Basic nutritional investigation

Vitamin D increases PPARγ expression and promotes beneficial effects of physical activity in metabolic syndrome

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ABSTRACT

Objectives: Peroxisome proliferator-activated receptor (PPAR) and vitamin D signaling pathways regulate a multitude of genes involved in different physiological functions. The aim of the present study was to examine the effects of vitamin D supplementation and aerobic training on metabolic syndrome and PPARγ expression.

Methods: Forty female ovariectomized rats were divided into five groups of aerobic training with high (OVX + Exe + HD), moderate (OVX + Exe + MD), and low dose of vitamin D (OVX + Exe + LD), aerobic training receiving vehicle (sesame oil; OVX + Exe + oil), and sham-operated control (sham) groups. After 2 mo of treatment, serum insulin, vitamin D, glucose, lipid profile, visceral fat, and liver PPARγ gene expression were measured.

Results: The combination of exercise and high doses of vitamin D significantly reduced insulin (P = 0.039), blood glucose (P = 0.024), and homeostatic model assessment for insulin resistance (P = 0.011), and elevated PPARγ gene expression (P = 0.032). Also, treatment with aerobic training and either high or moderate vitamin D, ameliorated overall metabolic syndrome Z scores (P = 0.001).

Conclusion: Findings from the present study suggested that a sedentary lifestyle and vitamin D deficiency accelerated the occurrence of metabolic syndrome probably by decreasing the expression of nuclear receptor PPARγ. Additionally, adequate levels of plasma vitamin D are necessary to achieve the beneficial metabolic effects of physical activity.

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Introduction

Obesity and metabolic syndrome (MetS) are two major clinical concerns in postmenopausal women [1]. It has been shown that low levels of serum vitamin D have the potential to worsen metabolic risk factors [2], cardiovascular diseases [3,4], diabetes [5], hypertension [6], dyslipidemia [7], and obesity [8,9]. Based on existing data, vitamin D deficiency is prevalent worldwide [2,8,10], especially in women [11,12].

It has been proven that regular physical activity as a nonpharmacologic tool prevents MetS partly by increasing anti-inflammatory adipokines [13], as well as peroxisome proliferator–activated receptors (PPARs) [14], which are nuclear receptors that play a crucial role in energy homeostasis and lipid and glucose metabolism. For example, PPARα is involved mostly in fatty acid oxidation [15], PPARβ elevates lipid catabolism [16,17], and PPARγ plays an important role in adipogenesis, lipid metabolism, and insulin sensitivity [18–21].

To our knowledge, the effects of vitamin D, PPARγ, and exercise on MetS indices are not fully understood. There are only two studies indicating elevation [22] and no significant change [23] in liver PPARγ mRNA expression after aerobic exercise training. Therefore, the present study was designed to examine the effect of vitamin D deficiency and further supplementation combined with aerobic exercise training on MetS and PPARγ expression. To approach these objectives, we used ovariectomized rats as a model of menopause and MetS [24].

Methods

Animal care

Forty female Wistar rats weighing 240 to 255 g were housed four per cage and fed standard-pellet rat chow (containing: 6% of calcium and 4% vitamin D)
and tap water ad libitum. Room temperature was maintained at 22 ± 2°C with a 12/12-h light/dark cycle (light on 07:00 h) and temperature (22 ± 1°C). The protocol was approved by the ethical committee of Guilan University of medical sciences, Rasht, Iran.

Surgery, food intake, body weight, and body mass index

To measure the food intake, an equal amount of food (20 g/d) was given to animals in each cage. Food consumption was measured by subtracting the weight of the uneaten food from the total given. After 8 wk of aerobic training (AT), rats were ovarioctomized and sham-operated under general anesthesia with an intraperitoneal injection of ketamine (Rotex Medica, Germany) and xylazine (Alfasan, Netherlands) in a 4:1 ratio, according to previous work [24]. Blood length (nose-to-anus) and body mass index (BMI) were calculated. All animals were weighed weekly, between 09:00 h and 11:30 h using a scale (Sartorius, Germany).

Groups and vitamin D supplementation

Two weeks after surgery, 16-wk-old rats were divided into four groups of ovarioectomy (OVX) and one sham-operated group (sham; n = 8 per group). OVX rats were subdivided into four groups: weekly cotreatment with AT and high (HD; 10 000 IU/kg food) or moderate (MD; 1000 IU/kg food) doses and vitamin D (LD; 100 IU/kg food) and sesame oil [25]. Based on the ingredients of animal's food (5000 IU/kg food), each animal in HD and MD groups received almost 100 IU vitamin D from food, and the remaining from a weekly injection. To balance the diet on isocaloric regimen, the LD group consumed almost 100 IU vitamin D from food (5000 IU/kg food,) each week.

