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Effect of consumption of fatty acids, calcium, Vitamin D and boron with regular physical activity on bone mechanical properties and corresponding metabolic hormones in rats

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The consumption of fatty acids, nutrients, and regular physical activity, individually influence bone mechanical properties in rats. To investigate their effects in combination, male rats were divided into the seven groups**:** G1: regular food and drinking water; G2: same as Gr.1 + physical activity (Whole body vibration; WBV); G3: same as Gr.2 + Calcium, Vit. D, Boron; G4: same as Gr.3 + canola oil; G5: same as Gr.3 + sunflower oil; G6: same as Gr.3 + mix of sunflower oil and canola oil; and G7: same as Gr.3 + coconut oil; and treated for 8 weeks. Analysis between the control with the groups 2 and 3 revealed that vibration in the G2 increased the body weight (P= 0.04), with no other major difference in plasma and bone indices. Comparison between the control with the G4-G7 (the oil groups) revealed that the rats in the G5 had a lower body weight (15 % less) and a significant increase in plasma levels of Estradiol in the G7 was noted. In addition, levels of Testosterone in the G4 and G7, and Free Testosterone in the G7 had a remarkable increase. Similar trend was observed for plasma levels of Vit. D in the G4 and G5. The stiffness and the breaking strength of the femur in the G7, and the breaking strength of the lumbar in the G7 compared to the control and the G4 and G5 was significantly higher and tended to increase in comparison to the G6. Better and stronger measurements observed for coconut oil is warranted to further study its effect on biomechanical properties of bones.

Keywords: Bone, Boron, Calcium, Fatty acids, Hormones, Vit D, Vibration

With the increase in the number of patients suffering from bone-related disorders worldwide, the medical as well as the socioeconomic impact of this problem becomes significant[1].

Physical activity as an effective strategy, recommended in general practice that can be used to increase lean mass and bone mass while decreasing fat mass and is also beneficial in improving health status of the individuals[2]. Recently, whole body vibration (WBV) as a non-pharmacological supportive treatment option has been regarded as an exercise training method with a potential of improving body composition[3]. In mice, reduced adipogenesis and factors associated with the onset of type II diabetes (e.g., triglycerides and nonesterified free fatty acid) is reported[4]. During exercise and recovery in young men, energy expenditure increased significantly with vibration and exercise training[5], and in a similar study group, energy expenditure, carbohydrate and fat oxidation rate, and oxygen consumption were increased by vibration[6]. WBV training improved isometric and dynamic muscle and muscle strength and cardio respiratory fitness[3,7,8]. Roelants *et al*.[7,9] reported a significant increase in fat-free mass and strength, and a significant increase in knee extensor strength with whole-body vibration. The treatment could increase bone mass density (BMD) in senior people, particularly in people with osteoporosis and women[10], and may increase spine and femoral BMD in older adults[11]. Early studies in

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humans, including some in children with disabilities, suggest that WBV holds promise as a technique for reducing fracture risk[12], and the training is effective for reducing the risk for osteoporosis by increasing lumbar BMD and leg strength[13,14].

Among dietary factors, evidence exists that boron may have antioxidants and anti-inflammatory properties. It may also reduce tissue damage from inflammation by hastening the destruction of reactive oxygen species by increasing activities of key antioxidant enzymes[15-18]. An increased level of steroid hormones after boron consumption and its potential to influence the bone metabolism has been reported[19-21]. It is suggested that boron and fish oil are beneficial to cortical bone strength, and showed to be beneficial for trabecular bone micro-architecture and influences the beneficial effects of fish oil on bone[22].

Better measurements of bone mechanical properties were observed for boron supplementation[23]. Boron deficiency resulted in altered bone healing because of a marked reduction in osteogenesis[24], and altered periodontal alveolar bone modeling and remodeling by inhibiting bone formation[25]. It is suggested that at the molecular level boron displays important roles on bone metabolism and may find novel usages at the regenerative medicine[26]. It may be interesting to study the above effects simultaneously with alterations in steroid hormone concentrations as a result of boron supplementation trials.

The role of Vitamin D in relation to Ca and bone metabolism is known as essential to build adequate bones and has long been under investigation. Recently the Vitamin D insufficiency was defined as serum level less than 20 ng/ml (50 nmoL/L) as it relates to bone[27]. Because calcium deficiency could deteriorate bone metabolisms and cardiovascular systems, adequate intakes of calcium and vitamin D are important for enhancing skeletal health[28].

The positive action of (n-3) fatty acids on bone formation[29] and dietary docosahexaenoic acid (DHA) lowers the ratio of 18:2n-6 (linoleic acid)/n-3 in bone compartments that favour bone conservation in mature ovariectomized (OVX) Sprague-Dawley rats[30].

High-fat diets, containing high levels of PUFA in the form of flaxseed or safflower oil, had a positive effect on bone strength when fed to male rats[31]. Consumption of ice cream containing 9% coconut oil is regarded as a good vehicle for delivery of calcium[32].

The rationale for combination therapy with antiresorptives and osteoanabolic agents and the clinically relevant advances observed in the fields of osteoporosis and metabolic bone disease is based on the fact that they work by different mechanisms and, thus together, should lead to an enhanced therapeutic effect[33].

