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Vitamin D is associated with metabotropic but not neurotrophic effects of exercise in ovariectomized rats

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Abstract

Purpose: Here, we studied the beneficial effects of aerobic exercise on metabolic syndrome components, cognitive performance, brain derived neurotrophic factor (BDNF) and irisin in ovariectomized rats with different serum vitamin D (Vit D) status.

Methods: Eighty female wistar rats were divided into 2 groups of sham operated (sham, n = 8), and ovariectomized (OVX, n = 72). Then OVX were divided into 9 groups of receiving combination of exercise protocol with low dose of Vit D (OVX + EXE + LD), high dose of Vit D (OVX + EXE + HD), Vit D deficiency (OVX + EXE - D), and (OVX + EXE + Veh). Also non exercised groups of OVX receiving high dose of Vit D (OVX + HD), low dose of Vit D (OVX + LD), Vit D deficiency (OVX - D), and Veh (OVX + Veh) were included. After 2 months of related interventions, spatial memory was assessed using Morris water maze (MWM), and then metabolic syndrome components were measured.

Results: High dose of Vit D supplementation showed significant reduction in weight ($p = 0.001$), lipid profiles ($p = 0.001$), visceral fat ($p = 0.001$) and waist circumference ($p = 0.001$) regardless of exercising or not, with no change in cognitive function. Serum BDNF level was significantly higher in Vit D deficient group ($p = 0.001$), and was decreased in the OVX + HD. In contrary, irisin did not show any significant relationship with serum concentration of Vit D, while it was significantly elevated in the exercised groups compared with non-exercised counterparts.

Conclusion: Vit D insufficiency deteriorates metabolic syndrome components, and elevates serum BDNF as a compensatory metabotropic factor, and further supplementation significantly attenuates these components parallel with reduction in BDNF. In addition, aerobic exercise successfully induces various metabolic benefits, provided optimum serum level of Vit D.

Keywords: Metabolic syndrome, Memory, Vitamin D, BDNF, Irisin, Aerobic exercise

Background

Metabolic syndrome (MetS) is a clinical challenge worldwide due to obesity, and sedentary life habits. Based on the previous studies, individuals with three criteria (central obesity, high triglycerides, low high-density lipoprotein cholesterol, hypertension, and hyperglycemia) are diagnosed with MetS [1–3]. During menopausal period, estrogen withdrawal predisposes women to cardiovascular [4] and cognitive dysfunctions [5].

On the other hand, chronic low serum Vit D concentration in the elderly women is an important public health concern due to its high prevalence of 50–80% [6].

It has been known that insufficient serum Vit D₃ alters metabolite function [7], causes insulin resistance [8, 9], and develops MetS components [3, 10]. Also Vit D insufficiency may leads to develop dementias, including Alzheimer's disease [11] and cognitive impairment [12]. Considering the fact that Vit D receptors (VDR) are located in the human cortex and hippocampus, which are key areas for cognition [13], Vit D is assumed to be important in learning and memory.

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One of the non-pharmacological tools to prevent both metabolic syndrome and cognitive deficit is aerobic exercise [14]. Some of the beneficial effects of aerobic exercise take place via elevation in serum BDNF [15, 16] and irisin [17]. It is postulated that exercised skeletal muscles secrete a myokine, named irisin, which passes blood brain barrier and secretes BDNF [14, 18, 19]. BDNF not only boosts learning and memory [14, 20, 21], as a neurotrophic factor [22, 23], but also it recently stands as a metabotropic factor too [24, 25]. It has not been understood why some of the obese individuals do not show any significant metabolic [26, 27] or cognitive [28] improvements after physical activities. Therefore, the present study was designed to clarify whether or not Vit D insufficiency might be the reason to prevent exercise beneficial effects? Thus, the objective of this study was co-treatment of Vit D supplementation with aerobic exercise on cognitive performance, metabolic syndrome components, serum BDNF and irisin level. To approach these objectives we used ovariectomized rats as a model of menopause and metabolic syndrome [29, 30].

Methods

Animals

Female rats (3 months age and 180–200 g weight) were housed four per cage and all rats (except Vit D deficient group) fed standard-pellet rat chow (Table 1) and tap water ad libitum. Room temperature was maintained at 22 ± 2 °C with a 12/12 h light/dark cycle (light on 7:00A.M.). All experiments were performed in accordance with National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) modified by ethical committee of Guilan University of medical sciences, Rasht, Iran, IR.GUMS.REC.1394.54.

Ovariectomy surgery

Eighty female wistar rats were divided into 2 groups of sham operated (sham, $n = 8$), and ovariectomized (OVX, $n = 72$). Then OVX were divided into 9 groups of receiving combination of exercise protocol with low dose of Vit D (OVX + EXE + LD), high dose of Vit D (OVX + EXE + HD), Vit D deficiency (OVX + EXE - D), and Veh (OVX + EXE + Veh). Also OVX receiving high dose of Vit D (OVX + HD), low dose of Vit D (OVX + LD), Vit D deficiency (OVX - D), and Veh (OVX + Veh) were included. Rats

were ovariectomized and sham-operated under general anesthesia with an intraperitoneal injection of 75 mg/kg ketamine (50 mg/ml, TRITTAU, Germany) and 5 mg/kg of xylazine (20 mg/ml, SciENcelab, Hoston) and ovaries were removed by one midline incision on the abdomen, then animals left for recovery. Three weeks after the surgery, animals displayed weight gain, dyslipidemia, high abdominal obesity and visceral fat.

