Visceral Fat ABCG1, ABCG5 and Visfatin Gene Expression in Response to a Treadmill Running Program with or without a Liquid Pistachio-atlantica (Bene) Extraction in Female Rats

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Abstract

ATP-binding cassette (ABC) transporters including ABCG1 and ABCG5, use the energy of ATP hydrolysis to translocate a wide variety of substrates across biological membranes. Visfatin, a novel adipokine, was revealed to be associated with obesity and to have insulin mimetic effect that is highly expressed in visceral adipose tissue. The aim of this study was to determine the visceral fat ABCG1, ABCG5 and visfatin relative gene expression.

Twenty wistar rats (6-8 weeks old and 125-135 g weight) were used. Animals were randomly assigned into saline-control (SC), saline-training (ST), and Bene-control (BC), and Bene-training (BT). Training groups was given exercise on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/day and 5 days/week for eight weeks. Subjects were fed oral, with Bene extraction and saline for four weeks. ABCG1, ABCG5 and visfatin relative genes expression was detected by Real-time PCR method. Results demonstrated that Bene extraction significantly reduces ABCG1 and ABCG5 relative gene expression and increase visfatin relative gene expression in visceral fat. Exercise training significantly reduces visfatin relative gene expression and increases ABCG1 and ABCG5 relative gene expression in visceral fat .

Keywords: ABCG1; ABCG5; visfatin; female rats; Treadmill exercise; Pistachia atlantica

List of abbreviations: Pistachia atlantica (Bene); saline-control (SC); Bene- control (BC); saline-training (ST); Bene-training (BT)

Introduction

Coronary artery disease (CAD) is one of the major causes of death in most societies that is associated with the concentration of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C lower) (1). Formation of HDL and its rearrangement process is complex and requires a variety of factors, such as lecithin cholesterol transferase (LCAT), cholesterol ester transport protein (CETP), phosphoinositide lipid carrier protein (PLTP), and ATP-binding cassette (ABC) transporters including ABCG1 and ABCG5 (2,3). ATP-binding cassette (ABC) transporters mediate the translocation of a wide variety of substrates such as ions, sugars, amino acids, vitamins, lipids, antibiotics and drugs to larger molecules (4). ABCG1 is the first member of the ABCG subfamily. The protein is expressed in many cell types (including macrophages, endothelial and epithelial cells, T and B cells, type II cells, astrocytes and neurons) and in numerous tissues including the brain, eye, kidney, spleen, lung, liver, and intestine (5-7). ABCG5 and ABCG8 are half-transporters that form heterodimers to become functional (8-10). Both proteins are expressed at a high level in the liver and intestine

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and at lower levels in the colon (11-15). In the intestine, ABCG5/G8 secrete absorbed plant sterols back into the intestinal lumen, while on the bile canaliculus ABCG5/G8 mediate biliary plant sterol as well as cholesterol secretion into bile (16, 17) .

In recent years, many researches have been done on the ABC family. One of these researches was carried out by Ghanbari-Niaki et al and investigated the effect of 6 weeks' endurance exercise on ABCA1 gene expression in rats. They reported that ABCA1 gene expression increases in rats' liver and that the plasma levels of high density lipoprotein- cholesterol (HDL-C), Pre-β HDL and lecithin cholesterol acyltransferase (LCAT), significantly increased (18). Khabazian et al showed that 12 weeks of aerobic exercise, increased mRNA expressions of ABCA1 gene expression in rat's small intestine (19). Also Zare-Kookandeh et al investigated that 8 weeks of endurance exercise, increased ABCG1 gene expression in small intestine and kidney tissue of rats (20).

Visfatin is an adipokine identified in 2004 (21), and thus named for the suggestion that it would be predominantly produced and secreted in visceral fat. Visfatin was found to be released predominantly from macrophages rather than from adipocytes in visceral adipose tissue (21). It is now believed that visfatin actions can be endocrine, paracrine, and autocrine as well. These autocrine effects of visfatin may play an important role in regulating insulin sensitivity in the liver (22). Plasma visfatin levels are elevated in patients with type2 diabetes and in obesity (23-25). Physical exercise is a metabolic and neuroendocrine stressor that mobilizes lipids for energy and is a cornerstone treatment for obesity and diabetes (26-28).