Therefore in a broad experiment, the effects of a non pharmacological combination therapy approach including the simultaneous impacts of a regular physical activity program [whole body vibration (WBV)], consumption of different fatty acids (polyunsaturated, monounsaturated and saturated fatty acids), plus selected nutrients (calcium, vitamin D, and boron) on the bone metabolic hormones and bone mechanical properties in rats have been investigated and results presented in this study.

Materials and Methods

Male Wistar rats weighing 140-180 g were obtained from the Animal House of Physiology Group, Baqiyattallah University of Medical Sciences. Eight rats in each group (control vs. six treatment groups) were randomly kept in plastic cages (four rats per cage) in a controlled environment with a 12:12 h light: dark cycle at a constant temperature (22ºC) and humidity (55-65%), with free access to food and water. Animals were weighed and provided clean cages weekly. Food intake was determined three times per week.

Vibration training—Vibration training started after a week of acclimatization and animals were placed in a compartment attached to a vibration platform. The vibration intervention for the groups was planned to be increased gradually over the time to acclimatize the animals to a new change of physical activity. Therefore, it consisted of one - three 5-min 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 for 60 min per set for the next 20 sessions until the end of 8 weeks of the experiment. In fact, it was a strategy to avoid any possible stress created by the vibration. Each training session was performed between 08.30 – 10.00 hrs. After each vibration cycle, the animals were given 1-2 min rest break between cycles. The vibration was performed at mode 1 with amplitude of 1-10 and at a frequency of 10-50 Hz. The speed of mode 1 in each cycle increases gradually and then decreases with the same trend within each specific time period. The control animals remained in their cages and placed over the vibration platform, without vibration treatment.

Diets and animal treatments— Rats in all groups were fed with standard chow from Pars Animal Food Co. (Tehran, Iran) and water *ad libitum* throughout the study. According to the manufacturer, the chow contained 650 mg Ca/100 g food and 80 I.U Vit.D3/100g food. The boron content was not analyzed, but it is reported to be kept at 70 µg/kg of the food. Food and water were provided in an identical manner for each group, except for the test lipid or fat source, which was added as commercial oil (w/w; 5%): canola oil (Co), sunflower oil (AF), mix of sunflower oil and canola oil (AC), and coconut oil (N) added to the diet of the groups 4-7, respectively. Commercial canola oil (as a rich source of monounsaturated fatty acids) and sunflower oil (as a rich source of polyunsaturated fatty acids) were manufactured by Khoramshahr Oil Co., (Varamin, Iran). Commercial coconut oil (C.B.C pure white coconut oil, as a rich source of saturated fatty acids) from Sime Darby Edible, Singapore was purchased locally. Rats in the groups 3-7 were supplemented with 100 mg Ca/d, 40 I.U Vit. D3 and 1 mg boron/d in their water, daily. Boric acid (Merck-Germany) was used as the source of boron and Ca+Vit.D3 tablets (Darou Paksh Co., Tehran, Iran) was used as the source of Ca and Vit.D3. Overall, the food and water provided 210 mg Ca, 55 I.U Vit. D3, and 1 mg boron/rat/day. Fresh food and water were provided three times per week and the consumption was monitored and recorded.

Details of treatment in various groups are as follows:

Group no.	Details
1 (control):	regular food and drinking water
2:	—do— + physical activity (WBW)
3:	as in Gr. 2+calcium+Vit. D+boron
4:	as in Gr. 3+canola oil
5:	as in Gr. 3+sunflower oil
6:	as in Gr. 3+mix of sunflower oil+canola oil
7:	as in Gr. 3+coconut oil

Eight weeks after training program, rats from all groups were weighed after 12 h fasting and anaesthetized for the collection of blood by cardiac puncture. Rats were restrained from food for 12 h but had access to drinking water. Since, parameters such as steroid hormones are subject to circadian rhythm, blood samples were collected at the peak time in the afternoon between 14.0°-16.0° hrs and plasma samples stored frozen until analysis.

Although, considering including a set of rats without WBV exercise was a possibility, it seems to be far beyond the aims of the present study. In fact, in the combination therapy approach, it was planned to investigate the simultaneous impacts of a regular physical activity program along with the consumption of different fatty acids and nutrients such as calcium, vitamin D, and boron on the bone properties in rats.

Commercially available assay kits were used to determine the blood parameter levels.

Alkaline phosphatase (Alk.Ph) activity in rat plasma was measured by kinetic photometric test using a kit from Pars Azmun, Tehran, Iran. Plasma 25-Hydroxy Vit. D was estimated by EIA method using reagent kit obtained from Immunodiagnostic System Ltd (IDS Ltd), Boldon, UK. Plasma rat calcitonin was recorded by ELISA method using reagent kit (Rat CT, USCN Life Science Inc. Wuhan, China). Plasma PTH was determined utilizing a two-site ELISA kit (Rat PTH, USCN Life Science Inc. Wuhan, China). The assays for plasma total testosterone (T), free testosterone (FT), and estradiol (E2) were performed by ELISA methods using reagent kits (Diagnostics Biochem Canada Inc., Ontario, Canada).

