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Research Article

Effect of Resistance Training with Palm Pollen and Testosterone on Runx2 Protein and Gene Expression Levels in Bone Tissue of Adult Male Rats

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Abstract

Background: A healthy lifestyle, nutrition, and exercise can improve bone mass via several mechanisms.
Objectives: This study assessed the effects of four weeks of palm pollen consumption along with resistance training on protein and gene expression levels of Runt-related transcription factor 2 (Runx2) in bone tissue of rats.
Methods: Thirty-six rats were selected and assigned into six groups, including (1) training + testosterone, (2) training + palm pollen, (3) testosterone, (4) palm pollen, (5) training and (6) sham. Then, 100 mg/kg of palm pollen was prescribed five days per week. Resistance training was performed five sessions per week, and 2 mg/kg of testosterone propionate was prescribed peritoneally. Gene expression and protein levels of Runx2 were measured via the real-time PCR and Western blot methods.
Results: Training had a significant effect on the increase in Runx2 protein levels ($P \leq 0.05$). Training + testosterone, training + palm pollen, testosterone, and palm pollen had significant effects on gene expression and protein levels of Runx2 ($P \leq 0.05$). Training + testosterone and training + palm pollen had more favorable effects on the increase of gene expression and protein levels of Runx2 than had testosterone, palm pollen, and training ($P \leq 0.05$).
Conclusions: Although training, palm pollen, and testosterone alone could increase the Runx2 protein levels in the bone tissue of rats, training with palm pollen and training with testosterone appeared to have more favorable effects on the increase of gene expression and protein levels of Runx2 than either alone.

Keywords: Training, Palm Pollen, Testosterone, Runx2, Bone

1. Background

Loss of spongy bone mass in humans begins in the early third decade of life before any change in the production of sex steroids (1). The loss of bone mass and strength in rodents with increasing age occurs along with an increase in the prevalence of osteoporosis and a decrease in the number of osteoblasts and the rate of bone formation. Testosterone is a 19-carbon steroid hormone that is metabolized in the liver, testicles, and prostate (2). Testosterone can play a potential role in the preservation of brain function, bone, and muscle mass. Studies have shown that testosterone therapy increases bone density; however, the effects of testosterone administration on bone markers depend on testosterone baseline levels, hypogonadism, and bone disorders (2). Animal studies have shown that osteoblasts in bone tissue are one of the targets of androgenic hormones, which increases the longitudinal growth, volumetric growth, and deposition of bone salts in different bones. Indeed, testosterone is one of the key factors in the growth of the skeletal structure and prevention of osteoporosis (2).

Runt-related transcription factor 2 (Runx2) is a transcription factor associated with osteoblast expression. Runx2 is known to be a major regulator of the differentiation and expression of osteoblasts (3). Indeed, the osteogenic activity of bone marrow stromal cells causes excessive expression of Runx2. Runx2 is regulated at both translation and post-translation levels (3). Mechanical bone stimulation can differentiate mechanical load-sensitive mesenchymal stem cells into osteoblasts. Past research has shown that mechanical loading is a powerful tool for preventing osteoporosis. Although osteoporosis is an age-related disease, lifestyle changes, nutrition, and exercise, especially during adolescence, can reduce the risk of osteoporosis. The exercise can enhance bone density via increasing phosphorus and calcium, which is facilitated by the secretion of the estrogen hormone, the increase of calcitonin, and the decrease of parathormone. Physical activity is a healthy way of manipulating people, with proven benefits, especially on bones of the elderly (4). However, the results of studies on the effect of exercise on bone markers are contradictory. In this regard, it has been reported that eight weeks of low, moderate, and high-intensity running significantly increased Runx2 levels in the bone marrow of male rats (5). Also, resistance training could induce a significant increase in Runx2 levels in rats (6). Nevertheless, eight weeks of resistance, intense endurance, and moderate endurance training did not change the gene expression levels of Runx2 in the femoral marrow of elderly rats (3). In line with sports activities, nutritional supplements have also been considered by various researchers in the prevention and treatment of bone disorders.

