BUN (mmol/L)	4.94±1.21	4.81±1.19	5.02±1.21
Cr (µmol/L)	60.45±9.44	59.93±9.16	59.59±10.30
P1NP (ng/mL)	52.98 (41.40–66.96)	55.93 (40.90–70.51)	52.00 (41.50–72.31)
β-CTX (ng/L)	370.00 (254.00–491.75)	402.00 (304.00–500.00)	451.00 (299.00–548.00) ^e
OC (ng/mL)	17.96 (15.14–21.85)	19.46 (15.53–22.92)	
PTH (pg/mL)	32.79 (25.77–41.59)	34.25 (25.79–40.82)	35.61 (27.58–45.24)
25(OH)D (ng/mL)	20.03 (16.01–24.15)	20.63 (16.39–24.45)	20.90 (17.83–27.88) ^{be}
Grip strength (kg)			
Left hand	22.79±4.75	20.27±5.30 ^b	20.06±5.34 ^b
Right hand	24.34±50.2	21.60±5.39 ^b	21.25±4.77 ^b
Chair rising test (s)	7.24±1.94	6.18±1.29 ^b	6.18±1.17 ^b
SF-36			
PF	95.00 (85.00–95.00)	80.00 (70.00–90.00) ^b	90.00 (75.00–95.00) ^b
RP	100.00 (100.00–100.00)	100.00 (50.00–100.00) ^b	100.00 (75.00–100.00) ^b
BP	93.24 (68.82–99.90)	79.92 (56.61–93.24) ^b	81.03 (56.61–99.90) ^b
GH	72.00 (52.00–80.00)	60.00 (45.00–70.00) ^b	65.00 (54.25–77.90) ^e
VT	80.00 (70.00–90.00)	65.00 (50.00–75.00) ^b	70.00 (65.00–85.00) ^{be}
SF	100.00 (75.00–100.00)	62.50 (50.00–100.00) ^b	87.50 (62.50–100.00) ^{be}
RE	99.80 (99.80–99.80)	99.80 (99.80–99.80) ^b	99.80 (99.80–99.80) ^b
MH	84.00 (76.00–100.00)	76.00 (68.00–88.00) ^b	76.00 (68.00–88.00) ^b

PF=Physical Functioning; RP=Role-Physical; BP=Bodily pain; GH=General Health; VT=Vitality; SF=Social Functioning; RE=Role-Emotional; MH=Mental Health.

(327.75–535.25) at the 24-month follow-up, whereas serum levels of PTH, P1NP and β-CTX significantly decreased in group C at the 12- and 24-month follow-ups (all *P*<0.05, Table 2, 4). No differences in serum levels of P1NP or β-CTX were observed between baseline and 24-month follow-up in group B. All of the levels of serum bone turnover markers that were measured were within a normal range.

In group C, the serum levels of 25(OH)D increased from 20.90 ng/mL (17.83–27.88) at baseline to 26.44 ng/mL (21.29–32.29) at the 24-month follow-up (*P*<0.05, Table 4). However, serum 25(OH)D levels were not significantly different between baseline and after the intervention in either group A or group B (*P*>0.05, Table 2, 3).

Changes in muscle strength and physical performance

Muscle strength and physical performance were significantly decreased at the 12- and 24-month follow-ups only in group A, as demonstrated by reduced grip strength in both hands and longer time of completion on the chair rising test. The

grip strength of the left hand was 22.79±4.75 kg at baseline and 21.04±4.18 kg after 24 months (*P*<0.05). The grip strength of the right hand was 24.34±5.02 kg at baseline and 23.45±4.98 kg and 23.41±4.44 kg after 12 and 24 months, respectively (*P*<0.05). The time of completion on the chair rising test was 7.24±1.94 s at baseline and 7.78±1.98 s and 7.82±2.19 s after 12 and 24 months, respectively (Table 2). The participants in group B exhibited no significant changes in muscle strength or physical performance after 12 months, as measurements of mean handgrip strength and completion times on the chair rising test were comparable with those that were taken at baseline (*P*>0.05). However, handgrip strength strikingly decreased, and the time of completion on the chair rising test significantly increased from 6.18±1.29 s at baseline to 6.82±2.54 s (*P*<0.05) at the 24-month follow-up (Table 3). The subjects in group C exhibited no significant changes in handgrip strength, although the completion time on the chair rising test was significantly increased (baseline: 6.18±1.17 s; 12 months: 7.06±1.17 s; 24 months: 7.78±2.01 s; *P*<0.05) at both

Table 2. Changes in biomarker levels, physical performance and SF-36 scores in group A. Continuous variables are presented as the means±SD. Non-normally distributed data are presented as the medians and inter-quartile ranges (in parentheses). ^b*P*<0.05 vs baseline. ^e*P*<0.05 vs 12-month follow-up.

