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High-fructose corn syrup causes vascular dysfunction associated with metabolic disturbance in rats: Protective effect of resveratrol

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ABSTRACT

High-fructose corn syrup (HFCS) is used in many prepared foods and soft drinks. However, limited data is available on the consequences of HFCS consumption on metabolic and cardiovascular functions. This study was, therefore, designed to assess whether HFCS drinking influences the endothelial and vascular function in association with metabolic disturbances in rats. Additionally, resveratrol was tested at challenge with HFCS. We investigated the effects of HFCS (10% and 20%) and resveratrol (50 mg/l) beverages on several metabolic parameters as well as endothelial relaxation, vascular contractions, expressions of endothelial nitric oxide synthase (eNOS), sirtuin 1 (SIRT1), gp91^{phox} and p22^{phox} proteins and superoxide generation in the aortas. Consumption of HFCS (20%) increased serum triglyceride, VLDL and insulin levels as well as blood pressure. Impaired relaxation to acetylcholine and intensified contractions to phenylephrine and angiotensin II were associated with decreased eNOS and SIRT1 whereas increased gp91^{phox} and p22^{phox} proteins, along with provoked superoxide production in the aortas from HFCS-treated rats. Resveratrol supplementation efficiently restored HFCS-induced deteriorations. Thus, intake of HFCS leads to vascular dysfunction by decreasing vasoprotective factors and provoking oxidative stress in association with metabolic disturbances. Resveratrol has a protective potential against the harmful consequences of HFCS consumption.

1. Introduction

Increased consumption of total sugar and fructose may be a contributing factor in the current widespread metabolic disorder. Fructose is found as a monosaccharide in many fruits and vegetables or as a disaccharide bounded to glucose in sucrose. High-fructose corn syrup (HFCS), usually containing 55% fructose and 42% glucose (HFCS-55), has largely replaced sucrose as a major sweetener in prepared foods and soft drinks in recent decades (Marriott et al., 2009). Currently, fructose accounts for 10% of daily caloric intake in the United States due to increased consumption of HFCS in the diet (Vos et al., 2008).

High-fructose consumption has been shown to cause insulin resistance, hypertriglyceridemia and hepatic steatosis, as well as endothelial dysfunction in rats (Hwang et al., 1987; Shinozaki et al., 1999, 2004; Nyby et al., 2007; Tran et al., 2009; Suwannaphet et al., 2010). Besides, high-sucrose in diet of rats leads to metabolic disturbances, which appeared after a relatively long period of exposure (Hafidi et al., 2001; Rubio et al., 2006; Tran et al., 2009). The experiments comparing the effects of equivalent amounts of sucrose and glucose–fructose mixture on rats showed that intake of free fructose could be more destructive than that of bound fructose, as evidenced by severe fatty liver and disrupted glucose homeostasis (Sanchez-Lozada et al., 2010; Sheludiakova et al., 2011). Therefore, the findings obtained with pure fructose or sucrose could not be completely adapted to HFCS. Administration of HFCS to rodents produced differential results such as increased or unchanged values in plasma triglyceride and insulin concentrations, body weight and hepatic steatosis (Figlewicz et al., 2009; Light et al., 2009; Collison et al., 2009; Bocarsly et al., 2010), while no data was reported on cardiovascular function. Therefore, studies are needed to determine the biological consequences of consumption of HFCS.

Resveratrol may contribute to the improvement of insulin resistance, probably by acting SIRT1-dependent mechanism, in diet-induced metabolic disorders (Baur et al., 2006; Lagouge et al., 2006).

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In a recent study, we documented that resveratrol protects endothelial function and integrity from diabetes-induced injury (Akar et al., 2011). The vasculoprotective effect of resveratrol, which determined by an improvement in endothelial reactivity, was ascribed to increased eNOS (Mattagajasingh et al., 2007; Zhang et al., 2009) or SIRT1 (Mattagajasingh et al., 2007; Csiszar et al., 2008, 2009) and decreased NADPH oxidase (Zhang et al., 2009; Akar et al., 2011) in animal models.

