Iranian Journal of Health and Physical Activity (2013) 4 (1), (9-16)

Effects of Aerobic Training, with or without Zizyphus Jujuba Water Extraction, on Fundus Nesfatin-1, ATP, HDL-C, and LDL-C Concentrations in Female Rats

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Received 20 August 2012 Accepted 19 February 2013

Abstract

Purpose: The aim of this study was to investigate the effects of six weeks of aerobic training, with and without extract of Jujuba, on fundus nesfatin-1, ATP concentration, plasma High-density lipoprotein (HDL), and Low-density lipoprotein (LDL) levels in female rats.

Material and Methods: 28 Wistar rats were randomly assigned to Saline-control, Saline-training, Jujuba-control and Jujuba-training groups. Training groups were given exercise on a motor-driven treadmill at 35 m/min (0% grade) for 60 min/day and 5 days/week for six weeks. Animals were fed orally, with Jujuba extraction and Saline (3 weeks, 60 mg per 100 g body weight). 72 hours after the last training session and after four hours of fasting, the rats were sacrificed, and their fundus tissue was excised. Some plasma was also collected for plasma variable measurements. All variables were compared using one way analyzes of variances. Correlations were calculated using the Pearson Product Moment correlation. All P values <0.05 were considered as significant.

Results: nesfatin-1 significantly increased in saline training group compared to saline control group ($P<0.028$). Exercise training ($P<0.091$) and extraction ($P<0.031$) independently increased plasma HDL-C and decreased fundus ATP ($P<0.002$). There was no significant difference between groups regarding plasma LDL concentration. There was also no correlation between fundus nesfatin-1 concentration and plasma HDL, LDL and fundus ATP.

Discussion and Conclusion: Exercise and using Jujuba extraction may prevent over-weight and cardiovascular diseases.

Key Words: Aerobic training, Nesfatin-1, Female rat, Zizyphus jujuba water extraction

Introduction

Energy homeostasis is controlled by a complex neuroendocrine system consisting of peripheral signals and central signals, in particular, neuropeptides [1, 2]. Nesfatin-1 is a protein molecule (neuropeptide) produced by the brains of mammals. High levels of nesfatin-1 in the brain lead to a loss of appetite, less frequent hunger, a sense of fullness and a drop in body fat and weight. [3-6]. lack of nesfatin-1 in the brain leads to an increase of appetite, more frequent episodes of hunger, an increase in body fat and weight, and the inability to feel full [7, 8]. This latter condition can be artificially induced by injecting an anti-nesfatin-1 antibody into the brain. Intra-cerebral-ventricular (ICV) and peripheral injection of nesfatin-1 elicits a dose-depended reduction of 4-h dark phase food intake [9, 10]. Neurons expressing nesfatin-1 are found in various areas including the brainstem (NTS, dorsal motor nucleus of the vagus: DMNX) and hypothalamic nuclei (ARC, PVN, SON) with proven roles in energy homeostasis. [3, 5, 11-13]. It has been shown that nesfatin-1 is expressed in different tissues including rats' gastric oxyntic mucosa or gastric X/A like cells, stomach, pancreatic beta cells and adipose tissue [14-17]. It has been suggested that changes in peptides and

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proteins that are released from hypothalamus and adipose tissue contribute to the pathogenesis of insulin resistance and its attending detrimental metabolic consequences including diabetes, dyslipidemia, hypertension, and cardiovascular disease [18, 19]. Previous study has shown that changes in nesfatin-1 expression and its levels are affected by several conditions such as fasting and refeeding [5, 20, 21], restraint stress [22, 23], abdominal surgery [14], high fat diet [17, 24], glycemic state [25] and physical exercise [24, 26].