Immediately, after blood sampling, the left and right tibiae, femurs, and the fifth lumbar vertebral bones were excised from all animals. The bones were kept in 0.9% saline. Mechanical properties of the fifth lumbar vertebral bones were determined with the axial compression test and bone mechanical properties of the left tibiae and femurs by three-point bending test using a Zwick materials testing-machine Z2.5, Germany. For three-point bending, the span of the two support points was 20 mm, and the deformation rate was 1 mm/min.

All measures of mechanical properties of the bones were determined on load deformation curve and consist of the extrinsic material properties of the bone samples, including linear stiffness, the maximal load or breaking strength (Fmax), energy to maximal load, and deformation to maximal load. Energy to maximal load was computed as the area under the load-deformation curve. Stiffness was computed as the slope of the linear portion of the load deformation curve. The maximal load or the yield point is the point on the curve after which plastic or permanent damage occurs to the bone. Prior to the yield point, the slope of the line is linear, and the bone is undergoing elastic deformation[34].

Statistical analysis—Data are expressed as mean±SD and a Statistical Package for the Social Sciences [(SPSS 17.0), New York: McGraw-Hill] was used to perform all comparisons. Analysis of variance (ANOVA) was used to evaluate the effects of training and treatments between the groups (determined by LSD test). A P-value of less than 0.05 was considered significant for the differences.

Results

The rats in the vibration groups adjusted to the vibration and tolerated well. Further, no major differences was observed in the amount of food and water intake between the control and the treatment groups, and all groups were consuming food and water normally (Table 1); except for the lower amount of food intake in the group 5 (sunflower oil) (15.5 g/d).

The analysis of body weight changes was not within the scope of the aim of the present study and we have only decided to report the observed changes as difference in body weight for each group in Table 1. No statistical analysis was performed for these data. However, the vibrated rats weighed more than the control group; except in the group 5, showing lower body weight. They weighed approximately 15 % less (Table 1).

Type and the amount of fatty acids available from various dietary oil sources with the relevant fatty acid ratio provided by the test fats are presented in Table 2.

The influence of different treatments in the groups 2 and 3 on the bone metabolic hormones (Table 3) indicates that vibration training with and without minerals plus Vit. D had no significant changes on the plasma level of hormones. There was only a higher trend in the level of Vit. D in the vibration group (Gr. 2) (P=0.09) and for E2 (P=0.07) compared to the control group.

Table 3—Effects of exercise training and dietary supplementation on plasma parameters groups 2 and 3

[Values are mean±SD from 8 observations in each group]

	Gr. 1 (control)	Gr. 2	Gr. 3
Alkaline phosphatase (U/L)	215.0±19.5	214.7±31.7	211.0±24.8
Vitamin D (nmol/L)	91.2±18.9	107.9±22.0*	106.7±14.8
Calcitonin (pg/ml)	80.3±11.5	81.0±10.3	79.8±10.8
Parathormone (pg/ml)	36.0±8.9	38.6±5.5	39.2±7.44
Testosterone (ng/ml)	1.84±0.40	1.88±0.63	1.83±0.77
Free testosterone (pg/ml)	0.44±0.19	0.44±0.17	0.38±0.15
Estradiol (E2; pg/ml)	8.35±1.32	9.82±2.22*	8.60±0.90

Nearly significant compared to control (P=0.07, E2), (P=0.09, Vit. D)

Table 1—Food and water consumption, body weight of control and treatment groups
[Values are mean±SD from 8 observations in each group]

	Gr. 1 (control)	Gr. 2	Gr. 3	Gr. 4	Gr. 5	Gr. 6	Gr. 7
Food intake (g/d)	17.1	17.8	17.8	16.6	15.5	16.3	16.7
Body weight (g)							
1st day	145.0±10.0	149.0±6.0	143.0±8.0	151.0±15.0	152.0±14.0	155.0±5.0	160.0±12.0
4 week	229.0±27.0	242.0±34.0	230.0±11.0	240.0±22.0	227.0±20.0	243.0±15.0	253.0±24.0
8 week	280.0±31.0	306.0±41.0	285.0±21.0	296.0±37.0	272.0±24.0	293.0±26.0	304.0±29.0
Difference in body weight (g)*	135.0±21.0	157.0±36.0	142.0±24.0	145.0±24.0	120.0±11.0	137.0±21.0	144.0±18.0

*Difference between 1st day and 8 weeks

Table 2—Fatty acid composition of added oils in the experimental diets (mg/d)

Fatty acid content (mg)	Gr. 4 (Canola)	Gr. 5 (Sunflower)	Gr. 6 (Canola+Sunflower)	Gr. 7 (Coconut)
Total saturated	60	80	71	713
Total monosaturated	523	150	332	48
Total polysaturated	232	509	380	15
S:M:P ratio	1:8.7:3.8	1:1.9:6.36	1:4.67:5.35	47.5:3.8:1

The influence of different treatments in the groups 2 and 3 on the bone mechanical properties (Table 4) revealed that the energy to maximal load for tibia bone was slightly higher (P=0.09) in the group 2 compared to the control, and the stiffness of the lumbar bone plus the maximal load or breaking strength (F_{max}) of the femur and lumbar bone was non-significantly higher than the control group.

