JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Dietary Supplementation of Grape Skin Extract Improves Glycemia and Inflammation in Diet-Induced Obese Mice Fed a Western High Fat Diet

Shelly Hogan,[†,‡] Corene Canning,[§] Shi Sun,[§] Xiuxiu Sun,[§] Hoda Kadouh,[§] and Kequan Zhou*[,†,§]

[†]Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, United States

[§]Department of Nutrition and Food Science, Wayne State University, Detroit, Michigan 48202, United States

[‡]Montana State University, Bozeman, Montana 59717

ABSTRACT: Dietary antioxidants may provide a cost-effective strategy to promote health in obesity by targeting oxidative stress and inflammation. We recently found that the antioxidant-rich grape skin extract (GSE) also exerts a novel anti-hyperglycemic activity. This study investigated whether 3-month GSE supplementation can improve oxidative stress, inflammation, and hyperglycemia associated with a Western diet-induced obesity. Young diet-induced obese (DIO) mice were randomly divided to three treatment groups ($n = 12$): a standard diet (S group), a Western high fat diet (W group), and the Western diet plus GSE (2.4 g GSE/kg diet, WGSE group). By week 12, DIO mice in the WGSE group gained significantly more weight (24.6 g) than the W (20.2 g) and S groups (11.2 g); the high fat diet groups gained 80% more weight than the standard diet group. Eight of 12 mice in the W group, compared to only 1 of 12 mice in the WGSE group, had fasting blood glucose levels above 140 mg/dL. Mice in the WGSE group also had 21% lower fasting blood glucose and 17.1% lower C-reactive protein levels than mice in the W group ($P < 0.05$). However, the GSE supplementation did not affect oxidative stress in diet-induced obesity as determined by plasma oxygen radical absorbance capacity, glutathione peroxidase, and liver lipid peroxidation. Collectively, the results indicated a beneficial role of GSE supplementation for improving glycemic control and inflammation in diet-induced obesity.

KEYWORDS: diet-induced obesity, grape skin extract, hyperglycemia, inflammation, oxidative stress

INTRODUCTION

Obesity rate in the US has doubled in the past 20 years with nearly one-third of adults being obese.[1] Particularly alarming is the equally marked increase in obesity among children.[2] Obesity has been associated with a low degree pro-inflammatory state, in which impairments in antioxidant mechanisms could be involved.[3] The chronic inflammation and oxidative stress may play a causal role in multiple forms of obesity-associated complications such as insulin resistance and type-2 diabetes.[4] It has been postulated that increased adiposity in obesity is a key mediator of inflammation and oxidative stress, contributing to the production of pro-inflammatory cytokines and reactive oxygen species.[5] Another common metabolic feature associated with obesity is hyperglycemia. Hyperglycemia has been significantly associated with the promotion of oxidative stress and inflammation.[6−10] For example, oxidative stress due to persistent and chronic hyperglycemia may result in diminished activity of antioxidant enzymes and increased reactive oxygen species generation, which further promotes diabetic complications.[9−14] Given the alarming prevalence of obesity in the USA and other developed countries, it is imperative to reduce the risk of obesity-induced diseases by identifying new strategies that can effectively address metabolic complications such as hyperglycemia, oxidative stress, and inflammation associated with obesity.

In this study, we proposed the use of grape skin extract with potential bioactive properties for treatment of obesity-induced hyperglycemia and the parallel oxidative and inflammatory stress. Grapes have been well characterized as a natural source of notable bioactive compounds with antioxidant and other health promoting qualities.[15−17] Our laboratory has recently discovered that a grape skin extract significantly inhibited intestinal α-glucosidase activity and reduced postprandial hyperglycemia in diabetic mice. The anti-hyperglycemic effect of grape skin extract is novel and is not related to its known antioxidant functions. Few studies have shown that grape extracts reduced oxidative stress and inflammation in animals and humans, and such beneficial effects have mainly been attributed to their antioxidant activities.[18,19] Grape skin extract may provide anti-hyperglycemic protection in addition to its antioxidant function and, therefore, may particularly benefit the prevention and treatment of metabolic complications such as hyperglycemia, elevated oxidative stress, and chronic inflammation associated with obesity. Research has shown that the high calorie, low fiber dietary pattern of the Western diet was significantly associated with the increased risk of obesity and diabetes in promotion of the disease related metabolic complications.[20] This study took an initial step to assess whether a red grape skin extract can be protective against metabolic complications in Western diet-induced obesity. The C57BLK/6J mouse is a widely used diet-induced obese animal

Received: November 3, 2010
Accepted: January 3, 2011
Revised: January 3, 2011
Published: March 09, 2011

model that develops obesity, insulin resistance, and eventually diabetes when fed a high fat diet formulated to approximate a typical human diet in Western cultures.[21] The purpose of this study was to evaluate the effect of grape skin extract supplementation on hyperglycemia, oxidative stress, and inflammation status in C57BLK/6J mice fed a Western high fat diet.

MATERIALS AND METHODS

Chemicals and Reagents. Folin−Ciocalteu reagent, Trolox, fluorescein, and 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) were purchased from Sigma Chemical Co. (St. Louis, MO). Phenolic standards including cyanidin 3-glucoside, catechin, epicatechin, quercetin, *trans*-resveratrol, caffeic acid, gallic acid, *p*-coumaric acid, syringic acid, and ferulic acid were also purchased from Sigma Chemical Co. The organic solvents for grape skin extraction and HPLC analysis were HPLC grade (Fisher Scientific Atlanta, GA). Mouse insulin enzyme linked immunoassay (ELISA) kit was purchased from Millipore Corporation (St. Charles, MO), mouse CRP ELISA kit was obtained from Immunology Consultants Laboratory Inc. (Newberg, OR), cytosolic glutathione peroxidase (cGPx) kit was purchased from Cayman Chemical Company (Ann Arbor, MI), and bicinchoninic acid (BCA) protein assay kit was purchased from Pierce Biotechnology (Rockford, IL).

