Original Article
Evaluation of the clinical efficacy of Zhuanggu Zhitong Recipe in treatment of postmenopausal osteoporosis and its correlation with the AMPK/mTOR autophagy signaling pathway

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Abstract: Objective: To evaluate the efficacy of bone strengthening and pain relieving capsules in the treatment of postmenopausal osteoporosis and its correlation with AMPK/mTOR autophagy signaling pathway. Methods: Between January 2021 and December 2022, 100 patients with postmenopausal osteoporosis of liver-kidney yin deficiency and qi stagnation and blood stasis type were retrospectively screened in the outpatient and inpatient departments of the Department of Orthopaedics and Traumatology of Liuzhou Hospital of Traditional Chinese Medicine, Guangxi Zhuang Autonomous Region, and were randomly divided into 2 groups of 50 patients each according to the order of the timing of the consultation, namely, the Strong Bone Pain Relief Capsules Treatment Group and the Alendronate Sodium Control Group. Bone mineral density of the left femur, Chinese medicine evidence points, serum AMPK level, E2 level before and after treatment, Ca^{2+} level, IL-17, serum phosphorus level, ALP, PTH, OC, PINP, NTX and TRAP-5b level before and after drug intervention were detected in the two groups. Results: Both groups achieved a lower TCM syndrome score, NRS score of low back pain, and AMPK level after treatment ($P<0.001$). Both groups had increased Ca^{2+} concentration and E2 level ($P<0.001$), and reduced IL-17 and serum P level ($P<0.001$). Both groups had significantly increased ALP, PTH, OC, PINP level, NTX and TRAP-5b level ($P<0.001$). The difference between the two groups was significant ($P<0.05$) and for TRAP-5b levels, ($P<0.001$). Efficacy for osteoporosis differed between the two groups ($P<0.05$). Conclusion: Strong Bone Pain Relief Capsules may negatively regulate the level of AMPK and increase the level of cellular autophagy so as to play a role in the improvement of osteoporosis.

Keywords: Evaluation, treatment, postmenopausal osteoporosis

Introduction

Postmenopausal osteoporosis (PMOP) is a common bone metabolic disease associated with aging, which is characterized by decreased bone mass, destruction of bone microstructure prone to fragility fracture [1], and is highly prevalent and harmful [2]. Various complications of postmenopausal osteoporosis affect women's healthy life and quality of life. Some studies have shown [3, 4] that the occurrence of postmenopausal osteoporosis is related to the imbalance of cellular autophagy, and autophagy and autophagy-related factors have a very important role in bone metabolic homeostasis, and among them, AMPK/mTOR autophagy sig-

naling pathway, which plays an important role in regulating the balance of bone metabolism, is one of the main converging points of multiple factors [5]. In the treatment of postmenopausal osteoporosis, there are many types of drugs used in western medicine. The most commonly used ones are calcitonin analogues, parathyroid hormone, and bisphosphonates [6], but the long-term use of these drugs has many side effects. A study has shown that ZGZT can also be used in the clinical treatment of sarcopenia and can effectively promote the formation of osteoblasts and inhibit the differentiation of stem cells into osteoclasts in the treatment of osteoporosis [7]. A study has shown that ZGZT can significantly improve the bone mineral den-

Table 1. TCM syndrome quantitative grading scale

Syndromes	None (0 point)	Mild (1 point)	Medium (2 points)	Severe (3 points)
Main symptoms				
Low back pain[a]	None	Visual analog scale for pain 1 to 3 minutes	Pain visual analog scale 4 to 6 points	Pain visual analog scale 7 to 10 points
Secondary symptoms				
Soreness and weakness in waist and knees[b]	None	Occasionally feeling soreness and weakness in waist and knees after walking (≥1000 m)	Feeling soreness and weakness in waist and knees after walking (300~1000 m)	Feeling soreness and weakness in waist and knees immediately after standing or walking (<300 m)
Cramps in lower limbs[b]	None	≤2 times per month	2 to 10 times a month	≥10 times per month
Hard steps[c]	None	Occasionally have difficulty walking, but no discomfort within 100 m	Difficulty walking short distances (10-100 m)	Difficulty walking, unable to exceed 10 m, or unable to stand
Difficult to be cautious[c]	None	Sober and weak	Difficult to be cautious	Unable to be prudent

Note: [a]The degree of pain is assessed by the patient under the guidance of a doctor, and the total score is calculated by ×3; [b]After the doctor and patient communicate with each other, the doctor will make an evaluation, and the total score will be calculated by ×2; [c]After the doctor and patient communicate with each other, the doctor will make an evaluation, and the score will be ×1 when calculating the total score.

sity of primary osteoporosis and relieve the symptoms of low back pain, but the mechanism of action is still not clear [8].

Therefore, the aim of this paper was to explore the correlation between factors associated with the AMPK/mTOR autophagy signaling pathway and postmenopausal osteoporosis.

Clinical data

Research subjects

The study subjects were from January 2021 to December 2022. 100 postmenopausal osteoporosis patients with liver and kidney yin deficiency, qi stagnation, and blood stasis were screened in the outpatient and inpatient departments of the Orthopedics and Traumatology Department of Liuzhou Traditional Chinese Medicine Hospital in Guangxi Zhuang Autonomous Region. All included patients signed informed consent forms. If allergies or adverse reactions occur during treatment, the drug was discontinued immediately.

The patient sample size estimation method uses the sample size estimation online tool (Power And Sample Size), with Power set to 0.8 and α set to 0.05. According to the research group's preliminary clinical pre-experiment evaluation, the effective rate of the test group is 60% and the control group is 85%. The calculated sample size was 47 per group. Considering that the dropout rate was approximately 10%,

the final sample size was determined to be 50 per group.

Diagnostic criteria

Guidelines for the Diagnosis and Treatment of Traditional Chinese Medicine for Postmenopausal Osteoporosis (Osteoporosis) (2019 Edition)" [2]. Western medicine diagnostic standards [9] (based on bone density): Bone density value is detected by dual-energy X-ray, and the result is expressed as T value. T value ≥-1.0 is normal; -2.5< T value <-1.0 is bone mass decline; T value ≤-2.5 can be used to diagnose postmenopausal osteoporosis. Enrolled patients should satisfy both Chinese and western medicine diagnostic criteria [10].

Inclusion criteria

1. Meets the diagnostic criteria for PMOP and liver and kidney yin deficiency, qi stagnation and blood stasis syndrome; 2. Female patients (age 50-60) within 5-10 years of menopause or with >12 months of natural menopause; 3. Voluntary acceptance of the treatments prescribed for this subject; 4. Has signed an informed consent form before the experiment.

Exclusion criteria

1. Parathyroid, gonads, adrenal glands, thyroid and other endocrine system diseases that affect bone metabolism; 2. Immune diseases or gynecological diseases affecting bone

Table 2. Osteoporosis efficacy evaluation criteria

Curative effect	Evaluation standard
Effective	Complete pain relief and improved bone density.
Efficient	The pain is relieved than before, and the bone density has not decreased.
Invalid	No significant improvement in pain or bone density.

Table 3. Criteria for evaluation of efficacy of TCM syndromes

| Curative effect	Evaluation standard
Got well	Symptoms such as waist and back pain are basically completely relieved, waist and knee soreness, lower limb cramps, difficulty in walking, and weight-bearing functions return to normal, and the syndrome score improvement rate is ≥95%.
Effective	Symptoms such as pain in the lower back were significantly relieved, soreness of the waist and knees, cramps in the lower limbs, difficulty in walking, and weight-bearing functions returned to normal, and the syndrome score improvement rate was ≥70%, <95%.
Efficient	Symptoms such as waist and back pain were mildly relieved, waist and knee soreness, lower limb cramps, difficulty in walking, and the ability to maintain balance were partially restored, and the syndrome score improvement rate was ≥30% and <70%.
Invalid	The clinical symptoms and waist and lower limb functions did not improve compared with before and after treatment, and the syndrome score improvement rate was <30%.
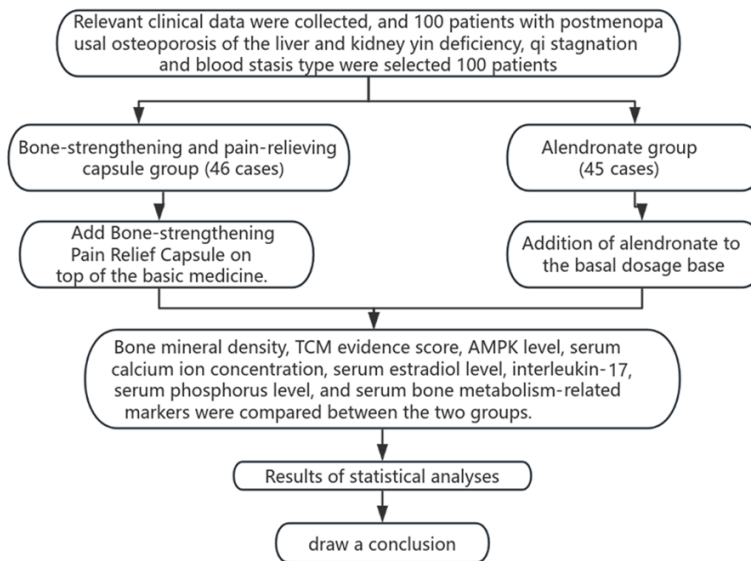
Figure 1. Flow chart.

metabolism; 3. Digestive and kidney diseases as well as affecting calcium and vitamin D absorption and metabolism.

