

## Research Article

# The effect of a period of Pilates and the supplementation of Royal Jelly on inflammatory indexes of diabetic obese women

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Pilates, Royal Jelly, TNF- $\alpha$ , CRP, IL-6, Type Two Diabetes

### Abstract

**Background:** The use of supplements, herbal extracts, and exercise training for the treatment of diseases and metabolic disorders has increased among people. Thus, the aim of this study was the study of The effect of a period of Pilates and the supplementation of Royal Jelly on inflammatory indexes of diabetic obese women.

**Materials and Methods:** In this quasi-experimental study 44 volunteer diabetic obese women participated and They were randomly divided into Four groups of 11 controls, Royal Jelly, Pilates, Pilates + Royal Jelly .Pilates exercise training was performed during 8 weeks (3 sessions per week, 60 min per session). The subjects consumed a supplement group of 1000 mg of royal Jelly daily for 8 weeks. covariance analysis test were used to examine the in-group differences and between the research groups.

**Results:** The results showed that eight weeks of Pilates and Royal Jelly Supplements significantly reduced the TNF- $\alpha$ , CRP and IL-6 diabetic women(P=0.001). In addition, serum glucose and serum insulin also decreased significantly in during eight weeks of Pilates and supplementation of the royal Jelly(P=0.001). Vo<sub>2</sub>max significantly Increase in training, training + supplement groups (P=0.001).

**Conclusion:** According to obtained results, it appears that Pilates training plus Royal Jelly extract consumption have better effect on serum inflammatory factors in obsess women with diabetes type 2

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## 1. Introduction

Type 2 diabetes is a manageable chronic disease that affects more than 285 million people in the world. According to the report of the World Health Organization, in the year 2000, almost two million Iranians had diabetes. It is predicted that the number of people with type 2 diabetes will increase to 6.4 million by 2030 (1). Environmental and genetic factors, insulin resistance and dysfunction of beta cells are involved in the development of this disease (2). Diabetes and obesity are closely related, and obesity, especially visceral obesity, is one of the important factors in type 2 diabetes (3). Inflammation is one of the most important causes of diabetes, which is associated with an increase in pro-inflammatory cytokines. The progression of type 2 diabetes with insulin resistance is influenced by anti-inflammatory mediators such as IL-6 and TNF- $\alpha$  (4). Tumor necrosis factor alpha (TNF- $\alpha$ ) as a pro-inflammatory cytokine that is produced by most cells of the body's defense system, endothelial cells, smooth muscle cells and adipose tissue and as a possible pathophysiological factor in atherosclerosis. Diabetes, cardiovascular disease, etc. are considered (5). IL-6 is another protein that interacts with TNF- $\alpha$  and IL-1 and is secreted from some body cells, especially muscle tissue and fat. Both cytokines TNF- $\alpha$  and IL-6 disrupt insulin signaling, and in laboratory studies, TNF- $\alpha$  injection induces insulin resistance through ERK1/2, JNK, and MAPK pathways, which destroys the mediated signaling pathway. insulin receptor substrates (IRS). On the other hand, the increase in IL-6 plasma levels leads to an increase in SOCS-3 and a decrease in the expression of IRS1(6). Longitudinal studies have shown that regular exercise reduces IL-6 levels and regular activity may suppress low-grade inflammation (7).

It has also been found that in inflammatory diseases such as diabetes, it is related to the increase of serum levels of IL-6 and CRP (8). CRP is a biomarker of general inflammation that is secreted in response to the accumulation of inflammatory cytokines (9). Increased CRP is associated with increased blood pressure, increased body mass, metabolic syndrome, diabetes, dyslipidemia, infection and chronic inflammation (10). On the other hand, research has shown that regular exercise has anti-inflammatory effects and suppresses low-grade systemic inflammation, which can play a role in diabetes management (11). Royal jelly is one of the natural supplements that has been traditionally used for some medical applications for a long time. This substance is secreted from the pharyngeal and submandibular glands of young worker bees and is used to feed the young larvae (for a few days) and the queen (until the end of life). Royal jelly has many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, salts (for example, iron and calcium) and vitamins (mainly thiamine, riboflavin and niacin) (12). It has been proven that royal jelly has activities such as relaxing blood vessels and reducing blood pressure, anti-tumor activity, blood lipid lowering effects and anti-inflammatory effects (13). In a research, Etemad and Zahali (2018) showed that royal jelly supplementation caused a significant decrease in the levels of inflammatory indicators such as TNF- $\alpha$ , CRP and IL-6(14). In another study, Panahi (2014) concluded that royal jelly caused a significant decrease in CRP levels in inactive women, which can be effective in preventing cardiovascular diseases (15).

