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.

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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 Tsallikian, 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
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-Danisidine 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 (Samut Sakhon, 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 Jaques, 1978). The content of procyanidins was calculated as the difference between total flavanols and monomeric flavanols (Saito et al., 2007). 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 protocols 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 diet supplemented with 0.5% and 1.0% GSE, respectively. The dry 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 (2500g), 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 (µIU/ml) divided by 22.5 (1 µIU/ml = 6.945 pmol/l).

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

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

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 15000 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
significantly higher than those of ND group at 15 and 30 min. The results showed that the incremental glucose and insulin of HF supplemented 1.0% GSE were significantly lower than the HF group at 15 and 30 min. There were no significant differences in the incremental glucose and insulin concentrations at 60, 90 and 120 min between the HF group and HF supplemented 1.0% GSE. In the group supplemented with 0.5% GSE, it was found that the incremental plasma glucose concentration was significant lower than that the HF group only 15 min after glucose administration. In addition, there were no significant differences in the incremental plasma insulin concentration between HF supplemented with 0.5% GSE and the HF group.

As shown in Fig. 2, the incremental AUCs (area under the curves) of plasma glucose concentration during OGTT of the HF group was elevated approximately 56% compared to ND group. The AUCs of glucose concentration in HF supplemented with 0.5% and 1.0% GSE groups were lower than that of the HF group by 19% and 28%, respectively. The incremental AUC values of insulin concentration during OGTT of the HF group were elevated by 42% compared to ND group, whereas the incremental AUCs of insulin concentration of HF supplemented with 0.5% and 1.0% GSE were decreased by 34% and 64%, respectively compared to that of the HF group.

Table 2
Body weight, food consumption, water intake, fasting plasma glucose and insulin concentration, and insulin sensitivity index of rats at the end of week 8.

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>HF</th>
<th>HF + 0.5% GSE</th>
<th>HF + 1.0% GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>388.0 ± 7.9</td>
<td>402.0 ± 10.4</td>
<td>369 ± 11.5*</td>
<td>363 ± 13.2*</td>
</tr>
<tr>
<td>Food consumption (g/d)</td>
<td>16.0 ± 0.5</td>
<td>16.0 ± 0.3</td>
<td>15.0 ± 0.2</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td>Water intake (ml/d)</td>
<td>29.0 ± 1.1</td>
<td>31.0 ± 1.7</td>
<td>28.0 ± 0.9</td>
<td>28.0 ± 0.4</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>128.7 ± 14.2</td>
<td>165.4 ± 15.8</td>
<td>139.4 ± 5.9</td>
<td>132.6 ± 11.4*</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.9 ± 0.2</td>
<td>2.8 ± 0.2*</td>
<td>2.3 ± 0.2</td>
<td>2.1 ± 0.3*</td>
</tr>
<tr>
<td>Insulin sensitivity index (HOMA-IR)</td>
<td>13.8 ± 0.6</td>
<td>24.0 ± 1.7</td>
<td>16.2 ± 1.9*</td>
<td>17.1 ± 1.1*</td>
</tr>
</tbody>
</table>

Results are expressed as means ± S.E.M., n = 6.
* P < 0.05 compared with ND (normal diet).
# P < 0.05 compared with HF (a high-fructose diet).

Fig. 1. The effects of GSE on incremental changes in plasma glucose (A) and insulin concentrations (B) at selected time intervals (15, 30, 60, 90 and 120 min). Results were expressed as means ± S.E.M., n = 6. *P < 0.05 compared with ND (normal diet), #P < 0.05 compared with HF (a high-fructose diet).

