SUPPLEMENT

Impact of Cranberries on Gut Microbiota and Cardiometabolic Health: Proceedings of the Cranberry Health Research Conference 2015[1–3]

Jeffrey B Blumberg,[4]* Arpita Basu,[5] Christian G Krueger,[6,7] Mary Ann Lila,[8] Catherine C Neto,[9] Janet A Novotny,[10] Jess D Reed,[6,7] Ana Rodriguez-Mateos,[11] and Cheryl D Toner[12,13]

[4]Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA; [5]Oklahoma State University, Stillwater, OK; [6]Complete Phytochemical Solutions, LLC, Cambridge, WI; [7]University of Wisconsin-Madison, Madison, WI; [8]North Carolina State University, Kannapolis, NC; [9]University of Massachusetts at Dartmouth, Dartmouth, MA; [10]USDA Beltsville Human Nutrition Research Center, Beltsville, MD; [11]University of Düsseldorf, Düsseldorf, Germany; [12]The Cranberry Institute, Carver, MA; and [13]CDT Consulting, LLC, Herndon, VA

ABSTRACT

Recent advances in cranberry research have expanded the evidence for the role of this *Vaccinium* berry fruit in modulating gut microbiota function and cardiometabolic risk factors. The A-type structure of cranberry proanthocyanidins seems to be responsible for much of this fruit's efficacy as a natural antimicrobial. Cranberry proanthocyanidins interfere with colonization of the gut by extraintestinal pathogenic *Escherichia coli* in vitro and attenuate gut barrier dysfunction caused by dietary insults in vivo. Furthermore, new studies indicate synergy between these proanthocyanidins, other cranberry components such as isoprenoids and xyloglucans, and gut microbiota. Together, cranberry constituents and their bioactive catabolites have been found to contribute to mechanisms affecting bacterial adhesion, coaggregation, and biofilm formation that may underlie potential clinical benefits on gastrointestinal and urinary tract infections, as well as on systemic anti-inflammatory actions mediated via the gut microbiome. A limited but growing body of evidence from randomized clinical trials reveals favorable effects of cranberry consumption on measures of cardiometabolic health, including serum lipid profiles, blood pressure, endothelial function, glucoregulation, and a variety of biomarkers of inflammation and oxidative stress. These results warrant further research, particularly studies dedicated to the elucidation of dose-response relations, pharmacokinetic/metabolomics profiles, and relevant biomarkers of action with the use of fully characterized cranberry products. Freeze-dried whole cranberry powder and a matched placebo were recently made available to investigators to facilitate such work, including interlaboratory comparability. *Adv Nutr* 2016;7(Suppl):759S–70S.

Keywords: cranberry, proanthocyanidins, microbiome, cardiometabolic, antimicrobial

Introduction

Dietary guidance is consistent in recommending greater consumption of fruit and vegetables to promote health. Indeed, the 2015 Dietary Guidelines Advisory Committee report noted that greater fruit and vegetable intake was the only characteristic of dietary patterns that was consistently identified in their report in every conclusion statement across health outcomes (1). Although the report does not recommend specific types of fruit, there has been a growing body of evidence that the phytochemical composition of berry fruit may differentiate them from other fruits and underlie some of their putative benefits. Recent advances in analytical methods have improved the characterization of polyphenols in berry fruit and subsequently the data in food-composition and metabolomics databases that are essential for observational studies. Furthermore, the development of a standard reference material (SRM)[14] and matched placebos for use in clinical trials has provided an important and innovative component for the design and conduct of new randomized clinical trials. This review, prepared from the proceedings of the Cranberry Health Research Conference held in conjunction with the Berry Health Benefits Symposium in Madison, Wisconsin, 12–15 October 2015, focuses particularly on advances in the field during the last 5 y with regard to the gut microbiota and cardiometabolic health.

