Grape Seed Procyanidins Improve Diabetic Symptoms in Mice with Streptozotocin-Induced Diabetes

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Abstract: Grape seed procyanidins (GSPCs) are bioflavonoid polymers that have been shown to have health benefits. We assessed the antidiabetic effect of GSPC in mice. Mice with streptozotocin(STZ)-induced diabetes were orally or intraperitoneally administered saline or 40-100 mg GSPC/kg BW daily for 7-10 d. We monitored body weight, blood glucose levels, amounts of food and water consumed, and amounts of urine and feces excreted. On the final day, we analyzed plasma chemistry and found that GSPC, but not structurally related monomers (e.g., catechin and epicatechin), reduced the glucose levels, food and water intake, and urine and feces excreted, all of which had increased due to STZ administration. This suggests a procyanidin-dependent effect of grape seed polyphenols on diabetes. Oral administration of GSPC was less effective within 9 d than was intraperitoneal administration of GSPC, suggesting that the effect is route-dependent. The decrease in diabetic blood glucose levels was reversible; when GSPC administration was stopped, glucose levels rose. However, although pretreatment with GSPC for 7 d did not completely prevent STZ-induced diabetic effects, it rapidly reduced them. Treatment with GSPC reduced fasting glucose levels and improved glucose tolerance in STZ-treated mice, in addition to decreasing STZ-stimulated levels of plasma triglyceride and cholesterol, creatinine, uric acid, and alkaline phosphatase activity. Moreover, GSPC suppressed the reduction in pancreatic islets and the decrease in plasma insulin hormone levels caused by STZ. Our findings indicate that GSPC improves hyperglycemia, polydipsia, polyuria, and polyphagia in mice with STZ-induced diabetes.

Keywords: Grape, diabetes, insulin, mice, procyanidins.

INTRODUCTION

Diabetes is commonly associated with obesity, cardiovascular disease, hypertension, high cholesterol, retinopathy, renopathy, and neuropathy [1]. The economic costs, prevalence, morbidity, and mortality associated with prediabetic conditions and diabetes are high [2-9]. The onset of diabetes is characterized by increased blood and urine glucose levels, excessive urination, thirst, hunger, and weight loss, all of which are regulated by genetic, endocrine, metabolic, pharmacological, environmental, and nutritional factors [1]. Accordingly, understanding how specific nutrients affect diabetic symptoms could help prevent the onset and progression of diabetes and associated diseases in humans.

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Grape seed procyanidins (GSPCs) are a family of bioflavonoid polymers [10] that were once known as "vitamin P" [11]. GSPCs have unique chemical structures comprising dimers or trimers of catechin and (-)-epicatechin (EC) and are found in red wine and grape seeds [10]. A number of studies have indicated that they have a variety of health benefits due to their antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and vasodilatory actions [10]. Further, procyanidins restore postischemic function in isolated rat hearts [12], stimulate the activities of enzymes, such as tyrosine kinases and phosphoinositide kinases [13-14], may prevent human metabolic syndrome [15], and possess antioxidant and free radical-scavenging activities [16-18]. Although recent studies have shown that oral administration of pharmacological doses (250 mg/kg body weight [BW]) of procyanidin-containing red wine and white wine extracts over a long period of time (6 wk) have an antidiabetic effect on Wistar rats with streptozotocin (STZ)-induced diabetes [19, 20], it was not clear from the results if procyanidin has a preventative effect on diabetic glucose levels or if it acts antidiabetically in mice. Further, it is unclear whether any of

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the *in vivo* effects of procyanidins are dependent on the duration of treatment and the route of administration.

In this study, we used mice with STZ-induced diabetes to assess possible effects of GSPC on diabetic symptoms (e.g., blood glucose and insulin levels, food intake, water consumption, body weight gain, and urine and feces excretions). We also investigated whether GSPC prevents increased blood glucose levels after STZ administration, and whether the effects of GSPC depend upon the route of administration.

MATERIALS AND METHODS

Chemical Reagents

GSPCs isolated from grape seed (*Vitis vinifera*) was a gift from Jaden polytechnic (Greer, SC, USA); the average percentage of polyphenolic compounds was 98%, as established by the vanillin-hydrochloric acid method. Other materials (e.g., STZ, sodium citrate, glucose, (+)-catechin, EC, and gallic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise noted.