To study the effect of different oils on the plasma variables (Table 5), a significant difference in the plasma concentration of E2 was noted in the coconut oil group (Gr. 7) ($P\leq$ 0.05). A non significant higher levels of Vit D. in the canola oil (Gr. 4) (P=0.09) and the sunflower oil (Gr. 5) (P=0.08) compared to the control and the coconut oil group; and for the plasma testosterone in the Gr. 4 and Gr. 7 compared to the other groups; and for the plasma free testosterone concentrations compared to the other groups were observed.

In the bone mechanical parameters, significant differences were observed for femur stiffness in the group 7 compared to the control, in the groups 5 and 6 (mix of sunflower oil and canola oil) compared to the group 7; and in the breaking strength (F_{max}) in the group 5 compared to the group 7, and in the group 7 compared to the control, indicating higher strength (Table 6).

The breaking strength (F_{max}) of the lumbar vertebral bones were significantly higher in the groups 4-6 compared to group 7 and in the group 7 versus to the control; and for the energy to maximal load in the group 4 compared to the group 7 ($P\leq$ 0.05).

Also, the stiffness of the femur bones in the group 4 in comparison with the controls (P=0.068); the stiffness of the tibia in the groups 6 (P=0.076) and 7 (P=0.067); and the energy to maximal load in the lumbar bones in the group 5 in comparison to the group 7 (P=0.075) tended to increase.

Discussion

Besides physical activity, other risk factors for bone diseases are well-established. Several variables such as healthy diet, physical activity or single

Table 4—Measures of extrinsic biomechanical properties of femur and tibia in 3-point bending and lumbar vertebra in compression test

[Values are mean±SD from 8 observations in each group]

		Gr.1 (control)	Gr. 2	Gr. 3
Stiffness (N/mm)	Femur	76.2±21.2	90.3±21.6	87.5±9.2
	Tibia	26.9±9.3	27.4±7.6	30.6±8.4
	Lumbar	333.4±98.0	305.6±111.3	345.5±91.5
Maximal load [F max] (N)	Femur	75.0±13.4	83.7±13.7	78.4±6.7
	Tibia	43.4±11.4	49.5±10.1	46.2±7.0
	Lumbar	256.4±39.4	283.9±47.8	257.4±32.4
Energy to maximum load (N mm)	Femur	47.3±12.4	55.9±8.5	49.9±8.5
	Tibia	37.7±16.5	48.5±11.5	43.6±5.6
	Lumbar	197.1±64.4	214.0±128.1	204.2±57.9
Deformation to maximal load (mm)	Femur	1.90±0.47	2.13±0.30	1.85±0.28
	Tibia	2.86±0.53	2.91±0.37	3.08±0.44
	Lumbar	1.83±0.27	1.83±0.63	1.83±0.31

Table 5—Effects of exercise training and dietary supplementation on plasma parameters in the groups (Control vs. the test oil groups)

[Values are mean±SD from 8 observations in each group]

	Control (Gr. 1)	Co (Gr. 4)	Af (Gr. 5)	Ac (Gr. 6)	N (Gr. 7)
Alkaline phosphatase (U/L)	215.0±19.5	231.7±34.8	232.0±34.6	236.6±29.5	243.4±42.4
Vitamin D (nmol/L)	91.2±18.9	108.2±15.3*	109.0±21.6	106.7±19.8	96.9±21.9
Calcitonin (pg/ml)	80.3±11.5	77.6±9.7	79.8v9.0	74.4±13.1	76.3±12.3
Parathormone (pg/ml)	36.0±8.9	37.7±5.4	34.3±5.8	30.9±15.0	34.7±8.3
Testosterone (ng/ml)	1.84±0.40	2.4±0.58	1.85±0.65	1.75±0.51	2.14±0.74*
Free testosterone (pg/ml)	0.44±0.19	0.42±0.16	0.39±0.16	0.43±0.24	0.51±0.26*
Estradiol (pg/ml)	8.35±1.32	8.32±1.47	8.7±0.94	8.82±0.99	10.01±0.87[a]

[a]: statistically significant; $P\leq$0.05

* Vitamin D shows 16% increase, Testosterone 16% increase, Free testosterone 14% increase, compared to the control.

Co= same as Gr.3 + canola oil; Af= same as Gr.3 + sunflower oil; Ac= same as Gr.3 + mix of sunflower oil and canola oil; N= same as Gr.3 + coconut oil

Table 6—Measures of extrinsic biomechanical properties of bones

[Values are mean±SD from 8 observations in each group]

		Control (Gr. 1)	Co (Gr.4)	Af (Gr.5)	Ac (Gr. 6)	N (Gr. 7)
Stiffness	Femur	76.2±21.2[a]	89.0±14.5	81.2±6.9[a]	79.8±14.3[a]	97.4±6.4
(N/mm)	Tibia	26.9±9.3	30.9±17.2	28.5±6.4	34.26±8.3	34.5±8.5
	Lumbar	333.4±98.0	311.3±74.7	423.5±157.6	319.3±84.8	397.0±233.4
Maximal load	Femur	75.0±13.4[a]	78.4±11.2	73.7±6.7	81.5±8.3	85.7±7.5
[F max] (N)	Tibia	43.4±11.4	46.9±8.4	43.7±6.3	45.2±7.4	48.7±5.9
	Lumbar	256.4±39.4[a]	265.9±30.2[a]	263.5±29.8[a]	268.8±42.3*	306.1±49.9
Energy to	Femur	47.3±12.4	46.6±9.1	48.7±6.7	55.3±8.3	52.7±10.0
maximum load	Tibia	37.7±16.5	44.4±9.2	41.3±10.1	38.2±7.5	42.6±9.3
(N mm)	Lumbar	197.1±64.4	166.4±51.0[a]	170.5±34.9	179.8±72.3	227.7±78.4
Deformation to	Femur	1.90±0.47	2.02±0.20	2.12±0.32	2.14±0.28	1.93±0.51
maximal load	Tibia	2.86±0.53	2.680.37	2.720.26	2.430.17	2.520.34
(mm)	Lumbar	1.83±0.27	1.74±0.41	1.74±0.40	1.71±0.29	1.97±0.51

