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ARTICLE in JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · JULY 2012

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# Muscadine Grape (*Vitis rotundifolia*) and Wine Phytochemicals Prevented Obesity-Associated Metabolic Complications in C57BL/6J Mice

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**ABSTRACT:** The objective of this study was to determine the effects of muscadine grape or wine (cv. Noble) phytochemicals on obesity and associated metabolic complications. Muscadine grape or wine phytochemicals were extracted using Amberlite FPX66 resin. Male C57BL/6J mice were given a low-fat diet (LF, 10% kcal fat), high-fat diet (HF, 60% kcal fat), HF + 0.4% muscadine grape phytochemicals (HF+MGP), or HF + 0.4% muscadine wine phytochemicals (HF+MWP) for 15 weeks. At 7 weeks, mice fed HF+MGP had significantly decreased body weights by 12% compared to HF controls. Dietary MGP or MWP supplementation reduced plasma content of free fatty acids, triglycerides, and cholesterol in obese mice. Inflammation was alleviated, and activity of glutathione peroxidase was enhanced. Consumption of MGP or MWP improved insulin sensitivity and glucose control in mice. Thus, consumption of muscadine grape and wine phytochemicals in the diet may help to prevent obesity-related metabolic complications.

**KEYWORDS:** muscadine grape, wine, obesity, C57BL/6J mice

## INTRODUCTION

Obesity is a multifactorial condition posing major health problems worldwide. It contributes to multiple metabolic abnormalities such as cardiovascular disease, insulin resistance, dyslipidemia, oxidative stress, inflammation, and fatty liver.<sup>1</sup> Due to its negative impact on public health, multiple campaigns have been launched to curb the increase of obesity in the United States. One of the effective strategies to prevent obesity is through dietary interventions, especially the use of phytochemicals.<sup>2</sup> Dietary polyphenols reduce obesity through different mechanisms such as suppression of adipocyte hypertrophy, inhibition of preadipocyte differentiation, stimulation of lipolysis, or induction of fat cell apoptosis.<sup>3</sup> For example, resveratrol decreased adipogenesis and viability of preadipocytes by transcriptional and post-transcriptional regulation of adipocyte-specific genes. In mature adipocytes, resveratrol decreased lipid accumulation through stimulation of lipolysis and apoptosis.<sup>4</sup> In vitro and in vivo studies demonstrated the synergistic effects of resveratrol, quercetin, and genistein in preventing weight gain and adipogenesis.<sup>5</sup> Experimental diets supplemented with a mixture of grape (*Vitis vinifera*) extract, green tea extract, and L-carnitine were also effective in preventing obesity and related metabolic disorders.<sup>6</sup>

Muscadine grapes (*Vitis rotundifolia*) are commonly grown and consumed in the southeastern United States. They contain a unique blend of bioactive phytochemicals with potential to improve symptoms of chronic metabolic diseases. Muscadine grapes are round, have either bronze- or purple-colored leather-like thick skin, and are well-adapted to warm, humid climates, which are not suitable for the growth of *V. vinifera*.<sup>7</sup> The phytochemical composition of muscadine is significantly

different from that of *V. vinifera*. For example, the skin of purple muscadine grapes contains exclusively anthocyanin 3,5-diglucosides, whereas *V. vinifera* contains only monoglucosides. The seeds and skin of muscadine grapes contain large amounts of ellagitannins and ellagic acid, which are absent in *V. vinifera*.<sup>8</sup> The resveratrol content of muscadine grapes is much lower compared to *V. vinifera*.<sup>9</sup> Phytonutrients with antioxidant properties are positively associated with disease prevention. A growing number of studies showed that muscadine grapes had anticancer,<sup>10</sup> antibacterial,<sup>11,12</sup> and anti-inflammatory properties.<sup>13</sup> To our knowledge, no study has evaluated the effects of muscadine grape or wine phytochemicals on the development of obesity and the associated metabolic complications.

A number of studies investigated the effects of supplementing high-fat diets with skin,<sup>14</sup> seed,<sup>15</sup> and pomace<sup>16,17</sup> extracts of *V. vinifera* on adiposity, inflammation, insulin resistance, and hyperlipidemia. For example, grape seed extracts supplemented in high-fat diets partially reversed obesity by affecting lipid metabolism in mice.<sup>18</sup> However, results from these studies cannot be extrapolated to muscadine grapes due to the difference in phytochemical composition. The objective of this study was to assess the effect of feeding muscadine grape or wine phytochemicals in high-fat diets on obesity and metabolic complications in C57BL/6J mice.

