A comparison of the effect of alendronate and You-Gui-Wan on osteoporosis
in female rats with kidney-yang deficiency

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Abstract

Background: In traditional Chinese medicine, You-Gui-Wan (YGW) is typically used to treat osteoporosis associated with kidney-yang deficiency. However, there have been few mechanistic studies on the effectiveness of kidney-yang deficiency-type osteoporosis with YGW. To further clarify the role of YGW in the effect of osteoporosis with kidney-yang deficiency, the study analyzed the therapeutic advantages of YGW by comparing the therapeutic effects of YGW and alendronate (ALN) on osteoporosis with kidney-yang deficiency.

Methods: SPF female SD rats were randomly divided into control, osteoporosis, osteoporosis with kidney-yang deficiency, osteoporosis with kidney-yang deficiency + YGW, and osteoporosis with kidney-yang deficiency + ALN groups. Except for the control group, osteoporosis was induced by the removal of bilateral ovaries. After 12 weeks, rats with osteoporosis in the kidney-yang deficiency group had kidney-yang deficiency syndrome triggered by hydrocortisone for 14 days. Rats were treated with YGW or ALN for 12 weeks. The weights of rats were recorded. Hematoxylin-eosin staining staining was used to observe pathological changes in bone trabeculae, liver, spleen, and kidneys of rats. Depletion of the growth plate cartilage of rats in different groups was observed by immunohistochemical staining. The expression of osteoclast key indices (ACP) and osteoblast key indices (ALP) in the bone tissue of rats in the different groups was observed by immunohistochemical staining. The expression of bone resorption-related indicators (TRAP and NNT) and bone formation-related indicators (BALP, BGP, and P1NP) and major indicators of kidney-yang deficiency (ACTH, T3, T4, cAMP, and cGMP) were observed using ELISA detection kit. The expression levels of the main indices of liver function (ALT and AST) were detected in different groups.

Results: The differences between the osteoporosis with kidney-yang deficiency group and osteoporosis group were that the weight of rats and the expression of ACTH, T3, T4, and cAMP decreased significantly, and the expression of cGMP increased in the osteoporosis with kidney-yang deficiency group. Moreover, both YGW and ALN effectively improved the symptoms of osteoporosis, including the injury of bone trabeculae and growth plates, as well as the expression of bone metabolism-related indicators. However, unlike ALN, YGW simultaneously ameliorated the expression of key indicators of kidney-yang deficiency and prevented weight loss in rats. In addition, YGW caused no obvious damage to the liver, spleen, or kidney, whereas ALN led to liver cirrhosis.

Conclusion: The results reveal that YGW plays a crucial part in osteoporosis with kidney-yang deficiency, increases bone mineral density, and improves bone metabolism indicators, and is safe and efficient for the efficacy of osteoporosis with kidney-yang deficiency. YGW might have a better therapeutic effect on osteoporosis in patients with kidney-yang deficiency. Therefore, alendronate should be used cautiously in patients with osteoporosis and poor liver function.

Keywords: osteoporosis; kidney-yang deficiency; You-Gui-Wan; alendronate
in the clinical treatment of TCM, YGW is often used for osteoporosis with kidney-yang deficiency [13]. However, there have been few studies on the mechanism of action of YGW in treating osteoporosis associated with kidney-yang deficiency. To further clarify the rationality of YGW in treating osteoporosis with kidney-yang deficiency, this study compared the efficacy and safety of YGW with alendronate (ALN), and demonstrated the advantages of YGW in treating osteoporosis with kidney-yang deficiency, providing a theoretical basis for its clinical application.

Materials & methods

Experimental animal

SPF female Sprague-Dawley rats (180–220 g, 6 months old) were provided by the Animal Experimental Center of Guangzhou University of Chinese Medicine, Guangzhou, China (certification No. SCXK (Yue) 2018-0034). All rats were housed in ventilated cages (5 rats per cage) with free access to pellet feed and water under 12 h light/dark cycle, 22 ± 1°C laboratory temperature, and 58% ± 4% relative humidity. Animal experiments were conducted in strict accordance with the ethical regulations of the Committee for Animal Care and Use at the Guangzhou University of Chinese Medicine and the Guide for the Care and Use of Laboratory Animals. The code of Ethics in Animal experiments was 20191129002.

