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Effects of fatty acids, nutrients and whole body vibration on bone histomorphometry, mechanical properties and metabolic parameters in male rat

Hossein Bahadoran[1], Soheila Salmasi [1], Mohammad Hassan Karimfar[2], Reza Shirazi[3], Mohammad Hossein Asadi[1], Mohammad Reza Naghii[4*], Giti Torkaman[5], Mahmoud Mofid[1]

[1]Department of Anatomy, Faculty of Medicine, Baqiyatallah University of Medical Sciences, Tehran, Iran
[2]Department of Anatomical Sciences, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
[3]Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
[4]Department of Nutrition, Health School, Baqiyatallah (a.s.) University of Medical Sciences, Tehran, Islamic Republic of Iran
[5]Department of Physical Therapy, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
*** Corresponding author:** Mohammad Reza Naghii,
Email: *naghiimr@yahoo.com*

Abstract

The aims of this study are to investigate the effects of consumption of fatty acids, nutrients and application of vibration on bone histomorphometry and mechanical properties in addition to metabolic bone parameters in rat. Fifty six male Wistar rats were divided into seven independent groups and treated for 8 weeks as followings: (1) Control (received regular rat chaw food and drinking water); (2) RV (regular food + vibration); (3) SPM (regular food + vibration + calcium + vitamin D + boron); (4) CA (SPM + canola oil); (5) SU (SPM + sunflower oil); (6) CA + SU (SPM + Canola oil + Sunflower oil); and (7) CO (SPM + coconut oil). After 8 weeks, plasma samples were analyzed for vitamin D, parathyroid hormone, calcitonin, testosterone, free testosterone, and estrogen levels. The right femur bone was excised for histomorphometric and geometric analyses. The left femur and fifth lumbar vertebra were also used for evaluation of mechanical properties.

Our results indicated that compact bone area and number of osteocytes in all treatment groups and energy to maximal load in RV compared to control group were significantly increased ($P<0.05$). The decline in trabecular separation area, femoral neck maximal load, and femoral neck stiffness in SPM compared to control, RV and both groups were significant ($P<0.05$). Also, several significant changes in spongy bone and trabecular separation areas, femoral neck maximal load and stiffness, lumbar maximal load and lumbar energy to maximal load between control and different treatment groups were detected ($P<0.05$). Decrease of trabecular separation area in CA, CA+SU, and CO groups compared to control group and femoral neck deformation in SU compared to CA group were significant. Finally, significant increase in estrogen level in CO group compared to all other groups was detected. Our findings demonstrated that combination of whole body vibration and coconut oil has beneficial effects on bone histomorphometry, mechanical properties and metabolic parameters.

Keywords: Fatty acids, nutrients, vibration, histomorphometry, mechanical properties, metabolic parameters, bone.

1. Introduction

Bone-related disorders are increasing worldwide and among them, osteoporosis represents the most common metabolic bone disease [1]. Osteoporosis is a growing health problem in the elderly populations, particularly in postmenopausal women [2], and some medications such as long-term glucocorticoid therapy can induce osteoporosis [3]. Therefore, maintenance of normal bone tissue and prevention of its degradation are necessary. Calcium is the most abundant mineral found in bone. Approximately 98% of the 1-2 kg of calcium in the adult human body is found in the skeleton [4].

Bone calcium homeostasis is closely regulated by some factors including vitamin D, parathyroid hormone and calcitonin, and is dependent to the calcium absorbance by the digestive system. Currently, hormone replacement therapy (HRT) is widely used for osteoporosis after menopause. HRT can reduce the rate of osteoporosis, however, large scale clinical research has shown that HRT was a factor in the growth of some cancers [5-6]. Although nutrition is only one of the many factors that influences bone mass, but, it has particular importance to bone health because it is modifiable [7]. Diet can be modified for the maintenance and development of bone mass. Proper nutrient intake has a crucial role in both prevention and treatment of osteoporosis [8]. The plants contain phytoestrogens such as isoflavones, lignans and coumestans, which are similar to mammalian estrogens. These phytoestrogens aid the cure of hormone-associated diseases such as osteoporosis [9].

