ORIGINAL ARTICLE

Effect of 6 weeks Pilates training along with dill supplementation on serum concentrations of nesfatin-1, lipocalin-2, and insulin resistance in females with overweight and obesity: A randomized controlled trial

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Background: This study aimed to investigate the effect of 6 weeks' pilates training along with dill supplementation on serum concentrations of nesfatin-1, lipocaline-2, and insulin resistance in females with overweight and obesity. **Materials and Methods:** In this randomized controlled trial study, 45 overweight and obese females are randomly assigned to four groups: Pilates training + dill group (PDG) (n = 12), pilates training + placebo group (PPG) (n = 11), dill supplementation group (DG) (n = 11), and placebo group (PG) (n = 11). Participants of PDG and PPG performed pilates training for 6 weeks (60 min, 3 sessions per week). PDG and DG received dill tablet (three times a day, 6 weeks). Anthropometric measurements, glycemic markers, and blood samples were assessed before (pretest) and after (posttest) 6 weeks of intervention. **Results:** Results showed a significant increase in serum concentrations of nesfatin-1 in PDG compared to pretest (P = 0.001). Differences in the serum concentrations of nesfatin-1 in PDG were greater than PPG, DG, and PG (P = 0.01). Furthermore, results found in significant reduction in serum concentrations of lipocalin-2, body mass index (BMI), and waist-hip ratio (WHR) in PDG, PPG, and DG as compared to pretest (P > 0.05). Fasting glucose plasma (FGP) was significantly decreased in all three intervention groups PDG (P < 0.001), PPG (P < 0.001), and DG (P < 0.001) as compared to pretest. Differences in FGP were significantly higher in PDG than PPG, DG, and PG (P = 0.001). A significant reduction was found for insulin only in PDG after 6 weeks of intervention as compared to pretest (P = 0.03). Insulin resistance significantly decreased in PDG (P = 0.03) and PPG (P = 0.04) as compared to pretest. Body fat percent (BFP) was significantly decreased in PDG (P = 0.003), PPG (P = 0.006), and DG (P = 0.01). However, there were no significant inter-group differences in insulin resistance, insulin, serum concentrations of lipocalin-2, BMI, BFP, and WHR after 6 weeks of Pilates training along with dill supplementation (P > 0.05). **Conclusion:** We concluded that 6 weeks of Pilates training along with dill may be beneficial for improvements in serum concentrations of nesfatin-1 and FGP.

Key words: Dill supplementation, insulin resistance, lipocalin-2, nesfatin-1, Pilates training

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INTRODUCTION

According to the reports of World Health Organization, obesity is one of the major public health concerns affecting 1.9 billion people globally.[1] Obesity is an world public health issue that affects the quality of life, increases the risk of illness, and raises health-care costs in countries in all parts of the world.[2] Adipose tissue is known as active endocrine organ that produces biologic substance called adipocytokines affecting glucose and lipid metabolism.[3] Nesfatin-1 is known as anti-inflammatory factor that improves insulin sensitivity by increasing insulin secretion from beta cells.[4] In addition to being secreted from hypothalamus, adipose tissue is described as another potential source