	Baseline (n=262)	12 months (n=257)	24 months (n=251)
Height (m)	1.54±0.05	1.54±0.05	1.54±0.05
Weight (kg)	59.08±9.25	59.49±9.85	60.44±9.45
BMI (kg/m ²)	24.75±3.36	24.99±3.65	25.15±3.54
Calcium (mmol/L)	2.43±0.10	2.22±0.10 ^b	2.31±0.11 ^{be}
Phosphorous (mmol/L)	1.08±0.11	0.94±0.15 ^b	1.20±0.16 ^{be}
ALP (U/L)	81.49±20.14	75.16±17.68 ^b	78.89±18.18 ^e
BUN (mmol/L)	4.94±1.21	4.67±1.15 ^b	4.77±1.17
Cr (μmol/L)	60.45±9.44	53.06±9.81 ^b	58.79±10.96 ^e
P1NP (ng/mL)	52.98 (41.4–66.96)	55.21 (44.48–67.86)	52.87 (41.89–66.68)
β-Ctx (ng/L)	370.00 (254.00–491.75)	417.00 (327.75–535.25) ^b	
OC (ng/mL)	17.96 (15.14–21.85)		18.81 (16.18–22.89)
PTH (pg/mL)	32.79 (25.77–41.59)	34.59 (26.55–42.29)	
25(OH)D (ng/mL)	20.03 (16.01–24.15)	19.56 (15.07–23.69)	
Grip strength (kg)			
Left hand	22.79±4.75	22.26±4.75	21.04±4.18 ^{be}
Right hand	24.34±5.02	23.45±4.98 ^b	23.41±4.44 ^b
Chair rising test (s)	7.24±1.94	7.78±1.98 ^b	7.82±2.19 ^b
SF-36			
PF	95.00 (85.00–95.00)	85.00 (65.00–95.00) ^b	85.00 (65.00–91.25) ^b
RP	100.00 (100.00–100.00)	100.00 (100.00–100.00)	100.00 (100.00–100.00)
BP	93.24 (68.82–99.90)	82.14 (57.72–99.9)	93.24 (57.72–99.00)
GH	72.00 (52.00–80.00)	62.00 (50.00–77.00)	62.00 (47.00–70.00) ^b
VT	80.00 (70.00–90.00)	75.00 (50.00–85.00) ^b	70.00 (60.00–85.00) ^b
SF	100.00 (75.00–100.00)	87.50 (50.00–100.00) ^b	87.50 (62.50–100.00) ^b
RE	99.80 (99.80–99.80)	99.80 (99.80–99.80)	99.80 (99.80–99.80)
MH	84.00 (76.00–100.00)	80.00 (68.00–91.00) ^b	80.00 (68.00–88.00) ^b

Abbreviations: PF=Physical Functioning; RP=Role-Physical; BP=Bodily pain; GH=General Health; VT=Vitality; SF=Social Functioning; RE=Role-Emotional; MH=Mental Health.

the 12-month and 24-month follow-ups (Table 4).

Changes in quality of life

In the current study, the subjects completed the SF-36 questionnaire to assess changes in health-related quality of life according to eight scales: PF, RP, BP, GH, VT, SF, RE and MH. In group A, the PF, VT, SF and MH scores were significantly decreased after 12 months, and the scores of all of the scales, except for RP, BP, and RE, were significantly reduced after 24 months compared to baseline (all *P*<0.05, Table 2). The mean PF, VT, SF, and MH scores of the participants in group B were significantly increased after 12 months (*P*<0.05). The mean PF, GH, VT and MH scores were lower after 24 months than after 12 months (all *P*<0.05) and were comparable with those at baseline (*P*>0.05, Table 3). In group C, none of the scores on any of the scales were significantly different after 12 or 24 months compared with baseline (all *P*>0.05), except for the SF score, which increased from 87.50 (62.50–100.00) at baseline to 100.00 (75.00–100.00) after 12 months (*P*<0.05, Table 4).

