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Combination of the anthocyanidins malvidin and peonidin attenuates lipopolysaccharide-mediated inflammatory gene expression in primary human adipocytes

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ABSTRACT

We recently demonstrated that California table grapes and a methanol-extractable, polyphenol-rich fraction decreased adiposity, insulin resistance, or markers of inflammation in high-fat fed mice. Malvidin and peonidin glycosides were the 2 most abundant anthocyanins in the polyphenol-rich fraction. We hypothesized that a blood borne combination of anthocyanidins malvidin and peonidin derived from intestinal β-glycosidase metabolism of these 2 anthocyanins are responsible, in part, for the beneficial health effects observed in vivo. Therefore, we supplemented primary human adipocytes with malvidin or peonidin, alone or together, followed by acute lipopolysaccharide (LPS) treatment. Neither peonidin nor malvidin alone consistently decreased the expression of several inflammatory genes. However, supplementing adipocytes with an equal combination of malvidin plus peonidin followed by LPS treatment decreased the mRNA levels of interleukin (IL)-6, IL-1β, IL-8, monocyte chemoattractant protein-1, toll-like receptor-2, tumor necrosis factor alpha, cyclooxygenase-2, and interferon gamma-induced protein-10. The highest combination dose of malvidin plus peonidin decreased or increased the expression of protein tyrosine phosphatase-1B and hormone sensitive lipase, respectively, genes encoding proteins associated with insulin resistance or lipolysis. These data indicate that a combination of malvidin plus peonidin have potentiating interactions that reduce inflammatory gene expression; however, in vivo studies are needed to support these in vitro data.

Abbreviations: ACC, acetyl-CoA carboxylase; aP2, adipocyte fatty acid binding protein; COX, cyclooxygenase; HF, high fat; HSL, hormone sensitive lipase; IP-10, interferon gamma-induced protein 10; IL, interleukin; JNK, c-Jun.-NH2 terminal kinase 1; LPS, lipopolysaccharide; MAPK, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; NF-κB, nuclear factor kappa B; PPAR, peroxisome proliferator activated receptor; PTP-1B, protein tyrosine phosphatase-1B; SCD, stearoyl-CoA desaturase; TBP, TATA-binding protein;; TG, triglyceride; TLR, toll-like receptor; TNF, tumor necrosis factor; WAT, white adipose tissue.

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1. Introduction

Obesity is a growing public health concern worldwide with significant metabolic consequences [1] such as atherosclerosis, type 2 diabetes, and hypertension [2]. Major risk factors contributing to obesity are the overconsumption of calories combined with a lack of physical activity [3]. The expansion of white adipose tissue (WAT) is associated with chronic, low-grade inflammation characterized by increased cytokine and chemokine production and markers of inflammation such as monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6, and tumor necrosis factor (TNF)α [4,5]. Another major contributor to inflammation is communication between microbes inhabiting the gut and the host. For example, intestinal dysbiosis and inflammation enhance the development and progression of inflammatory bowel diseases such as Crohn's disease and ulcerative colitis and systemic diseases like steatosis, atherosclerosis, and type 2 diabetes [6].

Lipopolysaccharide (LPS) is a component of the outer membrane of gram-negative bacteria that can enter the blood stream from the intestine when gut barrier integrity is compromised [7]. Recent studies have shown a positive association with plasma LPS and gut dysbiosis, obesity, and systemic inflammation [8,9]. Notably, stimulation of adipocytes with LPS causes lipolysis, insulin resistance, and secretion of proinflammatory cytokines and chemokines [10]. LPS binding to toll-like receptors (TLR)s triggers an inflammatory signaling cascade involving mitogen activated protein kinases (MAPK)s and nuclear factor kappa B (NF-κB) [11]. Activation of these proteins increases the transcription of proinflammatory genes. Therefore, decreasing blood LPS levels or inhibiting activation of these inflammatory pathways could be an effective dietary strategy for preventing obesity-related inflammation and their associated metabolic risks. Plant polyphenols are candidates due to their anti-inflammatory, antioxidant, and antimicrobial properties potential [12,13].