Boron is known as an essential element for plant nutrition, but it is not essential in animal nutrition. Improved growth and reduced plasma alkaline phosphatase activity after using of boron supplementation was reported in vitamin D deficient chickens. The interactions between boron and other nutrients were also speculated [10]. Also, vitamin D has been linked with many health outcomes, and its deficiency was related to rickets (in children) and osteomalacia (in adults) [11]. However, the quantity required for optimum health overall, is currently hotly debated with many researchers and clinicians [12]. During the past decade there has been increasing interest in the use of whole-body vibration (WBV) as a therapeutic modality. Many WBV treatment studies aim at improving some aspect of neuromuscular performance or at increasing bone mass or density [13].

Therefore, in the present study, the effects of nutrient supplementations and physical activity on bone microarchitecture in rats were investigated. For this purpose, bone histomorphometry, geometry and biochemistry analysis on bone and plasma samples was carried out, in order to obtain information about the bone structure.

2. Materials and Methods

2.1 Animals and housing

All the experimental procedures were approved by the Institution's Animal Welfare Committee (Baqiyatallah University of Medical Sciences, Tehran, Iran) and were conducted in accordance with the European's guidelines for the care and use of laboratory animals. All efforts were made to minimize suffering during the experimental period and all applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Fifty six male Wistar rats (150±30 g) were obtained from the Animal House of Physiology Department, Baqiyatallah University of Medical Sciences. The rats were weighed and randomly allocated to seven groups and 14 polycarbonate standard cages under a 12 hours light cycle with a constant temperature of 21±1 °C, and 65% relative humidity with free access to food and water.

2.2 Experimental grouping

Rats were given a 5-day acclimatization period with an access to regular rat chow and water ad libitum before the experiment. Experimental groups were categorized as follows:

Group no.	Details
1 (Control)	Regular food
2 (RV)	Regular food, drinking water + vibration
3 (SPM)	RV + calcium (100 mg/day) + vitamin D (40 IU/day) + boron (1 mg/ day) for each rat
4 (CA)	SPM + Canola oil
5 (SU)	SPM + Sunflower oil
6 (CA+SU)	SPM + Canola oil + Sunflower oil
7 (CO)	SPM + Coconut oil

Canola, sunflower and coconut oils were used as the sources of monounsaturated, poly unsaturated and saturated fatty acids, respectively and provided by commercial sources. The complete analysis of canola, sunflower, and coconut oils are presented as supplementary 1. Each oil source was added to the dietary pellet as 5% of daily diet weight and calcium, vitamin D and boron were provided in drinking water for 8 weeks.

2.3 Whole-body vibration procedure

For WBV, rats were placed in a plastic compartment attached to a vibration platform and the vibration was planned to be increased gradually over the time [14]. These included five minutes cycles of vertical sinusoidal whole body vibration for four sessions in the first week and increased gradually to 45 min until day 24 (with three sessions per week); followed by 60 min for the next 20 sessions until the end of the 8th week of the experiment. Each training session was performed between 8.30-10.00 a.m.

2.4 Sample collection

Food and water consumption were measured three times per week for 8 weeks. At the end of experimental period and after 12 hour fasting, blood sampling was done by cardiocentesis under anesthesia. Blood plasma was obtained by centrifugation (3000 rpm, 15 min) and stored at -20°C until use. All rats were sacrificed under deep ether anesthesia and bone sampling was performed from both femurs and 5th lumbar vertebra and kept in normal saline at -18°C until use.

2.5 Assessments

Right femur was used for histomorphometry analysis. Briefly, isolated bone tissue were dehydrated in graded alcohols (70 to 100%), cleared in xylene and embedded in paraffin. Parafinized tissue blocks were cut into 7 µm sections using a microtome (Letiz, Germany). In next step, the rat bone sections were stained with hematoxylin and eosin (H&E) and Masson's Trichrome. Morphometric analysis was performed using light microscopy and Motic Images 2000 Release 1.2 software in the neck of femur. Osteocyte count was done in three microscopic fields in each proximal, middle and distal part of femur neck. Geometry indices included length and anterior-posterior diameter of neck of left femur was calculated by digital calibrator. Mechanical tests were consisting of cantilever bending test and axial compression test and were performed using Zwick material testing-machine Z2.5 (Germany) in both left femur and fifth lumbar vertebra. The evaluated parameters are presented and defined in the Figure 1. These included Fmax (maximum bending strength of the bone), WRm (energy absorbed by the bone until Fmax), stiffness and εFmax (bending deformation until Fmax).