Visfatin has insulin-like performance and causes stimulation of glucose uptake in adipose cells and myocytes, Inhibits the release of glucose by the liver and accumulation of triglycerides. Haus et al investigated that 12 weeks' endurance exercise (intensity 80% HR max, duration: 60 min/session and 5 days a week), Causes weight loss along with the significantly reduction in plasma visfatin levels (29). Also Ghanbari-Niaki et al reported a single exercise session that consisted of a running-based high-intensity sprint test, Causes Plasma visfatin concentrations were significantly increased immediately after exercise and returned to baseline during the recovery period. An increase in plasma insulin was observed immediately after exercise. This response returned to baseline by 45 min and remained at this level at 90 min after the exercise. Similarly, glucose concentrations were significantly increased immediately after exercise and had returned to baseline at 45 and 90 min after exercise (30). However, no research has examined the effect of exercise on ABCG1, ABCG5 and visfatin genes expression in visceral fat. Also knowledge about the effect of Pistachia atlantica on these genes expression is lacking. Pistacia atlantica (Bene) are plants of Anacardiaceae family that is rich in antioxidants and unsaturated fatty acid. It is Shown that this plant leaves contain anti-oxidative compounds that reduce the amount of free radicals (31). The total amount of essential oil obtained from Pistachia atlantica is higher than any other species of the genus Pistachia (32). Also Pistachia has anti-inflammatory effect. In this study we investigated visceral fat ABCG1, ABCG5 and visfatin genes expression after 8 weeks of treadmill running program and Bene extraction in wild type female rats.

Material and methods

Plant material

The ripped fruit samples of Pistachiaatlantica (Bene) were collected from the fields of Maibod in the Yazd province of Iran, and were stored at –18 ° C until use. Plant material was identified by herbarium collection in department of physical education and sport science, university of Mazandaran, Baboulsar, Iran.

Preparation of the extracts

The extraction was prepared according to the Hamdan. et al (2004) (33). Briefly, the whole ripped and dried fruit of Pistachiaatlantica (Bene) (10g) was coarsely powdered and mixed with 150 ml of tap water and boiled for 45 min and then cooled at room temperature. After cooling, the mixture was filtered twice by using a Watman filter (No. 4 filter). The volume of the filtered solution was increased to 100 ml with tap water so that 1ml was equivalent to 100 mg of starting material. It has to be noted that we did not used distilled water on the basis of herbalist's recommendation. A fresh extraction was orally given at dose 100 mg/kg (7.5ul/g of body weight) immediately at the end of the training session for six week. The control groups have been treated at same manner and volume.

Animals

All experiments involving the animals were conducted

according to the policy of the Iranian convention for the protection of vertebrate animals used for experimental and other scientific purposes; and the protocol was approved by the Ethics Committee of the Sciences, University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS, Mazandaran, Iran. Twenty Wistar female rats (6-8 weeks old125-135 g weight) were acquired from Pasteur's Institute (Amol, Mazandaran) and maintained in the Central Animal House of Faculty of Physical Education and Sports Science of UMZ. Five rats were housed per cage (46-L volume) with a 12-hour: 12-hour light-dark cycle. Temperature was maintained at 22°C ± 1.4°C. Diets (a pellet form) and water were provided adlibitum. Animals were randomly assigned into control (n = 10) and training (n = 10) groups. Rats were divided further into saline-control (SC), saline-training (ST), and Bene-control (BC), and Bene-training (BT). The control group remained sedentary, whereas the training group underwent a moderate running exercise program.

Exercise training protocol

At first, the animals were familiarized with the rat treadmill apparatus, every day and for 4 days [(the 14-lane motorized-driven treadmill was designed by the primary author (UMZ, Baboulsar, Mazandaran, Iran)]. The exercise group was trained for 8 weeks using the same training methods previously described (18, 19). The rats run at 25 m/min for 60 minutes, 5 d/wk. The animals were killed 72 hours after the last exercise session. Food but not water was removed from the rat cages 4 hours before the sacrifices. The estrous cycle was determined in intact female rats by taking vaginal smears each morning by vaginal lavage. Smears were analyzed under a microscope to determine the type of cells present and the stage of the estrous cycle (34). Only female rats showing at least two consecutive 4- or 5-day estrous cycles were used. The established estrous cycle in each female was used to select the day of the experiment, at which time the estrous cycle stage was confirmed by vaginal smear (35).

Tissue biopsies

Seventy-two hours after the last training session, rats were anesthetized with intra peritoneal administration of a mixture of ketamine (30– 50 mg / kg body weight) and xylazine (3– 5 mg / kg body weight).The visceral fat was excised, cleaned, divided into two pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen and stored at − 80 ° C until RNA extraction.