Thus, in this study, we have investigated whether HFCS consumption alters endothelial and vascular reactivity, via affecting nitric oxide, SIRT1 and NADPH oxidase pathways, in association with the metabolic disturbances in rats. In addition, resveratrol was tested as a promising molecule against consequences of HFCS consumption.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Sigma Chemical Co., (St. Louis, MO) and Merck (Rahway, NJ) unless otherwise stated. HFCS (Cargill F55) was obtained from Cargill (Turkey). HFCS contains approximately 56% fructose, 37% glucose and 2% higher saccharides in the syrup of 76% total solids. It contains 72 g sugar, 23 g water and 5 mg sodium per 100 g syrup. *Trans*-resveratrol was obtained from Herb-Tech (ROC). The purity of resveratrol was determined by HPLC with LC–MS and 98% of the constituent was established to be *trans*-resveratrol.

2.2. Animals and diets

All animal procedures were approved by the Ethical Animal Research Committee of Gazi University (G.Ü.ET-09.047). Male Wistar rats aged 8 weeks were housed in temperature-controlled rooms (20–22 °C) under a 12-h light: dark cycle. The rats were fed with standard commercial chow diet ad libitum. The rat diet was composed of 62% starch, 23% protein, 4% fat, 7% cellulose, standard vitamins and salt mixture. After acclimation for 1 week, the rats were randomly divided into six groups: control; resveratrol; HFCS-10 (10% HFCS); HFCS-20 (20% HFCS); resveratrol-treated HFCS-10 and resveratrol-treated HFCS-20. Resveratrol (50 mg/l) or its vehicle (0.05% ethanol) was given to rats in drinking water under protection from light. The concentration of resveratrol was assigned from our previous *in vivo* observations (Soylemez et al., 2008; Akar et al., 2011). HFCS (55% fructose, 40% glucose) was prepared as 10% and 20% (w/v) solutions and administered to rats in drinking water for 10 weeks either in the presence or absence of resveratrol. These concentrations of HFCS were determined according to the sugar content of numerous soft drinks in which sugar ranges from 7% to 15% (Ventura et al., 2011). Animals consumed fluids ad libitum. Food and water intakes as well as body weights were recorded weekly during the follow-up. After 10 weeks, rats were euthanized by CO_2.

2.3. Measurement of systolic blood pressure

Systolic blood pressure (SBP) was measured by tail-cuff method (Tail-Cuff, BIOPAC Systems, NIBP200A) in the conscious, pre-warmed and restrained rats. The blood pressure readings were repeated four times between 9:00 a.m. and noon (12:00 a.m.).

2.4. Measurement of lipids, glucose and insulin in the serum

Cardiac blood samples of non-fasted rats were centrifuged at 4 °C and 10.000*g* for 30 min. Serum samples were immediately stored at −85 °C until the samples were assayed. High density lipoprotein (HDL), very low density lipoprotein (VLDL), total cholesterol, triglyceride and glucose levels were determined by standard enzymatic techniques. Serum insulin was measured by using commercially available specific ELISA kit (Cayman) according to the manufacturer's protocol.

2.5. Preparation of thoracic aortas and measurement of vascular reactivity

The thoracic aortas of the rats were isolated and immediately placed into cold Krebs solution of the following composition (mM): NaCl 118, KCl 4.73, KH_2PO_4 1.2, $MgSO_4{\cdot}7H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11 and EDTA 0.026. The aortic rings of 3–4 mm in length were mounted in a 5 ml organ bath containing Krebs solution at 37 °C and aerated with 95% O_2 and 5% CO_2. Four to six rings were prepared from each aorta and studied in parallel. Caution was exercised to preserve the endothelial layer during preparation of the aortic rings. The isometric forces of the rings were measured by using force displacement transducers (EMKA, Paris). In the aortic rings of rats, a passive stretch of 1 g was determined to be optimal tension for maximal responsiveness to phenylephrine (10^{-6} M). The preparations were allowed to equilibrate for approximately 1 h with an exchange of bathing solution every 15 min. The viabilities of the preparations were checked by KCl (40 mM) and the rings that produced a tension of less than 1 g were not included in the experiments. To prove the standardization of the aortic rings, two reproducible contractions were obtained with KCl (40 mM). The presence of the endothelium was tested functionally by applying acetylcholine (10^{-6} M) on phenylephrine (10^{-6} M)-precontracted aortic rings and preparations demonstrating <70% relaxations in control group were discarded (Soylemez et al., 2008; Akar et al., 2011).