Case withdrawal and dropout criteria

1. Subjects who cannot comply with the experiment; 2. Subjects who experience serious complications, special physiological changes or even adverse events; 3. Subjects who failed to complete the voluntary withdrawal as required;

4. Subjects who did not take medication as prescribed in the study protocol.

Research methods

Grouping and treatment

Two groups: the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group according to the order of visit time. In the end, 50 patients were included in the Zhuanggu Zhitong Recipe group and the alendronate sodium group. 50 cases. All included patients were given oral calcium carbonate D(II) (Wyeth Pharmaceuticals Co., Ltd., national drug approval number: H 10950029) 600 mg/time, once in the morning and evening. The treatment group was given oral Zhuanggu Zhitong Recipe on the basis of taking the above basic medicines (specific drug composition: 15 g of psoralen, 10 g of epimedium, 6 g of wolfberry, 6 g of Ligustrum lucidum, Drynariae 6 g, 6 g of dog's spine, and 6 g of Achyranthes bidentata. This recipe is processed into non-fried granules according to the technology. It is provided by the pharmacy of Liuzhou Traditional Chinese

Table 4. Basic information of the two groups of patients before treatment (χ±s)

	Zhuanggu Zhitong Recipe Group	Alendronate sodium group	P value
Age (years)	55.65±2.45	55.75±2.93	0.856
Height (cm)	158.34±2.59	157.93±2.66	0.454
Weight (kg)	51.93±2.91	51.34±2.62	0.315
NRS pain score	5.26±0.50	5.31±0.49	0.678
TCM syndrome score	14.43±2.39	13.62±2.47	0.114
Left femoral bone density (g/cm^2)	0.440±0.020	0.444±0.019	0.353
Blood calcium (mmol/L)	2.52±0.13	2.48±0.13	0.154
Blood phosphorus (mmol/L)	1.24±0.09	1.26±0.07	0.322
Serum iron (μmol/L)	17.60±2.10	17.63±1.74	0.926
E_2 (pg/mL)	15.47±0.74	15.49±0.71	0.895
IL-17 (ng/L)	141.83±7.48	141.96±6.13	0.927
IL-1 (ng/L)	89.98±4.48	90.85±5.00	0.385
ALP (U/L)	108.15±5.06	109.34±4.99	0.265
PINP (ng/ml)	40.31±1.40	39.84±1.29	0.100
L-NTX (nmol BCE/mmol Cr)	83.14±3.09	83.20±2.73	0.921
TRACP-5b (U/L)	3.79±0.47	3.87±0.50	0.426

Note: The age of the two groups of patients, the normality test results of the Zhuanggu Zhitong Recipe group and the alendronate sodium group were 0.216 and 0.072 respectively, obeying the normal distribution, the homogeneity of variance test L(1,89)=2.731, P=0.102, indicating homogeneous variances, using the independent sample T test.

Medicine Hospital. It was taken with warm water half an hour after breakfast and dinner), 200 ml/time, 2 times/day. The alendronate sodium group was given oral alendronate sodium tablets (Zhuozhou Dongle Pharmaceutical Co., Ltd., national drug approval number H20084165) 70 mg/time, once/week, on the basis of taking the above basic medications. One course of treatment was given every 4 weeks, for a total of 6 courses, for a total of 24 weeks (The research plan was approved by the Ethics Committee of Liuzhou Traditional Chinese Medicine Hospital. Ethics number: 2022 JAN - KY-YN-022-01).

Outcome measures

Security indicators: (1) Patients' electrolytes were monitored as well as hepatic and renal function before treatment and at 3, 6, and 12 months of treatment. (2) Occurrence of adverse event for prompt treatment of adverse reactions. (3) In the event of an adverse event, a case withdrawal is made promptly, followed by symptomatic treatment for the adverse reaction.

Bone density: A dual-energy X-ray bone density detector (American H OLOGIC Company, model: Horizon-A) as used to detect left femoral bone density before and after drug intervention.

Traditional Chinese Medicine syndrome score: The clinical symptoms of patients before and after treatment were graded and quantified according to the Quantitative Grading Scale of Chinese Medicine Symptoms (**Table 1**).

AMPK content: ELISA method was applied to detect the serum AMPK content of the two groups of sufferers before and after remedy, record it, and conduct linear correlation analysis with the clinical data analysis results.

Serum Ca^{2+}, P, Fe and E_2 levels: The o-cresolphthalein complex ketone method was used to detect the serum calcium ion concentration (Ca^{2+}) and serum iron (Fe) levels of the two groups of sufferers before and after therapy. The phosphine platinum acid reduction method was applied to detect the serum phosphorus (P) levels of the two groups before and after therapy. High-efficiency Liquid chromatography (HPLC) was used to detect the serum estradiol (E2) levels of the two groups of patients before and after therapy.

Serum IL-17 and IL-1 levels: The enzyme-linked immunosorbent assay (ELISA) method was applied to detect the levels of IL-17 and IL-1 in the two groups of patients before and after drug intervention.

Table 5. Comparison of bone density of left femur between two groups before and after treatment ($\overline{x}\pm s$)

	Group	Number of samples	Before treatment	After treatment	t	P
Left femoral bone density (g/cm²)	Zhuanggu Zhitong Recipe Group	46	0.440±0.020	0.637±0.024	-34.67	0.001
	Alendronate sodium group	45	0.444±0.019	0.655±0.024	-42.95	0.001

Note: The difference in bone density between the Zhuanggu Zhitong Recipe group and the alendronate sodium group before and after treatment was calculated separately. A normality test was performed on the differences between the two groups. The results were 0.776 and 0.266 respectively, which satisfies normality. Therefore, paired samples T test was used.
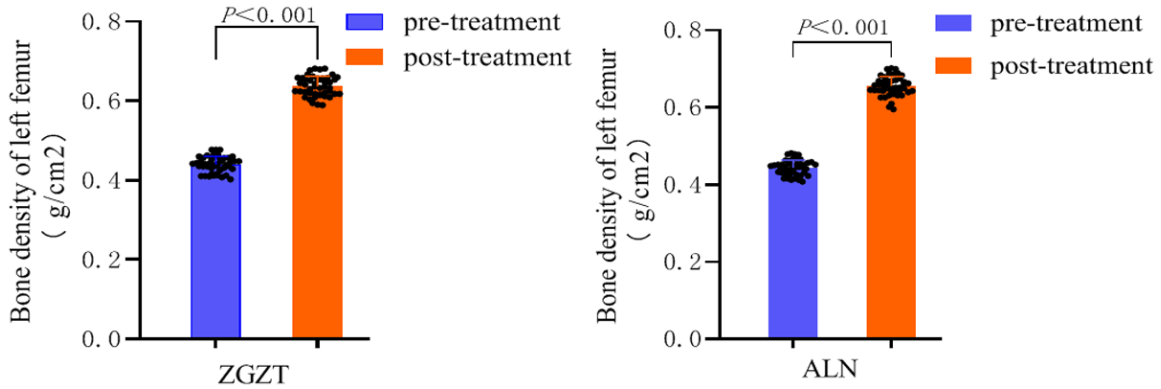

Figure 2. Comparison of femoral bone density between the two groups before and after treatment.

Table 6. Comparison of lumbar spine bone density difference between two groups before and after treatment ($\overline{x}\pm s$)

	Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
Left femoral bone density (g/cm²)	Zhuanggu Zhitong Recipe Group	46	0.188±0.021	-2.83	0.006	-0.022~-0.003
	Alendronate sodium group	45	0.201±0.022			

Note: The normality test was performed on the difference in bone density between the two groups before and after treatment. The results were 0.776 and 0.226 respectively, which satisfied the normality. The homogeneity of variance test L(1,89)=0.109, P=0.742, which satisfies the homogeneity of variances and uses the independent sample T test.
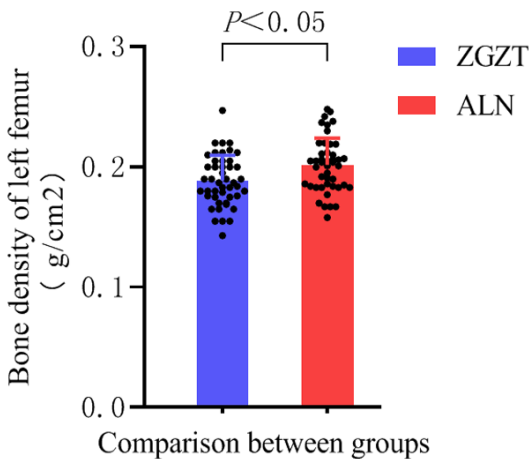

Figure 3. Comparison of differences in femoral bone density between the two groups before and after treatment.