[a]: statistically significant with Gr. 7; $P \leq 0.05$
$P<0.06$, compared to control

nutrients such as calcium, individually have been shown to reduce bone risks significantly. But, in the present study, a simultaneous combination effect of the known factors consisting of different fatty acids, Vitamin D, calcium and boron plus physical activity on bone strength was aimed to be determined and consequently was accepted by the scientific and ethics committee to be performed.

Recently, whole body vibration has been proposed as a potential alternatives, or adjuvant, to exercise[35]. Both acute and chronic alterations in peripheral vasculature occur with WBV and other reports indicate that hormonal fluctuations resulting from WBV vary considerably. A comprehensive review on the potential effects of WBV on several physiological systems is presented by Prisby *et al*[3]. It is reported that 24 weeks of WBV slightly increased lean mass in previously untrained females[9] and after 12 weeks reduced body fat accumulation and serum leptin levels with no alteration in food consumption[35]. Similar findings from human studies confirm the effectiveness of WBV in improving health status such as, improving pain and fatigue in women with fibromyalgia[36], reducing the risk of bone fracture more than walking[37], increase in the serum levels of testosterone and growth hormone[38-40], and representing no stressful stimulus for the neuroendocrine and neuromuscular systems[40,41]. Further, it would be well-advised to determine the influence of WBV on bone metabolism, mechanical properties and strength. In the present study the rats were healthy and treated well with the vibration with no signs of distress. The WBV group (Gr. 2) weighed approximately 14% more than control group after 8 weeks (weight gain: 135.0 vs 157.0 g), while both groups consumed similar amount of food (17.1 vs 17.8 g/d). Although, the mechanism responsible for the increased body weight was not studied, it is assumed that the observed body weight gain may be the result of higher muscle mass and bone mass and/or the result of higher blood flow leading to the alteration on the peripheral vascular and tissue perfusion. Maddalozzo *et al*.[35] reported that mature female vibrated rats weighed 10% less, with less body fat and serum leptin concentrations. Interestingly, in the sunflower oil group (Gr. 5) lower body weight was recorded despite 5% sunflower oil added in the diet. Roelants *et al*.[7] reported a significant increase in fat-free mass and strength with whole-body vibration.

This effect of sunflower oil seems to be mediated by a reduction of appetite or an increase in the feeling of satiety or partially by hormonal variations. Additionally, rats in this group consumed 12% less food. Silva *et al*.[42] reported that body weight of partially hydrogenated vegetable oil and saturated oil consuming groups was significantly higher than unsaturated oil groups. A lower body weight observed in the group 3 (SPM) compared to that in the group 2 (SP) could be attributed to the supplementation of Vitamin D, calcium and boron which requires further clarification. It is reported that dietary calcium plays an important role in body weight regulation[43-45].

The mean of testosterone and free testosterone levels was 15% higher in the group 7 which is consistent with the result of Hurtado de Catalfo *et al*[46]. They reported that coconut oil diet produced a high testicular level of antioxidants, testosterone, 3 beta- or 17 beta-hydroxysteroid dehydrogenase enzymes[46]. The activity of these two key enzymes involved in testosterone biosynthesis is enhanced by coconut oil[47].

A significant higher concentration of estradiol was found in the group 7 in comparison with the control and the groups 3, 4 and 6; the increase in the group 2 compared to the control and group 4 seems to be mediated by the vibration, alone. In general, estradiol concentration showed an increased level in most oil test groups and in particular after coconut consumption. Additionally, estradiol production is reported to be influenced by boron supplementation[21,48,49].

The 15% higher level of Vitamin D found in all treatment groups, except group 7 seems to be the result of Vitamin D and boron intake. The reported Vitamin D production by boron[19] is consistent with the finding of the present study. Vitamin D is best known for its effects on calcium regulation, but lately its beneficial effects on vascular health, blood pressure[50], association of the low level with higher risk of myocardial infarction[51] and manifestation as bone diseases have been recognized[52].

No major differences were observed in the plasma concentrations of alkaline phosphatase, parathormone and calcitonin. Measures of bone mechanical properties were exhibited better status in the groups 2 and 3 (Table 4). In both groups, the modulus of elasticity or stiffness at yield point of the femur bones and the lumbar vertebral samples tended to increase. The findings are consistent with the results of Yang *et al.*[53], reporting that the mechanical properties of the femur were influenced by WBV in the control femurs as well as in the unloaded bones, and improved bone strength in the proximal femur of the ovariectomized rats[54]. Moreover, Low-magnitude high-frequency vibration in rats accelerated fracture healing by enhancing bone remodeling and had a great potential in improving fracture outcome clinically[55]; and as an anabolic stimulus accelerated tendon healing[56].