Recently, herbal medicine and medical plants are widely used for the treatment of diseases and weight management [27, 28]. Zizyphus/Jujube/Red Date is plant of Khamancea family that contains different kinds of proteins and sugars. It has anti-inflammatory and anti-diabetic effects and is recommended for the digestive disorders, weakness, obesity and diarrhea. In a plant screening exercise, providing oleamide from a jujube extract for 3 weeks, attenuated scopolamine-induced amnesia in mice. Its positive role in improving cognitive impairment disorders, such as that seen in Alzheimer disease, was suggested, as the jujube extract appeared to increase the activation of choline acetyltransferase [29]. Water extracts of the jujuba fruit and bark, have exploited the potential cytotoxicity of jujuba. Apoptosis and differential cell cycle arrests are suggested to be responsible for the dose-dependent reduction of cell viability. Activity against certain human cancer cell lines has also been demonstrated in vitro [30-32]. Solati et al (2010) investigated the effects of water extracts of Ziziphus fruit on serum glucose, triglycerides, LDL-cholesterol, and HDL-cholesterol in diabetic adult male rats. They found that supplementation of this water extract significantly decreased fasting blood glucose and LDL-cholesterol and triglyceride levels after 14 days [28].

The effect of exercise on nesfatin-1 has not been investigated extensively. No research has examined the effect of physical exercise on nesfatin-1 in fundus tissue. Also the effect of Jujuba extraction on nesfatin-1 concentration is lacking. Thus, the current study was to investigate the effects of six weeks of aerobic training with and without Jujube extraction, on fundus nesfatin-1 concentrations in female rats.

Material and Methods

Plant material

The fruit of zizyphus Jujuba were purchased from the farmers of Birjand, Iran, and dried in shadow at 25° C. Plant Material was identified by herbarium collection in the department of biology, faculty of Science, University of Mazandaran, Iran.

Preparation of Jujube extraction

The extraction was prepared according to Svetlana et al (2006) [33]. Briefly, the whole dried fruit of Jujuba (4 g) was coarsely powdered and mixed with 60 ml of tap water and stay for 72 hours (soaked). Then the mixture was filtered by using a Whatman filter (No. 2 filter) and centrifuged. It has to be noted that we did not use distilled water according to the herbalist's recommendation. The fresh extraction was orally given to the rats (60 mg per 100 g body weight), immediately after the training session. The control groups have been treated with the 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 eight Wistar rats (6-8 weeks old, 125-135 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. Animals were randomly assigned to Saline-control (n=7), Saline-training (n=7), Jujube-control (n=7) and Jujube-training (n=7). Seven rats were housed per cage (46-L volume) with a 12-h: 12-h light-dark cycle. Temperature was maintained at 22°C ± 1.4°C. Food (a pellet form) and water were provided ad *libitum*. Training groups were given exercise on a motor-driven treadmill at 35 m/min (0% grade) for 60 min/day and 5 days/week for six weeks whereas the control groups remained sedentary. Supplementation was applied orally (Jujuba extraction and Saline, 3 weeks, 60 mg per 100 g of body weight).

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, Babolsar, Mazandaran, Iran). The exercise group was trained

for six weeks using the same training methods previously described [34]. The rats ran at 35 m/min for 60 minutes, five days/week. The animals were scarified 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. Only female rats showing at least two consecutive 4- or 5-day estrous cycles were used in the study. The established estrous cycle in each female was used to select the day of the experiment, that is when the estrous cycle stage was confirmed by vaginal smear [35, 36].

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). Fundus tissue was excised, cleaned, divided into two pieces, washed in ice-cold saline, and were immediately frozen in liquid nitrogen and stored at − 80 ° C. Blood samples were collected in EDAT test tubes (as anticoagulant) and were immediately processed for plasma preparation, during a 10-min centrifugation at 3000rpm. Plasma was also stored at -80C for future analysis.

Tissue ATP and nesfatin-1, plasma HDL and LDL concentrations measurements

Tissue nesfatin-1 levels were measured using a commercially available ELISA kit (USCN LIFE Science, variation: 7.1%, sensitivity: 0.09 ng/L) according to the manufacturer's protocol. Plasma high density lipoprotein cholesterol level (HDL) was determined by direct Immuno method (HDL-C Immuno FS, Pars Azmoun, Tehran, Iran), the Intra-assay coefficient of variation and sensitivity of the method were 1.2% and 0.03 mmol/L, respectively. LDLC was obtained by an enzymatic method (kit was purchased from Pars Azma another Iranian Com) according to the manufacturers' protocol. Fundus ATP concentration was determined using a Biaffin (Kassel, Germany) ATP-sensitive bioluminescence kit, and the amount of ATP in the samples was calculated according to the manufacturers' protocol.