Fig. 2. The effects of GSE on total area under the curves (AUCs) of plasma glucose (A) and insulin concentrations (B) by oral glucose tolerance test. Results were expressed as means ± S.E.M., n = 6. *P < 0.05 compared with ND (normal diet), #P < 0.05 compared with HF (a high-fructose diet).
P/C0 HF supplemented with 0.5% (higher concentrations of hepatic TBARS than ND group, whereas 3.4. Effect of GSE on hepatic oxidative markers

As shown in Table 3, the HF group (+18%) exhibited significantly higher concentrations of hepatic TBARS than ND group, whereas HF supplemented with 0.5% (~9%) and 1.0% GSE (~15%) groups displayed significantly lower concentrations of hepatic TBARS than the HF group. Moreover, the HF group significantly reduced the activities of CAT (~32%), SOD (~51%), GPx (~25%), and total glutathione level (~29%) when compared to the ND group. The results showed that HF supplemented with 0.5% GSE significantly increased hepatic total glutathione level (+9.01%) when compared to the HF group, whereas an increase was observed in CAT activity (+26) in HF supplemented with 1.0% GSE. Both groups supplemented with 0.5% GSE (+42%) and 1.0% GSE (+64%) increased activity of hepatic SOD. However, there was no significant difference between Gpx activity in the HF group and HF supplemented with GSE.

### Table 3
Effect of GSE on hepatic oxidative markers at the end of week 8.

<table>
<thead>
<tr>
<th></th>
<th>ND</th>
<th>HF</th>
<th>HF + 0.5% GSE</th>
<th>HF + 1.0% GSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS (nmol/mg protein)</td>
<td>0.66 ± 0.03</td>
<td>0.78 ± 0.03*</td>
<td>0.71 ± 0.04</td>
<td>0.67 ± 0.04*</td>
</tr>
<tr>
<td>Catalase (Unit/mg protein)</td>
<td>2.55 ± 0.10</td>
<td>1.73 ± 0.20*</td>
<td>1.93 ± 0.16</td>
<td>2.18 ± 0.11*</td>
</tr>
<tr>
<td>Superoxide dismutase (Unit/mg protein)</td>
<td>171.42 ± 12.97</td>
<td>84.14 ± 8.60*</td>
<td>120.38 ± 9.44*</td>
<td>138.31 ± 11.51*</td>
</tr>
<tr>
<td>Glutathione peroxidase (µmol/min/mg protein)</td>
<td>1.39 ± 0.10</td>
<td>1.04 ± 0.08*</td>
<td>1.19 ± 0.11</td>
<td>1.17 ± 0.07</td>
</tr>
<tr>
<td>Total glutathione (nmol/mg protein)</td>
<td>162.68 ± 16.67</td>
<td>114.85 ± 9.86*</td>
<td>126.50 ± 7.98*</td>
<td>139.78 ± 12.23</td>
</tr>
</tbody>
</table>

Results are expressed as means ± S.E.M., n = 6. *P < 0.05 compared with ND (normal diet). **P < 0.05 compared with HF (a high-fructose diet).

### 4. Discussion

Dietary fructose is a monosaccharide which can induce metabolic disorders including insulin resistance, hyperinsulinemia, hypertension, and dyslipidemia which is of pathophysiological importance for the development of diabetes and atherosclerosis (Schaeffer et al., 2009). There are many reports in the literature describing an increase in body weight, glycemia, and insulinemia with the consumption of high-fructose diets in both humans and animal models (Rizkalla et al., 1993; Tordoff and Alleva, 1990). Our results are consistent with previous studies which found that consumption of high-fructose diets markedly induces an increase in glycemia associated with hyperinsulinemia and, consequently, a reduction of insulin sensitivity. A significant increase in the incremental AUCs of glucose and insulin concentrations after glucose loading during OGTT are seen in rats fed HF diets, indicating that the ability of insulin to stimulate glucose disposal is markedly impaired in peripheral tissues associated with insulin resistance by fructose feeding. There is now much emerging evidence that chronic consumption of high-fructose diets contributes to excessive formation of reactive oxygen species (ROS). This leads to induced oxidative stress, and mediated insulin resistance (Houtis et al., 2006). Moreover, an increase in cellular ROS accumulation directly triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase, and nuclear factor-kappa B that, in turn, phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) proteins (Evans et al., 2005). Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerates the degradation of IRS-1, causing impaired glucose uptake in muscle, liver and adipose tissues (Evans et al., 2005).