Animals and Induction of Experimental Diabetes

Male ICR mice were obtained from the National Taiwan University Animal Center (Taipei, Taiwan). The mice were approximately 5 wk of age and weighed 18-20 g. The animals were maintained at an ambient temperature of 25±1 °C, 50%-60% humidity, and a photoperiod of 12 h light and 12 h darkness. Mice were given free access to a standard rodent chow diet (Hardwood Laboratory Bedding, Taipei, Taiwan) and water unless otherwise noted. Animal experimental protocols were reviewed and approved by the Laboratory Animal Ethics Committee, National Central University, Jhongli, Taiwan. Following the method reported by Junod *et al*. [21], diabetes was induced in mice that had fasted for 1 d by a single intraperitoneal (ip) injection of STZ (75 mg/kg BW) in 0.1 M sodium citrate buffer (pH 4.5). Diabetic animals with hyperglycemia (~300 mg/dL) 3-7 d after the injection of STZ were used in the experiments unless otherwise noted.

Experimental Treatments

Mice with STZ-induced diabetes were divided into several groups of five mice each. In the control group, mice with STZ-induced diabetes were administered saline, while in the treatment group, mice with STZ-induced diabetes were ip injected daily with GSPC. Normal mice were ip injected daily with or without GSPC for comparison. For the dose-dependent experiments, diabetic mice were ip injected daily with approximately 40 or 80 mg of GSPC/kg BW for the indicated time period in accordance with a method described by Kao *et al*. [22]. To assess the preventative effect of GSPC on diabetes, normal mice were daily pretreated with ~80 mg of GSPC/kg BW for 7 d or were untreated. After 1 d of fasting, animals were singly ip injected with or without STZ in the presence or absence of ~80 mg GSPC/kg BW for an additional 10 d. For experiments to study whether the effect of GSPC is route-dependent, diabetic mice were given 90-100 mg of GSPC/kg BW orally or by an ip injection daily for 10 d; GSPC was dissolved in water for oral administration and in sterile saline for the ip injection.

During the experimental period, the body weight, blood glucose levels, and amounts of food and water consumed and urine and feces excreted were monitored daily. Food and water consumption as well as urine and feces excretion were monitored in mice caged in groups of three to five animals. On the final day, mice were anesthetized with ether, and blood was collected in a heparinized syringe by heart puncture. Plasma was collected after centrifugation (5000 rpm for 30 min at 4 °C) for biochemical analyses. The liver, kidneys, testes, and spleen were collected and weighed. The pancreas was fixed in Bouin's solution and subsequently histologically examined by the Taipei Institute of Pathology (Taipei, Taiwan) using Harris' hematoxylin and eosin (H&E) staining method [23]. The pancreatic islets per section were counted from three different cross-sections.

Biochemical Analysis

For the blood biochemical analysis, a commercially available ELISA kit for insulin (Mercodia AB, Uppsala, Sweden) was used. Plasma chemistry (e.g., cholesterol and enzyme activity) tests were performed by Der-An Diagnostic (Jhong-li, Taiwan). Following the methods of Lee *et al*. [24], blood glucose levels were directly determined by a glucometer (Bayer Australia) using commercially available test strips for glucose.

Statistical Analysis

Data are expressed as the mean±SEM. Unpaired Student's *t* test was used to examine differences among the normal and STZ-treated groups or among the STZ- and GSPC-treated groups. One-way ANOVA followed by the Student-Newman-Keuls multiple-range test was used to examine differences among multiple groups. Differences were considered significant at $P<0.05$. Statistical analyses were performed using SigmaStat (Jandel Scientific, Palo Alto, CA, USA).

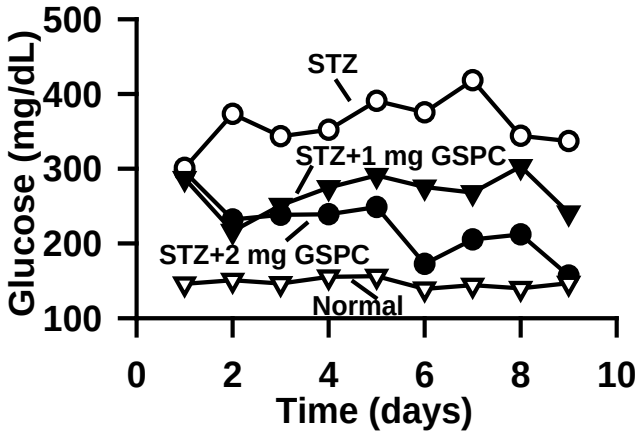

Fig. (1). The reduction by GSPC of STZ-increased blood glucose levels was dependent upon the dosage and duration of treatment. Decreased levels varied with the initial levels of blood glucose in mice with STZ-induced diabetes. Diabetes in 1 d fasting mice were induced by a single injection of 75 mg of STZ for 3-7 d, and were then ip injected with 1 or 2 mg of GSPC per mouse (40 or 80 mg/kg BW) daily for 9 d. Data are expressed as the means ± SEM from five animals in each group. The SE bars are not shown for clarity.