Grape Skin Extract (GSE) Preparation. Red wine Norton grapes (*Vitis aestivalis*) were harvested from the Blackstone vineyard (Blackstone, VA) and transported to the Enology Laboratory at Virginia Tech. The grape skins were hand peeled and then lyophilized. The dried grape skins were then milled to a fine powder with a Thomas Wiley minimill (Swedesboro, NJ) and extracted with 80% ethanol at 1:10 ratio (m/v). The extracts were then filtered to remove unwanted residues with a Whatman filter paper. After rotary evaporation of the organic solvent, the filtrates were frozen and lyophilized to obtain the grape skin extract (GSE). The gross energy of GSE was determined by an adiabatic bomb calorimeter (IKA, Wilmington, NC) in duplicate. The GSE was shipped to Research Diets Inc. (New Brunswick, NJ, USA) for incorporation into a pelleted high fat Western diet.

Total Phenolic Content (TPC) and Total Anthocyanin Content (TAC). The TPC of GSE was determined using Folin−Ciocalteu reagent with gallic acid as the standard and expressed as gallic acid equivalent per gram of GSE (mg GAE/g).[22] TAC of GSE was quantified using a pH differential method and expressed as mg of cyanidin 3-glucoside equivalents per gram of GSE (mg CGE/g).[23]

Determination of Phenolic Composition by High Performance Liquid Chromatography−Mass Spectrometry (HPLC−MS). GSE in methanol was injected onto a Luna C18 column (150 × 2.1 mm, 4 μm dp; Phenomenex, Torrance, CA) with a Thermo Survey autosampler (San Jose, CA) maintained at 10 °C. Mobile phase A consisted of 1% aqueous formic acid, and mobile phase B consisted 1% (v/v) formic acid in acetonitrile. The mobile phase was delivered to the HPLC column at a flow rate of 0.2 mL/min. The gradient elution program was as follows: 0 min, 97/3 A/B; 40 min, 70/30 A/B; 45 min, 0/100 A/B; 55 min, 0/100 A/B; 60 min, 97/3 A/B; 5 min postrun equilibration). The HPLC column effluent was pumped directly without any split into a Finnigan LCQDUO mass spectrometer (Thermo Scientific, Waltham, MA), which was operated in positive electrospray ionization at a temperature of 350 °C and a voltage of 4 kV. The mass spectrometer mass scan spectra were measured from *m*/*z* 150 up to *m*/*z* 1500 at 1000 amu/s. Individual phenolic compounds were then identified and quantified by comparing their MS spectra to the corresponding phenolic standards.

Oxygen Radical Absorbance Capacity (ORAC) of GSE. The ORAC assay was conducted to measure the peroxyl radical scavenging activity of GSE with Trolox as the antioxidant standard and ORAC value was expressed in micromoles of Trolox equivalents per gram of GSE (μmol TE/g).[24]

Table 1. Macronutrient and Selected Micronutrient Content of the Diet per 10 megajoule (MJ)*

nutrient	Western high fat diet (W and WGSE groups)	std diet (S group)
protein (g)	93.5 (17% of energy)	126.5 (23% of energy)
fat (g)	106.2 (41% of energy)	44.0 (17% of energy)
carbohydrate (g)	240.9 (43% of energy)	336.2 (60% of energy)
vitamin E (mg α-tocopherol equivalents)	1476.0	506.7

* Calculations were based on the diet composition data provided by Research Diets Inc. (New Brunswick, NJ).

Animals and Diets. Six week-old male C57BLK/6J mice (National Cancer Institute, Frederick, MD) were used. Mice were housed in cages (4 mice/cage) at a regulated temperature (20−23 °C) and alternating 12 h light/dark cycles with access to mouse diet and water *ad libitum*. After the two weeks of the acclimatization period, mice were randomly divided to three groups (12/group) receiving a standard diet (S), a Western high fat diet (W), or a Western high fat diet plus GSE (WGSE). The pelleted Western high fat diet (D12079B; Research Diets Inc., New Brunswick, NJ) resembles a typical high fat Western diet (Table 1) which has been shown to induce obesity and diabetes in mice.[25] The dosage of the GSE in the feed was incorporated into pellet form at a mixture of 2.4 g/kg of feed in order to dose each mouse at approximately 250 mg/kg body weight (bw) per day. Fresh feed, stored at 4 °C in light and air protected containers, was weighed and dispensed every three days to avoid potential degradation of GSE components. Body weight was determined at weeks 0, 2, 4, 6, 8, 10, and 12, and fasting blood glucose was measured at weeks 0, 2, 6, and 12. At termination, blood samples were collected into ice chilled heparinized tubes from the retro-orbital venous plexus while the mice were under anesthesia to obtain plasma for measurement of insulin and oxidative, and inflammatory biomarkers. All mice were then sacrificed with cervical dislocation under anesthesia. Liver was immediately excised, washed/chilled in ice-cold 0.9% NaCl, weighed, and then snap frozen with liquid nitrogen. The plasma and liver samples were stored for two weeks at −20 °C and −80 °C, respectively.[26]

Fasting Blood Glucose. After a 14 h fast, approximately 5 μL of whole blood was collected from the tail vein of each mouse to the blood glucose test strip and fasting blood glucose concentrations (mg/dL) were determined with a glucometer (ACCU-CHEK Meter, Roche Diagnostics, Kalamazoo, MI).