The use of medicinal plants has been of great importance throughout the history of medicine, and human beings have always used plants to treat their ailments throughout history. One of these herbs is date (Phoenix dactylifera), which has important pharmacological effects (7). Compounds such as proteins, fats, sugars, substances from the camphor family, plant sterols, and three types of coumarin have been identified in date flowers (7). Compounds such as zinc and cadmium in palm pollen have been reported to increase testosterone production and steroid metabolism by increasing the biosynthesis of 17β-hydroxysteroid dehydrogenases (7). Regarding the effects of testosterone on bone cells, it has been reported that testosterone consumption increases the rate of repair, bone tissue density, the number of osteoblasts, and the diameter and weight of bones, and reduces the number of osteoclasts (8). Therefore, the consumption of palm pollen can indirectly lead to the improvement of bone cells.

2. Objectives

In various studies, the effects of testosterone have been confirmed on bone size and density (1). Also, as noted, by increasing the biosynthesis of 17β-hydroxysteroid dehydrogenase, palm pollen enhances the steroid metabolism and testosterone production so that the effects of palm pollen consumption have been confirmed on increasing plasma levels of sex hormones in various studies. Therefore, the consumption of palm pollen can mimic the effects of testosterone (7). On the other hand, it has been reported that exercise has a positive role in increasing total bone marrow mesenchymal stem cells (MSCs), increasing osteogenesis, reducing bone MSC adipogenesis, and increasing the differentiation of load-sensitive MSCs to osteoblasts through Runx2 (6). Therefore, it seems that the consumption of palm pollen simultaneously with exercise can increase the effects of exercise on bone markers and exert similar effects to testosterone. Thus, in the present study, an attempt was made to investigate and compare the effects of palm pollen and testosterone administration along with exercise on Runx2. In fact, in the present study, testosterone was used as a positive control. Therefore, the effects of four weeks of palm pollen consumption along with resistance training, were determined on protein and gene expression levels of Runx2 in bone tissue of rats.

3. Methods

Thirty male rats were selected as the sample. After a one-week adaptation to the animal lab environment, they were divided into six groups, including (1) training + testosterone, (2) training + palm pollen, (3) testosterone, (4) palm pollen, (5) training, and (6) sham. The research lasted four weeks. During this period, the training groups performed five sessions per week of resistance training (9), and the palm pollen groups received 100 mg/kg (10) palm pollen via gavage five days per week. The testosterone groups received 2 mg/kg (11) testosterone propionate subcutaneously. At the end of the research period, all rats were anesthetized by xylazine 2% (10 mg/kg) and ketamine 10% (50 mg/kg) (12). Then, bone tissue was extracted and transferred to the laboratory. The real-time PCR and western blot methods were used for measuring the gene expression and protein levels of Runx2, respectively.

3.1. Testosterone Administration

In this study, 2 mg/kg testosterone as testosterone propionate (Iran Hormone Company) was dissolved in DMSO and injected as a positive control (11).

3.2. Preparation of Ethanolic Extract of Palm Pollen

For the preparation of the ethanolic extract of palm pollen, 250 g palm pollen was poured into a percolator. Extraction was performed with 670 ml of 90% ethanol. This was repeated three times. The yield of extract was 17.87%. After extraction, 100 mg/kg ethanolic extract of palm pollen was fed to rats five days per week (10).

3.3. Training Program

At the beginning of training, A weight equal to 50% of the weight of each rat was attached to the tail, and the rats climbed the ladder. In the case of the ability to climb, 30 grams were added again to the weights, and then rats tried to raise the ladder. Again, if rats could climb, another 30 grams were added. In the first session, the highest weight which rats could carry indicated the maximum strength of rats. In the training sessions, all rats performed four sets of climbing ladders; the weight was 50% of maximum strength in the first set, 75% in the second set, 90% in the third set, and 100% in the fourth set (9).