Boron has been shown in numerous studies to alter bone in different animal species. In pigs, supplementation level of 5 mg/kg diet increased bone bending moment in males, but not in females[57]. In male rats, boron supplementation did not change tibia or femur resistance to bending, but dietary levels of 200 mg/kg diet did increase vertebral resistance to crush force[58]. In addition, supplementation of 15 mg of boron/kg diet increased ultimate shear stress force of the fibula[59] and the basal diet supplemented with 5 mg/kg diet increased the measures of intrinsic and extrinsic strength of the femur[60].

The mean maximal load or the total applied force at the yield point of the tibiae, femora, and lumbar vertebras in the boron treatment group was reported to be greater, indicating higher mechanical property or strength of the bones with boron supplementation[23].

Boron supplementation markedly reduced the urinary excretion of calcium and magnesium and elevated the serum concentrations of 17 beta-estradiol and testosterone, suggesting a role in prevention of calcium loss and bone demineralization in postmenopausal[48].

Consumption of boron, calcium and Vitamin D along with WBV demonstrated higher femur and lumbar vertebral bone strength. These findings require firm confirmation in studies with longer times and different doses of these nutrients. Applications of other elements, in addition to histology and histomorphometry studies are recommended, as well.

Moreover, despite the high content of saturated fat in coconut oil, some health benefits were identified on bone parameters and hormones which make it as a better choice than other saturated fat if consumed in moderation. However, it seems that more research is needed in this issue and its relationship to bone health status. Health diets and more physical activity have been proven to reduce bone risks and disorders significantly. In the current study, a simultaneous combination effect of the known factors consisting of different fatty acids, Vitamin D, Calcium and boron plus physical activity has been determined and overall appeared to have beneficial effects on bon health.

There is no conflict of interest.

References

1 Günther KP, Hofbauer L, Defer A, Dreinhöfer K, Duda G, Goldhahn J, Jakob F, Kurth A, Linde I, Raschke MJ, Rueger JM, Steinbronn R, Stroszczynski C, Unger L & Zwipp H, Current application of osteoporosis guidelines--expert recommendations of the German Society of Orthopaedic Surgery and Traumatology, *Z Orthop Unfall*, 147 (2009) 542.

2 Cousins JM, Petit MA, Paudel ML, Taylor BC, Hughes JM, Cauley JA, Zmuda JM, Cawthon PM & Ensrud KE, Muscle power and physical activity are associated with bone strength in older men: The osteoporotic fractures in men study, *Bone,* 47 (2010) 205.

3 Prisby RD, Lafage-Proust MH, Malaval L, Belli A & Vico L, Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know, *Ageing Res Rev,* 7 (2008) 319.

