

Original Article

Combined effect of pulsed electromagnetic fields and narrowband ultraviolet B on bone metabolism in glucocorticoid-treated rats

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Abstract: Objective To investigate the combined effect of pulsed electromagnetic fields (PEMFs) and ultraviolet B (UVB) on bone metabolism in glucocorticoid-treated rats. Methods Thirty six female Sprague Dawley rats were randomly divided into control group, GIOP group and PEMFs+UVB group. The rats in GIOP group and PEMFs+UVB group were injected dexamethasone sodium phosphate injection (DXMT) twice a week for 12 weeks, meanwhile, the rats in PEMFs+UVB group were exposed to PEMFs and then UVB once a day for 12 weeks. After 12 weeks of intervention, bone mineral density (BMD) and bone mineral content (BMC) level of the whole body were measured by dual energy X-ray absorptiometry. The serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and urinary calcium (UCa) were determined by automatic biochemical analyzer. The serum tartrate resistant acid phosphatase (TRAP) was determined by TRAP assay kit. The serum $1,25(\text{OH})_2\text{D}_3$ and osteocalcin (OC) was determined by enzyme linked immunosorbent assay (ELISA). The serial sections of the fourth lumbar vertebral bodies were stained with Safranin-O/Fast green for histomorphometrical analysis. The mRNA and protein expressions of TRPV5 in bone and kidney tissues were detected by real time-PCR analysis and western blot analysis, respectively. Results After 12-week interventions, BMD and BMC levels of the whole body increased in the PEMFs+UVB group, serum ALP, OC and $1,25(\text{OH})_2\text{D}_3$ levels also increased in the PEMFs+UVB group. But serum TRAP, UCa levels decreased in PEMFs+UVB group. Histomorphometrical analysis showed that PEMFs+UVB intervention improved percentage of trabecular area (Tb.Ar), trabecular width (Tb.Wi) and trabecular number (Tb.N) by 75.44%, 16.55% and 31.07%, respectively, and reduced trabecular separation (Tb.Sp) by 49.65% compared with the GIOP group. Real time-PCR analysis and western blot analysis showed that PEMFs+UVB intervention increased the mRNA and protein expressions of TRPV5 in kidney tissues, and decreased the mRNA and protein expressions of TRPV5 in bone tissues in glucocorticoid-treated rats. Conclusion PEMFs stimulation combined UVB irradiation can prevent bone loss and improve bone metabolism disorders in glucocorticoid-treated rats.

Keywords: Pulsed electromagnetic fields, ultraviolet B, glucocorticoid-induced osteoporosis, TRPV5

Introduction

Glucocorticoid induced osteoporosis (GIOP) is a common metabolic bone disease, and one of the common causes of secondary osteoporosis [1], and it is also as a result of long-term use and (or) higher doses of glucocorticoids (GCs) [2]. GIOP is characterized by low bone strength and increased fracture risk, which results from both low bone mass and deteriorated bone microstructure. GCs play an important role in the treatment of many inflammatory disease

states, but the use of GCs brings significant side effects, including bone loss and fractures [3]. Even in children, GCs treatment also affects bone strength and growth [4, 5]. Bone mineral density (BMD) would increase and the number of fractures would go down after GCs treatment is terminated, however, many patients have to tolerate the long-term GCs treatment due to the treatment options are limited [6]. For example, it is estimated that 1% of the US population is treated with long-term GCs [7]. Unfortunately, about 30-50% of GCs-treated

patients will develop GIOP and osteonecrosis after receiving long-term GCs therapy [8]. Oral bisphosphonates were recommended as the preferred first-line therapy for GIOP in most clinical situations is at least in partly due to the long experience of treatment in postmenopausal osteoporosis [3, 9], but the risks of potential complications (e.g. osteonecrosis of the jaw, gastrointestinal complaints, and atypical subtrochanteric or diaphyseal femoral fractures) in long-term bisphosphonates treatment cannot be ignored. Calcium and vitamin D supplementation is needed for GCs-treated patients, whereas dietary intake of calcium and vitamin D needs to be optimized due to the potential harms of cardiovascular risks [10-12].

Pulsed electromagnetic fields (PEMFs) therapy is capable of producing satisfying therapeutic effects on a wide range of bone diseases (e.g. fresh fracture, delayed and nonunion fractures, osteoporosis, osteonecrosis, and osteoarthritis) [13, 14], and it have been suggested as an alternative noninvasive method for postmenopausal osteoporosis. Ultraviolet radiation has been used in the treatment of osteoporosis, and can get a better curative effect, especially for postmenopausal women with osteoporosis [15, 16]. The production of vitamin D in the skin by ultraviolet B (UVB) radiation can have beneficial effect on bone status [17], and the advantage of using UVB was helpful to reduce side effects of medication as well as low cost and easy application [15]. In the existing research, there is no study on the combined effect of PEMFs and UVB on prevention and cure of GIOP. Therefore, given the skeletal dynamics and pathogenesis of GIOP are distinctly different from postmenopausal osteoporosis [17], the curative effect of PEMFs combined UVB on bone metabolism in GC-treated rats need to investigate further.

In this present study, the GIOP SD rats model were established by intramuscular injection with dexamethasone sodium phosphate injection (DXMT) to investigate the combined effect of PEMFs and UVB on bone metabolism and further explored the expressions of TRPV5 in bone and kidney tissues of GC-treated rats.