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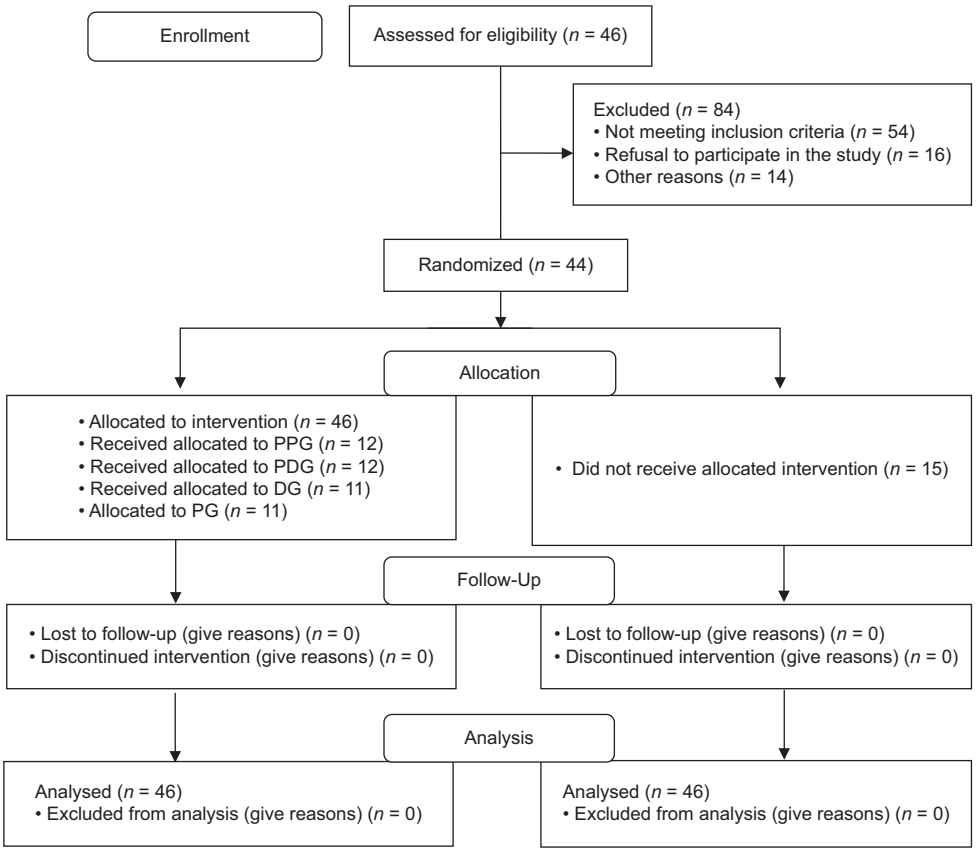

Figure 1: Participants Flow Diagram. Abbreviations: PPG; Pilates Training Group, DG = Dill Group; PDG = Pilates Training + Dill Group; PG = Placebo Group

of nesftain-1, with decreased levels of nesfatin-1 in obese people.[5] It was reported that any alternations in serum levels of nesfatin-1 are associated by alternations in weight and body mass index (BMI).[4] Nesfatin-1 regulated glucose, energy homeostasis, and insulin secretion.[6] Lipocalin- 2 was recently to be associated with obesity and insulin resistance in human and highly expressed by adipocytes.[7] Furthermore, circulation of lipocalin-2 was increased in obese individual and it has a positive correlation with variables such as insulin resistance and metabolic syndrome diseases. Low levels of lipocalin-2 decrease insulin resistance in adipose tissue by modulating of lipoxygenase-12 (as an enzyme involved in arachidonic acid metabolism) and tumor necrosis factor-α (as an inducer of insulin resistance).[7]

Various factors such as exercise and diet can alter expression of adipokines involved in energy regulation in normal and obese individuals.[8] Pilates training (body control science) is a method of exercise developed by Joseph Pilates in 1880 which includes low intensity exercises that used body weight as external resistance through isometric concentrations of the core muscles.[9] Accordingly, evidence indicates Pilates training increases strength, endurance, and flexibility, as well as improvement of physical conditions, psychological problems such as attention and concentration.[10] This type of training is not designed to reduce weight, but Pilates training can be considered a good option for people with overweight or obesity who have difficulty in adhering to those monotonous traditional physical exercises.[11] In addition to exercise, medical plants have been discovered and used in control and traditional treatment since prehistoric times. Dill (also called Anethum graveolens) is an herbal plant cultivated in various regions worldwide. It contains essential oil such as carvone and limonene, in addition to phenolics, such as trans-anethole and flavonoids that have hypolipidemic effects.[12] It was indicated that consumption of dill decreases serum levels of lipids concentration and has hypoglycemic and antioxidant activity.[13] In diabetic models, dill significantly decreases reduced triglycerides, total cholesterol, low-density lipoprotein cholesterol glucose levels, whereas it increased high-density lipoprotein cholesterol level.[13] However, to the best author's knowledge, this is the first study that examined the effect of Pilates training along with dill supplementation on serum concentrations of nesfatin-1, lipocalin-2 in overweight and obese females. Therefore, we conducted a randomized controlled trial design to investigate the role of 6 weeks Pilates along with dill supplementation on serum concentrations of nesfatin-1, lipocalin-2 in females with overweight and obesity. In addition, we evaluated the effects of 6 weeks Pilates along

