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EXPERIMENTAL STUDY

Effects of therapies for regulating and reinforcing lung and kidney on osteoporosis in rats with chronic obstructive pulmonary disease

Tian Yange, Li Ya, Li Jiansheng, Li Suyun, Jiang Suli, Wang Ying, Lu Xiaofan, Li Weiwei

Tian Yange, Department of Traditional Chinese Internal Medicine, Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing 100700, China; Gerontology Institute, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, China

Li Ya, Li Suyun, Department of Respiratory and Central Laboratory, The First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou 450000, China

Li Jiansheng, Jiang Suli, Wang Ying, Lu Xiaofan, Li Weiwei, Gerontology Institute, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, China

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Correspondence to: Prof. Li Jiansheng, Gerontology Institute, Henan University of Traditional Chinese Medicine, Zhengzhou 450046, China. li_js8@163.com

Telephone: +86-371-65676568

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Abstract

OBJECTIVE: To evaluate the efficacy and long-term effects of the three therapies for regulating and reinforcing lung and kidney (reinforcing lung and invigorating spleen, reinforcing lung and replenishing kidney, and supplementing *Qi* and nourishing kidney) in Traditional Chinese Medicine (TCM) on osteoporosis in rats with chronic obstructive pulmonary disease.

METHODS: Totally 120 rats were randomly divided into control, model, Bufeijianpi, Bufeiyishen, Yiqizishen, aminophyline groups. Repeated smoke inhalations and bacterial infections were used to duplicate the stable Chronic obstructive pulmonary disease rat model. Normal saline was given to the air control and model groups, while Bufeijianpi granule, Bufeiyishen granule, and Yiqizishen granule, and aminophylline were administrated to rats in the Bufeijianpi, Bufeiyishen, Yiqizishen, and aminophylline groups respectively from weeks 9 through 20. Another 12 weeks without medicines to observe the long-term effect. Rats were sacrificed at week 20 and week 32. Bone mass density (BMD), bone mineral content (BMC), morphology of the femoral head, lung function, and levels of serum interleukin (IL)-1β, IL-6, and tumor necrosis factor-α were detected.

RESULTS: At weeks 20 and 32, tidal volume, peak expiratory flow and expiratory flow at 50% tidal volume in the three TCM-treated groups were higher than those in the model group ($P < 0.05$). Femur weight, BMD, and BMC were significantly higher in the three TCM-treated groups and the aminophylline-treated group compared with the model group ($P < 0.01$), except for BMC in the Yiqizishen-treated group at week 20.

CONCLUSION: Bufeijianpi, Bufeiyishen, and Yiqizishen granules show good effects in the prevention and treatment of osteoporosis, which can alleviate airflow limitations and inflammation, improve BMD and BMC of the femur, and have favorable long-term effects.

Key words: Pulmonary disease, chronic obstructive; Osteoporosis; Medicine, Chinese traditional; Bufeijianpi granule; Bufeiyishen granule; Yiqizishen granule

INTRODUCTION

Chronic obstructive pulmonary disease (COPD), characterized by persistent and progressive airflow limitation, is a major cause of mortality worldwide.[1] Osteoporosis (OP) is one of the most common extrapulmonary complications of COPD.[2] OP is a bone metabolic disease characterized by low bone mass and degradation of bone tissue micro-structure, as well as increased bone fragility. The morbidity of OP in COPD patients is very high. A previous study indicated that at least 60% of COPD patients were also suffering from metabolic bone disease, 34% of them had low bone mass, while 29% of them had developed OP.[3] The prevalence of OP in COPD varies between 4% and 59%, depending on the diagnostic methods used, the population studied, and the severity of the underlying respiratory disease.[4] Graat-Verboom *et al* [5] found that the prevalence of OP in COPD patients increased from 47% to 61% in a period of 3 years. The causes of COPD, including smoking, chronic inflammation, hypoxia, vitamin D deficiency, malnutrition, and genetic sensitivity, are also considered as risk factors for OP. Patients with OP always suffer from decreased activity, an increased fracture incidence, and reduced vital capacity, which may further aggravate chest tightness, shortness of breath, dyspnea, and other symptoms. Moreover, unbearable body pain owing to OP may promote acute exacerbations of COPD.[6]

COPD patients may be at increased risk for vitamin D [25(OH)D] deficiency, then develop into OP, and treatments are available for those who are diagnosed early.[7] However, a retrospective cross-sectional study found that a large number of vertebral fractures in males with COPD were undiagnosed, and few anti-osteoporotic agent therapies were used for treatment of patients who were diagnosed.[8]

COPD is a lung-distention syndrome (Feizhang Bing) according to Traditional Chinese Medicine (TCM). In the stable phase, deficiency of lung-spleen *Qi*, deficiency of lung-kidney *Qi*, and deficiency of lung-kidney *Qi* and *Yin* are the three most common patterns.[9,10] Our previous clinical trials and animal experiments indicate that the representative prescriptions of the three therapies for regulating and reinforcing the lung and kidney, Bufeijianpi granule, Bufeiyishen granule, and Yiqizishen granule, can reduce the frequency and duration of acute exacerbations of COPD, improve 6-minute walk distance and quality of life, decrease pulmonary and system inflammation, and lessen histological impairments in the lung, and these good effects even exist at the 3-12-month follow-up.[11,12] However, there is limited evidence concerning its mechanism and the relationship with OP.