Vit D supplementation

Based on the ingredients of animal's food given by manufacture and subtracting consumed food from the total given per cage (Table 1), each animal of the LD group received 100 IU/kg/week Vit D (from food source, Tables 1, 2). HD group received 10,000 IU/kg/week Vit D [1000 IU/kg/week from food source and 9000 (10,000–1000) IU from injection]. Animals received Vit D₃ (drug factory of Abureyhan Birooni, Tehran, Iran) injection (s.c) once a week, and control group received sesame oil as vehicle of Vit D. To balance the diet on isocaloric regimen, Vit D deficient and low Vit D groups received wheat-based food based on nutrition software prepared in our lab [31]. The ingredients of this Vit D free diet is displayed in Table 2.

Aerobic exercise training

Aerobic training included running on a rodent treadmill (Daneshsalar Iranian Co, Tehran, Iran) with an incremental pattern (from 12 min/day at 12 m/min, 0% slope, up to 40 min/day at 25 m/min, 10% slope for the last 8 weeks) (Table 3) [32]. The regular endurance exercise used in this study was equivalent to 70–85% VO_{2max} [33].

Behavioral test

Protocol used for Morris water maze (MWM) included 5 days, each day consisted of 1 block of 4 trials, and each trial 60 s with an interval of 20 min. MWM consisted of a large circular pool with 148 cm diameter, divided to four directions (north east, north west, south east, south west), containing water at around 22 °C. A hidden platform with 10 cm diameter was kept 1.5 cm below the water surface in the target quadrant. A video camera was placed above the center of the pool to capture images of the swimming animal, and this connected to a video recorder [34, 35] and "Ethovision 7 Noldus" software. Times spent in the target quadrant and reach to the hidden platform as well as swimming speed were considered.

Table 1 Ingredients of Rodents standard diet per 100 g

Protein (g)	Fat (g)	Fiber (g)	Ashes (g)	Calcium (g)	Phosphorus (g)	Salt (g)	Moisture (g)	Energy (kcal)	Vitamin D (IU)
22.5–23.5	3.5	4–5	10	0.95–1	0.65–0.7	0.5–0.55	10	2850–2900	5000

Table 2 Ingredients of Vit D free diet per 100g

Energy (kcal)	Fat (g)	Saturated fat (g)	Sodium (mg)	Potassium (mg)	Carbohydrate (g)	Fiber (g)	Sugar (g)	Protein (g)	Vitamin A (IU)	Vitamin C (mg)	Calcium (mg)	Iron (mg)	Vitamin E (mg)
270	3.4	0.8	519	185	49.5	4.2	6.1	10.4	2	0.2	138	3.5	0.2
Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	B6 (mg)	Folate (µg)	Pantothenic acid (mg)	Magnesium (mg)	Zinc (mg)	Selenium (µg)	Phosphorus (mg)	Copper (mg)	Manganese (mg)	Water (g)	Vitamin D
0.5	0.3	5.9	0.1	85	0.8	45	1.2	28.8	153	0.2	1.2	34.5	0

Table 3 Treadmill exercise schedule during the 8 weeks

	Adaptation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Velocity (m/min)	10	12	15	15	20	20	23	25	25
Duration (min)	10	12	15	20	20	30	38	40	40
Treadmill slope (degree)	0	0	0	5	5	7	7	10	10

Morphometric and chemical assays

At the end of the behavioral test, animals were weighed and their waist circumference was measured on the largest zone of the rat abdomen using a centimeter [36].

Then animals were sacrificed by chloroform and blood was collected from the inferior vena cava by a syringe into the EDTA containing tubes. Finally, bloods were centrifuged for 15 min at 3000 rpm, and stored at -80°C for further analysis.

Visceral fat were completely removed from mesenteric, urogenital and retroperitoneal and weighed immediately to avoid evaporation using a weighing-scale (Doulton). The mesenteric fat included the adipose tissue surrounding the gastrointestinal tract. The urogenital fat included the adipose tissue surrounding the kidneys, ureters and bladder as well as ovaries, oviducts and uterus. The retroperitoneal fat consisted of the distinct deposit behind each kidney along the lumbar muscles.

Serum Vit D and BDNF were assessed using the ELISA (Immuno Diagnostics System Ltd., Boldon, UK) and (Bosterbio, Picokine, Canada) respectively. Irisin was assessed using ELISA (Zellbio Kit, GmbH, Germany) and lipids profile were measured using enzymatic analysis kits (Asan Pharmaceuticals, Hwasung, Korea).

Statistical analysis

Normality of variables was evaluated by Shapiro–Wilk test, then repeated measure and ANOVA with post hoc Tukey test were used for acquisition and memory retrieval respectively. Kruscalk–Wallis and Bonferroni post hoc were used to compare between groups differences of nonparametric variables (BDNF, BMI, Ca, Visceral fat, Waist circumference). HDL, nonHDL, TC, TG, Weight, Irisin and VitD were analyzed using ANOVA followed by post hoc Tukey test. p value < 0.05 were considered statistically significant, and results are expressed as the mean \pm SEM.

