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PYY (3-36) Gene Responses to Treadmill Running with or without *Pistachio-Atlantica* in Female Rats

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Abstract

Intrudaction: PYY (3-36) has an important role in satiety; reduce food intake and regulation of energy homeostasis. The aim of this study was to determine the small intestine and kidney PYY (3-36) relative gene expression in response to treadmill-running training in female rats.

Material and Methods: Twenty female wistarrats (6-8 weeks oldand 125-135 g weight) were used. Animals were randomly assigned into saline-control (SC), saline-training (ST), and Baneh-control (BC), and Baneh-training (BT). Training groups were given exercise on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/dayand5 days/week for eight weeks. Subjects were fed orally, withBaneh extraction and saline for four weeks. PYY (3-36) relative gene expression was detected by Real-time PCR method.

Results: Results demonstrated that Baneh extraction significantly increases PYY (3-36) relative gene expression in small intestine ($P < 0.013$) and kidney ($P < 0.025$).Also exercise reduces PYY (3-36) relative gene expression in and kidney ($P < 0.03$).

Discussion and Conclusion: Exercise training decreases PYY (3-36) relative gene expression in small intestine and kidney

Key Words: PYY (3-36), Female rats, Treadmill exercise, Pistachio atlantica

Introduction

There is, a complex physiological system called homeostasisin humans in whichthebalance between energy intake and energy expenditure is created [1]. Due to effects of appetite on energy homeostasis, its regulation plays an important role in energy balance[2].Appetite through the brain and sending signals from adipose tissue and Gastro-intestinal tract is set [1].Appetite regulation mechanisms are very complex, because the appetite of the integrated responses of the various factors[3]. Gastro-intestinal tract hormones include cholecystokinin (CCK), glucagon-like peptide 1(GLP-1), peptide YY (PYY) and ghrelin which are representative of potential regulation of appetite[4]. Peptide YY is structurally related to PP (Pancreaticpolypeptide)and NPY. This peptide is produced throughout the gut, with tissue concentrations that increase distally, reaching higher levels in the colon and rectum[5]. Peptide tyrosine – tyrosine (PYY), has an important role in satiety [6], reduce food intake and regulation of energy homeostasis [7]. The predominant form of PYY stored in intestinal cells (together with GLP-1) and released into the circulation is PYY3–36 [5].

Exercise is one of the factors in the energy equation [8]that often used to increase energy costs [9]. Physical activity and appetite can control appetite and can indirectly make adjustments food intake [10].The effect of exercise on PYY levels of heterogeneity of results is reported.Some studies have reported no change in PYY [9, 12], whereas others have observed both increase in PYY [2, 11, 13-15] and decrease in [16] after exercising.

More than two thousand years ago, doctors began to use herbs, such as walnut, pistachio, and silymarin to treat obesity.Veceraet al (2011) reported that silymarinpositively affects the plasma lipoprotein profile via up-regulation of ABC transporters involved in lipid metabolism[17]. High levels ofpolyunsaturated fatty acidsfound innutscanbecaused byloss of appetiteand its roleinweight lossandplasmalipidsis evident. It is shown that this plant leave contains anti-oxidative compounds that reduce the amount of free radicals [18].The total amount of essential oil obtained from Pistachiaatlanticais higher than any other species

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of the genus Pistachia [19].

To date, no research has been done on the effect of exercise on PYY (3-36) relative gene expression; nor has any study reported on the effect of Pistachiaatlantica on PYY(3-36) relative gene expression. As such, this research aims at studying; the effect of exercising and Pistachiaatlantica (Baneh) on relative gene expression of PYY(3-36).

Material and Methods

Plant material

The ripped fruit samples of Pistachiaatlantica (Baneh) 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 extracts were prepared by maceration (72h) of the coarsely powdered hull and kernel of Pistachiaatlantica, with 150 ml tap water for 45 min at room temperature and were filtered twice through filter paper. The volume of the filtered solution was increased to 100 ml with tap water so that one ml was equivalent to 100 mg of the preparatory material [20].The freshly prepared extracts were cooled and immediately used in the experiments. To the herbalists' recommendation, distilled water was not used for the extraction. After training, 100 mg/kg liquid extraction of Baneh was orally assigned to the Baneh groups and the same amount of saline was fed to saline groups.

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 Baneh-control (BC), and Baneh-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,everyday and for 4 days [(the 14-lane motorized-driven treadmill was designed by the primary author (UMZ, Babolsar, Mazandaran, Iran)]. The exercise group was trained for 8 weeks using the same training methods previously described (21, 22). 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 [23]. 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[24].

