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Citrus limonin glucoside supplementation decreased biomarkers of liver disease and inflammation in overweight human adults

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ABSTRACT

Mixtures of limonoid glucosides demonstrated health benefits in human and animal studies; however, the specific metabolic effects of purified citrus limonin glucoside (LG) in humans are unknown. We determined effects of LG on circulating biomarkers of chronic inflammatory diseases such as nonalcoholic fatty liver disease (NAFLD), diabetes, CVD, and cancer in a cross-over, placebo controlled, double-blind study in overweight/obese individuals. LG had no specific adverse effects. It did not alter circulating concentrations of blood lipids, lipoproteins or their particle sizes, glucose, insulin, hematological parameters, and markers of inflammation except MMP-9 and TNF-α which were decreased by 38.7% and 10.7%, respectively. LG significantly decreased concentrations of liver proteins: gamma-glutamyl transferase (33.8%), alanine aminotransferase (13.1%), alkaline phosphatase (10.1%), and complement C3 (6.4%). Since liver enzymes are elevated in metabolic syndrome, NAFLD, diabetes, CVD, and chronic kidney disease and liver cancer, LG may be useful in the prevention and/or treatment of those diseases.

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1. Introduction

Human epidemiological studies indicate that increased consumption of fruits and vegetables is associated with reduced risk of diabetes, cardiovascular disease (CVD), cancer and other chronic diseases (Joshipura et al., 2001; Kris-Etherton et al., 2002; Ness & Powles, 1997). Several human intervention studies investigated the health benefits of orange juice and reported improved lipid profile (Aptekmann & Cesar, 2010; Kurowska

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Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C3, complement component 3; CBC, complete blood cell count; CMP, comprehensive metabolic panel; CPE, citrus peel extract; CRP, C reactive protein; CVD, cardiovascular disease; GGT, gamma-glutamyl transferase; LG, limonin glucoside; MAP 1.6, multi-analyte panel 1.6; NAFLD, nonalcoholic fatty liver disease; PSA, prostate specific antigen; SAP, serum amyloid P; T2DM, type 2 diabetes mellitus; WHNRC, Western Human Nutrition Research Center

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et al., 2000), blood pressure, endothelial cell functions (Buscemi et al., 2012; Diaz-Juarez et al., 2009) and inflammation (Dalgard et al., 2009; Deopurkar et al., 2010; Ghanim et al., 2010). In other human intervention studies, orange juice or citrus flavonoids did not alter blood lipids or markers of inflammation (Demonty et al., 2010; Devaraj, Jialal, & Vega-Lopez, 2004). Inconsistencies among the results from these studies may have resulted from differences in many factors, including the amount of juice used, the duration of the study, the specific variety of oranges, specific bioactive components and their concentrations, the study population, and other factors.

In studies conducted with mice, orange juice prevented the high fat diet-induced weight gain and nonalcoholic fatty liver disease (NAFLD) (Salamone et al., 2012; Titta et al., 2010). In other animal studies, citrus peel extracts (CPE) improved liver functions, prevented obesity, increased adipose tissue lipolysis, and β oxidation (Green, Wheatley, McGrowder, Dilworth, & Asemota, 2013; Kang et al., 2012; Lee et al., 2011; Raasmaja et al., 2013; Tsujita & Takaku, 2007). Other preparations made from orange juice, seeds, peels, and molasses were reported to possess antioxidant and anti-inflammatory effects, and reduced the growth of cancer cells in several in vivo animal and in vitro cell culture models (Kim, Jayaprakasha, & Patil, 2013; Manners, 2007; Tundis, Loizzo, & Menichini, 2014). Overall, results from these reports suggest that orange juice and other citrus products have the potential to prevent several chronic human diseases.

