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Specific bioactive compounds in ginger and apple alleviate hyperglycemia in mice with high fat diet-induced obesity via Nrf2 mediated pathway

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ABSTRACT

Prolonged hyperglycemia activates the formation of advanced glycation end-products (AGEs). Major dicarbonyl compounds such as methylglyoxal or glyoxal are found to be the main precursors of AGEs and *N*(ε)-(carboxymethyl)lysine (CML) found to be predominantly higher in the diabetic population. We hypothesized that phloretin from apple and [6]-gingerol from ginger inhibit formation of AGEs and suppress the receptor for advanced glycation end products (RAGE) via nuclear factor erythroid-2-related-factor-2 (Nrf2)-dependent pathway. Phloretin and [6]-gingerol were supplemented at two different doses to C57BL/6 mice on high fat diet or standard diet for a period of 17 weeks. Phloretin or [6]-gingerol supplementation significantly reduced plasma glucose, alanine aminotransferase, aspartate aminotransferase, AGEs and insulin levels. Phloretin and [6]-gingerol also decreased the levels of AGEs and CML levels, via Nrf2 pathway, enhancing GSH/GSSG ratio, heme oxygenase-1 and glyoxalase 1 in liver tissue. These results suggest that phloretin and [6]-gingerol are potential dietary compounds that can alleviate diabetes-induced complications.

1. Introduction

Type 2 diabetes is characterized by hyperglycemia, in which the physiological environment activates several pathways, such as the hexosamine pathway, polyol pathway, protein kinase C isomers, the advanced glycation end products (AGEs) pathway, and the enediol pathway. These pathways contribute to the pathogenesis of diabetes-related complications (Yamagishi et al., 2012). During prolonged hyperglycemia, non-enzymatic glycation of reducing sugars with amino groups or lipids or nucleic acid generates irreversible AGEs in the form of fluorescent cross-linking (pentosidine [PENT]) and non-fluorescent cross-linking (methylglyoxal-lysine dimers; Nε-carboxymethyl-lysine [CML]) and non-fluorescent non-cross-linking (pyrraline), *via* intermediate dicarbonyl products like glyoxal (GO) and methylglyoxal (MGO) (Peng et al., 2008). MGO is the most volatile dicarbonyl compound reported to be 3–5 folds higher in diabetic patients compared with the healthy population (Khuhawar, Kandhro, & Khand, 2006). It has been shown that Kupffer and sinusoidal endothelial cells in liver are the active sites for AGEs uptake via receptor for AGEs (RAGE) during liver complications (Lee, Hsu, Hsu, & Pan, 2013). The best studied biomarkers of AGEs are CML, *N*ε-carboxyethyl-lysine (CEL), and PENT, which are reported from clinical studies (Perkins et al., 2012). In an *in vivo* study MGO infused to Sprague-Dawley (SD) rats resulted in increased plasma MGO, glutathione (GSH) levels and AGE formation in pancreas, adipose tissue, and skeletal muscle (Dhar, Dhar, Jiang, Desai, & Wu, 2011). It was well reported in cell culture studies that AGEs accumulation in the cells is found to be 14-folds faster in high glucose (30 mM) conditions (Giardino, Edelstein, & Brownlee, 1996). Nrf2 mediated pathway plays an important defense mechanism in adverse physiological conditions (Kumar, Kim, More, Kim, & Choi, 2014). Nrf2 activation mediates Nrf2 release from Keap1, which translocates from the cytosol to the nucleus enhancing the expression of phase II antioxidant enzymes (Itoh et al., 1999; Rushmore, Morton, & Pickett, 1991). To prevent/manage diabetic complications therapeutic strategies like antihyperglycemic agents such as sulfonylurea, thiazolidinedione (Kahn, Cooper, & Del Prato, 2014) or AGE inhibitors (aminoguanidine, carnosine and tenilsetam) have been investigated (Zhu, Zhao, Wang, Ahmedna, & Sang, 2015). However, these drugs have been proven to cause adverse side effects (Cornish, 2014; Huebschmann, Regensteiner, Vlassara, & Reusch, 2006).

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Therefore, interest in developing natural interventions combining higher levels of selectivity and safety for managing diabetes and its symptoms is growing. Many studies have been conducted to examine the antiglycation activity of phytochemicals. It has been established that these phytochemicals possess unique potential characteristics exerting their effects via several mechanisms which vary depending on the molecular structure of the compounds.

Flavonoids are polyphenolic compounds which possess potential health beneficial properties, reducing the risk of chronic diseases, such as cancer, cardiovascular disease, asthma, and diabetes. Flavonoids are found ubiquitously in fruits, vegetables, and beverages (tea, coffee, beer, wine and fruit drinks). Certain flavonoids have shown more effective trapping of AGE formation than aminoguanidine, a well-known AGE inhibitor (Wu & Yen, 2005). For example, luteolin (82%), rutin (78%), (−)-epigallocatechin-3-gal late (69%), and quercetin (65.5%) demonstrated significant inhibitory effects on MGO-mediated AGE formation (Totlani & Peterson, 2005). Bioactive chemical constituents such as (−)-epigal locatechin-3-gallate (EGCG; green tea), phloretin and phloridzin (apple) exhibited greater potency in inhibiting the transformation of arginine residue by MGO and genistein (soybean), by effectively trapping MGO to form mono- and di-MGO adducts which could help in the prevention of diabetic complications (Lv, Shao, Chen, Ho, & Sang, 2011; Sang et al., 2007; Shao et al., 2008). Zhu et al. (2015) reported for the first time, that the major active components of ginger ([6]-shogaol and [6]-gingerol) inhibit the formation of MGO *in vitro* and the that the major active site involved is the α-carbon of the carbonyl group in the side-chain of these active compounds.