Results

Three weeks after ovariectomy, animals showed an increase in body weight BMI ($p = 0.001$), visceral fat ($p = 0.018$) and waist circumference ($p = 0.001$) compared with SHAM group (Table 4). There was a statistically significant between groups difference in Vit D [$F(9,70) = 772.15$, $p = 0.001$]. The highest level of Vit D was detected in OVX + EXE + HD (117.04 ± 1.43 nmol/l, $p = 0.001$) and OVX + HD (107.06 ± 0.45 nmol/l, $p = 0.001$), but the lowest one in OVX – D (65.36 ± 0.92 nmol/l, $p = 0.001$) and

Table 4 The morphometric and hormonal variables after 8-week experimental period

Variables Groups	Weight (g)	BMI (g/cm ²)	Visceral fat (g)	Waist circumference (cm)	Vitamin D (nmol/l)	Calcium (mg/dl)
OVX + EXE + HD	**233.45 \pm 0.61	**0.54 \pm 0.001	**8.29 \pm 0.050	**10.75 \pm 0.231	**117.04 \pm 1.43	8.9 \pm 1.32
OVX + EXE + LD	245.14 \pm 0.26	0.574 \pm 0.009	9.20 \pm 0.029	12.875 \pm 0.26	102.1 \pm 1.12	***7.96 \pm 1.04
OVX + EXE – D	**259.25 \pm 2.22	**0.57 \pm 0.011	**15.43 \pm 1.29	**19 \pm 0	**67.13 \pm 1.07	8.84 \pm 0.92
OVX + EXE + Veh	271.61 \pm 0.59	0.64 \pm 0.001	9.54 \pm 0.024	13.76 \pm 0.451	90.35 \pm 1.28	9.42 \pm 0.24
OVX + HD	**240.73 \pm .048	**0.56 \pm 0.001	**8.76 \pm 0.009	**11.89 \pm 0.27	**107.09 \pm 1.27	10.11 \pm 0.75
OVX + LD	267.69 \pm 040	0.63 \pm 0.001	9.46 \pm 0.008	13.59 \pm 0.46	93.66 \pm 0.93	*9.06 \pm 0.47
OVX – D	**273.5 \pm 4.08	**0.64 \pm 0.03	**19.25 \pm 2.72	**20.38 \pm 0.71	**65.36 \pm 0.92	8.76 \pm 1.61
OVX + Veh	275.44 \pm 1.22	0.65 \pm 0.003	9.94 \pm 0.015	14.34 \pm 0.20	83.43 \pm 1.57	9.23 \pm 0.62
OVX	**247.86 \pm 0.31	**0.61 \pm 0.001	**10.5 \pm 0.42	#12.66 \pm 0.48	**82.31 \pm 0.37	**8.5 \pm 0.8
Sham	214.7 \pm 0.65	0.53 \pm 0.002	9.2 \pm 0.13	9.13 \pm 0.2	83.63 \pm 0.45	9.19 \pm 0.39

OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE – D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of VitD, OVX + LD, OVX – D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. OVX and SHAM group values are about 3 weeks after ovariectomy surgery. ** $p = 0.001$, compared with Veh group. # $p = 0.01$, compared with SHAM. ## $p = 0.001$, compared with SHAM. * $p = 0.046$, compared with Veh. *** $p = 0.003$, compared with Veh. Values are expressed as mean + SE, $n = 8$ rats per group

OVX + EXE - D (67.13 ± 1.07 nmol/l, $p = 0.001$) groups.

BMI showed statistically significant between groups difference as determined by ANOVA [$F(9,70) = 423.54$, $p = 0.001$]. The group of OVX + HD compared with OVX - D showed significant reduction in both weight ($- 3.16\%$ vs. 15.4%) and BMI ($- 9.8\%$ vs. 8.5%) ($p = 0.001$). In addition, OVX + EXE + HD showed significant reduction in weight ($- 5.9\%$ vs. 11.3%) and BMI ($- 13.11\%$ vs. $- 6.5\%$, $p = 0.003$) compared with OVX + EXE - D (Table 4).

There was a statistically significant between groups difference in waist circumference [$F(9,70) = 84.72$, $p = 0.001$] and visceral fat [$F(9,70) = 13.94$, $p = 0.001$].

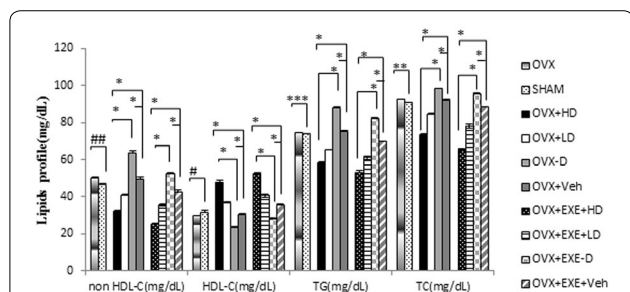


Fig. 1 Comparison of mean + Se lipids profile among different groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of Vit D, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. $n = 8$ rats per group. * $p = 0.001$, compared with Veh group, # $p = 0.001$, ** $p = 0.002$, *** $p = 0.01$, # $p = 0.03$, compared with sham group

Visceral fat in OVX + HD and OVX + EXE + HD weighed 8.75 g compared with 19.25 g in OVX - D and 15.43 g in OVX + EXE - D (Table 4).