Received: March 29, 2012

Revised: July 2, 2012

Accepted: July 12, 2012

Published: July 12, 2012

Table 1. Composition of Experimental Diets

ingredient	LF		HF		HF+MGP		HF+MWP	
	g/kg	kcal/kg	g/kg	kcal/kg	g/kg	kcal/kg	g/kg	kcal/kg
casein	200	800	200	800	200	800	200	800
L-cystine	3	12	3	12	3	12	3	12
corn starch	506.2	2025	0	0	0	0	0	0
maltodextrin	125	500	125	500	125	500	125	500
sucrose	68.8	275	68.8	275	68.8	275	68.8	275
cellulose	50	0	50	0	50	0	50	0
lard	20	180	245	2205	245	2205	245	2205
soybean oil	25	225	25	225	25	225	25	225
mineral mix	10	0	10	0	10	0	10	0
vitamin mix	10	40	10	40	10	40	10	40
MGP	0	0	0	0	3.09	0	0	0
MWP	0	0	0	0	0	0	3.09	0
total	1055	4057	774	4057	777	4057	777	4057
	g %	kcal %	g %	kcal %	g %	kcal %	g %	kcal %
carbohydrate	67	70	26	20	26	20	26	20
protein	19	20	26	20	26	20	26	20
fat	4	10	35	60	35	60	35	60
MGP	0	0	0	0	0.4	0	0	0
MWP	0	0	0	0	0	0	0.4	0

## MATERIALS AND METHODS

**Chemicals and Reagents.** Folin–Ciocalteu reagent, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ellagic acid, quercetin, myricetin, and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Standards of the 3-O- $\beta$ -glucosides of pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin (six mixed anthocyanin standard) were purchased from Polyphenols Laboratories (Sandnes, Norway). HPLC grade solvents were used for phytochemical extraction and analysis (Fisher Scientific, Atlanta, GA, USA). A mouse insulin enzyme-linked immunoassay (ELISA) kit was purchased from Crystal Chemicals (Downers Grove, IL, USA), and a mouse CRP ELISA kit was obtained from Immunology Consultants Laboratory Inc. (Newberg, OR, USA). A cytosolic glutathione peroxidase (cGPx) kit was purchased from Cayman Chemical Co. (Ann Arbor, MI, USA). Red muscadine wine (cv. Noble) was provided by Lakeridge Wineries (Clermont, FL, USA). Muscadine grapes (cv. Noble) were purchased from a local vineyard.

**Muscadine Grape and Wine Extract Preparation.** Grapes (15 kg) were crushed and extracted with acidified methanol (0.5% acetic acid, 20 L) in 10 batches. Extracts were filtered using Whatman no. 4 filter paper. Methanol in the extract was removed using a rotary evaporator. The concentrated extract was resuspended in water and loaded on a column (80 mm i.d.  $\times$  600 mm) (Ace Laboratories, NJ, USA) filled with Amberlite FPX66 resin (Rohm Hass, PA, USA). Prior to the sample loading, resin was conditioned with acidified water (0.5% formic acid), and the initial resin bed volume was 1 L. Noble muscadine wine (20 L) was loaded in 10 batches on the resin column without any treatment. After sample loading, the column was eluted with 4 L of acidified water to remove sugars and other compounds not adsorbed on the resins. Muscadine grape and wine phytochemicals were recovered from the column using 4 L of methanol elution. Extracts were dried using a SpeedVac concentrator (Thermo Scientific ISS110, Waltham, MA, USA). Approximately, 40 g of dried muscadine grape and 30 g of wine phytochemicals were obtained.

**Characterization and Phytochemical Analysis of Extracts.** The total phenolic content of the extracts was determined by using Folin–Ciocalteu reagent with gallic acid as a standard, and results were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg of GAE/g). ORAC assay was conducted to measure the peroxyl radical-scavenging activity of the extracts and was expressed in micromoles of Trolox equivalents per gram of the extracts ( $\mu$ mol TE/g).<sup>19</sup>

Anthocyanins were quantified using HPLC according to a published method.<sup>20</sup> Other phenolic compounds, including ellagic acid, quercetin, myricetin, and kaempferol, were quantified using HPLC after acid hydrolysis.<sup>7</sup> Briefly, dry muscadine grape or wine phytochemicals (150 mg) were dissolved in 5 mL of 1.2 N HCl in 50% methanol, vortexed, and incubated in a water bath at 90 °C for 80 min to hydrolyze flavonoid glycosides to aglycones. The hydrolyzed extracts were filtered through a 0.2  $\mu$ m syringe nylon filter and diluted. Chromatographic analyses were performed on an Agilent 1200 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with an autosampler/injector and diode array detector. A Zorbax SB-C<sub>18</sub> column (4.6 mm  $\times$  250 mm, 5  $\mu$ m, Agilent Technologies) was used for separation. Elution was performed using mobile phase A (0.5% formic acid aqueous solution) and mobile phase B (acetonitrile). UV-vis spectra were scanned from 220 to 600 nm on a diode array detector with detection wavelengths of 280, 360, and 520 nm.

**Animal Study.** Low-fat, high-fat, and high-fat diet with phytochemicals from muscadine grape or wine were prepared by Research Diets Inc. (New Brunswick, NJ, USA) using the formulations shown in Table 1. Thirty-six 6-week-old male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and housed in shoebox cages on corn-cob bedding (three per cage). Mice were acclimatized for 1 week and maintained on a 12 h light/dark cycle with access to food and water ad libitum. Following the adaptation period, mice were randomly divided into four experimental groups ( $n = 9$ ): low fat (LF, 10% kcal fat), high fat (HF, 60% kcal fat), high fat plus 0.4% muscadine grape phytochemical (HF+MGP), and high fat plus 0.4% muscadine wine phytochemical (HF+MWP). The LF and HF diets had energy densities of 3.85 and 5.24 kcal/g, respectively. The phytochemical dose in the diets was chosen on the basis of the effectiveness of resveratrol supplementation at 0.4% in diets for 15 weeks on body weight and obesity conditions.<sup>21</sup> Mice were maintained on experimental diets for 15 weeks. Body weight and food intake was recorded weekly. At the end of the study, animals were fasted for 7 h, and blood was taken by cardiac puncture from anesthetized mice. Plasma samples were isolated by centrifugation at 2000g for 15 min at 4 °C and stored at -80 °C. Livers and epididymal adipose tissue were harvested and weighed. Sections of liver and adipose tissue were frozen at -80 °C. This protocol was approved by the Institutional Animal Care and Use Committee of the University of Florida.

