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Differential Effects of Grape Powder and Its Extract on Glucose Tolerance and Chronic Inflammation in High-Fat-Fed Obese Mice

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ABSTRACT: The objective of this study was to determine the anti-inflammatory properties of grape powder (GP) or GP extract (GE) and examine (1) which polyphenol metabolites in GE were bioavailable, (2) the impact of GP and GE on glucose tolerance and inflammation in obese mice, and (3) if bioavailable polyphenols in GE decrease markers of inflammation in primary adipocytes. In experiment 1, C57BL/6J mice were gavaged with GE and serum polyphenols were measured. In experiment 2, mice were fed high-fat diets supplemented with 3% GP or 0.02% GE for 18 weeks and markers of inflammation were measured. In experiment 3, human adipocytes were treated with the bioavailable polyphenols quercetin 3-*O*-glucoside (Q3G) or quercetin 3-*O*-glucuronide (Q3GN) and markers of inflammation were measured. Serum Q3G and Q3GN increased at 1 h post-GE gavage and decreased thereafter. GP supplementation improved glucose tolerance at 5 weeks and decreased markers of inflammation ~20−50% in serum and adipose tissue at 18 weeks. Q3G, but not Q3GN, attenuated TNFα-mediated inflammatory gene expression ~30−40% in human adipocytes, possibly by suppressing c-Jun-NH_2 terminal kinase and c-Jun activation. In summary, (1) Q3G and Q3GN are bioavailable polyphenols in GE, (2) GP acutely improves glucose tolerance and chronically reduces markers of inflammation in obese mice, and (3) Q3G reduces several markers of inflammation in human adipocytes.

KEYWORDS: *grape powder, grape powder extract, glucose tolerance, inflammation, quercetin*

INTRODUCTION

Obesity is the most prevalent nutrition-related health problem worldwide, with 1.5 billion adults and 43 million children under the age of five classified as overweight and more than 500 million people classified as obese.[1] Obesity is caused by a positive energy balance mainly due to overconsumption of calories relative to energy expenditure that leads to the expansion of white adipose tissue (WAT) mass. Enlarged WAT is associated with a progressive infiltration and accumulation of macrophages that contribute to a chronic, low-grade inflammation.[2] Moreover, several studies have demonstrated that increased pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα)[3] or interleukin-6 (IL-6)[4] and chemokines such as monocyte chemoattractant protein-1 (MCP-1)[5] play an important role in the development of obesity-mediated insulin resistance and type 2 diabetes. For example, as WAT expands adipocytes begin to secrete TNFα, which stimulates (pre)adipocytes to produce MCP-1. MCP-1, in turn, recruits monocytes to WAT and induces their differentiation into activated macrophages, leading to chronic, low-grade inflammation and insulin resistance.[6]

Two strategies to suppress WAT expansion and obesity-mediated inflammation and insulin resistance are reduced caloric consumption and increased physical activity.[7] However, long-term lifestyle changes such as caloric restriction and exercise are usually poorly maintained. Also, pharmacological and surgical interventions to treat obesity and metabolic diseases, although effective, have considerable side effects and financial costs.[8] Therefore, alternative strategies for suppressing obesity-related conditions such as inflammation and insulin resistance are needed. One potential dietary strategy is consuming grapes or grape products (e.g., wine, grape juice, powder, extract, raisins), which are rich in phenolic compounds that possess antioxidant and anti-inflammatory properties. Indeed, human, rodent, and cell studies[9,10] support anti-inflammatory and antidiabetic effects of grape products including grape powder (GP) or grape powder extract (GE). However, the bioactive compounds in grape products are unclear, and the mechanisms by which these products prevent inflammation or insulin resistance are debatable.

Recently, our group reported that GE isolated from GP (lyophilized red, green, and blue-purple seeded and seedless California grapes) rich in quercetin obtained from the the California Table Grape Commission attenuated inflammation in human macrophages and adipocytes and insulin resistance in primary human adipocytes.[11,12] We also showed that quercetin

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aglycone repressed inflammation in human macrophages and adipocytes and insulin resistance in human adipocytes.[13,14] However, it is not known whether these in vitro effects could be reproduced in vivo. Also, GE was made by removing carbohydrates (e.g., sugars and fibers) from GP. This process of extraction might be responsible for differences in polyphenol content between GE and GP. Finally, the polyphenol metabolite profile in circulation following the consumption of our GE or GP was unknown. Therefore, the objective of this study was to determine (1) which polyphenols in GE were absorbed in mice, (2) the effect of two sources of grape polyphenols (i.e., GE and GP) on inflammation and insulin resistance in a diet-induced, obese mouse model, and (3) the extent to which bioavailable grape polyphenols suppressed markers of inflammation in primary human adipocytes.

In this study, we demonstrate that table grape powder acutely improves glucose tolerance and chronically decreases markers of inflammation in high-fat-fed mice and that quercetin 3-*O*-glucoside is a bioavailable polyphenol in grape powder that may contribute to grape powder's anti-inflammatory properties.

MATERIALS AND METHODS

Chemicals and Reagents. Lyophilized GP, obtained from red, green, and blue-purple seeded and seedless California grapes, was provided in aluminum bags by the California Table Grape Commission (Fresno, CA, USA). Polyphenol standards were obtained from Polyphenol Laboratories (Sandnes, Norway), Sigma-Aldrich (St. Louis, MO, USA), Caymen Chemical (Ann Arbor, MI, USA), or Extrasynthese (Genay, France). Carboxymethylcellulose sodium salt (CMC), ascorbic acid, glucose, isobutylmethylxanthine, quercetin 3-*O*-glucoside (Q3G), and quercetin 3-*O*-glucuronide (Q3GN) were purchased from Sigma-Aldrich. Aqueous formic acid was purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Apigenin was purchased from Chromadex (Irvine, CA, USA). TNFα and mouse MCP-1 enzyme-linked, immunosorbent assay (ELISA) kits were purchased from R&D Systems (Minneapolis, MN, USA). Mouse insulin ELISA kits were purchased from Crystal Chemicals (Downers Grove, IL, USA). Mouse Bio-Plex magnetic bead-multiplex immunoassays for serum TNFα and IL-6 were purchased from Bio-Rad (Hercules, CA, USA). RNeasy Plus Universal Kits, RNeasy Lipid Tissue Kits, RNase-Free DNase Sets, and RNeasy Mini Kits were purchased from Qiagen (Valencia, CA, USA). Agilent RNA 6000 Nano Kit was purchased from Agilent (Santa Clara, CA, USA). High-capacity cDNA Archive Kits and Taqman Gene Expression Assays were purchased from Applied Biosystems (Foster City, CA, USA). Adipocyte medium (AM-1) was purchased from Zen Bio Inc. (Research Triangle Park, NC, USA). Collagenase was purchased from Worthington (Lakewood, NJ, USA). Thiazolidinedione rosiglitazone (BRL 49653) was a gift from Dr. Per Sauerberg (Novo Nordisk A/S, Copenhagen, Denmark).

Preparation of GP and GE. The GP was extracted to remove the sugars (90 wt %/wt) in the laboratory of Dr. Wei Jia at the University of North Carolina at Greensboro (UNCG) Center for Translational Biomedical Research in North Carolina Research Campus, Kannapolis, NC, USA. To remove the sugars, a Diaion HP-20 anion resin column was used to elute the extract from the column using methanol, and then it was lyophilized to make the final GE.[15] Then, the polyphenol compositions of GP and GE (Table 1) were analyzed using an Agilent liquid chromatography time-of-flight mass spectrometry (HPLC-TOFMS; Agilent) as previously described.[11]

Experiment 1: Analysis of Serum Polyphenols after Gavaged GE by UPLC-MS/MS. Male, 12-week-old C57BL/6J mice (n = 32), weighing approximately 30 g, were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and housed individually in a 12 h light/12 h dark cycle, temperature-controlled room. Ethical treatment of animals was assured by the UNCG Institutional Animal Care and Use Committee (IACUC). Mice were acclimated to a low-fat purified diet (D12450B from Research Diets, New Brunswick, NJ, USA) for 10 days containing casein as the protein source devoid of phytoestrogens or other phytochemicals. Subsequently, mice were deprived of food for 6 h, and then 8 mice were gavaged with water only (1% CMC) for the 0 h time point or 24 mice were gavaged with GE (1 g/kg body weight, dissolved in a 1% CMC water solution; 1 g GE per 10 mL). Mice were then anesthetized by isoflurane inhalation at 0 (water control), 1, 3, or 8 h post-GE gavage. Blood was collected by orbital sinus puncture into a Vacutainer tube (BD Vacutainer Systems, Franklin Lakes, NJ, USA). Serum was obtained by centrifugation at 1100*g* for 10 min at 4 °C and immediately stored at −80 °C prior to polyphenol profile analysis.