Plasma Insulin. Fasting plasma insulin levels were measured at the final time point (week 12) in duplicates, using a mouse enzymatic ELISA kit according to the manufacturer's recommendations. The assay was measured spectrophotometrically (Victor^3 multilabel platereader, PerkinElmer, Turku, Finland) by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm and expressed as ng/mL.

Plasma C-Reactive Protein (CRP). An immunoperoxidase assay for determination of CRP at the termination time point (week 12) was used. Plasma CRP levels were measured by ELISA using a plate reader at an absorbance of 450 nm and expressed as ng/mL.

Plasma Cytosolic Glutathione Peroxidase. The plasma antioxidant enzyme glutathione peroxidase (GPx) activity was measured with an assay kit following the manufacturer's instructions. The GPx assay was measured spectrophotometrically with the plate reader at an absorbance of 340 nm (nmol/min/mL). The BCA protein assay was used for the determination of the total protein (μg/mL) in each plasma sample following manufacturer's instructions (Pierce Biotechnology, Rockford, IL, USA). GPx values were expressed as nmol/min/g protein.

Plasma Oxygen Radical Absorbance Capacity (ORAC). Plasma ORAC was measured to determine the influence of grape phenolic supplementation on oxidative stress status following the 12 week study. The ORAC assay was conducted using the appropriate dilutions of the plasma samples compared to the Trolox solutions.[27] The assay

Table 2. Phenolic Composition and Antioxidant Activity of GSE

phenolic compds and antioxidant activity	mg/g GSE
TPC[a]	215.6 ± 7.3
TAC[a]	94.8 ± 2.4
catechin	8.71 ± 0.27
epicatechin	3.45 ± 0.11
trans-resveratrol	0.21 ± 0.02
quercetin	0.16 ± 0.01
gallic acid	0.23 ± 0.02
caffeic acid	0.14 ± 0.01
syringic acid	0.06 ± 0.00
p-coumaric acid	0.11 ± 0.00
ferulic acid	0.26 ± 0.02
ORAC (μmol TE/g GSE)	3267 ± 79

[a] TPC and TAC were expressed as mg GAE and mg CGE per g GSE, respectively.

conditions were the same as described above for ORAC measurement of GSE. The ORAC value of each plasma sample was expressed as micromoles of Trolox equivalents per liter of the plasma sample (μmol TE/L).

Liver Lipid Peroxidation. Liver lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) and was expressed as micrograms of malondialdehyde (MDA) per gram of liver specimen.[28]

Statistical Analyses. All data are expressed as the mean ± standard deviation (SD). The data were analyzed using one-way analysis of variance followed by post hoc Duncan's multiple range tests for comparison of differences among all groups (SPSS for Windows, Version 13.0, SPSS Inc., Chicago, IL, USA). A significant difference was defined as a $P < 0.05$.

RESULTS

Phenolic Composition and Antioxidant Activity of GSE. As shown in Table 2, the TPC and TAC of GSE were 215 mg GAE/g and 94.8 mg CGE/g GSE, respectively, suggesting that GSE contained substantial amounts of phenolic compounds, particularly, anthocyanins. A number of individual phenolic compounds were also determined in GSE. The amounts of catechin and epicatechin in GSE were 8.71 and 3.45 mg/g GSE. Other phenolic compounds such as resveratrol, quercetin, gallic acid, and ferulic acid in GSE were determined to be 0.21, 0.16, 0.23, and 0.26 mg/g, respectively. The antioxidant activity of GSE was evaluated by ORAC. GSE exerted significant peroxyl radical scavenging activity with the ORAC value of 3267 μmol TE/g GSE.

Feed Intake and Dietary Exposure to GSE. Table 3 summarizes the average daily dietary intake of three treatment groups. The higher feed intake of the S group (148.8 g/kg bw/d) can be attributable to its lower caloric density than the high fat dietary pattern of the W and WGSE groups. GSE had an energy level of 0.94 joule (J) per gram. There was no significant difference between feed intake of the W (103.2 g/kg bw/d) and the WGSE groups (97.9 g/kg bw/d), suggesting that the GSE was well tolerated by the DIO mice. Based on the daily feed intake and GSE concentration in the WGSE diet (2.4 g/kg), the daily doses of GSE and catechin were 234.7 and 2.04 mg/kg body weight (bw), respectively. The ORAC value associated with the daily GSE intake was 766.84 μmol TE/g bw.

Table 3. Daily Feed Intake and Doses of GSE, Catechin, and ORAC Values in the Three Dietary Treatment Groups[a]

daily dietary intake	S group (n = 12)	W group (n = 12)	WGSE group (n = 12)
feed intake (g/kg bw/d)	148.8 ± 28.2 a	103.2 ± 28.9 b	97.9 ± 22.5 b
GSE (mg/kg bw/d)			234.7 ± 26.1
TPC from GSE (mg/kg bw/d)			50.6 ± 4.9
TAC from GSE (mg/kg bw/d)			22.2 ± 2.4
catechin from GSE (mg/kg bw/d)			2.0 ± 0.2
ORAC value from GSE (μmol TE/kg bw/d)			766.8 ± 85.3

[a] Daily feed intake was determined based on the total weekly intake per cage (4 mice/cage). Means for the measurements in three diet treatment groups with different superscripts are significantly different, $P < 0.05$. GSE dose was calculated based on its supplemented concentration in the WGSE diet (2.4 g/kg in diet). The content of catechin in GSE is based on LC/MS measurement.