Also statistically significant reduction was found in HDL [$F(9,70) = 208.84$, ($p = 0.001$)] LDL [$F(9,70) = 128.68$, ($p = 0.001$)], TG [$F(9,70) = 358.29$, ($p = 0.001$)] and TC [$F(9,70) = 394.02$, ($p = 0.001$)]. OVX + HD and OVX + EXE + HD showed elevation in serum HDL ($p = 0.01$), but reduction in non HDL and TG compared with OVX - D ($p = 0.001$, Fig. 1).

Behavioral results

Escape latency time measured in MWM in the 2nd, 3rd and 4th days of all groups showed trends of reduction (Fig. 2a, b) indicating that all animals learned the MWM task successfully. Since none of the groups differed in swimming speed [$F(10,70) = 1.31$, ($p = 0.24$)], the latency time to find the platform and total time spent in the target quadrant (TTS) were used here, as indicators of learning performance.

The OVX group displayed insignificant poor performance in acquisition ($p = 1.000$) and retrieval of memory ($p = 0.240$) compared with SHAM group. Vit D insufficiency had no significant effect neither on acquisition nor retrieval of memory compared with Veh group (Figs. 2a, 3 and 4). Also no significant change in TTS in the target quadrant in the OVX + EXE - D group was found compared with Veh group (14.95 ± 2.97 s vs. 16.7 ± 1.75 s, $p = 1.0$) (Fig. 3). As Figs. 2a, b, 3 and 4 show, Vit D supplementation for 8 weeks had no significant change on acquisition ($p = 0.644$, Fig. 2a) and retrieval of memory ($p = 0.90$, $p = 0.17$, Figs. 3, 4), compared with Veh group. Tukey's post hoc test showed that the co-treatment of aerobic training with high dose of Vit D insignificantly decreased escape latency time

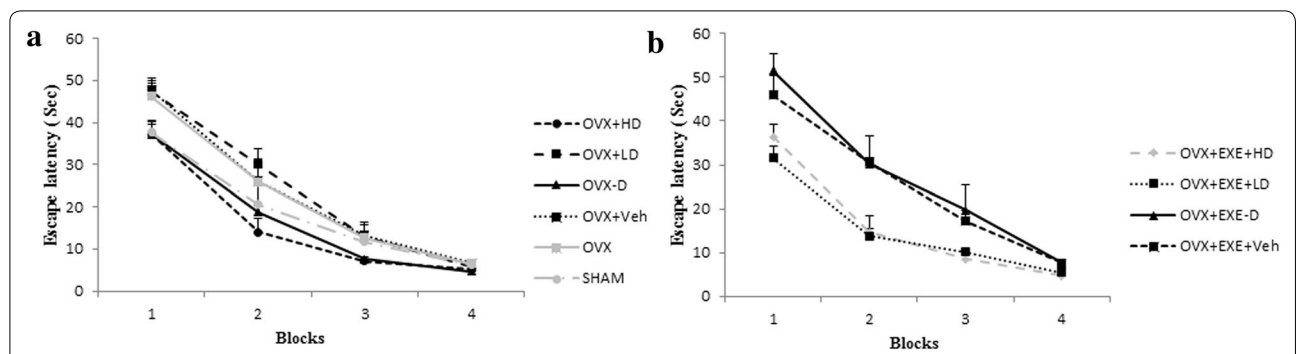


Fig. 2 a Comparison of escape latency in 4 blocks among OVX, sham, OVX + HD, OVX + LD, OVX - D and OVX + Veh groups. **b** Comparison of escape latency in acquisition phase of spatial memory in MWM among OVX + EXE + HD, OVX + EXE + LD, OVX + EXE - D and OVX + EXE + Veh groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of VitD, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. Values are expressed as mean \pm SEM, $n = 8$ rats per group

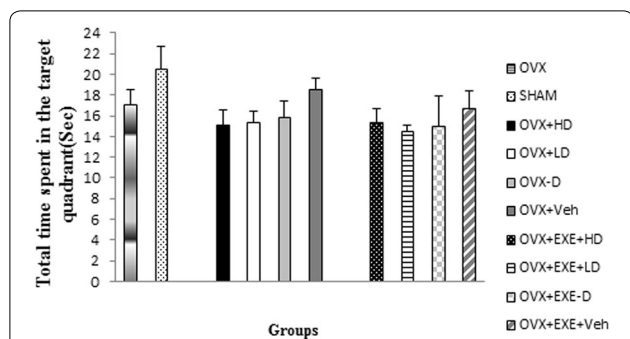


Fig. 3 Comparison of total time spent in the target quadrant in retrieval phase of spatial memory in MWM among different groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: Ovariectomy + low dose of VitD, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. Values are expressed as mean + Se, n = 8 rats per group

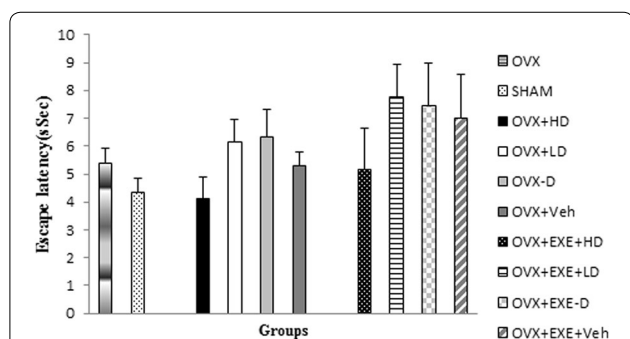


Fig. 4 Comparison of escape latency in retrieval phase of spatial memory in MWM among different groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of VitD, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. Values are expressed as mean + Se, n = 8 rats per group

compared with Veh group (5.2 ± 1.5 s vs. 7 ± 0.7 s, $p = 0.083$, Figs. 4, 5).