Table 1. Polyphenols in Grape Powder Extract (GE) and Grape Powder (GP)[a]

polyphenol	GE[b] (mg/kg)	GP[b] (mg/kg)
catechin	403.0 ± 24.9	8.1 ± 0.2
epigallocatechin	89.7 ± 4.7	
catechin gallate	102.5 ± 14.1	1.5 ± 0.1
oleanolic acid	1118.2 ± 20.4	
kaempferol	46.0 ± 2.8	
epicatechin	136.4 ± 5.0	4.1 ± 0.1
epicatechin gallate	230.9 ± 13.0	2.1 ± 0.1
kaempferol 3-*O*-glucoside	2536.6 ± 22.8	8.6 ± 0.3
rutin	2066.9 ± 28.8	8.2 ± 0.3
quercetin 3-*O*-glucoside	15465.5 ± 213.6	49.3 ± 0.3
trans-resveratrol	555.3 ± 152.1	
quercetin	N/A[c]	0.2 ± 0.01
procyanidin B2	5885.4 ± 102.0	58.4 ± 2.7
delphinidin 3-*O*-glucoside	1995.2 ± 34.9	32.7 ± 0.5
cyanidin 3-*O*-glucoside	2541.7 ± 98.5	27.8 ± 0.3
petunidin 3-*O*-glucoside	4555.1 ± 24.6	54.5 ± 1.7
peonidin 3-*O*-glucoside	33804.0 ± 110.1	226.9 ± 0.7
malvidin 3-*O*-glucoside	30601.3 ± 1182.6	207.3 ± 0.1
gallic acid		7.9 ± 0.4
total	100324.5	689.5

[a]1 kg of GP yielded 4 g of GE = 0.4% of initial weight. [b]Data are the mean ± SEM. [c]Quercetin in GE can be detected but was under the limit of detection.

Serum polyphenol levels were measured by a UPLC-MS/MS system (Acquity UPLC-Quattro Premier XE MS, Waters Corp., Milford, MA, USA) as described using a modification of the method of Day et al.[16] Briefly, each serum sample (200 μL) was acidified to pH 3 with 20% aqueous formic acid (10 μL). Then, 2 g/L ascorbic acid (20 μL) was added to prevent oxidation during sample preparation and 60 mmol/L apigenin (10 μL) was added to the serum as internal standard (I.S.). Acetonitrile (600 μL) was used to precipitate plasma proteins and extract flavonol metabolites. The samples were vortexed (VWR digital vortex mixer, VWR International, West Chester, PA, USA) for 30 s every 2 min over a 10 min period. The mixture was centrifuged at 13500 rpm for 20 min at 4.0 ± 0.5 °C (Microfuge 22R centrifuge, Beckman Coulter, Inc., Atlanta, GA, USA). The supernatant was collected and the pellet was re-extracted as described above but with methanol instead of acetonitrile. The acetonitrile and methanol supernatants were combined and reduced to dryness in a CentriVap Vacuum Concentrator (Labconco, Kansas City, MO, USA). Extracts were dissolved in methanol (100 μL) plus 1% formic acid (100 μL) and then centrifuged at 13500 rpm for 20 min (Allegra X-15R, Beckman Coulter, Inc.). The supernatant was filtered through a 13 mm syringe filter with a 0.2 μm PTFE membrane (Millipore Corp., Billerica, MA, USA) before LC-MS analysis.

A total of 69 standards were used. Each standard was individually dissolved in methanol or water and prepared as a stock solution at a concentration of 1 g/L. Each aliquot of standard stock solution was mixed to obtain a mixed stock solution. The resulting mixed solution was diluted at a series of concentration of 5.12, 2.56, 1.28, 0.64, 0.32, 0.16, 0.08, 0.04, 0.02, 0.01, or 0.005 mg/L. Apigenin (I.S.) was added to

Table 2. Compositions of Low-Fat (LF), High-Fat (HF), HF plus Grape Powder Extract (HFGE), HF with Modified Sugar Content (HS), and HF plus Grape Powder (HFGP) Diets

	LF		HF		HFGE		HS		HFGP	
ingredient	g/kg	kJ/kg	g/kg	kJ/kg	g/kg	kJ/kg	g/kg	kJ/kg	g/kg	kJ/kg
casein	200	3360	200	3360	200	3360	200	3360	200	3360
L-cystine	3	50.4	3	50.4	3	50.4	3	50.4	3	50.4
corn starch	506.2	8505	0	0	0	0	0	0	0	0
maltodextrin 10	125	2100	125	2100	125	2100	125	2100	125	2100
sucrose	68.8	1155	68.8	1155	68.8	1155	45.5	764.4	45.5	764.4
dextrose	0	0	0	0	0	0	11.65	195.7	0	0
fructose	0	0	0	0	0	0	11.65	195.7	0	0
cellulose, BW200	50	0	50	0	50	0	50	0	50	0
soybean oil	25	945	25	945	25	945	25	945	25	945
lard	20	756	245	9261	245	9261	245	9261	245	9261
mineral mixture	10	67	10	67	10	67	10	67	10	67
dicalcium phosphate	13	0	13	0	13	0	13	0	13	0
calcium carbonate	5.5	0	5.5	0	5.5	0	5.5	0	5.5	0
potassium citrate, $1{\cdot}H_2O$	16.5	0	16.5	0	16.5	0	16.5	0	16.5	0
vitamin mixture	10	164	10	164	10	164	10	164	10	164
choline bitartrate	2	0	2	0	2	0	2	0	2	0
GE	0	0	0	0	0.15	0	0	0	0	0
GP	0	0	0	0	0	0	0	0	23	386.4
FD&C yellow dye #5	0.025	0	0	0	0	0	0.025	0	0	0
FD&C red dye #40	0.025	0	0	0	0.025	0	0	0	0.05	0
FD&C blue dye #1	0	0	0.05	0	0.025	0	0.025	0	0	0
total	1055.05	17102.4	773.85	17102.4	774	17102.4	773.85	17103.2	773.55	17098.2
GE, %	0		0		0.02		0		0	
GP, %	0		0		0		0		3	

the diluted solutions, and the final concentration for I.S. was 150 μg/L. The calibration curve and the corresponding regression coefficients were obtained by I.S. adjustment. Quantification of polyphenols was performed by UPLC-MS/MS using a multiple reaction monitoring (MRM) mode. The concentration of each identified polyphenol was automatically calculated using the corresponding calibration curve in the MassLynx application manager, QuanLynx (Waters Corp., Milford, MA, USA).

Experiment 2: Supplementation of GP and GE in High-Fat-Fed Obese Mice. Male, 5-week-old C57BL/6J mice (n = 50) were obtained from Jackson Laboratories and housed as described above. Ethical treatment of animals was assured by the UNCG IACUC. Mice were randomly assigned to one of five dietary treatments (n = 10 mice per treatment; Table 2) for 18 weeks. Diets (Research Diets) were as follows: (1) a low-fat diet (LF) containing 10% of energy from fat, (2) a high-fat control diet (HF) containing 60% of energy from fat, (3) a HF diet supplemented with 0.02% (wt/wt) GE (HFGE), (4) another high-fat control diet with a modified sugar content (HS) to control for the amount and type of sugar in GP, and (5) a high-fat diet supplemented with 3% (wt/wt) GP (HFGP). The sugar composition in GP was ∼90% and consisted of dextrose and fructose at 50:50 ratio (wt/wt).[15] Energy from sugars including sucrose, dextrose, and fructose was adjusted in the LF, HF, and HS diets to be equivalent to energy from sucrose, dextrose, and fructose in the HFGE and HFGP. The amount of 0.02% GE in the HFGE was based on the amount of total polyphenols in 3% HFGP. Diets were packed under inert gas in individual 2.5 kg foil bags and stored at −20 °C until use. Fresh diet was provided twice per week to minimize oxidization. Mice had ad libitum access to both food and water. Food intake and body weight were measured weekly.