Materials and methods

Major reagents

Safranin-O/Fast green staining solution was purchased from Nanjing SenBeiJia Biological

Technology Co., Ltd. Rat 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) and osteocalcin (OC) ELISA Kit was purchased from Cloud-Clone Corp.. EDTA decalcifying solution was purchased from BOSTER. Trizol, tartrate resistant acid phosphatase assay kit, enhanced BCA protein assay kit, RIPA lysis buffer and SDS-PAGE gel quick preparation kit were purchased from Beyotime. Revert aid first strand cDNA synthesis kit and Super Signal West Pico Chemiluminescent Substrate Trial kit were purchased from Thermo Fisher Scientific Inc.. SYBR® Premix Ex Taq™ II was purchased from Takara Bio Inc.. TRPV-5 antibodies and GAPDH antibodies were purchased from Santa Cruz Biotech. SPF class Mouse feed (Ca: 1.0-1.8%, P: 0.6-1.2%) was purchased from Chengdu-Dossy.

Experimental protocols

All animal experimental protocol and care were approved by the Institutional Animal Care and Use Committee of Chengdu Medical College. Totally, 36 female Sprague-Dawley rats (aged 3 months, weighing 200±20 g) were employed and housed in feeding box individually under a controlled environmental conditions (12-hour light-dark cycle, temperature 22°C with humidity of 50±5%). All rats were unrestricted access to water and food. After one week of acclimatization, 12 rats were randomly divided into control groups and injected normal saline (2 ml/kg) into their right haunch muscles, twice a week, 12 weeks in a row. The rest of the rats were randomly divided into GIOP group and PEMFs+UVB group, and injected dexamethasone sodium phosphate injection (DXMT, 2.5 mg/kg) into their right haunch muscles to establish GIOP rat models (twice a week, 12 weeks in a row). As the same time, the rats in PEMFs+UVB group were exposed to PEMFs for 40 min, which was generated by the ZH-21 osteoporosis treatment system (Chongqing Zhonghan Electronic Technology Co., LTD, China) with a frequency of 50 Hz and an intensity of 4.0 mT, and the waveform is square wave with pulse width 200 μs. Subsequently, those rats were exposed to NB-UVB for 20 min at 1/4 MED, which was generated by CLS-UV ultraviolet ray treatment meter (Shenzhen CLS photoelectric equipment factory, China) with the wavelength of 311 nm and radiant power of 1.2 W. The above experimental protocols once a day, 12 weeks in a row. The rats in control group and GIOP group were also placed in the ZH-21 osteoporosis

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treatment system and under the CLS-UV ultra-violet ray treatment meter successively, but the treatment system was not running to provide sham PEMFs stimulation or NB-UVB.

Bone mineral density and bone mineral content measurement

The rats in each group were received intraperitoneal anesthesia of 10% chloral hydrate (3.5 mL/kg) after a period of 12 weeks' treatment, and positioned on the platform of dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare) in prone position. The Bone mineral density (BMD) and bone mineral content (BMC) of the whole body were detected and recorded [Supplementary Figure 1](#).

Blood and urine biochemical analysis

The fasting rats in each group were sequestered in metabolism cage (1 rat in each cage) after a period of 12 weeks' treatment, and the urine samples of 24 h were collected. Then the rats were received intraperitoneal anesthesia of 10% chloral hydrate (3.5 mL/kg), and positioned on the operation platform before they were executed. The fur on the abdomen was removed and skin was sterilized, and a midline 4-5 cm longitudinal incision was performed. The blood specimens were withdrawn from aorta abdominalis and then centrifuged to get serum. The serum calcium (Ca), phosphorus (P), alkaline phosphatase (ALP) and urinary calcium (UCa) were determined by automatic biochemical analyzer (TBA-120FR, Toshiba). The serum tartrate resistant acid phosphatase (TRAP) was determined by TRAP kit (Beyotime, China) according to the protocol of the manufacturer. The serum $1,25(\text{OH})_2\text{D}_3$ and osteocalcin (OC) was determined by enzyme linked immunosorbent assay (ELISA) according to the protocol of the manufacturer.

Histomorphometrical analysis

Safranin-O/Fast green solutions was used to histomorphometrical analysis. Briefly, after all rats were executed, the fourth lumbar (L4) vertebral bodies were collected, and each fourth lumbar was carefully cleaned and then decalcified by EDTA decalcifying solution (BOSTER, China) for 6 weeks. The vertebral body samples were put into the optimum cutting temperature compound (O.C.T. compound, Sakura) and quick freezing, then flatplaced in the cryostat

mould, and cut into serial sections with cryostat (15 μm per section). The serial sections were stained with Safranin-O/Fast green according to the protocol of the manufacturer. Histomorphometrical parameters were quantified by using the Image-Pro Plus 6.0 software (Media Cybernetics). The region of interest for trabecular bone was an area 1-3 mm below the growth plate of L4 vertebral body [Supplementary Figure 2](#). The static parameters are calculated using the following formula [14]: percentage of trabecular area (Tb.Ar) = trabecular area (Tb.Ar)/bone area (T.Ar) \times 100%. trabecular width (Tb.Wi) = $(2000/1.199) \times (\text{Tb.Ar}/\text{trabecular perimeter} [\text{Tb.Pm}])$. trabecular number (Tb.N) = $(1.199/2) \times (\text{Tb.Pm}/\text{T.Ar})$. trabecular number (Tb.N) = $(1.199/2) \times (\text{Tb.Pm}/\text{T.Ar})$. trabecular separation (Tb.Sp) = $(2000/1.199) \times (\text{T.Ar} - \text{Tb.Ar})/\text{Tb.Pm}$.