Statistically significant between groups difference was found in serum BDNF concentrations [$F(9,70) = 10.57$, ($p = 0.001$)]. Dunn-bonferroni post hoc test revealed the highest level of serum BDNF in the group with Vit D insufficiency ($p = 0.001$, Fig. 6). Co-treatment of aerobic training with high dose of Vit D, significantly decreased serum BDNF concentration compared with OVX + EXE + Veh ($p = 0.003$) and EXE - D ($p = 0.001$). Furthermore, significant negative correlation was found between Vit D and serum BDNF level (Fig. 6).

There was a statistically significant between groups difference in serum irisin [$F(9,70) = 5.62$, ($p = 0.001$)]. Irisin did not show any significant change in groups supplemented with different doses of Vit D (Fig. 7), while, combination of exercise with different doses of Vit D significantly increased irisin compared with non-exercised counterparts (OVX + EXE + HD vs. OVX + HD, $p = 0.001$, OVX + EXE + LD vs. OVX + LD $p = 0.046$, OVX + EXE - D vs. OVX - D, $p = 0.034$). No significant correlation was found between serum irisin and BDNF level (Fig. 8).

Discussion

Findings of the present study showed increase in visceral fat, BMI, body weight, waist circumference, TG and non-HDL cholesterol after ovariectomy surgery which was intensified by Vit D insufficiency. In line with these findings, two other reports [37, 38], showed that restoration with high dose of Vit D for 8 weeks significantly attenuates body weight, BMI, visceral fat, waist circumference, serum non-HDL cholesterol and TG.

It has previously reported that estrogen withdrawal leads to the progress of metabolic syndrome components [39–41]. Estrogen has been known to play an important role in the synthesis of particular enzymes such as lipoprotein lipase (LPL), hormone-sensitive lipase (HSL) and also apolipoproteins for HDL [39]. On the other hand, Vit D induces lipoprotein lipase activity [42] and decreases fatty acid absorption in the gut and increases

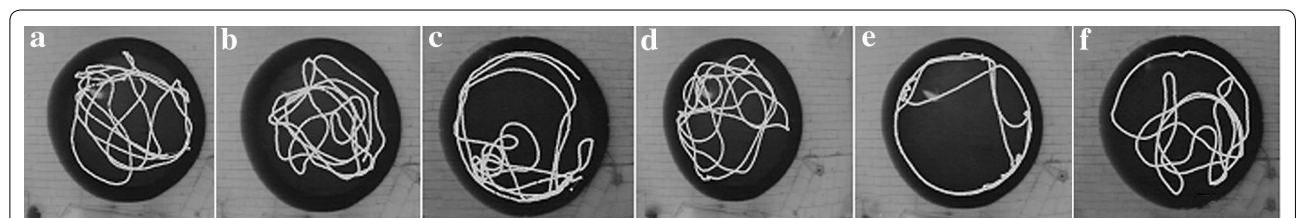


Fig. 5 Ethovision tracking from retrieval phase in MWM. **a** OVX + EXE + HD, **b** OVX + EXE + LD, **c** OVX + EXE - D, **d** OVX + HD, **e** OVX + LD, **f** OVX - D

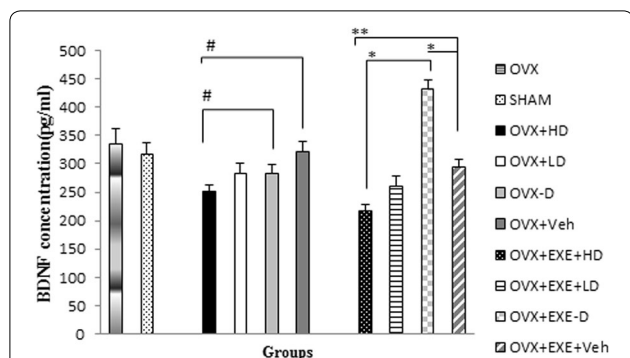


Fig. 6 Comparison of serum BDNF concentration among different groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of VitD, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. Values are expressed as mean + Se, n = 8 rats per group. *p = 0.001, compared with OVX + EXE - D and Veh. **p = 0.003, compared with Veh. #p = 0.046, compared with OVX-D and Veh

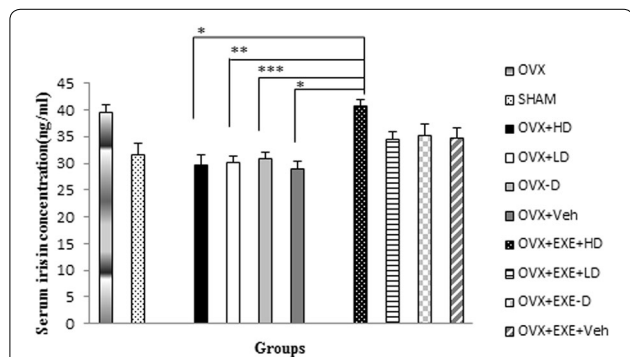


Fig. 7 Comparison of serum iris concentration among different groups. OVX + EXE + HD: ovariectomy + aerobic training + high dose of Vit D, OVX + EXE + LD: ovariectomy + aerobic training + low dose of Vit D, OVX + EXE - D: ovariectomy + aerobic training + Vit D deficiency, OVX + EXE + Veh: ovariectomy + aerobic training + sesame oil, OVX + HD: ovariectomy + low dose of VitD, OVX + LD, OVX - D: ovariectomy + VitD deficiency, OVX + Veh: ovariectomy + sesame oil, sham: sham-operated. OVX: ovariectomy. Values are expressed as mean + Se, n = 8 rats per group. *p = 0.001, **p = 0.002, ***p = 0.005 compared with OVX + EXE + HD

the conversion of cholesterol to bile acids and removes cholesterol from the circulation [43].

