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A polyphenol-rich fraction obtained from table grapes decreases adiposity, insulin resistance and markers of inflammation and impacts gut microbiota in high-fat-fed mice☆

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Abstract

The objective of this study was to determine if consuming an extractable or nonextractable fraction of table grapes reduced the metabolic consequences of consuming a high-fat, American-type diet. Male C57BL/6J mice were fed a low fat (LF) diet, a high fat (HF) diet, or an HF diet containing whole table grape powder (5% w/w), an extractable, polyphenol-rich (HF-EP) fraction, a nonextractable, polyphenol-poor (HF-NEP) fraction or equal combinations of both fractions (HF-EP+NEP) from grape powder for 16 weeks. Mice fed the HF-EP and HF-EP+NEP diets had lower percentages of body fat and amounts of white adipose tissue (WAT) and improved glucose tolerance compared to the HF-fed controls. Mice fed the HF-EP+NEP diet had lower liver weights and triglyceride (TG) levels compared to the HF-fed controls. Mice fed the HF-EP+NEP diets had higher hepatic mRNA levels of hormone sensitive lipase and adipose TG lipase, and decreased expression of c-reactive protein compared to the HF-fed controls. In epididymal (visceral) WAT, the expression levels of several inflammatory genes were lower in mice fed the HF-EP and HF-EP+NEP diets compared to the HF-fed controls. Mice fed the HF diets had increased myeloperoxidase activity and impaired localization of the tight junction protein zonula occludens-1 in ileal mucosa compared to the HF-EP and HF-NEP diets. Several of these treatment effects were associated with alterations in gut bacterial community structure. Collectively, these data demonstrate that the polyphenol-rich, EP fraction from table grapes attenuated many of the adverse health consequences associated with consuming an HF diet.

Keywords: Grapes; Obesity; Steatosis; Inflammation; Intestines; Microbiota

Abbreviations: *Agpat2*, acylglycerol-3-phosphate-O-acyltransferase 2; ANC, anthocyanin; *Atgl*, adipose triglyceride lipase; AUC, area under the curve; *B. wadsworthia*, *Bilophila wadsworthia*; BWG, body weight gain; *Cd36*, cluster of differentiation 36; *Cd11c*, cluster of differentiation 11c; *Cpt*, carnitine palmitoyltransferase; *Crp*, c-reactive protein; DEXA, dual-energy X-ray; DMAC, 4-dimethylaminocinnamaldehyde; DP, degree of polymerization; *DBB*, *Desulfobulbus* spp.; *DFM*, *Desulfotomaculum* spp; *DSB*, *Desulfobacter* spp; *dsrA*-Bw, *B. wadsworthia* specific dissimilatory sulfite reductase; *DSV*, *Desulfovibrio* spp; EPI, epididymal; *Erm1* (F4/80 human orthologue), epidermal growth factor-like module containing mucin-like hormone receptor 1; EP, extractable polyphenol; *Fas*, fatty acid synthase; *Fabp4*, fatty acid binding protein 4; FCE, food conversion efficiency; *Gpat2*, glycerol-3-phosphate acyltransferase; *Glp*, glucagon-like peptide; GP, grape powder; *Gpr*, G-protein receptor; GTT, glucose tolerance test; HF, high fat; HOMA-IR, homeostasis model assessment method for insulin resistance; *Hsl*, hormone sensitive lipase; ING, inguinal; IL, interleukin; LPS, lipopolysaccharide; LBP, LPS-binding protein; LF, low fat; *Mcp*, monocyte chemoattractant protein; MPO, myeloperoxidase; NKT, natural killer T; NEP, nonextractable polyphenol; NF-κB, nuclear factor kappa B; OTU, operational taxonomic units; PAC, proanthocanidin; PCA, primary component analysis; *Pck*, phosphoenolpyruvate carboxykinase; PPAR, peroxisome proliferator activated receptor; procyanidin A2; *Pyy*, peptide yy; *Scd1*, stearoyl-CoA desaturase 1; *Srebp*, sterol regulatory element binding protein; *Tbp*, TATA-binding protein (*TBP*); TG, triglyceride; *Tlr4*, toll-like receptor 4; *Tnf*, tumor necrosis factor; TFA, trifluoroacetic acid; TP, Total phenolics; WAT, white adipose tissue; ZO-1, zonula occludens-1.