Intraperitoneal Glucose Tolerance Tests (GTT). GTT was performed at baseline, 5, 10, and 15 weeks on nonanesthetized mice. Mice were deprived of food for 8 h and given an intraperitoneal glucose injection at a dose of 1 g/kg body weight. Blood was obtained from the tail vein, and glucose levels were determined at 0, 5, 15, 30, 60, and 120 min following glucose administration using a Contour blood glucose monitoring system (Bayer Diabetes Care, Tarrytown, NY, USA). Total GTT area under the curve (AUC) was calculated as described.[17]

Analysis of Serum Insulin and Inflammatory Cytokine and Chemokine Levels. Fasting serum insulin levels were determined at 12 weeks using ELISA kits according to the manufacturer's instructions. Serum TNFα and IL-6 levels were determined at 18 weeks using the Bio-Plex magnetic bead-multiplex immunoassay on the Bio-Plex 200 system according to the manufacturer's instructions. Serum MCP-1 levels were determined at 18 weeks using ELISA kits according to the manufacturer's instructions.

Tissue RNA Analysis and Real-Time Quantitative PCR (qPCR). Skeletal muscle or WAT was harvested at 18 weeks, and total RNA was extracted using RNeasy Plus Universal Kits or RNeasy Lipid Tissue Kits combined with RNase-Free DNase Sets, respectively. RNA integrity was assessed using an Agilent RNA 6000 Nano Kit on an Agilent 2100 Bioanalyzer (Agilent). For real-time qPCR, 2 μg of total RNA from mouse tissues was converted into first-strand cDNA by using a high-capacity cDNA Archive Kit. The qPCR was performed in a 7500 FAST Real Time PCR System (Applied Biosystems) by using Taqman Gene Expression Assays. Fold differences in gene expression were calculated as $2^{-\Delta\Delta Ct}$ using the endogenous reference gene TATA-binding protein (TBP).

Experiment 3: Primary Human Adipocytes Treated with Q3G or Q3GN. Abdominal WAT was obtained from nondiabetic and nonobese Caucasian and African-American women [age, 20−50 years; body mass index (BMI in kg/m^2) < 32.0] during abdominoplasty. Approval was obtained from the Institutional Review Board at the UNCG and the Moses Cone Memorial Hospital in Greensboro, NC, USA. Tissue was digested by using collagenase, and stromal vascular (SV) cells were isolated, proliferated, and induced to differentiate in AM-1 plus isobutylmethylxanthine (250 μmol/L) and thiazolidinedione rosiglitazone (1 μmol/L; BRL 49653) for 3 days. Cultures were then grown in AM-1 only for 4 days. Cultures containing ∼50% preadipocytes and ∼50% adipocytes, based on visual observations,

were treated at day 7 of differentiation. Each experiment was repeated at least three more times using a mixture of cells from three subjects.

Cell RNA Analysis and qPCR. Primary human SV cells were seeded in 35 mm dishes at 0.4×10^6 per dish and differentiated for 7 days. On day 7, human adipocyte cultures were pretreated with DMSO vehicle (0) or 1.5 nmol/L or 3, 10, or 30 μmol/L of Q3G or Q3GN for 1 h and subsequently treated with or without 20 ng/L of TNFα for 3 h. Total RNA from primary human adipocytes was extracted using RNeasy Mini Kits. RNA integrity was estimated as described above for tissue RNA. For real-time qPCR, 1 μg total RNA was converted into first-strand cDNA by using a high-capacity cDNA Archive Kit, and qPCR was conducted as described above for tissue RNA. Fold differences in gene expression were calculated as $2^{-\Delta\Delta Ct}$ using the endogenous reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Immunoblotting. Primary human SV cells were seeded in 35 mm dishes at 0.4×10^6 per dish and differentiated for 7 days. On day 7, primary human cultures were pretreated with DMSO vehicle (0) or 1.5 nmol/L or 10, 30, or 60 μmol/L of Q3G for 1 h and then treated with or without 20 ng/L of TNFα for 1 h. Immunoblotting was conducted as previously described.[18]

Statistics. Analyses for significant differences were conducted using one-way ANOVA. Student's t test were used to compute individual pairwise comparisons of least-squares means ($P < 0.05$). Data are expressed as the mean ± standard error of the mean (SEM). All analyses were performed using JMP version 8.0 software (SAS Institute, Cary, NC, USA).

RESULTS

Several Quercetin Metabolites Rapidly Appear in the Serum of Mice Gavaged with GE (Experiment 1). To determine the bioavailability of grape polyphenols, mice were gavaged with 1 g of GE/kg body weight based on work by Tsang et al.[19] GE traveled quickly through the gastrointestinal tract, as evidenced by visual examination of the movement of purple digesta through the gastrointestinal tract from 1 to 8 h and the appearance of entirely purple feces at 8 h (Figure 1A). Q3G (Figure 1B) was increased ($P < 0.0006$) at 1 and 3 h, and Q3GN (Figure 1C) was increased ($P < 0.0005$) at 1 h compared to baseline in circulation post-GE gavage. There was a trend ($P = 0.07$) for rutin (Figure 1D), another metabolite of quercetin, to be elevated in circulation at 1 h post-GE gavage.

GP Supplementation of a High-Fat Diet Improves Glucose Tolerance Acutely and Decreases Markers of Inflammation Chronically (Experiment 2). To determine the extent to which two sources of grape polyphenols (GP, GE) prevented insulin resistance and chronic inflammation associated with diet-induced obesity, mice were fed high-fat diets containing 3% GP (HFGP) or 0.02% GE (HFGE) for 18 weeks. The 3% GP diet was chosen on the basis of work by Seymour et al.[20,21] demonstrating anti-inflammatory and cardioprotective effects of 3% GP treatment for 18 weeks in a Dahl salt-sensitive rat model of hypertension. This level of GP is equivalent to nine daily human servings of fresh grapes, assuming 1 serving = 3/4 cup grapes weighing 126 g = 23 g of dried grape powder, and that a 30 g mouse eats ~3 g/d of food. The 0.02% GE diet contained the same amount of total polyphenols as 3% GP diet. A high-fat control diet (HF) contained the same amount of fat and sucrose as the HFGE diet, and the second high-fat control diet, HS, contained the same amount of fat and sugars (i.e., 50:50 ratio of dextrose/fructose) as the HFGP diet (Table 2). A low-fat diet (LF) was fed as a control for the HF, HFGE, HS, and HFGP diets (Table 2).

High-fat feeding impaired glucose tolerance at 5, 10, and 15 weeks (Figure 2A–C), decreased mRNA markers of glucose disposal rates in muscle and WAT at 18 weeks (Figure 2D,E),

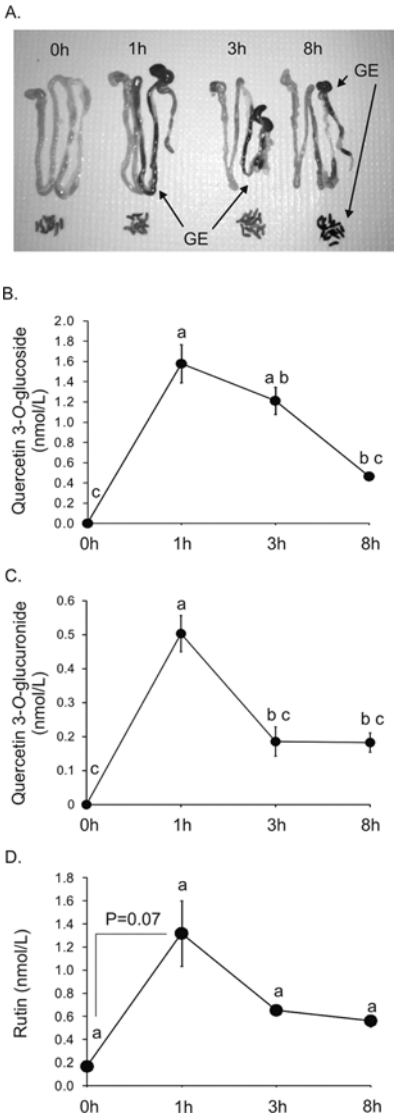

Figure 1. Several quercetin metabolites were detected in serum of mice following oral administration of grape powder extract (GE). Male C57BL/6J mice were gavaged with the water vehicle (controls, $n = 8$) or 1 g/kg body weight of GE ($n = 24$). Immediately, blood samples were collected from vehicle control mice (0 h). Then, blood samples were collected from mice that received the GE bolus at 1, 3, or 8 h postgavage. The movement and excretion of GE were indicated in purple/dark color by an arrow in the gastrointestinal tract and feces (A). The concentrations (nmol/L) of quercetin 3-*O*-glucoside (B), quercetin 3-*O*-glucuronide (C), and rutin (D) in mouse serum were measured using a UPLC-MS/MS system. Data were analyzed using one-way ANOVA and Student's t tests. Means ± SEM ($n = 8$) without a common letter differ, $P < 0.05$.