Real time-PCR analysis

The mRNA expressions of TRPV5 in rat kidney and bone tissues were analyzed by real-time PCR analysis. Briefly, after all rats were executed, the kidney tissues and right thighbones were collected. The total RNA of TRPV5 were extracted separately from kidney tissues and caput femoris using TRIzol reagent according to the manufacturer's instructions and quantified by spectrophotometry at a wavelength of 260 nm. Reverse transcription actions and PCR were performed using reverse transcriptase, oligo (DT) primers, and Taq DNA polymerase. The primers used were as follows: TRPV5 forward, 5'-AGCAGAAAAGTATTTGGGAGTC-3' and reverse, 5'-GGCTGTAGGACAAGTATGTGTA-3'; and GAPDH forward, 5'-CAGGAG GCATTGCTGATGAT-3' and reverse, 5'-GAAGGCTGGGGCT-CATTT-3'. The reaction was initiated with denaturation at 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec, 60°C for 60 sec (annealing), a terminal extension step at 95°C for 10 sec and a final holding stage at 4°C. GAPDH was used as an internal control, and relative mRNA expressions of TRPV5 were defined as the ratio of target genes expression to GAPDH expression.

Western blot analysis

The protein expressions of TRPV5 were analyzed by western blot analysis. Briefly, after all rats were executed, the kidney tissues and right thighbones were collected. The total pro-

Table 1. Bone mineral density (BMD) and Bone mineral content (BMC) of each group

Groups	BMD (g/cm ²)	BMC (g)
Control	0.151±0.004	8.27±0.31
GIOP	0.133±0.006*	7.11±0.34*
PEMFs+UVB	0.147±0.005#	8.02±0.29#

Compared with the control group: * $P < 0.01$; Compared with the GIOP group: # $P < 0.01$.

teins of kidney tissues and caput femoris were extracted using RIPA lysis buffer, respectively. Protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto a polycylinidene difluoride (PVDF) membrane, and then the PVDF membrane was blocked for 2 h at RT in TBS-Tween 20 (TBST) buffer containing skimmed milk, washed with TBST three times, and incubated overnight at 4°C with 1/500 dilution of TRPV5 antibodies and GAPDH antibodies, respectively. The PVDF membranes were incubated with horseradish peroxidase-labeled goat anti-rabbit immunoglobulin G (H+L) secondary antibody (1/4000 dilution) at 37°C for 1 h after being washed with TBST three times. Protein signals were detected using SuperSignal West Pico Chemiluminescent Substrate Trial kit and quantified by densitometry using Quantity One software (Bio-Rad).

Statistical analysis

The statistical analysis was conducted using SPSS 20.0 for Windows software. Data were present as mean ± standard deviation (SD). Differences in group were analyzed by using repeated measure analysis of variance (ANOVA). $P < 0.05$ was considered to be statistical significance.

Results

PEMFs+UVB intervention can improved BMD and BMC level of glucocorticoid-treated rats

After 12 weeks of different treatment, the body weights of rats in each group were recorded and the results of control group, GIOP group, and PEMFs+UVB group were 296.8±7.0 g, 291.1±6.6 g, and 294.1±7.9 g, respectively. There was no significant difference in body weight among the of three groups ($P > 0.05$). Then, the rats in each group were detected by

dual energy X-ray absorptiometry to record the values of BMD and BMC. As shown in **Table 1**, the BMD and BMC values of whole body of rats in GIOP group were significantly declined, compared with those of the normal rats in control group (all $P < 0.01$). These results demonstrated that the GIOP rats model was successfully established. The BMD and BMC values of rats in PEMFs+UVB group were lower than the normal rats in control group, but there was no significant difference between them (all $P > 0.05$), it indicated that PEMFs+UVB intervention can improved BMD and BMC level of glucocorticoid-treated rats

PEMFs+UVB intervention can improved blood and urine biochemical indexes of glucocorticoid-treated rats

As shown in **Table 2**, after 12 weeks of different treatment, the serum ALP, OC and 1,25(OH)₂D₃ levels of GIOP group were significantly declined, but the serum TRAP and urinary calcium (UcA) levels were significantly increased compared to those of the normal rats in control group (all $P < 0.01$). The serum ALP, OC and 1,25(OH)₂D₃ levels of PEMFs+UVB group were significantly higher than those in GIOP group (all $P < 0.01$), but the serum TRAP and urinary calcium (UcA) level were significantly lower than those in GIOP group (all $P < 0.01$). Moreover, there was no significant difference in the serum calcium (Ca) and phosphorus (P) between PEMFs+UVB group and GIOP group ($P > 0.05$).

Histomorphometrical analysis

After 12 weeks of different intervention treatment, the L4 vertebral bodies of each group were stained with Safranin-O/Fast green for histomorphometrical analysis and shown in **Figure 1A**. Bone tissue was stained in grayish-green or blue and cartilage tissue was stained in red. It found that GCs caused thinning of trabeculae and deteriorated architecture of trabecular bone in GIOP group, but the architecture of trabecular bone in PEMFs+UVB group were better than GIOP group. The results of histomorphometrical analysis were shown in **Table 3**. In contrast with the control group, Tb.Ar, Tb.N significantly declined and Tb.Sp significantly increased in GIOP group (all $P < 0.01$). However, the Tb.Ar, Tb.N of PEMFs+UVB group were significantly higher and Tb.Sp significantly lower than those in GIOP group (all $P < 0.05$).