Discussion

Vitamin D deficiency has been recognized as a major public health concern worldwide. Circulating vitamin D₃ (cholecalciferol) is predominately synthesized in the skin from a cholesterol derivative, 7-dehydrocholesterol, under the effect of sunlight and its UV radiation. It is then metabolised in the liver by the enzyme 25-hydroxylase and then in the kidneys by 1-alpha-hydroxylase. Oral administration of vitamin D₃, cholecalciferol and its analogues alphacalciferol and calcitriol are used for the treatment of vitamin D deficiency. The average serum 25(OH)D level in the current study was 21.86±6.82 ng/mL, which is defined as vitamin D insufficient. It is well known that low serum levels of 25(OH)D are associated with a significant decrease in the absorption of intestinal calcium and may induce secondary hyperparathyroidism, which could potentially stimulate the transformation of pre-osteoclasts into mature osteoclasts and lead to osteoporosis and a consequent increase in the risk of fractures^[20]. Kuwabara *et al*^[21] demonstrated that daily supplementation of 200 mg calcium and 800 IU vitamin D₃ for one month significantly reduced serum PTH levels compared to daily supplementation of only 200 mg calcium in an institutionalized, elderly Japanese population. Our results indicated that serum PTH levels significantly decreased after supplementation with 0.25 μg/d calcitriol and 600 mg/d calcium for 12 and 24 months. However, the responses of serum PTH levels to cholecalciferol supplementation appear

ciferol) is predominately synthesized in the skin from a cholesterol derivative, 7-dehydrocholesterol, under the effect of sunlight and its UV radiation. It is then metabolised in the liver by the enzyme 25-hydroxylase and then in the kidneys by 1-alpha-hydroxylase. Oral administration of vitamin D₃, cholecalciferol and its analogues alphacalciferol and calcitriol are used for the treatment of vitamin D deficiency. The average serum 25(OH)D level in the current study was 21.86±6.82 ng/mL, which is defined as vitamin D insufficient. It is well known that low serum levels of 25(OH)D are associated with a significant decrease in the absorption of intestinal calcium and may induce secondary hyperparathyroidism, which could potentially stimulate the transformation of pre-osteoclasts into mature osteoclasts and lead to osteoporosis and a consequent increase in the risk of fractures^[20]. Kuwabara *et al*^[21] demonstrated that daily supplementation of 200 mg calcium and 800 IU vitamin D₃ for one month significantly reduced serum PTH levels compared to daily supplementation of only 200 mg calcium in an institutionalized, elderly Japanese population. Our results indicated that serum PTH levels significantly decreased after supplementation with 0.25 μg/d calcitriol and 600 mg/d calcium for 12 and 24 months. However, the responses of serum PTH levels to cholecalciferol supplementation appear

Table 3. Changes in biomarker levels, physical performance and SF-36 scores in group B. Continuous variables are presented as the means±SD. Non-normally distributed data are presented as the medians and inter-quartile ranges (in parentheses). ^bP<0.05 vs baseline. ^eP<0.05 vs 12-month follow-up.