The cumulative concentration–response curves of phenylephrine (10^{-9}–10^{-4} M) and angiotensin II (10^{-10}–10^{-5} M) were constructed in aortic rings. The relaxant effects of acetylcholine (10^{-9}–10^{-4} M) and sodium nitroprusside (SNP, 10^{-10}–10^{-5} M) were studied in arterial rings constricted submaximally with phenylephrine (10^{-6} M). Moreover, relaxation to acetylcholine was also determined after L-NOARG preincubation.

2.6. Measurement of superoxide production in thoracic aorta

The superoxide generation in endothelium-intact aortic segments of rats was determined by use of lucigenin-enhanced chemiluminescence, at low concentration (5 μM) of lucigenin (Soylemez et al., 2008). Aortic segments (3–4 mm) were placed in Krebs-HEPES buffer and incubated in the dark for 10 min at 37 °C. HEPES buffer (200 μl) with lucigenin (5 μmol/l) was added in the wells of scintillation plates and equilibrated in the dark for 10 min at 37 °C. Chemiluminescence was then read every minute for 10 min in a scintillation counter (1450 Microbeta Wallac, TRILUX). The background values were subtracted from each reading. Superoxide production in aortic segments was measured in basal conditions (control) and in the presence of an inhibitor of NADPH oxidase, diphenylene iodinium (DPI, 10 μM, 20 min). Superoxide formation in the rat aortas was expressed as counts/min/mg dry tissue weight.

2.7. Western Blot Analysis in the rat aorta

Aortic homogenates were prepared in ice-cold lysis buffer containing 50 mM Tris–HCl (pH, 8.0), 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.26% sodium deoxycholate, 50 mM sodium fluoride, 10 mM beta-glycerophosphate, 0.1 mM sodium orthovanadate, 10 μg/ml leupeptin, and 50 μg/ml phenylmethylsulfonyl fluoride (PMSF), and incubated on ice for 40 min (Zhao et al., 2009). Eighty microliter of 10% Nonidet P-40 (NP-40) solution was added to the homogenates, and the mixture was then centrifuged for 2 min at 14,000*g* at 4 °C for removing the cellular debris and isolating total protein. The supernatant was collected as a cytosolic fraction for the analysis of proteins. Concentration of the protein was determined according to the procedure described by Lowry et al. (1951) using a protein assay kit supplied by Sigma (St. Louis, MO, USA). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer containing 2% beta-mercaptoethanol was added to the supernatant. Equal amounts of protein (50 μg) were electrophoresed and subsequently transferred to nitrocellulose membranes (Schleicher and Schuell Inc., Keene, NH, USA). Nitrocellulose blots were washed twice for 5 min each in PBS and blocked with 1% bovine serum albumin in PBS for 1 h prior to application of the primary antibody. The antibodies against eNOS, SIRT1, $gp91^{phox}$ and $p22^{phox}$ were purchased from Santa Cruz Biotechnology, Inc., (Santa Cruz, CA, USA). Primary antibody was diluted (1:1000) in the same buffer containing 0.05% Tween-20. The nitrocellulose membrane was incubated overnight at 4 °C with protein antibody. The blots were washed and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (Santa Cruz, CA, USA). Specific binding was detected using diaminobenzidine and H_2O_2 as substrates. Protein loading was controlled using a monoclonal mouse antibody against β-actin antibody (A5316; Sigma). Blots were performed at least three times to confirm the reproducibility of the results. Bands were analyzed densitometrically using an image analysis system (Image J; National Institute of Health, Bethesda, USA).

2.8. Statistical analysis

The results are given as mean ± standard error of the mean. Contractile responses to phenylephrine, and angiotensin II were expressed as percentage of the KCl (40 mM)-induced contraction. The relaxations to acetylcholine and SNP were expressed as percent decreases of the precontraction induced by phenylephrine. The maximal response (E_{max}) and potency (EC_{50}) of the agents were determined by non-linear curve fitting using the Prism 5.02 GraphPad programme. EC_{50} values are given as $-\log M$. Statistical analyses were performed by Student's *t*-test for unpaired data or ANOVA followed by the Bonferroni post hoc analysis where appropriate. Values were considered to be significantly different when the *P* value was less than 0.05.

3. Results

3.1. The effects of HFCS and resveratrol treatment on food and water consumption, body weight, blood glucose, insulin and lipid concentration of rats

The initial body weights of rats were comparable in all groups. After 10 weeks, HFCS-treated rats, especially HFCS-20-treated rats, gained less weight than the control group. Nevertheless, liquid