Serum bone metabolism related markers: The NPP substrate-AMP buffer method was applied to detect the alkaline phosphatase (ALP) levels of the two groups of patients before and after drug intervention. The ELISA method was used to detect the amino-terminal propeptide of type I procollagen (PINP) and urinary type I collagen in the two groups before and after treatment. Levels of cross-linked N-terminal peptide (L-NTX), tartrate-resistant acid phosphatase 5b (TRACP-5b).

Clinical efficacy evaluation standards [11]

1. Osteoporosis efficacy evaluation criteria (**Table 2**).

2. Criteria for evaluation of efficacy of TCM syndromes (**Table 3**).

Table 7. Comparison of TCM syndrome scores between the two groups before and after treatment ($\bar{x}\pm s$)

Group	Number of samples	Before treatment	After treatment	t	P
Zhuanggu Zhitong Recipe Group	46	14.43±2.39	3.82±1.74	41.85	0.001
Alendronate sodium group	45	13.62±2.47	4.28±1.56	30.28	0.001

Note: The difference in TCM syndrome scores before and after treatment in the Zhuanggu Zhitong Recipe group and the alendronate sodium group were calculated respectively. The normality test was performed on the difference in TCM syndrome scores between the two groups before and after remedy. The results were 0.095 and 0.084 respectively, which satisfies the normal distribution, so the paired sample T test is used.
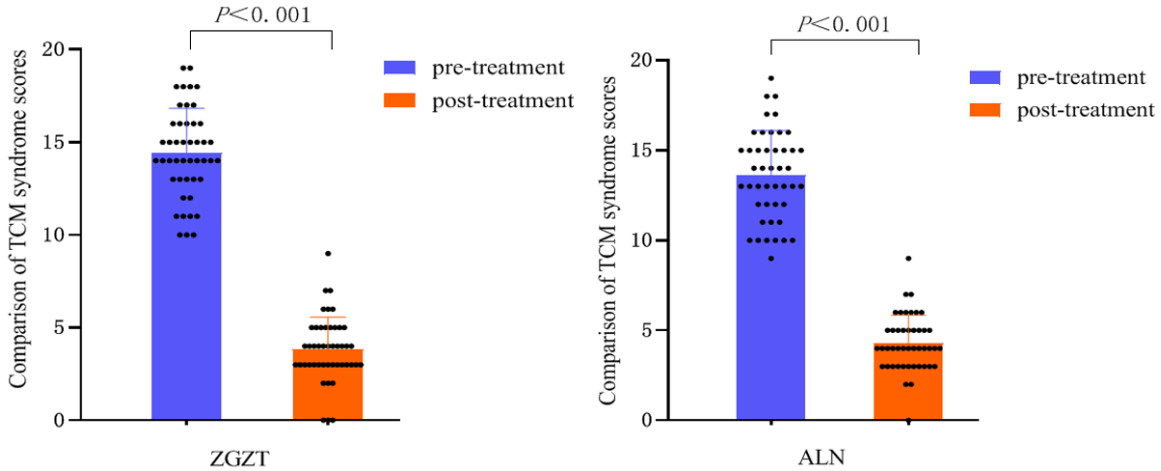

Figure 4. Comparison of TCM syndrome scores between the two groups before and after treatment.

Table 8. Comparison of the difference in TCM syndrome scores between the two groups before and after treatment ($\bar{x}\pm s$)

Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
Zhuanggu Zhitong Recipe Group	46	10.60±1.71	3.20	0.002	0.484~2.066
Alendronate sodium group	45	9.33±2.06			

Note: A normality test was conducted on the difference between the two groups of TCM syndrome scores before and after therapy. The results were 0.095 and 0.084 respectively, which satisfied normality. The homogeneity of variance test L(1,89)=2.578, P=0.112, homogeneity of variances, using independent samples T test.

The flow chart is as follows

The flow chart of this study is shown in **Figure 1**.

Statistical methods

Data analysis was carried out using SPSS 24.0 statistical analysis software. For normally distributed measured data, the mean and standard deviation are applied to describe the center position and degree of dispersion of the data. For two groups of normally distributed data, the independent samples T test was used to contrast. Non-normally distributed measured data used the median, 25th percentile, and 75th percentile to describe the location and degree of dispersion of the data. For two groups of non-normally distributed data, the rank sum test (Mann-Whitney U test) was applied for comparison. Analysis of bivariate relationships: Pearson linear correlation analysis was used to assess the relationship between two normally distributed measures. For non-normally distributed data, relationship analysis was performed using rank-sum correlation. Comparisons within groups before and after treatment were performed using paired T tests, comparisons between groups were performed using independent samples T tests, and counted data
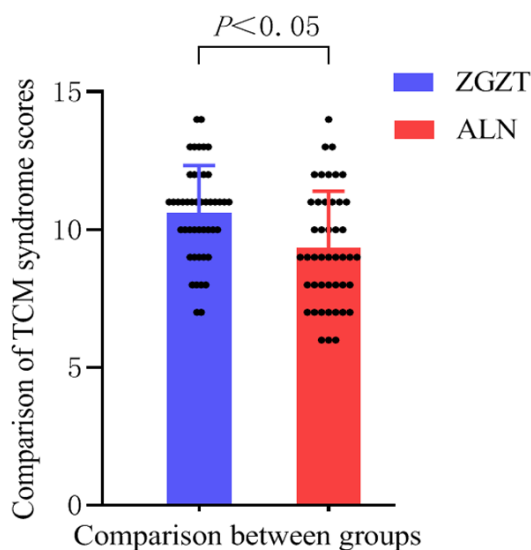
Figure 5. Comparison of the difference in TCM syndrome scores between the two groups before and after treatment.

were compared using rank-sum tests. $P<0.05$ was used to determine whether a difference was statistically significant. Graphs were drawn using GraphPad Prism 8.2.1 software.

Results

Statistical analysis of basic data

In this chapter of the study, using http://tools. medsci, patients were grouped according to the time of enrolment in order, and there were 50 cases in each of the two groups. During the study period there was 1 patient in the strong bone pain relief formula group who withdrew from the study due to gastrointestinal reactions after taking the drug, no obvious discomfort symptoms appeared in the follow-up, 1 case withdrew voluntarily, and there were 2 cases of loss of visits, shedding a total of 4 cases. In the alendronate group, 3 patients withdrew from the study due to gastrointestinal reactions after taking the drug, 1 withdrew voluntarily, and 2 cases were lost to follow-up, with a total of 5 cases falling off. Finally, 46 cases in the Bone Strengthening and Pain Relieving Formula group and 45 cases in the alendronate group completed this study.

Basic situation comparison

When comparing the basic information (height, age, weight, etc.) and clinical effect evaluation indicators (bone density, bone turnover markers, TCM syndrome scores) between the two groups of patients, no statistical significance had been found in the comparison between the two groups ($P>0.05$), **Table 4**.

Comparison of bone density

24 weeks of treatment, the bone density value results are shown in **Table 5** and **Figure 2**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group had a significantly increased left femur bone density value ($P<0.001$).

Comparison outcomes of the difference in femoral bone density before and after treatment are shown in **Table 6** and **Figure 3**: The alendronate sodium group had a better effect on increasing bone density than the Zhuanggu Zhitong Recipe group ($P<0.05$).

Comparison of TCM syndrome scores

The outcomes of TCM syndrome scores are shown in **Table 7** and **Figure 4**: Both the Zhuanggu Zhitong prescription treatment group and the alendronate sodium control group had lower TCM syndrome scores, indicating that Zhuanggu Zhitong prescription can effectively relieve soreness and weakness of the waist and knees, lower limb cramps, walking and weight-bearing functions, can effectively reduce the NRS score of low back pain, and improve TCM syndrome ($P<0.001$).

Results of the difference in TCM syndrome scores before and after treatment are shown in **Table 8** and **Figure 5**: Comparison of the difference in TCM syndrome scores before and after treatment. The treatment group of Zhuanggu Zhitong Recipe had a significantly better effect on reducing TCM syndrome scores than alendronic acid. The sodium control group ($P<0.05$) showed that Zhuanggu Zhitong Recipe had a better effect on improving TCM syndromes than alendronate sodium.

