

Effect of 12 Weeks of Intense Endurance Training and Bee Pollen Consumption on *ABCA1* Gene Expression in Small Intestine, Liver and Gastrocnemius Muscle of Male Rats

Amir Taghipoor Asramy (PhD candidate)

Department of Biochemistry, Faculty of Physical Education and Sport Sciences, University of Mazandaran, Mazandaran, Iran

Abbas Ghanbari-Niaki (PhD)

Department of Exercise Biochemistry, Faculty of Physical Education and Sport Sciences, University of Mazandaran, Mazandaran, Iran

Shirin Hakemi (PhD)

Department of Clinical Biochemistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Mehran Naghizadeh Qomi (PhD)

Department of Statistics, Faculty of Mathematics, University of Mazandaran, Mazandaran, Iran

Mohammad Mehdi Moghanny Bashi (PhD)

Department of Genetics, Faculty of Medicine, Kazeroon Branch, Islamic Azad University, Kazeroon, Iran

Corresponding author: Abbas Ghanbari-Niaki

Email: ghanbara@umz.ac.ir

Tel: +9891313314878

Address: University of Mazandaran, Mazandaran, Iran

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ABSTRACT

Background and Objectives: The aim of this study was to evaluate the effect of 12 weeks of intense endurance training and bee pollen consumption on *ABCA1* gene expression in small intestine, liver and gastrocnemius muscle tissues of male rats.

Methods: In this study, 24 male Wistar rats (aged 6-8 weeks and weighing 90-110 g) were randomly divided into four groups of saline-control (n=6), saline-training (n=6), bee pollen-control (n=6) and bee pollen-training (n=6). The training groups exercised on a treadmill for 12 weeks (30 m/min, 90 min/day, five days/week). The bee pollen groups were given bee pollen orally (500 mg/Kg) for 12 weeks. Data were analyzed using two-way ANOVA at significance level of 0.05.

Results: *ABCA1* gene expression was highest in the liver, gastrocnemius muscle and small intestine, respectively. The findings also revealed that the intense endurance training caused a non-significant increase in *ABCA1* gene expression in the small intestine and liver. However, the training caused a non-significant decrease in *ABCA1* gene expression in the gastrocnemius muscle. In addition, consumption of bee pollen significantly increased *ABCA1* gene expression in the small intestine and gastrocnemius muscle of male rats. However, the effect of bee pollen on the gene's expression in the liver was not statistically significant.

Conclusion: Based on our findings, it can be concluded that consumption of bee pollen has more beneficial effects on the *ABCA1* gene expression and reverse cholesterol transport compared with the intense endurance training.

Keywords: *ABCA1* protein, Pollen, exercise.

INTRODUCTION

Cardiovascular disease is the leading cause of death in industrialized countries. Fat gain, especially cholesterol, can be an important factor in worsening of the disease (1). In addition, coronary heart disease is directly influenced by high levels of low-density lipoprotein cholesterol (LDL-C) and has an inverse correlation with elevated levels of high-density lipoprotein cholesterol (HDL-C) (2). The preventive effect of HDL is attributed to its role in reverse cholesterol transport. ATP-binding cassette transporter A1 (ABCA1) is involved in the reverse cholesterol transport by facilitating cholesterol efflux from cells to the receptor of lipid-poor apolipoprotein A-I particles, which is then delivered to the liver for excretion as bile salts. Since Apo AI has been shown to bind specifically to ABCA1, the lipid-poor apo A-I (pre β -HDL) acts as a cholesterol and phospholipid receptor in an ABCA1-dependent manner, thus forming mature cholesteryl ester-rich spherical α -HDL particles following the lecithin cholesterol acyl-transferase enzyme activity (LCAT) (3-7). The health benefits of training, particularly on the cardiovascular system, have been well demonstrated. Researchers have shown that physical activity have beneficial effects on lipoprotein profile by reducing level of triglycerides, LDL and VLDL and increasing the level of HDL (8). Physical activity also improves some of the key processes involved in the reverse cholesterol transport, by increasing HDL composition (8), cholesterol efflux (9), APO-1 formation (10, 11), plasma pre β -HDL level (12, 13) and activity of LCAT (10, 14,15).