Our previous studies with California table grapes and their polyphenol-rich fractions found that (1) gavaging mice with a water-extractable, polyphenol-rich fraction (1 g/kg body weight) increased blood levels of quercetin-3-O-glucosides and glucuronides and rutin within 1 hour PO administration [14]; (2) grape powder supplementation (3% or 5%, w/w in the diet or approximately 9 or 15 human servings) improved glucose tolerance acutely and markers of inflammation chronically in mice fed a high-fat (HF) diet [14,15]; (3) quercetin-3-O-glucoside (30 μmol/L) reduced TNFα-mediated MCP-1 and IL-1β gene expression in human adipocytes [14]; (4) a water-extractable, polyphenol-rich fraction (10-60 μg/ml) attenuated TNFα-mediated inflammatory gene expression and insulin resistance in primary human adipocytes [16]; (5) quercetin (10–60 μmol/L) reduced TNFα-mediated inflammatory gene expression and insulin resistance in primary human adipocytes [17]; (6) a water-extractable, polyphenol-rich fraction (10–100 μg/ml) and quercetin (3–30 μmol/L) decreased makers of inflammation in human macrophages and adipocytes exposed to macrophage conditioned media [18,19]; and (7) a methanol-extractable, polyphenol-rich fraction (equivalent to approximately 15 human servings of grapes) reduced markers of inflammation and adiposity in high-fat fed mice [20]. However, we do not know the extent to which methanol-extractable anthocyanins in this fraction or deglycosylated anthocyanidins generated by intestinal metabolism attenuated inflammatory gene expression. Distinctly, malvidin and peonidin glycosides were the 2 most abundant anthocyanins found in the methanol-extractable, polyphenol-rich fraction and their instability, intestinal metabolism [21], and poor absorption [22-24] are well-documented.

Based on these data, we hypothesized that a combination of blood borne malvidin and peonidin derived from the intestinal β-glycosidase metabolism of malvidin and peonidin glycosides are responsible, in part, for the beneficial health effects of consuming anthocyanin-rich grapes. Therefore, the objective of this study was to investigate the extent to which malvidin and peonidin attenuated LPS-mediated inflammatory gene expression in primary cultures of newly differentiated human adipocytes. The rationale for using LPS to induce inflammation was because (1) obesity, gut dysbiosis, and impaired barrier function are associated with metabolic endotoxemia [7,8]; and (2) we previously found that serum levels of LPS binding protein were elevated in HF-fed mice and decreased in HF-fed mice supplemented with a combination of methanol extractable and non-extractable polyphenol fractions [20]. The rationale for using primary human adipocytes was because they (1) become inflamed when exposed to LPS; and (2) are a more appropriate (pre)adipocyte model than primary animal cells or cell lines when considering human application.

2. Methods and materials

2.1. *Cell culture supplies, anthocyanidins, and qPCR reagents*

All cell culture ware was purchased from Fisher Scientific (Norcross, GA). Adipocyte medium (AM-1) was purchased from Zen Bio Inc. (Research Triangle Park, NC). Gene-specific primers for toll-like receptor 2 (TLR-2; Hs00610101_m1), TLR-4 (Hs00374581_m1), TNFα (Hs99999043_m1), cyclooxygenase-2 (COX-2; Hs00153133_m1), interferon gamma-induced protein 10 (IP-10; Hs00171042_m1), MCP-1 (Hs00234140_m1), TATA-binding protein (TBP; Hs00427620_m1), IL-8 (Hs00174103), IL-6 (Hs00985639_m1), IL-1β (Hs01555410_m1), TLR4, peroxisome proliferator activated receptor gamma (PPARγ; Hs00234592_m1), stearoyl-CoA desaturase 1 SCD-1; Hs01682761_m1), acetyl-CoA carboxylase 1 (ACC-1; Hs01046047_m1), c-Jun.-NH2 terminal kinase 1 (JNK-1; Hs00177083_m1), JNK-2 (Hs00177102_m1), nuclear factor kappa B (NF-κB)-p65 (Hs00153294_m1), protein tyrosine phosphatase-1B (PTP-1B; Hs00182260_m1), hormone sensitive lipase (HSL; Hs000193510_m1), and adipocyte fatty acid binding protein (aP2; Hs00609791_m1) were purchased from Applied Biosystems (Foster City, CA). Malvidin (CAT# FM44901, ≥95% purity) and peonidin (CAT# FP34147, ≥95% purity) chlorides were purchased from Carbosynth Limited (Berkshire, UK). LPS (CAT# L4391) was purchased from Sigma Chemical Co. (St Louis, MO). All other reagents or chemicals were purchased from Sigma Chemical Co. unless otherwise stated.

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