Plasma samples were used for assaying of vitamin D (EIA, Immunodiagnostics System Ltd, UK), calcitonin and parathyroid hormone (PTH, ELISA, USCN Life Science Inc., China), testosterone, free testosterone, and estrogen (ELISA, Diagnostics Biochem Canada Inc., Canada) and alkaline phosphatase (ALP, Kinetic Photometric, Pars Azmun, Iran).

2.6 Statistical analysis

Values are expressed as mean ± SD. Differences between the experimental groups were determined by ANOVA, followed by LSD method for all pairwise multiple comparisons. P value <0.05 was considered as statistically significant.

3. Results

3.1 Daily food intake

Daily food intake and amount of different fatty acids which consumed in different groups are presented in Table 1. As demonstrated, the food intake in RV and SP groups is the highest amount (17.8 g/day) and in SU group is the lowest amount (15.5 g/day). Also, the highest consumption of saturated, monounsaturated and polyunsaturated fatty acids were belonged to the CO, CA, and SU groups, respectively.

Table 1. Food intake and total fatty acid content of food in different groups.

	Groups*						
Parameters	Control	RV	SPM	CA	SU	CA+SU	CO
Food intake (g/day)	17.1	17.8	17.8	16.6	15.5	16.3	16.7
Total FA content (mg)							
Saturated				60	80	71	713
Monounsaturated				523	150	332	48
Polyunsaturated				232	509	380	15
S:M:P ratio				1:8.7:3.8	1:1.9:6.4	1:4.7:5.3	47.5:3.8:1

* RV, regular food + vibration; SPM, RV + calcium (100 mg/day) + vitamin D (40 IU/day) + boron (1 mg/rat/day); CA, SPM+5% canola oil; SU, SPM+5% sunflower oil; CA+SU, SPM+5% Mix of canola & sunflower oil; CO, SPM+5% coconut oil.

3.2 Histomorphometry and geometric parameters

Comparison of mean and standard deviation of histomorphometry and geometric parameters in different groups are presented in Table 2. Osteocyte numbers were significantly increased in all experimental groups in comparison to control group ($P<0.05$). Also, some other significant differences between different groups in compact and spongy bone areas in addition to trabecular separation area were detected (Table 2).

3.3 Mechanical properties of the bones

Mechanical properties of femur neck and fifth lumbar vertebral bone in control and different experimental groups are presented in Figure 2. Lumbar vertebral bone had higher mechanical properties than femur neck in all parameters except εFmax. In addition, femoral neck stiffness and lumbar Fmax in CO group were significantly higher than control group ($P<0.05$). Other significant differences in four mechanical properties between experimental and control groups are also detected (Figure 2).

3.4 Plasma parameters

Plasma biochemical evaluation was performed by analysis of vitamin D, PTH, calcitonin, testosterone, free testosterone, estrogen and ALP as demonstrated in Table 3. Only significant increase in plasma estrogen level were detected in CO group in comparison to all other six groups ($P<0.05$).

4. Discussion

Preventive strategies based on both nutritional and physical plans must be designed to prevent or lower the risk of bone demineralization and osteoporosis. In this field, treatment of animal model especially rat and performing different studies with nutrients and physical activities can be useful. Moreover, sufficient calcium intake and vitamin D for its complete absorbance has been reported to support bone growth and prevent bone loss. In the present study, we evaluated the effects of eight weeks edible vegetable oils supplementation plus WBV, calcium/vitamin D and boron on bone microarchitecture in healthy rats. The analysis was based on bone histomorphometry, geometry, mechanical properties and plasma biochemistry. Histomorphometry is an important technique for evaluating the rate of bone turnover and for examining bone quality and architecture [15]. Totally, it was found that combination of WBV and coconut oil has beneficial effects on bone histomorphometry, mechanical properties and metabolic parameters.

