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Original Contribution

VOLUNTARY WHEEL RUNNING IS EFFECTIVE ON SUPPRESSING OF OBESITY BUT NOT ON BLOOD PRESSURE AND INSULIN RESISTANCE IN FEMALE RATS FED WITH HIGH FRUCTOSE DIET

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ABSTRACT

A fructose-rich diet has been known to cause metabolic syndrome effects such as body weight gain, increased blood pressure, blood lipids and glucose levels. The role of voluntary physical activity in these alterations is not known clearly. The aim of this study was to investigate the possible improving effects of voluntary physical activity in rats that were feeding with a fructose-rich diet.

Spraque-Dawley female rats were separated as control (C;n=7), voluntary physical activity (A;n=7), fructose (F;n=7) and fructose+activity (F+A;n=7) groups. A and FA groups were kept in cages with running wheels during six weeks. F and FA groups were fed with adding 20% fructose in drinking water. Body weight was measured weekly and Lee Index was used to determine obesity. At the end of the feeding period serum glucose, insulin and lipid levels were measured by enzymatic method and blood pressure was determined with the tail-cuff method.

Daily voluntary walking distance in F+A and A groups were similar during six weeks. Fructose intake induced to increase systolic blood pressure (p=0.001), diastolic blood pressure (p=0.002), glucose (p=0.041), insulin (p=0.001), cholesterol (p=0.001), triglyceride (p=0.001) and liver weight (p=0.035). The voluntary activity was found effective on the decrease of weight gain (p=0.018) however we did not observe a significant effect on blood pressure (p=0.917) and insulin resistance (p=0.565) following the fructose-rich diet.

We conclude that voluntary activity has preventive effect on obesity but may not to be effective on increased blood pressure and insulin resistance in female rats which were feeding fructose-rich diet during six weeks.

Key Words: Exercise, fructose, metabolic syndrome, voluntary physical activity

INTRODUCTION

Fructose-rich diet causes symptoms of metabolic syndrome such as obesity, dyslipidemia, and hypertension and insulin resistance (1, 2). The prevalence of metabolic syndrome in 20 years and older women was reported to be 31.9% (3). In addition, the frequency of metabolic syndrome is higher in persons who are doing less physical activity (4). Experimental studies have been reported that the effects of a long-term fructose-rich diet can be prevented by treadmill exercise (5, 6). According to a study by Mostarda et al. (6), treadmill walking exercise for five days a week for 10 weeks decreases weight gain, systolic and diastolic blood pressure, and insulin resistance results from the fructose-rich diet in rats. However, there are few studies on the preventive effects of voluntary wheel running of the weight gain, systolic and diastolic blood pressure, and insulin resistance.

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According to the data of the World Health Organization, 60% of the world population is not physically active and in recent years, it has led to further investigation of the effects of voluntary physical activity on the body in experimental studies (7). Voluntary activities are intrinsically motivating exercises in both humans and animals (8). In experimental studies, running wheels are used to perform voluntary exercises in rodents (9). The running wheel allows the experimental animals to perform physical activity in their living environments. Voluntarily exercising rats had lower body weight than non-exercising rats (10). In addition, voluntary exercise is reported to reduce cardiovascular complications associated with diabetes (11). A recent study by Rattanavichit et al. (12) showed that voluntary physical activity can increase insulin-mediated glucose transport in muscles and reduce adipose tissue content during a fructose-rich diet in male rats. However, it is stated that there may be gender related changes in this subject and voluntary exercise behavior in female rats could be varied (13). It is reported that this difference may be due to the fact that female rats tend to run more distance than male rats and that food consumption behaviors are different. In the present study, we aimed to investigate the possible improving effects of voluntary physical activity in female rats that were feeding with a high fructose diet. Thus, the effects of voluntary wheel running on body weight, blood pressure, serum lipid, glucose levels and insulin resistance were investigated in female rats receiving fructose in their drinking water (20% w/v) for six weeks in the present study.

