The Effect of Endurance Exercise and Adenosine Consumption on UCP-1 Gene Expression in the Visceral Adipose Tissue of Obese Male Rats

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Abstract

Objective: Reducing white adipose tissue is an important strategy for treatment of obesity and its related metabolic disorders. The purpose of this study was to determine the effect of endurance exercise and adenosine consumption on the expression of selected genes of thermogenic pathway in the visceral adipose tissue of obese male rats.

Materials and Methods: Twenty male wistar rats were fed on a high-fat diet (40 % fat) 12 weeks to get mean weight 319±30 gr. The rats were selected randomly in four groups; control-saline, control-adenosine, exercise-adenosine and exercise-saline. The adenosine groups received 0.2mgr/kg adenosine 12 weeks (seven days a week), 3 hours before exercise. The exercise schedule included running on the turning wheel without slope, performed 12 weeks, five sessions a week, with the speed of 20-25 meters per minute for 15-31 minutes. About 48 hours after the last exercise session, the rat's kidneys were removed and frozen immediately. In order to measure the relative expression of UCP-1 gene, the method of Real Time (RT) - PCR based on SYBR-Green dye was used. The gathered data was studied using the statistical method of two-way analysis of variance.

Results: Twelve weeks of endurance exercise with medium intensity and adenosine consumption resulted a significant increase of UCP-1 gene expression (P-value: 0.001 and P-value: 0.005 accordingly). Nevertheless, the collective effect of exercise and adenosine is indicative of insignificant difference in changes of the relative expression of UCP-1 gene in visceral adipose tissue.

Conclusion: Endurance exercise and adenosine consumption independently led to an increase in UCP-1 gene expression in the visceral adipose tissue of obese male rats Nevertheless, the collective effect of exercise and adenosine is indicative of insignificant difference in changes of the relative expression of UCP-1 gene in visceral adipose tissue. It is probable that some mechanisms be activated through the collective effect of exercise and adenosine that reduce the synergic effect of exercise and adenosine.

Keywords: Endurance exercise, UCP1 protein, White adipose tissue

Introduction

Obesity is a major health problem worldwide. Obesity and overweight increase the risk of cardiovascular diseases as well as hypertension, type II diabetes mellitus, dyslipidemia and different types of cancer (1).

The adipose tissue plays the fundamental role in balancing the relationships between nutrition, energy equilibrium and health (2). In mammals, two type of adipose tissue, white (WAT) and brown (BAT) were recognized with different function and structure (3). WAT is the main part of body fat tissue. It is the reservoir of the triglycerides and fatty acids which are used as energy substrates for ATP production through oxidative phosphorylation (4). BAT contains a high amount of mitochondria and transforms chemical energy through the expression of uncoupling protein 1 (UCP1) (5). It was demonstrated that high levels of BAT are accompanied by obesity, metabolic diseases and improvement of glucose homeostasis (6).

UCP1 is located in the inner membrane of the mitochondria of brown adipose cellules in brown adipose tissue (7), as well as in beige-like cells or Brite or Beige adipose cells (8). This protein forms a channel causing the diffusion of proton. As a result, the energy that could spent on ATP synthesis, would be excreted as heat (9). Therefore, this protein leads to higher energy consumption, metabolic incompetence and energy excretion through non-shivering thermogenesis in brown adipose tissue in addition to brown-looking white adipose tissue (10). Thus, UCP-1 protein synthesis in adipose tissue plays an important role in resisting and preventing lipid accumulation, weight gain and obesity (11).

Numerous nutritional and pharmaceutical factors are involved in UCP-1 gene expression in white adipose tissue including long exposure to cold and the effect of catecholamine and thyroid hormones and the hormone-like Irisin (12,13).

Recently, activity and exercise have been considered as appropriate stimulants for UCP-1 expression in white adipose tissue as some studies have demonstrated a significant increase in UCP-1 expression following a single session (14), 3 weeks (15), 5 weeks (14) and 8 weeks (16) of endurance exercise in the subcutaneous white fat tissue of the Inguinal region and the visceral white fat tissue of the epididymal region in rats.

