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Preventive effect of grape seed extract against high-fructose diet-induced insulin resistance and oxidative stress in rats

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ABSTRACT

The purpose of the present study was to investigate the preventive effect of grape seed extract (GSE) on insulin resistance and oxidative stress in rats fed a high-fructose diet. After 8 weeks of the experiment, the fasting plasma glucose, insulin concentrations, and the homeostasis model assessment of basal insulin resistance (HOMA-IR) of rats fed a high-fructose diet supplemented with 1% GSE were significantly lower than that of a high-fructose diet group. In the oral glucose tolerance test, rats fed a high-fructose diet supplemented with 1% GSE had a significantly reduced plasma glucose and insulin concentrations after 15 min of glucose loading, indicating that GSE improved glucose intolerance. In addition, fed rats fed a high-fructose diet supplemented with 1% GSE markedly increased activity of hepatic superoxide dismutase, catalase, and suppressed lipid peroxidation when compared to rats fed a high-fructose diet. However, rats fed a high-fructose diet supplemented with GSE were not found to have a significant change in the activity of hepatic glutathione peroxidase. In conclusion, intake of GSE may be a feasible therapeutic strategy for prevention of a high-fructose diet-induced insulin resistance and oxidative stress.

1. Introduction

The metabolic syndrome characterized by insulin resistance, dyslipidemia, and hypertension is associated with increased risk of type 2 diabetes and coronary heart disease, resulting in reduced quality of life and increased risk of mortality and morbidity. The prevalence of metabolic syndrome has dramatically increased worldwide due to a modern lifestyle and an increase of consumption of high-sugar diets especially fructose (Misra and Khurana, 2009). Recent findings support that the increased consumption of fructose may be an important contributor to the metabolic syndrome, typically resulting in hyperinsulinemia, insulin resistance, hypertension, and hypertriacylglycerolaemia (Gerrits and Tsalikian, 1993). Animal studies have shown that high-fructose diet-fed rats display hepatic oxidative damage and altered lipid metabolism due to hepatic stress as a result of the burden of fructose metabolism (Kelley et al., 2004).

Recently, plant foods have been used for prevention of diabetes mellitus because of the likelihood of high compliance and because they are largely free from side effects (Dimo et al., 2001; Kang et al., 2004; Wu et al., 2004). Grape seed extract (GSE), a well-known dietary supplement, contains important vitamins, minerals, and polyphenols including flavonoids, proanthocyanidins and procyanidins (Weber et al., 2007). It has recently become clear that GSE has shown various beneficial pharmacological effects such as its chemoprotective properties against reactive oxygen species (Nandakumar et al., 2008) and oxidative stress as well as being anti-inflammatory (Terra et al., 2009), anti-bacterial (Mayer et al., 2008), anti-cancer (Kaur et al., 2006), and anti-diabetic (Pinent et al., 2004). A recent study has shown that GSE reduces plasma cholesterol in rabbits fed a high-cholesterol diet, and that may reduce risk of atherosclerosis and coronary heart disease (Yamakoshi et al., 1999). It has been reported that grape seed procyanidin extract prevents high-fat diet-induced obesity in hamsters by improving adipokine secretion and reducing oxidative stress (Terra et al., 2009). In addition, proanthocyanidins, the bioactive flavonoid compounds from grape seed, possess insulinomimetic properties by stimulating glucose uptake in insulin-sensitive cell lines and decreases hyperglycemia in streptozotocin (STZ)-diabetic rats (Pinent et al., 2004). Although anti-hyperglycemic activities of GSE are well-documented, studies regarding its efficacy in the prevention of insulin resistance, hyperinsulinemia, and oxidative stress induced by consumption of a high-fructose diet have not been undertaken. Therefore, the aim of the study was to determine the

Abbreviations: GSE, grape seed extract; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; AUCs, area under the curves; OGTT, oral glucose tolerance test.

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protective effect of GSE on insulin resistance and hepatic oxidative markers in rats fed a high-fructose diet.

2. Materials and methods

2.1. Chemicals

O-Dianisidine dihydrochloride, PGO enzymes, thiobarbituric acid (TBA) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Catalase (CAT), glutathione, superoxide dismutase (SOD), glutathione peroxidase (GPx) reagent assay, and rat plasma insulin assay kit were purchased from Cayman chemical (MI, USA). All other chemical reagents used in this study were of analytical grade.

2.2. Preparation of grape seed extract

Grape seeds obtained from Siam Winery (Samutsakhon, Thailand) were washed with water at 60 °C for 2 h, crushed, and then extracted with distilled water at 90 °C for 2 h. The aqueous solution was freeze dried and GSE was kept at −20 °C (Saito et al., 1998). The amount of total flavanols was measured according to the vanillin method using (+)-catechin as a reference (Broadhurst and Jaues, 1978). The content of monomeric flavanols was obtained as the sum of each monomeric flavanol's amount such as (+)-catechin and epicatechin by an HPLC method using an Intertsil® ODS-3 C_{18} column (Li et al., 2008). The content of procyanidins was calculated as the difference between total flavanols and monomeric flavanols (Saito et al., 1998). In this study, the grape seed extract was composed of 50.1% total flavanols, 49.08% procyanidins, 1.02% monomeric flavanols.