MATERIAL AND METHODS

Animals and interventions: Twenty-eight Sprague-Dawley female rats (227.21 ± 12g) were supplied by the Experimental Animals Unit of Trakya University for this study. All rats were kept in an environment of 55% humidity, at 21±2°C, following a 12:12-h light-dark cycle (lights on from 7:00 AM to 7:00 PM) and each was in separate cages. Rats were randomly divided into four groups: control rats were given reverse osmosis water (C, n=7), voluntary physical activity rats given reverse osmosis water and doing voluntary physical activity (A, n=7), fructose rats receiving liquid fructose (20% w/v) (F, n=7) and fructose+voluntary physical activity rats receiving liquid fructose (20% w/v) (F+A, n=7). This study was approved by Trakya University Animal Experiments Local Ethics Committee (TUHADYEK-2016/21).

All animals were given free access to food (Optima Foods IND. And TRADE CO. INC Bolu, Turkey), and water for six weeks **(Table 1)**. Rats in the F and F+A groups were fed for six weeks by adding 20% fructose to drinking water passed through the reverse osmosis system. Amounts of fluid intake of all animals in the experimental groups were recorded daily and water consumption was calculated as mL/day per rat. Rats in the fructose groups were fed with fructose solution (200 g/L) prepared by dissolving standard feed and D-fructose (Sigma-Aldrich, St. Louis, MO, USA) in a ratio of 20% in drinking water. The fructose solution was prepared daily. In order to prevent bacterial growth in drinking water, both control and fructose groups' water bottles were cleaned with hot water every week. At the end of the feeding period, thiopental (I.E. Ulagay INC Istanbul, Turkey) was given to the rats intraperitoneally (100 mg/kg) for anaesthesia and afterwards their blood pressures were measured. Thereafter, blood samples were taken from the femoral vein for metabolic examinations.

Body weight measurement: In this study, body weight was measured weekly, and Lee index was used to determine obesity (6) Lee index was calculated by the formula of rats' body weight $(g)^{1/3}$x10/naso-anal length (mm). Total heart weight, right and left ventricular weights, liver and lung weights were measured in all groups. Heart weight (mg)/tibia length (mm) ratios were determined for cardiac hypertrophy.

Voluntary physical activity: For the voluntary physical activity of rats, a running wheel mounted on the cages and a device for recording the frequency of rotation was used (Atatek Automation End. Product. INC. Tekirdağ, Turkey). Similar to previous studies (14), the dimensions of the rotating wheel were 31.5 cm in diameter and 10 cm in width, with a cage height of 7 cm and a circumference of 1.081 meters. The wheel was removable from the cage and was cleanable. Daily wheel rotations were recorded as clockwise, counter clockwise and total number of revolutions. The rats in the activity groups were kept in cages with rotating wheels for one week before the experiment in order for them to recognize the wheel. Daily wheel activities were determined as km/day.

Table 1. *The ingredients of standard laboratory feed*

Crude protein (%)	24.0
Crude cellulose (%)	3.00
Crude fat (%)	5.55
Crude ash (%)	9.00
Lysine (%)	1.35
Methionine (%)	0.45
Calcium (%)	1.16
Phosphorus (%)	0.84
Sodium (%)	0.24
Vitamin A (I.U/kg)	22200
Vitamin D3 (I.U/kg)	4884
Iodine (calcium iodine anhydride) (mg/kg)	0.8
Cobalt (cobalt carbonate monohydrate) (mg/kg)	0.15
Copper (copper sulphate pentahydrate) (mg/kg)	10
Manganese (manganese oxide) (mg/kg)	50
Zinc (zinc oxide) (mg/kg)	50
Selenium (sodium selenite) (mg/kg)	0.15

Blood pressure determination and metabolic measurements: On the last day of the sixth week of the study, the blood pressure of rats was measured by the method of indirect *tail-cuff* plethysmography (MAY NIBP250, Ankara, Turkey). Systolic blood pressure, diastolic blood pressure, and heart rate averages were calculated by taking 5 measurements from each rat. The abdomens of rats were then cut open and blood samples were taken from femoral veins of the rats for metabolic examinations. Blood samples were centrifuged at 3000 rpm at +4°C for 15 mins (MPW 350R, Poland). Serum glucose, triglyceride, total cholesterol, high-density lipoprotein (HDL) levels were measured by the enzymatic method. Serum levels of insulin (EMD Millipore Corporation, Missouri, USA) were measured by ELISA method. Low-density lipoprotein (LDL) was calculated by Friedewald formula (16). Friedewald's formula is given as: LDL cholesterol=Total cholesterol-(HDL cholesterol+(TG/5)). Insulin resistance (HOMA-IR) values in rats were calculated by the formula of HOMA-IR: serum insulin (mmol/L)*blood glucose (mmol/L) /22.5 (16).