and raised fasting glucose and insulin levels at 12 weeks (Table 3) compared to the LF feeding. Notably, the HFGP group had improved GTT at 30 and 60 min and a lower AUC for the GTT ($P < 0.002$) compared to their HS control group at 5 weeks, suggesting a better glucose tolerance (Figure 2A). However, these beneficial effects of GP were not detected at 10 weeks (Figure 2B) or 15 weeks (Figure 2C), compared to their HS controls. Furthermore, fasting glucose and insulin levels (Table 3) at 12 weeks and the mRNA markers of glucose disposal rates in muscle (Figure 2D) and WAT (Figure 2E) at 18 weeks were not affected by feeding GP. Surprisingly, HFGE had no effect on

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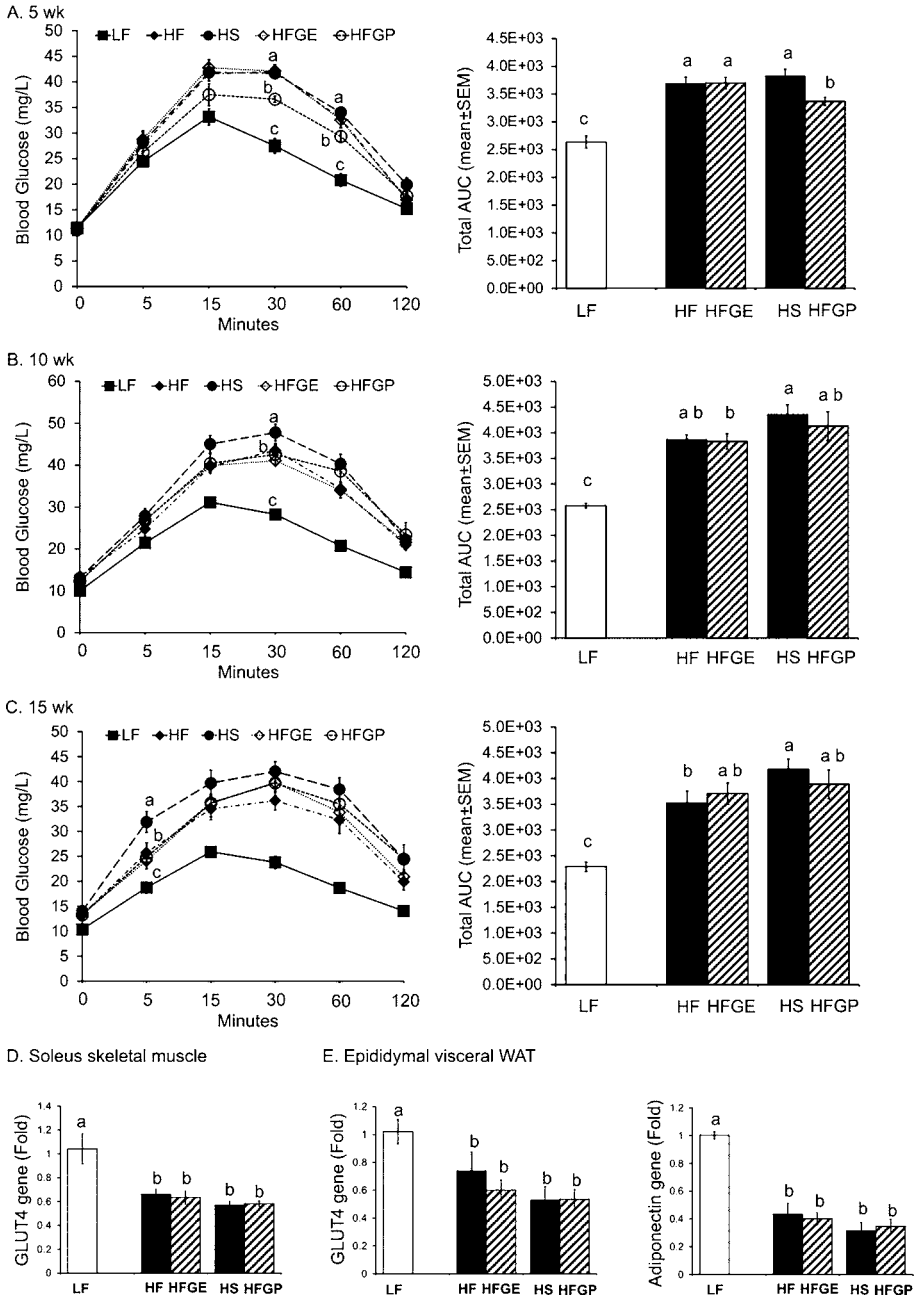

Figure 2. High-fat-fed obese mice supplemented with grape powder (GP), but not grape powder extract (GE), had better glucose tolerance acutely, but not chronically. Male C57BL/6J mice were fed for 18 weeks a low-fat (LF) diet, a high-fat (HF) diet, a high-fat diet plus GE (HFGE) diet, a high-fat diet with a modified sugar content (HS) similar to the HFGP group, or a high-fat diet plus GP (HFGP) diet. Intraperitoneal glucose tolerance tests (GTT) were performed, and total GTT area under the curve (AUC) was calculated at 5 weeks (A), 10 weeks (B), and 15 weeks (C). The mRNA levels of insulin-dependent glucose transporter 4 (GLUT4) in mouse soleus skeletal muscle at 18 weeks (D) was measured using qPCR. The mRNA level of GLUT4 and adiponectin in mouse epididymal WAT at 18 weeks (E) was measured using qPCR. Data were analyzed using one-way ANOVA and Student's *t* tests. Means ± SEM ($n = 7–9$) without a common letter differ, $P < 0.05$. Black squares or clear bars = LF; black diamonds or black bars = HF; clear diamonds or striped bars = HFGE; black circles or black bars = HS; clear circles or slashed bars = HFGP.

GTT at any time point (Figure 2A–C) or on mRNA markers of glucose disposal rates at 18 weeks (Figure 2D,E) as well as fasting glucose and insulin levels at 12 weeks (Table 3) compared to their HF control group.

Given the important role of macrophage recruitment and accumulation in WAT during the development of obesity-associated inflammation and metabolic diseases,[22] we sought to determine the extent to which grape products attenuated markers of macrophage recruitment/accumulation and inflammatory cytokine and chemokine levels in circulation and mRNA levels in WAT. We isolated four WAT depots including epididymal (bilateral intra-abdominal visceral depots attached to the epididymis), inguinal (bilateral superficial subcutaneous WAT depots between the skin and muscle fascia just anterior to the lower segment of the hind limbs), mesenteric (a glue-like visceral net located in the mesenterium of the intestines), and retroperitoneal (bilateral visceral depots in the abdominal cavity behind the peritoneum on the dorsal side of the kidneys) WAT. We found that the wet weights of the four WAT depots and liver at 18 weeks were lower in the LF group compared to all four

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Table 3. Effect of Grape Powder Extract (GE) or Grape Powder (GP) on Mice Fed a High-Fat (HF) or HF with Modified Sugar Content (HS) Diet, Respectively, Compared to Low-Fat (LF) Controls[a]

	LF		HF		HFGE		HS		HFGP	
	12 weeks	18 weeks	12 weeks	18 weeks	12 weeks	18 weeks	12 weeks	18 weeks	12 weeks	18 weeks
total body wt gain (g)		9.6 ± 1.0 b		22.5 ± 1.4 c		22.8 ± 0.7 c		24.0 ± 0.8 c		23.2 ± 0.9 c
total food intake (kJ)		6786.8 ± 376.1 b		5531.1 ± 328.9 c		5392.5 ± 457.3 c		5407.2 ± 354.1 c		5681.8 ± 450.2 c
adipose tissue wt (g)										
epididymal		1.1 ± 0.1 b		2.8 ± 0.2 c		2.8 ± 0.2 c		2.7 ± 0.2 c		2.6 ± 0.2 c
inguinal		0.5 ± 0.1 b		1.6 ± 0.2 c		1.9 ± 0.1 c		2.2 ± 0.1 c		2.1 ± 0.2 c
mesenteric		0.3 ± 0.0 b		1.0 ± 0.1 c		0.9 ± 0.1 c		1.2 ± 0.1 c		1.2 ± 0.1 c
retroperitoneal		0.4 ± 0.1 b		1.1 ± 0.1 c		1.0 ± 0.0 c		1.2 ± 0.0 c		1.3 ± 0.1 c
total fat depot wt (g)		2.3 ± 0.3 b		6.4 ± 0.4 c		6.6 ± 0.2 c		7.2 ± 0.4 c		7.2 ± 0.3 c
liver wt (g)		1.1 ± 0.1 b		1.7 ± 0.2 c		1.5 ± 0.1 c		1.9 ± 0.2 c		1.9 ± 0.2 c
fasting glucose (mg/L)	11.0 ± 0.3 b		13.0 ± 1.0 c		13.3 ± 0.6 c		14.8 ± 0.5 c		13.8 ± 0.6 c	
fasting insulin (μg/L)	0.8 ± 0.1 b		1.7 ± 0.3 c		1.6 ± 0.3 c		2.1 ± 0.4 c		2.2 ± 0.4 c	

[a]Data are the mean ± SEM. $P < 0.05$ when data (n = 7–9) in a row without a common letter differ.

high-fat groups (Table 3); however, there were no significant differences between the HF control and HFGE groups ($P > 0.07$) or the HS control and HFGP groups ($P > 0.1$) at 18 wk (Table 3). Thus, feeding GP or GE to high fat-fed mice did not reduce WAT depot or liver weights.