Drugs, reagents and antibodies

YGW was purchased from Zhongjing Wanxi Pharmaceutical Co., Ltd. (Nanjing, China). ALN was purchased from CSPC Pharmaceutical Co., Ltd. (Shijiazhuang, China). Hydrocortisone (Hyd) was purchased from CSPC Pharmaceutical Co., Ltd. (Tianjin, China). Safranin-O (G1053) was purchased from Service Bio Co., Ltd. (Wuhan, China). TRAP (ml1623177-2), NXT-1 (ml059218-2), BALP (ml003415-2), BGP (ml002885-2), P1NP (ml038224-2), CAMP (ml002907), cGMP (ml003133), and ACTH (ml002875) antibodies were purchased from Meiliian Co., Ltd. (Shanghai, China). T3 (CSB-E0508Sr) and T4 (CSB-E05082r) were purchased from Cusabio Co., Ltd. (Wuhan, China), and bicinechonic acid (BCA; P0010S) was purchased from Beyotime Biotechnology Co., Ltd. (Shanghai, China). ALT (CO09-2-1) and AST (CO10-2-1) were purchased from Nanjing Jiacheng Bioengineering Institute (Nanjing, China). Safranin-O (G1053) was purchased from Service Bio Co., Ltd. (Wuhan, China). ACP (11594-1-AP) and ALP (11187-1-AP) were purchased from PeproTech Co., Ltd. (Rockville, MD, USA).

Animal treatment and experimental protocol

Comparison of osteoporosis and osteoporosis with kidney-yang deficiency. SPF female Sprague-Dawley rats were randomly divided into control (n = 5), osteoporosis (n = 5), and osteoporosis with kidney-yang deficiency (n = 5) groups. After acclimatization for at least one week, rats in the control group underwent a sham operation, and other rats were anesthetized with pentobarbitone sodium injection, after which the bilateral ovaries were removed through a < 1 cm dorsal incision. The procedure of the sham operation, as in the control group, was the same as in the ovariectomized (OVX) rat group, but the ovaries were not removed. After 12 weeks, rats with osteoporosis in the kidney-yang deficiency group had kidney-yang deficiency syndrome triggered by Hyd for 14 days [14, 15]. The weights of rats were recorded. Hematoxylin-eosin (H&E) staining was applied to assess pathological changes in the bone trabeculae, liver, spleen, and kidney of rats in different groups. Depletion of the growth plate cartilage of rats in different groups was observed by safranine-O staining. The expression of osteoclast key indices (ACP) and osteoblast key indices (ALP) in the bone tissue of rats in the different groups was observed by immunohistochemical staining. The expression of bone resorption-related indicators (TRAP and NXT-1), bone formation-related indicators (BALP, BGP, and P1NP), and major indicators of kidney-yang deficiency (ACTH, T3, T4, CAMP, and cGMP) were observed using an ELISA detection kit purchased from MeilLian Co., Ltd. (Shanghai, China). The expression levels of the main...
indices of liver function (ALT and AST) were detected in different groups.

**Comparison of YGW and ALN on kidney-yang deficiency type osteoporosis.** SPF female Sprague-Dawley rats were randomly divided into control (n = 10), osteoporosis with kidney-yang deficiency (n = 10), YGW (n = 10) and ALN (n = 10) groups. After acclimatization for at least one week, rats in control group underwent sham operation and other rats were anesthetized with injection of pentobarbital sodium and bilateral ovaries were removed through a < 1 cm dorsal incision. The procedure of sham operation, as the control group, was the same as OXV rats group, but the ovaries were not removed. 12 weeks after OVX operation, OVX rats were randomly divided into four groups (n = 10): OVX, OVX + Hyd (20 mg/kg), OVX + Hyd + YGW (2.4 g/kg), and OVX + Hyd + ALN (1 mg/kg) [15].

Kidney-yang deficiency syndrome was induced by continuous injection of Hyd into rats for 15 days. In pharmacological studies, distilled water, YGW or ALN were administered once daily for 12 weeks. The body weight of the rats was recorded once per week. The weight change between the final and initial body weights was expressed as a percentage change relative to the initial body weight. After the rats were sacrificed, the serum levels of TRAP, NBT, BALP, BGP, P1NP, ACTH, T3, T4, cAMP, cGMP, ALT, and AST were measured. The tibia, and femur, liver, spleen, and kidneys were separated and fixed in the 4% paraformaldehyde. Samples were stored for subsequent histomorphometric analyses.