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1. Introduction

Obesity has been on the rise since the 1960s, affecting approximately one third of the adult population [1,2]. Obesity is associated with chronic inflammatory conditions that contribute to the metabolic syndrome (*e.g.*, Type 2 diabetes, hypertension and cardiovascular disease) [3]. Over consumption of calories coinciding with a lack of physical activity are the major risk factors for obesity development resulting in expansion of white adipose tissue (WAT). WAT expansion elicits inflammatory signals that recruit macrophages and other immune cells into the WAT [4]. This inflammatory scenario disrupts metabolic processes, resulting in impaired glucose and fatty acid uptake and metabolism, thereby contributing to the development of metabolic diseases.

Recently, the role that gut microbes play in the development of the metabolic syndrome has received attention due to their sensitivity to environmental changes that can trigger obesity [5], chronic inflammation [6–8] and insulin resistance [reviewed in 9]. Diets high in fat decrease gut microbial diversity and barrier-protecting bacteria while increasing the abundance of deleterious bacteria [reviewed in 10]. For example, an increase in the ratio of *Firmicutes* to *Bacteriodetes* is positively correlated with the development of obesity and insulin resistance [11]. Diets rich in saturated fat [11,12], particularly from milk [13], increase the abundance of sulfidogenic bacteria like *Bilophila wadsworthia* and *Desulfovibrionaceae* spp. These bacteria generate the genotoxic and cytotoxic gas hydrogen sulfide, which has been linked to ulcerative colitis, gut inflammation, irritable bowel syndrome, and colon cancer [13–15]. Notably, the effects of high fat (HF) diets on body weight gain are repressed in microbiota-free mouse models as well as in human fecal microbiota transplants from healthy donors into obese subjects [16].

The use of nondigestible carbohydrates, fiber, or polyphenols as prebiotics shows promise as potential interventions for the metabolic consequences of obesity [13, reviewed in 17–20]. Fiber, in particular inulin-type fructans, has been shown to increase the abundance of *Bifidobacteria* which was positively correlated with decreased hyperglycemia, endotoxemia, and systemic cytokine levels [21,22]. Similar effects have been reported in obese subjects with short-term supplementation of gluco-oligosaccharides [23], and are associated with the production of specific short chain fatty acids (SCFA) that regulate the synthesis of gastrointestinal (GI) peptides. These peptides influence energy intake and metabolism through interactions with G-protein receptors (Gpr) 41, 43 and 119 [reviewed in 20].

Polyphenols found in fruits and vegetables may improve GI health [24–26, reviewed in 27]. Absorption of polyphenols is poor in the upper gastrointestinal tract, leading to increased availability in the lower GI tract [reviewed in 27] that may influence microbiota taxa and their metabolites [reviewed in 28]. Indeed, the antiinflammatory, antioxidant and antimicrobial actions of polyphenols have been reported to positively influence gut microbes and host inflammation [reviewed in 29].

Grapes and other berries are rich in polyphenols including anthocyanins [reviewed in 29], which are known to have antiinflammatory and antioxidant effects [30]. These beneficial effects of grapes have been associated with reduced cytokine levels via suppression of nuclear factor kappa B (NFκB) and increased peroxisome proliferator-activated receptor (PPAR)α/γ [31]. Similarly, we demonstrated that C57BL/6J mice consuming an HF diet (*i.e.*, 60% kcals from lard) supplemented with whole powdered California table grapes (3%, w/w) had improved glucose tolerance after 5 weeks and decreased markers of inflammation ~20–50% in serum and WAT after 18 weeks [32]. We also showed that consuming a moderate fat diet (*i.e.*, 34% of kcals, primarily from butter fat) supplemented with whole powdered grapes (3% or 5% w/w) reduced adiposity, improved liver triglyceride (TG) levels, modestly reduced WAT inflammatory gene expression and lowered the cecum levels of sulfidogenic bacteria, while tending to increase the abundance of *Akkermansia muciniphila* and *Allobaculum* in the proximal colon and cecum, respectively [33].