**Intraperitoneal Glucose Tolerance Test.** Glucose tolerance tests were performed at week 12 on five mice per group. After mice were fasted for 6 h, 2 g/kg glucose was given intraperitoneally. Tail blood was collected at 0, 10, 20, 30, 60, and 120 min. Blood glucose concentration was determined with a hand-held Contour glucose monitor (Bayer Healthcare, Tarrytown, NY, USA). The area under curve (AUC; millimolar per minute) was calculated using a trapezoidal rule.<sup>22</sup>

**Measurement of Plasma Biomarkers Related to Glucose and Lipid Metabolism.** Plasma concentrations of total cholesterol, triglycerides, and free fatty acids were determined using commercial kits (Abcam, MA, USA). Fasting plasma insulin levels were measured with a murine ultrasensitive ELISA kit, measured spectrophotometrically at 450 nm and expressed as millimoles per liter. Plasma glucose levels (mmol/L) were measured using a glucose assay kit (Cayman Chemicals). Insulin resistance was evaluated by homeostasis model of insulin resistance (HOMA-IR) as previously described.<sup>23</sup>

**Measurement of Inflammatory and Oxidative Stress Biomarkers.** Plasma C-reactive protein levels were measured by ELISA using a plate reader at 450 nm and expressed as nanograms per milliliter. The plasma antioxidant enzyme glutathione peroxidase (GPx) activity was measured spectrophotometrically at 340 nm using commercial kits and expressed as nanomoles per minute per milliliter.

**Histology.** For the histological examination, epididymal fat tissues were fixed in 10% buffered formalin overnight and then embedded in paraffin, cut at a thickness of 5 µm, and stained with hematoxylin and eosin (H&E).<sup>21</sup> Frozen livers were sectioned into 8 µm thick sections using a microtome, and lipid deposition was detected by staining with Oil-red-O.<sup>6</sup> Digital images were acquired with a Leica DM 2500 phase contrast microscope and Magnafire and Image Pro 6.0 imaging software (Media Cybernetics, Silver Spring, MD, USA).

**Statistical Analyses.** All data are expressed as the mean ± standard error of the mean (SEM). One-way analyses of variance (ANOVA) with Tukey–Kramer HSD pairwise comparison of the means were performed using JMP (version 8.0, SAS Institute Inc., Cary, NC, USA). Fisher's least significant test was applied to analyze body weight gain of mice. Graphs were generated using Sigma-Plot software version 11.0. A  $p \leq 0.05$  difference was considered to be significant.

## RESULTS AND DISCUSSION

**Muscadine Grape and Wine Phytochemical Composition.** Phytochemical compositions of MGP and MWP are shown in Table 2. MGP contained a significantly higher amount of ellagic acid (18.2 mg/g) than MWP (1.1 mg/g). Ellagic acid has very low solubility in water. Ellagitannins hydrolyze to ellagic acid, and the ellagic acid forms sediment in muscadine wine.<sup>24</sup> The precipitated ellagic acid in muscadine is commonly removed by filtration. On the other hand, total anthocyanin content was higher in wine phytochemicals (124.8 mg/g) than in grape phytochemicals (67.9 mg/g). Red muscadine wine is commonly made by fermentation of crushed grapes (including skin). Anthocyanins were extracted from skins and concentrated in wine during fermentation. The ratio of individual anthocyanin 3,5-diglucosides in MGP was slightly different from that in MWP. For example, the major anthocyanin in MGP was delphinidin 3,5-diglucoside, whereas peonidin 3,5-diglucoside was the major anthocyanin in MWP. This difference was likely due to the change of anthocyanin profile during winemaking. Total phenolic content of phytochemicals in grape and wine were 620 and 389 mg of GAE/g, respectively (Table 2). The antioxidant activities of MGP and MWP, evaluated by ORAC, were 4824 and 3383 µmol TE/g, respectively.