Statistical analysis

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 ± SD. All variables were compared using one-way analyzes of variances. Correlations were calculated using the Pearson Product Moment correlation coefficient. All statistical analyses were performed using SPSS software (Version 19). All P values <0.05 were considered as significant.

Results

Data analysis revealed a significant difference in fundus nesfatin-1 concentration at the end of treadmill running program ($F= 5.290$, $P<0.006$). A suitable post-hoc test showed that fundus nesfatin-1 was up-regulated in Saline-trained group (in comparison to Saline-control group) (Fig.1) ($P<0.028$). Nesfatin-1 concentration was highest in Jujuba-trained group compared to other groups. Data analysis revealed no significant difference in plasma HDL-C concentration at the end of treadmill running program ($F=2.782$, $P<0.063$) (Fig.2). However, exercise ($P<0.09$) and supplementation ($P<0.031$) independently affected plasma HDL-C, and enhanced it concentrations (Fig.2). Data analysis revealed no significant difference in plasma LDL concentration at the end of treadmill running program ($F=0.493$, $P<0.691$) (Fig.3). Data analysis revealed a significant difference in fundus ATP concentration at the end of treadmill running program ($F= 11.309$, $P<0.001$) (Fig.4). A suitable post-hoc test showed that fundus ATP concentration was significantly lower in saline-trained group compared to saline-control group ($P<0.001$), also Jujuba extraction independently reduced fundus ATP concentration ($P<0.001$) (Fig.4). There was no significant correlation between fundus nesfatin-1 concentration and fundus ATP ($r = -0.375$; $P<0.062$), plasma HDL-C ($r = 0.252$; $P<0.195$) and LDL ($r = -0.062$; $P<0.753$) concentrations.

Discussion and Conclusion

The aim of this study was to investigate the effects of six weeks of aerobic training with and without extract of Jujuba, on fundus nesfatin-1 and ATP concentrations, plasma HDL and LDL concentrations in female rats. This is the first report to demonstrate alterations of female rat fundus

nesfatin-1 concentration in response to a treadmill running program at 35 m/min of intensity/speed. Previous study showed that nesfatin-1was expressed in different tissues including the rats

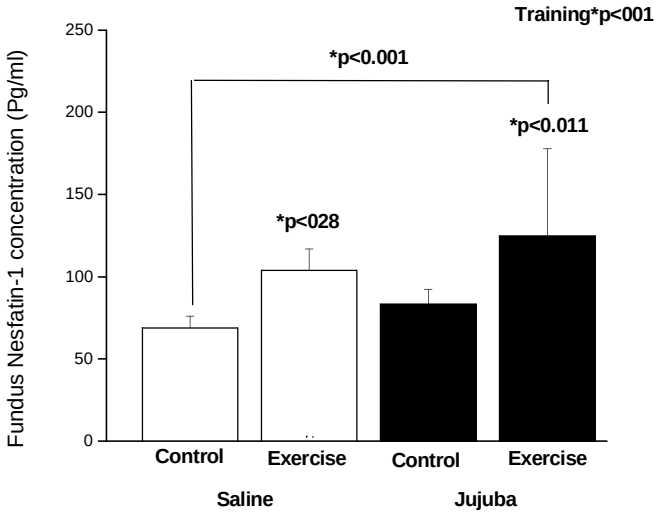

Figure 1: Fundus nesfatin-1 concentration in Saline-control, Saline-trained, Jujuba-control and Jujuba- trained groups of wild-type female rats. Data is expressed as mean ± SD. Each column represents one group with 7 rats.