RESULTS

Glucose Levels

Intraperitoneal injections of GSPC caused decreases in blood glucose levels in mice with STZ-induced diabetes within 9 d of treatment (Fig. **1**). The effect of GSPC was dependent upon the dosage and duration of treatment. A dose of 80 mg of GSPC/kg BW injected daily was significantly more effective at reducing blood glucose levels than was 40 mg/kg BW.

In the experiments determining the values of GSPC (Fig. **1**) required to significantly decrease blood glucose levels, we observed that the reduction in glucose levels was reversible; when GSPC administration was stopped, the reduced glucose levels rose (Fig. **2A**). Further, these mice gradually adapted within 10 d, and higher doses of GSPC (~200 mg/kg BW) were needed to reduce the increased glucose levels (Fig. **2B**).

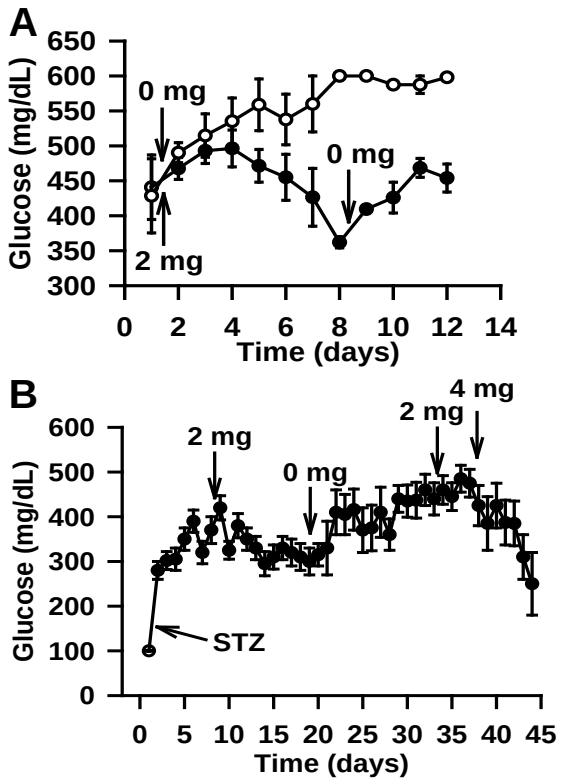

Fig. (2). Blood glucose levels in ICR mice with STZ-induced diabetes treated with GSPC. Mice with STZ-induced diabetes were ip injected daily with GSPC (number under arrow is the number of milligrams injected). Changes in the amount of GSPC injected are shown with arrows (**A** and **B**). Diabetic mice were injected with saline (○) or GSPC (●). In (**A**), decreases in diabetic mouse blood glucose levels were reversible; when GSPC administration was stopped, glucose levels rose. In (**B**), mice gradually adapted within 10 d, and higher doses (4 mg per mouse) of GSPC (~200 mg/kg BW) were needed to reduce the increased glucose levels. Values are the means ± SEM from five animals in each group.

To determine whether GSPC prevents diabetic induction by STZ in mice, we administered 80 mg GSPC/kg BW to normal mice (Fig. **3A**), or they received no treatment (Fig. **3B**). Seven days later, they were given a single injection of 75 mg of STZ/kg BW, and then provided daily with either GSPC or no treatment for an additional 10 d. Although pretreatment with GSPC for 1 wk did not completely prevent STZ-stimulated glucose levels in mice, it did rapidly reduce them within 2-10 d (Fig. **3A**). In normal mice pretreated with GSPC or untreated for 1 wk, the mean values of blood glucose levels were maintained at approximately 130~157 mg/dL or 151~174 mg/dL, respectively; after 1 d of fasting, the respective mean glucose levels were 74 and 78 mg/dL. When normal mice on an *ad libitum* diet were given two daily injections of GSPC for 10 d, the mean glucose levels were in the range of 125~146 mg/dL, compared with those of control mice with a range of 153~179 mg/dL. In contrast, when mice were not pretreated with GSPC but were simultaneously ip injected 7 d later with STZ for 10 d (Fig. **3B**), at least 4 d of GSPC injection were required to significantly reduce blood glucose levels in STZ-injected mice. Normal mice treated with GSPC exhibited mean glucose levels of 169~183 mg/dL and a 1-d fasting mean glucose levels of 60 mg/dL. Following 10 d of GSPC injections, blood glucose levels changed to a range of 134~174 mg/dL, compared with 150~187 mg/dL in control mice.