4 Rubin CT, Capilla E, Luu YK, Busa B, Crawford H, Nolan DJ, Mittal V, Rosen CJ, Pessin JE & Judex S, Adipogenesis is inhibited by brief, daily exposure to high-frequency, extremely low-magnitude mechanical signals, *Proc Natl Acad Sci U S A,* 104 (2007) 17879.
5 Da Silva ME, Fernandez JM, Castillo E, Nuñez VM, Vaamonde DM, Poblador MS & Lancho JL, Influence of vibration training on energy expenditure in active men, *J Strength Cond Res,* 21 (2007) 470.
6 Garatachea N, Jiménez A, Bresciani G, Mariño NA, González-Gallego J & de Paz JA, The effects of movement velocity during squatting on energy expenditure and substrate utilization in whole-body vibration, *J Strength Cond Res,* 21 (2007) 594.
7 Roelants M, Delecluse C, Goris M & Verschueren S, Effects of 24 weeks of whole body vibration training on body composition and muscle strength in untrained females, *Int J Sports Med,* 25 (2004) 1.
8 Bogaerts AC, Delecluse C, Claessens AL, Troosters T, Boonen S & Verschueren SM, Effects of whole body vibration training on cardiorespiratory fitness and muscle strength in older individuals (a 1-year randomised controlled trial), *Age Ageing,* 38 (2009) 448.
9 Roelants M, Delecluse C & Verschueren SM, Whole-body-vibration training increases knee-extension strength and speed of movement in older women, *J Am Geriatr Soc,* 52 (2004) 901.
10 Zha DS, Zhu QA, Pei WW, Zheng JC, Wu SH, Xu ZX, Li T & Chen JT, Does whole-body vibration with alternative tilting increase bone mineral density and change bone metabolism in senior people? *Aging Clin Exp Res*, Feb 21 (2011) [Epub ahead of print, DOI: 10.3275/7517].
11 Liu PY, Brummel-Smith K & Ilich JZ, Aerobic exercise and whole-body vibration in offsetting bone loss in older adults, *J Aging Res,* 2011 (2011) 379674.
12 Kasturi G & Adler RA, Mechanical means to improve bone strength: ultrasound and vibration, *Curr Rheumatol Rep,* 13 (2011) 251.
13 VON Stengel S, Kemmler W, Bebenek M, Engelke K & Kalender WA, Effects of whole-body vibration training on different devices on bone mineral density. *Med Sci Sports Exerc,* 43 (2011) 1071.
14 Verschueren SM, Bogaerts A, Delecluse C, Claessens AL, Haentjens P, Vanderschueren D & Boonen S, The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: a 6-month randomized, controlled trial, *J Bone Miner Res,* 26 (2011) 42.
15 Nielsen FH, The emergence of boron as nutritionally important throughout the life cycle, *Nutrition,* 16 (2000) 512.
16 Armstrong TA & Spears JW, Effect of boron supplementation of pig diets on the production of tumor necrosis factor-alpha and interferon-gamma, *J Anim Sci,* 81 (2003) 2552.
17 Mahabir S, Spitz MR, Barrera SL, Dong YQ, Eastham C & Forman MR, Dietary boron and hormone replacement therapy as risk factors for lung cancer in women, *Am J Epidemiol,* 167 (2008) 1070.
18 Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan A & Eryavuz A, The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats, *J Trace Elem Med Biol,* 24 (2010) 161.
19 Samman S, Naghii MR, Lyons Wall PM& Verus AP, The nutritional and metabolic effects of boron in humans and animals, *Biol Trace Elem Res,* 66 (1998) 227.
20 Sheng MH-C, Taper LJ, Veit H, Qian H, Ritchey SJ & Lau K-HW, Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats, *Biol Trace Elem Res,* 82 (2001) 109.
21 Naghii MR, Mofid M, Asgari AR, Hedayati M & Daneshpour MS, Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines, *J Trace Elem Med Biol,* 25 (2011) 54.
22 Nielsen FH & Stoecker BJ, Boron and fish oil have different beneficial effects on strength and trabecular microarchitecture of bone, *J Trace Elem Med Biol,* 23 (2009) 203.
23 Naghii MR, Torkaman G & Mofid M, Effects of boron and calcium supplementation on mechanical properties of bone in rats, *Biofactors,* 28 (2006) 195.
24 Gorustovich AA, Steimetz T, Nielsen FH & Guglielmotti MB, Histomorphometric study of alveolar bone healing in rats fed a boron-deficient diet, *Anat Rec*., 291 (2008) 441.
25 Gorustovich AA, Steimetz T, Nielsen FH & Guglielmotti MB, A histomorphometric study of alveolar bone modelling and remodelling in mice fed a boron-deficient diet, *Arch Oral Biol* 53 (2008) 677.
26 Hakki SS, Bozkurt BS & Hakki EE, Boron regulates mineralized tissue-associated proteins in osteoblasts (MC3T3-E1), *J Trace Elem Med Biol,* 24 (2010) 243.
27 Sai AJ, Walters RW, Fang X & Gallagher JC, Relationship between vitamin D, parathyroid hormone, and bone health, *J Clin Endocrin Metab,* 96 (2011) E436-46.
28 Anderson PH, Atkins GJ, Turner AG, Kogawa M, Findlay DM & Morris HA, Vitamin D metabolism within bone cells: Effects on bone structure and strength, *Mol Cell Endocrinol,* 347 (2011) 42.
29 Watkins BA, Li Y, Allen KGD, Hoffmann WE & Seifert MF, Dietary Ratio of (n-6)/(n-3) polyunsaturated fatty acids alters the fatty acid composition of bone compartments and biomarkers of bone formation in rats, *J Nutr,* 130 (2000) 2274.
30 Watkins BA, Li Y & Seifert MF, Dietary ratio of n-6/n-3 PUFAs and docosahexaenoic acid: actions on bone mineral and serum biomarkers in ovariectomized rats, *J Nutr Biochem*, 17 (2006) 282.
31 Lau BY, Fajardo VA, McMeekin L, Sacco SM, Ward WE, Roy BD, Peters SJ & Leblanc PJ, Influence of high-fat diet from differential dietary sources on bone mineral density, bone strength, and bone fatty acid composition in rats, *Appl Physiol Nutr Metab,* 35 (2010) 598.
32 Van der Hee RM, Miret S, Slettenaar M, Duchateau GS, Rietveld AG, Wilkinson JE, Quail PJ, Berry MJ, Dainty JR, Teucher B & Fairweather-Tait SJ, Calcium absorption from fortified ice cream formulations compared with calcium absorption from milk, *J Am Diet Assoc,* 109 (2009) 830.
33 Lewiecki EM, Bilezikian JP, Khosla S, Marcus R, McClung MR, Miller PD, Watts NB & Maricic M, Osteoporosis update from the 2010 santa fe bone symposium, *J Clin Densitom,* 14 (2011) 1.

34 Crenshaw TD, Peo ER, Lewis Jr. AJ, Moser BD & Olsen D, Influence of age, sex and calcium and phosphorus levels on the mechanical properties of various bones in swine, *J Anim Sci*, 52 (1981) 1319.

35 Maddalozzo GF, Iwaniec UT, Turner RT, Rosen CJ & Widrick JJ, Whole-body vibration slows the acquisition of fat in mature female rats, *Int J Obesity,* 32 (2008) 1348.

36 Alentorn-Geli E, Padilla J, Moras G, Lázaro Haro C & Fernández-Solà J, Six weeks of whole-body vibration exercise improves pain and fatigue in women with fibromyalgia, *J Altern Complem Med,* 14 (2008) 975.