Statistical analysis: Values were expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) was used to determine the main effects of fructose and activity and the fructose+activity interaction. The mean walking distances of F+A and A groups were compared using Student's t-test. A value of $p<0.05$ was considered statistically significant.

RESULTS

When the amount of fluid consumed was measured, it was observed that the rats in fructose groups (F and F+A) had higher fluid consumption than the other groups (C and A) **(Table 2)**. The difference in fluid intake between the first and sixth weeks was caused by fructose consumption ($p <0.001$). Running activity is shown in **Figure 1.** When the average walking distances of A and F+A group rats were measured in six weeks, there was no difference between F+A (5.44 ±3.03 km/day) and A (5.00 ±2.45 km/day) groups ($p=0.771$). The effects of fructose on the hemodynamics, metabolic and morphological values of four groups, the effects of activity and whether two factors together make any effects were examined by two-way ANOVA test. It was found that systolic and diastolic blood pressure values were higher due to a fructose-rich diet ($p=0.001$, $p=0.002$ respectively; **Table 3**). The decrease in heart rate was observed due to the effects of activity ($p=0.016$). When the metabolic findings of the groups were evaluated, it was found that glucose, insulin, cholesterol, triglyceride and HDL values were high due to the fructose-rich diet **(Table 4)**. No significant difference was found between HOMA-IR values of the groups, which are indicatives of insulin resistance **(Table 4)**. The change in Lee index, associated with weight gain, ($p=0.018$) was determined to be related to physical activity **(Table 2)**. In addition, liver weights were found to be high due to the effect of fructose-rich diet ($p=0.035$) **(Table 5)**. Total weight of heart, right and left ventricular

weights, heart weight/tibia length (HW/TL) ratio used to determine cardiac hypertrophy and lung weights were not significantly different between the groups **(Table 5)**.

Table 2. *The effect of fructose and exercise on Lee index and the difference in fluid intake between the first and the last day of feeding period*

Groups	C n=7	A n=7	F n=7	F+A n=7	Two-way ANOVA *p*		
					The effect of fructose	The effect of activity	The effect of fructose and activity
Lee Index	307±0.1	296±0.1	301±0.1	300±0.1	0.514	0.018	0.116
The difference in fluid intake (mL)	2.4±4.9	5.1±3.4	25.0±1.2	18.6±3.8	0.001	0.785	0.083

Data are expressed as mean ± standard deviation. C: Control group; A: Activity group; F: Fructose group; F+A: Fructose+Activity group.