Considering the conflicting results from past researches and the absence of a study that investigated the interactive effects of sports activities and royal jelly consumption on the concentration of TNF- $\alpha$ , CRP and IL-6, in this research the researcher sought to investigate the effect of Pilates training. And the use of royal jelly on the inflammatory indicators of obese diabetic women.

## 2. Materials and Methods

The method of the present study was semi-experimental with a pre-test-post-test design. The present study was conducted based on the ethical principles of the Islamic Azad University, Tehran East branch, and with the code of ethics committee. The subjects were 44 type 2 diabetic obese women with an age range of 25-35 years and a body mass index greater than or equal to 30, who were selected voluntarily. The final selection of the subjects was as follows: by the available sampling method, among 110 obese people with type 2 diabetes in the 8th district of Tehran who had applied based on a public call, after completing the questionnaire, 44 people were selected as a sample in Available and targeted were selected in this research. The criteria for entering the study were: obese diabetic women aged 25-35 years, body mass index greater than or equal to 30, fasting blood sugar 150 to 250 mg/dL and glycosylated hemoglobin 7 to 10.5, no diseases Cardio-vascular, skeletal-muscular and metabolic, non-menopausal of all subjects, not using insulin and not having any diabetic complications (neuropathy, nephropathy, retinopathy), not participating in regular sports activity more than one session a week during 6 months.

In the past, not smoking, not having a history of diabetes for more than 5 years, and not taking more than one type of oral anti-diabetic pill at night (all subjects were taking metformin in the same amount). Then these subjects were randomly divided into four control groups (11 people), Pilates training (11 people), Royal Gel (11 people), Pilates training + Royal Gel (11 people). Before the start of the study, in a briefing session of all programs, the correct way of performing exercises and possible risks were explained to the participants, and all participants filled out the consent form. In this form, it was emphasized that participating and withdrawing from the study by the applicant is completely free and optional, and all the information of the applicant is completely confidential, and the research results will be published in the form of general and group information. Also, due to the fact that one of the limitations of this study was the lack of strict control of the subjects' diet, so they were asked not to change their usual and daily diet and to avoid consuming any additional food and nutritional supplements.

The training groups did Pilates exercises for 8 weeks, three sessions per week and 60 minutes per session, but the control group and the supplement group were engaged in their daily activities during this time and did not participate in any sports activity program. Each training session included: 10 minutes of warming up, 40 minutes of Pilates exercises and 10 minutes of cooling down. Pilates exercises also included stretching exercises and strengthening exercises (activation).

Table 1: Pilates exercise protocol

movement	repetition	round	intensity	rest	Description	Type of exercise
-	-	-	9-10	-	minutes 10	General warm-up
<b>dogfish, color canvas, roll-up, single-leg stretch, cobra</b>	4-8	1	14-16	8 Inhale and exhale	Inhale and exhale one movement at a time	Stretching exercises
<b>Pull-ups, pull-ups, twisting pull-ups, hunched, criss-cross</b>	8 -4	1	16 -14	One ) 0 breath (only	Inhale and exhale one movement at a time	Strengthening exercises
-	-	-	9-10	-	minutes 10	(Activation)