Aerobic exercise training

At the beginning of the training, rats started to run on a motor-driven rodent treadmill (Iranian Model, seven lines, designed by Sports Sciences Research Center, Tehran, Iran) at the speed of 10 min/d, 3 d/week for 8 wk. They progressively ran from 15 min/d at 15 m/min, 0% slope, up to 40 min/d at 25 m/min, 10% slope for the last 4 wk [27]. The regular endurance aerobic exercise training used in this study was equivalent to 60% to 75% VO2 max [22].

Blood sampling and liver isolation

All trained rats were restrained from training 48 h before being sacrificed, and food was removed from the cage 12 h before being sacrificed. Blood samples were drawn from the inferior vena cava after anesthetizing of animals with ketamine and xylazine in a 4:1 ratio, into the EDTA-containing tubes and immediately were centrifuged for 15 min at 3000 g for further analysis. The liver was isolated and immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction.

Vitamin D assay

Serum vitamin D concentration was measured by rat vitamin D enzyme-linked immunosorbent assay (ELISA) kit (Immuno diagnostics system Ltd, Boldon, UK) [23]. Intra assay coefficient of variation and sensitivity of the method were 1.63% and 1.33 mg/dL, respectively.

Lipid profile measurement

Serum concentrations of triacylglycerol (TG) [28,29], total cholesterol (TC) [30], high-density lipoprotein cholesterol (HDL-C) [31] and low density lipoprotein cholesterol (LDL-C) [32] were measured using enzymatic analysis kits (Asan pharmaceutica ls, Hwasung, Korea). Serum glucose and insulin concentration were determined using enzymatic (GOD-PAP, glucose oxidase aminoanilide) colorimetric method (Pars Azo moun, Tehran, Iran) and rat insulin ELISA kit (DRG, Springfield Township, NJ, USA), respectively. The intraassay coefficient of variation and sensitivity of the method for insulin were 1.62% and 1.76 mg/dL, respectively. Homeostatic model assessment, as an index of insulin resistance (HOMA-IR) was calculated from the following equation [33]:

\[
\text{HOMA-IR} = \frac{\text{fasting insulin } (\mu \text{IU/mL}) \times \text{fasting glucose } (\text{mmol/L})}{22.5}
\]

Isolation of RNA and real-time PCR

The extraction of total RNA was carried out using liver tissue kits (Plus Mini RNeasy, TAKARA Co., Japan, Cat.No: 74134) according to the manufacturer’s instructions. RNA samples were converted to cDNA using an Applied Biosystems high-capacity cDNA archive kit and stored at −20°C. PPARG mRNA expression was analyzed on an Applied Biosystems 7500 real-time polymerase chain reaction (RT-PCR) system using Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen, Paisley, UK). All primers for the RT-PCR were designed using the Applied Biosystems Primer Express software V 2.0, and the following sequences was obtained: PPARG: 5’-CGTGCCGCGAGATTGGA-3’, 5’-CTCCAT TACGGAGATCCAC-3’; to give PCR product of 117 bp. Estimates of cDNA abundance were made using the portion of the curve for which the plot of the log input amount versus the cycle threshold (CT) differences resulted in a slope of −0, indicating that the ampiclon efficiency was approximately equal. Relative quantification of target genes in liver tissue was calculated using the 2−ΔΔCT formula, in which ΔCT equals the difference between CT values for both target genes.

Statistical analysis

Data were analyzed using SPSS-19 (version 19; SPSS Inc., Chicago, IL, USA). After confirming the normality of variables distribution by Kolmogorov–Smirnov (K–S) test, independent t test was used to compare OVX and SHAM group means, paired t test was used to compare intragroup quantitative variables and one-way analysis of variance (ANOVA) test followed by post hoc Bonferroni test, which was used to compare between groups differences. P < 0.05 was considered statistically significant.

Results

The percentage changes in body weight, body mass index (BMI), and waist circumference (WC) in the OVX (n = 32) and sham (n = 8) groups are shown in Figure 1. There was significant difference in body weight, BMI, and WC between the OVX and sham groups at the beginning of the intervention program.