In this study, we aimed to explore the short- and long-term effects of the three therapies for regulating and reinforcing lung and kidney on pulmonary function, cytokines, bone mass density (BMD), and bone mineral content (BMC) in COPD rats to provide evidence for its further study and clinical application.

MATERIALS AND METHODS

Animals

Sixty male and sixty female specific pathogen-free Sprague-Dawley rats, weighing (200 ± 20) g (2 months old), purchased from Laboratory Animal Center of Henan Province (SCXK [YU] 20050001), were housed in individual ventilated cages (Fengshi, China) for seven days before the experiment, with free access to sterile food and water. Experimental protocols were approved by the Experimental Animal Care and Ethics Committees of the First Affiliated Hospital of Henan University of Traditional Chinese Medicine, Zhengzhou, China (2012HLD-0001).

Bacteria

Klebsiella pneumoniae (strain: 46114) was purchased from the National Center For Medical Culture Collection (Beijing, China), and diluted with normal saline (Double-crane pharmaceutical Co., Ltd., Beijing, China) into the concentration of 6×10^8 colony forming units/mL before administration to animals.[13]

Cigarettes

Hongqiqu® filter cigarettes, flue-cured tobacco type, each containing tar 10 mg, nicotine 1.0 mg, and carbon monoxide 12 mg, was provided by Henan Tobacco Industry Co., Ltd. (Anyang, China).

Grouping of animals and preparation of COPD rat model

Overall, 120 rats were randomized into control, model, Bufeijianpi, Bufeiyishen, Yiqizishen, and aminophylline groups according to a random number table, with 20 in each group. Repeated smoke inhalations and bacterial infections were used to duplicate the stable COPD rat model.[13] COPD rats was evaluated according to the symptoms, pulmonary function and histological changes .[14]

Drugs and reagents

The three Chinese medicines are composed of the following herbs: (a) Bufeijianpi granule: Huangqi (*Radix Astragali Mongolici*) 15 g, Dangshen (*Radix Codonopsis*) 15 g, Baizhu (*Rhizoma Atractylodis Macrocephalae*) 12 g, Fuling (*Poria*) 12 g, Chuanbeimu (*Bulbus Fritillariae Cirrhosae*) 9 g, Dilong (*Pheretima Aspergillum*) 12 g; (b) Bufeiyishen granule: Renshen (*Radix Ginseng*) 9 g, Huangqi (*Radix Astragali Mongolici*) 15 g, Shanzhuyu (*Fructus Corni*) 12 g, Yinyanghuo (*Herba Epimedii Brevicornus*) 9 g, Gouqizi (*Fructus Lycii*) 12 g, Wuweizi (*Fructus Schisandrae Chinensis*) 9 g; (c) Yiqizishen granule: Renshen (*Radix Ginseng*) 9 g, Huangjing (*Rhizoma Polygonati Sibirici*) 15 g, Shudihuang (*Radix Rehmanniae Praeparata*) 15 g, Maidong (*Radix Ophiopogonis Ja-*

ponici) 15 g and Wuweizi (*Fructus Schisandrae Chinensis*) 9 g. The granules were prepared into fluid- extractum according to the standard operating procedures established by the Department of Pharmaceutics, the First Affiliated Hospital of Henan University of TCM, Zhengzhou, China. Aminophylline tablets (Xinhua, China) were crushed before being administrated to animals. Rat tumor necrosis factor-α (TNF-α), interleukin (IL-1β), and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Boster Biological Technology (Wuhan, China).

Administrations

From weeks 9 through 20, normal saline was administered to the rats in the control and model groups, 2 mL/animal, i.g., b.i.d. Bufeijianpi (4.84 $g \cdot kg^{-1} \cdot d^{-1}$), Bufeiyishen (4.44 $g \cdot kg^{-1} \cdot d^{-1}$), and Yiqizishen (4.84 $g \cdot kg^{-1} \cdot d^{-1}$) granules, and aminophylline (2.3 $mg \cdot kg^{-1} \cdot d^{-1}$) were respectively i.g. administrated in the Bufeijianpi, Bufeiyishen, Yiqizishen, and aminophylline groups, b.i.d. The dosages were adjusted every week according to body mass. Half of the rats in each group were sacrificed at week 20. The survivors were raised in normal conditions without exposure or treatment, and were sacrificed at week 32. The equivalent dosages were calculated by the formula: $D_{rat} = D_{human} \times (I_{rat}/I_{human}) \times (W_{human}/W_{rat})^{2/3}$, in which D means dose; I means body shape index; and W means body weight.

Pulmonary function tests

Tidal volume (V_T), peak expiratory flow, and expiratory flow at 50% V_T (EF50) were measured by an unrestrained whole body plethysmograph (Buxco) at weeks 0, 8, 20, and 32.

Femur weight and morphology

After the rats were sacrificed, all femurs were immediately sampled. The weight of the right femur was measured, then kept in a freezer at - 80° C for measurement of BMD and BMC. The left femur, including the femoral head, was cut and fixed in 4% paraformaldehyde solution for 72 h, and then decalcified with a formalin-nitric acid solution for 3 days. The samples embedded in paraffin were sliced into 6 μm sections, stained with hematoxylin-eosin, and then photographed using a PM-10AD optical microscope (Olympus, Japan).

