




AdipoR1 and AdipoR2 Receptor Gene Expression in Rats with Metabolic Syndrome Induced by Fructose Diet

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ABSTRACT

In this study, it was aimed to investigate serum adiponectin levels and AdipoR1 and AdipoR2 gene expressions in rats with metabolic syndrome with fructose diet. Twenty-four male Sprague Dawley rats were used for this purpose. The rats in the control group were fed only with water and rat food while the rats in the experimental group were fed with water containing 20% D-fructose and rat food for 16 weeks with *ad libitum*. In the control and metabolic syndrome groups, weight differences between the beginning and end of the experiment and the abdominal circumference measured at the end of the experiment were found statistically significant at the level of $p < 0.001$. Adiponectin levels were measured by ELISA in serum samples at the end of the experiment. Fructose administration caused a statistically significant decrease in serum adiponectin levels compared to the control group ($p < 0.001$). At the end of the period of fructose application time in liver tissue, quantitative change of AdipoR1 gene was observed as 1.97 fold decrease compared to control according to RT-PCR results, while a decrease of 3.11 fold was observed in the quantitative change of AdipoR2 gene.

As a result, high fructose consumption decreases serum adiponectin levels and significantly deteriorates adiponectin receptor expression in the liver.

Key Words: Adiponectin, AdipoR1, AdipoR2, Fructose, Metabolic syndrome

Fruktoz Diyetiyle Metabolik Sendrom Oluşturulan Ratlarda AdipoR1 ve AdipoR2 Reseptör gen Ekspresyonu

ÖZET

Bu çalışmada fruktoz diyetiyle metabolik sendrom oluşturulan ratlarda serum adiponektin düzeyleri ve AdipoR1 ve AdipoR2 gen ekspresyonlarının araştırılması amaçlandı. Bu amaçla 24 adet 8 haftalık erkek Sprague Dawley rat kullanıldı. Kontrol grubundaki ratlar sadece çeşme suyu ve rat yemi ile beslenirken deney grubundaki ratlar % 20'lik D-fruktoz içeren çeşme suyu ve rat yemi ile 16 hafta *ad libitum* beslendi. Kontrol ve metabolik sendrom grubundaki ratların deneme başlangıcı ve sonu arasındaki kilo farkları ve deneme sonunda ölçülen karın çevreleri gruplar arasında $p < 0,001$ düzeyinde istatistiksel olarak anlamlı bulundu. Deneme sonunda serum örneklerinde adiponektin düzeyleri ELISA yöntemi ile ölçüldü. Fruktoz uygulaması kontrol grubu ile karşılaştırıldığında serum adiponektin düzeyinde istatistiksel olarak anlamlı azalışa neden oldu ($p < 0,001$). Karaciğer dokusunda fruktoz uygulama süresi sonunda RT-PCR sonuçlarına göre AdipoR1 geninin kantitatif değişiminde kontrole göre 1,97 kat bir azalış gözlenirken, AdipoR2 geninin kantitatif değişiminde ise 3,11 katlık bir azalma belirlendi. Sonuç olarak yüksek fruktoz tüketimi, serum adiponektin düzeyini azaltırken karaciğerde adiponektin reseptörü ekspresyonunu belirgin şekilde bozduğu görüldü.

Anahtar kelimeler: Adiponektin, AdipoR1, AdipoR2, Fruktoz, Metabolik sendrom

Introduction

Fructose is a monosaccharide found naturally in fruits, grains, and root vegetables and is one of the essential energy sources for the body. Fructose, also known as fruit sugar, is the sweetest of all-natural sugars. High-fructose corn syrup is produced by converting glucose molecules into fructose using enzymes. It is used in food and beverage manufacturing. (Forshee et al., 2007).