Osteoporosis and fracture are multifactorial events, and no single risk factor can account for these conditions [16]. It had been reported that poor performance is associated with an increased risk of hip fracture over 5 years of follow-up in the older, community-dwelling men [16]. On the other hand, in some studies the beneficial effects of dietary oil supplementation from plant and animal origins on prevention of bone loss has been reported. Furthermore, Nadhanan and colleagues reported that emu oil (that is extracted from both the subcutaneous and retroperitoneal fat of the *Dromaiusnovaehollandiae*, traditionally native to Australia) could preserve osteoblasts, suppress osteoclast formation, and potentially be useful in preventing chemotherapy-induced bone loss[17]. Tagliaferri et al., reported that olive oil fortified with vitamin D prevented impaired bone mass and micro-architecture in addition to decreased oxidative stress and suggested a protective impact of olive oil as a source of polyphenols along vitamin D on bone metabolism[18]. It is shown that the consumption

Table 2. Mean and SD of bone geometry and histomorphometry parameters in different groups.

	Groups*						
Parameters	Control	RV	SPM	CA	SU	CA+SU	CO
Compact bone area (%)	41.0±0.50[a]	46.0±4.0[b]	47.0±3.1[b]	48.0±1.0[b]	48.0±7.0[b]	48.0±7.0[b]	51.0±1.3[b]
Spongy bone area (%)	30.0±3.2[a]	31.0±3.3[ab]	31.0±0.7[ab]	35.0±5.0[b]	31.0±2.4[ac]	33.0±2.7[ab]	32.0±3.2[ab]
Trabecular separation area (%)	26.0±4.0[a]	24.0±3.0[ab]	20.0±5.0[bc]	14.0±2.4[b]	21.0±6.80[ac]	19.0±6.8[bc]	17.0±3.0[bc]
Number of osteocyte(cell/unit)	18.0±7.0[a]	28.0±3.0[b]	29.0±4.0[b]	30.0±2.3[b]	30.0±3.0[b]	30.0±2.7[b]	31.0±3.0[b]
FNAPD (mm) #	2.37±0.13[a]	2.37±0.10[a]	2.34±0.12[a]	2.33±0.10[a]	2.35±0.13[a]	2.32±0.04[a]	2.33±0.05[a]
FNL (mm) ¥	2.26±0.13[a]	2.25±0.12[a]	2.26±0.10[a]	2.26±0.01[a]	2.25±0.13[a]	2.26±0.10[a]	2.27±0.07[a]

* RV, regular food + vibration; SPM, RV + calcium (100 mg/day) + vitamin D (40 IU/day) + boron (1 mg/rat/day); CA, SPM+5% canola oil; SU, SPM+5% sunflower oil; CA+SU, SPM+5% Mix of canola & sunflower oil; CO, SPM+5% coconut oil.
# FNAPD, femoral neck anterior-posterior diameter; ¥ FNL, femoral neck length.
Significant differences in each parameter are demonstrated by different superscript letters in each row (P<0.05).

Table 3. Mean and SD of plasma biochemical parameters in different groups.

	Groups*						
Parameters	Control	RV	SPM	CA	SU	CA+SU	CO
Vitamin D (nM)	91.20±18.90	107.0±22.0	106.70±14.80	108.20±15.30	109.0±21.60	106.70±19.80	96.90±21.90
Parathyroid hormone (pg/ml)	36.0±8.90	38.60±5.50	39.20±7.44	37.70±5.40	34.30±5.80	30.90±15.0	34.70±83.0
Calcitonin (pg/ml)	80.30±11.50	81.0±10.30	79.0±10.80	77.60±9.70	79.80±9.0	74.40±31.10	76.30±12.30
Testosterone (ng/ml)	1.84±0.40	1.88±0.63	1.83±0.77	2.04±0.58	1.85±0.65	1.75±0.51	2.14±0.74
Free T (pg/ml)	0.44±0.19	0.40±0.17	0.38±0.15	0.42±0.16	0.39±0.16	0.43±0.24	0.51±0.26
Estrogen (pg/ml)	8.35±1.32	9.82±2.22	8.60±0.90	8.32±1.47	8.70±0.94	8.82±0.99	10.0±0.87[a]
Alkaline phosphatase (U/L)	215.0±19.50	214.0±31.70	211.0±24.80	231.70±34.80	232.0±34.60	236.0±29.50	243.40±42.40