Fructose-induced hyperglycemia is one of the important factors to increase ROS, lipid peroxidation causing the depletion of the antioxidant defense status in various tissues (Reddy et al., 2009). The increase in catabolism of fructose causes the reduction of total glutathione levels and the suppression of hepatic antioxidant enzyme activities such as CAT, SOD, and GPx (Reddy et al., 2009; Oda et al., 1994). The findings of this study are in agreement with other investigations that reported a significant increase in lipid peroxidation and a significant decrease of hepatic antioxidant enzyme activities in fructose-induced diabetic rats (Reddy et al., 2009; Nandhini et al., 2005).

The present findings show that GSE prevents body-weight gain, hyperglycemia and hyperinsulinemia. In addition, GSE markedly attenuates the impairment of insulin-stimulated glucose disposal in rats with insulin resistance. GSE helps to protect hepatic antioxidant enzymes and decreases lipid peroxidation. Many studies have been reported that the monomers catechin and epicatechin (monomeric flavanols) are the major phenolic compounds in grape seeds that show antioxidant activity. For example, (+)-catechin shows antioxidant activity by inhibiting the oxidation of plasma lipids (Yilmaz and Toledo, 2004). Moreover, (+)-epicatechin is able to scavenge hydroxyl radicals, peroxyl radicals, superoxide radicals (Yilmaz and Toledo, 2004). Procyanidins are reported to have potent antioxidant activity both in vitro and in vivo (Simontii et al., 2002). In this study, (+)-catechin, (−)epicatechin and procyanidins were also found in GSE which is consistent with other studies (Fuleki and Ricardo da Silva, 1997; Escribano-Bailon et al., 1992; Oszmianski and Sapis, 1989). It is possible that these active principles in GSE may prevent insulin resistance, improve insulin sensitivity and increase the activity of antioxidant enzymes due to their antioxidant properties.

It has been reported that procyanidins in GSE exhibit insulinomimetic properties by stimulating glucose uptake in insulin-sensitive cell lines and decrease hyperglycemia in streptozotocin (STZ)-diabetic rats (Pinet et al., 2004). A recent study from our laboratory has demonstrated the inhibitory activity of GSE against intestinal α-glucosidase and pancreatic α-amylase, resulting in delayed carbohydrate digestion of absorbable monosaccharide (Adisakwattana et al., 2010). This mechanistic action is one of the therapeutic approaches for reducing postprandial hyperglycemia which contributes to a decrease in hemoglobin A1C (HbA1C) concentration and, consequently, reduces the incidence of chronic vascular complication (Baron, 1998). It is suggested that GSE exhibits the various mechanisms responsible for their anti-hyperglycemic effects and which may help to reduce insulin resistance and impair glucose intolerance. Décorde et al. (2009) reported the effects of a polyphenolic grape seed extract on obesity and insulin resistance in an animal model. After 12 weeks of the experiment, GSE markedly prevented hyperglycemia and reduced hyperinsulinemia, and increased adiponecin levels in hamsters fed a high-fat diet. Adiponecin is an adipokine that exerts a potent insulin-sensitizing effect by binding to its receptors such as AdipoR1 and AdipoR2, leading to activation of AMPK and PPARγ, consequently affecting the activation of glucose uptake and causing the reduction of insulin resistance (Kadowaki et al., 2006). The authors hypothesize that GSE may induce an increase
in adiponectin levels in rat fed a high-fructose diet which plays a vital role in improving insulin sensitivity. The cellular mechanisms which activate adiponectin gene expression in target tissues by GSE need to be a subject for future investigation.

5. Conclusion

The current study indicates that the supplementation of GSE in rats fed a high-fructose diet can prevent the development of hyperglycemia and hyperinsulinemia as well as reduce an oxidative stress. The present study also provides additional evidence in support of the use of GSE for prevention and/or management of diabetes and the pre-diabetic state of insulin resistance.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgement

This research was financially supported by the Ratchadaphiseksomphoth Endowment Fund and the Graduate School, Chulalongkorn University, Thailand. The authors gratefully acknowledge The Medical Food Research and Development Center, which has been financially and institutionally supported by Chulalongkorn University.

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