Since the discovery of the inverse relationship between HDL-C level and risk of cardiovascular disease, the origin of plasma HDL has been studied extensively. Moreover, several attempts have been made to raise plasma HDL levels and find the nature of HDL. The findings of these studies suggest that ABCA1 is critical for maintaining plasma HDL levels in rats. In addition, ABCA1 can be expressed in all body tissues, while only the small intestine and liver are able to produce APOA-1. Rats with gastrointestinal problems have significantly reduced amount of plasma HDL-C and HDL (5-7).

Khabazian et al. showed that short-term endurance training significantly increases

ABCA1 gene expression compared to a control group (16, 17). In addition, Ghanbari-Niaki stated that long-term endurance training improves *ABCA1* gene expression in the heart and gastrocnemius muscle of rats (18). Wellington et al. investigated the level of mRNA and ABCA1 in various tissues of rats and then divided them into three groups. The *ABCA1* gene expression was highest in the liver, kidneys, adrenal glands, heart, bladder, testes and brain; moderate in the lung, adipose tissue, esophagus, stomach and small intestine; and lowest in colon, skeletal muscle, thymus and spleen (19).

Over the past few decades, alternative therapies, especially herbal medicine and dietary supplementation has become more popular for treatment of several diseases including hyperlipidemia (20). Honey is a natural product that has been widely used for its therapeutic effects for almost 5,000 years. The use of honeybee and related products such as honey, venom, propolis, bee pollen and royal jelly became more popular in the 20th century (21). Bee pollen nutritional profile is one of the most complete in the world. To this day, 200 substances including proteins, amino acids, carbohydrates, lipids, fatty acids, phenolic compounds, enzymes, co-enzymes and vitamins have been found in bee pollen from different plant species (20, 22, 23).

Experimental studies on rats and rabbits have demonstrated that bee pollen has lipid lowering activity and causes a decrease in level of plasma lipids and triglycerides. Moreover, bee pollen has been reported to increase HDL and reduce LDL level. Clinical studies confirm that bee pollen causes serum lipids loss, 20-35% decrease in blood lipids and cholesterol and 30% reduction in platelet aggregation, and improves atherosclerosis (24). The liver and gastrocnemius muscle can use lipids as primary fuel during rest and prolonged endurance activity. In the human body, cholesterol is mainly synthesized by the small intestine (18). Bee pollen offers 15% lecithin by volume, which is thought to increase metabolic rate (25). There is currently no study available on the role of training along with bee pollen supplementation in the mechanism of reverse cholesterol transport. Therefore, this study was conducted to determine the effect of 12 weeks of intense endurance training and bee pollen

consumption on *ABCA1* gene expression in the small intestine, liver and gastrocnemius muscle of male rats.

MATERIAL AND METHODS

In the present semi-experimental study, 24 white male Wistar rats aged 8 weeks (mean weight: 90 ± 20 g) were obtained from the Pasteur Institute of Amol, Iran. The rats were kept in a 6×5 meters room under 12:12-h light:dark cycle, at 22 ± 3 °C and 45% humidity. One week was given to the subjects to become familiar with the laboratory environment and manipulations. Using simple random sampling, the subjects were divided into four groups of saline-control, bee pollen-control, saline-training and bee pollen-training. The rats in all groups were matched in terms of body weight.

The training groups exercised on a treadmill at intensity of 30 m/min, five days a week, for 12 weeks. The rats were subjected to run at low intensity (15 m/min, for 20 min), but later, speed and duration of exercise were gradually increased. In the first three weeks, the subjects were able to run at 30 m/min for 90 min, five days/week. The subjects ran on the treadmill at a fixed intensity and duration for the next nine weeks. Rats in the control group walked freely on a treadmill, three sessions per week, for 10 minutes (26).

Immediately after training, 500 mg aqueous extract per Kg of body weight were administered orally to the rats in the control and bee pollen-training groups, five times a week for 12 weeks. Equal amount of saline solution (normal saline) was given to the saline group.