Table 2. The results of blood and urine biochemical analysis

Group	Ca (mmol/L)	P (mmol/L)	UCa (μ mol/L)	OC (ng/L)	1,25(OH) ₂ D ₃ (ng/L)	ALP (U/L)	TRAP (U/L)
Control	2.35±0.11	1.89±0.31	16.32±1.41	175.83±6.94	27.13±0.90	175.50±18.48	1.61±0.08
GIOP	2.42±0.08	2.12±0.29	46.43±4.74*	155.00±6.83*	18.33±1.49*	85.42±10.53*	1.85±0.05*
PEMFs+UVB	2.38±0.11	2.09±0.40	30.95±6.75*#	162.75±5.24*#	26.30±1.42#	135.17±23.42*#	1.71±0.10*#

Compared with the control group: * $P < 0.01$; Compared with the GIOP group: # $P < 0.01$.

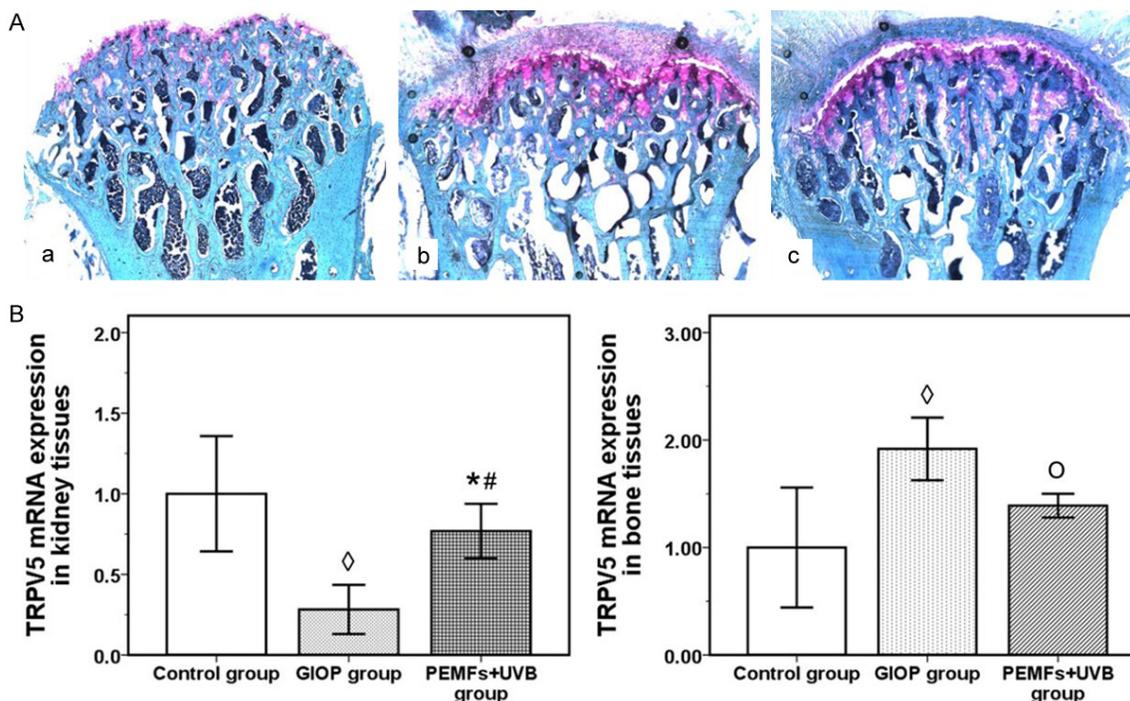


Figure 1. The results of Safranin-O/Fast green staining and real-time PCR analysis. (A) The L4 vertebral bodies were stained with Safranin-O/Fast green: (a) control group, (b) GIOP group, (c) PEMFs+UVB group; bone tissue was stained in grayish-green or blue; cartilage tissue was stained in red. (B) The TRPV5 mRNA expressions were estimated using real-time PCR analysis. Compared with the control group: * $P < 0.05$, $\diamond P < 0.01$; Compared with the GIOP group: # $P < 0.05$, $^{\circ} P < 0.01$.

Those results indicated that PEMFs+UVB intervention improved percentage of trabecular area (Tb.Ar), trabecular width (Tb.Wi), and trabecular number (Tb.N) by 75.44%, 16.55% and 31.07%, respectively, and reduced trabecular separation (Tb.Sp) by 49.65% compared with the GIOP group.

PEMFs+UVB intervention can decreased the expression of TRPV5 mRNA in bone tissues, but increased the expression of TRPV5 mRNA in kidney tissues of glucocorticoid-treated rats

The relative mRNA expressions of TRPV5 were estimated using real-time PCR analysis. As shown in **Figure 1B**, in contrast with control group, the mRNA expressions of TRPV5 in GIOP

group were significantly increased in bone tissues ($P < 0.01$) and significantly decreased in kidney tissues ($P < 0.01$). However, the mRNA expressions of TRPV5 of PEMFs+UVB group were significantly higher than GIOP group in kidney tissues ($P < 0.05$), and significantly lower than GIOP group in bone tissues ($P < 0.01$).