2.3. Animals and treatments

Male Sprague Dawley rats (180–200 g) were obtained from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand. All animal experiments were conducted according to the ethical guidelines outlined in the Guide for Care and Use of Laboratory Animals. The animal facilities and protocol were approved by the Laboratory Animal Care and Use Committee at Faculty of Veterinary Science, Chulalongkorn University, Thailand. Sprague Dawley rats were housed in individual stainless steel cages in a room maintained at 25 ± 1 °C on a 12:12-h light–dark cycle. They were fed standard laboratory chow with water ad libitum and fasted overnight before the experiments. Rats were randomly assigned to five groups of six animals. As shown in Table 1, the composition of the normal diet (AIN-93 diet; ND) and a high-fructose diet (HF) were prepared following the procedures of Guo et al. (2007). Diets were freshly mixed in small amounts every 2–3 days. Group 1 received ND for 8 weeks. Group 2 received HF, Groups 3 and 4 received a high-fructose (HF) diet supplemented with 0.5% and 1.0% GSE for 8 weeks, respectively. Body weight, food intake, and water intake were monitored weekly. Blood samples were obtained after an overnight fast from the tail vein of all the animals. Heparin-containing blood samples were immediately centrifuged (2500*g*), and the plasma was separated and frozen at −20 °C until analyzed for glucose and insulin concentrations.

2.4. Biochemical analysis

The plasma glucose concentration was determined by the glucose oxidase method. The plasma insulin concentration was estimated by using an enzyme immunoassay (EIA) kit. The homeostasis model assessment of basal insulin resistance (HOMA-IR) was used to calculate an index from the product of the fasting concentrations of plasma glucose (mmol/L) and plasma insulin (μU/mL) divided by 22.5 (1 μIU/mL = 6.945 pmol/L).

Table 1
Composition of the experimental diets (g/kg diet).

Ingredients	Normal diet (ND)	High-fructose (HF) diet
Casein	200	200
Corn starch	530	–
Sucrose	100	–
Fructose	–	630
Soybean oil	70	70
Mineral mixture	35	35
Vitamin mixture	10	10
Cellulose powder	50	50
L-Cystine	3	3
Choline bitartrate	2.5	2.5

2.5. Oral glucose tolerance test

One day before the termination of the experiment, animals were subjected to an oral glucose tolerance test. Briefly, after overnight fasting, animals received a glucose load (2 g/kg) orally. Blood samples were collected from the tail vein at 0 (before glucose administration), 15, 30, 60, 90 and 120 min after glucose administration. Plasma glucose and insulin concentrations were determined by using the glucose oxidase method, and EIA kit, respectively. Plasma glucose and insulin concentrations were expressed as incremental plasma glucose and insulin. Incremental glucose and insulin concentration were calculated by using each postprandial plasma concentration minus fasting concentration in individual animals (Wolever et al., 1991). The incremental plasma glucose and insulin curves were plotted as the change in incremental plasma glucose and insulin over time. The integrated area under the postprandial glucose and insulin response curves (AUCs) was calculated by the trapezoidal method.

2.6. Hepatic markers of oxidative stress

The animals were euthanized by cervical dislocation and the liver was removed immediately, frozen in liquid nitrogen and stored at −70 °C. The tissues (0.4 g) were homogenized in 50 mM phosphate buffer, pH 7.0, containing 1 mM EDTA. The homogenates were centrifuged at 10,000*g* for 15 min at 4 °C. The supernatant was used for the determination of glutathione (Baker et al., 1990), CAT (Johansson and Borg, 1988), and GPx activity (Forstrom et al., 1978). Lipid peroxidation (LPO) was determined by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA), a product formed due to the peroxidation of lipids (Ohkawa et al., 1979). The tissues (0.4 g) were homogenized in 50 mM phosphate buffer, pH 7.0, containing 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mannitol, and 70 mM sucrose. The homogenates were centrifuged at 1500*g* for 5 min at 4 °C. The supernatant was used for the determination of SOD activity (Maier and Chan, 2002). Protein content was determined according to the method of Bradford (1976) using bovine serum albumin as the standard.

2.7. Statistical analysis

Data were expressed as means ± S.E.M. Statistical analysis was performed by one-way ANOVA. The least significant difference (LSD) test was used for mean comparisons and $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of GSE on body weight, food consumption, and water intake

As shown in Table 2, the rats fed a high-fructose diet showed a slight increase in body weight, as compared to the normal diet (ND) group at week 8. It was found that body weight was significantly reduced by feeding HF supplemented with 0.5% and 1.0% GSE as compared to feeding HF alone. The mean food consumption and water intake were not significantly different between the HF group and HF supplemented with GSE.

3.2. Effect of GSE on plasma glucose, insulin concentrations, and insulin sensitivity index

After 8 weeks of the experiment, the rats fed with HF had up to a 1.29-fold increase in plasma glucose concentration, and a 1.47-fold increase in plasma insulin concentration (Table 2). The plasma glucose and insulin concentrations of HF supplemented with 1.0% GSE were significantly lower than the HF group by 20% and 24%, respectively. The degree of insulin resistance (HOMA-IR) was found to be higher in the HF group at week 8. The HOMA-IR score in HF supplemented with 0.5% and 1.0% GSE showed a decrease to approximately 32% and 30% of the score when compared to the HF group, respectively.

3.3. Effect of GSE on the oral glucose tolerance test

Fig. 1 shows the incremental changes in plasma glucose and insulin concentrations of rats following an oral glucose challenge. After animals received a glucose load orally, incremental plasma glucose and insulin concentrations peaked at 15 min. The incremental glucose and insulin concentrations of the HF group were