Comparison of NRS scores for low back pain

The results of the NRS score of low back pain are shown in **Table 9** and **Figure 6**: Both the Zhuanggu Zhitong prescription treatment group and the alendronate sodium control group showed lower NRS scores of low back pain ($P<0.001$), indicating that both Zhuanggu

Table 9. Comparison of changes in waist pain NRS scores before and after treatment ($\bar{x} \pm s$)

Group	Number of samples	Before treatment	After treatment	t	P
Zhuanggu Zhitong Recipe Group	46	5.26±0.50	2.84±0.76	22.92	0.001
Alendronate sodium group	45	5.31±0.49	3.53±0.54	19.40	0.001

Note: The difference in NRS scores of cold waist pain before and after treatment in the Zhuanggu Zhitong Recipe group and the alendronate sodium group were calculated respectively. The normality test was performed on the difference in NRS scores of cold waist pain before and after remedy in the two groups. The outcomes were 0.202 and 0.233, satisfying normality, using paired sample T test.
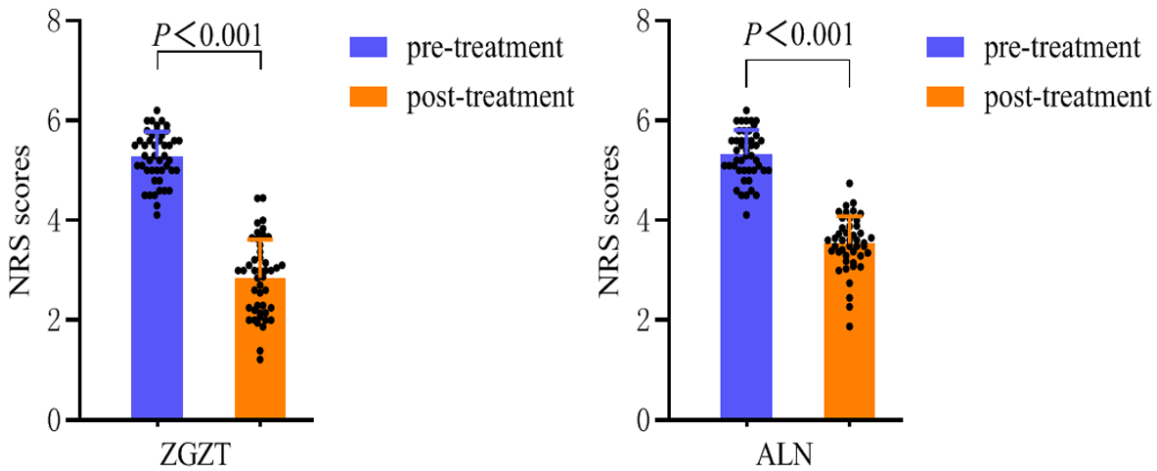

Figure 6. Comparison of NRS scores of cold waist pain in the two groups before and after treatment.

Table 10. Comparison of the difference in waist pain NRS scores between the two groups before and after treatment ($\bar{x} \pm s$)

Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
Zhuanggu Zhitong Recipe Group	46	2.42±0.71	4.68	0.019	0.365~0.922
Alendronate sodium group	45	1.76±0.61			

Note: The normality test was performed on the difference in NRS scores of cold waist pain between the two groups before and after treatment. The results were 0.202 and 0.233 respectively, which satisfied the normality. The homogeneity of variance test L(1,89)=1.082, P=0.301, indicating homogeneous variances, using the independent sample T test.
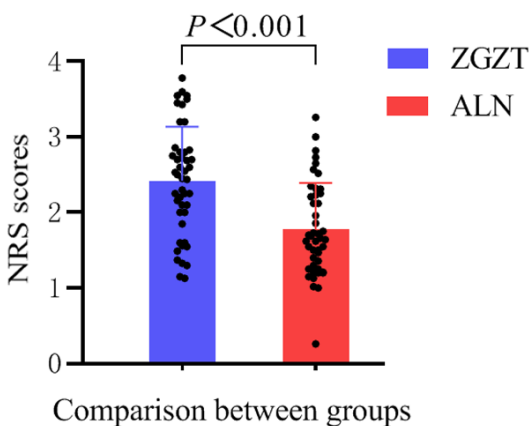

Figure 7. Comparison of the differences between the two groups of low back pain NRS scores before and after treatment.

Zhitong Recipe and alendronate sodium have good effects on relieving low back pain.

The outcomes are shown in **Table 10** and **Figure 7**. After treatment, the difference in NRS score of cold pain in the waist was compared between the two groups. The improvement of NRS score in the alendronate sodium group was more significant than that of the Zhuanggu Zhitong Recipe group.

AMPK content comparison

The AMPK content results are shown in **Tables 11**, **12** and **Figure 8**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group of postmeno-

Table 11. Changes in AMPK content in the two groups before and after treatment ($\overline{x}\pm s$)

	Group	Number of samples	Before treatment	After treatment	t	P
AMPK (pg/mL)	Zhuanggu Zhitong Recipe Group	46	11.31±0.44	14.04±0.62	-28.10	0.001
	alendronate sodium group	45	11.26±0.38	14.12±0.61	-31.52	0.001

Note: The difference in AMPK expression levels between the Zhuanggu Zhitong Recipe group and the alendronate sodium group before and after treatment was calculated respectively. A normality test was performed on the differences between the two groups. The results were 0.274 and 0.951 respectively, which met normality. Adopt Paired samples T-test.

Table 12. Comparison of AMPK content differences between the two groups before and after treatment ($\overline{x}\pm s$)

	Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
AMPK (pg/mL)	Zhuanggu Zhitong Recipe Group	46	2.73±0.65	-0.939	0.350	-0.318-0.139
	Alendronate sodium group	45	2.85±0.60			

Note: The difference in AMPK expression levels between the Zhuanggu Zhitong Recipe group and the alendronate sodium group before and after treatment was calculated respectively. A normality test was performed on the differences between the two groups. The results were 0.274 and 0.951 respectively, which satisfied the normality. The differences between the two groups were tested for homogeneity of variances, L(1,89)=0.357, P=0.552, which satisfied the homogeneity of variances, and the independent sample T test was used.
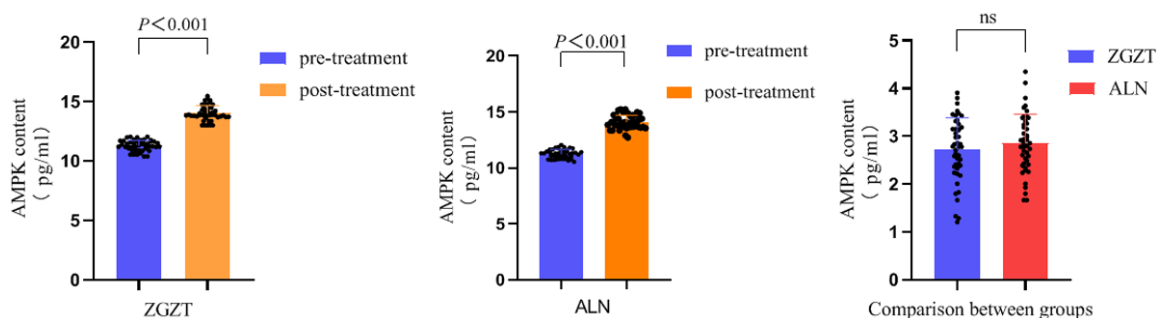

Figure 8. Comparison of AMPK content changes before and after treatment and AMPK content difference before and after treatment.

Table 13. Correlation analysis between BMD and E$_2$ and AMPK amount respectively

	M	SD	1	2	3
1 AMPK	11.28	0.415	1		
2 BMD	0.442	0.197	0.532**	1	
3 E2	15.48	0.729	0.514**	0.435*	1

Note: The normality test results of AMPK, BMD and E2 are 0.142, 0.08 and 0.097 respectively. The two sets of data conform to the normal distribution. ** indicates comparison with AMPK group, P<0.05. * indicates comparison with BMD group, P<0.05.

pausal osteoporosis patients had increased AMPK content in the serum. (P<0.001). This indicated that both Zhuanggu Zhitong Recipe and alendronate sodium could effectively reduce AMPK content levels. In order to clarify whether there was a correlation between AMPK levels and postmenopausal osteoporosis, this study further analyzed the linear correlation between AMPK levels in serum and clinical characteristics of patients.

Correlation analysis between AMPK content and patient clinical information

The results of Pearson's correlation analysis are shown in **Table 13**. The correlation coeffi-

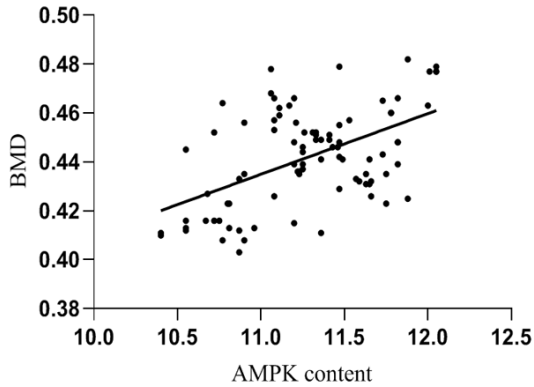**Figure 9.** Correlation analysis between BMD and AMPK content.

Figure 10. Correlation analysis between E$_2$ and AMPK content.

cient between AMPK content and BMD in the serum of postmenopausal osteoporosis patients is $r=0.296$, $P<0.05$, which illustrates a relationship between AMPK content and bone density in postmenopausal osteoporosis patients. There was a significant positive correlation between them. The correlation coefficient between AMPK content and E$_2$ was $r=0.280$, $P<0.05$, indicating that there is a positive correlation (**Figures 9**, **10**).

Comparison of serum Ca^{2+}, P, Fe and E$_2$ levels

The results of serum Ca^{2+}, P, Fe and E$_2$ contents are shown in **Table 14** and **Figure 11**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group were able to increase Ca^{2+}, P, Fe and E$_2$ levels ($P<0.001$), indicating that both Zhuanggu Zhitong Recipe and alendronate sodium can significantly increase Ca^{2+}, P, Fe and E$_2$.