PEMFs+UVB intervention can decreased the expression of TRPV5 protein in bone tissues, but increased the expression of TRPV5 protein in kidney tissues of glucocorticoid-treated rats

The protein expressions of TRPV5 were estimated using Western blot analysis. As shown in **Supplementary Figures 3, 4, 5, 6**, in contrast to control group, the protein expressions of TRPV5

Table 3. Histomorphometrical analysis of the fourth lumbar (L4) vertebral bodies

Group	Tb.Ar (%)	Tb.Wi (μm)	Tb.N (n/mm)	Tb.Sp (μm)
Control	59.72 \pm 3.63	55.41 \pm 5.69	10.42 \pm 0.38	38.77 \pm 4.91
GIOP	30.74 \pm 3.09 ^o	52.70 \pm 4.67	5.89 \pm 0.30 ^o	117.92 \pm 11.13 ^o
PEMFs+UVB	53.93 \pm 1.37 ^{*#}	61.42 \pm 2.63 ^{o,*}	7.72 \pm 0.45 ^{o,*}	59.37 \pm 3.97 ^{*o}

Data were expressed as mean \pm SD. Tb.Ar: trabecular area; Tb.Wi: trabecular width; Tb.N: trabecular number; Tb.Sp: trabecular separation. Compared with the control group: ^{*} $P < 0.05$, ^o $P < 0.01$; Compared with the GIOP group: [#] $P < 0.05$, ^{*} $P < 0.01$.

in GIOP group were significantly increased ($P < 0.01$) in bone tissues and significantly decreased ($P < 0.01$) in kidney tissues. However, the protein expressions of TRPV5 of PEMFs+UVB group were significantly higher than GIOP group in kidney tissues ($P < 0.01$), and significantly lower than GIOP group in bone tissues ($P < 0.01$) (Figure 2).

Discussion

GIOP is a common secondary osteoporosis and as a complication of long-term use and (or) higher doses of GCs for organ transplantation or many inflammatory disease [18]. Anti-osteoporosis therapy should be a necessary choice for patients receiving long-term GCs therapy [19]. After treating the GCs treatment had ended, anti-osteoporosis therapy may be considered to stop. However, in consideration of the potential side effects and/or high cost of currently anti-osteoporosis drugs, it is necessary to seek a proper prevention method for GIOP with a lower cost and minor side effects.

Bone metabolism disorder is a key involved in the pathogenesis of GIOP [20], and it is the result of the imbalance between bone formation and bone resorption which are coordinated by osteoblastic bone formation and osteoclastic bone resorption [1]. Serum ALP is a marker of early stage of osteoblast differentiation, and osteocalcin (OC) is a marker of osteoblast activity. ALP and OC are used to measure the changes in bone formation [15], whereas TRAP is used to measure the changes in bone resorption as a marker of osteoblast activity [14]. In the present study, the results of BMC and BMD testing and histomorphometrical analysis suggested that GIOP rats were successfully induced by intramuscular injection with DXMT, and the serum ALP and OC significantly declined and serum TRAP significantly increased in

GIOP rats. However, in contrast with the GIOP rats, the levels of BMC, BMD, serum ALP and OC were improved, and trabecular was close-packed and significantly restored by increasing Tb.Ar and Tb.N., reducing Tb.sp in the glucocorticoid-treated rats of PEMFs+

UVB group. The results of the above mentioned indicated that PEMFs stimulation combined UVB irradiation can improve the imbalance between bone formation and bone resorption, and ultimately prevent bone loss and improve bone metabolism disorders. Our previous study suggested that PEMF stimulation can promote bone marrow mesenchymal stromal cells (BMSCs) to differentiate into osteoblasts for bone formation with no apparent side effects in GIOP rats [14]. However, the disadvantageous effects of GCs on bone occur through multiple pathways, including renal calcium loss, intestinal calcium absorption decrease, sex steroid levels decline, and muscle weakness [1], especially the regulation of calcium homeostasis by kidney and bone.

The regulation of calcium homeostasis in renal is manifested by the re-absorption and excretion of calcium through the renal tubules [21]. High levels of urinary calcium are frequently accompanied by osteoporosis, but the causal relationship between the high levels of urinary calcium and osteoporosis remains unclear. Some scholars suggested that the treatments for high levels of urinary calcium can improve bone mass to a certain degree [22]. In this present study, the urinary calcium levels of GIOP group were significantly higher than those in control group. However, in contrast with GIOP group, the urinary calcium levels significantly decreased, and the $1,25(\text{OH})_2\text{D}_3$ levels significantly increased in PEMFs+UVB group. Vitamin D plays an very important role on bone health due to it promotes the reabsorption of calcium and phosphorus in renal tubules to reduce urinary calcium and urine phosphorus, and increase bone mineralisation [23]. $1,25(\text{OH})_2\text{D}_3$ is a highly active form of vitamin D, and the main target organ of it include renal tubules, small intestine mucosa and skeleton. More than 90% of vitamin D production in vivo is