Furthermore, researchers are seeking new pathways of brown fat transfer and activation. Adenosine is a new beige and brown fat activator which acts through A2A receptors. Adenosine bonds to four receptors of protein G including the inhibiting receptors A1, A3 and the stimulating receptors A2A, A2B. Activation of stimulating receptors could increase adenylyl cyclase activity and increase cAMP while having contrary effects. The distribution of adenosine receptors varies in different tissues and depends on the expression of the receptor, although adenosine activity is quite dependent on tissue (17). Kaartinen et al. (1994) reported in an article that the signaling pathway of AR1A is more active in the adipocytes of fat Zucker rats which leads to increased sensitivity to inhibition by AR1A agonists. In addition, the inhibition of adenylyl cyclase by AR1A in the adipocytes of fat Zucker rats is increased and the tonic activity of AR1A on lipolysis has also been higher in fat rats (18). AR1A agonists are strong inhibitors of adipose tissue lipolysis and have the potential to be utilized as clinically effective antilipolytics (19).

Although the mentioned researches studied the effect of exercise and adenosine on adipose tissue, there are few articles studying the specific effect of exercise training on UCP-1 gene expression in perirenal visceral fat tissue. It is imagined that as a result of exercise training and adipose intake, the expression of this certain gene would change in this area of white fat, as in other areas of adiposity in the body. Thus, the aim of this study was to evaluate the effect of 12 weeks of endurance exercise and adenosine on UCP-1 expression in the perirenal visceral white adipose tissue in obese male rats, in order to clarify the effect of exercise training and adenosine stimulants on the expression of these proteins.

Materials and Methods

Twenty male wistar rats aged between 4-5 weeks with the initial weight range of 128 ±

32 grams were provided by the Pastor Institute of Amol and transferred to the research center. The subjects gained weight through high-fat diet (containing 40% fat) over 12 weeks. The rats were randomly divided into 4 groups of saline-control, saline-exercise, adenosine-control and adenosine-exercise.

The rats were kept in 4 groups with 5 rats, inside clear polycarbonate cages with a temperature of 22° (±2) C, humidity level of 55% (±5) percent and 12:12 light cycle with suitable air conditioning during their one-week before the start of study as well as the period of the methods execution.

The food was produced by Dam Beh Parvar Company in Karaj and for each 100 grams of their weight which was measured each week, 5 grams of food was placed inside the cage once a week. In this study, the water required by each animal was freely provided to them in 500 ml bottles designed for laboratory animals. The food ration was made of 40% fat, 13% protein and 47% carbohydrates and during twelve weeks, all 20 rats used this diet and after the obesity criteria, the exercise phase continued with the high-fat diet.

Endurance Exercise Protocol

The exercise group subjects were involved in twelve weeks of endurance exercise with moderate intensity. The exercise groups trained 5 days each week. The exercise period contained two parts: the first part (introduction): including 5 sessions (one week) of walking and running on the treadmill with the speed of 15 meters per minute, zero degrees of inclination and for 10 minutes.

The second part (gradual increase of intensity): in this pas, rats run on the treadmill for 15 minutes with the speed of 20 meters per minute. Gradually, in two weeks of exercise, time and intensity of the activity was increased and reached the final time period of 31 minutes and speed of 25 meters per minute. In addition, the first 5 minutes of the 40-minute session was spent warming up and the last 5 minutes for cooling off (Table 1).

Adenosine Consumption Dosage in Rats

The exercise-adenosine and control-adenosine groups received 2 milligrams of adenosine for each kilogram of body weight for 12 weeks (seven days a week) intraperitoneally 3 hours before the exercise. Saline groups also received physiological serum in the same manner and amount of adenosine-receiving groups.

Laboratory Sampling and Measurements

Forty-eight hours after the last training session and one night of fasting, the animals were anaesthetized via intraperitoneal injections of Xylazine (10 mg/kg) and Ketamine (75mg/kg), (21), and their perinephric adipose tissue was quickly removed and frozen through liquid nitrogen in the temperature of -70 degrees Centigrade.

In order to establish UCP-1 gene expression, RT-PCR technique was used. Initially, the tissue RNA was extracted using the Kit protocol (Qiagen, Germany). Then, the quality of the extracted RNA was measured by a spectrophotometer (Qiagen, DPI-1). In order to synthesize cDNA, the primer Oligo dt and reverse transcriptase enzyme were used according to the concerned protocol (MWG-Biotech, Germany). The sequences are as follows (Fermentas):

UCP-1:

Forward: GCCTCTACGATACGGTCCAA

Reverse: CTGACCTTCACCACCTCTGT

PCR reaction was performed using PCR master mix (Applied Biosystems) and SYBER

Table 1. Endurance exercise protocol

Variable	Introduction	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12
Speed (m/min)	15	20	20	21	21	22	22	23	23	24	24	25	25
Time (min)	10	15	16:30	18	19:30	21	22:30	24	25:30	27	28:30	30	31

Green in the ABI Step One installation (Applied Biosystems, Sequence Detection Systems, Focter City CA) according to the company protocols. 40 cycles were considered for each Real-Time PCR process and the temperature for each cycle was adjusted to 94 degrees Centigrade for 20 seconds, 58-60 degrees Centigrade for 30 seconds and 72 degrees Centigrade for 30 seconds.