In order to measure the anthropometric variables, the weight was measured with minimum clothing and without shoes and using a digital balance with an accuracy of 100 grams. Height was measured using an inflexible tape measure while standing next to a wall. Body mass index was calculated with the ratio of weight (kilograms) to the square of height (meters). The maximum oxygen consumption (VO<sub>2</sub>MAX) of the subjects was evaluated using the Rockport test. The subjects in the supplement groups also consumed 1000 mg of Royal Oral Gel manufactured by S.A. Martine Nieto Pharmaceutical Company in Spain daily for 8 weeks (16). To measure biochemical variables, blood sampling was done 24 hours before and after the last training session at 7-8 in the morning and after 8-10 hours of fasting. In each step, 5 ml of blood was taken from the subjects' brachial vein. To separate the serum, the blood samples were kept in an incubator at 37 degrees for 30 minutes and centrifuged at 3000 rpm for 10 minutes and finally the serum was extracted. Then the extracted serum was frozen at -20°C to measure TNF- $\alpha$ , CRP, IL-6, glucose and serum insulin. The levels of IL-6 and TNF- $\alpha$  were measured using a human kit (manufactured by BOSTER, China) with a sensitivity of less than 3.0, respectively.

Pico grams per milliliter and 1/. Pico grams per milliliter was determined using the ELISA measurement method with a Biotek ELISA reading device made in the United States. CRP was measured using a bionic laboratory kit made in Iran with a sensitivity of 1.95 mg/liter using a mindray Bs-800 device. Serum insulin level using Mercodia AB lab kit made in Sweden by ELISA method with 15/ sensitivity. micro units per milliliter and the percentage of intra-subject variation was measured as 6.48. Fasting glucose was also measured by glucose oxidase enzyme method (Pars Azmoun, Iran) with a sensitivity of 5 mg/dL and a percentage of intra-subject variation of 1.19.

The Shapiro-Wilk test was used to determine the normality of data distribution. Also, analysis of covariance (Ancova) test was used to check the significant difference of each of the research variables between the training and control groups. The significance level was considered for all calculations ( $P < 0.05$ ). All statistical operations were performed using SPSS version 24 software.

### 3. Results

Individual characteristics of subjects (anthropometric characteristics and body composition) are given in Table 2. First, the research data has been described using descriptive statistics including mean and standard deviation. The results of the analysis of covariance between the experimental and control groups in the values of TNF- $\alpha$ , CRP, IL-6, glucose and insulin in the subjects' serum before and after 8 weeks of Pilates training with the use of royal jelly are presented in Table 3. As a result of 8 weeks of Pilates training with royal jelly in obese diabetic women, the levels of TNF- $\alpha$ , CRP, IL-6, glucose and serum insulin decreased significantly ( $P < 0.05$ ). The results of Ben Feroni's post hoc test revealed that there was a significant difference in TNF- $\alpha$  and IL-6 between the training + supplement group and the control group ( $P = 0.000$ ). The results of Bonferroni's post hoc test showed that in the CRP variable, this significant difference was related to the supplement group with the exercise + supplement group ( $P = 0.045$ ), the exercise and exercise + supplement group with the control group ( $P = 0.000$ ). Also, the results of Bonferroni's post hoc test indicated that there was a significant difference in serum glucose and insulin between the control group and other groups ( $P = 0.000$ ). As a result of 8 weeks of Pilates training with royal jelly in obese diabetic women,  $Vo_{2max}$  values increased significantly ( $P < 0.05$ ). The results of Bonferroni's post hoc test showed that in the  $Vo_{2max}$  variable, this significant difference was related to the supplement group with the exercise group ( $P = 0.017$ ) and the exercise group and supplement exercise with the control group ( $P = 0.000$ ).

**Table 2: Average anthropometric characteristics and body composition**

Groups		Age (Year)	Height (cm)	Weight (kg)	Body mass index ( $kg/m^2$ )
Control	Pre test	31.2±45.65	1±165.82	84.64 ±03.2	75.30±0.469
	Post test			85.2±41.02	02.31 ±0.481
supplement	Pre test	31.3±54.44	164±1.07	84.1±36.88	08.31±0.706
	Post test			83.1±05.91	65.30±0.666
Exercise	Pre test	32.3±18.06	165±1.35	85.2±68.16	60.31±0.752
	Post test			84.1±27.73	70.30±0.703
+ Exercise supplement	Pre test	31.3±81.06	164±1.75	82.2±34.01	35.30±0.335
	Post test			78.1±60.78	99.28±0.420
Significance level	F			Sig=0.000 F=171.05	Sig=0.000 F=148.79

**Table 3: Variables of the research stage before and after eight weeks of Pilates practice and consumption of royal jelly with covariance test**

\* A significant level ( $P \geq 0.05$ ) is considered.

