



Effects of Pectin Supplementation and 14 Weeks of Endurance Training on the Serum Atrial Natriuretic Peptide Levels and Lipid Profile of Obese Pregnant Mice

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p>	<p>Introduction: The present study aimed to evaluate the effects of pectin supplementation and 14 weeks of endurance training on the serum atrial natriuretic peptide (ANP) levels and lipid profile of obese pregnant mice.</p>
<p><i>Article History:</i> Received: 23 Oct 2019 Accepted: 04 Dec 2019 Published: 01 Jan 2020</p>	<p>Methods: This experimental study was conducted on 40 female laboratory mice (mean weight: 30±5 g), which were purchased from Tehran Pasteur Institute, Iran and received a high-fat diet with canola oil. The animals were randomly divided into four groups of 10, including control, pectin intake, endurance training, and pectin intake with endurance training. Training was performed for 11 weeks (five sessions per week) with the intensity of 30-65% MEA on the treadmill. After becoming pregnant, the exercise groups were trained on a treadmill for three weeks five days per week with the intensity of 20-60% MEA. In the pectin groups, 400 milligrams of pectin per every kilogram of the body weight was injected gavage during days 2-19 of pregnancy. Data analysis was performed using the Kolmogorov-Smirnov test, analysis of covariance (ANCOVA), and least significant difference (LSD) test.</p>
<p><i>Keywords:</i> Pectin Endurance Training Lipid Profile ANP</p>	<p>Results: Maternal blood sampling was performed on day 19 of pregnancy to measure serum ANP, total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL), LDL/high-density lipoprotein (HDL) ratio, reduced carbohydrate (RC), and HDL levels. The ANP levels decreased in the pectin group, while it increased in the exercise and exercise with pectin groups (P=0.056); however, the difference was not considered significant. Moreover, the serum levels of TC (P=0.012), TG (P=0.029), LDL (P=0.049), LDL/HDL (P=0.011), and RC (P=0.043) significantly decreased in all the experimental groups (P=0.05), while the HDL levels significantly increased in these groups.</p> <p>Conclusion: According to the results, pectin consumption and exercise could effectively decrease serum TC, TG, LDL, LDL/HDL ratio, and RC and increase HDL.</p>

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Introduction

Pregnancy is associated with various physiological changes, such as weight gain, diabetes, and cardiovascular diseases (1). With maternal health care before and during pregnancy, pregnant women and their newborns will be protected against the potential risk of excessive illnesses (2). Overweightness and obesity are important risk factors for women before pregnancy (3), which also increase the risk of miscarriage.

Congenital heart and neurological deficits are mainly caused by the changes in maternal hormone levels, which also appear in the fetus due to obesity (4). Increased prenatal illnesses lead to the birth of infants with severe health issues, while imposing substantial costs on families (5). Brain-type natriuretic peptide (BNP) and atrial natriuretic peptide (ANP) are the natriuretic brain and atrial peptides, which are considered in the diagnosis of heart failure and other such problems. ANP is a 28-amino acid

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chain, which is synthesized from a polypeptide and is often secreted from the vascular heart cells in a naturally healthy heart (6). Increased ANP secretion is an important neutralizing mechanism, which causes sodium excretion and vascular expansion through the inhibition of renin-angiotensin-aldosterone secretion, as well as sympathetic nerve blockade. Moreover, ANP inhibits aldosterone synthesis through the inhibition of cholesterol transfer to cytochrome P450 in the inner mitochondrial membrane (7). Natriuretic peptides are involved in the regulation of blood pressure and blood volume, while their physiological effects are diverse. On the other hand, their concentration varies depending on physiological conditions, such as pregnancy. Considering the role of this hormone in the regulation of the systemic function and homeostasis, the assessment of the changes in its levels due to various factors (e.g., obesity, physical activity, and type of physical activity) could provide valuable data on the prevention and control of cardiovascular diseases (8). Impaired lipid metabolism is considered to be an independent risk factor for the changes in the serum levels of triglyceride (TG), low-density lipoprotein (LDL), and high-density lipoprotein cholesterol (HDLc)(9).