The relative expression of genes studied in this research was calculated by Livak formula (2001), (20).

This study was dane according medical research ethics, also it should be hofired the principle of valuatarine and informed consent was dane.

Statistical methods

Descriptive statistics were used for grouping and dispersion index determination. The Shapiro-Wilk test revealed natural distribution in the data. In order to study the changes among the four groups, two-way analysis of variance was used in SPSS 16 software with the significance level of $P<0.5$. Levene's test was used to assess the equality of variances among the research groups.

Results

According to the Shapiro-Wilk test, the data for all variables in each group were of natural distribution. In Table 2 the data of weight and UCP-1 for each of the four groups is presented.

By analyzing the data related to the final weight of the subjects using the two-way variance analysis test, it was noted that adenosine intake alone had not led to a significant discrepancy in the final weight changes of the subjects among the groups (P=0.16 and F=2.164). However, endurance exercise alone showed a significant difference in the final weight of the subjects among the groups (P=0.002 and F=13.617), while the interactive effects of exercise and adenosine were not significant, either (P=0.40 and F=0.740). (Figure 1)

In addition, by analyzing the data related to the relative expression of UCP-1 gene using the two-way variance analysis test, both variables of endurance exercise and adenosine intake (each independently) showed significant difference in UCP-1 gene expression changes in visceral adipose tissue (P=0.001, F=33.535 and P=0.005, F= 10.498 respectively). However, the interactive effects of exercise and adenosine indicated insignificant difference in the relative expression of UCP-1 gene in visceral adipose tissue (P=0.982 and F=0.001). (Figure 2)

Discussion

The data from this research indicate that endurance exercise alone shows a significant difference in the final weight of subjects among the groups while the interactive effect of exercise and adenosine and adenosine alone do not exhibit a significant difference. It may be concluded that endurance exercise has a more competent effect on weight loss.

The data gathered in this study show that endurance exercise and adenosine intake (independently) result in significant difference in UCP-1 gene expression changes in visceral adipose tissue. Nevertheless, the collective effect of exercise and adenosine is indicative of insignificant difference in changes of the relative expression of UCP-1 gene in visceral adipose tissue. Bostrom et al, 2012, reported that the expression of UCP-1 was multiplied 25 times in subcutaneous white adiposity after 3 weeks of treadmill exercise (15).

Table 2. Descriptive indicators main variables of the research

Groups / Variable	Control-saline (X ± SE)	Control-adenosine (X ± SE)	Exercise-adenosine (X ± SE)	Exercise-saline (X ± SE)
Final weight (gr)	396.9±57.24	385.98±16.24	304.35±45.15	346.17±29.15
UCP-1	1	4.50±2.23	10.83±2.03	7.28±3.81