Serum Ca^{2+}, P, Fe and E$_2$ levels are shown in **Table 15** and **Figure 12**: Calcium ions and blood phosphorus concentrations between the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group with no statistical significance in the difference ($P>0.05$). The comparison of serum Fe levels between groups showed that Zhuanggu Zhitong Recipe was more significant in increasing the patient's serum iron level ($P<0.001$). The intergroup comparison of E$_2$ levels in the Zhuanggu Zhitong Recipe treatment group was increased more than that of the alendronate control group ($P<0.001$), indicating that both groups can significantly increase the patient's blood calcium concentration and E$_2$ content, but in the
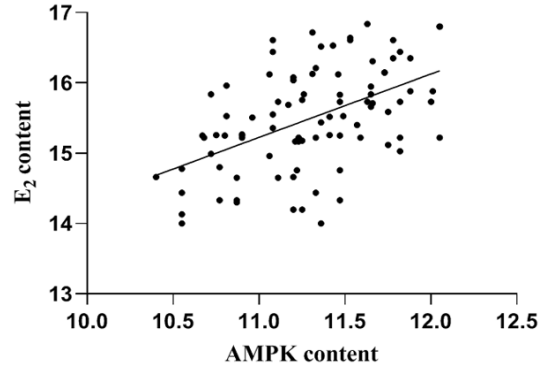
improvement The difference in E$_2$ levels in the Zhuanggu Zhitong Recipe treatment group was more significant than that in the alendronate sodium control group.

Comparison of serum IL-17 and IL-1 levels

The results of serum IL-17 and IL-1 levels before and after treatment are shown in **Table 16** and **Figure 13**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group had reduced IL-17 and IL-1 levels ($P<0.001$), indicating that both Zhuanggu Zhitong Recipe and alendronate sodium had a good effect on reducing inflammatory indicators.

Serum IL-17 and IL-1 contents before and after treatment are shown in **Table 17** and **Figure 14**. There was no difference between IL-17 and IL-1 before and after treatment in the two groups ($P>0.05$).

Comparison of bone formation markers

The results of bone formation markers are shown in **Table 18** and **Figure 15**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate control group had improved bone formation markers ALP and P in postmenopausal osteoporosis patients. The INP level ($P<0.001$) shows that both Zhuanggu Zhitong Recipe and alendronate sodium had a good effect on promoting bone formation.

The difference outcomes of bone formation-related marker levels between the two groups before and after therapy are shown in **Table 19** and **Figure 16**: After treatment, the bone for-

Table 14. Comparison of changes in Ca^{2+}, P, Fe and E_2 between the two groups before and after treatment ($\bar{x}\pm s$)

	Group	Number of samples	Before treatment	After treatment	t	P
Ca^{2+} (mmol/L)	Zhuanggu Zhitong Recipe Group	46	2.52±0.13	2.65±0.13	-31.64	0.001
	alendronate sodium group	45	2.48±0.14	2.60±0.14	-36.34	0.001
P (mmol/L)	Zhuanggu Zhitong Recipe Group	46	1.24±0.09	1.09±0.08	51.88	0.001
	alendronate sodium group	45	1.26±0.07	1.11±0.08	64.15	0.001
Fe (μmol/L)	Zhuanggu Zhitong Recipe Group	46	17.60±2.10	18.67±2.13	-32.70	0.001
	alendronate sodium group	45	17.63±1.74	18.48±1.76	-28.31	0.001
E_2 (mmol/L)	Zhuanggu Zhitong Recipe Group	46	15.47±0.74	18.77±0.70	-48.51	0.001
	alendronate sodium group	46	2.52±0.13	2.65±0.13	-36.51	0.001

Note: We calculated the difference of Ca^{2+}, P, Fe and E_2 content before and after treatment between the Strong Bone Pain Relieving Formula group and the alendronate group, and carried out the normality test on the difference of the two groups. The results were 0.112 and 0.119, 0.107 and 0.100, 0.107 and 0.156, 0.119 and 0.248, respectively, and the difference of the data in the two groups satisfied the normality. The paired samples t-test was used.

mation-related indicators (ALP, PINP) were compared between the two groups. No significant difference was found in the improvement of ALP ($P>0.05$), but for the improvement of PINP, the Zhuanggu Zhitong Recipe group was sharply better than the alendronate sodium group ($P<0.05$), indicating that the Zhuanggu Zhitong Recipe is effective in promoting PINP. The production effect was better than that of alendronate sodium.

The two groups based on the levels of bone resorption markers (L-NTX, TRACP-5b) are shown in **Table 20** and **Figure 17**: Both the Zhuanggu Zhitong Recipe treatment group and the alendronate sodium control group had lower bone resorption markers (L-NTX, TRACP-5b) levels ($P<0.001$), indicating that both Zhuanggu Zhitong Recipe and alendronate sodium have the regulatory effect of significantly reducing the levels of L-NTX and TRACP-5b.

The difference in results of L-NTX and TRACP-5b between the two groups before and after therapy are shown in **Table 21** and **Figure 17**. The alendronate control group had an excellent improvement effect on the bone resorption markers L-NTX and TRACP-5b as did the Zhuanggu Zhitong Recipe treatment group ($P<0.05$).

Comparison of osteoporosis effect assessment

Osteoporosis efficacy evaluation criteria: The results of the two groups in improving osteoporosis are shown in **Table 22**: Comparison of the efficacy showed a significant difference

($P<0.05$). The alendronate sodium improvement of bone density value was better better than Zhuanggu Zhitong Recipe.

TCM syndrome efficacy evaluation criteria: The results are shown in **Table 23**. The comparison of the efficacy of traditional Chinese medicine syndrome between the two groups shows that the Zhuanggu Zhitong Recipe group is better than alendronate sodium in terms of systemic care for postmenopausal osteoporosis patients.

Discussion

Improvement of bone turnover marker levels and bone density

We found that bone resorption markers NTX and TRAP-5b were reduced after treatment in the Strong Bone Pain Relieving Capsules group, and 25-OH-VD was significantly increased in both groups after treatment, but the magnitude of the elevation was not significantly different between the two groups. Bone mineral density is the gold standard for the diagnosis of osteoporosis [12], and by using dual-energy X-ray absorptiometry to measure femoral bone mineral density and dual-utilizing bone mineral density (BMD) before and after treatment, this suggests that the Strong Bone Relief Formula has a promising role to play in the treatment of postmenopausal osteoporosis.

Improvement of TCM syndromes

Traditional Chinese medicine considers "deficiency" and "stasis" as the main pathogenesis
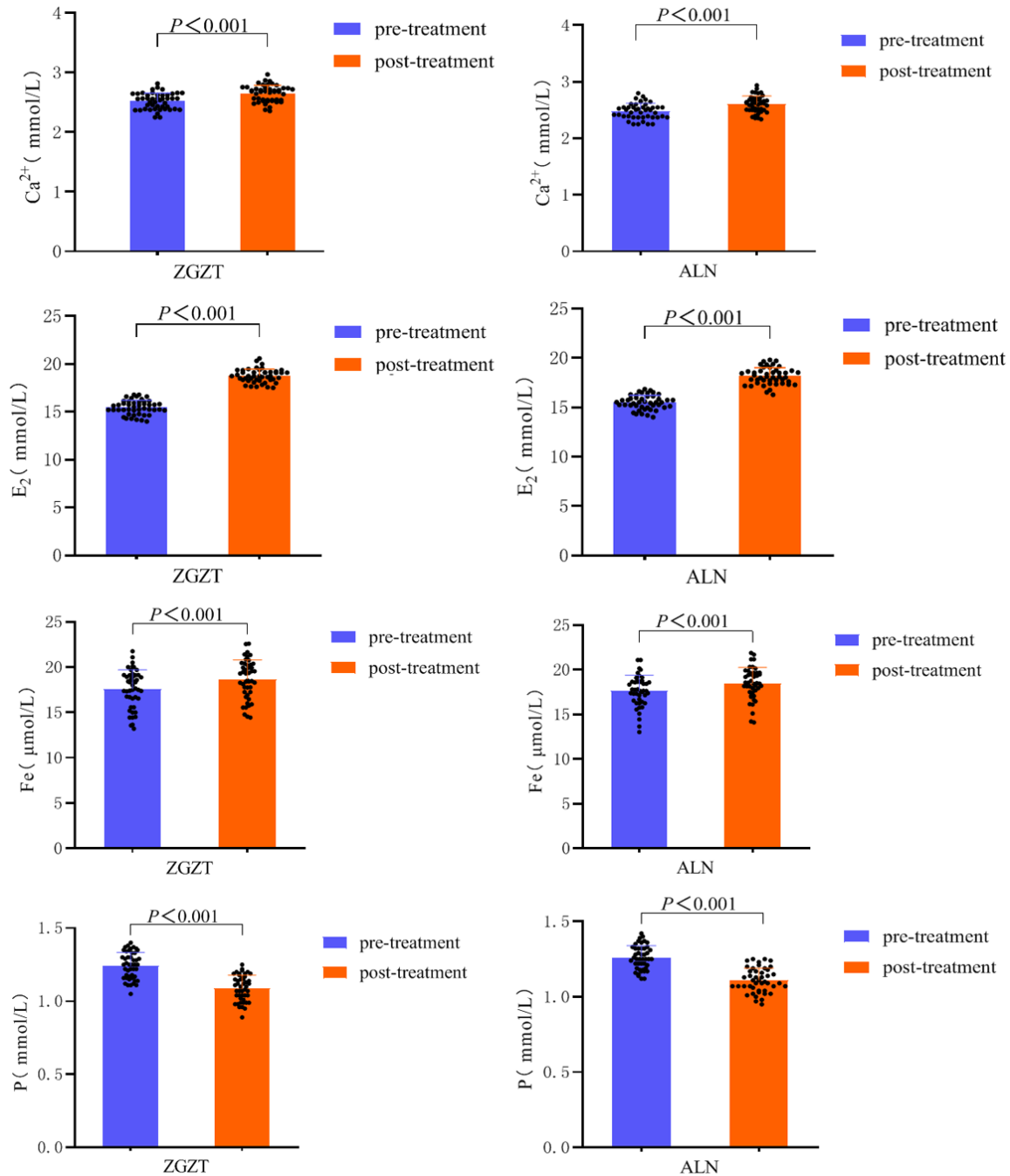
Figure 11. Changes in Ca^{2+}, P, Fe and E_2 contents in the two groups before and after treatment.