Orange juice and CPE contain a number of health promoting factors including ascorbic acid, flavonoids, and limonoids. Results from animal and cell culture studies conducted with purified or enriched individual components prepared from citrus juice, molasses, seeds, and peel have demonstrated health benefits for each of the above three citrus ingredients (Kim et al., 2013; Manners, 2007; Marti, Mena, Canovas, Micol, & Saura, 2009; Toh, Tan, Lim, Lim, & Chong, 2013; Tundis et al., 2014). A few intervention studies monitored the health benefits of ascorbic acid and flavonoids in human subjects; however, to the best of our knowledge there are no published reports regarding the long term safety and health benefits of purified limonoids in humans. Limonoids are unique, naturally occurring, highly oxygenated triterpenoid compounds that have significant biological activity (Kim et al., 2013; Manners, 2007; Tundis et al., 2014). Citrus limonoids appear in high concentrations in citrus juice and tissues as water-soluble limonoid glucosides (350–400 ppm) (Ozaki et al., 1995) or in seeds as water insoluble limonoid aglycones (>1% dry wt.) (Fong, Hasegawa, Miyake, & Ozaki, 1993; Ozaki et al., 1995). Certain limonoid aglycones, in particular limonin, are responsible for the development of delayed bitterness in citrus (Maier, Bennett, & Hasegawa, 1977). In a previous human study we reported that a single dose of purified LG served as a drink (0.25, 0.5, 1.0, or 2.0 g) was absorbed and metabolized by human subjects; highest blood limonin concentration was reached within 6 h of consumption and it was detectable at 24 h in 30% of the subjects (Manners, Jacob, Breksa, Schoch, & Hasegawa, 2003). Chemical structures of limonin and limonin glucoside (LG) are presented in Fig. 1.

Based upon the results from previous studies, we hypothesized that long term consumption of LG by human subjects would reduce the plasma concentrations of markers for chronic inflammatory diseases. In a cross-over design, placebo

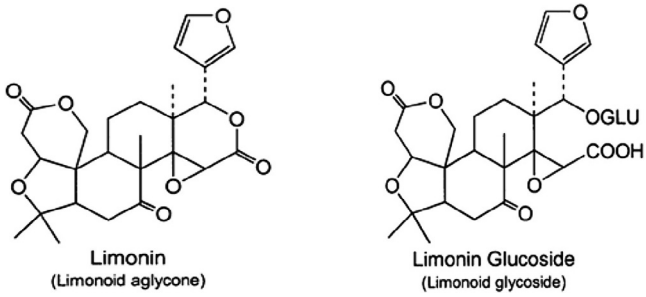

Fig. 1 – Chemical structures of limonin (limonoid aglycone) and limonin glucoside (limonoid glucoside).

controlled, double-blind study we examined the effects of LG consumption on blood lipids, lipoproteins, liver enzymes, and risk factors for diabetes, CVD, and cancer. Primary endpoints of interest were blood lipids, liver enzymes, inflammation and insulin resistance, while secondary endpoint was the adverse effects of LG. Response variables selected were based on the results with orange juice, CPE or isolated citrus components in human and animal studies. Standard clinical laboratory analyses and targeted proteomic arrays were used to determine the effects of LG consumption on the risk factors for chronic diseases.

2. Materials and methods

2.1. *LG and beverages*

The LG used in this study was isolated from molasses and orange juice as described (Breksa & Dragull, 2009; Breksa, Dragull, & Wong, 2008) with purity in excess of 99.93%. This was a pilot study and the number of study subjects was limited by the amount of purified LG available; high purity LG was necessary to conduct this study in order to determine LG-specific metabolic effects. The dose of LG (about 6.5 mg/kg) for this study was based on our previous human study (Manners et al., 2003) and results from animal studies. LG concentrations of more than 100 times/kg body weight used in this study were reported to be safe in studies with mice and hamsters (Guthrie, Morley, Hasegawa, Manners, & Vandenberg, 2000; Miller, Taylor, Berry, Zimmerman, & Hasegawa, 2000).

2.2. *Subjects*

This study was conducted between April 2007 and March 2010 at the Western Human Nutrition Research Center (WHNRC), Davis, CA, and was approved by the Human Subjects Review Committee of the University of California, Davis. Potential study subjects were screened for health history, lifestyle, dietary habits, physical and clinical evaluation including blood and urine analysis. Subjects displaying signs of infection who consumed more than 2 oz (59 mL) of alcohol/day or more than 32 oz (946 mL) of orange or grape fruit juice/day and who smoked were excluded. Also excluded were those taking cholesterol lowering drugs, prohibited substances, or who had conditions that made blood drawing by venipuncture unusually difficult. Subject