**Histomorphometric analysis**

Tibial and femoral samples were decalcified in 10% EDTA for three weeks. The samples were dehydrated and embedded in paraffin. Histological sections were prepared, and H&E and safranin-O staining were conducted for observation.

**Calculation of bone trabecular area**

**Graphics segmentation.** ITK-SNAP3.6 software was used to roughly segment the areas of interest in the graph. We dragged the Image into ITK-Snap3.6 software and select Generic ITK Image format to open the file (Supplementary Figure S1), adjusted the image color to distinguish the border, selected “Color Map Editor” on the “Tools” toolbar to adjust the color (Supplementary Figure S2), circled the area to be analyzed (Supplementary Figure S3), and saved the picture (Supplementary Figure S4–S6).

**Calculation of contour area of epiphysis.** We used the cv2.imread function in python software to read the above roughly segmented image area, converted the read image to grayscale (Supplementary Figure S7), used the findContours function in cv2 to find the contour and returned the properties of the contour. The contourArea function in cv2 was used to calculate the area of each aperture contour. The area calculation is based on the number of pixels. The algorithm is to take the center point of the pixel at the boundary of the connected domain, connects it to form a contour, and calculates the area of the hole.

**Calculation of the area of each small pore.** We used the cv2.imread function in python software to read the above roughly segmented image area, converted the read image to grayscale (Supplementary Figure S8), used the findContours function in cv2 to find the contours of each hole and returned the properties of each contour (Supplementary Figure S9). The contourArea function in cv2 was used to calculate the area of each aperture contour (the area calculation is based on the number of pixels). The area calculation is based on the number of pixels. The algorithm is to take the center point of the pixel at the boundary of the connected domain, connects it to form a contour, and calculates the area of the hole. The trabecular area is the area of the epiphysial contour minus the total area of each small pore in the contour.

**Immunohistochemistry**

Tibia and femur sections (4 μm) were deparaffinized in xylene and rehydrated in a graded series ethanol (100%, 95%, 85%, and 75%) for 5 min each, followed by antigen recovery. Slices were incubated at room temperature with 3% H2O2 for 15 min, washed with 1× PBS, incubated with goat serum at 37 °C for 30 min, and incubated overnight with primary anti-ACP or anti-ALP antibody at 4 °C. Whereafter, the slices were incubated with secondary antibody at 37 °C for 30 min and subsequently incubated with HRP at 37 °C for 30 min and colored with DAB.

**ALT, AST and ELISA assays**

The activity of ALT and AST in plasma was determined according to the manufacturer’s instructions. ELISA kits (MeiLian Co., Ltd., Shanghai, China) were used to detect levels of TRAP, NBT, BALP, BGP, P1NP, ACTH, T3, T4, cAMP, cGMP, ALT, and AST were measured. The tibia, and femur, liver, spleen, and kidneys were separated and fixed in the 4% paraformaldehyde. Samples were stored for subsequent histomorphometric analyses.

**Statistical analysis**

Results are presented as mean ± standard deviation (SD). Statistical analyses were performed using one-way analysis of variance with Dunnett’s multiple comparison test to analyze the significance among multiple groups, and an unpaired Student’s-t test was used to analyze the significance between two groups. The Kolmogorov-Smirnov test was used to determine whether the samples conformed to a normal distribution. Statistical significance was set at P < 0.05.

**Results**

**Comparison of bone histopathology between osteoporosis and osteoporosis with kidney-yang deficiency**

Osteoporotic manifestations developed in all rats following bilateral ovary removal, as evidenced by the reduction in bone trabeculae (Figure 1A, 1B). Furthermore, safranine-O staining showed that the ACP markedly increased, and the expression of the osteoblast index ALP notably decreased in both the osteoporosis group and osteoporosis with kidney-yang deficiency group (Figure 1E–1H). These results are consistent with the fact that there was no significant area of the growth plate in both the osteoporosis group and osteoporosis with kidney-yang deficiency group was notably reduced (Figure 1C, 1D). In addition, the expression of the osteoclast index difference in bone injury between the osteoporosis and kidney-yang deficiency groups.