An increase in the consumption of high-fructose corn syrup has occurred nowadays. Since fructose increases the taste and delays the feeling of satiety, it reveals health risks with excessive food consumption (Angelopoulos et al., 2009). The digestion, absorption, and metabolism of fructose are different from glucose. Fructose is absorbed from the intestines with the glucose transporter GLUT5 and then diffused into blood vessels via GLUT2. Unlike glucose, the absorption of fructose from the intestines does not require ATP hydrolysis and is independent of sodium absorption. This results in excessive fructose uptake by the liver (Rizkalla, 2010). It has been demonstrated that a diet high in fructose leads to glucose intolerance and insulin resistance, type 2 diabetes, obesity, hypertension, and cardiovascular diseases (Ross et al., 2009).

Adiponectin is among the many proteins secreted by adipocytes that are involved in the regulation of glucose and lipid metabolism and can change insulin sensitivity and energy balance. It increases the sensitivity of tissues to insulin, has protective impacts against the development of type 2 diabetes mellitus and has several anti-inflammatory and antiatherogenic features (Berg et al., 2002; Brochu-Gaudreau et al., 2010).

Adiponectin exerts its metabolic impacts, at least partially, by stimulating protein kinase activated with adenosine 5-monophosphate, which increases both glucose uptake and fatty acid oxidation rates (Tomas et al., 2002) and nuclear receptor peroxisome proliferator-active receptor (PPAR) and promotes lipid oxidation. All these impacts are thought to be mediated by two receptors, called adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) expressed in most tissues, such as the liver, adipose tissue, skeletal muscles (Chinetti et al., 2004), pancreatic β -cells (Kharroubi et al., 2004), and macrophages (Tomas et al., 2002).

AdipoR1 is commonly expressed in skeletal muscle and AdipoR2 in the liver. AdipoR1 receptors provide AMP-activated protein kinase (AMPK) activation in skeletal muscle, increase glucose uptake and fatty acid oxidation, and AdipoR2 causes AMPK activation in the liver (Ma et al., 2015). AdipoR1 and AdipoR2 are integral membrane proteins, in contrast to G-protein-coupled receptors, the N-terminal part is intracellular, and the C-terminal part is extracellular. Globular and full-length adiponectin binds to AdipoR1/R2, and AMP-kinase affects PPAR α

ligand activity, fatty acid oxidation. The N-terminal cytoplasmic part of AdipoR1 interacts with adaptor protein part with pleckstrin, phosphotyrosine-binding part and leucine zipper motif (APPL). The interaction of APPL with AdipoR1 is stimulated by the binding of adiponectin to the C-terminal part of AdipoR1. The mentioned interaction takes an essential part in adiponectin signalling and adiponectin-mediated lipid oxidation and glucose uptake (Kadowaki et al., 2006).

Obesity decreases AdipoR1/R2 expression as well as adiponectin levels. This decreases adiponectin sensitivity in obese patients and leads to insulin resistance. The expression of AdipoR1 and AdipoR2 in muscle and adipose tissue and AMP-kinase activation in skeletal muscle decreased in ob/ob insulin-resistant mice. A vicious circle develops by increasing hyperinsulinemia. It has been revealed that adiponectin receptor expression also decreases in type 2 diabetic patients. In a study, AdipoR1 mRNA expression was determined to be an independent determinant of first-phase insulin secretion independent of insulin sensitivity and body fat. Since AdipoR1/R2 is also expressed in pancreatic β cells, it is thought that it may also be involved in insulin secretion (Mao et al., 2006; Tsuchida et al., 2004).

The disruption of the expression of the said receptors affects the whole-body metabolism and the metabolic impacts of adiponectin (Yamauchi et al., 2003). Thus, the expression level of AdipoR1 and R2 in target tissues may take part in controlling metabolism and insulin sensitivity. Little is known about regulating AdipoR1/R2 expression. Studies have shown that the expression of adiponectin receptors decreases in ob/ob and db/db mice (Tsuchida et al., 2004; Inukai et al., 2005) and skeletal muscles of individuals with a family history of type 2 diabetes mellitus (Civitarese et al., 2004) but does not change in obese Zucker rats (Beylot et al., 2006). Thus, the exact role of AdipoRs in insulin-resistant situations continues to be discussed, and it is thought that it may differ depending on the model studied and the cause of insulin resistance. On the other contrary, Debard et al. (2004) revealed no decrease in AdipoR1 and AdipoR2 messenger mRNA concentrations in the skeletal muscle of type 2 diabetic patients in comparison with healthy subjects. Furthermore, taken together, the above-mentioned findings support the idea that the increased expression of AdipoR1/R2 increases insulin sensitivity and reduces the risk of developing type 2 diabetes mellitus.