Also Vit D increases PPAR- α , PPAR- γ and CPT-1 (carnitine palmitoyl transferase I) expression and promotes β -oxidation and reduces TG levels [44, 45]. CPT-1 also helps FFA transport into mitochondrion for further oxidation and thus decreases lipid deposition [45].

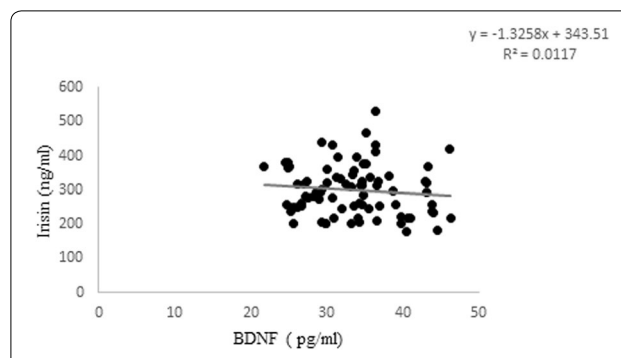


Fig. 8 Correlation between serum concentration of BDNF and Irisin. There was no significant correlation between BDNF and Irisin. $p = 0.34$, $R^2 = 0.0117$, $n = 8$

Therefore, these findings suggest that both estrogen and Vit D play important roles in lipid metabolism.

Unlike to the metabolic improvements, Vit D had no significant change in cognitive function. This finding is consistent with the previous studies in ovariectomized [46], aged [47] and Alzheimer model of rats [48] but in contradictory with some other studies on Alzheimer [49], obese [50] and ovariectomized rats [51]. It should be noticed that the dose and duration of Vit D supplementation in our study were sufficient, because serum concentration of Vit D was significantly correlated with supplementation. However, the weakness of this study was using Vit D free diet parallel with keeping animals away from UV light to induce Vit D deficient rats. As biochemical measurements showed, this method induced Vit D insufficiency rather than deficiency, possibly due to the fact that stored Vit D in other tissues such as adipose tissue and macrophages could release Vit D into the circulation [42, 46]. Taken into account that Vit D insufficiency in OVX animals did not cause significant memory impairment, thus further Vit D restoration, didn't show significant improvement compared to the baseline values. Although Adham et al. reported a better performance in MWM after Vit D supplementation in rats [49].

Furthermore, Vit D insufficient group displayed more BDNF concentration and Vit D supplementation reduced it to the basic level. The inverse significant correlation between Vit D and BDNF is consistent with Flavio et al. [52] study in which they reported reduction in plasma nerve growth factor (NGF) and BDNF following Vit D supplementation in healthy postmenopausal women [52]. Elevation in circulating level of BDNF in Vit D insufficiency status might reflect BDNF-mediated metabotropic compensatory effects rather than neurotrophic role in order to maintain cardio metabolic homeostasis [53, 54].

Based on our findings, although 8 weeks of aerobic exercise significantly improved dyslipidemia compared with non-exercised counterparts. Animals receiving co treatment of Vit D supplementations and exercise, showed much more improvement than their counterparts. Unlike BDNF insignificant response to exercise, irisin level was raised significantly. Irisin has been proposed to be secreted by myocytes and adipocytes during exercise [17, 55] and modulates energy expenditure [17] insulin resistance [17] and dyslipidemia [56, 57]. It appears from our data that elevation in irisin takes place in response to exercise after muscle contraction regardless of serum Vit D level.

In addition, the present study didn't show any significant change in working and reference memory assessed by MWM after aerobic exercise in OVX groups with different status of Vit D. However study carried out by Kaidah et al. [58] showed contradictory result [58]. Inconsistency might be related to difference in exercise protocols which consisted of five times per week for 12 weeks in ovariectomized rats in their work [58].

Finding, no correlation between irisin, BDNF, and cognitive performance challenges the previous theory indicating irisin mediated exercise-induced cognitive performance [19]. Therefore, irisin cannot be an essential pathway from exercise toward central nervous system to boost learning and memory, as Timmons et al. [59] stated before [59].

In conclusion, Vit D insufficiency deteriorates metabolic syndrome components, and further supplementation significantly attenuates them parallel with reduction in BDNF. In addition, aerobic exercise successfully induces various metabolic benefits, provided optimum serum level of Vit D.

Authors' contributions

PB designing the experiments and supervising and preparing manuscript. SGS and RH doing the experiments. BST helped doing experiments and preparing manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All data from experiments are available.

Ethics approval and consent to participate

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) modified by ethical committee of Guilan University of medical sciences were completely followed.