**Food Intake and Body and Tissue Weights in Mice.** Average food intake (g/mouse/day) in mice was estimated and reported in Table 3. Animals fed low-fat diet consumed more

**Table 2. Antioxidant and Phytochemical Composition of Muscadine Grape or Wine Extracts<sup>a</sup>**

phytochemical	MGP (mg/g dry extract)	MWP (mg/g dry extract)
quercetin	0.5 ± 0.1	0.5 ± 0.02
myricetin	1.4 ± 0.3	2.1 ± 0.1
kaempferol	0.2 ± 0.06	0.3 ± 0.01
ellagic acid	18.2 ± 0.15*	1.1 ± 0.3
delphinidin 3,5-diglucoside	19.0 ± 1.1	25.5 ± 1.5
cyanidin 3,5-diglucoside	7.9 ± 2.5	19.1 ± 1.1*
petunidin/pelargonidin 3,5-diglucoside <sup>b</sup>	16.3 ± 1.1	27.2 ± 1.5*
peonidin 3,5-diglucoside	14.6 ± 1.6	35.2 ± 2.2*
malvidin 3,5-diglucoside	10.1 ± 1.8	17.8 ± 1.1
total anthocyanins	67.9 ± 5.7	124.8 ± 3.2*
total phenolic content (mg of GAE/g)	620 ± 58	389 ± 32
ORAC (µmol/TE/g)	4824 ± 6.8	3383 ± 7.1

<sup>a</sup>\*, significant differences at  $p \leq 0.05$  using *t* test. <sup>b</sup>Peaks coeluted on HPLC. Samples were analyzed in duplicate. Data are the mean ± SEM for duplicate tests.

feed compared to mice in the high-fat diet group (3.3 vs 2.7 g/mouse/day;  $p < 0.05$ ). The energy intake of mice on the low-fat diet was not significantly different from that of mice on the high-fat diet (12.8 vs 14.3 kcal/mouse/day). Grape or wine phytochemical supplementation in high-fat diets did not affect estimated food intake and caloric intake in animals (Table 3). The body weights of mice from week 1 to 15 are shown in Figure 1A. The net body weight gains at weeks 7 and 15 are depicted in Figure 1B. As expected, mice fed a high-fat diet gained more weight than mice fed a low-fat diet. At the end of the experiment, mice in the HF group had gained 37% more weight ( $p < 0.05$ ) than mice in the LF group. Supplementing high-fat diets with grape or wine phytochemicals did not affect the cumulative body weights in mice at week 15 (Figure 1B). However, during the seventh week of the feeding period, HF fed controls had gained 45% more weight ( $p < 0.05$ ) than LF mice. Mice fed HF+MGP showed a 12% decrease in body weights ( $p < 0.05$ ) compared to high-fat fed mice. The suppression of body weight gain caused by MGP in animals was not due to decreased food intake. Similar observations were made in mice fed resveratrol (0.4%) for 15 weeks,<sup>25</sup> grape seed extract (250 mg/kg bw) for 12 weeks,<sup>5</sup> and dietary wine pomace extract (0.45%) for 4 weeks.<sup>17</sup>

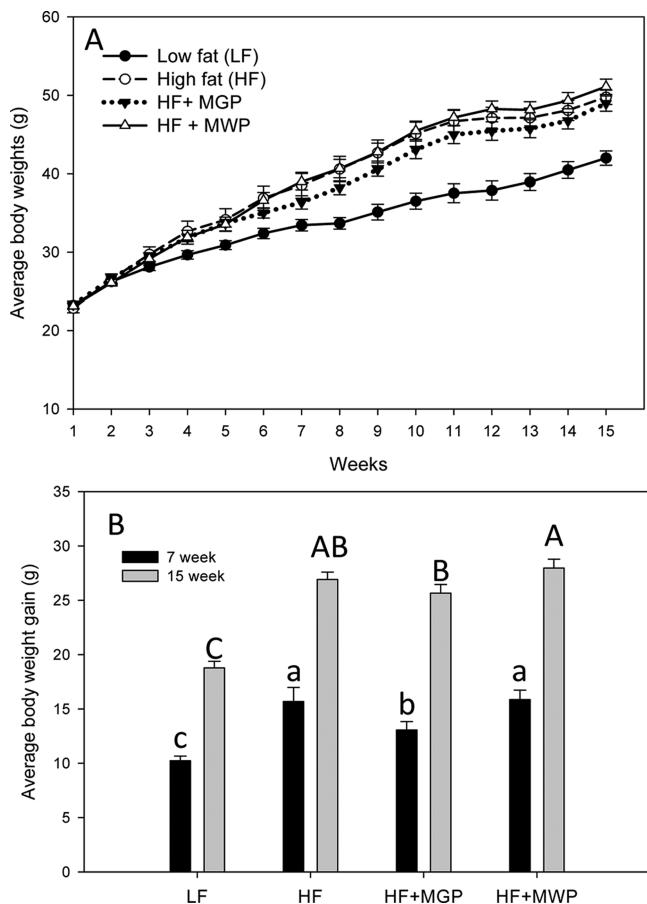
Feeding mice with HF diet increased the weight of epididymal adipose tissue by 67% compared to the fat mass in their lean counterparts on LF diets (Table 3). Supplementation of MGP and MWP in the HF diet significantly reduced fat mass by 29 and 12.5%, respectively, as compared to the HF group. MGP and MWP supplementation reduced liver weights by 41.6 and 37.5%, respectively, when compared to mice fed the HF diet alone (Table 3). Hepatic tissue in lean mice weighed 54% less than that of obese mice. In previous studies, rodents fed HF diets showed more body weight, higher fat deposition and total liver weight, and increased energy intake compared with those on the standard-fat diet.<sup>26</sup> Similar results were obtained in this study.