* RV, regular food + vibration; SPM, RV + calcium (100 mg/day) + vitamin D (40 IU/day) + boron (1 mg/rat/day); CA, SPM+5% canola oil; SU, SPM+5% sunflower oil; CA+SU, SPM+5% Mix of canola & sunflower oil; CO, SPM+5% coconut oil.
[a] Statistically significant; P≤ 0.05.

Figure 1. Cantilever bending test parameters and definitions (Fmax: maximum bending strength of the bone; WRm: energy absorbed by the bone until Fmax; stiffness; εFmax: bending deformation until Fmax).

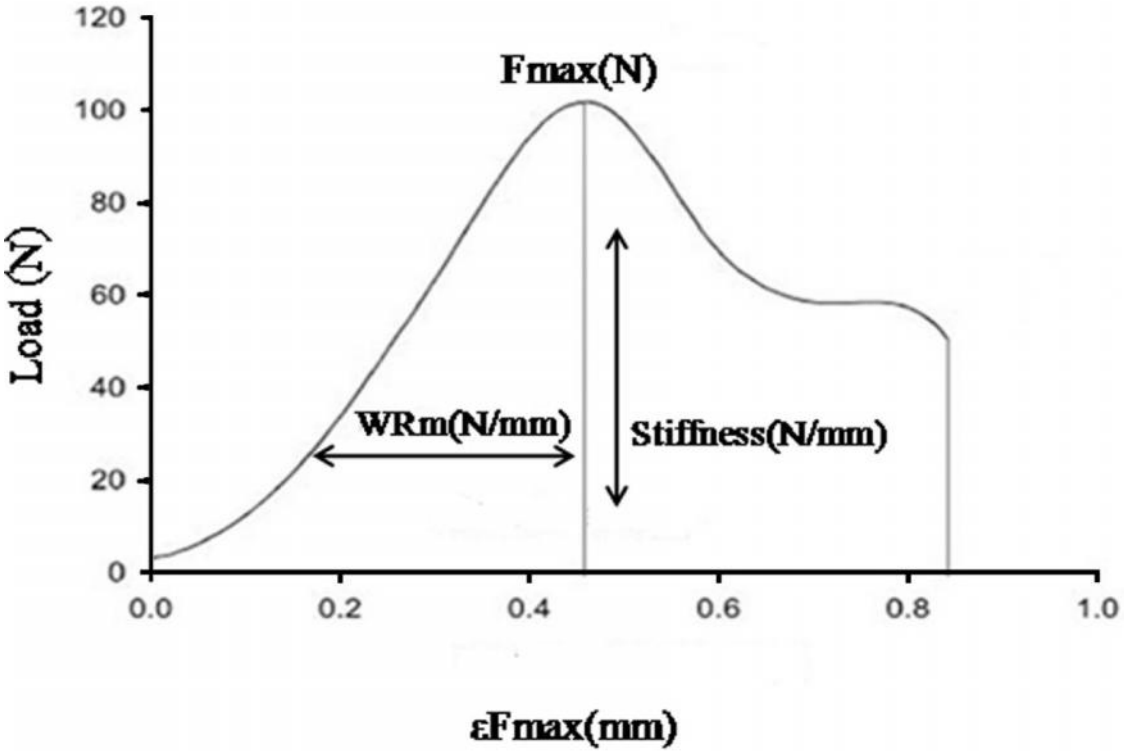
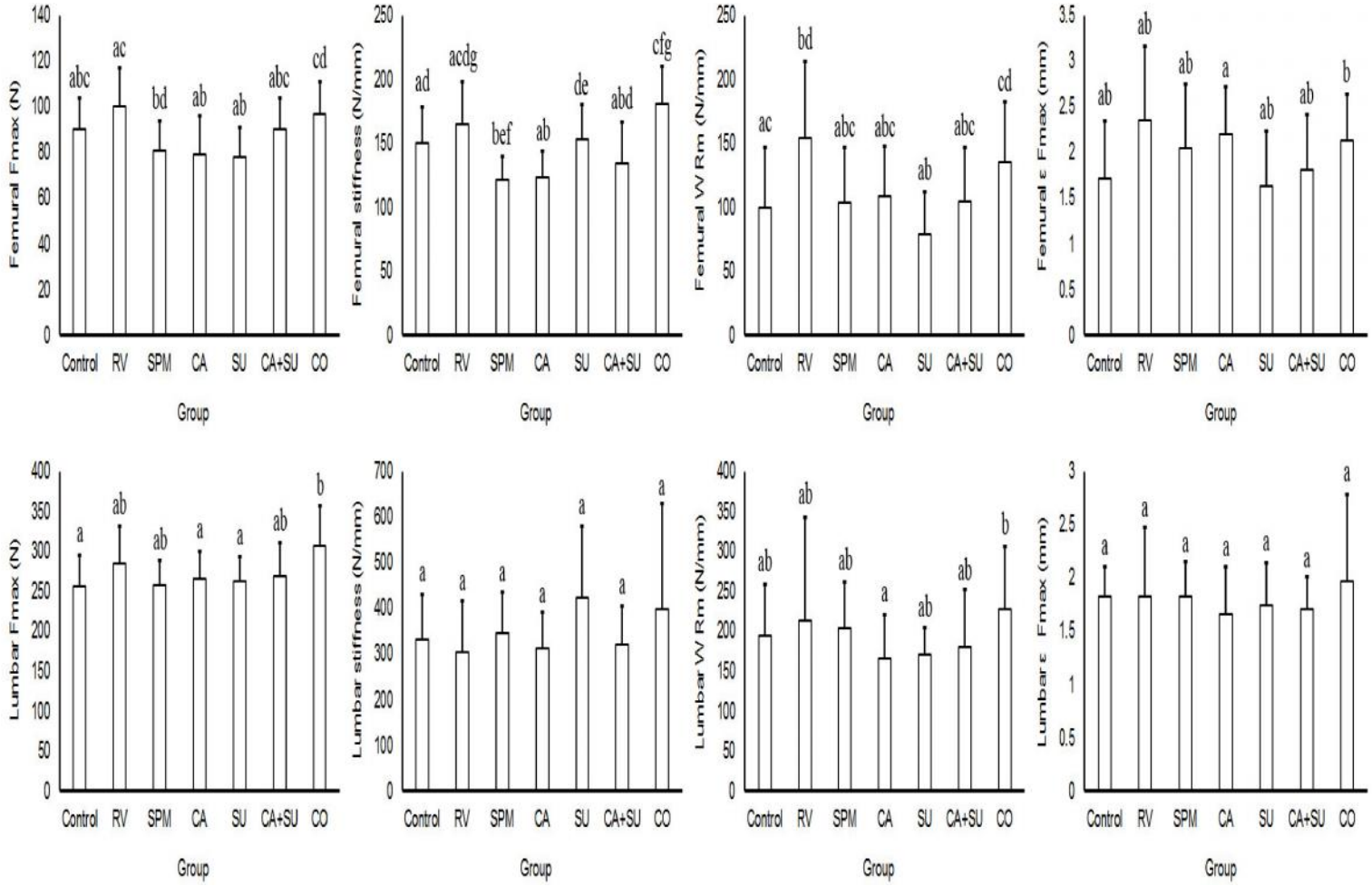


Figure 2. Comparison of the mean ± SD of mechanical properties of femoral neck and lumbar bone in different experimental groups.



of experimental oils (canola, sunflower and coconut) play similar roles in increase of bone mass and strength. Moreover, the measurement of polyphenol and active contents of these oils for clarifying the underlying mechanisms is highly recommended.

