Original Article

MOLECULAR METABOLISM

A polyphenol-rich cranberry extract reverses insulin resistance and hepatic steatosis independently of body weight loss

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ABSTRACT

Objective: Previous studies have reported that polyphenol-rich extracts from various sources can prevent obesity and associated gastro-hepatic and metabolic disorders in diet-induced obese (DIO) mice. However, whether such extracts can reverse obesity-linked metabolic alterations remains unknown. In the present study, we aimed to investigate the potential of a polyphenol-rich extract from cranberry (CE) to reverse obesity and associated metabolic disorders in DIO-mice.

Methods: Mice were pre-fed either a Chow or a High Fat-High Sucrose (HFHS) diet for 13 weeks to induce obesity and then treated either with CE (200 mg/kg, Chow + CE, HFHS + CE) or vehicle (Chow, HFHS) for 8 additional weeks.

Results: CE did not reverse weight gain or fat mass accretion in Chow- or HFHS-fed mice. However, HFHS + CE fully reversed hepatic steatosis and this was linked to upregulation of genes involved in lipid catabolism (e.g., PPARα) and downregulation of several pro-inflammatory genes (eg, COX2, TNFα) in the liver. These findings were associated with improved glucose tolerance and normalization of insulin sensitivity in HFHS + CE mice. The gut microbiota of HFHS + CE mice was characterized by lower Firmicutes to Bacteroidetes ratio and a drastic expansion of *Akkermansia muciniphila* and, to a lesser extent, of *Barnesiella spp*, as compared to HFHS controls.

Conclusions: Taken together, our findings demonstrate that CE, without impacting body weight or adiposity, can fully reverse HFHS diet-induced insulin resistance and hepatic steatosis while triggering *A. muciniphila* blooming in the gut microbiota, thus underscoring the gut-liver axis as a primary target of cranberry polyphenols.

Keywords *Akkermansia*; *Barnesiella*; Obesity; *Vaccinium macrocarpon*; Flavonoids

1. INTRODUCTION

Obesity has reached pandemic proportions worldwide, significantly contributing to reduce life quality and lifespan at a global scale [1]. This condition is characterized by abnormal and excessive fat accumulation and is influenced by both genetic and environmental determinants. While several genetic loci have been associated with obesity, they explain only a fraction of the total variance within populations; moreover, genes deemed obesity-predisposing interact with environmental factors to regulate, for instance, satiety and energy expenditure [2]. Among the environmental determinants of obesity and its associated dysmetabolic conditions, dietary habits play a central role. Diet also strongly influences our "other genome" (*i.e.*, the metagenome), modeling gut microbial community structure [3] and impacting host metabolism and energy partitioning [4]. Research conducted throughout the last decade has revealed a clear association between obesity and gut microbial dysbiosis, which is generally characterized by a reduction in bacterial richness and by major taxonomic and functional changes [5].

The consumption of fiberless diets rich in simple sugars and saturated fat (often referred to as Western diets) generates well-known detrimental metabolic consequences, leading to insulin resistance and glucose intolerance in the early-term, which later evolves to overt obesity, type 2 diabetes, and cardiovascular complications. NAFLD and NASH are highly prevalent diseases occurring in the setting of obesity and type 2 diabetes; they may eventually progress to hepatocellular

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carcinoma and contribute to dysregulate glucose and lipid homeostasis [6]. The gut exerts major influences on liver physiology as both organs are anatomically and functionally connected by means of the portal circulation, but also since bacteria and bacteria-derived molecules can translocate from the gut to the liver and can potentially contribute to diet-induced insulin resistance and liver disease [7,8].

Plant-rich diets are abundant in fruits and vegetables and strongly linked to lean and healthy phenotypes [9], which prompts the search for bioactive phytonutrients to treat or prevent obesity and its related dysmetabolic conditions. The use of polyphenol-rich fruit extracts or isolated polyphenols as strategies to alleviate obesity-linked diseases have been demonstrated in humans [10,11] and in animal models [12,13], but the mechanisms of action are not yet fully elucidated. Several dietary polyphenols are generally poorly bioavailable and build up in the colon, where they are modified by gut microbial enzymes and, in turn, reshape gut microbial communities [14]. We have previously demonstrated that a polyphenol-rich cranberry extract prevents diet-induced obesity in high fat high sucrose-fed mice, and these findings were linked to improved gut-liver homeostasis and expansion of *Akkermansia muciniphila* population in the gut microbiota [12]. Similar effects were reported by others using a polyphenol-rich extract of concord grape [15] and apple proanthocyanidins [16], but no studies have yet tested whether polyphenols can reverse an already established obesity and more severe metabolic alterations, including hepatic steatosis and inflammation. In the present study, we investigated the potential of a polyphenol-rich cranberry extract to reverse an already established obesity, insulin resistance, and NAFLD, and whether such effects may be linked to the reshaping of the gut microbiota and blooming of *A. muciniphila*, a well-known target of food polyphenols.