of postmenopausal osteoporosis [13]. Bone-strengthening and pain-relieving capsule is one of the effective drugs for the treatment of post-menopausal osteoporosis under the guidance of the theory of replenishing deficiency and regulating stasis. This study showed that the group of Bone-strengthening and Pain Relieving Capsules could significantly improve the TCM evidence points and significantly reduce the clinical symptoms such as low back pain, lumbar and knee soreness, and calf cramps. Although there were some differences between the group and alendronate group in improving bone density, it was significantly better than the alendronate group in improving patients' clinical symptoms and systemic care effects.

Table 15. Comparison of differences in Ca^{2+}, P, Fe, and E_2 between the two groups before and after treatment ($\bar{x} \pm s$)

	Group	Number of samples	Difference before and after treatment	t	P	95% *CI of difference*
Ca^{2+} (mmol/L)	Zhuanggu Zhitong Recipe Group	46	0.126±0.027	0.779	0.434	-0.006~0.014
	Alendronate sodium group	45	0.122±0.019			
P (mmol/L)	Zhuanggu Zhitong Recipe Group	46	0.152±0.015	0.711	0.479	-0.004~0.010
	Alendronate sodium group	45	0.149±0.152			
Fe (μmol/L)	Zhuanggu Zhitong Recipe Group	46	1.07±0.22	5.191	0.001	0.141~0.316
	Alendronate sodium group	45	0.84±0.19			
E_2 (mmol/L)	Zhuanggu Zhitong Recipe Group	46	3.29±0.46	6.467	0.001	0.445~0.841
	Alendronate sodium group	45	2.65±0.48			

Note: For Ca^{2+}, P, Fe, and E_2, the difference between the contents of the two groups before and after treatment was calculated respectively, and the normality test was performed on the difference between the two groups respectively. Results of Ca^{2+}, P, Fe, and E_2 were 0.301 and 0.420, 0.085 and 0.333, 0.107 and 0.156, and 0.119 and 0.248, respectively. For the test of chi-square L(1,89)=2.486, P=0.118, L(1,89)=2.276, P=0.135, test L(1,89)=0.038, P=0.845, L(1,89)=0.159, P=0.691, variance chi-squaredness was satisfied, and independent samples t-test was used.
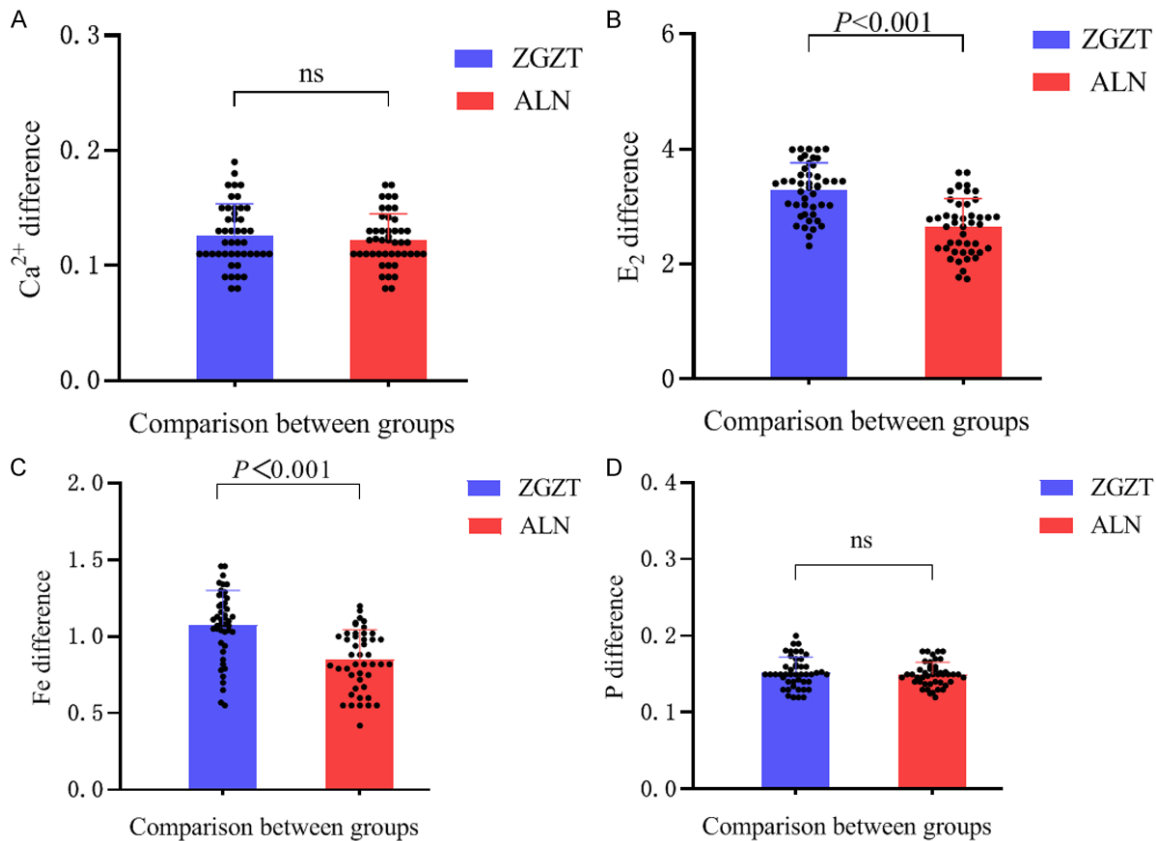

Figure 12. Comparison of differences between the two groups of Ca^{2+}, P, Fe and E_2 before and after treatment. Note: A is the comparison of the Ca^{2+} difference between the two groups, B is the comparison of the E_2 difference between the two groups, C is the comparison of the serum iron difference between the two groups, and D is the comparison of the blood phosphorus difference between the two groups.

Improvement of serum Ca^{2+}, P, Fe and E_2

As an important estrogen for women [14], E_2 can promote the proliferation and differentia-

tion of osteoblasts to promote bone formation. It can also inhibit the formation and activity of osteoclasts and reduce bone resorption [15]. In this study, after 24 weeks of treatment,

Table 16. Changes in IL-17 and IL-1 levels in the two groups before and after treatment (χ±s)

	Group	Number of samples	Before treatment	After treatment	t	P
IL-17 (ng/L)	Zhuanggu Zhitong Recipe Group	46	141.83±7.48	118.59±8.17	39.78	0.001
	Alendronate sodium group	45	141.96±6.13	119.52±7.82	30.42	0.001
IL-1 (ng/L)	Zhuanggu Zhitong Recipe Group	46	89.98±4.48	76.46±4.50	137.78	0.001
	Alendronate sodium group	45	90.85±5.00	77.33±5.01	121.36	0.001