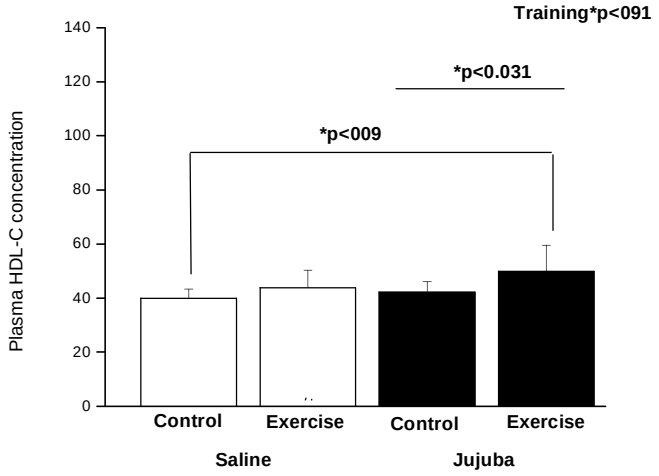

Figure 2: Plasma HDL-C concentration in Saline-control, Saline-trained, Jujuba-control and Jujuba- trained groups of wild-type female rats. Data is expressed as mean ± SD. Each column represents one group with 7 rats.

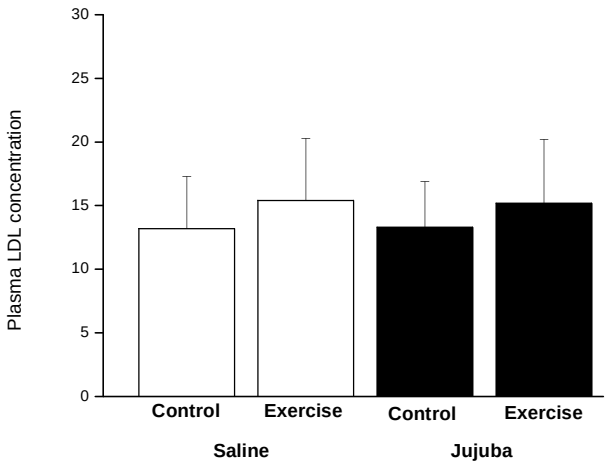

Figure 3: Plasma LDL concentration in Saline-control, Saline-trained, Jujuba-control and Jujuba- trained groups of wild-type female rats. Data is expressed as mean ± SD. Each column represents one group with 7 rats.

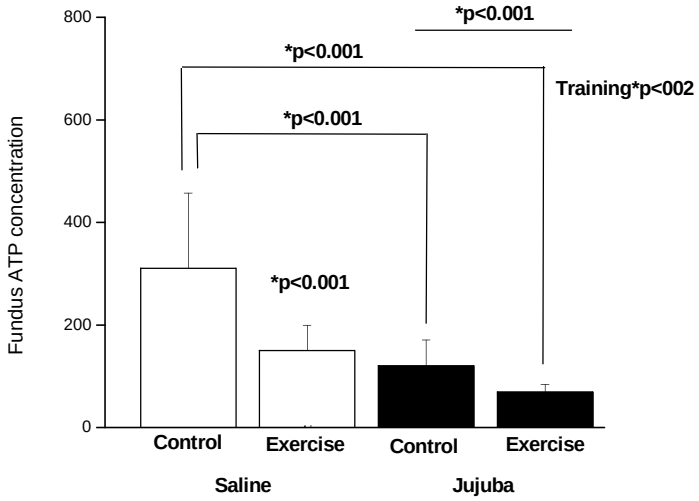

Figure4: Fundus ATP concentration in Saline-control, Saline-trained, Jujuba-control and Jujuba- trained groups of wild-type female rats. Data is expressed as mean ± SD. Each column represents one group with 7 rats.