Adiponectin and adiponectin receptors are multiple potential therapeutic targets for combating obesity-related diseases that are characterized by insulin resistance (Kadowaki et al., 2006). The present research aimed to examine serum adiponectin levels and liver adiponectin receptor (AdipoR1 and AdipoR2) gene expressions in rats with metabolic syndrome under different nutritional conditions (high-fructose diet).

Table 1 Rat qPCR primer sequences.

Gene	Primers
Gapdh	Forward ATGGTGAAGGTCGGTGTGAAC
	Reverse GGTCAATGAAGGGGTCGTT
AdipoR1	Forward GTACCAGCCAGATGTCTTCCC
	Reverse CGCTTACCCTTCTCTCCAG
AdipoR2	Forward GTAACAATGACAACCACCACGG
	Reverse TCCCACACCTTACAAACAACTC

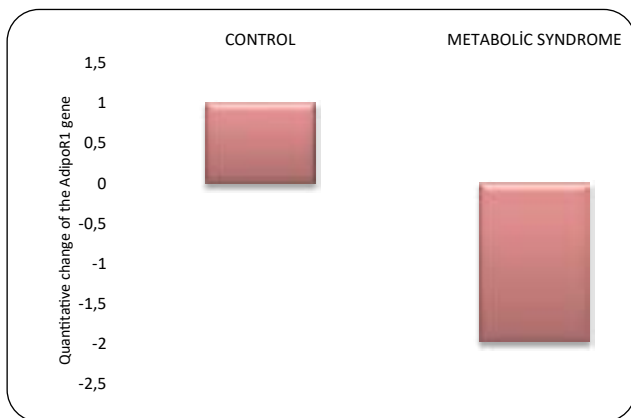


Figure 1 Change in AdipoR1 gene expression in liver tissue.

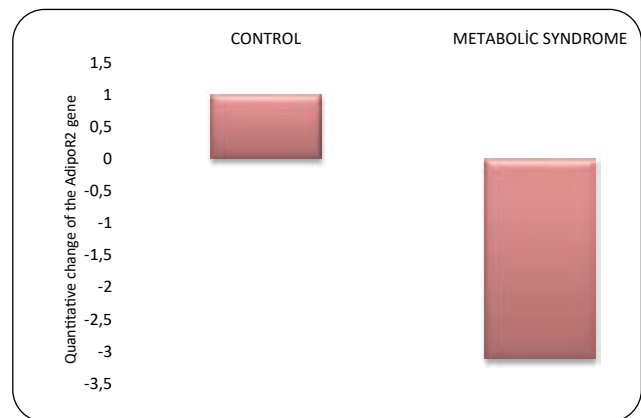


Figure 2 Change in AdipoR2 gene expression in liver tissue.

Material and Method

Animals

For this study, 24 male Sprague Dawley rats, 8 weeks old (~245 g b.w.) were used. They were kept in the animal house of Aydın Adnan Menderes University, Faculty of Veterinary Medicine. They were kept at $22 \pm 1^\circ\text{C}$ temperature at 12 h dark-light cycles. Food and water were given as *ad libitum*. The experiments were conducted by Ethical Guidelines for investigations of laboratory animals and were approved by the Ethical Committee of Aydın Adnan Menderes University (2015/081).