BMD and BMC

After placement into a Plexiglas box and immersion in distilled water, the excised femurs were scanned by DPX-NT dual-energy X-ray absorptiometry (GE, Fairfield, CA USA), and then analyzed by en-CORE software. BMD and BMC were expressed as absolute values (g/cm^2). The coefficient of variation was less than 3%.

Cytokines

The levels of TNF-α, IL-1β, and IL-6 in serum were measured by ELISA kits (Boster, Wuhan, China) according to the instructions strictly.

Statistical analysis

SPSS 19.0 software (IBM; Armonk, NY, USA) was used for data analysis. Data are expressed as mean ± standard deviation ($\bar{x} \pm s$). One-way analysis of variance was employed for multiple comparisons. A paired sample *t*-test was used to analyze the difference between weeks 20 and 32. The Pearson correlation analysis method was used to analyze the correlation of lung function and BMD, BMC, as well as cytokines and BMD and BMC. $P < 0.05$ was considered statistically significant.

RESULTS

General conditions

From the third week, the fur of COPD model rats withered and became yellow. The rats gradually became weak and asthenic with mucous hypersecretion, anorexia, body weight reduction, polyuria, and diarrhea. The three TCM therapies, especially Bufeiyishen granule, could relieve these symptoms.

Short- and long-term effects of the therapies for regulating and reinforcing lung and kidney on pulmonary functions

At week 8, V_T (Figure 1A), PEF (Figure 1B), and EF50 (Figure 1C) decreased significantly in COPD rats compared with those in the control group ($P < 0.01$). There were no statistical differences in any treatment group compared with the model group. At weeks 20 and 32, V_T, PEF, and EF50 decreased significantly in the model rats compared with the control group ($P < 0.01$). PEF, V_T, and EF50 in the three TCM-treated groups were higher than those in the model group ($P < 0.05$, $P < 0.01$), while PEF in the aminophylline-treated group was higher than that in the model group only at week 20 ($P < 0.05$), with no significant differences among the three TCM-treated groups. Compared with that of week 32, PEF at week 20 in the Bufeijianpi and Bufeiyishen-treated groups was higher ($P < 0.01$).

Short- and long-term effects of the therapies for regulating and reinforcing lung and kidney on femur morphology

As shown in Figure 2, thinning of the femoral cortex, expansion of the marrow cavity, thinning of the bone texture and decreased density of the trabecular bone were observed in model rats at week 20. At week 32, pathological impairments of the femur were found in more COPD rats than non-COPD rats, especially in the model group, while there were improvements in the three TCM-treated groups.