RNA isolation, cDNA synthesis and Real-time PCR

Total RNA was extracted from 80 to 100 mg of tissue using RNA purification kits (AccuZol, Bioneer, Cat.No: k3090). Complementary DNA (cDNA) was extended from 1µl oligo-(dt)18 primers (0.25 µg per reaction) using cDNA synthesis kit (Accu Power RT PreMix, Bioneer, Cat.No: k2041-B) according to the manufacturer's instructions. Complementary DNA concentration was 1 to 2 ng/25µl reaction. Real-time quantitative PCR was performed using Quanti Fast SYBR Green PCR Kit (Cat. No. 204052; Qiagen, GmbH, Germany) in using 15 µl reaction containing 0.5µl single-strand cDNA,7.5µlMaster Mix, 1µl of the each forward and reverse primers (5 pmol/µl) and 5µl dH2O. The primers for ABCG1, ABCG5, visfatin and β-actin (as normalizer) were taken from Sporstøl et al, 2007, Yu et al., 2003, Josephs et al., 2007 and Gao &Yuan, 2010, respectively (Table 1) (36-39). Real-time PCR reactions were performed using the Rotor Gene 3000 real time PCR system from Corbett using following program: step1:95 °C for 5 min and step2:40 cycle of 95 °C for 10 sec and 60°Cfor 30 sec. The last heating step in phase 2 was carried out for generation of a melting curve of the product. The amplicons were melted at the rate of 0.1°C/s to generate the high resolution melting profile.

Statistical analysis

The relative levels of mRNA were analyzed by the 2–ΔΔCtmethod. CT for each sample determined using Rotor-Gene 3000 Software. Briefly, Δ-CT value was calculated by taking the CT of the ABCG1, ABCG5 and visfatin gene and subtracting it from CT of β-actin. The ΔΔ-CT was calculated by subtracting the Δ-CT (sample) values from the Δ-CT (control). The relative quantification was then calculated by the expression 2-ΔΔCT (40).

The Kolmogorov-Smirnov test was used to determine the normality of distribution, and variables were found to be normally distributed. All results are expressed as means ± SEM. Statistical analysis were performed using a one way analysis of variance. Least significant difference (LSD) post hoc test was used in the event of a significant ($P < .05$) F ratio. All statistical analysis was performed with SPSS (Version 13; SPSS, Chicago, IL).

Table1. Oligonucleotide primer sequences and real-time PCR amplification parameter

Gene	Forward and reverse primer sequences	Annealing temperature (°C)	Amplicon size (bp)	Gene accession no.
ABCG1	F:5′-GAAGGTTGCCACAGCTTCTC-3′ R:5′-CATGGTCTTGGCCAGGTAGT-3′	55	339	NM_053502
ABCG5	F:5′-AGGCTCAGTTACAGGCTCAGAG-3′ R:5′-GTCCCACTTCTGCTGGCATGAT-3′	60°C	118	AF312714
Visfatin	F:5′-AGCGGCAGAGCACAGTACCATA-3′ R:5′-CCACAGACACAGGCACTGATGA-3′	60°C	101	NM_177928
β-actin	F:5′-TATCGGCAATGAGCGGTTCC-3′ R:5′-AGCACTGTGTTGGCATAGAGG-3′	55-60°C	145	NM_031144

Results

ABCG1, ABCG5 and visfatin relative gene expression in visceral fat were determined in female rats. Data analysis revealed a significant difference in visceral fat ABCG1 mRNA relative abundance between groups (F=12.53, P<0.001) (Fig.1). Using a suitable following post hoc test, data were showed that visceral fat relative expression of ABCG1 was higher in ST group when compared with other groups at the end of program (Fig.1). A significant difference was also found in visceral fat relative mRNA expression of ABCG5 at the end of treadmill running program (F=3.75, P<0.034). In this regard, the ABCG5 mRNA relative abundance was lower in Bene treated animals when compared with Bene-Control group (Fig.2). Consider to Fig 3, the visceral fat visfatin, was higher in bene groups when compared with saline groups (F=4.731, P<0.015) (Fig.3).