Although GP and GE did not prevent high fat-induced adiposity, we found that the HFGP group had lower serum levels of TNFα ($P = 0.001$) and MCP-1 ($P = 0.03$) compared to the HS controls (Figure 3A). However, there were no differences in serum TNFα ($P = 0.09$) and MCP-1 ($P = 0.2$) levels in the HFGE group compared to HF group at 18 weeks (Figure 3A). Moreover, there were no differences in circulating IL-6 levels in the HFGP group compared to the HS control group or HFGE group compared to HF control group at 18 weeks (data not shown). Consistent with serum levels, the HFGP group had lower mRNA levels of TNFα ($P = 0.05$), MCP-1 ($P = 0.002$), and two markers of macrophage recruitment and accumulation, CD11c ($P = 0.03$) and F4/80 ($P = 0.05$), in epididymal WAT compared to the HS control group (Figure 3B). In contrast, there were no differences in the expression of these markers in epididymal WAT in the HFGE group compared to the HF control group at 18 weeks (Figure 3B). Finally, the HFGP group had lower mRNA levels of IL-6 ($P = 0.03$), MCP-1 ($P = 0.02$), interferon γ-induced protein 10 (IP-10; $P = 0.02$), and F4/80 ($P = 0.003$) in inguinal WAT compared to the HS controls (Figure 3C). Again, there were no significant reductions in any of these markers in inguinal WAT in the HFGE group compared to the HF controls (Figure 3C).

Q3G, but Not Q3GN, Attenuates Several Inflammatory Markers in Primary Human Adipocytes Treated with TNFα (Experiment 3). To investigate the potential mechanism by which bioavailable grape polyphenols attenuate inflammation, we pretreated primary cultures of newly differentiated human adipocytes with Q3G or Q3GN followed by acute treatment with TNFα, an inflammatory cytokine elevated in the circulation and WAT of obese individuals[3] and in obese mice in this study. Cultures were treated with 1.5 nmol/L or 3, 10, or 30 μmol/L Q3G for 1 h and subsequently treated with 20 ng/L of TNFα for 1 h to determine the activation of inflammatory mitogen-activated protein kinases including extracellular signal-related kinase (ERK) and c-Jun-NH_2 terminal kinase (JNK) and their downstream transcription factors including nuclear factor (NF)-κB and cJun, respectively, or for 3 h to determine inflammatory gene expression. The 1.5 nmol/L of Q3G and 20 ng/L of TNFα were chosen on the basis of the results in experiments 1 and 2, respectively, showing serum levels of Q3G increased to 1.5 nmol/L at 1 h postgavage with GE (Figure 1B) and serum levels of TNFα increased to 20 ng/L in mice fed high-fat diets for 18 weeks (Figure 3A). We found that Q3G at 10 or 30 μmol/L, but not at lower levels, attenuated TNFα-induced MCP-1 and IL-1β gene expression (Figure 4A) and modestly decreased JNK and c-Jun phosphorylation (Figure 4B). However, Q3G did not suppress TNFα-mediated induction of other inflammatory genes (e.g., IL-6) or activation of ERK and NF-κB (data not shown). In contrast, Q3GN (1.5 nmol/L or 3, 10, or 30 μmol/L) did not attenuate TNFα-mediated MCP-1, IL-1β, and IL-6 gene expression in primary human adipocytes (data not shown).

DISCUSSION

In this study, we reported that Q3G and Q3GN levels increased in the serum of mice at 1 h postgavage with GE and decreased thereafter. We found that high-fat-fed mice supplemented with GP, but not GE, had better glucose tolerance at 5 weeks, but not

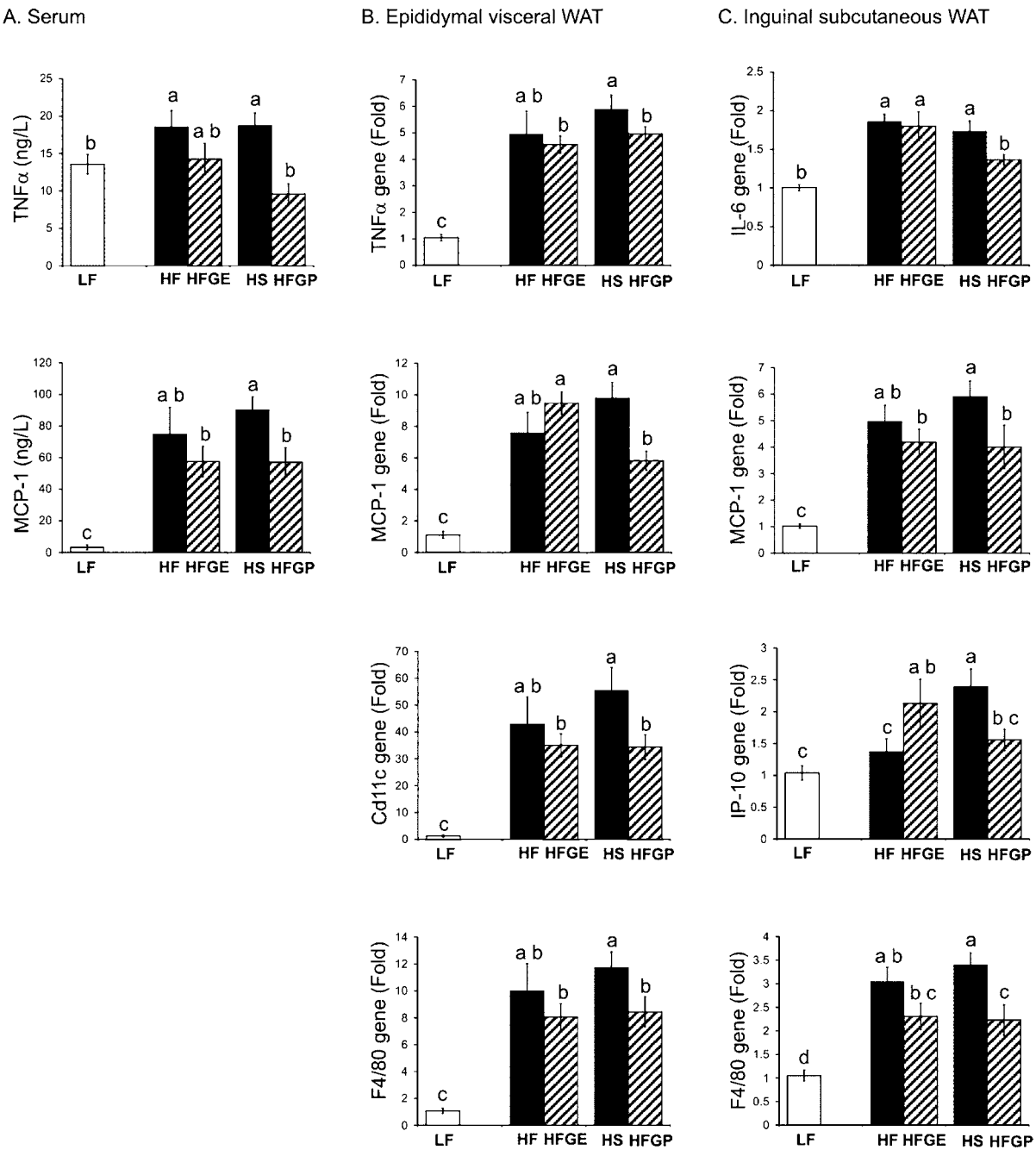

Figure 3. High-fat-fed obese mice supplemented with grape powder (GP), but not grape powder extract (GE), had lower systemic inflammation. Male C57BL/6J mice were fed for 18 weeks a low-fat (LF) diet, a high-fat (HF) diet, a high-fat diet plus GE (HFGE) diet, a high-fat diet with a modified sugar content (HS) similar to the HFGP group, or a high-fat diet plus GP (HFGP) diet. At 18 weeks, blood was collected and serum was harvested to measure the levels of an inflammatory cytokine (e.g., TNFα) and chemokine (e.g., MCP-1). Also, WAT depots were harvested to measure the mRNA levels of inflammatory cytokines (e.g., TNFα, IL-6) and chemokines (e.g., MCP-1, IP-10) and macrophage markers (e.g., CD11c, F4/80). The concentrations (ng/L) of TNFα and MCP-1 in mouse serum (A) were measured using magnetic bead-multiplex immunoassay and enzyme-linked immunosorbent assay, respectively. The mRNA levels of TNFα, MCP-1, CD11c, and F4/80 in epididymal WAT (B) were measured using qPCR. The mRNA levels of IL-6, MCP-1, IP-10, and F4/80 in inguinal WAT (C) were measured using qPCR. Data were analyzed using one-way ANOVA and Student's *t* tests. Means ± SEM (n = 7−9) without a common letter differ, $P < 0.05$. Clear bars = LF; black bars = HF or HS; slashed bars = HFGE or HFGP.