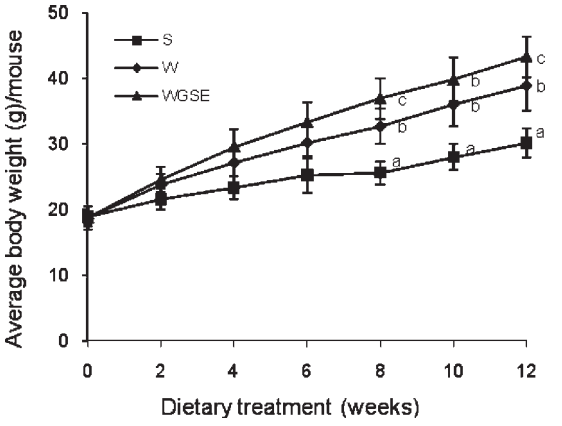

Figure 1. Effect of the three dietary treatments on mouse body weight. Mouse body weight was expressed as the mean ± SD (n = 12). Means of body weight at each time point with different letters are significantly different, $P < 0.05$.

Effect of GSE Supplementation on Body Weight. Figure 1 shows the body weight changes of mice by three diet treatments. The fat content of the diet exerted notable effect on mouse body weight. The Western high fat diet in the W and WGSE groups induced significantly higher weight gain than the normal diet (the S group) after 8 weeks of dietary treatment. By week 12, the WGSE group had gained significantly more weight (24.6 g) than the W and S groups (20.2 and 11.2 g, respectively). The results indicate that the incorporation of the GSE in the Western high fat diet significantly affected mouse body weight.

Effect of GSE Supplementation on Fasting Blood Glucose and Insulin Levels. Fasting blood glucose levels among all three groups were not significantly different from the initial dietary treatment (Figure 2). However, after 6 weeks of the dietary treatments, mice in the W and WGSE groups had significantly higher fasting blood glucose levels (111 and 103 mg/dL, respectively) compared to the S group (84 mg/dL), suggesting a significant role of high fat diet in deteriorating blood glucose control. By week 12, the average fasting blood glucose levels in

the S, W, and WGSE groups were 110.3, 144.6, and 119.3 mg/dL, respectively. The fasting blood glucose in the W group ranged from 120 to 175 mg/dL, and 8 of 12 mice in this group had fasting blood glucose levels above 140 mg/dL.; mice in the WGSE group had fasting blood glucose levels of 93−156 mg/dL, and only 1 of 12 mice had fasting blood glucose above 140 mg/dL. The average fasting blood glucose level in the WGSE group was 21.2% lower than that of the W group ($P < 0.05$), suggesting that GSE supplement can significantly improve blood glucose control in mice fed with the Western high fat diet. In addition, no significant difference was detected between the fasting glucose levels of the WGSE and S groups, indicating that the 3 month supplementation of GSE with a Western high fat diet could maintain fasting blood glucose levels similar to the levels of the mice fed with the standard diet. The lower concentration of fasting blood glucose may be linked to an improvement of insulin sensitivity or insulin resistance in mice in response to the treatment with grape extract. However, there was no significant difference in the insulin concentrations detected between any of the groups when measured at the 12 week time point (Table 4).

Effect of GSE Supplementation on Oxidative Stress Biomarkers. Following 12 weeks of diet treatments, a variety of antioxidant and oxidative stress biomarkers including plasma ORAC, cGPx, and liver lipid peroxidation were measured (Table 4). Plasma ORAC determined the peroxyl radical scavenging activity of the plasma in the mice. The average ORAC values of the S, W, and WGSE groups were 43.1, 49.8, and 52.6 μmol TE/mg, respectively. Mice fed the standard diet (S group) and Western high fat diet (W group) had similar ORAC values ($P > 0.05$). However, mice in the WGSE group had significantly higher ORAC values than mice in the S group. The GSE supplementation (the WGSE group) was associated with a trend of improved plasma antioxidant capacity compared to the W group but the difference was not significant ($P = 0.06$). The plasma cGPx values of the S, W, and WGSE groups were 4.0, 4.3, and 3.9 nmol/min/g, respectively, and were not significantly influenced by any of the dietary treatments (Table 4). Liver lipid peroxidation in the mice was measured and expressed as mg malondialdehyde (MDA) per kg liver. Unexpectedly, the S group had the highest levels of liver lipid peroxidation (8.2 mg MDA/kg liver) ($P < 0.05$). The WGSE group had a nonsignificant 19.5% reduction in MDA level compared to the W group (4.05 vs 5.03 mg MDA/kg liver, respectively, $P = 0.06$).

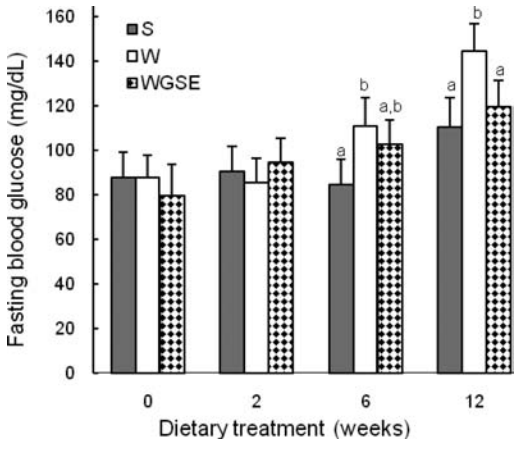

Figure 2. Effect of the three dietary treatments on fasting blood glucose. Fasting blood glucose was expressed as the mean ± SD ($n = 12$). Means of fasting blood glucose at each time point with different superscripts are significantly different, $P < 0.05$.