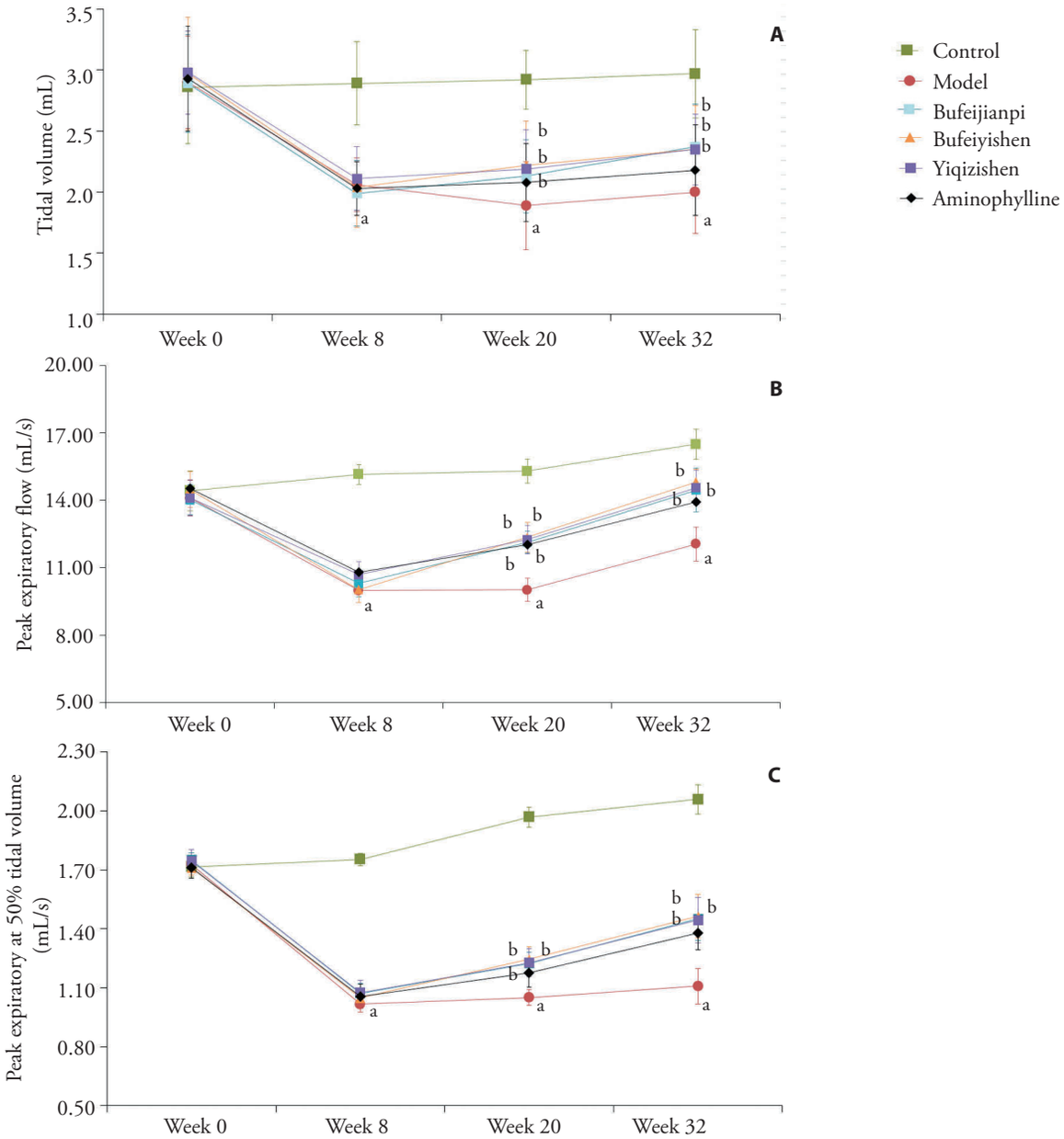

Figure 1 Time course changes of tidal volume, peak expiratory flow, and peak expiratory at 50% tidal volume in Bufeijianpi-, Bufeiyishen-, Yiqizishen- and aminophylline-treated rats

A: tidal volume; B: peak expiratory flow; C: peak expiratory at 50% tidal volume. The control and model groups were treated with normal saline (2 mL); the Bufeijianpi group with Bufeijianpi granules (4.84 g • kg $^{-1}$ • d $^{-1}$); the Bufeiyishen group with Bufeiyishen granules (4.44 g • kg $^{-1}$ • d $^{-1}$); the Yiqizishen group with Yiqizishen granules (4.84 g • kg $^{-1}$ • d $^{-1}$); and the aminophylline group with aminophylline (2.3 mg • kg $^{-1}$ • d $^{-1}$). The data are expressed as mean ± standard deviation. Significant differences compared with the control group at the parallel time point are designated, as $^{a}P < 0.05$, and with the model group, as $^{b}P < 0.05$.

Short- and long-term effects of the therapies for regulating and reinforcing lung and kidney on body mass and femur mass

As shown in Figures 3A and B, the femur mass, but not body mass, decreased significantly in the model group at both week 20 and 32 compared to the control group ($P < 0.01$). The femur mass was significantly higher in the three TCM- and aminophylline-treated groups than that in the model group ($P < 0.01$), and it was significantly higher in the Bufeijianpi and Bufeiyishen-treated groups than in the aminophylline group ($P < 0.01$), with no statistical differences among the three TCM-treated groups or between week 20 and 32 ($P > 0.05$).

Short- and long-term effects of the therapies for regulating and reinforcing lung and kidney on BMD and BMC

At weeks 20 and 32, BMD (Figure 3C) and BMC (Figure 3D) of femurs decreased significantly in the model group compared with the control group ($P < 0.01$), while they were higher in the three TCM- and aminophylline-treated groups than those in the model group ($P < 0.05$, $P < 0.01$). BMD in the Bufeijianpi- and Bufeiyishen-treated groups was higher than that in the aminophylline group ($P < 0.05$, $P < 0.01$). At week 20, BMD in the Bufeiyishen group was higher than that in the Yiqizishen group ($P < 0.05$). BMC in the Bufeijianpi and the Bufeiyishen groups was significantly higher