	Baseline (n=107)	12 months (n=105)	24 months (n=101)
Height (m)	1.54±0.05	1.54±0.06	1.54±0.07
Weight (kg)	58.20±9.34	58.7±98.1	59.14±9.86
BMI (kg/m ²)	24.54±3.52	24.57±3.60	24.81±3.56
Calcium (mmol/L)	2.38±0.08	2.32±0.09 ^b	2.35±0.11 ^b
Phosphorus (mmol/L)	1.11±0.12	1.14±0.15	1.31±0.20 ^{be}
ALP (U/L)	82.21±15.11	74.96±19.27 ^b	79.27±21.49
BUN (mmol/L)	4.81±1.19	5.08±1.11	5.79±1.29 ^{be}
Cr (μmol/L)	59.93±9.16	62.36±10.56	62.51±10.60
P1NP (ng/mL)	55.93 (40.90–70.51)	53.53 (38.84–66.44)	55.25 (37.61–72.99)
β-CTX (ng/L)	402.00 (304.00–500.00)		379.00 (268.25–498.00)
OC (ng/mL)	19.46 (15.53–22.92)		18.78 (14.99–24.15)
PTH (pg/mL)	34.25 (25.79–40.82)		31.76 (26.06–40.70)
25(OH)D (ng/mL)	20.63 (16.39–24.45)		20.43 (16.04–25.15)
Grip strength (kg)			
Left hand	20.27±5.30	20.45±5.32	18.97±5.37 ^e
Right hand	21.60±5.39	21.92±4.99	19.87±5.52 ^{be}
Chair rising test (s)	6.18±1.29	6.50±1.91	6.82±2.54 ^b
SF-36			
PF	80.00 (70.00–90.00)	90.00 (80.00–95.00) ^b	85.00 (68.75–95.00) ^e
RP	100.00 (50.00–100.00)	100.00 (81.25–100.00)	100.00 (75.00–100.00)
BP	79.92 (56.61–93.24)	78.81 (56.61–99.9)	79.92 (45.51–93.24)
GH	60.00 (45.00–70.00)	61.00 (50.00–75.00)	55.00 (45.00–65.00) ^e
VT	65.00 (50.00–75.00)	75.00 (60.00–85.00) ^b	62.50 (50.00–75.00) ^e
SF	62.50 (50.00–100.00)	87.50 (75.00–100.00) ^b	87.50 (75.00–100.00) ^b
RE	99.80 (99.80–99.80)	99.80 (99.80–99.80)	99.80 (99.80–99.80)
MH	76.00 (68.00–88.00)	84.00 (72.00–92.00) ^b	80.00 (64.00–92.00) ^e

PF=Physical Functioning; RP=Role-Physical; BP=Bodily pain; GH=General Health; VT=Vitality; SF=Social Functioning; RE=Role-Emotional; MH=Mental Health.

to vary^[20, 22, 23]. In the present study, no changes were detected in serum PTH levels in subjects who were treated with 600 mg/d elemental calcium and 800 IU/d cholecalciferol. Patel *et al*^[24] examined the effect of cholecalciferol on bone metabolism in healthy adult women who ranged in age from 23 to 70 years (mean age 45.3 years). The results revealed that supplementation with 800 IU/d cholecalciferol for 12 months increased serum 25(OH)D levels, whereas no effects on serum PTH or bone turnover marker levels were detected. Serum levels of PTH are also influenced by dietary calcium intake, and the optimal dose of cholecalciferol supplementation is influenced by calcium intake; thus, this interdependence must be taken into account. With respect to bone resorption, high serum concentrations of β-CTX indicate increased bone resorption, bone fragility and fracture risk^[25]. Our results showed that serum levels of β-CTX significantly decreased after supplementation with 0.25 μg/d calcitriol and 600 mg/d calcium for 12 and 24 months. These results indicate that supplementation with 0.25 μg/d calcitriol effectively reduced bone resorption. Furthermore, the results of the current study revealed that serum levels of P1NP, a metabolic marker of bone formation, were significantly decreased after treatment with calcium and calcitriol for 24 months. Hurst *et al*^[26] found that women who

were deficient in vitamin D and who were administered 4000 IU of cholecalciferol daily for 6 months exhibited a suppression of age-induced bone turnover and bone resorption. However, our results did not indicate that cholecalciferol supplementation had any effect on the levels of bone turnover markers, which is consistent with data from previous studies^[21, 27]. These results might have occurred because the participants in our study received inadequate doses of vitamin D supplementation.

Sarcopenia was first defined in 1989 by Rosenberg as an age-related reduction in muscle or lean body mass^[28]. The techniques that are used to assess sarcopenia include BIA and DXA measurements for muscle mass, handgrip strength for muscle strength, the Short Physical Performance Battery (SPPB) for typical gait speed, and the get-up-and-go test for physical performance^[29]. The Leiden 85-plus Study examined Dutch individuals who were older than 85 years of age and found that low handgrip strength was a predictor of accelerated decline in activities of daily living (ADL) score and cognition^[30]. Menopausal status is considered to significantly affect the maintenance of muscle mass, and a transition into menopause has been associated with a reduction in muscle mass in women^[31]. In the participants of the present study, assess-

Table 4. Changes in biomarker levels, physical performance and SF-36 scores in group C. Continuous variables are presented as the means±SD. Non-normally distributed data are presented as the medians and inter-quartile ranges (in parentheses). ^bP<0.05 vs baseline. ^eP<0.05 vs 12-month follow-up.