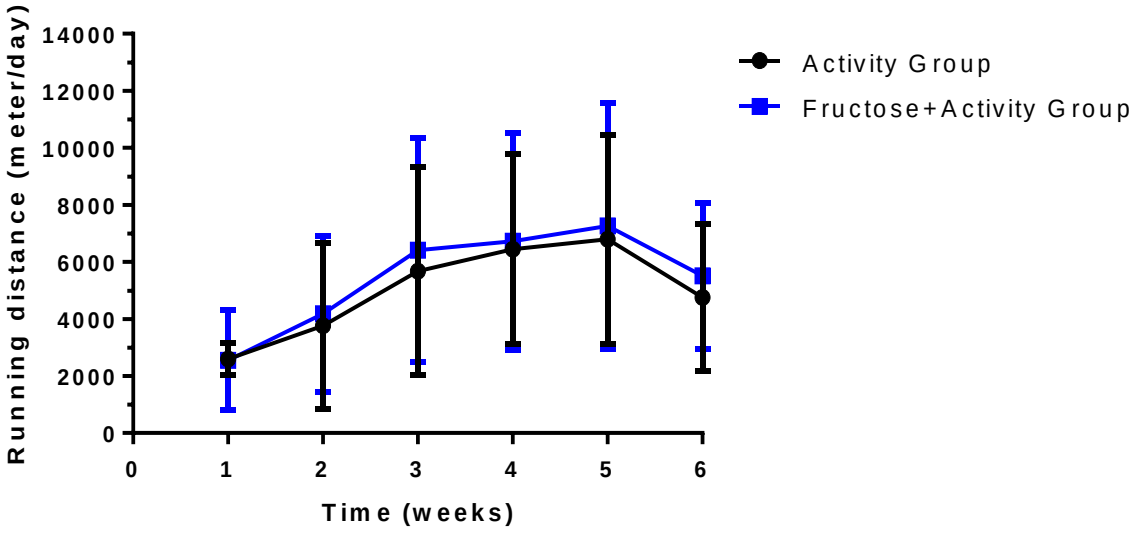

Figure 1. Measurements of wheel activity in the activity groups

Table 3. *Blood pressures and heart rates of the groups*

	C n=7	A n=7	F n=7	F+A n=7	Two-way ANOVA *p*		
					The effect of fructose	The effect of activity	The effect of fructose and activity
Systolic pressure (mmHg)	132.9±18.5	129.4±16.5	155.2±22.5	153.1±13.8	0.001	0.917	0.569
Diastolic pressure (mmHg)	107.0±16.7	105.9±17.4	113.5±13.1	122.9±14.4	0.002	0.369	0.269
Heart rate (beat/min)	395.2±35.2	356.2±46.9	418.1±31.9	374.9±45.3	0.366	0.016	0.864

Data are expressed as mean ± standard deviation. C: Control group; A: Activity group; F: Fructose group; F+A: Fructose+Activity group.

Table 4. *Metabolic findings of the groups*

	C n=7	A n=7	F n=7	F+A n=7	Two-way ANOVA *p*		
					The effect of fructose	The effect of activity	The effect of fructose and activity
Glucose (mg/dl)	104.0±16.4	86.9±18.5	117.4±21.0	104.0±13.5	0.041	0.266	0.426
Insulin (ng/mL)	0.8±0.3	0.8±0.3	1.1±0.25	0.8±0.3	0.046	0.061	0.290
HOMA-IR	4.84±2.16	4.44±2.14	3.07±2.99	4.67±2.6	0.466	0.565	0.190
Total cholesterol (mg/dl)	58.0±12.9	42.1±5.0	72.4±6.1	75.6±13.4	0.001	0.363	0.198
Triglyceride (mg/dl)	42.1±21.8	61.7±35.3	127.7±46.9	90.6±45.5	0.001	0.204	0.446
HDL (mg/dl)	33.9±7.6	25.9±4.1	40.4±3.0	42.6±6.0	0.001	0.618	0.272
LDL (mg/dl)	14.7±9.1	3.9±5.5	6.4±9.8	14.9±7.8	0.201	0.854	0.066

Data are expressed as mean ± standard deviation. C: Control group; A: Activity group; F: Fructose group; F+A: Fructose+Activity group. HDL: High-density lipoprotein; LDL: Low-density lipoprotein. HOMA-IR: Homeostasis assessment model of insulin resistance.

Table 5. *Morphological evaluations of the groups regarding heart, liver and lung*

	C n=7	A n=7	F n=7	F+A n=7	Two-way ANOVA *p*		
					The effect of fructose	The effect of activity	The effect of fructose and activity
Total heart weight (g)	0.92±0.11	0.92±0.07	0.88±0.06	0.95±0.10	0.193	0.303	0.377
Right ventricle weight (g)	0.17±0.32	0.18±0.04	0.17±0.05	0.18±0.02	0.564	0.912	0.480
Left ventricle weight (g)	0.63±0.06	0.67±0.65	0.64±0.08	0.69±0.09	0.454	0.207	0.240
Liver weight (g)	9.0±0.9	9.5±1.2	10.7±0.8	10.9±2.4	0.035	0.919	0.964
Lung weight (g)	1.37±0.36	1.53±0.84	1.45±0.05	1.39±0.12	0.583	0.959	0.431
HW/TL (mg/mm)	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.048	0.503	0.373

Data are expressed as mean ± standard deviation. C: Control group; A: Activity group; F: Fructose group; F+A: Fructose+Activity group. HW: Heart weight; TL: Tibia length.

DISCUSSION

In this study, voluntary physical activity was found to be effective in decreasing the weight gain and resting heart rate in the development of metabolic syndrome induced by adding fructose to drinking water for six weeks in female rats. However, voluntary physical activity in female rats did not show any significant change in blood pressure, blood glucose and lipid levels due to a fructose-rich diet.