at 10 or 15 weeks, compared to controls. Notably, feeding GP, but not GE, reduced markers of inflammation in circulation and in two WAT depots at 18 weeks. Finally, the bioavailable polyphenol Q3G attenuated TNFα-mediated JNK and c-Jun activation and MCP-1 and IL-1β gene expression in primary human adipocytes.

Bioavailability of Polyphenols in Foods or Beverages. Several studies indicate that Q3G is not commonly present in the circulation, because of β-glucosidase hydrolysis of quercetin glucosides in the intestine and liver.[16,23] However, we detected an increase in Q3G levels in the mouse serum at 1 h postgavage with GE. Indeed, Manach et al. reviewed 97 human bioavailability studies and reported that quercetin glucosides might not be detected in plasma after oral administration at nutritionally relevant levels.[24] Therefore, it is possible that the peak in Q3G after 1 h of administration was due to the high dose

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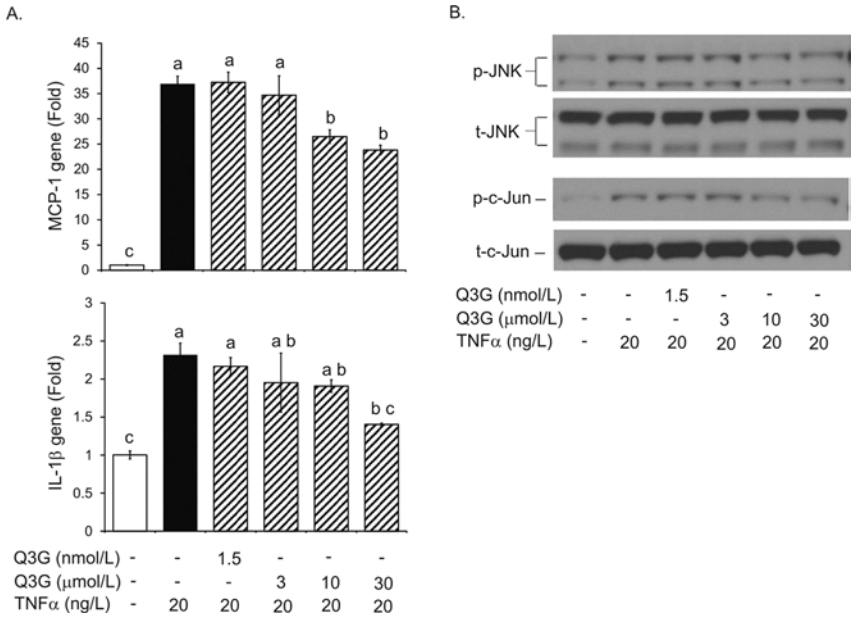

Figure 4. Quercetin 3-*O*-glucoside (Q3G) attenuated TNFα-mediated MCP-1 and IL-1β gene expression and JNK and cJun activation in primary human adipocytes. Cultures of newly differentiated primary human adipocytes (day 7) were pretreated with DMSO vehicle (−) or 1.5 nmol/L or 3, 10, or 30 μmol/L Q3G for 1 h and then treated without (−) or with 20 ng/L TNFα for 3 h to measure inflammatory gene expression or 1 h to measure inflammatory kinase and transcription factor activation. The mRNA level of MCP-1 and IL-1β (A) was measured using qPCR. The protein levels of phosphorylated JNK, total JNK, phosphorylated c-Jun, and total c-Jun (B) were determined using immunoblotting. Data in panel A were analyzed using one-way ANOVA and Student's *t* tests. Means ± SEM (n = 3) without a common letter differ, $P < 0.05$. Data in panels A and B are representative of three independent experiments. Clear bars = DMSO vehicle controls; black bars = TNFα alone; slashed bars = TNFα + Q3G.

of GE administered as a bolus. As a bolus, quercetin glucosides may have passively diffused across or between enterocytes, escaping intestinal as well as hepatic β-glucosidase hydrolysis. Consistent with our data, several groups have reported that the intact form of the polyphenols such as Q3G and rutin are bioavailable.[25,26] Moreover, cyanidin 3-*O*-glucoside and its metabolites such as methylated cyanidin 3-*O*-glucosides were detected in rat plasma at 30−60 min postgavage with cyanidin 3-*O*-glucosides (MW 484) at 0.9 mmol/L (0.44 g)/kg body weight.[27] Interestingly, anthocyanins including cyanidin 3-*O*-glucosides or malvidin 3-*O*-glucoside were present in relatively high and varied amounts in our GE and GP (Table 1). However, anthocyanins or their metabolites were undetectable in our mouse serum samples within 1−8 h after GE gavage. Consistent with our data, several intervention studies reported that anthocyanins such as malvidin 3-*O*-glucoside were undetectable in the plasma of adult subjects after a single oral administration of red wine and red grape juice.[28,29] One possible explanation for this lack of detection of these metabolites is that they can be rapidly degraded or metabolized by the intestinal microflora or by freezing of urine or blood samples for storage.[30]

Effects of Polyphenol-Rich Foods on Glucose Tolerance. The rapid rise in obesity is accompanied by a similar increase in insulin resistance and type 2 diabetes. For instance, ∼90% of people with type 2 diabetes are overweight and ∼50% are obese, suggesting a strong positive relationship between the two diseases.[31] Here, we showed that mice fed a high-fat diet (i.e., 60% energy from fat) supplemented with GP had improved glucose tolerance acutely at 5 weeks (Figure 2A), but not chronically at 10 weeks (Figure 2B) or 15 weeks (Figure 2C). Consistent with our data, consumption of muscadine grape wine or dealcoholized grape wine (150 mL/day) for 4 weeks improved fasting blood glucose and insulin levels in subjects with type 2 diabetes.[32] In contrast to our study, resveratrol, a polyphenol found in grapes and red wine, has been reported to improve glucose and lipid homeostasis in high-fat-fed C57BL/6J obese mice during long-term supplementation at 0.04% in the diet (wt/wt) for 12−48 weeks or at 2.5−400 mg/kg body weight/day for 15−16 weeks.[33,34] Moreover, a recent study reported that supplementation of 0.4% muscadine grape phytochemicals or 0.4% muscadine wine phytochemicals for 15 weeks decreased fasting glucose and insulin levels in high-fat-fed, obese mice.[35] A critical difference between those studies and our study is that they used a higher dose (i.e., 2−20-fold) of grape polyphenols in diets to achieve the inhibitory effect on obesity-associated insulin resistance. Such a dose of grape polyphenols is not physiologically possible from the consumption of fresh grapes, as this would be equivalent to 18−90 servings of grapes for humans per day. In contrast, our level of GP was equivalent to 9 human servings of grapes daily, which is already quite high. Overall, multiple studies suggest that grape products have antidiabetic effects due to their abundant polyphenol content and low glycemic index.[36]