**Comparison of bone metabolism index and kidney-yang deficiency index between osteoporosis and osteoporosis with kidney-yang deficiency**

The weight of rats with osteoporosis with kidney-yang deficiency group decreased markedly compared with the control and osteoporosis groups (Figure 2A, 2B). In addition, compared with the control and osteoporosis groups, the expression of kidney-yang deficiency indicators ACTH, T3, T4, and cAMP decreased significantly, and cGMP increased observably in osteoporosis with kidney-yang deficiency group (Figure 2C–2G). In addition, the levels of the bone resorption indicators TRAP and NBT were obviously elevated in the osteoporosis group and osteoporosis with kidney-yang deficiency group compared to the control group (Figure 2H, 2I). However, the levels of bone formation indicators, BALP, BGP, and P1NP, markedly decreased in the osteoporosis group and in the kidney-yang deficiency group compared to the control group (Figure 2J–2L). The above results show that there was no obvious difference in osteoporosis symptoms between the osteoporosis group and the kidney-yang deficiency group. However, compared to the rats in the osteoporosis group, the rats in the osteoporosis with kidney-yang deficiency group showed obvious symptoms of kidney-yang deficiency.

The injury of liver, spleen, kidney between osteoporosis and osteoporosis with kidney-yang deficiency
Figure 1 Comparison of bone histopathology between osteoporosis and osteoporosis with kidney-yang deficiency. (A, B) Observation and quantitative analysis of H&E stained sections of bone tissue. (C, D) Observation and quantitative analysis of safranine-O stained sections of bone tissue. (E-H) Observation and quantitative analysis of immunohistochemical staining with ACP/ALP in bone tissue. Data were shown as mean ± SD. Hyd, hydrocortisone; H&E, hematoxylin-eosin staining; OVX, ovariectomized; Con., Control; ns, no significance.
Figure 2 Comparison of bone metabolism index and kidney-yang deficiency index between osteoporosis and osteoporosis with kidney-yang deficiency. (A, B) The body weight changes of rats. (C–L) The expression of ACTH, T3, T4, cAMP, cGMP, TRAP, NXT-1, BALP, BGP and P1NP in serum. Data were shown as mean ± SD. Hyd, hydrocortisone; OVX, ovariectomized; Con., Control; ns, no significance.

The H&E staining results showed that all rats had fatty liver lesions, but rats in the osteoporosis with kidney-yang deficiency group had more severe lesions (Figure 3A, 3B). However, there was no obvious difference in the expression of ALT or AST between the groups (Figure 3G, 3H). In addition, there was no obvious damage to the spleen or kidneys in either group (Figure 3C-3F).

Effect of YGW or ALN on improving the injury of trabecular bone and growth plate
Bone injury caused by osteoporosis in kidney-yang deficient rats was relieved by YGW or ALN treatment (Supplemental Figure S10). Compared with the osteoporosis with kidney-yang deficiency group, the trabecular bone (Figure 4A, 4B) and growth plate (Figure 4C, 4D) areas increased in the YGW and ALN groups, and the expression of ACP (Figure 4E, 4F) in osteoclasts decreased, whereas the expression of ALP (Figure 4G, 4H) in osteoblasts increased. Both YGW and ALN improved osteoporosis symptoms.

Effect of YGW or ALN on improving the imbalance of bone metabolism index and kidney-yang deficiency index
Figure 3 The injury of liver, spleen, kidney between osteoporosis and osteoporosis with kidney-yang deficiency. (A–F) Observation and quantitative analysis of H&E stained slices of liver, spleen and kidney. (G, H) The expression of ALT and AST in serum. Data were shown as mean ± SD. Hyd, hydrocortisone; H&E, hematoxylin-eosin staining; OVX, ovariectomized; Con., Control; ns, no significance.
It was reported that YGW, but not ALN, increased the body weight of kidney-yang deficiency-type osteoporotic rats (Figure 5A, 5B). In addition, YGW effectively improved the kidney-yang deficiency index (ACTH, T3, T4, cAMP, and cGMP) of osteoporosis in kidney-yang-deficient rats. Compared with the rats with osteoporosis in the kidney-yang deficiency and ALN groups, YGW significantly increased the expression of T3, T4, cAMP, and ACTH, and decreased the expression of cGMP (Figure 5C–5G). Nevertheless, both YGW and ALN decreased the levels of the bone resorption indicators TRAP and NKT-1 and increased the levels of the bone formation indicators BALP, BGP, and P1NP (Figure S1–S5L). These results revealed that both YGW and ALN could effectively relieve the symptoms of osteoporosis, but only YGW could simultaneously improve the symptoms of kidney-yang deficiency at the same time.