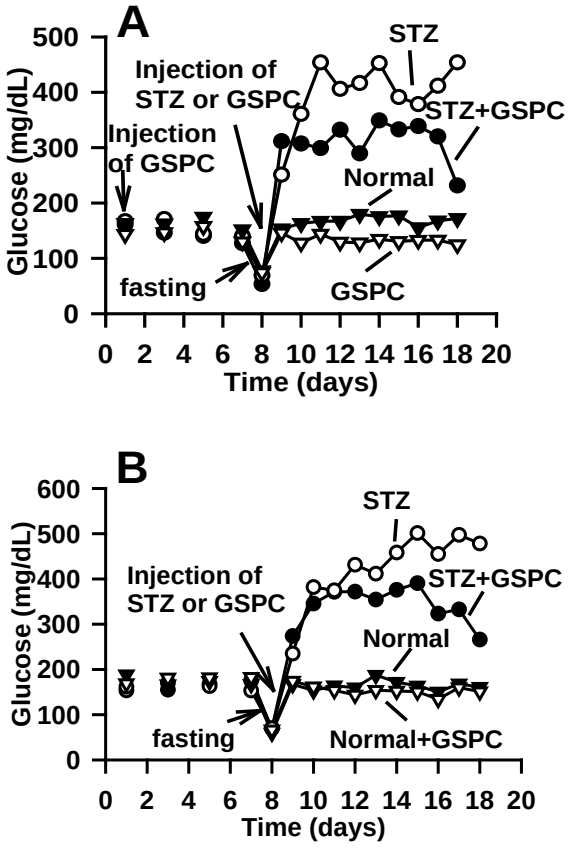

Fig. (3). GSPC rapidly decreased but did not completely prevent the stimulatory effect of STZ on blood glucose levels when normal mice were treated with GSPC (80 mg/kg BW) 1 wk before the STZ injection (**A**) or were given GSPC simultaneously with STZ (**B**). Data are expressed as the means ± SEM from five animals in each group. The SE bars are not shown for clarity.

We next used 80 mg GSPC/kg BW to examine its affect on fasting glucose levels and oral glucose tolerance in mice

with STZ-induced diabetes (Fig. **4**). Mice with diabetes induced by a single injection of STZ were ip injected with 2 mg of GSPC (= 80 mg/kg BW) per mouse or were given no treatment for 7 d. After 1 d of starvation, animals were orally administered a single dose of 1 g glucose/kg BW and then monitored for 4 h (Fig. **4**). We observed that the GSPC injection significantly reduced the 1-d fasting mean glucose levels in STZ-diabetic mice from 135 to 73 mg/dL, which is close to the normal fasting glucose level of 64 mg/dL. During the oral glucose tolerance test in normal mice, the rise in blood glucose levels produced by consuming the glucose solution was reduced to 106 and 72 mg/dL, respectively, within 2 h and 4 h of glucose ingestion. In contrast, blood glucose concentrations remained at 271 and 122 mg/dL, respectively, 2 h and 4 h after the oral glucose challenge in diabetic mice that did not receive GSPC injections, while they were 131 and 69 mg/dL at 2 h and 4 h, respectively, in mice with STZ-induced diabetes treated with GSPC. Peak values of glucose levels in all groups of mice occurred 30 min after glucose ingestion, and were 165, 361, and 239 mg/dL, respectively, in normal treated, diabetic untreated, and diabetic treated mice.

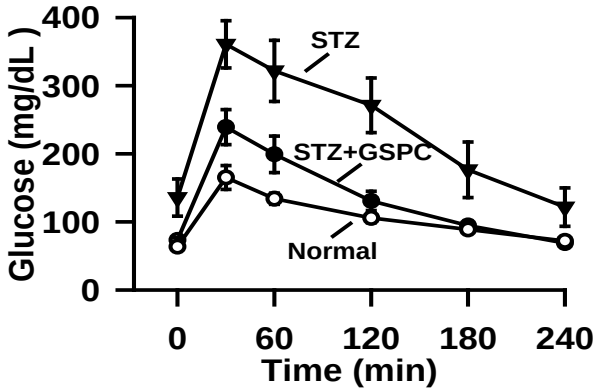

Fig. (4). GSPC improved the oral glucose tolerance in mice with STZ-induced diabetes. Mice with STZ-induced diabetes were ip injected daily with 80 mg GSPC/kg BW or were untreated for 7 d. One day after the last treatment mice were fasted for 1 d and were then orally administered a single dose of 1 g glucose/kg BW. Blood glucose levels were measured in intervals of 30 or 60 min for 4 h. Data are expressed as the means ± SEM from five animals in each group.