Effects of Polyphenol-Rich Foods on Chronic Inflammation. A feature of obesity and type 2 diabetes is their linkage with chronic, low-grade inflammation that begins in WAT and eventually becomes systemic. For instance, obese patients with type 2 diabetes have elevated levels of TNFα[3] and MCP-1[5] in their WAT and blood. Our study showed that 3% GP, but not 0.02% GE, attenuated the levels of TNFα and MCP-1 in circulation (Figure 3A) and WAT (Figure 3B) at 18 weeks. Consistent with our data, Dahl salt-sensitive, hypertensive rats supplemented with 3% GP in the diet (wt/wt) for 18 weeks had lower plasma and cardiac levels of TNFα.[20,21] Nevertheless, we were surprised that GE had no effect on glucose tolerance acutely or on markers of inflammation chronically in obese mice, considering that the GE diet had the same amount of total polyphenols as the GP diet. Furthermore, our in vitro studies

using GE[11,12] and other in vivo studies using grape seed extract[37,38] reported inhibitory effects on inflammation or insulin resistance. This discrepancy may be due to loss of ~10% water-insoluble polyphenols (e.g., quercetin aglycone) during the preparation of GE from GP.[39,40] Indeed, quercetin aglycones were undetectable in our GE (Table 1) as well as in circulation following GE gavage, and we previously demonstrated that they are rapidly taken up by adipocytes and attenuate inflammation and insulin resistance.[14] Thus, this suggests that whole fruits such as GP contain a greater variety of polyphenols or other phytochemicals than GE that may play an important role in attenuating inflammation chronically and improving glucose tolerance acutely in diet-induced obese mouse models. Another explanation regarding the discrepant effect between GP and GE is that GP, but not GE, contains fiber, which can modulate gut microbiota. Studies reported that diet-induced obesity and diabetes are associated with increases in sulfate-reducing bacteria, which compromise barrier function through their production of the pro-inflammatory and genotoxic gas hydrogen sulfide[41] and prebiotic-induced protection from metabolic dysfunction in genetic (ob/ob) and diet-induced obese and diabetic mice.[42] Therefore, we speculate that GP, but not GE, restores barrier function through selection for mucosal populations of butyrate-producing bacteria via prebiotic components such as constituent fiber or polyphenols or selective diminution of sulfate-reducing bacteria through polyphenols or perhaps some combination of both mechanisms, thereby reducing inflammatory signaling and insulin resistance in high-fat-fed mice.

In Vitro Effects of Q3G and Q3GN. In our cell studies, we chose to examine the direct effects of Q3G or Q3GN in human primary adipocytes, because of their application to human obesity versus rodent obesity. However, using murine primary adipocytes or immortalized adipocyte cultures (i.e., 3T3-L1) might have been more applicable to our mouse studies. Sakurai et al. reported that oligomerized grape seed polyphenol attenuated inflammatory TNFα and MCP-1 production in HW mouse white adipocytes cocultured with a murine macrophage cell line (RAW 264 cells).[43] Here, we reported that Q3G, but not Q3GN (data not shown), attenuated inflammatory MCP-1 and IL-1β gene expression in human primary adipocyte cultures treated with TNFα (Figure 4A), although the effective levels in vitro were much higher than those detected in circulation, possibly due to the nonphysiological conditions of in vitro experiments. Moreover, direct comparisons of blood levels of nutrients or compounds to tissue or cellular levels is controversial, given the differential mechanisms that regulate uptake, metabolism, and excretion in each compartment. Moreover, dietary compounds such as polyphenols usually work synergistically rather than individually, and therefore it is likely that Q3G or even Q3GN works synergistically with multiple compounds in grapes to suppress inflammatory signaling.

It has been demonstrated that TNFα-induced MCP-1 gene expression in adipocytes or other cell types occurs via activation of the transcription factors NF-κB or AP-1 (i.e., c-Jun, c-fos, ATF), because the promoter of the MCP-1 gene contains NF-κB and AP-1 binding sites.[44,45] Indeed, we found that Q3G reduced TNFα-mediated phosphorylation of JNK and downstream c-Jun activation (Figure 4B), but not ERK activation or IκBα degradation (data not shown), in human primary adipocyte cultures. Further investigations examining upstream activators of TNFα-mediated inflammatory signaling are needed to understand how Q3G suppresses this pathway.

In summary, our findings suggest that feeding GP equivalent to nine daily human servings to high-fat-fed mice improves glucose tolerance acutely (i.e., 5 weeks) and markers of inflammation chronically (i.e., 18 weeks) without affecting body fat levels. In addition, Q3G may be one of the polyphenols in GP that contributes to these beneficial outcomes. However, conjugates of epigallocatechins and other potentially bioavailable polyphenol conjugates were not measured and, thus, is a limitation of this study. Further mouse studies feeding a high-fat diet that more closely resembles a typical Western diet (i.e., 34% of energy from fat) and clinical studies with overweisght or obese individuals are needed to determine if these effects of GP or other grape products can be reproduced in humans.

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Notes

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ABBREVIATIONS USED

AUC, area under the curve; ERK, extracellular signal-related kinase; GE, grape powder extract; GP, grape powder; GTT, glucose tolerance test; HF, high-fat diet; HS, high-fat diet with a modified sugar content; IL, interleukin; LF, low-fat diet; JNK, c-Jun-NH2 terminal kinase; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor-κB; Q3G, quercetin 3-*O*-glucoside; Q3GN, quercetin 3-*O*-glucuronide; SV, stromal vascular; TNF, tumor necrosis factor; WAT, white adipose tissue

REFERENCES

(1) Obesity and overweight data from World Health Organization; http://www.who.int/mediacentre/factsheets/fs311/en/index.html (accessed June 28, 2012).

(2) Gregor, M. F.; Hotamisligil, G. S. Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* **2011**, *29*, 415−445.

(3) Hotamisligil, G. S.; Arner, P.; Caro, J. F.; Atkinson, R. L.; Spiegelman, B. M. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. *J. Clin. Invest.* **1995**, *95*, 2409−2415.

(4) Bastard, J. P.; Maachi, M.; Van Nhieu, J. T.; Jardel, C.; Bruckert, E.; Grimaldi, A.; Robert, J. J.; Capeau, J.; Hainque, B. Adipose tissue IL-6 content correlates with resistance to insulin activation of glucose uptake both in vivo and in vitro. *J. Clin. Endocrinol. Metab.* **2002**, *87*, 2084−2089.

(5) Kanda, H.; Tateya, S.; Tamori, Y.; Kotani, K.; Hiasa, K.; Kitazawa, R.; Kitazawa, S.; Miyachi, H.; Maeda, S.; Egashira, K.; Kasuga, M. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J. Clin. Invest.* **2006**, *116*, 1494−1505.

(6) Wellen, K. E.; Hotamisligil, G. S. Obesity-induced inflammatory changes in adipose tissue. *J. Clin. Invest.* **2003**, *112*, 1785−1788.

(7) Ahmadi, N.; Eshaghian, S.; Huizenga, R.; Sosnin, K.; Ebrahimi, R.; Siegel, R. Effects of intense exercise and moderate caloric restriction on