Variables		Control	supplement	Exercise	Exercise + supplement	F	Sig	Effect size
<b>TNF-<math>\alpha</math></b> (pico gram per milliliter)	<b>Pre test</b>	21.3 $\pm$ 23.23	22.3 $\pm$ 14.84	21.4 $\pm$ 37.30	21.4 $\pm$ 40.09	4.27	*0.011	0.248
	<b>Post test</b>	21.2 $\pm$ 13.82	21.3 $\pm$ 10.82	20.4 $\pm$ 32.66	19.3 $\pm$ 86.20			
<b>CRP)</b> milligrams per liter)	<b>Pre test</b>	5.3 $\pm$ 13.04	5.4 $\pm$ 11.04	4.3 $\pm$ 98.51	4.3 $\pm$ 97.42	7.05	*0.001	0.352
	<b>Post test</b>	5.3 $\pm$ 15.07	4 $\pm$ 5.30	4.3 $\pm$ 82.72	4.3 $\pm$ 66.13			
<b>IL-6</b> (pico gram per milliliter )	<b>Pre test</b>	25.3 $\pm$ 0.412	21.3 $\pm$ 0.311	$\pm$ 0.378	06.3 $\pm$ 0.433	8.08	*0.000	0.383
	<b>Post test</b>	25.3 $\pm$ 0.304	$\pm$ 82/2./236	20.3 41.2 $\pm$ 0.715	35.2 $\pm$ 0.483			
<b>Glucose</b> ( Millimol/liter)	<b>Pre test</b>	76.10 $\pm$ 0.728	55.10 $\pm$ 0.751	74.10 $\pm$ 0.766	14.11 $\pm$ 0.910	205.97	*0.000	0.941
	<b>Post test</b>	97.10 $\pm$ 0.795	86.9 $\pm$ 0.779	58.9 $\pm$ 0.777	72.8 $\pm$ 0.796			
<b>Insulin</b> (Millimol/liter)	<b>Pre test</b>	35.7 $\pm$ 0.773	16.7 $\pm$ 0.744	36.7 $\pm$ 0.448	47.7 $\pm$ 0.533	30.85	*0.000	0.704
	<b>Post test</b>	71.7 $\pm$ 0.388	66.6 $\pm$ 0.793	57.6 $\pm$ 0.551	96.5 $\pm$ 0.578			
<b>Vo2max</b> (M1.kg/min)	<b>Pre test</b>	27.1 $\pm$ 83.11	29.1 $\pm$ 15.67	19.28 $\pm$ 1.41	26.28 $\pm$ 0.566	9.86	*.0000	0.523
	<b>Post test</b>	27.2 $\pm$ 11.98	29.1 $\pm$ 94.35	31.2 $\pm$ 45.61	30.1 $\pm$ 52.22			

## 4. Discussion

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced TNF- $\alpha$  in obese diabetic women. Etemad and Zahli (2018) reported that eight weeks of aerobic training and 500 mg of royal jelly significantly reduced TNF- $\alpha$  in overweight women (14). Moulai et al. (2018) showed that 6 weeks of aerobic training and 1000 mg of royal jelly caused a significant decrease in TNF- $\alpha$  in patients with MS (16), which is consistent with the results of the present study. The possible mechanism related to the findings of the present research is related to the effect of sports activity on the amount of cortisol and catecholamines; So that the results of research indicate that sports activity can affect the amount of cortisol, catecholamines, carbohydrate reserves, and these changes themselves lead to an increase in TNF- $\alpha$ . However, cortisol level was not measured in the current research, which can be one of the limitations of the current research. Researchers reported that the administration of royal jelly inhibits capillary permeability in the acute phase of inflammation and reduces the formation of granulation tissue in the chronic phase of inflammation (17). In the present study, it was seen that royal jelly supplement inhibits pro-inflammatory cytokines (TNF- $\alpha$  and IL-6) and CRP by stimulating macrophages from LPS (lipopolysaccharides) and TNF- $\gamma$  (interferon gamma). This type of inhibition does not appear to be caused by cytotoxic effects on macrophages; Rather, it is caused by the effects of royal jelly (18). It has also been seen that TNF- $\alpha$  can bind to oromodulin glycan and this binding is inhibited by oligosaccharides.