Most of the medicines that are used during pregnancy could have short-term or long-term effects on the fetus. Moreover, some birth defects have been reported to be caused by natural remedies although they have not been disruptive. Consequently, congenital defects could be due to hereditary and environmental factors (10). Reports have suggested that approximately 42-66% of the population in Tehran city (Iran) use at least one complementary medical treatment method, and 38-54% of these individuals use herbal medications (11).

Pectin is an herbal medicine that may have important effects on the maternal and fetal immune system. Pectin is a natural polymer, which forms the cell wall in most plants, approximately 70% of which is galacturonic acid. Pectin is widely used in the food industry as a thickener and stabilizer (12). The main sources of pectin extraction are citrus peel, apple pap, and sugar beet (13). According to the literature, the annual consumption of pectin is 30,000 tons, which increases by 1-5% each year (14). Pectin has cholesterol-reducing properties and lowers the risk of cardiovascular diseases (15).

Physical exercise during pregnancy is an important health implication for pregnant women, which has not been investigated adequately. Many pregnant women refuse to participate in physical exercises or decrease such activities, while some others increase the intensity and duration of their physical activities during this pregnancy. The latest instructions issued by the American College of Obstetricians and Gynecology in December 2015 highlighted the importance of physical activity during pregnancy (16). Physical activity may enhance fetal growth by increasing the maternal and fetal plasma volume, as well as the cardiac output and placental-fetal uterine blood flow (17). Therefore, the consumption of pectin as a dietary supplement during pregnancy along with exercise activities are considered to be extreme physiological changes, which may not only give rise to favorable conditions for the mother and fetus, but they may also be risky to the health of the mother and fetus.

Considering the effects of endurance training on pregnant women and lack of research data on pectin consumption during pregnancy, the present study aimed to investigate the effects of pectin supplementation, endurance training, and their synergistic effects on the serum ANP, total cholesterol (TC), TG, LDL, LDL/HDL ratio, reduced carbohydrate (RC), and HDL in obese pregnant women.

Materials and Methods

This experimental study was conducted on 40 female laboratory mice (mean weight: 30 ± 5 g), which were purchased from Tehran Pasteur Institute, Iran. After transferring the mice to the animal laboratory, they were kept in special cages at the temperature of 20-24°C with 45-55% humidity within a 12-hour light/dark cycle for one week, so that they could adapt to the new environment.

Laboratory rats were pellet-fed with food produced from livestock food production centers, which contained 10 grams of pellets every day per 100 grams of the body weight. In this study, the animal feed was obtained from Karaj Pasteur Institute (Iran) and placed in each cage depending on the weekly weight gain of the animals. In addition, the mice needed 10-12 milliliters of water per 100 grams of the body weight.

The animals had free access to water, which was provided to them in 500-milliliter bottles. For the

induction of a high-calorie diet, the pellets were mixed with canola oil, so that the mice would become obese. It is notable that the research plan was in accordance with the instructions on animal care and use as issued by the National Academy of Sciences Laboratory.

After initial adaptation to the laboratory environment, the animals were randomly divided into four groups of 10, including pectin intake, endurance training, pectin intake with endurance training, and control. The pectin intake groups were injected with 400 mg/kg of pectin via gavage relative to each kilogram of the body weight during days 2-19 of pregnancy (31). An electrical magnetizing mixer (model: Stirrer 110 Electromagnet, Alfa Co., Germany) was used for the solubilization and homogenization of the supplements with water, and the 900-rpm magnetic turbulence was used to completely dissolve the supplements in water to obtain a homogeneous solution.

To measure the speed of the laboratory mice on the treadmill, the treadmill pace was initially set at 18 m/min and increased by 1 m/min every minute until the rats were no longer able to run. This protocol was performed three times, and the average speed was considered as the maximum running speed of the animals (32 m/min). Moreover, a shocker was placed at the end of the treadmill in order to prevent the mice from stopping (the shocker was not used in pre-pregnancy and during pregnancy to stop the mice).