Note: For IL-17, the difference between the two groups before and after therapy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.108 and 0.404 respectively, both of which were greater than 0.05. Normality was satisfied, and the Paired samples T-test was used. For IL-1, the difference between the two groups before and after therapy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.224 and 0.623 respectively, both of which were greater than 0.05. Normality was satisfied, and the Paired samples T-test was used.
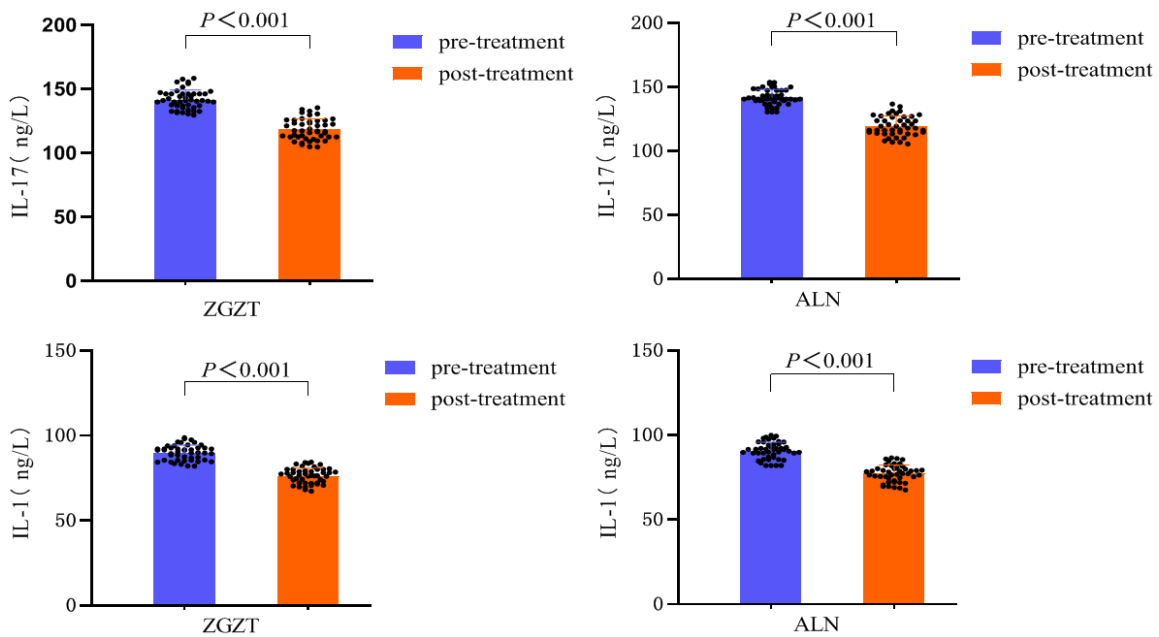

Figure 13. Changes in IL-17 and IL-1 levels in the two groups before and after treatment.

Table 17. Comparison of the differences between the two groups of IL-17 and IL-1 before and after treatment ($\overline{x}$±s)

	Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
IL-17 (ng/L)	Zhuanggu Zhitong Recipe Group	46	23.23±3.96	0.852	0.396	-1.065~2.664
	Alendronate sodium group	45	22.32±4.65			
IL-1 (ng/L)	Zhuanggu Zhitong Recipe Group	46	13.52±0.66	0.019	0.985	-0.292~0.297
	Alendronate sodium group	45	13.51±0.74			

Note: For IL-17, the difference between the two groups before and after remedy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.108 and 0.404 respectively, both of which were greater than 0.05, satisfying normality and having homogeneous variances. The homogeneity test L(1,89)=2.464, P=0.120, meets the homogeneity of variances, and uses the independent sample T test. For IL-1, the difference between the two groups before and after remedy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.224 and 0.623 respectively, both of which were greater than 0.05, satisfying normality and homogeneity of variance. The test L(1,89)=1.129, P=0.291, met the homogeneity of variances, and the independent sample T test was used.
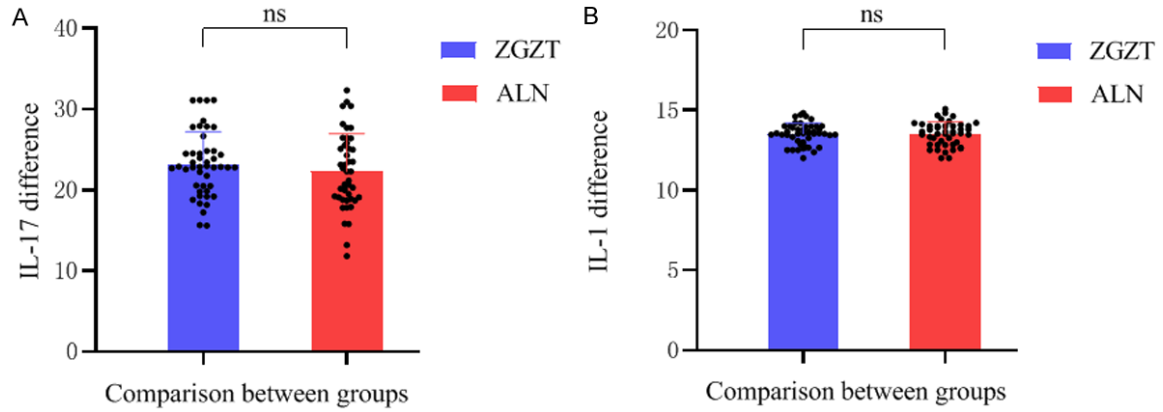
Figure 14. Comparison of differences in IL-17 and IL-1 levels between the two groups before and after treatment. Note: (A) The image is the comparison of the IL-17 difference between the two groups, and (B) the image is the comparison of the IL-1 difference between the two groups.

Table 18. Comparison of bone formation-related marker levels before and after treatment (χ±s)

	Group	Number of samples	Before treatment	After treatment	t	P
ALP (U/L)	Zhuanggu Zhitong Recipe Group	46	108.15±5.06	122.05±5.56	-38.20	0.001
	Alendronate sodium group	45	109.34±4.99	123.06±5.42	-30.99	0.001
PINP (ng/ml)	Zhuanggu Zhitong Recipe Group	46	40.31±1.40	45.71±1.57	-33.47	0.001
	Alendronate sodium group	45	39.84±1.29	44.30±1.46	-24.72	0.001

Note: The difference between the two groups before and after treatment was calculated for ALP, and the normality test was performed on the difference between the two groups. The results were 0.152 and 0.257, respectively, both greater than 0.05, which satisfied normality. Paired sample T was used test. For PINP, the difference between the two groups before and after remedy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.108 and 0.113 respectively, both of which were greater than 0.05, which satisfied normality. Paired sample T was used test.
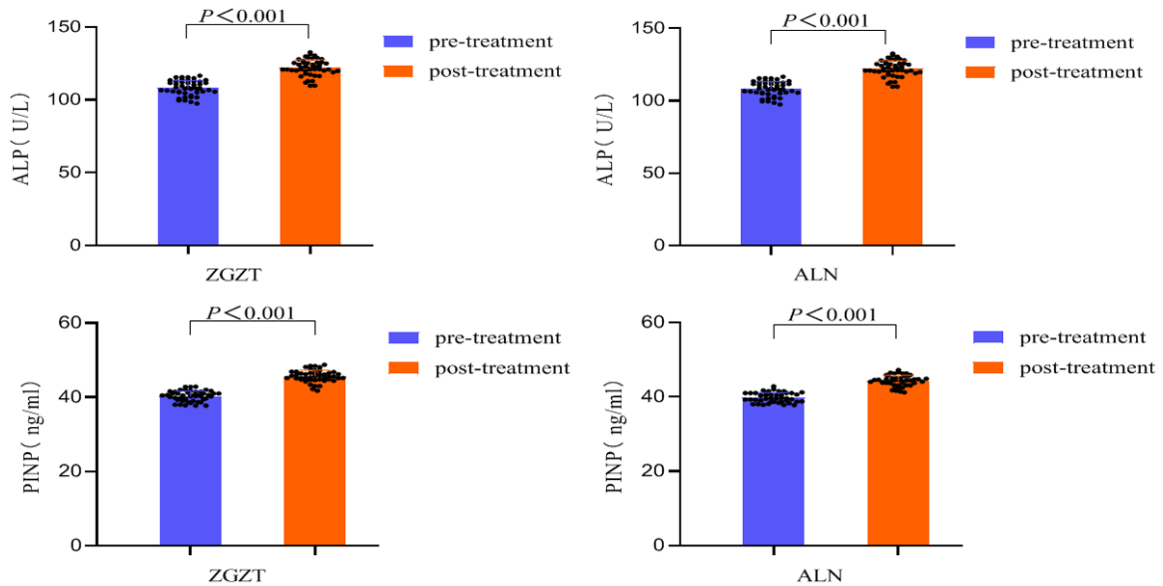

Figure 15. Intra-group comparison of ALP and PINP levels between the two groups before and after treatment.

Table 19. Comparison of differences between 19 groups of bone formation related marker levels before and after treatment ($\bar{x}\pm s$)

	Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
ALP (U/L)	Zhuanggu Zhitong Recipe Group	46	13.85±2.45	0.242	0.809	-0.996~1.272
	Alendronate sodium group	45	13.71±2.96			
PINP (ng/ml)	Zhuanggu Zhitong Recipe Group	46	5.39±1.09	3.870	0.001	0.455~1.415
	Alendronate sodium group	45	4.46±1.21			

Note: For ALP, the difference between the two groups before and after therapy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.152 and 0.257 respectively, both of which were greater than 0.05, which satisfied normality and homogeneity of variance test. Result: L(1,89)=2.393, P=0.125, which meets the homogeneity of variances, and the independent sample T test is used. For PINP, the difference between the two groups before and after therapy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.108 and 0.113 respectively, both of which were greater than 0.05, which satisfied normality and homogeneity of variance test. Result: L(1,89)=0.538, P=0.465, meeting the homogeneity of variances, using the independent sample T test.
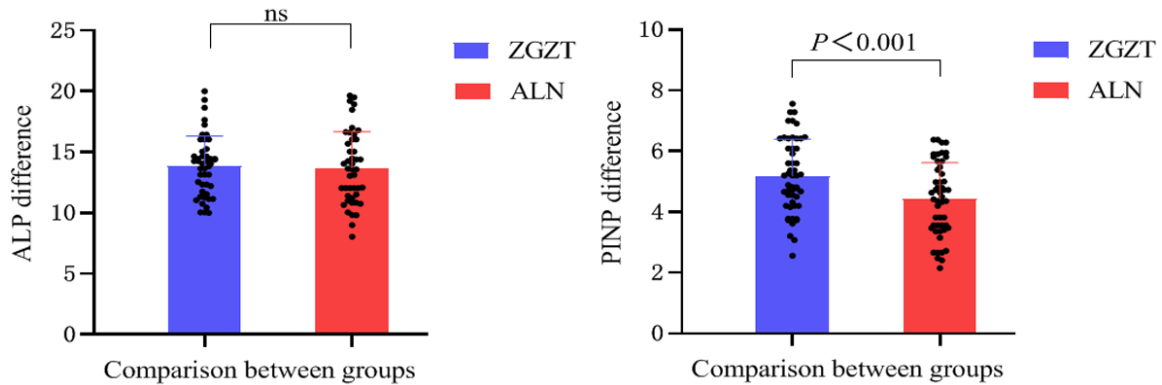

Figure 16. Comparison of bone formation-related marker levels between the two groups before and after treatment.