Because royal jelly contains large amounts of mannose-rich sugar chains, it acts directly on activated macrophages to inhibit the production of pro-inflammatory cytokines (18). Among the pro-inflammatory cytokines, inhibition of TNF- $\alpha$  by royal jelly is more effective. TNF- $\alpha$  is able to stimulate the cascade of pro-inflammatory cytokines. Effective inhibition of TNF- $\alpha$  production also reduces IL-6 production, which may depend on the effective anti-inflammatory action of royal jelly.

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced CRP in obese diabetic women. Physical activity reduces interleukin-6 and necrosis factor alpha by reducing fat, leptin, adiponectin and insulin sensitivity, and as a result, reduces CRP (19). Also, the evidence shows that the increase in nitric oxide from the endothelial and the improvement of the function of the endothelial wall reduces systemic and local inflammation, and as a result, reduces the production of inflammatory cytokines from the smooth muscles of the endothelial wall, and their final effect is probably the reduction of the production of the inflammatory index CRP from the liver. (20). The reduction of CRP can be attributed to the effect of royal jelly in addition to exercise. Royal jelly contains important compounds such as proteins, sugars, lipids, amino acids, vitamins and minerals; It also has a wide range of medicinal functions such as antioxidant, anti-edematous, antimicrobial, anti-allergic and anti-tumor properties and protective effects on immune, inflammatory and nervous systems (21). Nora et al. (2020) reported a significant decrease in CRP as a result of eight weeks of endurance training and consumption of royal jelly (100 mg/kg) in the muscle tissue of rats with Alzheimer's disease (22).



The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation significantly reduced IL-6 in obese diabetic women. The long-term increase of IL-6 in type 2 diabetic patients, obesity is related to their inactive lifestyle and may be related to the increase in the number of macrophages located in adipose tissue (23). While exercise causes an acute increase in circulating IL-6 from muscles during contraction, which stimulates the release of glucose from the liver. Therefore, the source of IL-6 in adipose tissue versus skeletal muscle and chronic response versus pulsatile response in healthy individuals and diabetic or obese individuals, respectively, may explain the contradiction related to the role of IL-6 in healthy and diseased humans (24). In other words, it can be said that the sources of IL-6 production in obese diabetics are adipose tissue and in healthy people skeletal muscle. It is well established that insulin resistant and obese subjects with low-grade chronic inflammation recover in concert with a decrease in cytokine levels after interventions such as weight loss (23). However, reducing IL-6 levels may be ineffective in the absence of adipose tissue inflammation (24). Recent studies have shown that the release and abundant production of IL-6 from adipose tissue in obese and overweight people causes a negative regulation of metabolism and may play a role in the development of insulin resistance (25). Research has shown that royal jelly has antimicrobial activity. The ability of royal jelly's antibacterial properties can be attributed to its unique fatty acid. HDA-10 has high antibacterial activity against animal and human pathogens. HDA-10 has been reported to have potential medicinal functions due to its antitumor properties, angiogenesis inhibition, and immunogenetic activities.

Inflammatory cytokines such as TNF- $\alpha$ , IL-8, IL-6, IL-1 $\beta$  and TGF- $\beta$  may cause inflammatory diseases. HDA-10 prevents the production of inflammatory cytokines (26). The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation caused a significant decrease in serum glucose and insulin in obese diabetic women. Studies have shown that consuming royal jelly can improve glucose and insulin levels in diabetic subjects. Askari et al. (2016) reported a significant decrease in serum glucose in type 2 diabetic rats due to the consumption of royal jelly (27). Yilaghi Ashrafi et al. (1400) also reported a significant decrease in glucose and insulin resistance index as a result of eight weeks of intense intermittent exercise and consumption of royal jelly (28), which is in line with the results of the present study. Royal jelly contains large amounts of phenolic compounds from the flavonoid family, the most important of which are quercetin, kaempferol, apigenin, and luteolin. Flavonoids affect diabetes in several ways, these compounds regulate carbohydrate and lipid metabolism and reduce hyperglycemia, dyslipidemia, and insulin resistance, and prevent oxidative stress and inflammatory responses. Flavonoids (especially quercetin) also prevent weight loss in diabetes (29). Therefore, royal jelly with its flavonoid content may have prevented the weight loss of obese diabetic women in the present study. Apigenin and quercetin inhibit the oxidative stress caused by streptozotocin in beta cells, liver and kidney and reduce free radicals (30) Apigenin and kaempferol have hypoglycemic effect in diabetic rats and can reduce fasting glucose. This result was also observed in this study (30).