The morphology of adipose tissue and liver of mice from different diet groups is depicted in Figure 2. Feeding mice with obesogenic HF diets resulted in increased fat mass with crown-like structures in epididymal adipose tissue (Figure 2A) and

**Table 3.** Food Intake, Energy Intake, and Weight of Epididymal Adipose Tissue and Liver<sup>a</sup>

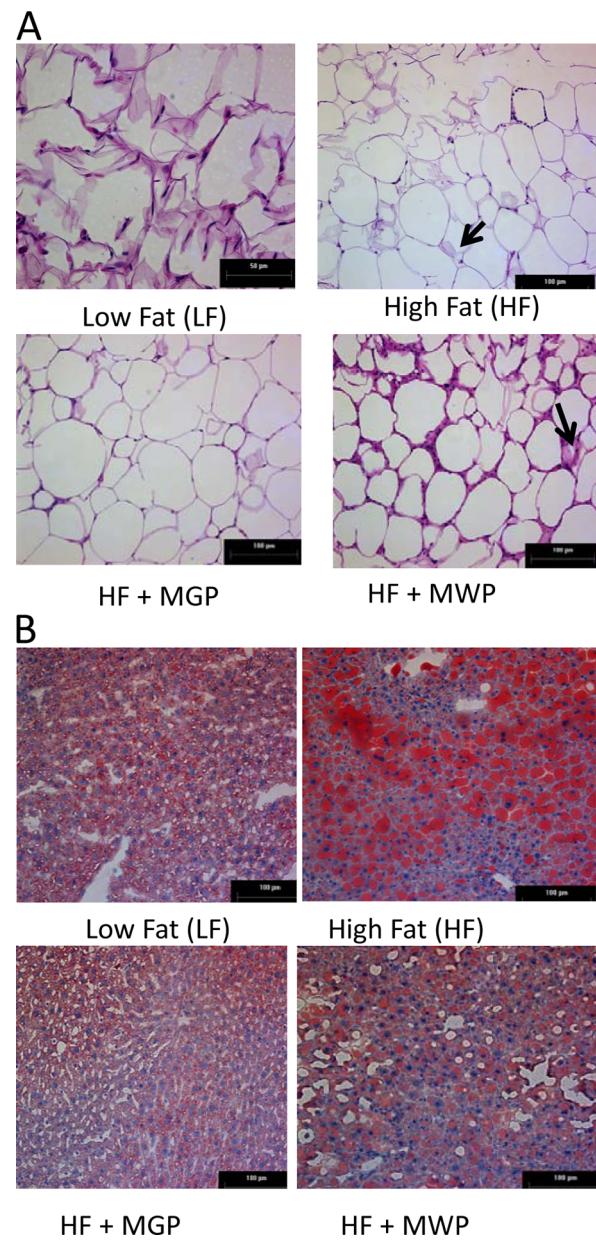
group	food intake (g/mouse/day)	energy intake (kcal/mouse/day)	adipose wt (g)	adipose % body wt	liver wt (g)	liver % body wt
LF	3.3 ± 0.1 a	12.8 ± 0.4 a	1.67 ± 0.03 c	3.98 ± 0.12 bc	1.16 ± 0.07 c	2.76 ± 0.18 b
HF	2.7 ± 0.1 b	14.4 ± 0.7 a	2.42 ± 0.13 a	4.90 ± 0.30 a	2.45 ± 0.08 a	4.93 ± 0.19 a
HF+MGP	2.7 ± 0.06 b	14.3 ± 0.3 a	1.70 ± 0.05 c	3.48 ± 0.13 c	1.40 ± 0.04 b	2.86 ± 0.09 b
HF+MWP	2.7 ± 0.1 b	14.1 ± 0.4 a	2.12 ± 0.05 b	4.16 ± 0.13 b	1.51 ± 0.05 b	2.96 ± 0.10 b

<sup>a</sup>Values are the mean ± SEM. HF, n = 8; other groups, n = 9. Columns not sharing a common letter are significantly different (*p* ≤ 0.05).



**Figure 1.** Cumulative body weights of CS7BL/6J mice (1–15 weeks): net body weight gains at weeks (A) 7 and (B) 15. HF, n = 8; other groups, n = 9.

increased lipids in hepatic tissues (Figure 2B). However, MGP supplementation altered adipose tissue morphology and reduced lipid accumulation in the liver. Epididymal adipose tissue sections by H&E staining show adipocytes from mice fed the different diets; although difference in adipocyte size may exist, this parameter was not measured. High-fat diets have shown to increase lipid accumulation in adipocytes. This causes an expansion of adipose tissue, initiating elevation in adipose expression of inflammatory genes such as TNF- $\alpha$  and monocyte chemoattractant protein-1 (MCP-1).<sup>27</sup> Inflamed and insulin-resistant adipocytes, characterized by low lipogenic and high lipolytic capacity, cause increased release of free fatty acids (FFA). FFA may activate Toll-like receptor 4 (TLR4), resulting in cytokine or chemokine secretion propagating inflammatory signals to the systemic level.<sup>28</sup> MGP and MWP may attenuate adipose inflammation by reducing adipose tissue macrophage (ATM) infiltration and suppressing TLR4 mediated pro-inflammatory signaling cascade, thereby lowering plasma FFA in mice fed HF diets.<sup>28</sup> Quercetin, one of the