The animals were adapted to the environment by placing them in standard cages in the experimental animal unit one week before the study. Animals were randomly divided into 2 groups (each, $n = 12$): (1) the control group received regular rodent diet (2) Metabolic syndrome group was fed on a regular rodent diet and 20 % of drinking water was composed of fructose solution. The fructose solution was prepared fresh each day. The body weight of each animal was measured every week from the start of the experiment. The body weight difference between control and metabolic syndrome group animals was calculated statistically with the data obtained at the end of the experiment. The abdominal circumference of the animals was measured and recorded on the experiment start and end day to determine the weight change of each animal during the experiment period. The average abdominal circumference measurement difference between the control group and the metabolic syndrome group with the data obtained at the end of the experiment was statistically calculated.

After 16-weeks feeding period, rats were euthanized under anaesthesia by taking blood from the heart. The samples were centrifuged and stored at -20°C until time of assay of serum levels of Adiponectin. The animals were sacrificed by a blow to the head and tissue samples from the liver were dissected, frozen in liquid nitrogen and kept at -80°C until they were used to assess the AdipoR1 and AdipoR2 gene expression.

Biochemical Analysis

Serum level of Adiponectin was determined by using rat adiponectin ELISA kit (Boster Biological Technology, USA) according to the manufacturer's instruction.

Total RNA was isolated from the livers using a Total RNA extraction Kit (Geneall, Cat no: 305-101), according to the manufacturer's protocol. The samples were stored at -80°C until they were used. RNA concentrations were determined by optical density measurement at 260 and 280 nm. Purity was assessed by the 260/280 nm ratio. Complementary deoxyribonucleic acid was synthesized from total RNA with the cDNA Synthesis

kit (Transcriptor High Fidelity cDNA Synthese kit, Roche, Version 8, 05091284001).

Real-time PCR analysis was performed using SYBR green (Roche, Fast Start Essential DNA Green Master), by the manufacturer's procedure using a Light Cycler Nano Real-Time PCR. All primers were design program Primer3 and synthesized by Atlas Biotechnology (Ankara/Turkey).

Primers are listed in Table1. Gapdh mRNA levels were measured as internal standard and the data were expressed as relative units as a ratio of Gapdh mRNA concentrations. RT-PCR condition was an initial incubation at 40°C for 10 minutes that was followed by a 10- minutes incubation at 95°C then 40 cycles at 95°C (10 s), 60°C (10 s) and 72°C (15 s), and 20-second melting at 58°C . The Pfaffl method was used to determine the relative levels of AdipoR1 and AdipoR2 gene expression. The fold of AdipoR1 and AdipoR2 was normalized about the reference genes expressed (Livak and Schmittgen, 2001).

Statistical Analysis

Data were processed using the statistical package SPSS version 22 (Statistical Package for the Social Sciences). The compliance of the data to normal distribution was evaluated using the Shapiro Wilk test. Difference between groups showing normal distribution was made by Student's T test. Values of $p < 0.05$ were considered significant from the results obtained from the statistical analysis. Data were given as mean \pm standard deviation. Significance levels were shown as *** $p < 0.001$.

Results

At the beginning and end of the experiment, the weights of threats in the control and metabolic syndrome groups were called and weight differences between the weightings were taken. Accordingly, the difference between the weights of threats in the control group was 36.84 ± 2.64 g and in the metabolic syndrome group was 40.93 ± 1.27 g. The weight difference values between the groups were found to be statistically significant ($p < 0.001$). At the end of the experiment, abdominal circumference measurements of rats in the control and metabolic syndrome groups were taken and the abdominal circumference of threats in the control group was 18.59 ± 1.44 cm and the abdominal circumference of threats in the metabolic syndrome group was measured as 21.28 ± 1.3 cm. Statistical significance was found at the $p < 0.001$ between the groups. Serum adiponectin levels were measured as 11.72 ± 0.56 ng/ml in the control group and 8.34 ± 0.33 ng/ml in the experimental group. Fructose administration caused a statistically significant decrease in serum adiponectin level compared to the control group ($p < 0.001$).

At the end of the fructose application period in the liver tissue, according to the RT-PCR results, in the quantitative change of the AdipoR1 gene compared to the control was observed a 1.97-fold decrease, while a 3.11-fold decrease was observed in the quantitative change of the AdipoR2 gene (Figure 1 and 2).