With its strong antioxidant properties, royal jelly fights against reactive oxygen species such as hydroxyl radicals and superoxide anions and significantly reduces lipid peroxidation and increases antioxidants in the pancreatic tissue of type 2 diabetic patients, which according to According to the mentioned materials, some of these effects are probably due to the presence of flavonoids in royal jelly. The hypoglycemic effect of royal jelly can also be attributed to the vitamins in it (31). Studies have shown that vitamins B, C, D, E, biotin and niacin are abundantly found in royal jelly. Vitamin C reduces the level of serum glucose in type 2 diabetes (31) and in many chemical reactions, it competitively replaces glucose and prevents the glycosylation of proteins, especially hemoglobin and lipoproteins. Vitamins B1, B6, B12, D and E, biotin and niacin also strengthen the function of beta cells and by stimulating the production of glycogen and inhibiting gluconeogenesis, it reduces the glucose level in patients with diabetes. Therefore, part of the role of royal jelly in reducing glucose and insulin can be attributed to the vitamin compounds in it (32).

The results of the present study showed that eight weeks of Pilates training and royal jelly supplementation caused a significant increase in  $Vo_{2max}$  of obese diabetic women. Regular physical activities lead to an increase in plasma volume, which increases venous return to the heart and ventricular preload, and as a result, the stroke volume increases for a certain intensity of exercise. An increase in stroke volume at a similar heart rate increases output, blood flow to skeletal muscles increases, and muscle tissue oxygen is provided to a greater extent than before, as a result, maximum oxygen consumption increases.

Antioxidant supplements also increase oxygen supply to the brain, reduce fatigue, prevent blood clots and blood vessels from clogging, for this reason it improves blood flow and oxygen supply to the skeletal muscles, and probably increases the body's oxygen consumption. (33).

## Conclusion

In general, it can be said that eight weeks of pilates training and royal jelly supplementation significantly reduced TNF- $\alpha$ , CRP, IL-6, glucose and serum insulin in obese diabetic women. The findings of this research showed the beneficial role of Pilates exercises and the use of royal jelly supplements on the level of inflammation and blood sugar control in obese women with type 2 diabetes. Also, according to the results of the research, it can be said that the use of two intervention methods, regular exercise and consumption of royal jelly together, will make blood sugar control more effective in the form of a reduction in serum glucose and insulin in these people. The existence of a significant difference in TNF- $\alpha$ , CRP, and IL-6 indicates the effective inhibitory role of pilates exercises and consumption of royal jelly in the production of pro-inflammatory cytokines and CRP from stimulated macrophages. Therefore, performing Pilates exercises along with royal jelly supplement is recommended as an effective method in treating diabetes and reducing inflammation for obese diabetic women.

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## **Author contributions**

Conceptualization: **N.R.**; Methodology: **N.R.**; Software: **N.R.**; Validation: **N.R.**; Formal analysis: **N.R.**; Investigation: **N.R.**; Resources: **N.R.**; Data curation: **N.R.**; Writing - original draft: **N.R.**; Writing - review & editing: **N.R.**; Visualization: **N.R.**; Supervision: **N.R.**; Project administration: **N.R.**; Funding acquisition: **N.R.**

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## **Compliance with ethical standards**

**Conflict of interest** None declared.

**Ethical approval** The Ethics Committee of Islamic Azad University, East Tehran Branch approved the study.

**Informed consent** Informed consent was obtained from all participants.

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