Mean body weight, BMI, food intake (FI), and WC are shown in Table 1. There was significant difference between mean body weight, BMI, FI, and WC after 8 wk of intervention; mean body weight, BMI, and WC were significantly lower in the OVX + Exe + HD and OVX + Exe + MD groups at the end of the study compared with the beginning (P < 0.01). Mean FI did not show significant change at the end of the study when compared with the beginning of the study in the OVX + Exe + HD and OVX + Exe + MD groups, whereas mean FI in the OVX + Exe + LD and OVX + Exe + oil groups significantly increased.

As Table 2 shows, there was a statistically significant difference between groups in serum 25-hydroxyvitamin D and HDL at the end of the intervention, with the highest level in OVX + Exe + HD and the lowest in the oil-receiving group. Inversely, OVX + Exe + HD showed the lowest serum LDL-C concentration compared with OVX + Exe + oil (P = 0.001), OVX + Exe + LD (P = 0.011), and OVX + Exe + MD (P = 0.023). Additionally, the mean visceral fat, TC and TGs were significantly lower at the end of the study in the OVX + Exe + HD,
Values are calculated using independent implementation; OVX exercise with moderate-dose vitamin D supplementation; OVX calculated using paired values with superscript P

ovariectomized
PPAR difference in glucose, insulin, HOMA-IR, and shown in Figures 2

Comparison of mean

Table 2

Comparison of mean ± SD of visceral fat, lipid profile, vitamin D, and calcium before the intervention among the OVX groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>n</th>
<th>$P$ value $^a$</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OVX + Exe + HD</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OVX + Exe + MD</td>
<td>8</td>
<td></td>
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<tr>
<td></td>
<td>OVX + Exe + LD</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OVX + Exe + Oil</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>Before</td>
<td>248.31 ± 0.96</td>
<td>246.72 ± 0.97</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>233.45 ± 1.72</td>
<td>237.21 ± 0.98</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Before</td>
<td>0.58 ± 0.0040</td>
<td>0.60 ± 0.0093</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>0.54 ± 0.0021</td>
<td>0.58 ± 0.0034</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>Before</td>
<td>14.33 ± 0.96</td>
<td>14.30 ± 0.056</td>
</tr>
<tr>
<td></td>
<td>After</td>
<td>14.33 ± 0.026</td>
<td>14.35 ± 0.022</td>
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</table>
| BMI mass index; FI, food intake; OVX + Exe + HD, ovariectomized—exercise with high-dose vitamin D supplementation; OVX + Exe + MD, ovariectomized—exercise with moderate-dose vitamin D supplementation; OVX + Exe + LD, ovariectomized—exercise with low-dose vitamin D supplementation; OVX + Exe + Oil, ovariectomized—exercise with oil group; WC, waist circumference

$P$ values with superscript “a” are calculated using one-way analysis of variance test followed by post hoc Bonferroni test; superscript letter “b” indicates values are calculated using paired t test

$^a$ P < 0.05 vs OVX + Exe + oil group.

$^b$ P < 0.05 vs OVX + Exe + MD group.

$^c$ Significantly different in comparison with pre- and post-test between groups.

$^d$ Significantly different in comparison with pre- and post-test within the groups.

Finally, one-way ANOVA showed a significant difference in Z scores for MetS between all groups (Fig. 6). The cotreatment of AT with either HD or MD vitamin D could ameliorate overall Z scores of MetS compared with the OVX + Exe + oil (P = 0.001 and P = 0.021, respectively).

Discussion

Results of the present study showed that serum vitamin D was elevated according to the supplemenations, however, serum calcium did not show any significant change between groups probably due to strong hormonal regulation. HD and MD vitamin D supplementation combined with AT led to significant reduction in visceral fat, BMI, body weight, serum glucose, insulin, TC, TG, LDL-C, and HOMA-IR compared with exercised rats receiving the vehicle [2]. Additionally, the strongest expression in PPARα was found in the group receiving HD vitamin D

Table 2

Comparison of mean ± SD of visceral fat, lipid profile, vitamin D, and calcium before the intervention among the OVX groups

<table>
<thead>
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<td></td>
<td>OVX + Exe + LD</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>OVX + Exe + Oil</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Visceral fat (g)</td>
<td>8.29 ± 0.014</td>
<td>8.55 ± 0.034</td>
<td>9.20 ± 0.083</td>
</tr>
<tr>
<td>TC (mg/dL)</td>
<td>65.56 ± 1.56</td>
<td>69.25 ± 1.25</td>
<td>78.18 ± 2.32</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>53.25 ± 1.98</td>
<td>55.01 ± 1.77</td>
<td>61.01 ± 2.08</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>25.31 ± 1.41</td>
<td>30.31 ± 1.13</td>
<td>35.87 ± 1.24</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>32.56 ± 1.52</td>
<td>50.18 ± 1.79</td>
<td>40.68 ± 1.41</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>117.38 ± 1.43</td>
<td>112.79 ± 0.928</td>
<td>102.71 ± 1.12</td>
</tr>
<tr>
<td>Calcium (mg/mL)</td>
<td>8.90 ± 1.32</td>
<td>8.74 ± 1.17</td>
<td>8.25 ± 1.61</td>
</tr>
</tbody>
</table>