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 kidney and small intestinewere 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, 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 1loligo-$(dt)_{18}$primers (0.25 µg per reaction) using cDNA synthesis kit (AccuPower RT PreMix, Bioneer, Cat.No: k2041-B) according to the manufacturer's instructions. 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,7.5µl Master Mix, 1µl of the each forward and reverse primers (5 pmol/µl) and 5µl dH2O.The primers for PYY(3-36)and β-actin (as normalizer) were taken from Reimer et al, 2010 and Gao, &Yuan, 2010, respectively [25, 26]. Expected fragment size and gene bank accession numbers are listed in Table1. The PCR was carried out on the RotorGene 3000 Real time PCRsystem from Corbett is listed in Table 2. Product specificity was confirmed in the initial experiments by 3% agarose gel electrophoresis and routinely by melting curve analysis.

Table1: Oligo nucleotide primer sequences and real-time PCR amplification parameters

Gene	Forward and reverse primer sequences	Annealing temperature (°C)	Amplicon size (bp)	Gene accession no.
PYY(3-36)	F:AGCGGTATGGGAAAAGAGAAGTC R: ACCACTGGTCCACACCTTCTG	55 °C	111	-
β-actin	F:5′-TATCGGCAATGAGCGGTTCC-3′ R:5′-AGCACTGTGTTGGCATAGAGG-3′	55 °C	145	NM_031144

Table 2: Real-time Cycler conditions

step		Time	Temperature
PCR initial activation step		5 min	95 °C
Two-step cycling			
Denaturation		10s	95 °C
35-45 Cycle	Combined annealing/extension	30s	55 °C
Melting Curve		5 min	62 to 95°C
Cooling		End of reactions	40°C

Statistical analysis

The relative levels of mRNA were analyzed by the $2^{-\Delta\Delta Ct}$ method [27]. 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 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).

Results

PYY (3-36) relative gene expression in small intestine and kidney weredetermined in female rats.Data analysis revealed a significant difference in small intestine PYY (3-36) mRNA relative abundance between groups ($F=3.509$, $P<0.042$) (Fig.1). Using a suitable following post hoc test, datawere showed that small intestine relativeexpression of PYY (3-36) was highest in BC group(2.9849±1.095) compared to other groups at the end of program (Fig.1). In this regard Baneh increases the PYY (3-36) relative mRNA expression ($P<0.013)($ only for separate P of post hoc test with univariate p that used for Baneh and saline groups) (Fig.1).

Our findings show that PYY (3-36) relative gene expression in kidney in SC group significantly was lower than BC group (2.8725±0.9426) ($P<0.031$) (Fig. 2) and PYY (3-36) relative gene expression in BT group (1.0521±0.3012) significantly was lower than BC group ($P<0.03$) (Fig. 2). In this regard Baneh increases the PYY (3-36) relative mRNA expression ($P<0.025$) and exercise decrease the PYY (3-36) relative mRNA expression ($P<0.03$) (Fig.2).

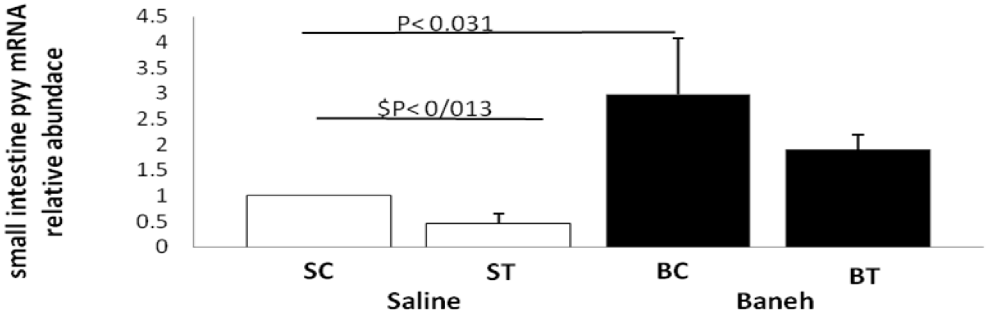

Figure1: Real-time PCR of small intestine PYY (3-36) relative mRNA expression in saline-control (SC), saline-training (ST), Baneh-control (BC), and Baneh-training (BT) wild-type female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per each group. SC vs BC, (P < 0.031)

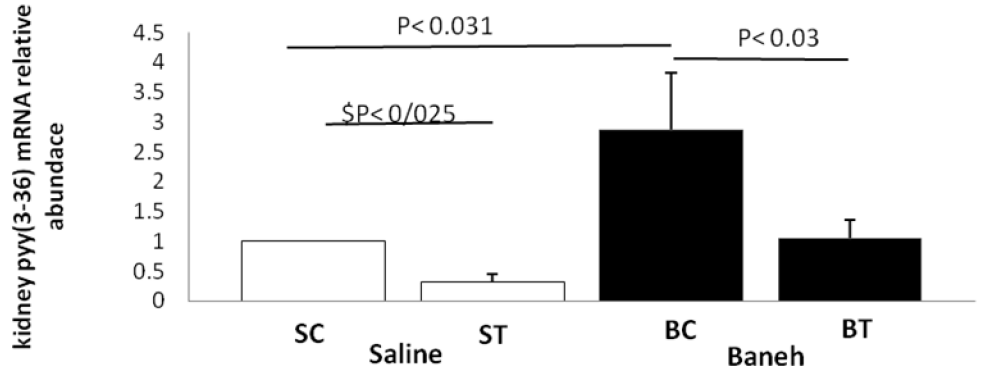

Figure2: Real-time PCR of Kidney PYY (3-36) relative mRNA expression in saline-control (SC), saline-training (ST), Baneh-control (BC), and Baneh-training (BT) wild-type female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per each group. SC vs BC, (P < 0.031) - BC vs BT, (P < 0.03)