37 Gusi N, Raimundo A & Leal A, Low-frequency vibratory exercise reduces the risk of bone fracture more than walking: a randomized controlled trial, *BMC Musculoskelet Disord,* 7 (2006) 92.

38 Bosco C, Iacovelli M, Tsarpela O, Cardinale M, Bonifazi M, Tihanyi J, Viru M, De Lorenzo A & Viru A, Hormonal responses to whole-body vibration in men, *Eur J Appl Physiol,* 81 (2000) 449.

39 Cardinale M & Pope MH, The effects of whole body vibration on humans: dangerous or advantageous? *Acta physiol Hung,* 90 (2003) 195.

40 Kvorning T, Bagger M, Caserotti P & Madsen K, Effects of vibration and resistance training on neuromuscular and hormonal measures, *Eur J Appl Physiol,* 96 (2006) 615.

41 Erskine J, Smillie I, Leiper J, Ball D & Cardinale M, Neuromuscular and hormonal responses to a single session of whole body vibration exercise in healthy young men, *Clin Physiol Funct Iimaging,* 27 (2007) 242.

42 Silva AP, Guimaraes DE, Mizurini DM, Maia IC, Ortiz-Costa S, Sardinha FL & et al., Dietary fatty acids early in life affect lipid metabolism and adiposity in young rats, *Lipids,* 41 (2006) 535.

43 Davies KM, Heaney RP, Recker RR, Lappe JM, Barger-Lux MJ, Rafferty K & et al., Calcium intake and body weight, *J Clin Endocrin Metab,* 85 (2000) 4635.

44 Pereira MA, Jacobs DR, Jr., Van HL, Slattery ML, Kartashov AI & Ludwig DS, Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study, *JAMA,* 287 (2002) 2081.

45 Zemel MB, Shi H, Greer B, Dirienzo D & Zemel PC, Regulation of adiposity by dietary calcium, *The FASEB J,* 14 (2000) 1132.

46 Hurtado de Catalfo GE, de Alaniz MJ & Marra CA, Dietary lipids modify redox homeostasis and steroidogenic status in rat testis, *Nutrition,* 24 (2008) 717.

47 Hurtado de Catalfo GE, de Alaniz MJ & Marra CA, Influence of commercial dietary oils on lipid composition and testosterone production in interstitial cells isolated from rat testis, *Lipids,* 44 (2009) 345.

48 Nielsen FH, Hunt CD, Mullen LM & Hunt JR, Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women, *The FASEB J,* 1 (1987) 394.

49 Naghii MR & Samman S, The effect of boron supplementation on its urinary excretion and selected cardiovascular risk factors in healthy male subjects, *Biol Trace Elem Res,* 56 (1997) 273.

50 Giovannucci E, Liu Y, Hollis BW & Rimm EB, 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study, *Arch Intern Med,* 168 (2008) 1174.

51 Lee JH, Gadi R, Spertus JA, Tang F & O'Keefe JH, Prevalence of vitamin D deficiency in patients with acute myocardial infarction, *Am J Cardiol,* 107 (2011) 1636.

52 Thacher TD & Clarke BL, Vitamin D insufficiency, *Mayo Clin proc* 86 (2011) 50.

53 Yang P, Jia B, Ding C, Wang Z, Qian A & Shang P, Whole-body vibration effects on bone before and after hind-limb unloading in rats, *Aviat Space Envir Med,* 80 (2009) 88.

54 Tezval M, Biblis M, Sehmisch S, Schmelz U, Kolios L, Rack T, Stuermer KM & Stuermer EK, Improvement of femoral bone quality after low-magnitude, high-frequency mechanical stimulation in the ovariectomized rat as an osteopenia model, *Calcif Tissue Int,* 88 (2011) 33.

55 Chow DH, Leung KS, Qin L, Leung AH & Cheung WH, Low-magnitude high-frequency vibration (LMHFV) enhances bone remodeling in osteoporotic rat femoral fracture healing, *J Orthop Res,* 29 (2011) 746.

56 Sandhu E, Miles JD, Dahners LE, Keller BV & Weinhold PS, Whole body vibration increases area and stiffness of the flexor carpi ulnaris tendon in the rat, *J Biomech,* 44 (2011) 1189.

57 Armstrong T A, Spears J W, Crenshaw T D & Nielsen F H, Boron supplementation of a semi purified diet for weaning pigs improves feed efficiency and bone strength characteristics and alters plasma lipid metabolites, *J Nutr*, 130 (2000) 2575.

58 Chapin R E, Ku WW, Kenney M A, McCoy H, Gladen B, Wine R N, Wilson R & Elwell M R, The effects of dietary boron on bone strength in rats, *Fundam Appl Toxicol,* 35 (1997) 205.

59 Armstrong T A & Spears J W, Effect of dietary boron on growth performance, calcium and phosphorus metabolism, and bone mechanical properties in growing barrows, *J Anim Sci,* 79 (1997) 3120.

60 Armstrong T A, Flowers W L, Spears J W & Nielsen F H, Long-term effects of boron supplementation on reproductive characteristics and bone mechanical properties in gilts, *J Anim Sci,* 80 (2002) 154.