Effect of GSE Supplementation on Inflammation. Systemic inflammatory status of mice in each group was also determined by measuring the plasma acute phase CRP level at the termination of the 12 week study. The average plasma CRP levels of the S, W, and WGSE groups were 22.2, 28.6, and 23.7 ng/mL, respectively (Table 4). Mice in the WGSE and S groups had similar plasma CRP levels ($P > 0.05$), that were significantly lower than those of mice in the W group. The result showed that the GSE supplementation significantly suppressed the plasma CRP of Western high fat diet-fed mice by 17.1%.

DISCUSSION

This feeding study was designed to assess whether the 12 week GSE supplementation can improve metabolic complications associated with a Western high fat diet-induced obesity. The DIO mice fed the high fat diet allowed for the natural progression of obesity and eventually developed type 2 diabetes. Indeed, we showed that the 6 week old DIO mice fed the Western diet for 12 weeks developed significant obese and diabetic conditions. GSE was selected for this study due to two considerations: first, GSE is a well-known natural source of antioxidants. Research has shown significant antioxidant and anti-inflammatory capacity of GSE *in vitro* and *in vivo*. However, to the best of our knowledge, no study has investigated the potential effects of GSE supplementation on obesity associated oxidative stress and inflammation in an extended period, especially when the obesity was induced by a typical Western high fat diet. Moreover, our previous *in vitro* and acute animal investigations have demonstrated that GSE had a unique antipostprandial hyperglycemic effect. GSE specifically inhibited intestinal α-glucosidase activity and consequently reduced digestion and absorption of dietary starch, thereby improving blood glucose control. This novel function of GSE is independent of its antioxidant activity. Controlling blood glucose is essential to improve obese and diabetic conditions. Therefore, the antioxidant

Table 4. Effect of 12 Week Supplementation of Grape Derived Extracts Incorporated into a High Fat Diet on Antioxidant and Inflammatory Biomarkers[a]

biomarkers	S group ($n = 12$)	W group ($n = 12$)	WGSE group ($n = 12$)
plasma ORAC (μmols TE/L)	43.1 ± 6.1 a	49.8 ± 12.5 a,b	52.6 ± 3.4 b
plasma CRP (ng/mL)	22.2 ± 0.4 a	28.6 ± 0.8 b	23.7 ± 0.6 a
plasma cGPx (nmol/min/g)	4.0 ± 0.4 a	4.3 ± 0.7 a	3.9 ± 0.5 a
plasma insulin (ng/mL)	0.3 ± 0.1 a	1.5 ± 1.7 a	0.8 ± 0.7 a
liver TBARS (mg MDA/kg)	8.2 ± 2.0 a	5.0 ± 1.8 b	4.1 ± 1.4 b

[a] Values were expressed as the mean ± SD. Means for the measurements of each biomarker in three diet treatment groups with different superscripts are significantly different, $P < 0.05$.

and anti-hyperglycemic activities of GSE may provide dual protection against metabolic complications associated with obesity and diabetes.

In this study, we showed that the Western high fat diet significantly affected glucose homeostasis in DIO mice and 83% of mice in the W group developed diabetes after 12 week treatment (Figure 2). This is in agreement with previous feeding studies in which higher fasting blood glucose levels were detected in mice fed a high fat diet compared to a control diet.[39] However, GSE supplementation in the Western diet significantly suppressed fasting blood glucose levels by 22% in DIO mice, and after the 12 week treatment, only 25% of mice in the WGSE group had diabetes (Figure 2). The antidiabetic effect by GSE supplementation may be a result of the α-glucosidase inhibitory activity of GSE. α-Glucosidase inhibition was shown to improve blood glucose control by reducing dietary starch digestion and suppressing postprandial blood glucose spikes.[29] Grape derived agent has been shown to exert an antidiabetic activity after being administered to rats for 6 weeks.[30] Similarly, grape seeds rich in procyanidins have been reported to have an anti-hyperglycemic effect in streptozotocin-induced rats; the authors suggested that this response was a consequence of a delay in intestinal glucose absorption and/or through an insulin signaling pathway.[31] Improvements in insulin sensitivity can help to maintain blood glucose homeostasis and prevent the detrimental effects of hyperglycemia.[32] However, our study did not show a significant effect of GSE supplementation on insulin response, suggesting that the suppressed blood glucose in DIO mice by GSE supplementation is independent of insulin response.

In agreement with previous studies, the selected GSE in our study exerted significant *in vitro* antioxidant activity with an ORAC value of 3267 μmol TE/g GSE. The TPC of GSE was 215.6 mg GAE/g. Based on average daily feed intake and GSE concentration in the diet, we determined that the mice in the WGSE group consumed 50.6 mg total phenolic compounds and 2.0 mg catechin from GSE per day per kg body weight. GSE supplementation contributed the daily ORAC value of 766.8 μmol TE/kg bw (Table 3). We measured three biomarkers including plasma ORAC, cGPx, and liver lipid oxidation to determine the effect of GSE supplementation on oxidative stress status in DIO mice. Lipid peroxidation is an indicator of oxidative stress associated with membrane damage.[33] Enzymatic antioxidant activity, such as glutathione peroxidase (GPx), is a biomarker of lipid peroxidation and reported to be lower in animals with diabetes.[34] Similarly, enhanced antioxidant activity (ORAC) reflects the potential to reduce oxidative deterioration of lipid macromolecules. The 12 week supplementation of GSE showed a trend of reducing oxidative stress in the Western diet-fed mice, but the improvement was not significant (Table 4). A previous study showed enhanced plasma ORAC values in humans after 4 weeks of daily consumption of golden raisins, sun-dried raisins, or grapes.[35] Other studies also showed that grape wine flavonoids improved diabetes associated oxidative stress.[36,37] However, in our study, GSE supplementation showed no significant improvement in plasma ORAC values in Western diet-fed DIO mice. Similarly, the levels of plasma GPx and liver lipid oxidation in the mice from the WGSE group were not different from those in the W group. By contrast, mice in the S group had higher levels of liver lipid oxidation than those in the W and WGSE groups. Similar findings were reported by a previous study showing a decrease in liver lipid peroxidation after feeding mice a high fat diet (72% fat) for 4 weeks as compared to a control diet.[38] It has been suggested that the lower lipid oxidation with the high fat diet could be partly due to the 90.8% higher vitamin E concentration in the high fat diet (153 μg/g) than the control diet (14 μg/g).[38] In our study, the standard diet (S group) contained 100 mg vitamin E/kg compared to 3350 mg vitamin E/kg found in the Western high fat diet (W and WGSE groups). Therefore, the significant higher levels of liver lipid oxidation in the S group could be associated with the remarkably lower level of vitamin E content in its diet.