cardiovascular risk factors and inflammation. *Am. J. Med.* **2011**, *124*, 978−982.
(8) Hussain, S. S.; Bloom, S. R. The pharmacological treatment and management of obesity. *Postgrad. Med.* **2011**, *123*, 34−44.
(9) Vislocky, L. M.; Fernandez, M. L. Biomedical effects of grape products. *Nutr. Rev.* **2010**, *68*, 656−670.
(10) Chuang, C. C.; McIntosh, M. K. Potential mechanisms by which polyphenol-rich grapes prevent obesity-mediated inflammation and metabolic diseases. *Annu. Rev. Nutr.* **2011**, *31*, 155−176.
(11) Overman, A.; Bumrungpert, A.; Kennedy, A.; Martinez, K.; Chuang, C. C.; West, T.; Dawson, B.; Jia, W.; McIntosh, M. Polyphenol-rich grape powder extract (GPE) attenuates inflammation in human macrophages and in human adipocytes exposed to macrophage-conditioned media. *Int. J. Obes. (London)* **2010**, *34*, 800−808.
(12) Chuang, C. C.; Bumrungpert, A.; Kennedy, A.; Overman, A.; West, T.; Dawson, B.; McIntosh, M. K. Grape powder extract attenuates tumor necrosis factor α-mediated inflammation and insulin resistance in primary cultures of human adipocytes. *J. Nutr. Biochem.* **2011**, *22*, 89−94.
(13) Overman, A.; Chuang, C. C.; McIntosh, M. Quercetin attenuates inflammation in human macrophages and adipocytes exposed to macrophage-conditioned media. *Int. J. Obes. (London)* **2011**, *35*, 1165−2272.
(14) Chuang, C. C.; Martinez, K.; Xie, G.; Kennedy, A.; Bumrungpert, A.; Overman, A.; Jia, W.; McIntosh, M. K. Quercetin is equally or more effective than resveratrol in attenuating tumor necrosis factor-α-mediated inflammation and insulin resistance in primary human adipocytes. *Am. J. Clin. Nutr.* **2010**, *92*, 1511−1521.
(15) Fuhrman, B.; Volkova, N.; Coleman, R.; Aviram, M. Grape powder polyphenols attenuate atherosclerosis development in apolipoprotein E deficient (E0) mice and reduce macrophage atherogenicity. *J. Nutr.* **2005**, *135*, 722−728.
(16) Day, A. J.; Mellon, F.; Barron, D.; Sarrazin, G.; Morgan, M. R.; Williamson, G. Human metabolism of dietary flavonoids: identification of plasma metabolites of quercetin. *Free Radical Res.* **2001**, *35*, 941−952.
(17) Potteiger, J. A.; Jacobsen, D. J.; Donnelly, J. E. A comparison of methods for analyzing glucose and insulin areas under the curve following nine months of exercise in overweight adults. *Int. J. Obes. Relat. Metab. Disord.* **2002**, *26*, 87−89.
(18) Brown, J. M.; Boysen, M. S.; Jensen, S. S.; Morrison, R. F.; Storkson, J.; Lea-Currie, R.; Pariza, M.; Mandrup, S.; McIntosh, M. K. Isomer-specific regulation of metabolism and PPARγ signaling by CLA in human preadipocytes. *J. Lipid Res.* **2003**, *44*, 1287−1300.
(19) Tsang, C.; Auger, C.; Mullen, W.; Bornet, A.; Rouanet, J. M.; Crozier, A.; Teissedre, P. L. The absorption, metabolism and excretion of flavan-3-ols and procyanidins following the ingestion of a grape seed extract by rats. *Br. J. Nutr.* **2005**, *94*, 170−181.
(20) Seymour, E. M.; Singer, A. A.; Bennink, M. R.; Parikh, R. V.; Kirakosyan, A.; Kaufman, P. B.; Bolling, S. F. Chronic intake of a phytochemical-enriched diet reduces cardiac fibrosis and diastolic dysfunction caused by prolonged salt-sensitive hypertension. *J. Gerontol. A: Biol. Sci. Med. Sci.* **2008**, *63*, 1034−1042.
(21) Seymour, E. M.; Bennink, M. R.; Watts, S. W.; Bolling, S. F. Whole grape intake impacts cardiac peroxisome proliferator-activated receptor and nuclear factor κB activity and cytokine expression in rats with diastolic dysfunction. *Hypertension* **2010**, *55*, 1179−1185.
(22) Shaul, M. E.; Bennett, G.; Strissel, K. J.; Greenberg, A. S.; Obin, M. S. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet-induced obesity in mice. *Diabetes* **2010**, *59*, 1171−1181.
(23) Sesink, A. L.; O'Leary, K. A.; Hollman, P. C. Quercetin glucuronides but not glucosides are present in human plasma after consumption of quercetin-3-glucoside or quercetin-4′-glucoside. *J. Nutr.* **2001**, *131*, 1938−1941.
(24) Manach, C.; Williamson, G.; Morand, C.; Scalbert, A.; Rémésy, C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am. J. Clin. Nutr.* **2005**, *81*, 230S−242S.
(25) Manach, C.; Morand, C.; Demigné, C.; Texier, O.; Régérat, F.; Rémésy, C. Bioavailability of rutin and quercetin in rats. *FEBS Lett.* **1997**, *409*, 12−16.
(26) Reinboth, M.; Wolffram, S.; Abraham, G.; Ungemach, F. R.; Cermak, R. Oral bioavailability of quercetin from different quercetin glycosides in dogs. *Br. J. Nutr.* **2010**, *104*, 198−203.
(27) Tsuda, T.; Horio, F.; Osawa, T. Absorption and metabolism of cyanidin 3-*O*-β-D-glucoside in rats. *FEBS Lett.* **1999**, *449*, 179−182.
(28) Bub, A.; Watzl, B.; Heeb, D.; Rechkemmer, G.; Briviba, K. Malvidin-3-glucoside bioavailability in humans after ingestion of red wine, dealcoholized red wine and red grape juice. *Eur J Nutr.* **2001**, *40*, 113−120.
(29) Garcia-Alonso, M.; Minihane, A. M.; Rimbach, G.; Rivas-Gonzalo, J. C.; de Pascual-Teresa, S. Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and antioxidant capacity of plasma. *J. Nutr. Biochem.* **2009**, *20*, 521−529.
(30) Felgines, C.; Talavéra, S.; Gonthier, M. P.; Texier, O.; Scalbert, A.; Lamaison, J. L.; Rémésy, C. Strawberry anthocyanins are recovered in urine as glucuro- and sulfo-conjugates in humans. *J. Nutr.* **2003**, *133*, 1296−1301.
(31) Garber, A. J. Obesity and type 2 diabetes: which patients are at risk? *Diabetes Obes. Metab.* **2012**, *14*, 399−408.
(32) Banini, A. E.; Boyd, L. C.; Allen, J. C.; Allen, H. G.; Sauls, D. L. Muscadine grape products intake, diet and blood constituents of non-diabetic and type 2 diabetic subjects. *Nutrition* **2006**, *22*, 1137−1145.
(33) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **2006**, *444*, 337−342.
(34) Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell* **2006**, *127*, 1109−1122.
(35) Gourineni, V.; Shay, N. F.; Chung, S.; Sandhu, A. K.; Gu, L. Muscadine grape (*Vitis rotundifolia*) and wine phytochemicals prevented obesity-associated metabolic complications in C57BL/6J mice. *J. Agric. Food Chem.* **2012**, *60*, 7674−7681.
(36) Zunino, S. Type 2 diabetes and glycemic response to grapes or grape products. *J. Nutr.* **2009**, *139*, 1794S−1800S.
(37) Cheah, K. Y.; Howarth, G. S.; Yazbeck, R.; Wright, T. H.; Whitford, E. J.; Payne, C.; Butler, R. N.; Bastian, S. E. Grape seed extract protects IEC-6 cells from chemotherapy-induced cytotoxicity and improves parameters of small intestinal mucositis in rats with experimentally-induced mucositis. *Cancer Biol. Ther.* **2009**, *8*, 382−390.
(38) Kar, P.; Laight, D.; Rooprai, H. K.; Shaw, K. M.; Cummings, M. Effects of grape seed extract in type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. *Diabetes Med.* **2009**, *26*, 526−531.
(39) Chow, C. K. Grape powder polyphenols and atherosclerosis development. *J. Nutr.* **2006**, *136*, 2272.
(40) Fuhrman, B. Grape powder polyphenols and atherosclerosis development. *J. Nutr.* **2006**, *136*, 2273.
(41) Zhang, C.; Zhang, M.; Wang, S.; Han, R.; Cao, Y.; Hua, W.; Mao, Y.; Zhang, X.; Pang, X.; Wei, C.; Zhao, G.; Chen, Y.; Zhao, L. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J.* **2010**, *4*, 232−241.
(42) Everard, A.; Lazarevic, V.; Derrien, M.; Girard, M.; Muccioli, G. G.; Neyrinck, A. M.; Possemiers, S.; Van Holle, A.; François, P.; de Vos, W. M.; Delzenne, N. M.; Schrenzel, J.; Cani, P. D. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* **2011**, *60*, 2775−2786.

(43) Sakurai, T.; Kitadate, K.; Nishioka, H.; Fujii, H.; Kizaki, T.; Kondoh, Y.; Izawa, T.; Ishida, H.; Radák, Z.; Ohno, H. Oligomerized grape seed polyphenols attenuate inflammatory changes due to antioxidative properties in coculture of adipocytes and macrophages. *J. Nutr. Biochem.* **2010**, *21*, 47−54.

(44) Martin, T.; Cardarelli, P. M.; Parry, G. C.; Felts, K. A.; Cobb, R. R. Cytokine induction of monocyte chemoattractant protein-1 gene expression in human endothelial cells depends on the cooperative action of NF-κB and AP-1. *Eur. J. Immunol.* **1997**, *27*, 1091−1097.

(45) Asamizu, S.; Urakaze, M.; Kobashi, C.; Ishiki, M.; Norel Din, A. K.; Fujisaka, S.; Kanatani, Y.; Bukahari, A.; Senda, S.; Suzuki, H.; Yamazaki, Y.; Iwata, M.; Usui, I.; Yamazaki, K.; Ogawa, H.; Kobayashi, M.; Tobe, K. Angiotensin II enhances the increase in monocyte chemoattractant protein-1 production induced by tumor necrosis factor-α from 3T3-L1 preadipocytes. *J. Endocrinol.* **2009**, *202*, 199−205.