with dill supplementation on insulin resistance in the same study participants. It was hypothesized that Pilates training along with dill supplementation may provide additive benefits on concentrations of adipokines in overweight and obese females.

METHODS AND DESIGN

Study design and participants

In this randomized controlled trial study, 45 overweight and obesity females (BMI ≥25) recruited in the present study. All participants were classified according to BMI which was calculated in standard way. According to CONSORT statement, Figure 1 shows the flowchart of this study. Participants are randomly divided to four following groups: Pilates training + dill group (PDG), Pilates training + placebo group (PPG), dill supplementation group (DG), and placebo group (PG). Written informed consent was obtained from all subjects. Inclusion criteria were: Having 25 and more BMI, age between 25 and 45 years old, lack of professional training for last 6 month, free of chronic diseases, and being woman. Exclusion criteria were: Medical drug history for lose weight, doing special diet for weigh lose, a history of active sport, regular exercise for last 6 month. Randomization was performed by used a digital tool available at www.randomizer.org.

Furthermore, the calculation of the sample size was carried out using G * Powers software (HeinrichHeineUniversität, Düsseldorf, Germany). The present study was approved by the Ethics Committee Birjand University of Medical Science (IR.BUMS.REC.1397.182) and registered at the Iranian Registry of Clinical Trials (IRCT20220107053655N1).

Anthropometric measurement

Anthropometric measurements were performed at the beginning and end of 6th week intervention. Height was measured barefoot by using digital staodiometer (to the nearest 0.5 cm). Body compositions of each participant such as weight, BMI, body fat percent (BFP), and waist-hip ratio (WHR) were assessed by using body composition analyzer (ACCUNIQ BC360, South Korea).

Blood measurement and processing

After 12 h of fasting (48 h before and after the first and the last intervention session), 10 mL blood sample was collected from antecubital vein between 8 AM and 10 AM. For the separation of serum, blood samples were centrifuged at 3000 rpm for 10 min, followed by storage of aliquots at-80°C. Serum concentrations of nesfatin-1 and lipocalin-2 were evaluated by ELISA human kit (Eastbiopharm, China-USA) with a sensitivity 0.15 ng/ml and 10.12 ng/ml, respectively. The intra-assay coefficient of variations of nesfatin-1 and lipocalin-2 were CV <12% and CV <12%, respectively. The inter-assay coefficient of variations nesfatin-1 and lipocali-2 were CV <10% and CV <10% percent, respectively. Pars test kit (manufacturer Japan-Germany) was used to measure serum concentrations fasting glucose plasma (FGP) by the Auto analyzer system. Also, serum concentrations fasting insulin were evaluated by ELISA human kit (Diaplus Q-1) manufactured Japan-USA. Insulin resistance index was measured by following equation:

HOMA-IR = (FGP mmol/ μ) fasting insulin× U

Dill intervention

In the present study, Participants in PDG and DG received the pills that contained dill powder supplementation (Three times daily, 6 weeks 1.1 g). Participants in PG also received (Three times daily, 6 weeks) pills which contained 1 gr starch in similar shape to dill pills.[14] Participants were instructed to refrain from exercise activities or any particular diet during intervention. Compliance with dill and any complication about it were checked through weekly telephone interviews.