After the animals became familiar with the treadmill, those in the exercise groups exercised on the treadmill for 11 weeks (five sessions per week) at the intensity of 30-65% MEA. At the beginning of the 12th week, each female mouse was placed in a cage with a male rat for pregnancy induction. After 24 hours, vaginal plaques were assessed, and one-day pregnancy was declared. The exercise groups practiced on the treadmill for three weeks (five days per week) at the intensity of 20-60% MEA (32). The obese mice in the control group did not participate in any activities during the study.

On day 19 of pregnancy, blood samples were obtained from the pregnant mice. The obtained blood was poured into test tubes with no anticoagulant, which were placed in an incubator (model: INB400, Memmert, Germany) at the temperature of 37°C for 12 minutes. After

coagulation, the tubes were placed in a centrifuge (model: EBA280, Hettich, Germany) for 12 minutes at 5,000 rpm. Following that, the serum was separated using a sampler, transferred to another test tube, and stored at the temperature of 80°C in a freezer in order to measure the serum levels of ANP, TC, TG, LDL, LDL/HDL ratio, and RC.

In accordance with the instructions of the manufacturer, serum ANP levels were measured using the CK-E91504 laboratory kit (made in USA), and the lipid profiles were measured using the Pars Azmoonkit (made in Iran). All the ethical issues were observed in this study, and the surgeries and biopsies were performed under complete anesthesia. Furthermore, it was attempted to use the least acceptable sample size, and the ethical considerations of working with laboratory animals were approved by the Ethics Committee of Islamic Azad University in 2018.

Statistical Analysis

Data analysis was performed in SPSS version 22 using the Kolmogorov-Smirnov test to determine the normal distribution of the data in the study groups. In addition, the analysis of covariance (ANCOVA) and least significance difference (LSD) test were applied to compare the serum ANP variables between the study groups. The obtained results were expressed as mean and standard error of mean (SEM). In all the statistical analyses, the significance level was considered to be 0.05.

Results

Compared to the control group, the ANP levels decreased in the pectin consumption group and increased in the exercise only and exercise with pectin consumption groups (Table 1). However, the difference was not considered significant ($P=0.056$). According to the obtained results, the experimental groups had significantly lower serum levels of TC, TG, LDL, LDL/HDL ratio, and RC compared to the control group ($P=0.05$).

According to the obtained results, the HDL levels increased in the experimental groups ($P=0.043$), and the difference in this regard was considered significant. Furthermore, a significant difference was denoted in the serum levels of TG ($P=0.029$) LDL ($P=0.049$), LDL/HDL ratio ($P=0.011$), and RC ($P=0.043$).

Table 1. Comparison of ANP and Lipid Profile between Study Groups

Group/ Variable	Pectin (N=10)	Exercise (N=10)	Exercise with Pectin (N=10)	Control (N=10)	F	P- value
ANP(pg/m)	240.73±19.81	402.63±78.86	330.115±65.75	307.77±02.485	3.112	0.056
TC(mg/dl)	❖ 73.6±20.41	❖ 75.6±40.76	❖ 73.6±20.41	❖ 87.5±60.59	5.039	0.012
TG(mg/dl)	❖ 81.5±20.06	❖ 83.5±20.06	❖ 81.5±20.06	❖ 94.9±01.61	3.903	0.029
LDL(mg/dl)	❖ 24.3±80.27	❖ 26.3±40.84	❖ 24.3±80.27	❖ 33.6±20.64	3.268	0.049
HDL(mg/dl)	❖ 157.5±40.59	❖ 154.4±40.15	❖ 157.5±40.59	❖ 147.5±40.59	3.425	0.043
LDL/HDL ratio	❖ 0.0±15.02	❖ 0.0±16.03	❖ 0.0±15.02	❖ 0.0±22.04	5.192	0.011
RC(mg/dl)	❖ 32.6±03.02	❖ 35.6±60.02	❖ 32.6±60.02	❖ 43.5±80.06	3.422	0.043