2. MATERIAL AND METHODS

2.1. Animals

All animal experiments reported in this manuscript comply with the Animal Research: Reporting of *In Vivo* experiments (ARRIVE) guidelines. Eight week-old C57Bl/6J male mice (Jackson, USA) were housed 2−3 animals per cage, kept on Sani-chips bedding and in controlled environment (12 h daylight cycle, lights off at 18:00) with food and water *ad libitum* in the animal facility of the Québec Heart and Lung Institute (Québec, Canada). After two weeks of acclimatization, mice were pre-fed either a healthy Chow (Teklad 2018, Harlan) or a High-Fat/High-Sucrose (HFHS) diet for 21 weeks. Diet composition was previously published [12] and, although the abbreviation HFHS particularly refers to the enriched presence of saturated fat and simple sugars, it is important to stress that the lack of soluble fibers is a major obesogenic component of this diet [17]. During the last 8 weeks of the study (i.e. from the beginning of week 13 to the beginning of week 21), control groups (Chow, $n = 8$ and HFHS, $n = 8$) were orally administered the animal facility's drinking water whereas the treated groups (Chow + CE, $n = 11$ and HFHS + CE, $n = 10$) received a cranberry extract (CE, 200 mg/kg, Nutra Canada, Québec, Canada). The polyphenolic profile of CE was published elsewhere [12,18]. Body weight gain and food intake were assessed twice weekly. At week 21, animals were anesthetized in chambers saturated with isoflurane and then sacrificed by cardiac puncture. Organs and tissues were carefully collected and blood was drawn in tubes containing 2 IU of heparin and immediately centrifuged in order to separate plasma from cells. All interventions were carried out during the animals' light cycle. All procedures strictly followed the National Institutes of Health (NIH)'s Guide for the Care and Use of Laboratory Animals and were previously approved by the Laval University Animal Ethics Committee.

2.2. Glucose homeostasis

At week 17, mice were fasted for 6 h and insulin tolerance tests (ITT) were performed after intraperitoneal injections of insulin (0.75 UI/kg body weight). Glycemia was measured with an Accu-Check glucometer (Bayer) before (0 min) and after (10, 20, 30, 60, and 90 min) insulin injection. At the end of week 19, mice were fasted overnight (12 h) and oral glucose tolerance tests were carried out (OGTT, 1 g of glucose/kg body weight). Blood was collected before (0 min) and after (15, 30, 60, 90, and 120 min) glucose challenge for glycemia determination. Blood samples (~30 μL) were collected at each time point during OGTT and insulinemia was determined using an ultra-sensitive ELISA kit (Alpco, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the following formula: fasting insulinemia (μUI/mL) x fasting glycemia (mM)/22.5.

2.3. Oil red O staining

During necropsies, mouse livers were embedded in Tissue-Tek® OCT, immediately snap-frozen in liquid nitrogen, and stored at −80 °C. Staining of neutral lipids was based on the methods described by Mehlem et al. with some adaptations [19]. Briefly, 12 μm liver sections were allowed to equilibrate at room temperature for 5 min and then post-fixed with a Formalin (10%)/Calcium (2%) solution for 15 min. The sections were then incubated with oil red O (ORO) working solution at room temperature for 5 min, followed by a 5- minute clearing in 60% isopropyl alcohol and a counterstaining of 15 s with Mayer's hematoxylin.

2.4. Q-PCR and antioxidant enzymes

Gene mRNA expression analysis by q-PCR and quantification of antioxidant enzymes were carried out as previously described [12]. Primer sequences used were: (5′→ 3′) PPARα F- CGACCTGAAAGATTCGGAAA, R- GGCCTTGACCTTGTTCATGT; PPARγ F- CAGGCCTCATGAAGAACCTT, R- GCATCCTTCACAAGCATGAA; SREBP1c F- GACCCTACGAAGTGCACACA, R- TCATGCCCTCCATAGACACA; SREBP2 F- CGACCAGCTTTCAAGTCCTG, R- CCTGTACCGTCTGCACCTG; LXRα F- GGAGTGTCGACTTCGCAAAT, R- CTTGCCGCTTCAGTTTCTTC; LXRβ F- AAACGATCTTTCTCCGACCA, R- ATGGCTAGCTCGGTGAAGTG; COX2 F- GCTGTACAAGCAGTGGCAAA, R- CCCCAAAGATAGCATCTGGA; TNFα F- GAACTGGCAGAAGAGGCACT, R- AGGGTCTGGGCCATAGAACT; NFκB F- AGCTTCACTCGGAGACTGGA, R- ACGATTTTCAGGTTGGATGC; IκB F- TGGCCAGTGTAGCAGTCTTG, R- GACACGTGTGGCCATTGTAG.

2.5. Cecal mucin determination

Cecal contents were collected at week 21, snap-frozen in liquid nitrogen and stored at −80 °C. Cecal feces were freeze-powdered and the presence of mucins was determined using a fluorometric assay kit (Cosmo Bio, Japan) that discriminates O-linked glycoproteins (mucins) from N-linked glycoproteins.

2.6. Fecal samples

Fecal samples were freshly collected at baseline (week 13) and week 21 and immediately stored at −80 °C. Bacterial genomic DNA was extracted from approximately 50 mg of fecal material collected from each cage. Samples were resuspended in lysis buffer containing 20 mg/ml lysozyme and incubated for 30 min at 37 °C. Further lysis was performed by adding 10% SDS and proteinase K to 350 μg/ml followed by incubation for 30 min at 60 °C. Samples were homogenized using a bead beater and 0.1 mm zirconium beads and then