Training intervention

Pilates training was performed by 60%–70% of maximal heart rate. The intensity of Pilates training was measured by Carvonen by the following method:

Max. Heart Rate = 220–Age

Heart Rate Reserve = HR max–HR Rest

(Resting Heart Rate)

%60 Target Heart Rate = (0.60 ′ HRR) + HR rest

All exercise (PDG and PPG) groups participated in the 6 weeks Pilates training intervention (60 min, three sessions per week). All sessions included 3 phases: 10 min warm-up, 40 min Pilates training, and 10 min cool-down. Pilates training program was shown in Table 1.[15]

Statistical analysis

Data were analyzed using SPSS 26 (version 26, IMB-SPSS Inc., Chicago, IL, USA) and are presented as mean ± Standard deviation. Kolmogorov-Smirinov (K-S) test was used to verify the normality of data distribution. Paired-samples *t*-test and Analysis of covariance were applied to examine the intra-and inter-group differences, respectively. $P < 0.05$ were considered as statistically significant.

RESULTS

The demographic data of participants are shown in Table 2. Paired-sample *t*-test shown significant increase

Table 1: Pilates training program

Warm-up	Pilates training	Cool-down
Neck stretch	The shoulder bridge	Spine stretch
Cat stretch	one leg stretch	The saw
Leg stretch	Double leg stretch	Double leg stretch
Spin rotation	Single leg circle	Child's pose
Back flexion and extension	The hundred	Double-leg kick hands-clasped
Kneeling arm and leg reach	The saw	Double-leg kick-hands spread open
Roll over	swimming	Side bend
Rolling like a ball	Criss-cross	Mermaid stretch
Pelvic curl	Teaser	
	Dart	
	Neck roll	
	Side leg lift	
Repletion gradually increased from 8-12		

Table 2: Demographic data of participants

Variable	PPG (*n*=12)	DG (*n*=11)	PDG (*n*=12)	PG (*n*=11)
Age (year)	31.75±5.13	31.54±6.71	30.50±5.99	31.36±7.57
Height (cm)	155.58±5.14	160.45±4.98	160.25±5.87	157.27±5.15
Weight (kg)	76.74±8.58	77.24±9.40	77.90±4.56	76.91±8.45

Values are peresented as mean±SD. PDG=Pilates training+ dill suplammentation group; PPG=Pilates training + placebo; DG=Dill supplementation group; PG=Placebo group; SD=Standard deviation

in serum concentrations of nesfatin-1 compare pretest in PDG ($P = 0.001$), but not in PPG ($P = 0.29$), DG ($P = 0.21$), and PG ($P = 0.93$) [Table 2]. Lysergic acid diethylamide (LSD) test indicated that serum concentrations of nesfatin-1 was greater in PDG as compared with PPG ($P = 0.002$), DG ($P = 0.02$), and PG ($P = 0.02$) [Figure 2a]. Also, results indicated no significant decreased in serum concentrations of lipocalin-2 in all intervention groups ($P = 0.91$) [Figure 2b]. FGP was significantly decrease in all three intervention groups PDG ($P < 0.001$), PPG ($P < 0.001$), and DG ($P < 0.001$) as compared to pretest. Also, according to LSD's results, there were significant alterations in FGP in PDG as compared to PPG ($P = 0.001$), DG ($P = 0.01$), and PG ($P = 0.01$). Furthermore, insulin resistance significantly decreased in PDG ($P = 0.03$) and PPG ($P = 0.04$) as compared with pretest [Table 3]. BFP significantly decreased in PDG ($P = 0.003$), PPG ($P = 0.006$), and DG ($P = 0.01$) compared to pretest [Table 2]. Insulin significantly decreased only in PDG after 6 weeks of intervention as compared to pretest ($P = 0.03$). However, no significant differences were observed in insulin resistance ($P = 0.13$), insulin ($P = 0.57$), BMI ($P = 0.67$), BFP ($P = 0.90$), and WHR ($P = 0.13$) between all groups [Table 3].

DISCUSSION

This is a randomized controlled trail study designed to evaluated effect of 6 weeks of Pilates training along with dill supplementation on adipokines, glycemic markers, and anthropometric measurements in females with overweight and obesity. The results of the present study demonstrated that Pilates training and dill supplementation can significantly increase serum concentrations of nesfatin-1 and can improved insulin resistance, FGP, and BFP in overweight and obese females.