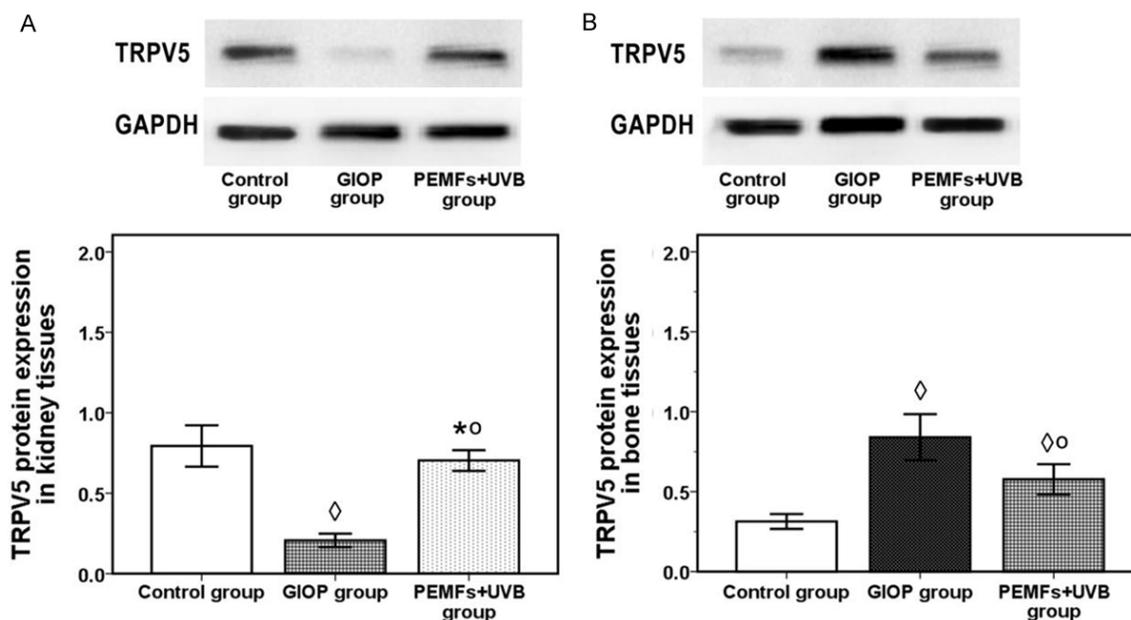


Figure 2. The results of western blot analysis. The TRPV5 protein expressions in kidney (A) and bone tissues (B) were estimated using western blot analysis. Compared with the control group: * $P < 0.05$, $\diamond P < 0.01$; Compared with the GIOP group: # $P < 0.05$, $^{\circ}P < 0.01$.

from exposure to sunlight and only approximately 10% of vitamin D requirement derives from food intake [15]. Previous studies reported artificial UVB radiation accelerated vitamin D formation in the skin, and UVB lamps was the most effective method of maintaining the serum concentration of $1,25(\text{OH})_2\text{D}_3$ [24, 25]. $1,25(\text{OH})_2\text{D}_3$ not only promotes the synthesis of calcium binding proteins (CaBP) in small intestinal mucosa cells to increase intestinal absorption of calcium, but also increase renal calcium reabsorption function through affects the expressions of TRPV5 in kidney tissues.

TRPV5 is one of the members of TRPV subfamily in transient receptor potential channel superfamily, and it is an important channel protein mediating Ca^{2+} transmembrane transport. TRPV5 plays as a major channel for calcium influx in the process of calcium transport, and it is a ion channel gating for renal Ca^{2+} transmembrane absorption [26]. Some studies found that GCs increase bone calcium release by affecting calcium reabsorption in the kidney, resulting in a decrease in bone mineral density, and increased urinary excretion of calcium. High levels of urinary calcium is mainly due to low expression of TRPV5 in the renal tubular epithelium [27]. TRPV5 is the main target of $1,25(\text{OH})_2\text{D}_3$ during the active reabsorption of

tubular Ca^{2+} . Moreover, the TRPV5 is expressed in osteoclasts in humans and mice, and exists in the cellular area in contact with the bone surface, whereas there is no expression of TRPV5 in osteoblasts. TRPV5 plays an important role in osteoclasts differentiation, and it is closely related to bone resorption and Ca^{2+} transport function in bones tissues [28, 29]. In this present study, in contrast with control group, the mRNA and protein expressions of TRPV5 in GIOP group were significantly increased ($P < 0.05$) in bone tissues and significantly decreased ($P < 0.05$) in kidney tissues. It indicated that the expressions of TRPV5 in kidney and bone tissues are one of the important pathological mechanisms of GIOP. However, the mRNA and protein expressions of TRPV5 of PEMFs+UVB group in kidney tissues were significantly higher than GIOP group ($P < 0.05$), and significantly lower than GIOP group in bone tissues ($P < 0.05$). It suggested that improvement of renal calcium reabsorption function and inhibition of bone resorption were mainly affected by the expressions of TRPV5 in the rats receiving UVB irradiation, whereas the direct effect of PEMFs on TRPV5 expression had not been confirmed in the present study.

In conclusion, our study suggests that PEMFs stimulation combined UVB irradiation have a

good comprehensive therapeutic effect on prevent bone loss and improve bone metabolism disorders. PEMFs combined UVB may be a suitable therapeutic method for prevention and cure of GIOP, and it would offer some potential benefits for patients receiving long-term GCs therapy.

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Disclosure of conflict of interest

None.