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References

- Haffner SM, Valdez RA, Hazuda HP, et al. Prospective analysis of the insulin-resistance syndrome (syndrome X). *Diabetes*. 1992;41:715–22.
- Meigs JB, Nathan DM, Wilson PW, et al. Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance. The Framingham offspring study. *Ann Intern Med*. 1998;128:524–33.
- Reilly MP, Rader DJ. The metabolic syndrome: more than the sum of its parts? *Circulation*. 2003;108:1546–51.
- Rosano GMC, Vitale C, Marazzi G, et al. Menopause and cardiovascular disease: the evidence. *Climacteric*. 2007;10:19–24.
- Henderson VW. Cognitive changes after menopause: influence of estrogen. *Clin Obstet Gynecol*. 2008;51:618–26.
- Kennel KA, Drake MT, Daniel L, Hurley vitamin D deficiency in adults: when to test and how to treat. *Mayo Clin Proc*. 2010;85:752–8.
- Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007;357:266–81.
- Pittas AG, Lau J, Hu FB, et al. The role of vitamin D and calcium in type 2 diabetes. A systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2007;92:2017–29.
- Liu S, Song Y, Ford ES, et al. Dietary calcium, vitamin D, and the prevalence of metabolic syndrome in middle-aged and older US women. *Diabetes Care*. 2005;28:2926–32.
- Tinkler GP, Voytko ML. Estrogen modulates cognitive and cholinergic process in surgically menopausal mokeys. *Prog Neuropsychopharmacol Biol Psychiatry*. 2005;29:423–31.
- Banerjee A, Khemka VK, Ganguly A, et al. Vitamin D and Alzheimer's disease: neurocognition to therapeutics. Hindawi Publishing Corporation International Journal of Alzheimer's Dis. 2015. <https://doi.org/10.1155/2015/192747>.
- Annweiler C, Fantino B, Schott AM, et al. Vitamin D insufficiency and mild cognitive impairment: cross-sectional association. *Eur J Neurol*. 2012;19:1023–9.
- Eyles DW, Smith S, Kinobe R, et al. Distribution of the vitamin D receptor and 1[alpha]-hydroxylase in human brain. *J Chem Neuroanat*. 2005;29:21–30.
- Babaei P, Azali AK, Soltani TB, Damirchi A. Effect of 6 weeks of endurance exercise and following detraining on serum brain derived neurotrophic factor and memory performance in middle aged males with metabolic syndrome. *J Sports Med Phys Fitness*. 2013;53:437–43.
- Babaei P, Damirchi A, Mehdiipoor M. Long term habitual exercise is associated with lower resting level of serum BDNF. *Neurosci Lett*. 2014;566:304–8.
- Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *EJN*. 2004;20:2580–90.
- Boström P, Wu J, Jedrychowski MP, et al. A PGC1-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*. 2012;481:463–8.