Pilates training significantly lowered FGP and insulin resistance in PPG group. It has been found that Pilates improves FPG and insulin resistance by neutralizing reactive oxygen species and increasing the activity of antioxidant enzymes.[16] In this regard, Mir and Mir[17] showed Pilates training significantly decreased insulin resistance and insulin in obese women. Also, Saremi, Bahrami,[18] found significant decrease in insulin resistance following of 8 weeks of Pilates training in polycystic ovary syndrome women. The main mechanism of Pilates training on glycemic parameters have been not well characterized. In fact, exercise training improves insulin sensitivity by improvements in glucose transportation and enhancement of oxidative capacity. Also, evidences demonstrated that Pilates training through the increase of glucose transporter type 4 (GLUT4), insulin receptor substrates and increase muscle mass induces improvement in glycemic control and substrate metabolism in skeletal muscle.[17,19] Other finding of the present study was significant decreased in FPG and in significant reduction in insulin resistance in DG group. Madani, Ahmady Mahmoodabady[20] investigation showed a significant reduction in blood glucose with receiving 300 mg/kg/w hydroalcoholic extract of dill supplementation for 10 day (200–250 g) in male diabetic rats. In contrast to our findings, Haidari, Zakerkish[21] reported significant improvement insulin resistance, insulin, and FPG following 8 weeks of dill supplementation in patient with T2DM. Also, Supplementation of patients with T2DM with 3.3 g/day of dill powder for 8 weeks could significantly reduce levels of insulin.[22] The hypoglycemia effect of dill is due to the flavonoid (quercetin).[23] Flavonoid has an antioxidants effect and reduces the absorption of glucose in the intestine.[23,24] Dill inhibits the transfer of glucose from the small bowel and delays the gastric emptying into the small bowel by inhibiting digestive enzymes such as alpha amylase and alpha glucosidase, which are involved in carbohydrate hydrolysis.[24] In addition to the antioxidant effects of dill, the bioactive compounds in dill can be effective in repairing damaged beta cells and insulin secretion.[24] The lack of positive effects of regular dill consumption in insulin resistance may be due to the lower amounts of dill, which highlights the importance of higher amounts of dill supplementation to induce significant effects on glycemic parameters. Other finding of the present study was significant reduction in FPG in PDG compared to PPG,

Table 3 The pre-posttest of biochemical and anthropometric factors of participants in all groups

Variables	Group	Pre-test	Post-test	*P* Paired sample *t*-test	*P* ANCOVA
BMI (kg/m^2)	PPG	32.07±1.91	31.89±1.64	0.40	0.67
	DG	30.09±1.84	30.06±1.85	0.72	
	PDG	30.99±1.60	30.77±1.52	0.34	
	PG	31.48±2.37	31.12±1.85	0.14	
BFP (%)	PPG	41.04±4.43	40.39±4.68	0.006*	0.90
	DG	44.90±5.60	44.37±5.66	0.01*	
	PDG	42.45±3.55	46.88±3.83	0.003*	
	PG	43.16±4.35	42.78±4.28	0.42	
WHR	PPG	0.97±0.25	0.97±0.28	0.16	0.13
	DG	0.92±0.37	0.92±0.33	0.17	
	PDG	0.97±0.47	0.97±0.42	0.83	
	PG	0.91±0.54	0.91±0.49	0.81	
FPG (mg/dl)	PPG	80.75±8.09	85.75±5.10	<0.001*	0.001*
	DG	80.75±8.09	85.75±5.10	<0.001*	
	PD	90.25±5.46	83.16±5.20	<0.001*	
	PG	89.38±8.49	91.45±10.50	0.26	
Insulin (μu/mL)	PPG	9.16±2.77	7.00±3.01	0.11	0.57
	DG	10.62±2.85	9.33±3.49	0.24	
	PDG	16.08±5.69	12.24±5.58	0.03*	
	PG	8.63±4.33	8.28±4.76	0.78	
Insulin resistance (μu/mL)	PPG	38.8±13.95	42.2±20.58	0.04*	0.13
	DG	43.42±13.68	43.62±18.60	0.94	
	PDG	61.84±26.97	47.02±20.58	0.03*	
	PG	31.18±12.36	32.02±19.97	0.83	