Cranberries and the Gut Microbiota

Molecular mechanisms. Much of the attention regarding the impact of cranberries on the gut microbiota has been directed to studies of the effect of cranberry extracts or juice on uropathogens and urinary tract infections (UTIs) (2, 3). However, this focus has expanded to encompass a broader range of the cranberry's antimicrobial, antifungal, and antiviral actions against *Helicobacter pylori* (4–6), *Streptococcus mutans* (7), *Porphyromonas gingivalis* (8), *Staphylococcus aureus* (9), *Pseudomonas aeruginosa* (10), *Cryptococcus neoformans* (6),

Haemophilus influenzae (11), *Candida albicans* (12, 13), and extraintestinal pathogenic *Escherichia coli* (ExPEC) (14). Cranberry constituents, particularly the proanthocyanidins, flavonols, and hydroxycinnamic acids, may act against these pathogens by preventing bacterial adhesion and coaggregation, decreasing biofilm formation and/or reducing inflammation rather than via bactericidal activity. This expanding body of research includes in vitro, ex vivo, and animal studies that have suggested potential clinical effects and have helped to elucidate mechanisms of action as well as human studies that have shown physiologic effects (3–5, 14).

The antimicrobial properties of cranberry proanthocyanidins have been generally associated with their degree of polymerization (DP) and ratio of A- to B-type linkages. For example, by using an in vitro broth microdilution assay for growth inhibition of several yeast species, treatment of cultures with cranberry fractions of varying composition showed that cranberry proanthocyanidin fractions with a larger DP were found to be more effective than those with a smaller DP at inhibiting the growth of *Candida* spp. (12). In comparing primarily A-type proanthocyanidins from cranberries with primarily B-type proanthocyanidins from apples, Feliciano et al. (15) found that, although both increased agglutination and reduced epithelial cell invasion by ExPEC, the strongest effects were associated with a higher percentage of A-type linkages. This observation is consistent with other research that showed that A-type proanthocyanidins interact most strongly with bacterial virulence factors and more effectively decrease bacterial motility (16, 17).

Microbiota biofilm. The prevention of biofilm formation, an early step in the development of infection, through interference in the coaggregation of bacteria is a well-documented antimicrobial mechanism of cranberry proanthocyanidins. The extensively hydroxylated structure of proanthocyanidins encourages intermolecular hydrogen bonding, allowing smaller molecules to aggregate and interact with receptors on cell surfaces. Thus, many studies of high-molecular-weight nondialyzable material from cranberry juice concentrate reveal potent antiadhesion activity with microbial species, including those found in the oral cavity, stomach, small intestine, and colon (6, 11, 14, 18, 19). However, although purified cranberry proanthocyanidins are more effective in some antimicrobial assays than are crude or mixed extracts, several studies suggest that other compounds in cranberry possess antibacterial properties that alone or in combination with proanthocyanidins may enhance overall protection against infection. For example, Pinzón-Arango et al. (20) exposed *E. coli* to cranberry juice cocktail (CJC) or cranberry proanthocyanidins over 48 h and found that the proanthocyanidins reduced whereas the CJC completely eliminated biofilm formation. Candidate CJC constituents may include nonphenolic compounds such as isoprenoids like ursolic acid and xyloglucans, hemicellulose oligosaccharides found in high-molecular-weight nondialyzable fractions (21). Hotchkiss et al. (22) found that arabinoxyloglucans isolated from pectinase-treated cranberry hulls prevented the adhesion of *E. coli* strains to bladder and colonic epithelial cells in vitro.

Bacterial adhesion to cells and other surfaces involves basic physical forces such as electrostatic and steric interactions, van der Waals forces, and surface charge, as well as both specific and nonspecific interactions of surface proteins and carbohydrates such as glucans, adhesins, and sugar-specific lectins (23–25). Using atomic force microscopy, Liu et al. (26) found that exposure to cranberry juice decreased the adhesion forces of P-fimbriated *E. coli* (HB101pDC1) and altered the conformation and length of the P-fimbriae. Pinzón-Arango et al. (24) found that these fimbrial changes were reversible, even for cultures grown in the presence of cranberry juice. de Llano et al. (27) showed the efficacy of colonic metabolites of cranberry polyphenols, including hydroxylated benzoic and phenylacetic acids, in inhibiting the adhesion and biofilm formation of uropathogenic *E. coli* to bladder epithelial cells, a relation that underscores the critical need to elucidate the role of the gut microbiota in transforming cranberry polyphenols to bioactive and bioavailable compounds.

Gut microbiota metabolism and function. The gut microbiota is now appreciated as a critical factor in nutrition and health, influencing the bioavailability and metabolism of food components and affecting body systems, including brain and immune functions. The integrity of the gut mucosal barrier is essential for maintaining a chemical and physical barrier against food, environmental antigens, and microbes (28, 29). Goblet cells migrate up the villi after differentiating from crypt stem cells and turn over with the epithelial layer every 3–5 d. Goblet cells secrete mucins, particularly mucin 2 (Muc-2), that contribute substantially to

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*To whom correspondence should be addressed. E-mail: jeffrey.blumberg@tufts.edu.