Discussion and Conclusion

To our knowledge, this is the first report to demonstrate alterations of female rat small intestine and kidney PYY (3-36)relative gene expression in response to a treadmill running and Baneh crud extraction regime. The major finding of the present study wasthe observation of a higher PYY (3-36)relative gene expression in control groups of small intestine and kidney than trained groups. Another finding wasthe observation of a lower relative gene expression ofPYY (3-36) in Saline-control small intestine and kidney of rats when compared with Baneh-control animals. PYY (3-36) is produced throughout the gut, with tissue concentrations that increase distally, reaching higher levels in the colon and rectum[5].

In this study we detected PYY (3-36) relative gene expression by Real- time PCR method that is concurrent with previous reports in tissues PYY (3-36) gene expression

In recent years, several studies have beendone on PYY. The effect of exercise on PYY levels of heterogeneity are controversy.Some studies have reported no change in [9, 12] YY, whereas others have observed both increase in [2, 11, 13-15] YY and decrease in [16] after exercising. Interestingly, recent studies have revealed inhibitory effects of exercise on the hunger associated with these hormones in healthy subjects.Martin et al (2007) were the first who that looked at the impact of activity on the concentration of circulating PYY. The researchers observed that total PYY concentrations during a 60-minute sessions of moderate intensity activity (66% HRmax) significantly increased [8]. Broom and et al (2009), reported the peak blood concentrations of total PYY in response to aerobic and not resistance activities.In this study, total plasma PYY concentrations during treadmill running activity (69% Vo2max) increased significantly 30 minutes after the above sedentary control values, remained [9]. Jones and et al (2009) examined the effect of exercise on hormones associated with appetite control and insulin sensitivity. 12 young obese

middle-aged, did aerobic activity (85 - 60% Vo2max) three days a week for 32 weeks, then the total PYY concentrations during fasting before and after the activity was measured. Overall PYY after the intervention (23%) was increased significantly[28]. The findings of the studies examining the effect of exercise on PYY, it has been suggested that aerobic activity increases the concentration of PYY [8, 9, 29]. On the other hand it has been shown that PYY concentration after fatty meals, high-carbohydrate meal is higher than [30].

Previous research showed that pistachios are rich in essential oils [31]. Analysis of the PistachiaatlanticavarMutica 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 was higher than any other species of the genus [19]. GC-MS analysis of Pistachiaatlantica in our study shows that it is composed of α-Pinene (0.71%), Limonen (0.54%), Hexadecenoic acid (7.52%), Palmitinic acid (28.86%), trans-Oleic acid (49.28%), n-Octadecanoic acid (3.87%), Oleic acid (0.2%), 3-pentadecyl-Phenol (2.69%), Phenol, 3-pentadecyl (0.84%), 3-pentadecyl- Phenol (1.58%), 5-dihydroo xazoleCinnamylcinnamate (0.36%), Phenol, 3-pentadecyl- Acetic acid, 4-methylphenyl ester Acetic acid, 4-methylphenyl ester (0.62%), Phenol, 3-pentyl (0.36%), Ribitol, pentaacetate N-Propenyl-2-methoxy-6-methylbenza mide 6, 7-Dimethoxyisatin (0.15%).

The important point of this research was the significant increase of PYY (3-36) gene expression by Baneh in small intestine and kidney that was probably due to high levels of fatty acid in the Baneh (Figures 1, 2).

Besides, Cooper and et al (2011) reported that the cycling activity of 2 hours daily for 3 days with VO2max 45% increase in PYY concentrations; onthe other hand, PYY concentrations increased significantly in exercise with a diet rich in unsaturated fatty acid group as compared to the group that received only diet rich in unsaturated fatty acid [32].

In conclusion, the present study showed a significantly lowerrelative expression of PYY (3-36) mRNA in small intestine and kidney following treadmill running program in female rats. Also, it revealed a significant difference inPYY (3-36) relative gene expression in animals fed with Baneh compared to saline groups. Our results showed that Pistachiaatlantica (Baneh) mayincreasePYY (3-36) relative gene expression in small intestine, and kidney tissue due to its essential fatty acids. This finding confirms previous results showing that PYY is reduced after exercise and increases in response to receiving fatty foods.

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