Figure 1: Real-time PCR of Visceral fat ABCG1 mRNA relative expression in saline- control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per group. SC vs. ST, (P < 0.001), ST vs. BT, (P < 0.001)

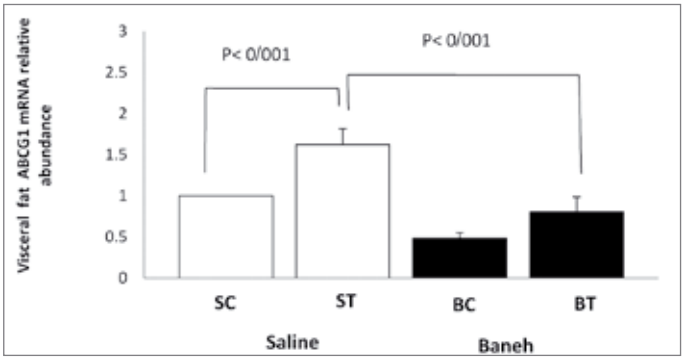


Figure 2: Real-time PCR of Visceral fat ABCG5 mRNA relative expression in saline- control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per group. BC vs. BT, (P < 0.029)

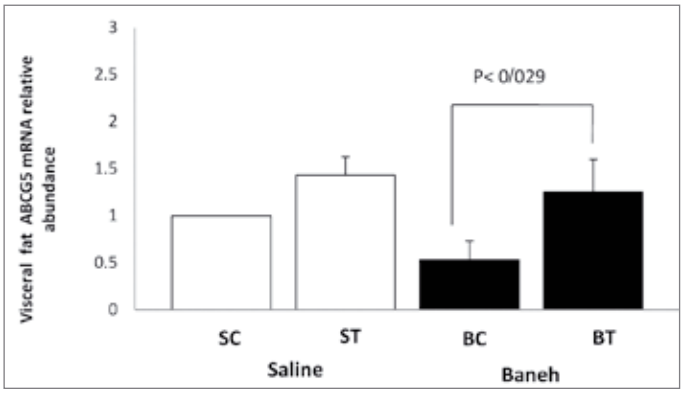
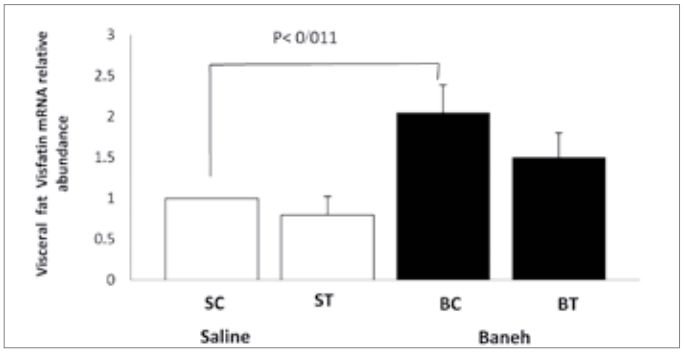


Figure 3: Real-time PCR of Visceral fat Visfatin mRNA relative expression in saline- control (SC), saline-training (ST), Bene-control (BC), and Bene-training (BT) female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per group. SC vs. BC, (P < 0.011)



Discussion

The present investigation revealed a significant effect of treadmill running program with or without a liquid pista-

chio-atlantica (Bene) extraction on visceral fat ABCG1, ABCG5 and visfatin relative genes expression. To our knowledge, this is the first report to demonstrate alterations of visceral fat ABCG1, ABCG5 and visfatin relative genes expression in response to a treadmill running and Bene crud extraction regime. The major finding of the present study was a higher ABCG1 and ABCG5 relative genes expression in trained visceral fat than control groups. Also a lower relative genes expression of ABCG1 and ABCG5 was observed in Bene-treated visceral fat of rats when compared with saline-treated animals. Another finding was a higher visfatin relative genes expression in Bene groups when compared with saline groups. mRNA expression of ABCG1 were observed in several tissues including the brain, eye, kidney, spleen, lung, liver, and intestine (6, 7). The expression of ABCG5 gene has been reported in liver and small intestine (15), colon and choroid plexus (11-15).

ABCG facilitates the efflux of cholesterol from cells to HDL, rather than to free apoA-I (6, 41, 42). In recent years, several studies were done on ABCA1 transporter. Ghanbari-Niaki et al investigated the effect of 6 weeks' endurance exercise (intensity: 25 m/min, duration: 90 min/session and five days a week), that resulted ABCA1 gene expression increase in rat's liver. Also the plasma levels of high density lipoprotein-cholesterol (HDL-C), Pre-β HDL and lecithin cholesterol acyltransferase (LCAT) significantly increased (18). Khabazian et al showed that 12 weeks of aerobic exercise (intensity: 25 m/min, duration: 90 min/session and five days a week, 60 minutes a day, five days a week), increase mRNA expressions of rats' small intestinal ABCA1 gene expression (19). In human subjects, a low intensity exercise training (walking) for 8 weeks has been shown to increase the levels of ABCA1 and ABCG1 expression in peripheral blood lymphocytes (43). Ghanbari-Niaki et al. (2012) who found that given a high dose of aqueous extraction of pistachia atlantica (Bene) extraction reduced and increased small intestine and kidney ABCG8 expression, respectively (44). Recently Côté et al. (2012) who studied the effect of atherogenic diet (high fat/high cholesterol) and a progressive exercise training (15-26m/min on 0%-10% slope, 15-60min/day, 5times/week, and for 6 weeks) on liver and small intestine ABCG5 and ABCG8 gene expression (45).