[14] Abbreviations used: AIEC, adherent-invasive *Escherichia coli*; BP, blood pressure; CAD, coronary artery disease; CEACAM, carcinoembryonic antigen-related cell adhesion molecule; CJC, cranberry juice cocktail; c-PAC, cranberry proanthocyanidin standard; CRP, C-reactive protein; CVD, cardiovascular disease; DP, degree of polymerization; EEN, elemental enteral nutrition; ExPEC, extraintestinal pathogenic *Escherichia coli*; FimH, protein FimH; FMD, flow-mediated vasodilation; FWCP, freeze-dried whole-cranberry powder; Muc-2, mucin 2; Nrf2, nuclear factor E2-related factor 2; PapG, fimbrial adhesin PapG; ProA2, procyanidin A2; sIgA, secretory IgA; SRM, standard reference material; STAT6, signal transducers and activators of transcription 6; Th2, T-helper 2; T2D, type 2 diabetes; UTI, urinary tract infection.

the maintenance of mucosal integrity (30). Mucin secretion is regulated by a complex network of cholinergic stimulation and T-helper 2 (Th2) cytokines IL-4 and IL-13 (31–35).

Dysfunction of the gut barrier and dysbiosis have been associated with typical Western diets high in saturated fat and low in fiber and phytochemicals, patterns that may lead to increased permeability of bacterial LPS and a pathogen-associated molecular pattern that stimulates innate immune responses in macrophages, neutrophils, endothelial cells, and adipocytes. LPS plays a role in acute infection-related inflammatory responses and is found in blood and tissues with both postprandial and chronic inflammation (36–39). With the use of mice (CEABAC10) that express human carcinoembryonic antigen-related cell adhesion molecules (CEACAMs), Martinez-Medina et al. (37) found that a high-fat, high-sugar diet increased intestinal permeability and TNF-α secretion, which resulted in a greater ability of adherent-invasive *E. coli* (AIEC) to colonize gut mucosa and induce inflammation. This diet also induced gut barrier dysfunction reflected by reduced levels of Muc-2 mRNA, increased permeability of 4-kDa fluorescein isothiocyanate-dextran, and decreased numbers of goblet cells. It is worth noting that AIEC may contribute substantially to the etiology of Crohn disease, an inflammatory bowel disease in which CEACAM6 is overexpressed on the apical surface of ileum epithelium (40). Furthermore, variant AIEC type 1 pili adhere to CEACAM6, a key step in the colonization of the ileum and chronic inflammation present in Crohn disease. In addition, after feeding mice a high-fat, high-sugar diet, Anhê et al. (41) reported that the addition of a cranberry extract attenuated the consequent chronic inflammation associated with gut barrier dysfunction, including reductions in plasma LPS, cyclooxygenase-2, and TNF-α. Furthermore, the ratio of NF-κB to inhibitor κB was significantly lower in the jejunal tissue of the mice fed cranberry extract relative to the mice fed the high-fat, high-sugar diet. Also suggesting the capacity of cranberry polyphenols to reduce intestinal oxidative stress and inflammation, in vitro experiments with Caco-2/15 intestinal cells by Denis et al. (42) revealed positive but differential effects of low-, medium-, and high-molecular-mass polyphenols from cranberries on oxidative stress, proinflammatory cytokines, NF-κB activation, and nuclear factor E2-related factor 2 (Nrf2) downregulation, as well as PPAR-γ coactivator 1α.