Pancreatic Islets and Plasma Insulin Levels

To investigate whether GSPC reduces the inhibitory effects of STZ on murine pancreas, we examined the histology of pancreatic islets using H&E staining methods (3), and measured changes in circulating levels of insulin (Fig. **5**). We found that injection of 80 mg of GSPC/kg BW for 9 d markedly increased the mean weight of the pancreas in mice with STZ-induced diabetes from 1 mg to 1.7 mg (data not shown). Additionally, the number of pancreatic islets per tissue section in mice with STZ-induced diabetes was increased from 2.3±0.6 to 4.3±0.8 after an injection of 80 mg GSPC/kg BW, while those in normal mice without GSPC treatment were 10.8±1.7 (Fig. **5A** and **B**). Finally, the mean circulating levels of insulin in mice with STZ-induced diabetes significantly increased from 0.29 to 0.61 ng/ml following

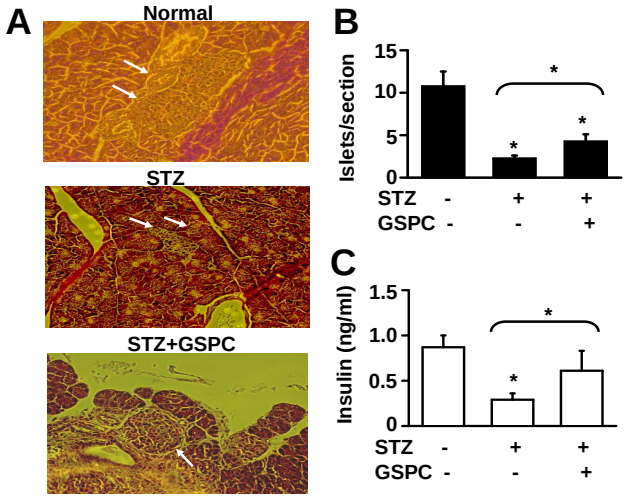

Fig. (5). GSPC reduced the suppressive effects of STZ on the number of pancreatic islets (**A** and **B**) and plasma insulin levels (**C**) in mice. Mice with STZ-induced diabetes were ip injected daily with 80 mg GSPC/kg BW or were untreated for 9 d. The pancreas were microscopically examined using the H&E staining method, while plasma insulin levels were examined with ELISA. Photos in (**A**) show representative histological sections. Data in (**B**) and (**C**) are expressed as the means± SEM from five animals per group.

an injection of 80 mg GSPC/kg BW, compared with 0.87 ng insulin/ml in normal mice without GSPC treatment (Fig. **5C**).

Other Blood Chemistry and Organs

Mice with STZ-induced diabetes were treated with GSPC for 10 d, and their plasma was analyzed for various components (Table **1**). Treating normal mice with GSPC did not cause significant changes in plasma levels of total protein, albumin, triglyceride, blood urea nitrogen, creatinine, or enzymes that are indicative of severe damage to the liver and other organs, such as alanine aminotrasferase, aspartate aminotransferase, and lactate dehydrogenase. However, significant changes in the amount of blood cholesterol (-39%) and the activity of blood alkaline phosphatase (-28%) were observed. In contrast, treatment with STZ alone caused a significant decrease in the amount of blood albumin (-17%), increased levels of blood triglyceride (+25%) and creatinine (+48%), and increased blood alkaline phosphatase activity (+98%). Ten days after injection of 80 mg GSPC/kg BW into mice with STZ-induced diabetes, significant decreases in blood triglyceride (-21%) and cholesterol (-27%) levels and alkaline phosphatase activity (−43%) were observed.

We next examined whether GSPC can reduce the inhibitory effect of STZ on the weight of murine liver, kidney, spleen, and testes (Table **2**). In normal mice, the liver, kidneys, spleen, and testes did not exhibit any significant changes in organ weight 10 d after injecting 80 mg GSPC/kg BW. However, treating mice with STZ alone tended to increase the weight of both the liver and kidneys compared with those of normal mice. Further, injecting 80 mg but not 40 mg GSPC/kg BW suppressed the STZ-increased weight of the liver and kidneys.

Table 1. Effects of GSPC on Plasma Chemistry in Normal and STZ-Diabetic Mice

Plasma/Organ	Normal	Normal+ GSPC (2 mg)	STZ	STZ+GSPC (1 mg)	STZ+GSPC (2 mg)
Glucose (mg/dL)	163±6	135±6*	550±22	490±40	290±30*
Protein (g)	5.2±0.1	5.0±0.2	5.1±0.1	4.9±0.1	4.7±0.2
Albumin (g)	2.4±0.1	2.1±0.1	2.0± 0.1*	2.1±0.1	1.9±0.1
Triglyceride (mg/dL)	114±10	102±9	143±14*	120±10	113±17*
Cholesterol (mg/dL)	123±5	75±6*	129±6	121±8	94±9*
BUN (mg/dL)	25±1	24±1	35±6	36±3	32±3
Creatinine (mg/dL)	0.29±0.02	0.30±0.02	0.43±0.02*	0.43±0.02	0.38±03
Uric acid (mg/dL)	2.2±0.4	1.8±0.3	2.9±0.5	2.9±0.3	2.1±0.3
ALT (U/L)	103±24	90±27	183±64	130±28	181±42
AST (U/L)	290±36	373±31	272±44	304±32	286±40
ALKP (U/L)	126±6	91±9*	249±32*	171±23	143±23*
LDH (U/L)	1606±173	1968±391	1951±368	1455±178	1784±220