Inflammatory biomarker CRP levels have been shown to be significantly higher in obese populations compared to lean humans.[39,40] The average plasma CRP levels of the S, W, and WGSE groups were 22.2, 28.6, and 23.7 ng/mL, respectively. Mice fed with the Western high fat diet (the W group) had significantly higher CRP levels than the mice fed with the standard diet (the S group). However, GSE supplementation significantly reduced the elevated CRP levels in the Western diet-induced obese mice by 17.1%. CRP levels in WGSE group were not significantly different from those in the S group, suggesting that GSE supplementation was able to restore the elevated CRP levels in the obese mice to a normal range found in the lean mice. This anti-inflammatory effect may reflect potential dual bioactive properties of dietary GSE in regard to anti-hyperglycemic and free radical scavenging activities. Similar to our findings, improvements in inflammatory immune response have been reported with grape seed extracts. For instance, 19 weeks of high fat diet supplementation with daily procyanidins from grape seed (7 mg) resulted in a decrease in plasma CRP.[41] Over time, the better blood glucose control and potential antioxidant protection by dietary GSE may cause a lower systemic inflammatory response by the innate immune system as reflected by lower plasma levels of CRP.

An unexpected result from this study was the weight gain identified in the WGSE group. In particular, there was an 11.2% increase in body weight gain in the WGSE group compared to the W group. The alteration of body weight disagreed with some feeding studies that administered phenolic extracts or oral-hypoglycemic agents in which no significant increases in body weight were detected.[42] However, similar to our findings, there are reports of anti-hyperglycemic agents increasing weight gain. For instance, rats administered green tea extracts (300 mg/kg bw/d) for 6 weeks had significant body weight increases as compared to the control group.[43] The authors suggested that this effect may be due to improved glycemic control elicited by the green tea extract in the rats. Additionally, mice with diabetes and supplemented daily with an anti-hyperglycemic aqueous extract from a Vietnamese edible plant (*Cleistocalyx operculatus*) at 500 mg/kg for 8 weeks had increased body weight as compared to the control group.[44] In our study, the mice in the W and WGSE groups were estimated to consume similar amounts of dietary calories. However, unless the animals were housed singly we do not definitely know how much food was consumed per mouse. Another explanation for the weight gain may be attributed to the carbohydrates or other components in GSE which may have contributed to a higher caloric value in the diet.

In conclusion, this dietary supplementation study demonstrated a significant beneficial effect of GSE in reducing fasting blood glucose and inflammation in obese mice induced by a typical Western high fat diet. GSE was also associated with a developing protective trend of improved oxidative status in mice fed a Western diet. These results indicate that dietary GSE may play a protective role in improving metabolic complications associated with diet-induced obesity.

AUTHOR INFORMATION

Corresponding Author

*Tel: (313) 577- 3444. Fax: (313) 577-8616. E-mail: kzhou@wayne.edu.

Funding Sources

This work was supported in part by the Macromolecular Interfaces with Life Sciences (MILES) Integrative Graduate Education and Research Traineeship (IGERT) at Virginia Tech from the National Science Foundation (NSF) under Agreement No. DGE-0333378.

ACKNOWLEDGMENT

We acknowledge the assistance of Dave Gemmell and colleagues for animal care and procedure expertise at the laboratory animal facility at Virginia Tech.

REFERENCES

(1) National Center for Health Statistics. *Prevalence of overweight and obesity among adults*; Centers for Disease Control and Prevention: Atlanta, GA, United States, 2007.

(2) Rocchini, A. P. Childhood Obesity and a Diabetes Epidemic. *N. Engl. J. Med.* **2002**, *346*, 854–855.

(3) Dandona, P.; Aljada, A.; Bandyopadhyay, A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol.* **2004**, *25*, 4–7.

(4) Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* **2006**, *444*, 860–867.

(5) Mohamed-Ali, V.; Pinkney, J. H.; Coppack, S. W. Adipose tissue as an endocrine and paracrine organ. *Int. J. Obes. Relat. Metab. Disord.* **1998**, *22*, 1145–1158.

(6) Dandona, P.; Mohanty, P.; Ghanim, H.; Aljada, A.; Browne, R.; Hamouda, W.; Prabhala, A.; Afzal, A.; Garg, R. The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 355–362.

(7) Patel, C.; Ghanim, H.; Ravishankar, S.; Sia, C. L.; Viswanathan, P.; Mohanty, P.; Dandona, P. Prolonged reactive oxygen species generation and nuclear factor-kappaB activation after a high-fat, high-carbohydrate meal in the obese. *J. Clin. Endocrinol. Metab.* **2007**, *92*, 4476–4479.