Interestingly, the effects of high-fat, high-sugar diets on gut barrier function in mice are similar to those observed in animal models of parenteral nutrition and elemental enteral nutrition (EEN) (43, 44). EEN induces dysfunction of gut-associated lymphoid tissue, including decreased lymphocytes in Peyer's patch and reduced tissue Th2 cytokines, and suppresses mucosal barrier function when compared with normal nutrition (43, 45–48). The addition of cranberry proanthocyanidins to EEN was found to increase ileal tissue IL-4 and IL-13 concentrations, goblet cell number and size, and the secretion of intestinal Muc-2, attenuating the impairment of the mucosal barrier integrity after EEN alone (44). Pierre et al. (43) reported that the addition of cranberry proanthocyanidins significantly supported other indexes of gut-associated lymphoid tissue function impaired by EEN in mice, indicated in part by decreased Peyer's patch lymphocytes and lower concentrations of tissue Th2 cytokines. Cranberry proanthocyanidins also helped to restore the EEN-induced decreases in polymeric Ig receptor, a transport protein involved in enterocyte transcytosis of secretory IgA (sIgA) from B cells in the lamina propria into the intestinal lumen. EEN decreases in luminal concentrations of sIgA were attenuated by cranberry proanthocyanidins; intestinal sIgA opsonizes bacterial antigens such as the virulence factors of pathogenic *E. coli*, rendering them less viable and more susceptible to killing by lymphocytes. The addition of cranberry proanthocyanidins also significantly prevented EEN-induced decreases in tissue IL-4 and phosphorylated signal transducers and activators of transcription 6 (STAT6).

Clinical studies are necessary to determine whether the results from these mouse models can be translated to the capacity of cranberry phytochemicals to reduce diet-induced intestinal inflammation in humans. Interestingly, there is limited evidence suggesting an effect of cranberry on systemic immune function in humans, which may be partly mediated via gut metabolism of cranberry polyphenols. For example, a randomized, double-blind placebo-controlled study documented increased ex vivo proliferation of γδ-T cells, immune cells located within the epithelium of the gastrointestinal and reproductive tracts, after the consumption of a cranberry beverage for 10 wk (49).

ExPEC in the gut. Although ExPEC generally do not cause acute enteric disease, their colonization in the gut increases the risk of subsequent extraintestinal infection, including UTIs, septicemia, surgical wound infections, and neonatal meningitis (50, 51). ExPEC attach to and invade epithelial cells through adhesins expressed on type I pili (protein FimH) and P fimbriae (fimbrial adhesin PapG) and persist inside the host cell in vacuoles where they may evade immune detection. ExPEC, uropathogenic *E. coli*, and the AIEC associated with Crohn disease have similar virulence factors and are within the same *E. coli* phylogroups (B2 and D) (40). These phylogroups differ from enteropathogenic *E. coli* and Shiga toxin–producing *E. coli*, such as O157:H7, because enteropathogenic *E. coli* and Shiga toxin–producing *E. coli* cause acute intestinal disease and produce attaching and effacing lesions of the intestinal epithelium. Gut colonization by ExPEC is a likely cause of a chronic inflammatory state because ExPEC may evade immune detection and colonize enterocytes. The continuous presence of *E. coli* LPS in the gut mucosa may cause chronic intestinal inflammation. Although ExPEC have a meaningful impact on public health via their consequences on morbidity and mortality, they have not received concordant attention because they have been highly susceptible to antibiotics. However, 20–45% of ExPEC have become resistant to first-line antibiotics such as cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole (52, 53).

Thus, it is becoming critical to appreciate and further investigate the potential role for dietary bioactive components in reducing such infections.

Recently, Feliciano et al. (15) showed that A-type proanthocyanidins have greater bioactivity than B-type proanthocyanidins for increasing ExPEC agglutination and decreasing their invasion (and subsequent colonization) of gut epithelial cells, an important observation for the elucidation of the effect of cranberry proanthocyanidins on UTIs. As suggested by Feliciano et al. (15) and other studies described above, decreasing intestinal colonization and associated inflammation may be achieved by usual serving sizes of cranberry juice without the requirement for absorption of its constituent proanthocyanidins into the circulation or their appearance in the urine. It is important to note that recent randomized clinical trials have confirmed and extended the body of evidence showing cranberry's bacterial antiadhesion activity in urine ex vivo (3, 54), its capacity to reduce the recurrence of UTIs (55), and its therapeutic efficacy in preventing UTIs in gynecologic surgery patients after catheter removal (56). Nonetheless, additional research that uses similarly relevant ex vivo and in vivo models can be used to substantiate the structure-function relation of A-type proanthocyanidins to intestinal and extraintestinal infections and to develop preventive and therapeutic strategies against increasingly antibiotic-resistant classes of pathogens (57, 58). Such an effort could be advanced by the availability of a cranberry SRM as discussed below.