The daily dose of GSPC per mouse injected ip was 1 or 2 mg (~40 or 80 mg/kg BW) for10 d. Mice in normal group were injected with saline. Mice in STZ group were singly ip injected with 75 mg/kg BW. Values are the mean ± SEM, n =8. *P<0.05, GSPC *vs.* the normal group, STZ *vs.* the normalgroup,or GSPC + STZ *vs*. STZ. BUN, blood urea nitrogen; ALT, alanine transaminase; AST, aspartate transaminase; ALKP, alkaline phosphatase; LDH, lactate dehydrogenase.

Table 2. Effects of GSPC on Organ Weight, Food Intake, and Water Consumption Innormal and STZ-Diabetic Mice

Measurement	Normal	Normal+ GSPC (2 mg)	STZ	STZ+GSPC (1 mg)	STZ+GSPC (2 mg)
Initial BW (g)	23.63±0.41	23.30±0.62	23.0±0.6	23.1±0.2	22.1±0.9
Final BW (g)	29.85±0.49	26.50±0.81*	27.0±0.9*	24.2±0.2*	21.0±0.1*
Liver (g)	1.81±0.05	1.70±0.08	2.0±0.06*	1.84±0.10	1.77±0.08*
Kidney (g)	0.57±0.04	0.51±0.05	0.62±0.02	0.61±0.03	0.54±0.02*
Spleen (g)	0.17±0.01	0.19±0.02	0.16±0.01	0.16±0.02	0.15±0.01
Testis (g)	0.24±0.02	0.21±0.02	0.21±0.01	0.23±0.02	0.21±0.01
Food (g/mice/day)	4.95±0.16	3.83±0.24*	7.00±0.30*	5.80±0.50*	4.75±0.31*
Water (g/mice/day)	8.40±0.18	6.00±0.40*	21.20±0.30*	13.7±2.42*	9.73±1.20*

The daily dose of GSPC per mouse injected ip was 1 or 2 mg (~40 or 80 mg/kg BW) for10 d. Mice in normal group were injected with saline. Mice in STZ group were singly ip injected with 75 mg/kg BW. Values are the mean ± SEM, n =8.*P<0.05, GSPC *vs*. the normal group, STZ *vs*. the normal group, or GSPC + STZ *vs*. STZ.

Body Weight, Food and Water Intake, and Urine and Feces Excretion

Persistent thirst (polydipsia), frequent urination (polyuria), and nagging hunger (polyphagia) are common symptoms of diabetes [1]. Accordingly, we examined whether GSPC could regulate the effect of STZ on body weight, food and water consumption, and urine and feces excretion (Tables **2** and **3**). Treating normal mice with 75 mg STZ/kg BW alone caused a significant decrease in body weight and significant increases in food intake and water consumption. Ten days after injection of 40 or 80 mg GSPC/kg BW, STZ-increased food intake (-17% and -33%, respectively) and water consumption (-35% and -54%, respectively) was significantly suppressed, and STZ-induced decreases in body weight gain (-10% and -22%, respectively) were also suppressed. Moreover, an ip injection of 80 mg GSPC/kg BW suppressed STZ-induced increases in urine and feces excretion by 78% and 41%, respectively (Table **3** and Supplemental Fig. **1**). Notably, injecting normal mice with 80 mg GSPC/kg BW also caused significant decreases in body weight gain, food intake, and water consumption.

To determine whether GSPC depends upon the route of administration to mediate the effects of STZ on body weight, food and water intake and urine and feces excretion, we orally administered or ip injected mice with 90-100 mg GSPC/kg BW once daily for 10 d and then measured changes in these parameters (Table **3**). The results of oral administration of GSPC were similar to those of GSPC ip injection with regard to suppressing STZ-increased blood glucose levels (-28%), food (-25%) and water (-31%) consumption, urination (-33%) and feces (-44%) excretion, and enhancing the STZ-induced decrease in body weight (-12%).