gastric oxyntic mucosa or gastric X/A like cells [14], stomach [15], pancreatic beta cells [16], and adipose tissue [17]. The present study showed that nesfatin-1 concentration increased in training groups compared to the control groups. The effect of exercise on nesfatin-1 has not been investigated extensively. No research has examined the effect of exercise on tissue nesfatin-1 concentrations. However, Ghanbari-Niaki et al (2010) focused on the effects of two different anaerobic exercise sessions (anaerobic sprint test RAST: 7 sets of 6 × 35 m every 10 s with 1 min rest between sets and a non-combat kickboxing session: 7 sets of 6 techniques, 20 s per technique with 1 min rest between sets) on plasma nesfatin-1 concentrations in human subjects [26]. In that study, plasma nesfatin-1 concentrations did not change significantly. They also showed that lack of nesfatin-1 in response to the exercise protocols may be partially due to the fasting condition. Ghanbari-Niaki et al (2012, unduplicated data), investigated the effects of eight weeks of aerobic training on tissue nesfatin-1/nucleobindin-2 expression and its plasma concentration. They showed that tissue nesfatin-1/NUCB2 mRNA expression and plasma HCL-C concentration were affected (up regulated) by physical exercise while plasma nesfatin-1 remained unchanged. However they did not find any significant correlation between tissues nesfatin-1 expression and plasma nesfatin-1 concentration. Another existing study by the same researchers focused on the effect of endurance exercise and supplementation of Pistachio-Atlantic on intestine nesfatin-1/NUCB2 gene expression. In that study they showed that aerobic exercise (60 min/day and 5 days/week for eight week) affected nesfatin-1/NUCB2 expression but the change was not significant. They suggested the same trend for exercise-induced increase in intestine expression of nesfatin-1 mRNA expression. Also they found that Pistachio-Atlantica supplementation suppressed intestinal nesfatin-1/NUCB2 gene expression [24].

The mechanisms by which endurance exercise training could change nesfatin-1 are still unknown. However, fasting has been shown to affect nesfatin-1 concentrations in serum and refeeding has been reported to increase the activity of nesfatin-1 neurons in the hypothalamus and nesfatin-1 mRNA expression in the supraoptic nucleus of the hypothalamus [3, 5].

Endurance training has an important role in changing the tissue ATP concentrations. Referring to pervious researches that investigated the relationship between endurance exercise and tissue ATP concentration, several variables with critical roles were found including length, and intensity of physical exercise, and the time between the last training session and sacrifices [37-40]. Therefore, it possible that the long time between the last training session and rat sacrifices (72hours), and also the intensity of exercise training (moderate: 20-35 m/min) have contributed to fundus ATP

reduction in the present study.

According to nutrition sciences, jujuba consists of different nutrient and materials including sugars (Starch 21.8%, fructose 16%, glucose 9.6%, and sucrose 21.8%), Fat (19%), various amino acids (Glycine, histidine, leucine, iso-leucine, phenylalanine, proline, serine, threonine and etc.), glutamic acid, protein (4.5-5.6%), various minerals (Iron, sodium, potassium, zinc, manganese, sulfur and etc.) and vitamins (C, B_1, B_2). Generally, jujuba fruit is high in carbohydrates, especially fructose and glucose, which account for about 77% of its weight. Vitamins C, B complex, and A, as well as calcium, potassium, and other mineral elements, have also been identified in jujuba fruit [41, 42]. It has been reported that it consists of short-medium chain fatty acids including stearic, oleic, palmitic and linoleic. In addition jujuba has glycoside complexes, including phenols (Querectin and Kaemferol) as well as flavonoids and triterpenes [42-47]. Solati et al (2010) investigated the effects of water extracts of Ziziphus vulgaris L. fruit on serum glucose, triglycerides, LDL-cholesterol, HDL-cholesterol and activities of aminotransferase enzymes inreptozocin-induced in diabetic adult male rats. They found that supplementation of this water extract by gavage (feeding)/tube feeding at doses of 0.25, 0.5, 1, 1.5 and 2 g/kg in 0.5 ml distilled water in diabetic rats resulted in a significant decrease of fasting blood glucose, LDL-cholesterol and triglyceride levels after 14 days. The levels of HDL-cholesterol and insulin, and activities of serum aminotransaminase(AST) , alanine aminotransferase(ALT), and aspartate aminotransferase did not change significantly in the extract-supplemented groups compared to the control group [28]. In the present study jujuba extraction did not have any significant effect on fundus nesfatin-1 concentration. However there was a trend of jujuba-induced increase in fundus nesfatin-1 concentration that was accompanied with an increase in plasma HDL-C concentration.

Acknowledgements

We wish to thank the students of Mazandaran Biochemistry lab (Department of Physical Education and Sport Science, Mazandarn, Baboulsar, Iran) for their helpful comments and guides.

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