Cranberries and Cardiometabolic Health

A limited but growing number of clinical research studies (59–72) have focused on cardiometabolic health (**Tables 1** and **2**). The most commonly examined risk factors for cardiometabolic conditions in these studies have included serum lipid profiles, blood pressure (BP), endothelial function, glucoregulation, and a variety of biomarkers of inflammation and oxidative stress. Although the results of this research have generally been promising, a clear and consistent picture of this emerging area is confounded by sometimes marked differences in the cranberry products (cranberry juices, dried cranberries, and cranberry extracts) and doses used, as well as the characteristics of the study populations (2, 73). Although few animal model studies have examined this topic, Kim et al. (74–76) reported that 5% cranberry powder added to atherogenic diets with or without intraperitoneal LPS administration produced positive effects on serum lipids, proinflammatory cytokines, oxidative stress, and antioxidant capacity in rodents.

Lipid profile. Early reports by Ruel et al. (66–68) found that interventions with low-calorie cranberry juice were associated with increases in plasma HDL cholesterol as well as with reductions in plasma oxidized LDL cholesterol, adhesion molecules, and matrix metalloproteinase 9. Lee et al. (59) showed a reduction in both LDL cholesterol and total cholesterol in a trial in 30 patients with type 2 diabetes (T2D) who consumed cranberry extract supplements daily for 12 wk. In a double-blind, placebo-controlled trial, Shidfar et al. (60) reported that 58 men with T2D who consumed 1 cup cranberry juice/d for 12 wk experienced decreases in apoB and increases in apo A-1 and paraoxonase-1, although data on LDL, HDL, and total cholesterol were not reported. In an 8-wk randomized clinical trial of low-calorie cranberry juice consumption by 56 healthy adults, Novotny et al. (61) found that TGs were significantly decreased in the cranberry group whereas other elements of the lipid profile were unchanged.

BP. Previous studies of the effect of cranberry juice on BP suggested a potential benefit on BP (67, 69). More recent studies also examined changes in BP after cranberry intake (59, 61–65). The durations of these studies ranged from 1 to 4 mo and tested intakes of total polyphenols ranging from 346 to 835 mg/d; and study populations were heterogeneous, including subjects with obesity, metabolic syndrome, T2D, coronary artery disease (CAD), and risk factors for cardiovascular disease (CVD), as well as healthy volunteers. With daily doses of CJC increasing every 4 wk from 0–125 to 250–500 mL, systolic BP decreased by 3 mm Hg with the 500-mL intervention compared with baseline in obese men (67). Of the more recent studies, only the study performed in healthy individuals and with the lowest dose of polyphenols showed an improvement in BP, with a reduction of 4.7 mm Hg in diastolic BP achieved after 8 wk of daily supplementation (61).

Endothelial function. Endothelial dysfunction, often characterized by a decrease in nitric oxide production and impaired flow-mediated vasodilation (FMD), is a critical factor underlying the development and progression of atherosclerosis (77). In a randomized controlled trial with a crossover design, Dohadwala et al. (63) found that daily supplementation with cranberry juice for 4 wk did not improve FMD or peripheral artery tonometry in 44 patients with CAD, although an uncontrolled pilot study in a subset of the same population showed a modest improvement in FMD 4 h after an acute dose of cranberry juice. In a 4-wk trial with a cranberry juice drink, Flammer et al. (64) found no significant changes in peripheral artery tonometry in individuals with endothelial dysfunction and other CVD risk factors. Further research on the effect of cranberries on measures of vascular reactivity is required in healthy individuals examining both the dose-response and time course of the intervention.

Recently, in a clinical study of 10 healthy adults, Feliciano et al. (78) identified and quantified by ultra-performance liquid chromatography/quadrupole-time-of-flight mass spectrometry analysis a total of 60 cranberry-derived phenolic metabolites in plasma and urine after the acute ingestion of cranberry juice containing 787 mg polyphenols. These metabolites included sulfates of pyrogallol, valerolactone, benzoic acids, phenylacetic acids, and glucuronides of flavonols, as well as sulfates and glucuronides of cinnamic acids. Their concentrations ranged from in the low nanomolars to the high micromolars depending on the compound. Among these 60 phenolic metabolites, 12 were found to be independent

TABLE 1 Summary of randomized placebo-controlled trials on the cardiometabolic effects of cranberry[1]