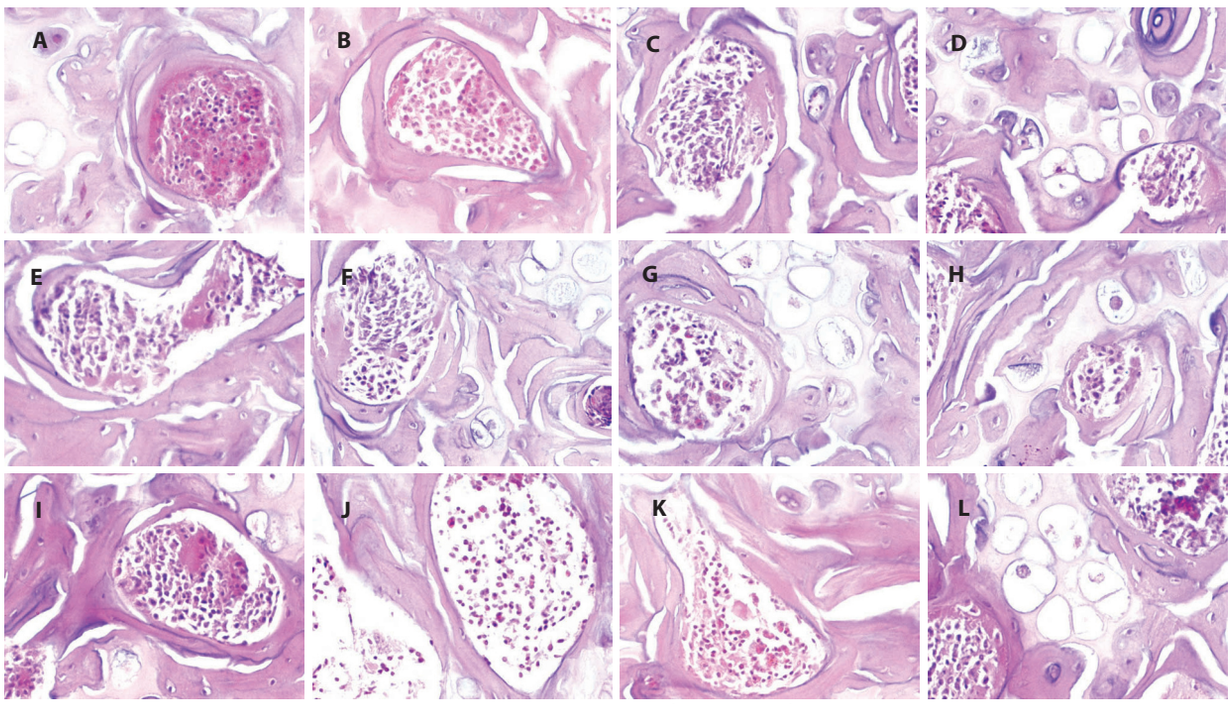

Figure 2 Femoral pathological changes in the rats treated with Bufeijianpi, Bufeiyishen, Yiqizishen granules, or aminophylline at weeks 20 and 32 (HE staining, ×400)

A1: control group at week 20; A2: control group at week 32; B1: model group at week 20; B2: model group at week 32; C1: Bufeijianpi group at week 20; C2: Bufeijianpi group at week 32; D1: Bufeiyishen group at week 20; D2: Bufeiyishen group at week 32; E1: Yiqizishen group at week 20; E2: Yiqizishen group at week 32; F1: aminophylline group at week 20; F2: aminophylline group at week 32. The control and model groups were treated with normal saline (2 mL); the Bufeijianpi group was treated with Bufeijianpi granule (4.84 g·kg^{-1}·d^{-1}); the Bufeiyishen group with Bufeiyishen granule (4.44 g·kg^{-1}·d^{-1}); the Yiqizishen group with Yiqizishen granule (4.84 g·kg^{-1}·d^{-1}); and the aminophylline group with aminophylline (2.3 g·kg^{-1}·d^{-1}). HE: hematoxylin-eosin.

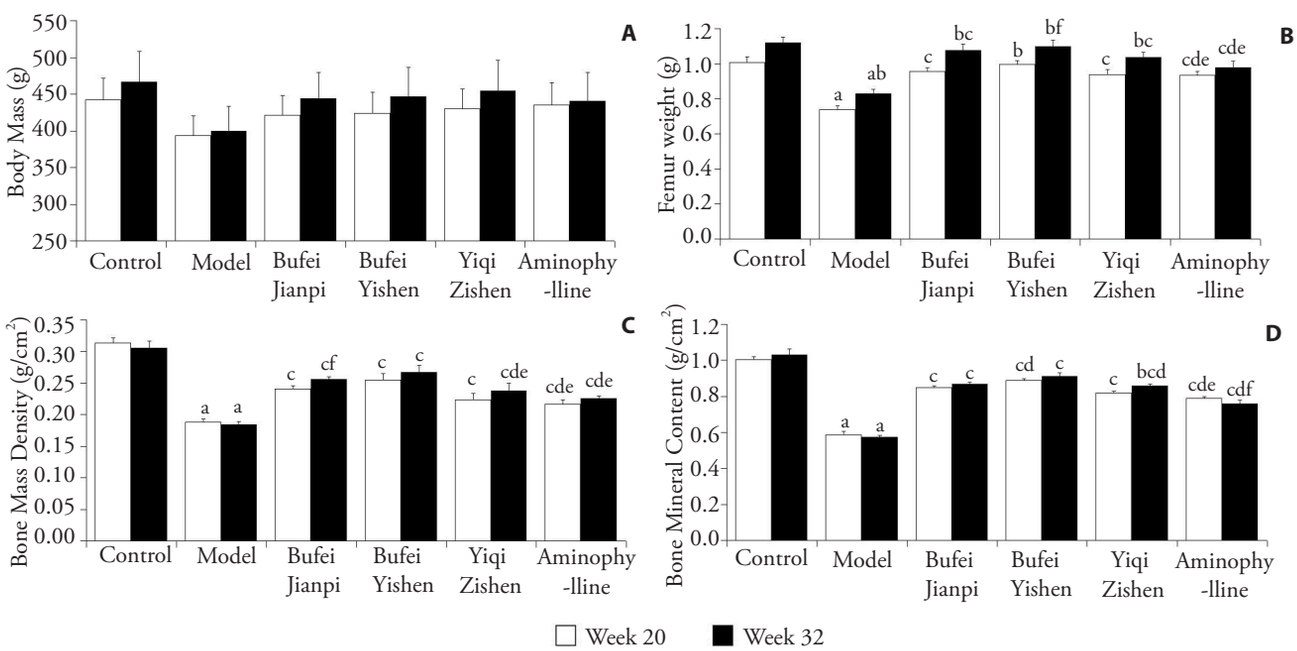

Figure 3 Change of body mass, femur mass, bone mass density and bone mineral content in each group