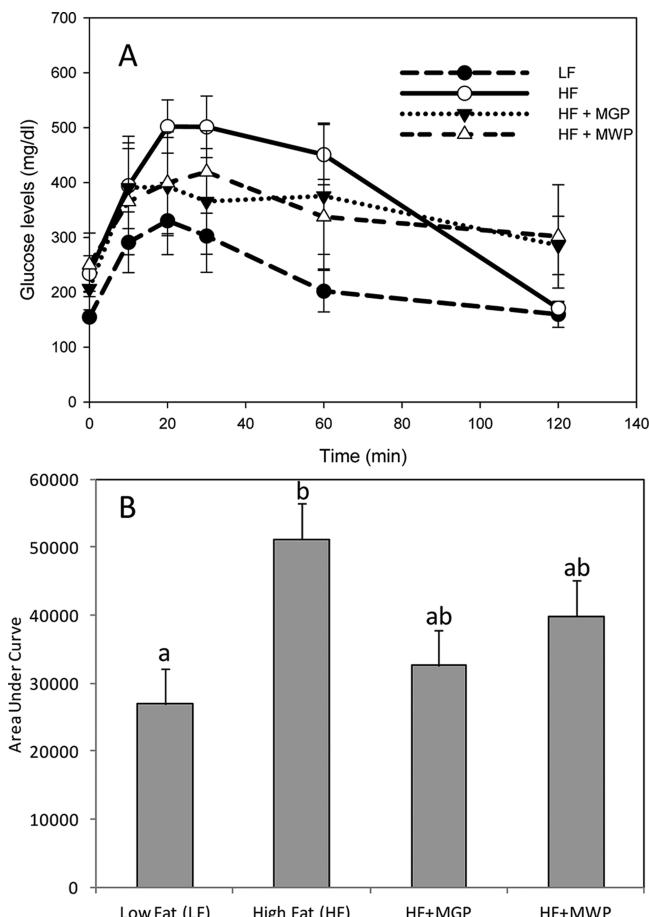


**Figure 2.** (A) Histology of the epididymal adipose tissue, stained with hematoxylin and eosin; (B) histology of liver tissue, stained with Oil-red-O. Arrows indicate crown-like structures in adipose tissue.

polyphenols available in grape powder extract, was found to be effective in mitigating macrophage-mediated inflammation in adipocytes.<sup>29</sup> Figure 2B shows grape or wine phytochemical effects on reducing hepatic lipidosis. Obesity accompanies liver lipid accumulation, which leads to hepatic steatosis and increased glucose production in liver. The livers of obese controls had numerous lipid droplets compared to lean counterparts, whereas MGP fed mice had lower lipids in

hepatic tissues as similarly seen in mice fed LF diets. These data are parallel with the liver tissue weights in mice fed HF and grape- or wine-supplemented diets. Grape or wine polyphenols may target hepatic defensive enzymes and adipogenic gene expression, as seen in mice fed grape extract.<sup>6</sup>

**Glucose Metabolism.** The time course for plasma glucose levels is shown in Figure 3A. A marginally delayed glucose



**Figure 3.** (A) Blood glucose levels in intraperitoneal glucose tolerance tests at week 12; (B) area under curve of blood glucose for 2 h after a glucose challenge ( $n = 5$ ).

clearance was noted in all mice (including the LF group). In contrast to this study, an earlier study showed that feeding mice strawberry powder supplemented in high-fat diets lowered nonfasting blood glucose levels but did not alter responses during intraperitoneal glucose challenge.<sup>30</sup> Strawberries or blueberries did not improve glucose tolerance in mice.<sup>31</sup> The incremental AUC of plasma glucose concentration during intraperitoneal glucose tolerance test is shown in Figure 3B. The AUC of glucose levels in LF group was 47.4% lower ( $p < 0.001$ ) compared to HF controls (Figure 3B). The AUC levels in the mice fed HF+MGP and HF+MWP were lower than that of HF group by 36.3 and 22.2%, respectively, but not statistically different from either LF- or HF-fed mice. At the end of the feeding study, fasting blood glucose and insulin levels were measured in all mice ( $n = 9$ ), and the homeostasis model assessment of basal insulin resistance (HOMA-IR) was calculated (Table 4). Lower HOMA-IR values indicate greater insulin sensitivity, whereas higher HOMA-IR values show lower insulin sensitivity or insulin resistance. MGP significantly