The running wheel apparatus used for voluntary exercise enables experimental animals to perform physical activity in their living environments. Voluntary wheel running is preferred because it is self-motivating, allows for long-term activity, and can be performed without stress. In the studies using the rotating wheel, different animal species can perform activities at various levels (7). Previously reported that rats were able to walk-run for a maximum of 43 km, wild mice for 31 km, laboratory mice for 16 km, golden hamsters for 9 km, and gerbil for 8 km per 24 hours (9). In our study, the rats walked between 1.2 and 9 km per day. This walking distance covers a fairly wide range. The results of a recent study in female rats for the cause of this wide range of voluntary exercise behaviors suggest that voluntary exercise with a greater running distance is associated with an increase in the function of N-Methyl-D-aspartate receptors in the nucleus accumbens (17). In recent years, some studies conducted to evaluate the voluntary exercise of rats in different groups with similar activity levels. For example, in a study by Rattanavichit et al. (12), rats that covered an average distance of 5-8 km per day were selected for evaluating voluntary physical activity levels. In our study, all rats were evaluated without limitation in terms of voluntary activity. Thus, voluntary physical activity at all levels of rats was evaluated in this study.

Metabolic syndrome findings such as increased glucose intolerance, insulin resistance, hyperinsulinemia, hypertension, dyslipidemia have been shown in laboratory animals fed with a fructose-rich diet (18). The amount of fructose, the mode of administration or the duration of administration were different in the previous experimental studies. For example, male Spraque-Dawley rats fed with 60% fructose diet showed significant metabolic effects than the rats were given 10% fructose in drinking water in a previous study (19). However, no difference has been observed in serum glucose, insulin and lipid values rats fed with 10% fructose in drinking water between the control group in the study by Moura et al. (18). Similar to this finding, we did not find a significant change in blood glucose and lipid levels when we added 10% fructose to drinking water, in our previous study (20). In the present study, we observed increased serum glucose, insulin, cholesterol, triglyceride and HDL levels after fructose-rich diet for six weeks. However, we observed that voluntary physical activity did not cause a significant change in increasing metabolic parameters except of body weight.

In our study, the difference in fluid intake between the first and the last day of the experiment has significantly increased in the fructose groups (F and F+A group) (Table 2). This increase may be related with the using appropriate amount of fructose in drinking water. In a study by Mamikutty et al. (21) which was given male rats 20% and 25% fructose in drinking water showed that rats consumed a higher amount of 20% fructose in drinking water than 25% fructose. In addition, rats fed with 20% fructose in drinking water consumed more water than the rats in the control group. This was suggested as that the rats liked the taste of 20% fructose in drinking water than 25% fructose. In previous studies, the amount of fructose given by drinking water was varied between 5-40% (20-23). In our study, fructose was given by adding 20% to drinking water and the same feed content in a diet was used in all groups (Table 1). However, although the fluid intake was determined, daily feed intake and energy values could not be determined. Therefore, the effect of feed intake on the process of weight gain could not be shown between groups.

According to the findings of our study, no significant changes were detected between the groups in terms of insulin resistance, HOMA-IR values due to a fructose-rich diet or exercise. In a previous study, it was suggested that voluntary activity had a decreasing effect on HOMA-IR in diabetic rats, caused a decrease in insulin resistance, and had a protective effect on cardiac tissue by increasing the protein expression of vascular endothelial growth factor-A (24). A lower limit was determined for voluntary exercise and the findings of rats covering a shorter distance were excluded in some of the studies. For example, regarding the effect of voluntary exercise on insulin resistance, Rattanavichit et al. (12) did not evaluate the rats covering less than 5 km per day. In rats fed with 10% fructose in drinking water, it was shown that voluntary exercise decreased the amount of visceral fat and was reported to be a contributing factor in regulating insulin sensitivity. In our study, the reason of not detecting a significant change in HOMA-IR levels may be due to the evaluation of all the

rats without any lower limit for voluntary physical activity in present study.

In conclusion, according to the results of this study, voluntary physical activity decreases obesity and heart rate however, it does not cause important changes in blood pressure, blood glucose and lipid homeostasis during a fructose-rich diet in female rats.

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