A: changes in body mass; B: femur weight; C: bone mass density; D: bone mineral content. The control and model groups were treated with normal saline (2 mL); the Bufeijianpi group was treated with Bufeijianpi granule (4.84 g·kg^{-1}·d^{-1}); the Bufeiyishen group with Bufeiyishen granule (4.44 g·kg^{-1}·d^{-1}); the Yiqizishen group with Yiqizishen granule (4.84 g·kg^{-1}·d^{-1}); and the aminophylline group with aminophylline (2.3 g·kg^{-1}·d^{-1}). Data are expressed as mean ± standard deviation. Significant differences compared with the control group at parallel time points are designated as $^{a}P < 0.05$ and with the model group, the Bufeijianpi group, the Bufeiyishen group, the Yiqizishen group at parallel time points as $^{c}P < 0.05$, $^{d}P < 0.05$, $^{e}P < 0.05$, and $^{f}P < 0.05$, respectively. $^{b}P < 0.05$ indicates week 32 *vs* week 20 in the same group.

than that in the aminophylline group ($P < 0.01$), and it was higher in the Bufeiyishen group than that in the Bufeijianpi and Yiqizishen groups ($P < 0.05$, $P < 0.01$). At week 32, BMD in the Bufeijianpi- and Bu-

feiyishen-treated groups was higher than that in the Yiqizishen group ($P < 0.05$, $P < 0.01$), while BMC in the three TCM-treated groups was significantly higher than that in the aminophylline group ($P < 0.01$).

Short- and long-term effects of therapies for regulating and reinforcing lung and kidney on cytokines

As shown in Figure 4A, IL-1β was significantly higher in the model group than that in the control group at both weeks 20 and 32 ($P < 0.01$). However, IL-1β was lower in the three TCM- and the aminophylline-treated groups than that in the model group ($P < 0.01$). At week 20, it was lower in the three TCM-treated groups than that in the aminophylline group ($P < 0.01$), and also lower in the Yiqizishen group than that in the Bufeiyishen group ($P < 0.01$). At week 32, there were no statistical differences among all the treated groups or compared to week 20.

As shown in Figure 4B, IL-6 was significantly higher in the model group than that in the control group at week 20 ($P < 0.01$), while it was lower in the three TCM- and aminophylline-treated groups than that in the model group ($P < 0.01$). Among the four treated groups, IL-6 was lower in the three TCM-treated groups than that in the aminophylline group ($P < 0.01$). Additionally, it was lower in the Bufeijianpi group than that in the Bufeiyishen and Yiqizishen groups ($P < 0.05$, $P < 0.01$). IL-6 was also lower in the Yiqizishen group than that in the Bufeiyishen group ($P < 0.01$). At weeks 32, IL-6 was significantly higher in the model group than that in the control group ($P < 0.01$), but lower in the Bufeijianpi and Bufeiyishen groups than that in the model, Yiqizishen, and aminophylline groups ($P < 0.05$, $P < 0.01$). There were no significant differences between weeks 20 and 32.

As shown in Figure 4C, TNF-α was significantly higher in the model group than that in the control group at both weeks 20 and 32 ($P < 0.01$), while it was lower in the three TCM-treated groups than that in the model group ($P < 0.05$, $P < 0.01$). At week 20, TNF-α was significantly lower in the three TCM-treated groups than that in the aminophylline group. It was also lower in the Bufeijianpi group than that in the Bufeiyishen and Yiqizishen groups ($P < 0.05$, $P < 0.01$). At week 32, TNF-α in the Bufeijianpi and Bufeiyishen groups was lower than that in the Yiqizishen group and the aminophylline group ($P < 0.05$, $P < 0.01$), with no significant difference between weeks 20 and 32.

Correlations between pulmonary function and BMD and BMC

As shown in Table 1, V_T, PEF, and EF50 were positively correlated with BMD and BMC at both weeks 20 and 32.

Correlations between cytokines and BMD and BMC

As shown in Table 2, IL-1β, IL-6, and TNF-α were negatively correlated with BMD and BMC at both weeks 20 and 32.

DISCUSSION

COPD is characterized by persistent airflow limitation and pulmonary function decline, and is accompanied by various extra-pulmonary morbidities, such as skele-