Discussion

It was reported that insulin resistance and hypertension were observed in laboratory animals fed with a high-fructose diet, glucose metabolism and uptake pathways were turned upside down, triglyceride synthesis and lipogenesis increased significantly, and the mentioned results were largely parallel to the metabolic syndrome criteria in humans. Thus, diets containing high fructose are critical for inducing changes in metabolic syndrome in experimental animals, identifying the causative mechanisms, and developing new strategies for disease prevention/treatment (de Moura et al., 2009; Cardinali et al., 2013).

It is known that the pathophysiological changes observed in fructose-mediated metabolic syndrome models show differences that may arise from the study plan, such as the breed, age of the animal used, the amount of fructose, the route and duration of administration (Dai and McNeill, 1995; Roglans et al., 2007). In our study, the use of Sprague Dawley rats, which were reported to be more sensitive to the administration of fructose via drinking water, was preferred, and 20% fructose was added to their drinking water for 16 weeks.

Fructose and glucose added to the diet cause weight gain and a significant increase in fat mass. However, while there is an increase in visceral adipose tissue in those fed with a fructose-added diet, an increase in subcutaneous fat accumulation is observed in those fed with a glucose-added diet. Important data are suggesting that an increase in visceral adipose tissue is associated with cardiovascular diseases and metabolic diseases such as type 2 diabetes compared to subcutaneous fat accumulation (de Moura et al., 2009).

In the study conducted by Bocarsly et al. (2010), mice were given high-fructose corn syrup and sucrose for certain periods, and their effect on the body weight, fat and triglyceride of the mice was investigated. As a result of the study, it was stated that mice fed with high-fructose corn syrup showed abnormal weight gain, high triglyceride level, and fat accumulation. Therefore, it was emphasized that the excessive consumption of high-fructose corn syrup is an essential factor in obesity.

In a study in which the metabolic effects of diets containing free or bound fructose were compared, Sheludiakova et al. (2012) reported that although the total energy intake of animals was 25% higher, there was no change in body weight, whereas, in animals fed with fructose predominantly, there was abdominal fat, increased plasma triglyceride level and decreased glucose tolerance. Novelli et al. (2007) observed a significant increase in body weight after 30 days when they gave 30% sucrose to rats.

In our study, the differences in weight gain at the beginning and the end of the experiment and abdominal circumference measurements were found to be higher in rats fed with fructose diet. This suggests that a diet high in fructose may be an important risk factor for obesity.

Adiponectin secreted from adipose tissue is inversely proportional to adipose tissue mass and visceral adipocytes, unlike other adipocytokines, and its amount in the circulation decreases in obese individuals. Metabolic syndrome is a condition characterized by obesity, hyperinsulinemia, hyperlipidaemia, and hypertension and represents a serious risk factor for cardiovascular diseases and type II diabetes. The studies conducted have shown that a decrease in serum adiponectin level plays

a role in the development of insulin resistance and metabolic syndrome (Matsuzawa et al., 2004; Okamoto et al., 2006). It has been determined that the adiponectin form, which is in the form of a high molecular weight complex, is the active form in the serum and is better correlated with metabolic syndrome (Hanson et al., 2002).

Adiponectin takes an essential part in energy haemostasis by regulating glucose and fatty acid metabolism in peripheral tissues, including muscles and the liver (Berg et al., 2002). The biological functions of adiponectin depend on the function and expression of its specific receptors (AdipoR1, AdipoR2) as well as its serum levels. Some studies have demonstrated that the lack of adiponectin receptors causes hyperglycaemia and hyperinsulinemia (Parker-Duffen et al., 2014). It has been observed that the loss of metabolic effects of adiponectin in AdipoR1 and AdipoR2 knockout mice causes an increase in the tissue triglyceride content, inflammation, oxidative stress, insulin resistance, and glucose intolerance (Yamauchi et al., 2007).