Table 3. A Comparison of Orally and ip Administered GSPC on Glucose Levels, BW, Food and Water Intake, and Feces and Urine Excretion in STZ-Diabetic Mice

Measurement	Normal	Oral		ip	
		STZ	STZ+GSPC	STZ	STZ+GSPC
Glucose (mg/dL)	168±7	509±36	368±3*	520±22	298±46*
BW (g)	29.0±1.7	22.5±1.1	19.7±0.1*	26.8±1.0	20.5±0.1*
Food (g/mice/day)	6.6±0.2	6.8±0.3	5.1±0.4*	7.4±0.3	3.3±0.3*
Water (g/mice/day)	8.9±0.2	23.5±1.3	16.2±1.5*	19.3±0.3	6.5±0.7*
Feces (g/mice/day)	2.3±0.1	3.4±0.3	1.9±0.3*	2.7±0.2	1.6±0.2*
Urine (g/mice/day)	1.5±0.2	8.1±0.5	5.4±0.7*	6.7±0.8	1.5±0.2*

The daily dose of GSPC per mouse administered orally (~100 mg/kg BW) or ip (~90 mg/kg BW) was 2 mg for 10 d. Mice in STZ group were ip injected with 75 mg/kg BW.Values are the mean ± SEM, n = 5. The initial average values of plasma glucose measured in normal, oral (STZ and STZ+GSPC) and ip (STZ and STZ+GSPC) groups were 153 ± 11, 503 ± 13, 502 ± 12, 426 ± 15, and 423 ± 13 mg/dL, respectively. The initial average body weights of the normal, oral (STZ and STZ+GSPC) and ip (STZ and STZ+GSPC) groups were 20.7 ± 1.0, 19.5 ± 0.7, 19.8 ± 1.2, 22.7 ± 0.6, and 22.3 ± 0.9 g, respectively. *P<0.05, GSPC + STZ *vs*. STZ inoral or ipgroups.

Except for feces excretion, the changes in these parameters induced by oral GSPC intake were smaller than those induced by ip GSPC administration.

DISCUSSION

The present study describes how GSPC decreases diabetic symptoms induced by STZ in mice. The effects of GSPC were dose and time dependent. In general, concentrations exceeding 80 mg/kg BW were more effective than a lower concentration of 40 mg/kg BW. Although the antidiabetic effects of GSPC on mice with STZ-induced diabetes were dependent upon the dosage and duration of treatment, the route of administration, and the presence of procyanidins, these results are similar to those observed previously in rats [13-14, 16, 19-20]. In our study, it is likely that GSPC decreased glucose levels in STZ-diabetic mice by increasing circulating insulin levels. This conclusion is supported by the findings that treatment with GSPC reduced STZ-decreased insulin levels and the number of pancreatic islets. Another possible explanation for the antidiabetic effect of GSPC is that it possesses antioxidant and free radical-scavenging activities [16-18] which reduce the destructive effect of the nitrosourea moiety of STZ on pancreatic islets. This explanation is indirectly supported by the fact that treatment with pycnogenol reduced the STZ-increased activity of hepatic catalase and elevated STZ-reduced levels of hepatic glutathione and glutathione redox enzyme activity [16]. The antioxidant and free-radical-scavenging properties of GSPC [16-18] may explain our findings that 1-wk of pretreatment with GSPC caused an earlier decrease in diabetic glucose levels in mice with STZ-induced diabetes than did the simultaneous administration of STZ and GSPC (Fig. **3**), which reduced glucose levels 1-2 d faster than did a post-STZ injection of GSPC (Fig. **1B**).

In our study, GSPC suppressed STZ-stimulated food intake by mice. This observation is consistent with *in vivo* findings that the procyanidin-related polyphenol, (-)-epigallocatechin gallate (EGCG), reduced food intake in obese Zucker rats [22] and Sprague-Dawley rats [26]. Thus, the effects of GSPC on the various diabetic symptoms of mice with STZ-induced diabetes may be secondary effect of GSPC on food intake. For example, the large decrease in urine and feces excretion in GSPC-treated STZ-diabetic mice could have been caused by diminished water consumption due to low food intake in these mice. Firm conclusions will require more thorough studies.