The rats were anesthetized 48 h after the last training session by intraperitoneal injection of ketamine (80 mg/Kg) and xylazine (10 mg/Kg) while fasting for three hours (water was available). Approximately 50 mg of tissues were powdered by incubation. The powders were homogenized in 800 μ L of TRizol (Invitrogen, USA) for extraction of total RNA. The samples were centrifuged at 13000 g for 15 min at 4 °C to separate protein components and phenolic compounds. One ml of chloroform was added to the supernatant, and the mixture was vortexed for 15 seconds. The mixture was centrifuged again at the mentioned conditions. Mineral and aqueous phases were separated. The supernatant containing RNA was mixed with 800 μ L of

isopropanol, and the mixture was incubated for 15 min at room temperature, and centrifuged at the mentioned conditions. The extracted RNA was dissolved in 50 μ L of RNAase-free water and stored at -80 °C for future testing. RNA integrity was checked by agarose gel electrophoresis and detection of RNA band between 18s and 28s. The RNA quality and concentration were assessed using Nanodrop (Pharmacia, Sweden), and absorption at ratio of 260/280 nm was between 1.6 and 1.8 for all samples.

All procedures were performed under a laminar hood (Zal Tajhiz Co., Iran) previously sterilized with alcohol (75%) and UV. Revert Aid H Minus First Strand cDNA kit (Product code: K1631) was used for cDNA synthesis in a thermocycler (Bio Rad, USA), according to the manufacturer's instructions and using random hexamer primers. The relative mRNA level of *ABCA1* gene was measured in small intestine, liver and gastrocnemius muscle tissues by semi-quantitative RT-PCR (Bio Rad, USA). All samples were analyzed in duplicate. After obtaining the binary CT for each sample, mean values were determined. Data were entered into Microsoft Excel and the level of *ABCA1* expression was calculated according to delta-delta CT formula.

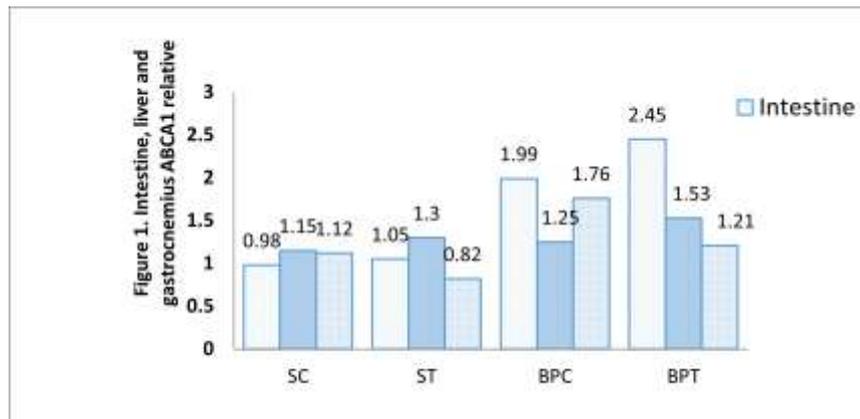
Data were analyzed in SPSS (version 20). Normality of data distribution was verified using the Shapiro-Wilk test. Levene's test was used to assess the equality of variances. Two-way analysis of variance and Scheffe post hoc test were used to evaluate differences between the groups. All statistical analyses were performed at 95% confidence level ($P < 0.05$).

RESULTS

After the 12 weeks of intense endurance training, *ABCA1* gene expression increased in the small intestine tissues from the training group ($P > 0.05$). *ABCA1* gene expression in the small intestine of pollen-control group increased significantly compared to the control group (Figure 1).

Moreover, the training and bee pollen supplement had no statistically significant effect on the mRNA content of *ABCA1* in the liver (Figure 1). Supplementation of bee pollen caused a significant increase in *ABCA1* gene expression in gastrocnemius muscle, while the endurance training decreased *ABCA1* gene expression in the gastrocnemius muscle ($P > 0.05$).

Figure 1- Real time PCR of Intestine, liver and gastrocnemius ABCA1 relative mRNA expression in Saline-Control (SC), Saline-Training (ST), Bee pollen-Control (BPC), and Bee pollen-Training (BPT) wild type male Rats Data expressed as mean±SE.



DISCUSSION

The results of the present study showed that the expression of *ABCA1* gene was highest in the liver, gastrocnemius muscle and small intestine of rats, respectively. However, there was no significant difference in the expression of *ABCA1* gene between the study groups. This is somewhat similar to the findings of Farke et al., demonstrating that *ABCA1* gene expression is highest in the skeletal muscle, liver and small intestine of cows, respectively (27). It should be noted that the difference in *ABCA1* gene expression between skeletal muscle and other tissues could be attributed to the structure of skeletal muscle (oxidative and glycolytic). However, our study investigated the male rat gastrocnemius muscle.