18. Coelho FG, Vital TM, Stein AM, et al. Acute aerobic exercise increases brain-derived neurotrophic factor levels in elderly with Alzheimer's disease. *J Alzheimer's Dis*. 2014;39:401–8.
19. Wrann CD, White JP, Salogiannis J, et al. Exercise induces hippocampal BDNF through a PGC-1 α /FNDC5 pathway. *Cell Metab*. 2013;18:649–59.
20. Binder DK, Scharfman HE. Brain-derived neurotrophic factor. *Growth Factors*. 2004;22:123–31.
21. Kumar S, Parkash J, Kataria H, et al. Interactive effect of excitotoxic injury and dietary restriction on neurogenesis and neurotrophic factors in adult male rat brain. *Neurosci Res*. 2009;65:367–74.
22. Gomez PF, Ying Z, Roy RR, Edgerton VR, et al. Voluntary exercise induces aBDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol*. 2002;88:2187–95.
23. Vaynman S, Ying Z, Gomez PF. Interplay between brain derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. *Neuroscience*. 2003;122:647–57.
24. Boyuk B, Degirmencioglu S, Atalay H, et al. Relationship between levels of brain-derived neurotrophic factor and metabolic parameters in patients with type 2 diabetes mellitus. *J Diabetes Res*. 2014. <https://doi.org/10.1155/2014/978143>.
25. Nakagawa T, Kishino MO, Sugar E. Brain-derived neurotrophic factor (BDNF) regulates glucose and energy metabolism in diabetic mice. *Diabetes Metab Res Rev*. 2002;18:185–91.
26. Pontzer H, Durazo AR, Dugas LR, et al. Constrained total energy expenditure and metabolic adaptation to physical activity in adult humans. *Curr Biol*. 2016;26:1–8.
27. Pontzer H. Constrained total energy expenditure and the evolutionary biology of energy balance. *Exerc Sport Sci Rev*. 2015;43:110–6.
28. Saadati H, Babri SH, Ahmadiasl N, et al. Effects of exercise on memory consolidation and retrieval of passive avoidance learning in young male rats. *Asian J Sports Med*. 2010;1:137–42.
29. Okura T, Nakata Y, Ohkawara K, et al. Effects of aerobic exercise on metabolic syndrome improvement in response to weight reduction. *Obesity*. 2007;15:2478–84.
30. Damirchi A, Mehdizade R, Ansari MM, et al. Effects of aerobic exercise training on visceral fat and serum adiponectin concentration in ovariectomized rats. *Climacteric*. 2010;13:171–8.
31. Shinoda M, Latour M, Lavoie J. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. *Int J Obes Relat Metab Disord*. 2002;26:335–43.
32. Gauthier MS, Couturier K, Latour J-G, Lavoie J-M. Concurrent exercise prevents high-fat-diet-induced macrovesicular hepatic steatosis. *J Appl Physiol*. 2003;94:2127–34.
33. Latour MG, Shinoda M, Lavoie J-M. Metabolic effects of physical training in ovariectomized and hyperestrogenic rats. *J Appl Physiol*. 2001;90:235–41.
34. Hooge RD, Deyn PPD. Applications of the Morris water maze in the study of learning and memory. *Brain Res Rev*. 2001;36:60–90.
35. Richard GM. Morris. Morris water maze. *Scholarpedia*. 2008;3:6315.
36. Gerbaix M, Metz L, Ringot E, et al. Visceral fat mass determination in rodent: validation of dual-energy X-ray absorptiometry and anthropometric techniques in fat and lean rats. *Lipids Health Dis*. 2010;9:140.
37. Carretero JIB, Blasco FA, Villafuella JJ, et al. Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. *Clin Nutr*. 2007;26:573–80.
38. Trowman R, Dumville JC, Hahn S, et al. Asystematic review of the effects of calcium supplementation on body weight. *Br J Nutr*. 2006;95:1033–8.
39. Szafran H, Smielak KW. The role of estrogens in hormonal regulation of lipid metabolism in women. *Prz Lek*. 1998;55:266–70.
40. Yoshida T, Takahashi K, Yamatani H, et al. Impact of surgical menopause on lipid and bone metabolism. *Climacteric*. 2011;14:445–52.
41. Weinberg ME, Manson JE, Buring JE, et al. Low sex hormone-binding globulin is associated with the metabolic syndrome in postmenopausal women. *Metabolism*. 2006;55:1473–80.
42. Ford ES, Ajani UA, McGuire LC, et al. Concentrations of serum vitamin D and the metabolic syndrome among U.S. adults. *Diabetes Care*. 2005;28:1228–30.
43. Chen HI, Lin LC, Yu L, et al. Treadmill exercise enhances passive avoidance learning in rats: the role of downregulated serotonin system in the limbic system. *Neurobiol Learn Mem*. 2008;89:489–96.
44. Rastegar H, Damirchi A, Babaei P. Vitamin D increases PPAR γ expression and promotes beneficial effects of physical activity in metabolic syndrome. *Nutrition*. 2017;36(54–5919):173–82.
45. Ning C, Liu L, Lv G, et al. Lipid metabolism and inflammation modulated by vitamin D in liver of diabetic rats. *Lipids Health Dis*. 2015. <https://doi.org/10.1186/s12944-015-0030-5>.
46. Varga H, Németh H, That T, et al. Weak if any effect of estrogen on spatial memory in rats. *Acta Biol Szeged*. 2002;46:13–6.
47. Latimer CS, Brewer LD, Searcy JL, et al. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. *PNAS*. 2014;111:E4359–66.
48. Taghizadeh M, Djazayeri A, Salami M, et al. Vitamin-D-free regimen intensifies the spatial learning deficit in Alzheimer's disease. *Int J Neurosci*. 2011;121:16–24.
49. Adham R, Mohamed A, Gehan Y, et al. Neuroprotective role of vitamin D₃ in colchicine-induced Alzheimer's disease in rats. *Alex J Med*. 2014;51:127–36.
50. Hajiluan G, Nameni G, Shahabi P, et al. Vitamin D administration, cognitive function, BBB permeability and neuroinflammatory factors in high-fat diet-induced obese rats. *Int J Obes*. 2017. <https://doi.org/10.1038/ijo.2017.10>.
51. Su J, Sripanidkulcha K, Hu Y, et al. The effect of ovariectomy on learning and memory and relationship to changes in brain volume and neuronal density. *Int J Neurosci*. 2012;122:549–59.
52. Flavio P, Luigi A, Giovanni VF, et al. Vitamin D (calcifediol) supplementation modulates NGF and BDNF and improves memory function in postmenopausal women: a pilot study. *Res Endocrinol*. 2013. <https://doi.org/10.5171/2013.552758>.
53. Chaldakov GN. The metabotropic NGF and BDNF: an emerging concept. *Arch Ital Biol*. 2011;149:257–63.
54. Damirchi A, Soltani TB, Azali AK, et al. Influence of aerobic training and detraining on serum BDNF, insulin resistance, and metabolic risk factors in middle-aged men diagnosed with metabolic syndrome. *Clin J Sport Med*. 2013;24:513–8.
55. Moreno NJM, Ortega F, Serrano M, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab*. 2013;98:E769–78.
56. Xin C, Liu J, Zhang J, et al. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. *Int J Obes (Lond)*. 2016;40:443–51.
57. So WY, Leung PS. Irisin ameliorates hepatic glucose/lipid metabolism and enhances cell survival in insulin-resistant human HepG2 cells through adenosine monophosphate-activated protein kinase signaling. *Int J Biochem Cell Biol*. 2016;78:237–47.
58. Kaidah S, Soejono SK, Partadiredja G. Exercise improves hippocampal estrogen and spatial memory of ovariectomized rats. *Bratisl Med J*. 2016;117:94–9.
59. Timmons JA, Baa K, Davidsen PK, et al. Is irisin a human exercise gene? *Nature*. 2012;488:E9–10.