However, the identities of the bioactive fractions in whole table grapes and the role that gut microbiota play in improvements in diet-induced obesity in mice fed with grapes are unknown. Therefore, the objective of this study was to determine the extent to which consuming extractable, polyphenol-rich and nonextractable, polyphenol-poor fractions of table grapes improved intestinal and systemic health in mice fed an HF, American-type diet. The lyophilized whole-grape powder was extracted with acidified methanol, and sugars were removed to afford the extractable polyphenol-rich (EP) fraction. The residual plant material after methanol extraction constituted the nonextractable, polyphenol-poor (NEP) fraction. This separation scheme allowed us to compare the effects of the more soluble polyphenols in the EP fraction compared to insoluble polyphenols typically bound to fiber in the NEP fraction. These fractions were incorporated into an HF, American-type diet [34]. This HF diet was fed alone or in combination with the EP fraction, the NEP fraction, both fractions (EP+NEP), or whole-grape powder (GP; 5%, w/w) for 16 weeks.

2. Materials and methods

2.1. Plant materials and chemicals

The lyophilized (*i.e.*, powdered) table grapes were kindly provided by the California Table Grape Commission and consisted of a mixture of red, green and purple-seeded and seedless grapes. Reference compounds procyanidin B2 (PAC-B2), catechin and epicatechin were purchased from Chromadex (Irvine, CA, USA). 4-dimethylaminocinnamaldehyde (DMAC), Folin–Ciocalteu reagent and trifluoroacetic acid (TFA) were purchased from Sigma-Aldrich Inc. (St. Louis, MO, USA). All organic solvents were HPLC grade and obtained from VWR International (Suwanee, GA, USA).

2.2. Extraction and polyphenol enrichment

Freeze-dried grape powder (200 g × 5 batches) was blended each with 1 L 50% acidified methanol (0.1% TFA). The mixture was centrifuged (Sorvall RC-6 plus, Asheville, NC, USA) at 3452 ×*g* for 10 min, and the supernatant was collected. The combined supernatants (2 L) were evaporated to remove the organic solvent, and then loaded to Amberlite XDA-7 resin. The resin was washed with water to get rid of all free sugars and organic acids. The polyphenols were eluted from the resin with 100% methanol, organic solvent was evaporated under vacuum and the remaining aqueous extract was freeze dried to afford the EP fraction. The pelleted material (plant debris after extraction) was put in a vacuum oven (45 °C) to get rid of the organic solvent before freeze-drying to afford the NEP fraction.

2.3. Alkaline hydrolysis of NEP fraction

Alkaline hydrolysis of the NEP fraction was performed according to Yang *et al.* [35] with some modifications. In 15-mL centrifuge tube, 2.0 mL of 4-mol/L NaOH were added to 0.5-g NEP, flushed with nitrogen, closed and incubated for 1 h at room temperature. The mixture was adjusted to pH 7 with drops of concentrated hydrochloric acid, then loaded onto a column packed with celite at a ratio 1:2 v/w. The hydrolyzed polyphenols were eluted with 30-mL methanol-ethyl acetate (20:80 v/v) and evaporated to dryness.

2.4. Determination of total phenolics, anthocyanins and proanthocyanidins

Total phenolics (TP) were determined in EP and NEP hydrolysate fractions with Folin–Ciocalteu reagent [36]. Concentrations were expressed as mg/L gallic acid equivalents. Total monomeric anthocyanin (ANC) content was measured in EP using the pH differential spectrophotometric method [37] and expressed as cyanidin3-*O*-glucoside equivalents. Total proanthocyanidin content (PAC) was determined in EP using the DMAC method as previously described [38] and quantified as procyanidin B2 equivalents.

2.5. HPLC profile analyses of anthocyanins and proanthocyanidins

HPLC analyses were conducted according to the previously reported protocols [39].

2.6. Animals

Four-week old, male C57BL/6J mice were obtained from The Jackson Laboratories (Bar Harbor, ME, USA) and acclimated on a standard chow diet for 1 week. Mice were housed in pairs, maintained at a temperature of 22 °C with 50% humidity and exposed to