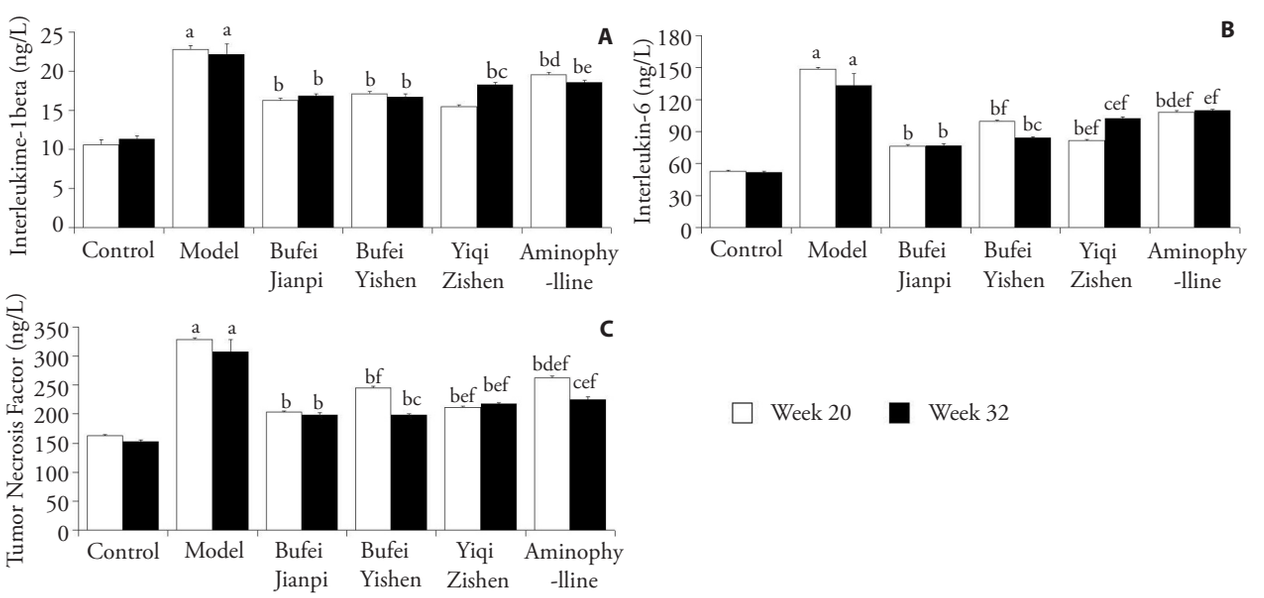

Figure 4 Levels of serum interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α in rats treated with Bufeijianpi, Bufeiyishen, Yiqizishen granules, or aminophylline at weeks 20 and 32

A: IL-1β; B: IL-6; C: TNF-α. The control and model groups were treated with normal saline (2 mL); the Bufeijianpi group was treated with Bufeijianpi granule (4.84 $g \cdot kg^{-1} \cdot d^{-1}$); the Bufeiyishen group with Bufeiyishen granule (4.44 $g \cdot kg^{-1} \cdot d^{-1}$); the Yiqizishen group with Yiqizishen granule (4.84 $g \cdot kg^{-1} \cdot d^{-1}$); and the aminophylline group with aminophylline (2.3 $g \cdot kg^{-1} \cdot d^{-1}$). The data are expressed as mean ± standard deviation. Significant differences compared with the control group at parallel time points are designated as $^{a}P < 0.05$ and with the model group, the Bufeijianpi group, the Bufeiyishen group, and the Yiqizishen group at parallel time points as $^{b}P < 0.05$, $^{d}P < 0.05$, $^{e}P < 0.05$, $^{f}P < 0.05$, respectively. $^{c}P < 0.05$, indicates week 32 *vs* week 20 in the same group.

Table 1 Analysis on correlativity between pulmonary function and BMD and BMC

		Week 20					Week 32				
		BMD	BMC	V_T	EF50	PEF	BMD	BMC	V_T	EF50	PEF
BMD	Cor	1	0.854[a]	0.757[a]	0.606[a]	0.621[a]	1	0.846[a]	0.637[a]	0.535[a]	0.575[a]
	P value	-	0.000	0.000	0.000	0.000	-	0.000	0.000	0.001	0.000
BMC	Cor	-	1	0.730[a]	0.635[a]	0.607[a]	-	1	0.622[a]	0.606[a]	0.600[a]
	P value	-	-	0.000	0.000	0.000	-	-	0.000	0.000	0.000
V_T	Cor	-	-	1	0.634[a]	0.634[a]	-	-	1	0.500[a]	0.544[a]
	P value	-	-	-	0.000	0.000	-	-	-	0.002	0.001
EF50	Cor	-	-	-	1	0.352[a]	-	-	-	1	0.516[a]
	P value	-	-	-	-	0.000	-	-	-	-	0.001
PEF	Cor	-	-	-	-	1	-	-	-	-	1

Notes: Cor: pearson coefficient of correlation P: significance (2-tailed). [a]Correlation is significant at the 0.01 level. BMD: bone mass density; BMC: bone mineral content; V_T: tidal volume; PEF: peak expiratory flow; EF50: expiratory flow at 50% tidal volume.

Table 2 Correlations between cytokines and BMD and BMC

		Week 20					Week 32				
		BMD	BMC	IL-1β	IL-6	TNF-α	BMD	BMC	IL-1β	IL-6	TNF-α
BMD	Cor	1	0.854[a]	- 0.809[a]	- 0.835[a]	- 0.841[a]	1	0.846[a]	- 0.742[a]	- 0.804[a]	- 0.756[a]
	P value	-	0.000	0.000	0.000	0.000	-	0.000	0.000	0.000	0.000
BMC	Cor	-	1	- 0.843[a]	- 0.924[a]	- 0.921[a]	-	1	- 0.783[a]	- 0.821[a]	- 0.802[a]
	P value	-	-	0.000	0.000	0.000	-	-	0.000	0.000	0.000
IL-1β	Cor	-	-	1	0.921[a]	0.922[a]	-	-	1	0.649[a]	0.778[a]
	P value	-	-	-	0.000	0.000	-	-	-	0.000	0.000
IL-6	Cor	-	-	-	1	0.999[a]	-	-	-	1	0.776[a]
	P value	-	-	-	-	0.000	-	-	-	-	0.001
TNF-α	Cor	-	-	-	-	1	-	-	-	-	1

Notes: Cor: pearson coefficient of correlation P: significance (2-tailed). [a]Correlation is significant at the 0.01 level. BMD: bone mass density; BMC: bone mineral content; IL: interleukin; TNF-α: tumor necrosis factor-α.