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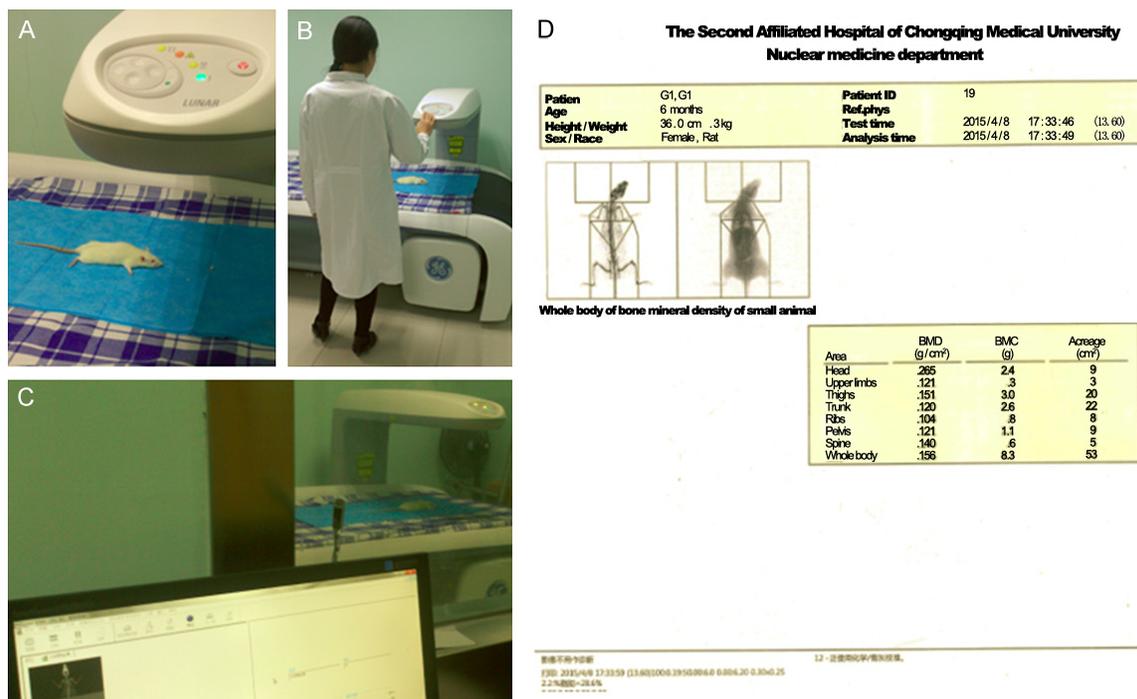
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Bone mineral density and bone mineral content measurement



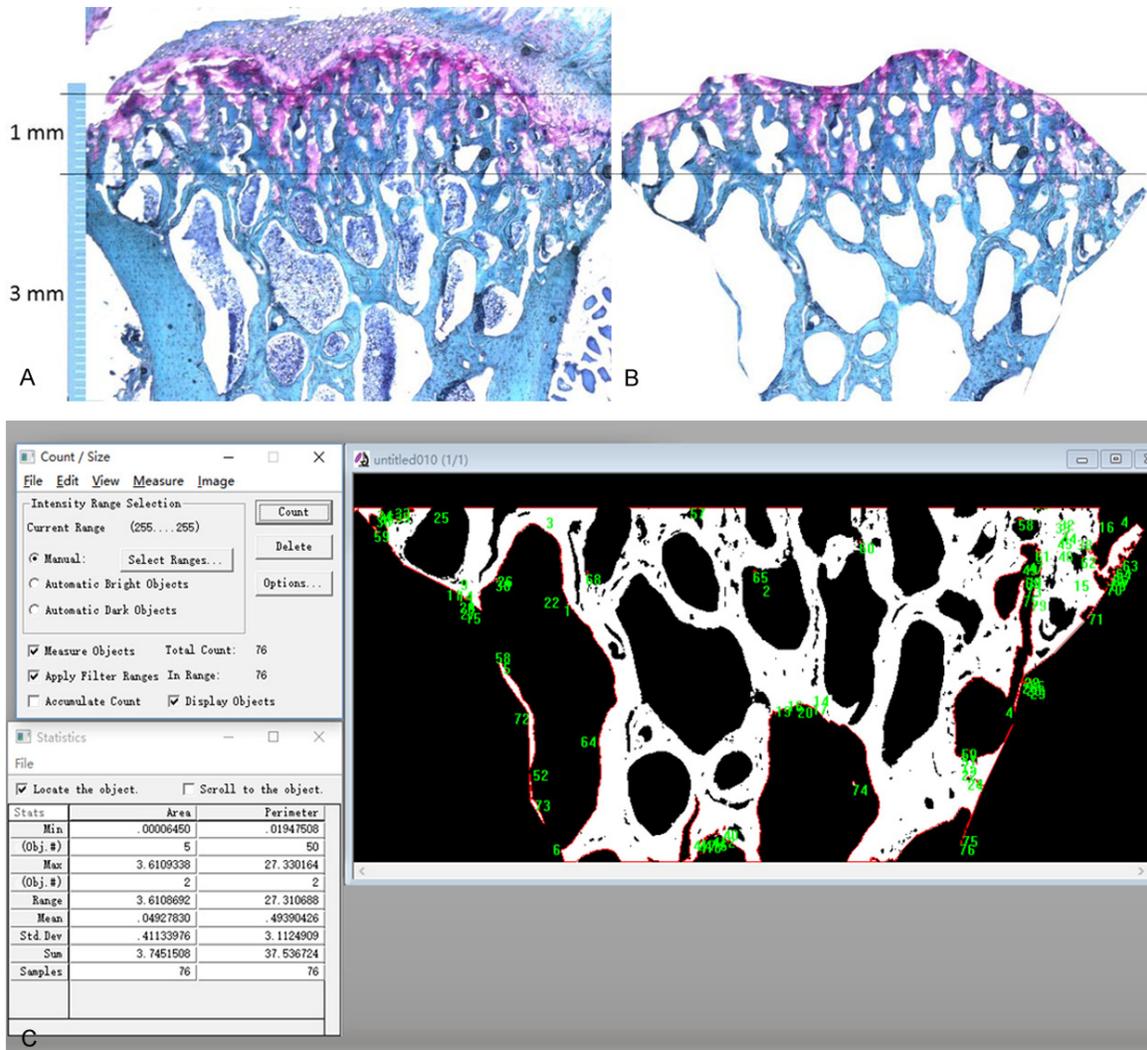
Supplementary Figure 1. A-C. Bone mineral density and bone mineral content measurement by dual energy X-ray absorptiometry. D. The report of bone mineral density and bone mineral content measurement.