3.4. Western Blotting Method for Runx2 Measurement

To measure the protein levels of Runx2, the Western blotting method was used. Images obtained from the studied bands of each protein were analyzed using ImageJ software. To ensure equal amounts of protein at the time of measurement, the protein content was determined by the lowry method before testing. The GAPDH protein was used as an internal control.

3.5. Runx2 Measurement by Real-Time PCR

For measuring the gene expression of Runx2, the real-time PCR method was used. Table 1 shows the sequence of Runx2 and GAPDH as the control gene.

Table 1. The Sequence of Forward-Reverse Primers of Runx2 and GAPDH

Gene	Forward	Reverse
Runx2	5'-GCCGTAGAGAGTAGGGAAGAC-3'	5'-CCACAAGGTGCCAGGAATG-3'
Gapdh	5'-CAT ACT CAG CAC CAG CAT CAC C-3'	5'-AAG TTC AAC GGC ACA GTC AAG G-3'

3.6. Analysis of Data

The Shapiro-Wilk test and one-way ANOVA with Tukey's post hoc tests were used in SPSS software for statistical analysis of data ($P \leq 0.05$).

4. Results

Figures 1 and 2 represent the protein and gene expression levels. The results showed that training + testosterone (P = 0.001), training + palm pollen (P = 0.001), testosterone (P = 0.001), and palm pollen (P = 0.006) had significant effects on the increase of Runx2 gene expression levels. Besides, training + testosterone (P = 0.001), training + palm pollen (P = 0.001), and testosterone (P = 0.02) had more effects on the increase of Runx2 gene expression levels than training, and training + testosterone and training + palm pollen had more effects on the increase of Runx2 gene expression levels than testosterone and palm pollen (P = 0.001) (Figure 1).

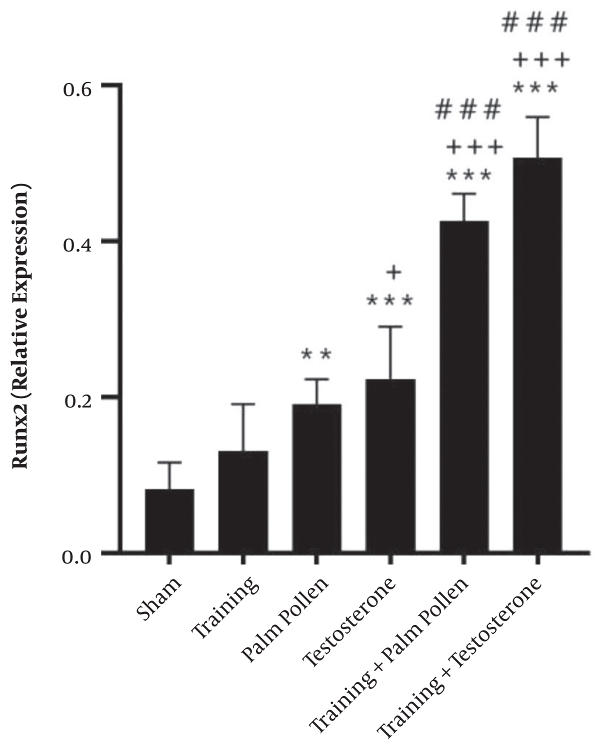

Figure 1. Runx2 gene expression (relative to GAPDH expression) levels in six groups of study. Statistical analyses were performed using one-way ANOVA with Tukey's post hoc tests. ***$P < 0.001$ and **$P < 0.01$ significant increases compared to the sham group; $^{+++}P < 0.001$ and $^{+}P < 0.05$ significant increases compared to the training group; $^{###}P < 0.001$ significant increases compared to the palm pollen and testosterone groups

Training + testosterone, training + palm pollen, testosterone, palm pollen, and training had significant effects on the increase of Runx2 protein levels (P = 0.001). Training + testosterone, training + palm pollen, and testosterone had more effect on the increase of Runx2 protein levels than training and palm pollen (P = 0.001). Training + testosterone and training + palm pollen had more effects on the increase of Runx2 protein levels than testosterone (P = 0.001), and training + testosterone had more effects on the increase of Runx2 protein levels than training + palm pollen (P = 0.001) (Figure 2).