Among the active component of the dietary plant sources, boron has reported to have the potential beneficial effects on vitamin D synthesis and function and on bone metabolism. Enhancement of plasma level of vitamin D and testosterone was reported after boron supplementation [19]. Also, it can affect other hormone concentrations, in addition to serum and bone levels of calcium, magnesium and phosphorous [20]. In other studies, it has been reported that boron can induce some special protein expression which is required for the differentiation of mesenchyme cells to osteoblasts [21-22]. Also, increase of serum 17 β-estradiol after administration of boron was reported in a human study [23]. Nielsen and colleagues reported that boron deficiency can decrease lumbar bone mineral concentration and trabecular space [24]. Our results are in accordance with the finding of the above study and some other reports by stressing on the role of boron in bone metabolism, functions and structures.

In ovariectomized rats, bone loss can be induced by estrogen deficiency and exposure to xenoestrogens is known to affect the musculoskeletal system [25]. It is clear that estrogen affects articular cartilage and intervertebral disc turnover[26]. In a study performed by Rowas and colleagues, it had been reported that diethylstilbestrol, a synthetic non-steroidal estrogen, significantly affected the musculoskeletal system of adult mice. Its effects included increase of lumbar and femoral bone mineral content, bone area and trabecular bone area [25]. Phytoestrogens, as another source of xenoestrogens are considered to be an effective alternative estrogenic substance that can prevent bone loss caused by the deficiency of estrogen [27]. Existence of these phytoestrogens are reported in different type of plants such as soybean [28-29] and *Puerariaspp* [30-33]. We found that in the group of coconut oil, the plasma estrogen increased even more than other groups using oils, physical activity and the control group. This finding is in agreement with the previous report, demonstrating that coconut juice could increase the estrogen level in andropause men [27]. In addition, these oils contain different levels of α-tocopherol in the range of 0.9 ppm for coconut oil to 410 ppm for sunflower oil. It had been reported that α-tocopherol could affect bone structure in rat [34] and some of the observed effects may be attributed to the α-tocopherol content of the plant oils.

Moreover, in addition to nutritional strategies, alternative methods are recommended for prevention and treatment of osteoporosis. Physical activities and exercise have been reported to maintain bone mass density[35], and exercise has long been used for the prevention of osteoporosis [36]. It can maximize and maintain the bone mass, and also reducing the bone loss after menopause [37]. Bone gain consist of bone mass increase in both hip and spine after high impact activity, which is recorded in systematic reviews and meta-analysis studies [38]. Accordingly, bone loss reduction after exercise is also reported in postmenopausal women [39]. WBV can offer some fitness and health benefits. In this method, a pivotal machine running on a low frequency would be mainly used for physiotherapy applications and strength and it is reported to increase serum vitamin D level, enhance bone mineralization [40], and induce bone matrix synthesis by osteoblasts [41]. Studies indicated that vibration can increase the serum vitamin D [42] and testosterone levels[43]. Both vitamin D and testosterone have the anabolic effects on the bone metabolism and estradiol, as a product of testosterone can stimulate osteoblast formation; and increase the intestinal absorption of calcium which is considered as a beneficial factor for bone function and metabolism. Previously, we reported that supplementation of boron could enhanced mechanical properties of tibia, femur and lumbar vertebral bones in rats [43], and consequently along in investigating the combined effect of the consumption of fatty acids, nutrients, and regular physical activity on bone mechanical properties and hormones in rats[44]. The findings of the present study are comparable with other reports using different intensity and duration of WBV and nutrients plus different sex and gender.

Conclusion

Although, the known risk factors of bone loss such as aging, gender, and menopause cannot be prevented or changed in human, but alteration of life style particularly physical activity and nutritional status can influence the prevention of bone disorders and osteoporosis. The above findings demonstrated that continuous physical activity as performed by vibration, in addition to consumption of oil with known fatty acids, especially coconut oil, could provide the synergistic effects on the bone structure and mechanical properties of the bone.

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Conflict of interests

The authors declared that there are no conflicts of interests.

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