	Baseline (n=116)	12 months (n=113)	24 months (n=109)
Height (m)	1.55±0.05	1.56±0.05	1.56±0.05
Weight (kg)	59.50±8.46	58.79±8.74	58.94±8.84
BMI (kg/m ²)	24.69±3.32	24.23±3.24	24.35±3.30
Calcium (mmol/L)	2.40±0.09	2.29±0.08 ^b	2.37±0.09 ^{be}
Phosphorous (mmol/L)	1.10±1.12	1.14±0.16 ^b	11.8±0.16 ^b
ALP (U/L)	80.18±18.54	71.68±21.27	90.75±56.53 ^{be}
BUN (mmol/L)	5.02±1.21	5.22±1.12	5.54±1.34 ^b
Cr (μmol/L)	59.59±10.30	62.94±0.53 ^b	60.55±13.39
β-CTX (ng/L)	451.00 (299.00–548.00)	312.00 (210.00–458.00) ^b	358.50 (274.50–476.75) ^b
P1NP (ng/mL)	52.00 (41.50–72.31)	41.31 (32.20–55.57) ^b	47.73 (35.78–62.29) ^b
PTH (pg/mL)	35.61 (27.58–45.24)	29.41 (23.09–36.18) ^b	28.13 (19.60–35.02) ^b
25(OH)D (ng/mL)	20.90 (17.83–27.88)	23.16 (18.97–28.42)	26.44 (21.29–32.29) ^{be}
Grip strength (kg)			
Left hand	20.06±5.34	20.93±4.52	19.85±4.40
Right hand	21.25±4.77	21.98±4.76	21.09±4.64
Chair rising test (s)	6.18±1.17	7.06±1.17 ^b	6.78±2.01 ^b
SF-36			
PF	90.00 (75.00–95.00)	90.00 (80.00–95.00)	85.00 (75.00–95.00)
RP	100.00 (75.00–100.00)	100.00 (100.00–100.00)	100.00 (75.00–100.00)
BP	81.03 (56.61–99.90)	82.14 (57.72–99.90)	79.92 (57.72–93.24)
GH	65.00 (54.25–77.90)	62.00 (52.00–77.00)	60.00 (47.00–72.00)
VT	70.00 (65.00–85.00)	75.00 (55.00–90.00)	65.00 (55.00–85.00)
SF	87.50 (62.50–100.00)	100.00 (75.00–100.00) ^b	87.50 (62.50–100.00) ^e
RE	99.80 (99.80–99.80)	99.38 (99.80–99.80)	66.50 (66.50–99.80)
MH	76.00 (68.00–88.00)	88.00 (76.00–96.00)	80.00 (60.00–88.00)

PF=Physical Functioning; RP=Role-Physical; BP=Bodily pain; GH=General Health; VT=Vitality; SF=Social Functioning; RE=Role-Emotional; MH=Mental Health.

ments of handgrip strength and chair rising tests were performed to measure muscle strength and physical performance, respectively. The subjects in group A exhibited significantly reduced muscle strength and physical performance over time, as demonstrated by reductions in grip strength in both hands and by increasingly long completion times on the chair rising test. These results indicated that muscle strength deteriorates and physical performance is reduced in postmenopausal women as they age. In a long-term study on healthy older men and women who exhibited serum 25(OH)D levels <30 ng/mL, daily supplementation with 800 IU vitamin D₃ and 1000 mg calcium significantly improved physical performance over a 12- to 20-month period compared to supplementation with calcium alone^[8]. In the current study, supplementation with cholecalciferol for 12 months or calcitriol for 12 or 24 months maintained baseline levels of handgrip strength. However, there were significant increases in the completion times of the chair rising test in all of the groups. The causative factor behind the lack of improvement in physical performance in the current study could be a gender-specific effect of vitamin D supplementation; an observational study of older Italians demonstrated a relationship between vitamin D status and measures of frailty in men but not in women^[4]. Additionally, in a study that focused on the effects of cholecalciferol

and calcium supplementation on skeletal muscle strength in males and females, a sex-stratified analysis revealed an improvement in handgrip strength in males but not in females^[32]. However, a study that was conducted in Hong Kong revealed that in older Chinese men with adequate levels of vitamin D [mean serum 25(OH)D level of 77.9 nmol/L] and high levels of baseline physical function, vitamin D status may not play an important role in physical function or muscle mass based on cross-sectional or longitudinal analysis^[33].