Covariance and LSD tests were used at significance level of $P \leq 0.05$

❖ Significant difference compared to control group

Discussion

According to the results of the present study, ANP levels increased in the endurance training and pectin consumption and endurance training groups, which is consistent with the results obtained by Gharakhanloo and Habibi (18), who reported increased venous return and catecholamine levels through exercise as the proposed mechanisms of increased ANP (18). After secretion into the plasma, ANP binds to the type A (b) receptors that are located in the brain, blood vessels, kidneys, and adrenal glands. The binding of ANP to these receptors activates guanylyl cyclase, which in turn activates the cGMP signal cascade. As a result, ANP opposes the effects of the aldosterone-angiotensin-renin system (19).

Various studies have reported the sympathetic nerve stimulation and intensity of physical activity as the triggers of ANP release during physical exercise (20). Beta-adrenergic and ANP receptors interact with cardiovascular and metabolic regulation during physical exercise. ANP affects the cardiovascular system through the vascular tone, renal sodium control, and cardiac hypertrophy, which are partially mediated by the stimulation of adrenergic beta-blockers (21).

There have been reports on increased ANP secretion in resistance training. Resistance and speed exercises cause vasodilation and increase the heart power by affecting the blood vessels and heart muscle. As a result, the concordance in the results of speed exercises seems reasonable. Furthermore, vasodilation in speed athletes is likely to expand, rapidly resulting in increased dilation. After physical exercise, venous return increases, and further dilation is applied to the heart muscle due to the mechanism of the muscular and respiratory pumps, which in turn affects ANP secretion (22).

A notable observation in the current research was the miscarriage of all the fetuses in the endurance training group, which indicated the inadequate severity of the physical exercise corresponding to the high level of ANP in endurance training. Some studies have also shown reduced ANP levels following aerobic exercise (23); these studies have been mainly focused on the effects of physical exercise on patients with cardiovascular disorders.

Since the resting levels of ANP are elevated in these patients due to the increased cardiac output, physical exercise is likely to decrease ANP levels through the modulation of this pressure (24). Increased atrial expansion during physical exercise may be due to the central blood volume as the atrial pressure increases the ANP hormone (25). According to our findings, ANP levels reduced in the pectin consumption group, which could be due to the effect of pectin on cholesterol reduction, thereby improving the cardiovascular status.

Approximately 50% of the pectin in apples affects lipid metabolism and reduces plasma cholesterol in the presence of polygalacturonic acid in the pectin structure (26). In a study by Noura et al. (2016), which was conducted on adult male Wistar rats, the therapeutic effects of apple pectin on obesity were examined. The findings of the mentioned study confirmed the anti-obesity effects of the pectin molecule (27), which is consistent with the results of the present study.

Pectin disrupts the uptake cycle of cholesterol through developing a complex of fiber with bile salts to reduce the blood cholesterol. Moreover, the binding of bile salts to polysaccharide compounds plays a key role in the reduction of blood cholesterol. As pectin molecules bind to cholesterol during digestion in the body, they slow down the glucose uptake through trapping carbohydrates (28).

Another research in this regard Šeřčíková et al. (2016) aimed to investigate the effects of pectin on the intestine, and the obtained results indicated that diets containing pectin could significantly decrease the fat content in the body of rats (29). In order to determine the effects of high-intensity and low-intensity physical exercise (e.g., walking), Okura et al. (2012) divided 90 healthy obese women (aged 34-60 years) into three groups of diet only, diet with low-intensity exercise, and diet with high-intensity exercise. In the mentioned study, the subjects exercised three days per week for 14 weeks, and the findings demonstrated that all the risk factors improved significantly in all the groups ($P=0.05$) (30); this is in congruence with the results of the present study. Therefore, it could be inferred that improved blood lipid levels depend on the type, intensity, and duration of physical exercise.

Conclusion

According to the results, the consumption of pectin in the pregnant obese mice could cause positive changes in the cardiovascular status, as well as the serum levels of TC, TG, LDL, LDL/HDL ratio, RC and HDL.

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