*P<0.05; Values are peresented as mean±SD. PDG=Pilates training+dill suplammentation group; PPG=Pilates training+placebo; DG=Dill supplementatin group; PG=Placebo group; BMI=Body mass index; BFP=Body fat precent; WHR=Weist-hip ratio; FPG=Fasting plasma glucose; ANCOVA=Analysis of covariance

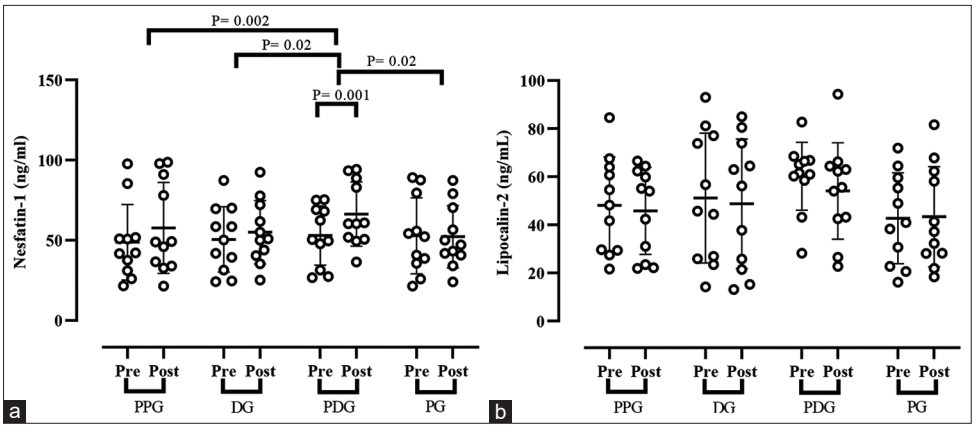

Figure 2: Changes in nesfatin-1 and lipocalin-2 in response to 6 weeks of Pilates training and dill supplementation. PPG = Pilates Training Group; DG = Dill Group; PDG = Pilates Training + Dill Group; PG = Placebo Group

DG, and PG. Based on the best authors' knowledge this is the first study that evaluated effect of Pilates training along with dill supplementation on glycemic parameters. It seems that Pilates training increases glucose uptake, which is the main factor in the improvement of FPG, insulin concentrations through increased glucose uptake capacity, muscular blood flow, and GLUT4. These findings indicate that there may be an additive effect of Pilates training and dill supplementation in improving FGP in overweight and obese females.

Various studies indicated that exercise training improves insulin resistance by bioactive factors secreted from adipose tissues. Adipose tissue is the main source in expression of nesfatin-1 and subsequently it released in bloodstream.[25] Gene expression and release of nesfatin-1 are regulated by nutritional status.[26] Not only nesfatin-1 suppressed food intake and appetite, but also played an important role in energy expenditure and glucose homeostasis.[27] Also, There are significant positive correlations between nesfatin-1 and insulin sensitivity