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OVX + Exe + HD, ovariectomized—exercise with high dose vitamin D supplementation; OVX + Exe + MD, ovariectomized—exercise with moderate dose vitamin D supplementation; OVX + Exe + LD, ovariectomized—exercise with low dose vitamin D supplementation; OVX + Exe + oil, ovariectomized—exercise with oil group; TC, total cholesterol; TG, triacylglycerol; Vit D, vitamin D

$^a$ P < 0.05 vs OVX + Exe + oil group.

$^b$ P < 0.05 vs OVX + Exe + MD group.

$^c$ Significantly different in comparison with pre- and post-test between groups.

$^d$ P < 0.05 vs OVX + Exe + oil group.
supplementation in addition to AT. The synergistic effects of vitamin D and aerobic exercise training on metabolism has been previously addressed in OVX rats [4].

Similar to previous studies [34–38], all OVX rats showed some of the MetS symptoms such as weight gain, dyslipidemia, and visceral fat accumulation due to estrogen reduction. An interesting finding from the present study was that simultaneous regular AT in the group with lower vitamin D not only did not lead to a reduction in weight, but it also significantly increased weight compared with the group with an adequate level of serum vitamin D. Because AT with HD vitamin D supplementation led to significant weight loss, the weight gain observed in the group receiving LD vitamin D supplementation could not be related to free fat mass increases after aerobic exercise training. Therefore, an optimum level of vitamin D is necessary to achieve the beneficial effects of aerobic exercise training on weight loss. The association between weight and vitamin D might be related to the effect of vitamin D on lipoprotein lipase synthesis, uncoupling protein 2 (UCP2), and further resting energy expenditure (REE) in adipose tissue [39]. For example, vitamin D induces lipoprotein lipase activity [40] and decreases fatty acid absorption via the formation of insoluble Eum–fatty complexes in the gut [41]. Moreover, parallel with weight loss in the exercised group receiving HD vitamin D, the cardiovascular risk factors of TG, TC, LDL-C were decreased compared with the exercised group receiving LD vitamin D. Similar to our findings, two other studies reported an inverse correlation between serum concentration of lipid profile and vitamin D [42,43]. It has been shown that vitamin D increases the conversion of cholesterol to bile acids [44], and therefore decreases its concentration in serum. It is obvious that aerobic exercise training per se led to improvement in lipid profile, which is in line with previous studies on patients.
who are obese and those with diabetes participating in endurance exercise [45,46]. Exercise appears to enhance the ability of skeletal muscles to use lipids [47] and increases lecithin cholesterol acyltransferase; the enzyme responsible for ester transfer to HDL-C [48]. Taken together, treatment with exercise and vitamin D supplementation has an advantage in controlling weight gain and improving lipid profiles in animal models of MetS.

Additionally, another major finding of the present study was a 51.25% increase in liver PPARγ expression after a combination of aerobic exercise and HD vitamin D compared with the vehicle control group. To our knowledge, this is the first study to indicate the intervention of PPARγ and vitamin D in mediating exercise metabolic effects. In agreement with our results, an earlier study indicated a twofold increase in liver PPARγ mRNA after endurance training [49]. In the present study, elevation in PPARγ expression was inversely associated with insulin resistance and serum lipid improvement in the group receiving HD vitamin D and parathyroid hormone with mortality among middle-aged and older European men. Age Ageing 2014;43:528–35.

Fig. 6. Z scores for metabolic syndrome in the study. Values calculated using one-way analysis of variance followed by post hoc Bonferroni test. OVX + Exe + HD, ovariectomized—exercise with high dose vitamin D supplementation; OVX + Exe + MD, ovariectomized—exercise with moderate dose vitamin D supplementation; OVX + Exe + LD, ovariectomized—exercise with low dose vitamin D supplementation; OVX + Exe + oil, ovariectomized—exercise with oil group.

Conclusion

From a clinical point of view, the results of the present study suggest that the optimum level of vitamin D is required to achieve beneficial effects of AT on cardiovascular risk factors and insulin resistance.

References