**Effect of YGW or ALN on liver, spleen and kidney**

The H&E staining results showed that all rats had fatty liver lesions; however, those in the ALN group were more serious and had cirrhosis (Figure 6A, 6B). However, there was no significant difference in the expression of ALT or AST between groups (Figure 6G, 6H). In addition, there was no obvious damage to the spleen or kidneys in either group (Figure 6C–6F). These results suggested that ALN should be used with caution in patients with osteoporosis associated with liver dysfunction.

**Discussion**

Osteoporosis is a progressive degenerative bone disease associated
with aging and is characterized by the loss of bone mass and deterioration of bone tissue architecture, resulting in bone fragility and an increased risk of fracture. Osteoblast and osteoclast dysfunction is believed to be the main pathological cause of osteoporosis [16]. In postmenopausal women, excessive osteoclast activity due to estrogen deficiency leads to reduced bone mass and decreased bone strength, resulting in osteoporosis [17–19].

Based on TCM theory of traditional Chinese medicine, the fundamental cause of osteoporosis is kidney deficiency. The Yellow Emperor’s Classic of Internal Medicine mentioned: “kidney dominated bones and generated marrow”. Therefore, kidney deficiency leads to the weakening of myelopoietic function, which causes bone marrow emptiness, weakened bone strength, and ultimately osteoporosis [20]. There is a relationship between the classification of kidney deficiency syndrome and the degree of osteoporosis, and osteoporosis with kidney-yang deficiency is more serious [9]. Therefore, kidney yang-tonifying drugs are often used to treat osteoporosis. YGW has the function of regulating kidney function, improving hematopoietic function, and enhancing body immunity [21]. Its clinical effects on osteoporosis have been confirmed; however, its therapeutic mechanism has not yet been fully elucidated.

To better introduce scientific experimental methods into the research field of TCM, researchers established a rat model of TCM syndrome of kidney-yang deficiency by injecting hydrocortisone under the guidance of TCM theory, based on the clinical manifestation of TCM syndrome of kidney-yang deficiency, combined with modern etiology and pathogenesis studies [22]. In addition, the rats were injected with hydrocortisone after bilateral ovariectomy to establish an osteoporosis rat model with kidney-yang deficiency [23]. Modern medical research has shown that the damage and dysfunction of hypothalamic cells are the main pathological basis of kidney-yang deficiency syndrome, and the changes in different links and degrees of

Figure 5: Effect of YGW or ALN on improving the imbalance of bone metabolism index and kidney-yang deficiency index. (A, B) The body weight changes of rats. (C-L) The expression of ACTH, T3, T4, cAMP, cGMP, TRAP, NXT-1, BALP, BGP and P1NP in serum. Data were shown as mean ± SD. Hyd, hydrocortisone; OVX, ovariectomized; YGW, You-Gui-Wan; ALN, alendronate; Con., Control; NS, normal saline; ns, no significance.
the hypothalamic-pituitary-target gland axis (thyroid axis, adrenal axis, and gonadal axis) are the main pathological links of kidney-yang deficiency syndrome, and the indicators revealing the functional changes of these axes were the specific indicators of kidney-yang deficiency [24-27]. The decreased level of ACTH (adrenal coefficient) reflected the dysfunction or inhibition of the hypothalamic-pituitary-adrenal axis [28, 29]. The decreased levels of T3 and T4 (thyroid coefficient) indicated the disorder of the hypothalamic-pituitary-thyroid axis to varying degrees [30, 31]. In addition, cAMP and cGMP are secondary messengers in the body and are important factors regulating physiological functions, metabolism, cell proliferation, and differentiation [32]. The ligands of cAMP include polypeptide hormones, which have regulatory effects on endocrine hormones, including adrenocortical and thyroid hormones, and their changes are related to a certain extent. cAMP and cGMP are specific indicators of kidney–yang or kidney–yin deficiency. cAMP levels decrease and cGMP levels increase during yang deficiency [33].