The mechanism of bioactivity exerted by these natural and dietary compounds is attributed to an Nrf2-mediated defense pathway which enhances Nrf2 mRNA and protein level or induces its nuclear translocation *via* phosphorylation of serine, which mitigates cardiovascular complications, diabetic nephropathy, regulates liver glucose homeostasis, and inhibits hyperglycemic-induced damage (Jiménez-Osorio, González-Reyes, & Pedraza-Chaverri, 2015). For instance, dietary garlic supplementation to fructose-fed rats was found to enhance the translocation of Nrf2 in the nucleus by decreasing Keap1 protein levels, in the heart (Padiya et al., 2014). Curcumin was also found to elevate the protein levels of Nrf2 and heme oxygenase-1 (HO-1) in cells of normal rat kidney, tubular epithelial cells incubated at high glucose concentrations, promoting anti-fibrotic effects (Zhang et al., 2015). In rats with experimental diabetic nephropathy induced by STZ, curcumin could restore renal function by normalizing levels of glutathione (GSH) as well as the activity of key enzymes such as superoxide dismutase (SOD), glucose-6-phosphate dehydrogenase, lactate dehydrogenase, aldose reductase (AR), sorbitol dehydrogenase and the ratio of fatty acids in the membrane (Suresh Babu & Srinivasan, 1998). It was demonstrated that the α,β-unsaturated carbonyl entity and catechol moiety of [6]-shogaol derivatives separates Keap1-Nrf2 complex activating Nrf2 mediated pathway (Zhu et al., 2016).

Several *in vitro* studies documented the capacity of phloretin and [6]-gingerol to trap dicarbonyl species but to date there is no *in vivo* mechanism for evaluation of their potency (Chen et al., 2014; Shao et al., 2008). The authors previously have reported that bioactive compounds from apple, green tea and ginger have inhibited MGO-induced cell toxicity (Sampath, Zhu, Sang, & Ahmedna, 2016). Hence, the aim of this study was to investigate the potency of phloretin and [6]-gingerol in an *in vivo* model system deigned to simulate high fat diet-induced obesity leading to type 2 diabetes and related diabetic complications.

2. Materials and methods

2.1. Animals and diets

Bioactive [6]-gingerol was isolated from dietary ginger and characterized by members of the research team at North Carolina A&T State University, USA (Sang et al., 2009), while phloretin, was purchased from Sigma Chemicals (St. Louis, MO). All compounds were >95% pure.

All the experiments were performed according to the policies and guidelines of the Institutional Animal Care and Use Committee of the Qatar University, Al-Tarfa, Qatar (approval No. # 014-2013). Animals were purchased from Harlan (San Pietro Al Natisone, IT). Male C57BL/6J mice (4–5 weeks old) were housed in cages in a vivarium under standard conditions and allowed access to food and water *ad libitum*. The diet consisted of either a standard diet (10% energy as fat, 12% protein and 76% carbohydrate, 8604 Teklad Rodent Diet®, Harlan) or a high fat diet (45% energy as fat, 19% protein, and 36% carbohydrate, HFD; TD 110716, Teklad Research Rodent Diet®, Harlan) keeping sucrose content constant in both diets.

2.2. Experimental design

Mice were randomly assigned to one of six treatment groups (n = 11 per group): (i) standard diet, (ii) HFD, (iii) HFD + phloretin [25 mg/kg], (iv) HFD + phloretin [75 mg/kg], (v) HFD + [6]-gingerol [25 mg/kg], (vi) HFD + [6]-gingerol [75 mg/kg]. All compounds were suspended in a 5% DMSO aqueous solution (carrier solution). All the solutions or carrier solution (DMSO) were prepared fresh on the day of intraperitoneal (i.p.) administration three times a week over a period of seventeen weeks. The food and fluid intake and body weight were measured regularly. At Week 17, food-deprived mice (8 h) were anesthetized by administration of ketamine/xylazine (at 100 mg/kg and 10 mg/kg) via i.p. and whole blood was drawn from heart by cardiac puncture and collected in heparinized tubes. Liver, adipose tissue and kidneys were removed, rinsed, weighed; snap frozen and stored at −80 °C. Plasma was isolated by refrigerated centrifugation at 700g for 15 min and stored at −80 °C.

2.3. Fasting blood glucose

Fasting blood glucose was monitored at Week 0, 3, 5, 7, 9, 11, 13, 14, 15, 16 and 17 of the study period. Prior to the blood glucose measurements, mice were deprived of food for 8 h and the cage bedding was changed to minimize the interference from coprophagy. Blood was collected from the saphenous vein and glucose level was measured with a One Touch Ultra® 2 glucose monitor (LifeScan Inc., Milpitas, CA).

2.4. Biochemical analysis of plasma samples

Fasting plasma insulin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were spectrophotometrically measured at Week 17 of the study upon sacrificing of test mice using Infinity ALT and AST Reagent commercial kit (Thermo Scientific, Waltham, MA). The insulin level was measured by ELISA (Millipore, Billerica, MA) as described in the manufacturer's protocol. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to Bose et al. (2008).

$$Insulin\ resistance = \frac{\text{glucose}\ (mg/dL) \times insulin\ (mU/L)}{450}$$