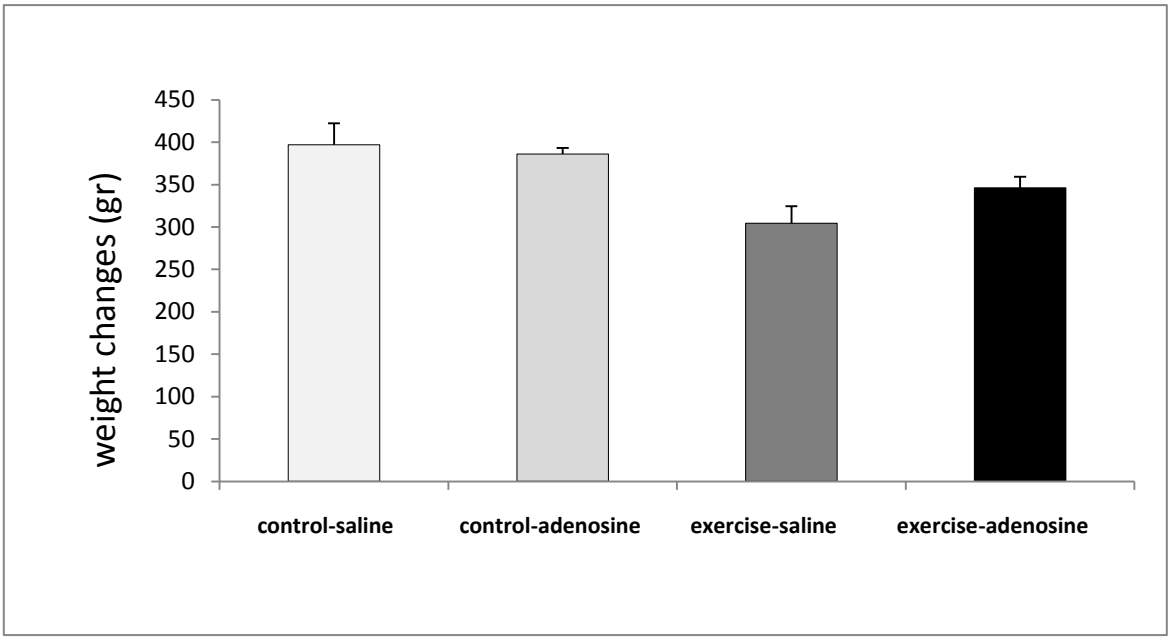

Figure 1. Weight change of subjects in study groups

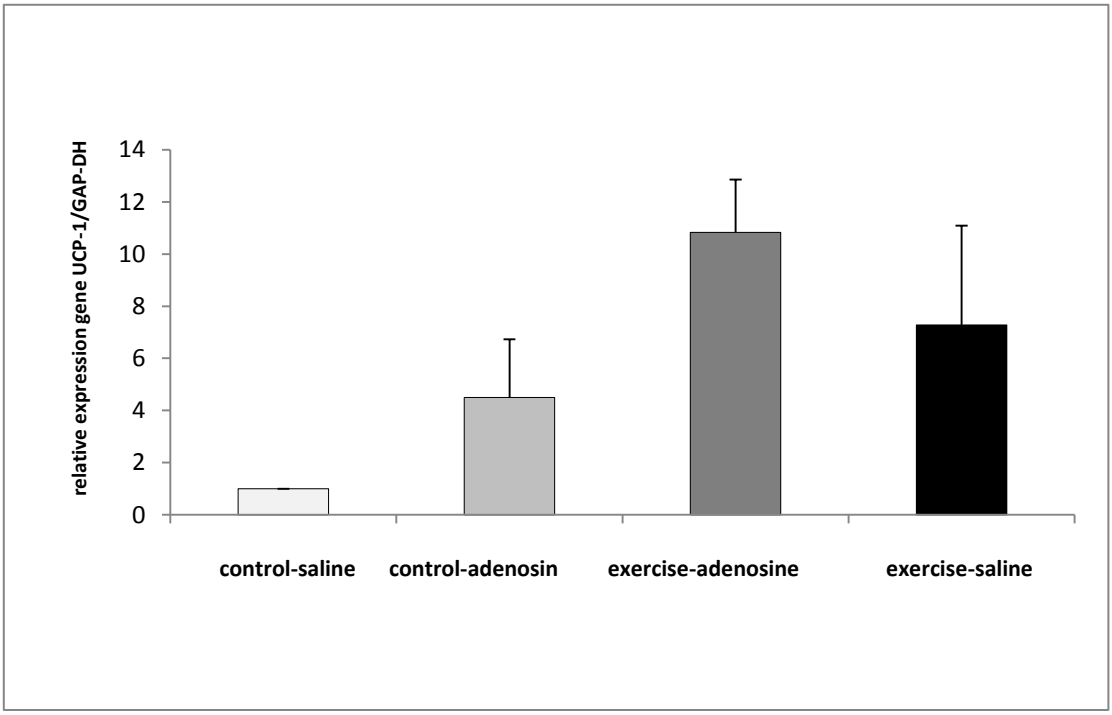

Figure 2. Changes in UCP-1 gene expression among study groups

Exercise training leads to in increase in beige adipocyte expression in subcutaneous fat tissue. In a research involving rats running on the wheel for 3 to 4 weeks, beige cellules appeared in subcutaneous adiposity indicated by increased PGC-1α, PRDM16, UCP-1 and other markers of brown or beige adipose tissue (15). Recent studies show that only eleven days of exercise training involving voluntary running on the wheel lead to distinctively higher regulation in beige and brown adipocyte marker genes including PGC-1α, PRDM16 and UCP-1.

A conception concluded from these studies is that the adaptations induced by exercise training in subcutaneous adipose tissue lead to an improvement in systemic metabolic homeostasis, which occurs through regular activity.