In addition, previous papers have shown that exercise increases the gene expression of G1 and G8 in the liver, small intestine and kidney and Bene extract reduced the expression of these genes (20, 44).

It has been shown that a high-fat diet suppresses ABCA1, ABCG4 and ABCG8 gene expression (46). Previous research showed that pistachios are rich in essential oils (47). Analysis of the Pistachia atlantica var Mutica essential oil by GC-MS method, showed that it is composed of α-pinene (70%), β-pinene (1.94%), 3-carene (0.2%), carveol (2.18%), epoxypinene (2.15%), limonene oxide (9%), myrtenol (5.31%), limonene (0.62%),citral (5.72%), α-phellandrene (0.2%), and β-myrcene (0.3%). The total amount of essential oil

obtained was 22% v/w which is higher than any other species of the genus (32).

Although we did not work on some of nuclear receptors whose are involved in cholesterol efflux such as peroxisome proliferator-activated receptor (PPAR), liver X receptor (LXR), and farnesoid X receptor (FXR), but It might be also possible that Bene administration reduced ABCG4 expression via these nuclear receptors (45, 48, 49). Physical exercise has been shown to have impact on nuclear receptors. Butcher et al. (2008) reported that LXRα, PPAR α and PPARγ were significantly increased following an 8 weeks of low-intensity exercise program (43).

In the past decade, other finding was a lower visfatin mRNA relative abundance in saline-treated visceral fat of rats when compared with Bene-treated animals. Revollo et al found that mouse brown adipose tissue, liver, and kidney had the highest levels; mouse heart had intermediate levels; mouse white adipose tissue, lung, spleen, testis, and muscle had low levels; and mouse brain and pancreas had no visfatin protein expression levels (50).

Evidence suggests that visfatin is an adipokine that exerts insulin-like action. Visfatin is able to mimic insulin function and lower plasma concentrations of glucose through binding to the insulin receptors (51). Other studies found that the serum concentrations of visfatin increased in diabetic patients, suggesting that visfatin may act as a compensatory factor in glucose metabolism (52).

Visfatin may be involved in improving insulin sensitivity (50). Contrary to previous findings, mRNA levels of visfatin in visceral fat tissue, after correction of BMI, was not associated with indices of insulin resistance (50).

In recent year several studies was made on visfatin Lee et al. investigated the effect of 12 weeks' Aerobic exercise (intensity: 300 - 400 cal energy expenditure, duration: 45

- 50 min/session and four days a week), that resulted visfatin significantly decrease in plasma of adolescents and Obese women (53). Domieh et al. reported that 8 weeks' Aerobic exercise (intensity 65% - 80% HR max, duration: 20 - 34 min/session and 3 days a week), Causes visfatin significantly decrease in plasma of Middle-aged men and a positive relationship between visfatin and plasma triglyceride levels and body fat were observed (54). Also Haus et al. investigated that 12 weeks' endurance exercise (intensity 80% HR max, duration: 60 min/session and 5 days a week), Causes Weight loss Along with the significantly reduction in plasma visfatin levels (55).

Data collected by using a GC-MS has shown that our used material had main following compositions; hexade-cenoic acid (7.52%), Palmitinic acid (28.86%), trans- Oleic acid (49.28%), n-Octadecanoic acid (3.87%). It is possible that the existence of a higher trans oleic acid and Palmitinic acid contents were enough to act as a high fat liquid extraction to increase visfatin expression in Bene groups. In pre-adipocytes, visfatin expression decreased by 50% with palmitate and 30% with oleate (56). One existing study has focused on the effects of unsaturated free fatty acids on plasma concentration of adipokine peptide: Cooper et al showed that dietary fatty acid composition significantly reduce plasma PYY concentration and can increases plasma ghrelin concentration that are not significant (57).

In summary, this is the first study demonstrating the effect of exercise training on visceral fat ABCG1, ABCG5 and visfatin genes expression. The present study also clearly shows that treadmill exercise increase ABCG1, ABCG5 and decrease visfatin gene expression. Also bene causes decrease in ABCG1, ABCG5 and increase visfatin gene expression.

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