The rats in each group were received intraperitoneal anesthesia of 10% chloral hydrate (3.5 mL/kg) after a period of 12 weeks' treatment, and positioned on the platform of dual energy X-ray absorptiometry (Lunar iDXA, GE Healthcare) in prone position. The BMD and BMC of the whole body were detected and recorded.

Histomorphometrical analysis

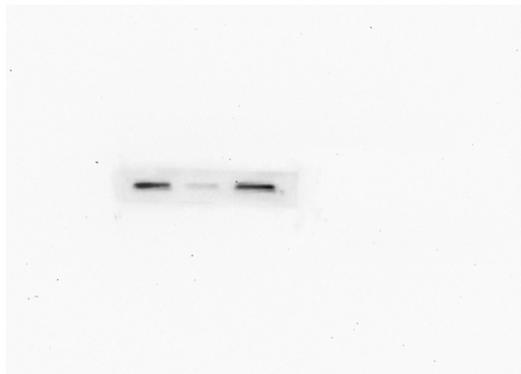
The serial sections of the fourth lumbar (L4) vertebral bodies were stained with Safranin-O/Fast green. The region of interest for trabecular bone was an area 1-3 mm below the growth plate of L4 vertebral body. Histomorphometrical parameters were quantified by using the Image-Pro Plus 6.0 software (Media Cybernetics). The values of trabecular area, trabecular perimeter and bone area were detected and recorded. The static parameters are calculated using the following formula [14]: percentage of trabecular area (Tb.Ar) = trabecular area (Tb.Ar)/bone area (T.Ar) × 100%. trabecular width (Tb.Wi) = (2000/1.199) × (Tb.Ar/trabecular perimeter [Tb.Pm]). trabecular number (Tb.N) = (1.199/2) × (Tb.Pm/T.Ar). trabecular number (Tb.N) = (1.199/2) × (Tb.Pm/T.Ar). trabecular separation (Tb.Sp) = (2000/1.199) × (T.Ar - Tb.Ar)/Tb.Pm.

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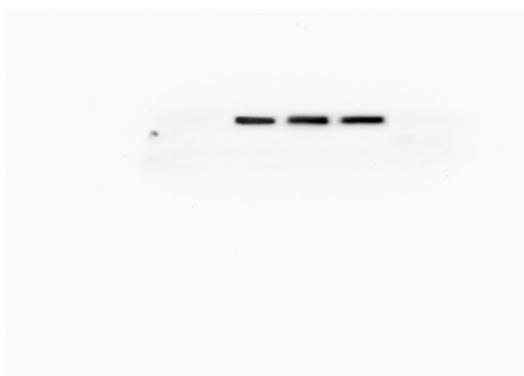


Supplementary Figure 2. A, B. The serial sections of L4 vertebral bodies were stained with Safranin-O/Fast green. C. Histomorphometrical parameters were quantified by using the Image-Pro Plus 6.0 software.

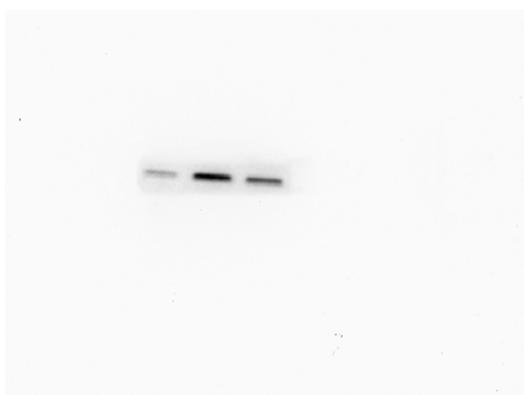
Riginal WB membrane



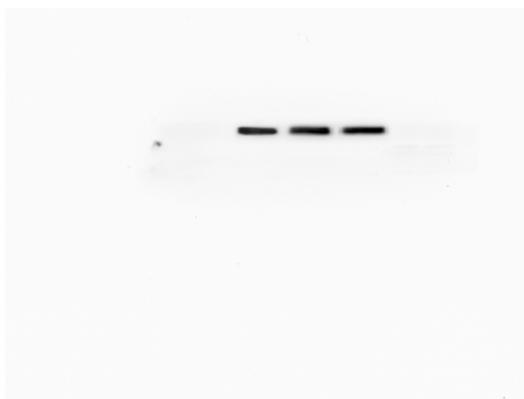
Supplementary Figure 3. The protein expressions of TRPV5 in kidney tissues.



Supplementary Figure 4. The protein expressions of GAPDH in kidney tissues.



Supplementary Figure 5. The protein expressions of TRPV5 in bone tissues.



Supplementary Figure 6. The protein expressions of GAPDH in bone tissues.