We detected a non-significant *ABCA1* overexpression in the small intestine following the intense endurance training. Although several studies have clarified the role of liver in the synthesis and expression of *ABCA1*, few studies have investigated the level of *ABCA1* gene expression in the small intestine.

An investigation revealed that *ABCA1* in the small intestine is responsible for about 30% of plasma HDL synthesis. *ABCA1* in the small intestine is directly involved in conversion of cholesterol into HDL (28, 29). A study by Khabazian et al. showed that endurance training improves *ABCA1* gene expression in the small intestine (17).

We found that supplementation of bee pollen significantly increased *ABCA1* gene expression in the small intestine. Empirical studies conducted on rats and rabbits show that pollen has lipid lowering activity, which can contribute to reduction of total lipid content and triglycerides (24). Bee pollen also reduces

the level of hormones such as insulin and testosterone, which are responsible for lipid synthesis (23). In addition, bee pollen increases lipid efflux from adipose tissue because of its high niacin content. It also improves functional bowel disorders by reducing reactive oxygen species and elevating excess cholesterol efflux to macrophages via *ABCA1* (30).

The intense endurance training and bee pollen supplementation caused a non-significant increase in *ABCA1* gene expression in the liver tissues. *ABCA1* is largely expressed in the liver and has a key role in cholesterol and phospholipids efflux from the cells into lipid-free/lipid-poor apolipoproteins. Our findings are consistent with results of some previous studies (16). Based on our results, regular endurance training could increase *ABCA1* gene expression in the liver of rats. Physical activity can elevate HDL and cause LXR and PPAR overexpression in the liver, which in turn regulate the *ABCA1* gene activity (31). On the other hand, high glucocorticoids and low insulin levels following long-term endurance activities promote *ABCA1* gene expression in hepatocytes.

The intense endurance training reduced *ABCA1* gene expression in gastrocnemius muscle. However, this effect was not statistically significant. In recent years, several studies have been conducted on the effects of exercise on *ABCA1* gene expression. According to these studies, endurance training increases *ABCA1* gene expression in the liver, small intestine and gastrocnemius muscle (32). Our results regarding the expression of *ABCA1* in the gastrocnemius muscle are inconsistent with findings of Ghanbari et al., which could

be due to difference in the type of training and method of *ABCA1* expression assessment (semi-quantitative). It is also possible that another transporter, such as *ABCG1* (unreported), is more active than *ABCA1* in the gastrocnemius muscle (33). We demonstrated that taking bee pollen significantly improves *ABCA1* expression in the gastrocnemius muscle of male rats. It is well demonstrated that bee pollen has a beneficial role in skeletal muscle protein metabolism due to the synergistic effect of various nutrients such as leucine and antioxidants (34). Moreover, some of the components of pollen such as flavonoids increase gene expression and protein synthesis in muscle cells (35). In addition, bee pollen improves mitochondrial function in skeletal muscle by increasing citrate synthase (25). Although apoA1 cannot be expressed in the skeletal muscle (unreported), it can enter the skeletal muscle from other tissues. ApoA1/HDL ratio affects glucose uptake in skeletal muscles through *ABCA1*. Therefore, it can be concluded that *ABCA1* is an important transporter in the reverse cholesterol transport from other tissues to the liver, and plays a role in activation of AMPK in skeletal muscles, which is essential for glucose homeostasis, lowering blood sugar and increasing lipid oxidation (33). On the other hand, bee pollen consumption caused a significant increase in expression of *ABCA1*

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gene in the gastrocnemius muscle.

CONCLUSION

The intense endurance training could increase *ABCA1* gene expression in the small intestine and decrease the expression of the gene in the gastrocnemius muscle, but had no effect on the liver. Moreover, bee pollen consumption increases *ABCA1* gene expression in the small intestine and gastrocnemius muscle. Bee pollen also caused a non-significant overexpression in the *ABCA1* gene in the liver.

It can be concluded that consumption of bee pollen has more beneficial effects on the *ABCA1* gene expression and reverse cholesterol transport compared with the intense endurance training.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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