The mechanisms that cause low adiponectin concentrations in insulin resistance are unclear. TNF- α is one of the molecules thought to cause insulin resistance. The studies conducted in adipose tissue and adipose culture cells have shown that (IL)-6 and TNF- α inhibit adiponectin secretion and expression (Bruun et al., 2003; Macda et al., 2001).

In a screening study carried out in India, it was observed that the rate of type 2 diabetes was low in individuals with high blood adiponectin levels and high in those with low blood adiponectin levels (Lindsay, 2002). In their study performed on obese individuals, Koca et al. (2006) reported that adiponectin levels decreased. Arita et al. (1999) reported a negative correlation between the decrease in adiponectin levels and body mass index in obese individuals. They stated that the reason for this might be related to the increase in insulin resistance, increase in the arteriosclerotic lesion and/or adipose tissue, and the increased TNF- α level.

Although mice with adiponectin deficiency showed almost normal insulin sensitivity when fed with a standard diet, they developed severe insulin resistance when fed with a high-fat and high-sucrose diet for two weeks (Kubota et al., 2002; Maeda et al., 2002). Another group of researchers observed increased fatty acid oxidation in the skeletal muscle of mice with adiponectin deficiency but did not observe any impact on insulin sensitivity or glucose tolerance in mice fed with a standard or high-fat diet (Ma et al., 2002).

Atanasovska et al. (2009) found that chronic fructose administration caused a significant increase in systolic blood pressure, body weight, serum triglyceride, free fatty acids, and insulin levels and decreased HDL adiponectin concentrations compared to the control group. The researchers reported that rosiglitazone treatment applied for four weeks could reverse these results and increase serum adiponectin levels two-fold.

Alzamendi et al. (2009) reported that plasma free fatty acids, leptin, and adiponectin levels increased and insulin resistance developed in laboratory animals fed with fructose for three weeks. Nakagawa et al. (2006) found that metabolic syndrome developed in rats fed with fructose, but not in rats fed with the same amount of glucose. Aksoy et al. (2016) observed that adiponectin levels increased significantly in rats fed with high-fructose and high-sucrose diet for eight weeks. They concluded that a diet rich in fructose was an important risk factor for obesity and mediated changes in Na⁺/K⁺-ATPase activity. Meeprom et al. (2011) reported that a high-fructose diet caused insulin resistance by considerably reducing insulin receptor, GLUT4 protein expression and adiponectin, AdipoR1 mRNA expression in the skeletal muscle. Mostafa-Hedeab et al.

(2017) showed that decreased AdipoR1/R2 expression levels in the liver, pancreas, kidney, and heart tissues of rats fed with a high-fructose diet for eight weeks were accompanied by an increase in blood sugar and insulin levels and that adiponectin activity, which led to insulin resistance, decreased.

Bonnard et al. (2008) found that AdipoR1 mRNA levels in the liver tissues of mice fed with a high-fructose diet for 16 weeks and AdipoR2 mRNA levels in the muscle tissues decreased and were inversely correlated with insulin levels. Choi et al. (2017) showed that AdipoR2 gene expression decreased in the liver tissues of mice fed for three weeks by increasing the fructose rate (35%) in the diet. Our results are in line with reports stating that high fructose consumption significantly impairs adiponectin receptor expression. Furthermore, AdipoR1 and AdipoR2 expression and the agonism of these receptors may be new targets in the treatment of insulin resistance and type 2 diabetes.

In our study, a decrease was observed in the serum adiponectin levels and the expression of AdipoR1 and AdipoR2 receptor genes expressed in the liver tissue of rats administered with a 20% fructose diet with drinking water for 16 weeks. Our results will contribute to the literature to examine the effects of the longer-term consumption of fructose as in humans and to develop new treatment strategies in future studies.

To obtain more comprehensive data on this subject, it is recommended to conduct in vivo and in vitro studies to compare the consumption of different amounts of sugar and sugar products in the long term and to better explain the mechanism of action of fructose.

Conflict of interest

The authors declare that they have no competing interests.

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