To assess the effects of vitamin D supplementation on the quality of life of our participants, the subjects completed SF-36 questionnaires both before and after the intervention. The responses that are provided in the PF, RP, BP, and GH scales of the SF-36 questionnaire reflect physical health. The responses to the RE, MH, SE, and VT scales of the questionnaire reflect mental health^[34]. In a cohort of subjects with fibromyalgia, vitamin D deficiency was reported to be associated with depression and anxiety^[35]. Jugdeep *et al*^[36] found no significant changes in the SF-36 scores of subjects who received a single intramuscular injection of 600 000 IU ergocalciferol, whereas a different study demonstrated an improvement in seasonal affective disorder as a result of vitamin D supplementation^[37]. In our study, there were significant decreases in PF, VT, SF, and MF scores on the SF-36 at the 12-

and 24-month follow-ups, indicating that a degeneration of health status occurs, especially with respect to mental health, in postmenopausal women who are supplemented with only 600 mg/d calcium. PF, VT, SF, and MH scores significantly increased after cholecalciferol supplementation for 12 months and were identical to baseline levels after 24 months, indicating that 800 IU/d cholecalciferol improved the health status of these subjects, particularly with respect to mental health. Calcitriol supplementation at 0.25 µg/d maintained comparable physical and mental health levels based on the scores on all of the scales of the SF-36 between baseline and after 12 months and 24 months of the intervention, except for a significant increase in SF at the 12-month follow-up. The results of our study demonstrated a beneficial effect of 800 IU/d vitamin D supplementation on quality of life, especially mental health, in postmenopausal women. The underlying mechanism might involve the permeation of the blood-brain barrier and the choroid plexus by the lipophilic molecule vitamin D via both active transport and passive diffusion facilitated by vitamin D-binding protein^[38]. *In situ*, vitamin D exerts its effects via vitamin D receptors, which are nuclear hormone receptors that are expressed in cerebral neurons. Importantly, vitamin D is considered a “neurosteroid hormone” due to its various neural activities in rodents, including its neurotrophic, immunoregulatory, and antioxidant effects^[14-16]. Moreover, Annweiler *et al*^[39] found that serum deficiency of 25(OH)D was associated with larger lateral cerebral ventricles; these findings provide a scientific basis for vitamin D replacement in older adults.

The strengths of this study included that it was a prospectively designed study that was conducted for 2 years and that the study sample was relative large, with most of the subjects (approximately 95%) completing the 2-year follow-up. However, there were also several limitations. First, the study sample was limited to women because no data on men were available. A previous study showed that there might be a sex-specific association between serum vitamin D levels and physical performance and muscle mass^[40]. Therefore, the effects of this supplementation on older men remain to be investigated. Second, serum levels of 25(OH)D and biochemical bone turnover markers (β-CTX) were not measured at the 12-month follow-up for groups A and B; therefore, the effects of treatment with calcium only or with calcium and cholecalciferol for 12 months on bone metabolism was not evaluated. Additionally, in this study, urinary calcium excretion was not measured in any of the groups.

Based on the results of this 2-year prospective study, we concluded that postmenopausal Chinese women exhibited significant decreases in muscle strength, physical performance and quality of life, which were accompanied by increased bone turnover marker levels, as they aged. Supplementation with 800 IU/d cholecalciferol and 600 mg/d calcium for 24 months maintained and even improved quality of life; however, this treatment exerted no significant effect on muscle strength, physical performance, bone turnover marker levels or PTH concentrations. After 24 months of intervention, supplementation with 0.25 µg/d calcitriol and 600 mg/d calcium exerted

a protective effect on muscle strength, physical performance and health status, along with a suppression of bone turnover marker levels and PTH.

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Author contribution

Li-hong GAO conducted statistical analysis and drafted the manuscript. Zhen-lin ZHANG and Ling XU conceived and designed the study and revised the manuscript. Wen-jun ZHU, Yu-juan LIU and Jie-mei GU performed the study. Ou WANG and Xiao-ping XING gave valuable suggestions on the performance of the study. All of the authors read and approved the final manuscript.

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