**Table 4. Effects of MGP and MWP on Glucose Homeostasis in C57BL/6J Mice<sup>a</sup>**

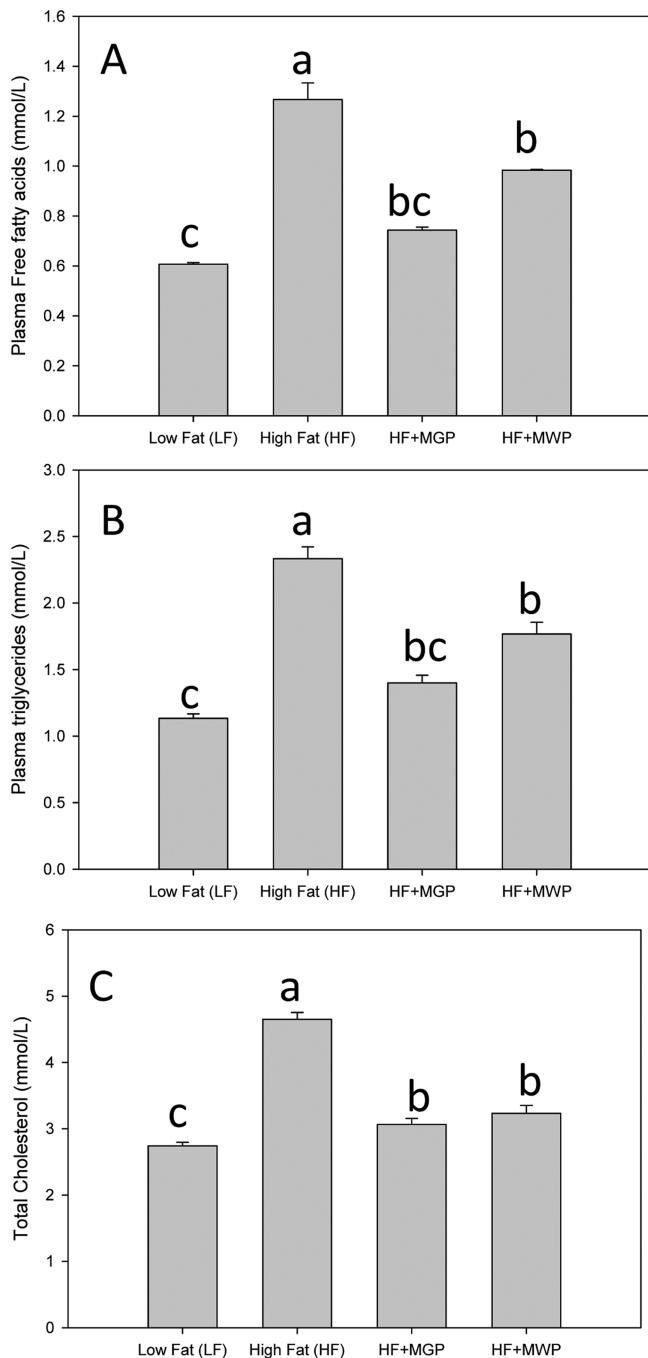
group	plasma glucose (mg/dL)	plasma insulin ( $\mu$ units/L)	HOMA-IR
LF	132.4 $\pm$ 0.4 d	10.8 $\pm$ 0.02 d	3.5 $\pm$ 0.02 d
HF	176.0 $\pm$ 0.3 a	27.9 $\pm$ 0.08 a	12.2 $\pm$ 0.02 a
HF+MGP	140.8 $\pm$ 0.4 c	15.0 $\pm$ 0.04 c	5.1 $\pm$ 0.02 c
HF+MWP	154.0 $\pm$ 0.5 b	18.1 $\pm$ 0.04 b	6.9 $\pm$ 0.01 b

<sup>a</sup>Values are the mean  $\pm$  SEM. HF,  $n = 8$ ; other groups,  $n = 9$ . Columns not sharing a common letter are significantly different ( $p \leq 0.05$ ).

reversed HF diet induced elevations in plasma glucose ( $-21.3\%$ ) and plasma insulin ( $-47.7\%$ ) levels compared to obese controls, whereas MWP reduced plasma glucose and insulin levels by 12.3 and 36.9%, respectively. Insulin resistance was found to be higher in HF obese controls at week 15. Compared to the HF group, insulin sensitivity was increased significantly by muscadine grape or wine supplementation as indicated by lower HOMA-IR. Our results were consistent with an early study that showed the antihyperglycemic effects of grape seed extracts.<sup>32</sup> Various mechanisms and pathways may be responsible for insulin-sensitizing effects by grape seed extract<sup>32</sup> and grape powder,<sup>33</sup> such as insulin-signaling pathway, decreased hepatic lipid accumulation, and/or FFA-induced oxidative stress.

**Muscadine Phytochemicals on Plasma Lipid Profile.** Dyslipidemia is associated with higher risk for cardiovascular disease, insulin resistance, and nonalcoholic fatty liver. The influence of HF diets supplemented with grape or wine phytochemicals on systemic markers of lipid metabolism were measured. When compared to LF control group, the serum free fatty acids (Figure 4A), triglycerides (Figure 4B), and total cholesterol (Figure 4C) were elevated by  $>2$ -fold in mice fed high-fat diets. There was a  $>40\%$  reduction in plasma free fatty acids, triglycerides, and total cholesterol in mice fed MGP in HF diets. MWP supplementation resulted in lower plasma lipid levels in mice, but no significant differences were noted between MGP and MWP feeding groups. Similar effects of resveratrol and grape seed proanthocyanidins on lipid homeostasis were reported earlier,<sup>21,34</sup> suggesting grape compounds regulate hepatic lipogenesis and adipose lipolysis simultaneously. Consumption of lyophilized grape powder over 4 weeks improved plasma lipid profiles and reduced oxidative stress in women.<sup>35</sup> According to Boer et al.,<sup>36</sup> triglycerides are synthesized in the liver and are then transported by very low-density lipoproteins to adipose tissues. Here, we noted that serum free fatty acids, total triglyceride, and total cholesterol levels were significantly higher in the obese control group compared to their lean counterparts. Muscadine polyphenol supplementation improved plasma lipid profile, which was correlated with lower adipose tissue and liver mass.