In this study, we found that there were no obvious differences in the levels of bone tissue injury and bone metabolism indices (ACP, ALP, TRAP, NRTX-1, BALP, BGP, and P1NP) between the osteoporosis and kidney-yang deficiency groups; however, the symptoms of kidney-yang deficiency were obvious in osteoporosis in kidney-yang deficient rats. Compared with the normal group and the osteoporosis group, the body weight of the rats in the osteoporosis with kidney-yang deficiency group was sharply decreased, the expression of kidney-yang deficiency index (ACTH, T3, T4, cAMP) was sharply decreased, and the level of cGMP was significantly increased.

Based on the above findings, osteoporosis in the kidney-yang deficiency group was used as a model to explore the therapeutic effects of YGW and ALN on osteoporosis with kidney-yang deficiency. The results displayed that both YGW and ALN effectively ameliorated the symptoms of osteoporosis, including weakening the injury of the

Figure 6 Effect of YGW or ALN on liver, spleen and kidney. (A–F) Observation and quantitative analysis of H&E stained sections of liver, spleen and kidney. (G, H) The expression of ALT and AST in serum. Data were shown as mean ± SD. Hyd, hydrocortisone; H&E, hematoxylin-eosin staining; OVX, ovariotomized; YGW, You-Gui-Wan; ALN, alendronate; Con., Control; NS, normal saline; ns, no significance.
bone trabecula and growth plate, increasing the expression of the bone formation index, and decreasing the expression of the bone resorption index. However, ALN could not simultaneously relieve the symptoms of kidney-yang deficiency compared to YGW, including increased body weight and improved expression of ACTH, T3, T4, cAMP, and cGMP in rats. These results show that YGW might have a better therapeutic effect in osteoporosis patients with kidney-yang deficiency syndrome. In addition, with the increase in age, the liver of rats gradually showed a fatty liver phenomenon, and the symptoms were slightly aggravated after hydrocortisone induction, cirrhosis occurred after long-term administration of ALN [34–37]. These findings suggest that ALN should be used with caution in patients with osteoporosis and liver dysfunction. In subsequent studies, we will further explore whether YGW improves osteoporosis with kidney-yang deficiency by regulating the function of the hypothalamic-pituitary-adrenal axis, and what factors downstream of the signaling axis affect the related activities of bone metabolism to exert a therapeutic effect.

Conclusions

In summary, both YGW and ALN effectively improved bone tissue injury and bone metabolism indices in the treatment of osteoporosis in kidney-yang deficient rats; however, YGW also increased body weight and improved kidney-yang deficiency indices in rats. In addition, ALN has a damaging effect on the liver. Therefore, YGW may be more effective in patients with osteoporosis and kidney-yang deficiency syndromes. This study only conducted pharmacodynamic and some drug toxicity experiments and did not further explore the mechanism of effect. Therefore, there are still many problems that cannot be explained clearly, and the specific mechanism of the effect will be analyzed in detail in further research.

References

13. Liang Z, Dong B, Yuan PW, et al. Clinical study of Yougupill on postmenopausal osteopenia with kidney yang deficiency. Chin J Osteoporos. 2022;22(8);1164–1168. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=r-3vL8v-lgqnm-B1rhyhql4mfrFl4cDBO3CHnHyW548l0qJgB7ZUpuna3A3_3Q5nCa2cuwqYzyjw14q-lmVFCKBAPYPQorrElkXtwTJGLsVRK_0PSvRh+nvPeCnDiJhkuG1uwvKiiNvAnDrk9P0h1YhwTA1ygw==&uniplatfo?rn=NXKK%26language=CHS
15. Li Y, Xu HT, Li HQ, et al. Establishment of animal model of osteoporosis with kidney yang deficiency syndrome in rats. Drug Eval Res. 2015;38(2):136. (Chinese) Available at: https://kns.cnki.net/kcms2/article/abstract?v=r-3vL8v-lgqnm-70h6oXoBybfVuytCutfatFCk20uMKb1ZpFZt367Ug323CTzLs8e5xFp965FQZhukhmnf25TDiReds-uksl777jXgC1qA81vGw2FQBRYwdek3hjok5sLrdXo6vwLgwYUEBWA5rSlsQcNg==&uniplatfo?rn=NXKK%26language=CHS
19. Almeida M, Laurent MR, Dubois V, et al. Estrogens and androgens in skeletal physiology and pathophysiology. Physiol...