in response to exercise in overweight women.[28] Various factors such as exercise can alter nesfatin-1 secretion involved in energy regulation. Based on the results of the present study, serum concentrations of nesfatin-1 didn't significantly increased following of 6 weeks of Pilates training in PPG group. To the best of author's knowledge, this is the first study that evaluated effect of Pilates training on serum concentrations of nesfatin-1. The response of nesfatin1 to Pilates training is ambiguous. In contradiction to our results, Haghshenas, Ravasi[29] found a significant increase in the levels of nesfatin-1 after 12 weeks of resistance training. In a study by Mogharnasi, TaheriChadorneshin[30] showed significant increase in the levels of nesfatin-1 following both endurance and resistance trainings in overweight and obese women. In agreement to our study, Hajipour, Shabkhiz[31] reported nonsignificant increase following aerobic training in the levels of nesfatin-1 in obese men.[31] These disagreements in the literature may be due to different intervention duration, exercise intensity, diversity in the type of exercise, and characteristics of participants. In the present study, the lack of positive effects of regular Pilates training on nesfatin-1 expression may be due to insufficient intensity and volume of Pilates training intervention. Also, previous evidence suggested serum concentrations of nesfatin-1 decreased 18% in fasting and it returns to normal levels after 12 h of re-feeding. Other findings of the present study was significant increase in the serum concentrations of nesfatin-1 following 6 weeks of Pilates training along with dill supplementation which was similar to study of Chaolu, Asakawa,[32] who showed serum levels of nesfatin-1 significantly increased following 4 weeks of endurance training and high-fat diet. Also, Golestani *et al*.[33] found that high intensity interval training and spirulina supplementation significantly increased levels of nesfatin-1 in overweight and obese females.[33] Niaki *et al*.[34] reported significant enhancement in the levels of nesfatin-1 following of 6 weeks of aerobic training with *Zizyphus jujuba* water extraction in females rats.[34] Based on the best authors' knowledge, the present study is the first one which evaluated effects of dill supplementation on serum concentration of nesfatin-1 in females with overweight and obese females. Nesfatin-1 reduces blood glucose by activating AMP-activated protein kinase, up-regulating the phosphorylation of protein kinase B in the pancreas, and by increasing the glucose transporter-4 membrane translocation in skeletal muscle and adipose tissue that in turn improves insulin sensitivity.[35] In line with our findings, a study demonstrated that nesfatin-1 and fat percentage are likely to be improved significantly in response to exercise.[36] On the other hand, increased levels of the adipocytokine induced change by exercise can act as stimuli in reducing visceral and subcutaneous fat. In agreements with our findings, a negative correlation has been reported between levels of nesfatin-1 and BFP in previous studies.[8,37] Based on the results of previous studies, levels of nesfatin-1 is highly sensitive to significant loss of fat percentage.[30,38]

Recently, Lipocalin-2 had been potentially described as connected to obesity and adipose tissue inflammation which has a direct correlation with inflammatory markers and insulin resistance index.[39] Findings of the present study revealed that training and interaction effect of Pilates training and dill supplementation did not have significant effect on serum concentrations of lipocalin-2. Mehraba, Damirchi[40] demonstrated that serum concentrations of lipocalin-2 were affected by intensity exercise training. Choi, Kim[7] compared effect of two aerobic exercises intensity in obese men; they reported that 12 weeks of moderate aerobic exercise (45%–55% $VO_{2\ max}$) had no significant effect in reduction of BFP and lipocalin-2, whereas they observed significant decrease in serum concentrations of lipocalin-2 and BFP in high intensity aerobic exercise (65%–75% $VO_{2\ max}$). Also, Choi, Kim[7] investigated effect of 12 weeks combine training on obese women; results showed that despite of high volume of exercise, no significant decreased was observed in levels of lipocalin-2 due to low intensity of training. Both intensity and volume of training are essential for reduction of BFP (especially visceral fat) and afterward reduction of lipocalin-2. Some studies indicated that higher intensity and volume of exercise are more effective in reduction of lipocalin-2. On the other hand, in significant alterations in serum concentrations of lipocalin-2 maybe due to volume and short duration or lower dosage of dill.

Our results should be interpreted taking into consideration the following limitations. One limitation of the preset study was lack of controlled participants' diet. However, there is no evidence to suggest that participants kept exactly the same normal dietary habits. Calories consumption and nutrition can alter circulation of nesfatin-1, lipocalin-2, and body composition, levels of nesfatin-1 influences food intake.

CONCLUSION

In general, the additive effect of Pilates training along with dill supplementation can significantly increase serum concentrations of nesfatin-1 and improve FGP in females with overweight and obesity. Pilates training plays an important role in regulating energy balance and reduction appetite by increasing serum concentrations of nesfain-1. Therefore, Pilates training can be used as a preventive and therapeutic strategy in improvement of anthropometric measurement and reduction of obesity-related diseases in overweigh and obese individuals.

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Conflicts of interest

There are no conflicts of interest.

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