5. Discussion

In the present study, four-week resistance training did not significantly change the gene expression levels of Runx2 in the bone tissue of rats; however, it significantly increased the Runx2 protein levels in the bone tissue. Various studies have been conducted on the effect of exercise on Runx2, which have reported contradictory results. In contrast with the findings of the present study, it was shown that resistance training in vivo led to an increase in Runx2 gene expression and BMD in mice (6) and running led to an increase in Runx2 gene expression in bone tissue (13). Also,

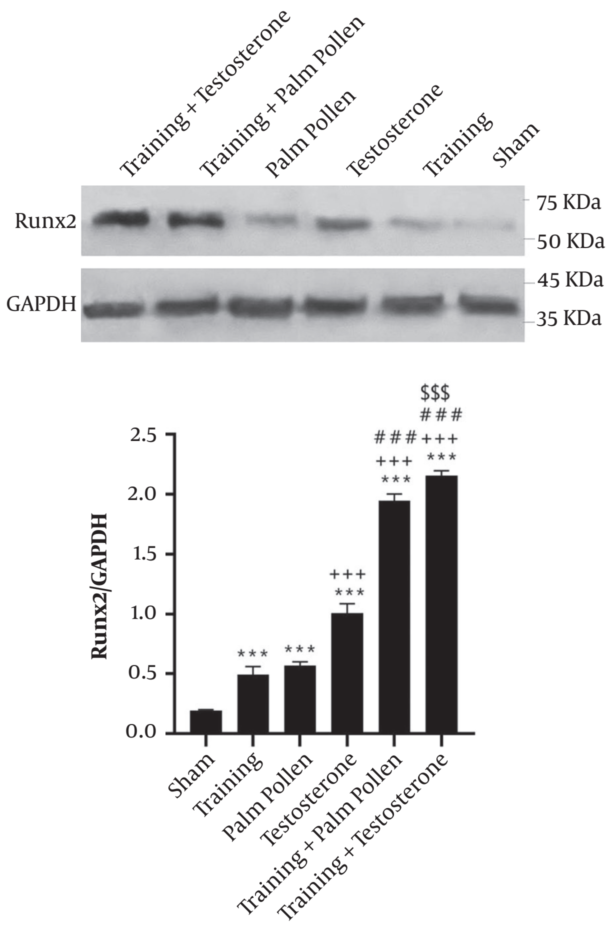

Figure 2. Runx2 protein levels in six groups of study. Statistical analyses were performed using one-way ANOVA with Tukey's post- hoc tests. ***P < 0.001 Significant increases compared to the sham group; +++P < 0.001 Significant increases compared to the training and palm pollen groups; ###P < 0.001 Significant increases compared to the testosterone group; $$$P < 0.001 Significant increases compared to the training + palm pollen group