The GSPC used in this study consisted mostly of polyphenolic dimers of catechin and EC [10]. These grape seed polyphenols included 65% procyanidins, 30% catechin and EC monomers, 3% gallic acid, and 2% other compounds. Accordingly, we attempted to examine whether the polyphenolic monomers in the GSPC extract used in this study contributed to the suppression of the diabetic effects of STZ. Because amount of GSPC that had an antidiabetic effect was 2 mg per mouse (~80 mg/kg BW), which should contain 0.6 mg catechin and EC and 0.06 mg gallic acid, we used 0.5 mg of each monomeric compound as the test concentration for daily ip administration to each mouse (~20 mg/kg BW). We found that when the monomers (i.e., catechin, EC, and gallic acid) were given individually to mice with STZ-induced diabetes for 9 d, none of them alone significantly reduced STZ-induced diabetic blood glucose levels after 8 d of treatment (data not shown). A combination of catechin and EC did not significantly alter blood glucose levels in STZ-injected mice, while the combination of catechin, EC, and gallic acid slightly reduced STZ-induced diabetic glucose levels by 10% (data not shown). However, neither EC nor gallic acid nor catechin caused significant changes in the circulating levels of insulin hormones or pancreas weight in mice with STZ-induced diabetes compared to mice treated with STZ alone (data not shown). Further, neither EC nor catechin nor gallic acid at the same concentrations and duration of treatment significantly decreased STZ-increased amounts of food intake, water consumed, or urine and feces excretions (data not shown). Taken together, these observations suggest that the effects of grape seed polyphenols on diabetic symptoms in mice with STZ-induced diabetes are due to procyanidins. This is consistent with previous findings that green tea modulates blood glucose levels in rats in polyphenol-specific ways [12], that the inhibitory effect of procyanidins on 12-O-tetradecanoylphorbol-13-acetate-stimulated ornithine decarboxylase activity in mouse epidermis increases with the degree of polymerization (dimmers have a greater effect than monomers) [27], and that apple procyanidins inhibit pancre-

atic lipase activity according to the degree of polymerization [28]. Procyanidin dimers contain a greater number of hydroxyl groups on the aromatic rings than do catechin or EC monomers, and these hydroxyl groups may be important for hydrogen bonding and free radical-scavenging activities [16-18]. In addition, some GSPCs have more gallyl or galloyl group, which exhibit some conformational flexibility, and may also be important for interactions with other molecules. Further investigations of the chemical basis of the antidiabetic activity of GSPC in mice with STZ-induced diabetes are required to understand how procyanidin activity is different from that of EC and catechin.

The effects of GSPC on diabetic symptoms appear to depend upon the route administration. The effects of GSPC, like green tea EGCG [22], were weaker when a corresponding amount of GSPC was orally administered to mice for 9 d. This may have been due to inefficient absorption of GSPC [29-31], and suggests that the effects of GSPC administered ip were not caused by interactions of between GSPC and food or by a reaction involving GSPC within the gastrointestinal tract.

The injected dose of GSPC (40-90 mg/kg BW) used in this study did not appear to be toxic to the liver or kidney of normal mice, because GSPC did not cause significant changes in the plasma levels of total protein, albumin, blood urea nitrogen, creatinine, uric acid, or enzymes that are indicative of severe damage to the liver and other organs. Furthermore, GSPC had no significant effect on the weights of mice liver and kidneys. Although we did not observe a statistically significant elevation of serum aspartate aminotransferase or lactate dehydrogenase activity in normal GSPC-treated mice, the small increase in the activities of these enzymes in the plasma could be indicative of a slight effect of GSPC on the liver, or could be related to lowered food intake. Significant changes in plasma alkaline phosphatase activity in normal GSPC-injected mice could also have been related to diet restriction. However, GSPC did not cause significant alterations in the number of pancreatic islets or circulating levels of insulin. In contrast, 80 mg GSPC/kg BW partially reduced the toxic effects of STZ on the liver and kidneys, as reflected by the fact that GSPC tended to reduce STZ-stimulated levels of plasma triglyceride, cholesterol, blood urea nitrogen, creatinine, uric acid, and alkaline phosphatase and lactate dehydrogenase activities, as well as the the STZ-stimulated weights of both the liver and kidneys. Decreased plasma triglyceride and cholesterol levels in mice with STZ-induced diabetes by GSPC may be attributable to the reduction in food intake and/or intestinal lipid absorption. This explanation is indirectly supported by reported findings that GSPC inhibits pancreatic lipase activity [28].

CONCLUSION

GSPC improve diabetic symptoms (e.g., increased urine and feces excretion; polyphagia; and blood glucose, triglyceride, and cholesterol levels) in mice with STZ-induced diabetes. Although oral administration of GSPC was less effective within 9 d than was ip administration of GSPC, long-term oral consumption of grape or procyanidin-containing extracts may mimic some of the acute GSPC effects described in this report and may be beneficial to health. The results of our study may provide some insight into the beneficial effects of procyanidin-containing beverages and the relationship between the high consumption of grapes and the low incidence of diabetes in some countries [10].

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SUPPLEMENTARY MATERIAL

Supplementary material can be viewed at
www.bentham.org/open/tophyj

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