Table 20. Comparison of L-NTX and TRACP-5b levels between the two groups before and after treatment ($\chi\pm s$)

	Group	Number of samples	Before treatment	After treatment	t	P
L-NTX (nmolBCE/L)	Zhuanggu Zhitong Recipe Group	46	83.14±3.09	61.96±4.82	31.41	0.001
	Alendronate sodium group	45	83.20±2.73	59.70±4.60	44.58	0.001
TRACP-5b (U/L)	Zhuanggu Zhitong Recipe Group	46	3.79±0.47	3.42±0.47	889.96	0.001
	Alendronate sodium group	45	3.87±0.50	3.48±0.50	943.13	0.001

Note: The differences of L-NTX and TRACP-5b content before and after treatment were calculated for the two groups respectively, and the normality test was conducted for the differences of the two groups. Results were 0.353 and 0.142, 0.260 and 0.134 respectively, which were all greater than 0.05, which satisfied normality and the paired samples t-test was used.

Zhuanggu Zhitong Recipe can significantly increase E_2 levels. Compared to the alendronate sodium group, its effect was more significant. This is consistent with the results of some studies confirming that traditional Chinese medicine has an estrogen-like effect.

The imbalance of calcium and phosphorus metabolism will lead to increased secretion of parathyroid hormone (PTH), thereby increasing bone resorption [16]. This study found that the serum Ca^{2+} concentration of postmenopausal osteoporosis patients was significantly increased after treatment with Zhuanggu Zhitong Recipe, and there was no statistical difference between the increase in serum calcium concentration and the alendronic acid group.
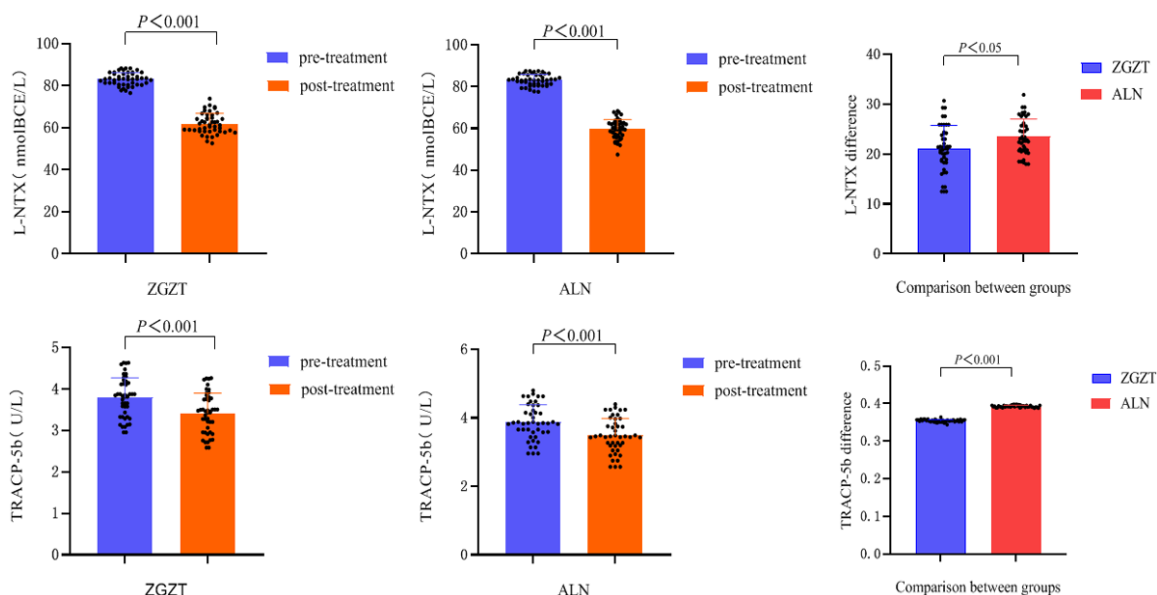
Figure 17. Intra-group comparison of L-NTX and TRACP-5b levels before and after treatment between the two groups and inter-group comparison of the differences before and after treatment.

Table 21. Comparison of differences between the two groups before and after treatment with L-NTX and TRACP-5b (χ±s)

	Group	Number of samples	Difference before and after treatment	t	P	95% CI of difference
L-NTX (nmolBCE/L)	Zhuanggu Zhitong Recipe Group	46	21.17±4.57	-2.71	0.008	-4.033~-0.623
	Alendronate sodium group	45	23.50±3.53			
TRACP-5b (U/L)	Zhuanggu Zhitong Recipe Group	46	0.374±0.002	-31.98	0.001	-0.019~-0.017
	Alendronate sodium group	45	0.392±0.002			

Note: L-NTX content between the two groups before and after treatment was calculated separately, and the normality test was performed on the difference between the two groups. The results were 0.353 and 0.142 respectively, both greater than 0.05, which satisfied normality and having homogeneous variances. The results of the homogeneity test: L(1,89)=1.252, P=0.266, satisfying the homogeneity of variances, using the independent sample T test. TRACP-5b content between the two groups before and after remedy was calculated respectively, and the normality test was performed on the difference between the two groups. The results were 0.260 and 0.134 respectively, both greater than 0.05, satisfying normality and variance. Homogeneity test results: L(1,89)=0.024, P=0.876, which meets the homogeneity of variances and used the independent sample T test.

Serum iron plays a crucial role in bone health and maintenance [17]. In this study, serum iron concentration was slightly elevated after treatment in both groups, but the degree of elevation was significantly higher in the group of The Strong Waist Tong Fang than in the group of Alendronic Acid.

Improvement in serum IL-17 and IL-1

Autophagy can inhibit osteoclast proliferation and differentiation by regulating T cells and suppressing the release of inflammatory factors [18]. Liu [19] et al. found that ozone can regulate autophagy in OA chondrocytes through the AMPK/mTOR signaling pathway, thereby inhibiting the release of inflammatory factors, suggesting that inflammatory factors are the main factors causing articular cartilage pain [20]. This study found that after 24 weeks of drug treatment, The Zhuanggu Zhitong Recipe treatment group significantly reduced IL-17 and IL-1 levels, indicating that Zhuanggu Zhitong Recipe has good efficacy in improving osteoporotic pain and slowing bone loss.

Correlation analysis between AMPK content and bone density

We propose a hypothesis: Zhuanggu Zhitong Recipe may play a role in treating postmeno-

Table 22. Comparison of osteoporosis therapeutic effects (N%)

Group	Number of samples	Effective	Efficient	Invalid	Z	P
Zhuanggu Zhitong Recipe Group	46	33 (71.7)	13 (28.3)	0	-2.042	0.041
Alendronate sodium group	45	40 (88.9)	5 (11.1)	0		

Note: Using the rank sum test, the average rank of the Zhuanggu Zhitong Recipe group was 42.14, and the average rank of the alendronate sodium group was 49.94; Z=-2.042, P=0.041.

Table 23. Comparison of efficacy of 5 TCM syndromes (N%)

Group	Number of samples	get well	Effective	efficient	invalid	Z	P
Zhuanggu Zhitong Recipe Group	46	5 (11.8)	39 (84.5)	2 (3.7)	0	-2.906	0.004
alendronate sodium group	45	2 (4.4)	30 (66.7)	13 (28.99)	0		

Note: Using the rank sum test, the average rank of the Zhuanggu Zhitong Recipe group is 57.10, and the average rank of the alendronate sodium group is 44.88; Z=-2.906, P=0.004.

pausal osteoporosis by regulating autophagy through the AMPK signaling pathway.

Clinical study summary

Zhuanggu Zhitong Recipe combined with calcium carbonate D(II) can significantly increase the AMPK content. AMPK has a significant positive correlation with bone density. Zhuanggu Zhitong Recipe may improve the level of autophagy by positively regulating AMPK levels in osteoporosis.

Acknowledgements

Disclosure of conflict of interest

None.

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