in the bone tissue of ovariectomized rats, the gene expression levels of Runx2 significantly increased after 12 weeks of running on a treadmill (14). One of the reasons for the inconsistency of the findings of the present study and the mentioned studies can be the type of subjects; Wang et al. study used ovariectomized rats (14), and regarding the effects of ovariectomy on bone mass and osteoporosis, it appears that exercise has a greater effect on gene expression levels of Runx2 in ovariectomized rats than in healthy rats. However, consistent with this research, Hemati Farsani et al. reported that eight weeks of moderate and high-intensity resistance training did not enhance the gene expression levels of Runx2 in the bone tissue of elderly rats (3). Considering that the intensity of exercise and the type of activity prescribed were similar in these two studies, it seems that the reason for the similarity of Runx2 gene expression findings and the inconsistency of Runx2 protein level findings can be the method of measuring variables. Runx has been reported to increase the expression of osteocalcin and COL1A1 genes. Therefore, Runx2 is a primary osteogenic marker in the cell. Researchers have found that Runx2 is involved in bone formation in animal samples. Studies on the role of gene expression have shown that Runx2 in bones is specifically involved in bone formation (osteoblasts). Research on mice also has shown that Runx2 deficiency causes bone density and bone mass loss, and this gene is essential for bone formation. It has been reported that by increasing miR-378-5p, exercise leads to an increase in AKT, which subsequently increases Runx2 and osteoblasts (15).

In the present study, testosterone and palm pollen consumption for four weeks enhanced gene expression and protein levels of Runx2. Although testosterone had a greater effect on the Runx2 protein levels than had palm pollen, palm pollen and testosterone had a similar effect on increasing the Runx2 gene expression levels. In this regard, it has been reported that palm pollen contains flavonoid, steroid, saponin, and lipid compounds that stimulate the secretion of LH and androgenic hormones. The palm pollen seed extract also contains compounds such as ß- sitosterol, quercetin, and rutin, which have estrogenic activity (7). Compounds such as palmitic acid and stearic acid in palm pollen prevent the conversion of testosterone to dihydrotestosterone in tissues via inhibiting the activity of the 5-alpha reductase enzyme; as a result, less testosterone is converted to dihydrotestosterone, which ultimately leads to an increase in blood testosterone levels (7). Therefore, it seems that palm pollen can have effects similar to testosterone by increasing the testosterone levels and mimicking the effects of testosterone (7). Testosterone has been reported to increase the expression of the Runx2 gene and osteocalcin (16).

Regarding the simultaneous effects, in the present study, palm pollen consumption with training could enhance gene expression and protein levels of Runx2; besides, palm pollen consumption along with training could enhance gene expression and protein levels of Runx2 more than did palm pollen and training alone; therefore, the results of the present study indicated the synergistic effects of palm pollen consumption and training so that they had similar effects to testosterone administration. Regarding the effects of exercise on bone cells, resistance training has been reported to reduce bone degradation (by reducing the number of osteoclasts) and increase bone formation (by increasing the number of osteoblasts, optimal mechanical load, and high levels of strain in resistance training) (17). However, palm pollen appears to apply its effects on improving bone marrow by increasing testosterone so that saponin in palm pollen increases pituitary gonadotropins (18). Therefore, palm pollen and resistance training with separate cellular mechanisms can improve the conditioning of bone cells.

In the present study, as training could not increase Runx2 gene expression but increased protein levels of Runx2, it can be noted that the transcription and translation can be differently regulated. Actually, there are four main steps in the process of regulating protein levels: (i) mRNA synthesis, (ii) mRNA degradation, (iii) protein synthesis, and (iv) protein degradation. Different synthesis and degradation of mRNA and proteins can cause a discrepancy between mRNA and protein levels. For instance, the protein's half-life could increase due to the reduced rate of degradation. Another possibility is that mRNA is more preferentially translated during analysis. Also, many studies have confirmed this discrepancy. (19).

5.1. Conclusion

Although training, palm pollen, and testosterone alone can increase Runx2 protein levels in the bone tissue of rats, training with palm pollen and training with testosterone appear to have more favorable effects on the increase of Runx2 gene expression and protein levels than either alone.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

Footnotes

Authors' Contribution: Laboratory studies and tests: N. P; study and review: M. P and SA. H; analysis and interpretation of data: M. P, MA. A, and SA. H.

Conflict of Interests: The authors declare that they have no conflict of interest.

Ethical Approval: Researchers received introduction letters from the Marvdasht Branch of Islamic Azad University with a code of IR.IAU.M.REC.1399.022.

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