(8) Lee, Y. A.; Cho, E. J.; Tanaka, T.; Yokozawa, T. Inhibitory activities of proanthocyanidins from persimmon against oxidative stress and digestive enzymes related to diabetes. *J. Nutr. Sci. Vitaminol. (Tokyo)* **2007**, *53*, 287–292.

(9) Lapolla, A.; Piarulli, F.; Sartore, G.; Ceriello, A.; Ragazzi, E.; Reitano, R.; Baccarin, L.; Laverda, B.; Fedele, D. Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. *Diabetes Care* **2007**, *30*, 670–676.

(10) Rameshwar, J.; Anand, K. Antihyperglycaemic and antiperoxidative roles of acarbose in type 2 diabetes mellitus are possibly mediated through changes in thyroid function. *Clin. Exp. Pharmacol. Physiol.* **2006**, *33*, 1104–1106.

(11) Ihnat, M. A.; Thorpe, J. E.; Kamat, C. D.; Szabo, C.; Green, D. E.; Warnke, L. A.; Lacza, Z.; Cselenyak, A.; Ross, K.; Shakir, S.; Piconi, L.; Kaltreider, R. C.; Ceriello, A. Reactive oxygen species mediate a cellular 'memory' of high glucose stress signalling. *Diabetologia* **2007**, *50*, 1523–1531.

(12) Ceriello, A.; Lush, C. W.; Darsow, T.; Piconi, L.; Corgnali, M.; Nanayakkara, N.; Frias, J. P.; Maggs, D. Pramlintide reduced markers of oxidative stress in the postprandial period in patients with type 2 diabetes. *Diabetes Metab. Res. Rev.* **2008**, *24* (2), 103–108.

(13) Satoh, N.; Shimatsu, A.; Yamada, K.; Aizawa-Abe, M.; Suganami, T.; Kuzuya, H.; Ogawa, Y. An alpha-glucosidase inhibitor, voglibose, reduces oxidative stress markers and soluble intercellular adhesion molecule 1 in obese type 2 diabetic patients. *Metabolism* **2006**, *55*, 786–793.

(14) Ihara, Y.; Toyokuni, S.; Uchida, K.; Odaka, H.; Tanaka, T.; Ikeda, H.; Hiai, H.; Seino, Y.; Yamada, Y. Hyperglycemia causes oxidative stress in pancreatic beta-cells of GK rats, a model of type 2 diabetes. *Diabetes* **1999**, *48*, 927–932.

(15) Louli, V.; Ragoussis, N.; Magoulas, K. Recovery of phenolic antioxidants from wine industry by-products. *Bioresour. Technol.* **2004**, *92*, 201–208.

(16) Shi, J.; Yu, J.; Pohorly, J. E.; Kakuda, Y. Polyphenolics in grape seeds—biochemistry and functionality. *J. Med. Food* **2003**, *6*, 291–299.

(17) Zhang, F. L.; Gao, H. Q.; Shen, L. Inhibitory effect of GSPE on RAGE expression induced by advanced glycation end products in endothelial cells. *J. Cardiovasc. Pharmacol.* **2007**, *50*, 434–440.

(18) Vitseva, O.; Varghese, S.; Chakrabarti, S.; Folts, J. D.; Freedman, J. E. Grape seed and skin extracts inhibit platelet function and release of reactive oxygen intermediates. *J. Cardiovasc. Pharmacol.* **2005**, *46*, 445–451.

(19) Greenspan, P.; Bauer, J. D.; Pollock, S. H.; Gangemi, J. D.; Mayer, E. P.; Ghaffar, A.; Hargrove, J. L.; Hartle, D. K. Antiinflammatory Properties of the Muscadine Grape (Vitis rotundifolia). *J. Agric. Food Chem.* **2005**, *53*, 8481–8484.

(20) van Dam, R. M.; Rimm, E. B.; Willett, W. C.; Stampfer, M. J.; Hu, F. B. Dietary Patterns and Risk for Type 2 Diabetes Mellitus in U.S. Men. *Ann. Intern. Med.* **2002**, *136*, 201–209.

(21) Merat, S.; Casanada, F.; Sutphin, M.; Palinski, W.; Reaven, P. D. Western-type diets induce insulin resistance and hyperinsulinemia in LDL receptor-deficient mice but do not increase aortic atherosclerosis compared with normoinsulinemic mice in which similar plasma cholesterol levels are achieved by a fructose-rich diet. *Arterioscler. Thromb. Vasc. Biol.* **1999**, *19*, 1223–1230.

(22) Zhou, K.; Su, L.; Yu, L. L. Phytochemicals and antioxidant properties in wheat bran. *J. Agric. Food Chem.* **2004**, *52*, 6108–6114.

(23) Hogan, S.; Zhang, L.; Li, J.; Zoecklein, B.; Zhou, K. Antioxidant properties and bioactive components of Norton (Vitis aestivalis) and Cabernet Franc (Vitis vinifera) wine grapes. *LWT—Food Sci. Technol.* **2009**, *42*, 1269–1274.

(24) Zhou, K.; Hao, J.; Griffey, C.; Chung, H.; O'Keefe, S. F.; Chen, J.; Hogan, S. Antioxidant Properties of Fusarium Head Blight-Resistant and -Susceptible Soft Red Winter Wheat Grains Grown in Virginia. *J. Agric. Food Chem.* **2007**, *55*, 3729–3736.

(25) Abu-Elheiga, L.; Oh, W.; Kordari, P.; Wakil, S. J. Acetyl-CoA carboxylase 2 mutant mice are protected against obesity and diabetes induced by high-fat/high-carbohydrate diets. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10207–10212.