**Phytochemical Supplementation on Antioxidant Status and Inflammation.** Inflammation and oxidative stress are the junction points between increased adiposity, insulin resistance, and dyslipidemia. This complex of obesity-related metabolic abnormalities is known as metabolic syndrome.<sup>37</sup> The plasma C-reactive protein level is a measurable inflammatory biomarker in obesity conditions. We found that grape and wine phytochemical supplementation normalized this inflammatory biomarker protein levels with respect to LF-fed lean controls and increased the activity of glutathione peroxidase. The obese control group had a  $>2.5$ -fold increase



**Figure 4.** Plasma lipid levels of free fatty acids (A), total triglycerides (B), and total cholesterol (C) in C57BL/6J mice. HF,  $n = 8$ ; other groups,  $n = 9$ .

in plasma C-reactive protein levels, as compared to lean controls (Table 5). Mice fed muscadine grape or wine phytochemicals in high-fat diets over 15 weeks had 50 and 23% reductions in plasma C-reactive protein, respectively. Glutathione peroxidase is an antioxidative enzyme that is down-regulated in chronic disease conditions such as obesity and insulin resistance.<sup>38</sup> Dysregulation in redox homeostasis leads to adipocyte dysfunction and finally increased oxidative stress. Glutathione peroxidase activity was significantly lower in mice fed HF diets (Table 5). The MGP-supplemented group had a >4-fold increase in glutathione peroxidase activity, and MWP-fed animals showed 2-fold increased antioxidant enzyme levels

**Table 5. Effects of MGP and MWP on Plasma CRP and Glutathione Peroxidase Activity<sup>a</sup>**

group	plasma C-reactive protein (CRP) (ng/mL)	plasma glutathione peroxidase activity (nmol/min/mL)
LF	$23.1 \pm 0.02$ d	$1.1 \pm 0.02$ b
HF	$61.8 \pm 0.14$ a	$0.3 \pm 0.03$ d
HF +MGP	$30.1 \pm 0.03$ c	$1.2 \pm 0.02$ a
HF +MWP	$47.6 \pm 0.01$ b	$0.7 \pm 0.05$ c

<sup>a</sup>Values are the mean  $\pm$  SEM. HF,  $n = 8$ ; other groups,  $n = 9$ . Columns not sharing a common letter are significantly different ( $p \leq 0.05$ ).

in plasma. Dietary polyphenols have the capacity to increase the expression of anti-inflammatory and antioxidative genes via the activation of nuclear factor- $\kappa$ B and nuclear factor erythroid related factor-2.<sup>39</sup> An inverse correlation between C-reactive protein and glutathione peroxidase activity in HF-fed experimental groups suggests a beneficial role of muscadine grape or wine phytochemicals in mitigating oxidative stress and inflammation in obese animals.

A high-fat diet induces obesity in C57BL/6J mice and the associated metabolic syndrome characterized by hyperlipidemia, hyperglycemia, oxidative stress, and inflammation. Muscadine grape and wine phytochemicals were able to restore obesity-triggered pathological conditions in C57BL/6J mice. These effects are comparable to those of the extracts from *V. vinifera*.<sup>40</sup> Major phytochemicals in muscadine grapes were anthocyanins. Purified anthocyanins from purple potato, blueberries, and purple corn were reported to reduce the pathogenesis of obesity.<sup>41</sup> The benefits of anthocyanins were attributed to their capacity to activate the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway. Anthocyanins were also reported to down-regulate the levels of sterol regulatory element-binding protein 1 and its target genes including acetyl-coenzyme A carboxylase and fatty acid synthase.<sup>41</sup> MGP supplementation in diets appeared to be more effective in reducing animal body weights and lipid accumulation than MWP. Such differences were likely due to differences in phytochemical compositions. MGP contained a higher amount of ellagic acid than MWP. An ellagittannin-rich extract from pomegranate leaf was effective in preventing obesity in mice.<sup>42</sup> Ellagic acid suppressed resistin secretion by reducing its intracellular protein levels.<sup>43</sup> The synergistic effect between anthocyanins and ellagic acid may be possible by affecting targets in different pathways. Resveratrol in *V. vinifera* was shown to prevent obesity.<sup>21</sup> However, resveratrol was not likely to be a major bioactive compound in muscadine grapes, because its level was extremely low.<sup>9,44</sup> Resveratrol was below the detection limit in the extracts used in this study. Clearly, relative levels of ellagic acid, resveratrol, and other phenolics in any specific extract derived from specific fruits will affect the specific metabolic impacts that extract provides.

In summary, our findings support the promising role of muscadine grape and wine phytochemicals as dietary means in obesity management. Additional studies are needed to determine the effective dose levels of muscadine grape or wine phytochemicals required to affect relevant biomarker of chronic disease.

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### Funding

This research is supported in part by a grant from the Florida Department Agriculture and Consumer Service, viticulture research program.

### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS USED

cGPx, cytosolic glutathione peroxidase; HF, high fat; HOMA-IR, homeostatic model assessment—insulin resistance; LF, low fat; MGP, muscadine grape phytochemicals; MWP, muscadine wine phytochemicals.

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