Saeid Daneshyar et al (2015), alanyzed the effect of eight weeks of endurance exercise on UCP-1 gene expression in visceral white adipose tissue in the retroperitoneal region of mice in their study and realized that long-term endurance exercise training results in little weight loss and body mass index and increased gene expression of the thermogenic protein UCP-1 in the white adipose tissue in the retroperitoneal area (21). The data from the mentioned studies are consistent with the present study in indicating increased expression of UCP-1 in white adipose tissue as a result of exercise training.

It appears that exercise training plays a role in increasing the expression of UCP-1 through many important endocrine mechanisms: 1) in response to exercise training, sympathetic activity and the amount of norepinephrine in the circulation increase distinctively (22). Norepinephrine bonds to Beta-3-adrenergic receptors, leading to the induction of the signaling pathway resulting in UCP-1 and nuclear factor PGC-1α in the subcutaneous adiposity and the brown adipose tissue (12), which in long term cause the increased accumulation of this protein in white adipose tissue. 2) In response to exercise training, thyroid hormone levels in the circulation (23) and the activity and amount of DIO2 enzyme in white adipose tissue (24) increase, which probably leads to the induction of UCP-1 gene expression in long term (12). 3) In response to exercise training, Irisin secretion from muscles and adipose tissue increases considerably (25), which may induce UCP-1 expression in white adipose tissue through an unknown mechanism (12). Apart from endocrine factors, intracellular regulatory agents (26) such as PGC-1α, also play an essential role in the induction of UCP-1 expression (14).

On the subject of the role of adenosine in UCP-1 gene expression, Rosen et al (2014) demonstrated that adenosine increases the expression of thermogenic markers in brown and white adipocytes in humans and also induces the expression of thermogenic markers in white and brown adipocytes in mice. Adenosine, A_{2A} and A_{2B} agonists as well as A_1 antagonist result in higher oxygen consumption and circular AMP. In addition, adenosine and A_{2A} agonist, increase lipolysis. In the mentioned study, Western Blot showed that A_{2A} and A_{2B} protein levels are many times higher in mature brown adipocytes than in white adipocytes while A_1 receptors of adenosine were higher in white adipocytes in mice. Furthermore, the expression of A_{2A} receptor was distinctively higher in brown adipocytes of humans than in white adipocytes while the expression of A_1 receptors was higher in white adipocytes. In brown adipose tissue, A_{2A} receptors were the most expressed adenosine receptors. In general, the mentioned study indicated that adenosine receptors are expressed differently in brown and white adipose tissue, as well as among different species. This may explain the reason why adenosine has contrary effects in different species and in different types of fat tissue. Rosen et al revised the signal of A_{2A} adenosine to counteract the obesity resulted from diet continuing their research. Mice with a high-fat diet (HFD) that were treated by an A_{2A} agonist demonstrated a significant weight loss and a 26 percent reduction in the relative fat mass and a 13 percent increase in fat-free mass. In addition, the weight of the inguinal white adipose tissue and the gonadal white adipose tissue was reduced 48 and 71 percent respectively. Also, mice treated with A_{2A} agonist, showed an increase in the expression of thermogenic markers in white and brown adipose tissue with more than 7 times increased UCP-1 in white adipose tissue. The browning of white-looking adipose tissue protects mice from obesity caused by diet and could be induced by beta-adrenergic agonists and many other stimuli (27,28).

In the white adipose tissue of the mice treated with A_{2A} agonist for 10 days, the two brown adipocyte markers, PPAR and PGC-1α lead to increased UCP-1, which shows that selective activation of A_{2A} receptors in white adipose tissue is able to induce browning (29).

Conclusions

According to the studies mentioned above, it was observed that adenosine, A_{2A} and A_{2B} agonists and A_1 antagonist cause the activation of Adenylyl cyclase and transformation of AMP into cyclic AMP by bonding to the related receptors in the membrane of adipose cells and thus activate the PGC-1α /P38MAPK/PKA pathway and lead to UCP-1 gene expression in visceral adiposity. The reason for increased UCP-1 in the adenosine group could possibly be one of the mechanisms mentioned above. On the insignificance of the collective effect of exercise and adenosine on UCP-1 gene expression, it may be noted that the average in this group had increased compared to the exercise and adenosine groups separately but the increase was statistically insignificant. It is probable that some mechanisms be activated through the collective effect of exercise and adenosine that reduce the synergic effect of exercise and adenosine. Thus, there is a need for further research in this field.

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