(26) Wennlof, A. H.; Yngve, A.; Nilsson, T. K.; Sjostrom, M. Serum lipids, glucose and insulin levels in healthy schoolchildren aged 9 and 15 years from Central Sweden: reference values in relation to biological, social and lifestyle factors. *Scand. J. Clin. Lab. Invest.* **2005**, *65*, 65–76.

(27) Moore, J.; Hao, Z.; Zhou, K.; Luther, M.; Costa, J.; Yu, L. L. Carotenoid, tocopherol, phenolic acid, and antioxidant properties of Maryland-grown soft wheat. *J. Agric. Food Chem.* **2005**, *53*, 6649–6657.

(28) Hogan, S.; Canning, C.; Sun, S.; Sun, X.; Zhou, K. Effects of Grape Pomace Antioxidant Extract on Oxidative Stress and Inflammation in Diet Induced Obese Mice. *J. Agric. Food Chem.* **2010**, *58*, 11250–11256.

(29) Bischoff, H. Pharmacology of alpha-glucosidase inhibition. *Eur. J. Clin. Invest.* **1994**, *24* (Suppl. 3), 3–10.

(30) Al-Awwadi, N.; Azay, J.; Poucheret, P.; Cassanas, G.; Krosniak, M.; Auger, C.; Gasc, F.; Rouanet, J. M.; Cros, G.; Teissedre, P. L. Antidiabetic activity of red wine polyphenolic extract, ethanol, or both in streptozotocin-treated rats. *J. Agric. Food Chem.* **2004**, *52*, 1008–1016.

(31) Pinent, M.; Blay, M.; Blade, M. C.; Salvado, M. J.; Arola, L.; Ardevol, A. Grape seed-derived procyanidins have an antihyperglycemic effect in streptozotocin-induced diabetic rats and insulinomimetic activity in insulin-sensitive cell lines. *Endocrinology* **2004**, *145*, 4985–4990.

(32) Barnard, R. J.; Wen, S. J. Exercise and diet in the prevention and control of the metabolic syndrome. *Sports Med.* **1994**, *18*, 218–228.

(33) Halliwell, B.; Gutteridge, J. M. Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet* **1984**, *1*, 1396–1397.

(34) Ananthan, R.; Latha, M.; Ramkumar, K. M.; Pari, L.; Baskar, C.; Narmatha Bai, V. Modulatory effects of Gymnema montanum leaf extract on alloxan-induced oxidative stress in Wistar rats. *Nutrition* **2004**, *20*, 280–285.

(35) Parker, T. L.; Wang, X. H.; Pazmino, J.; Engeseth, N. J. Antioxidant capacity and phenolic content of grapes, sun-dried raisins, and golden raisins and their effect on ex vivo serum antioxidant capacity. *J. Agric. Food Chem.* **2007**, *55*, 8472–8477.

(36) Landrault, N.; Poucheret, P.; Azay, J.; Krosniak, M.; Gasc, F.; Jenin, C.; Cros, G.; Teissedre, P. L. Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. *J. Agric. Food Chem.* **2003**, *51*, 311–318.

(37) Ceriello, A.; Bortolotti, N.; Motz, E.; Lizzio, S.; Catone, B.; Assaloni, R.; Tonutti, L.; Taboga, C. Red wine protects diabetic patients from meal-induced oxidative stress and thrombosis activation: a pleasant approach to the prevention of cardiovascular disease in diabetes. *Eur. J. Clin. Invest.* **2001**, *31*, 322–328.

(38) Sohet, F. M.; Neyrinck, A. M.; Dewulf, E. M.; Bindels, L. B.; Portois, L.; Malaisse, W. J.; Carpentier, Y. A.; Cani, P. D.; Delzenne, N. M. Lipid peroxidation is not a prerequisite for the development of obesity and diabetes in high-fat-fed mice. *Br. J. Nutr.* **2009**, 1–8.

(39) Devries, M. C.; Hamadeh, M. J.; Glover, A. W.; Raha, S.; Samjoo, I. A.; Tarnopolsky, M. A. Endurance training without weight loss lowers systemic, but not muscle, oxidative stress with no effect on inflammation in lean and obese women. *Free Radical Biol. Med.* **2008**, *45*, 503–511.

(40) Block, G.; Dietrich, M.; Norkus, E.; Jensen, C.; Benowitz, N. L.; Morrow, J. D.; Hudes, M.; Packer, L. Intraindividual variability of plasma antioxidants, markers of oxidative stress, C-reactive protein, cotinine, and other biomarkers. *Epidemiology* **2006**, *17*, 404–412.

(41) Terra, X.; Montagut, G.; Bustos, M.; Llopiz, N.; Ardevol, A.; Blade, C.; Fernandez-Larrea, J.; Pujadas, G.; Salvado, J.; Arola, L.; Blay, M. Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. *J. Nutr. Biochem* **2009**, *20* (3), 210–218.

(42) Puls, W.; Keup, U.; Krause, H. P.; Thomas, G.; Hoffmeister, F. Glucosidase inhibition. A new approach to the treatment of diabetes, obesity, and hyperlipoproteinaemia. *Naturwissenschaften* **1977**, *64*, 536–537.

(43) Babu, P. V.; Sabitha, K. E.; Shyamaladevi, C. S. Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. *Chem.-Biol. Interact.* **2006**, *162*, 114–120.

(44) Mai, T. T.; Chuyen, N. V. Anti-hyperglycemic activity of an aqueous extract from flower buds of Cleistocalyx operculatus (Roxb.) Merr and Perry. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 69–76.