tal muscle atrophy/dysfunction, and OP. OP, one of the most common complications, has been reported in several studies.[1] The National Health and Nutrition Examination Survey 1999-2008 (14 828 subjects aged 45+, including 995 with COPD) showed that 16.9% of the subjects aged 45+ with COPD suffered from OP, and the incidence increased with age.[15] Cigarette smoking, chronic systemic inflammation, inactivity, malnutrition, and other factors are involved in this process. Cigarette smoking, which is the main cause of COPD, is also considered to be one of the risk factors leading to OP.[16] Cigarette smoking can reduce the thickness of the cortical bone and gene expression of the bone matrix.[17] BMD and BMC are always measured to diagnose OP and judge the severity. Otherwise, hypoxia and emphysema can cause muscle atrophy, and reduce BMD through protease hydrolysis or load increases, which then result in OP.[18] A previous study showed that vitamin D intake in COPD patients was less than the recommended intake, and a lack of vitamin D can reduce calcium absorption, and eventually result in OP.[19] However, systemic corticosteroids are the most common cause of drug-related OP. A meta-analysis concludes that the use of more than 6.25 mg prednisone daily can lead to the risk of decreased of BMD and increase in fracture.[20] In contrast, the effects of the long-term use of inhaled corticosteroids on BMD remain controversial.[21,22] Some studies demonstrated a relationship between BMD and airflow limitations. Forced expiratory volume in 1 second (FEV_1) was found to be a significant predictor of low BMD aside from BMI and the COPD stage.[23] Therefore, airflow obstruction is an important risk factor for OP.

OP is an atrophic debility of bones (Guwei) or bone rheumatism (Gubi) syndrome in TCM. Deficiency of the kidney and spleen are considered to be the cause of OP.[24] Based on our previous studies, there are three common patterns of stable COPD, lung-spleen *Qi* deficiency, lung-kidney *Qi* deficiency, and lung-kidney *Qi-Yin* deficiency. Patients with lung-spleen *Qi* deficiency generally have poor digestion, which may cause less appetite, vitamin D deficiency, malnutrition, and

result in OP. Patients with lung-kidney *Qi* deficiency may directly generate osteopenia and increase bone fragility. Lung-kidney *Qi-Yin* deficiency often occurs in severe COPD. Kidney deficiency is believed to be the key pathogenesis of OP for COPD. Therefore, regulating and reinforcing lung and kidney should be used as early as possible.[25]

COPD patients with OP have significantly lower body mass index (BMI) and leptin expressions.[26] Studies have identified a direct positive relationship between serum leptin and bone mass in nonobese women.[27] Leptin enhances differentiation of bone marrow cells into osteoblasts, and reduces osteoclast formation and bone resorption.[28] Decreases in BMI increase the odds ratio for OP, while high BMI reduces the risk of OP.[29] In this study, most COPD rats had low body mass and femur weight, while the Bufeijianpi, Bufeiyishen, Yiqizishen granules and aminophylline treatments could increase femur weight. Bufeijianpi and Bufeiyishen granules showed more benefits in increasing femur weight than aminophylline. The primary means of diagnosing OP is BMD by dual energy absorptiometry scanning. A low BMD value in COPD indicates the occurrence of OP.[2] Whole-body BMD is affected by BMI, COPD stage, and leptin.[30] Smoke exposure can decrease BMC and BMD, and increase bone turnover (inhibiting bone formation and stimulating its resorption), as well as affect bone histomorphology. Epimedium pubescen flavonoid has a beneficial effect on preventing bone loss in passive smoking rats, which is shown by maintained BMD and BMC at the femur neck and lumbar vertebrae.[17] Our results indicate that BMD and BMC decreased significantly in COPD rats, which is in line with previous studies. Bufeijianpi, Bufeiyishen, Yiqizishen granules, and aminophylline could improve BMD, but Bufeijianpi and Bufeiyishen granules are superior to aminophylline. Bufeiyishen granules showed more benefits in improving BMC than Bufeijianpi and Yiqizishen granules, and had better long-term effects after a three-month course of treatment.

As one of the common complications of COPD, OP is closely related to limited airflow. A study with the NHANES Ⅲ data demonstrates that airflow obstruction is independently associated with reduced BMD.[31] OP causes a reduction in rib mobility and decrease in respiratory muscle function, which can lead to reductions in $FEV_1$and FVC, and then aggravation of airflow.[32] In this study, it was found that tidal volume, peak expiratory flow, and peak expiratory at 50% tidal volume were positively correlated with BMD and BMC of the femur, which is in accordance with the current literature.

Systemic inflammation in COPD is possibly a contributing factor to OP. In addition, physical inactivity may be a major cause for systemic inflammation, and lead to exacerbations of OP.[33] Recently, the relationship between high levels of systemic inflammation and a high risk of osteoporotic fractures in COPD was identified. The serum levels of C-reactive protein, TNF-α, and IL-6 in patients with OP are higher than those without OP.[34] Our study found that the three TCM granules could more significantly decrease the expressions of IL-1β, IL-6, and TNF-α than that of aminophylline after a 12-week treatment. We also found that IL-6 and TNF-α remained lower in the Bufeijianpi- and Bufeiyishen-treated rats than those in the aminophylline-treated rat after a 12-week follow-up.

Bufeijianpi, Bufeiyishen, and Yiqizishen granules can improve BMD and BMC of femur in COPD rats, alleviate airflow limitations and systemic inflammation, and help prevent and treat OP, with favorable long-term effects.

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