Study, year (ref)	Intervention	Population	Age,[2] y	*n*	Study design	Duration, wk	Dose/d	Polyphenol content	Outcomes
Lee et al., 2008 (59)	Cranberry extract	Adults with T2D	65 ± 1	30	Parallel	12	500 mg × 3	Not reported	↓: TC, LDL-C; NC: FBG, HbA1c, SBP, DBP, TGs, HDL-C, oxLDL-C, insulin, HOMA-IR, CRP
Shidfar et al., 2012 (60)	CJ	Adults with T2D	55 ± 9	58	Parallel	12	240 mL	Not reported	↓: FBG, apoB; ↑: paraoxonase-1 activity; NC: apo A-1, Lp(a)
Novotny et al., 2015 (61)	LC CJ	Healthy adults	50 ± 11	56	Parallel	8	480 mL	346 mg TPs, 21 mg ACNs, 235 mg PACs	↓: DBP, FBG, HOMA-IR, TGs, CRP; NC: SBP, insulin, HOMA-β, TC, LDL-C, HDL-C, apo A-1, apo A-2, apoB, ICAM, VCAM
Basu et al., 2011 (62)	LC CJ, 27%	Adults with metabolic syndrome	52 ± 8	31	Parallel	8	480 mL	458 mg TPs, 25 mg ACNs	↓: oxLDL-C, malondialdehyde, 4-hydroxynonenal; NC: SBP, DPB, FBG, TC, LDL-C, HDL-C, VLDL-C, TGs, IL-6, CRP, plasma antioxidant capacity
Dohadwala et al., 2011 (63)	CJ, 54%	Adults with CAD	63 ± 9	44	Crossover	4	480 mL	835 mg TPs, 94 mg ACNs	↓: HDL-C, carotid femoral PWV; NC: SBP, DPB, FBG, insulin, HOMA-IR, TC, LDL-C, TGs, carotid radial PWV, CRP, ICAM-1
Flammer et al., 2013 (64)	LC CJ, 54%	Adults with CVD risk factors	49 ± 16	69	Parallel	16	460 mL	800 mg TPs, 69 mg ACNs, 1224 mg PACs	NC: SBP, DBP, TC, HDL-C, TGs, AIX, pulse pressure, heart rate, reactive hyperemia index, CRP, ICAM, VCAM, IL-6, TNF-α, oxLDL-C
Ruel et al., 2013 (65)	LC CJ, 27%	Healthy overweight men	45 ± 10	35	Crossover	4	500 mL	400 mg TPs, 21 mg ACNs	NC: SBP, DBP, mean arterial pressure, heart rate, AIX, global endothelial function, NOx, uric acid, oxLDL-C, ICAM-1, VCAM-1, E-selectin

[1] ACN, anthocyanin; AIX, augmentation index; CAD, coronary artery disease; CJ, cranberry juice; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; HDL-C, HDL cholesterol; HOMA-β, homeostatic model assessment of β cell function; ICAM, intercellular adhesion molecule; Lp(a), lipoprotein a; LC, low-calorie; LDL-C, LDL cholesterol; NC, no significant change; NOx, nitrate/nitrite; oxLDL-C, oxidized LDL cholesterol; PAC, proanthocyanidin; PWV, pulse-wave velocity; ref, reference; SBP, systolic blood pressure; TC, total cholesterol; TP, total polyphenol; T2D, type 2 diabetes; VCAM, vascular cell adhesion molecule; VLDL-C, VLDL cholesterol; ↓, significantly different decrease from placebo group; ↑, significantly different increase from placebo group.

[2] Values are means ± SDs.

predictors of time- (0–6 h) dependent increases in FMD after an acute dose (range: 409–1909 mg total polyphenols) (79). These results indicate that cranberry polyphenols can acutely increase endothelial function in healthy individuals. Arterial stiffness, commonly assessed by pulse-wave velocity or the augmentation index (a measure of the enhancement of central aortic pressure by a reflected pulse wave), is an established risk factor for CVD (80–82). In their randomized clinical trial of patients with CAD, Dohadwala et al. (63) found that a 4-wk intervention with cranberry juice significantly reduced the carotid-femoral pulse-wave velocity. However, no changes in the augmentation index were observed by Ruel et al. (65) after 4 wk of supplementation with cranberry juice in 35 volunteers presenting with obesity and other cardiovascular risk factors.

Glucoregulation. Berry fruit polyphenols have been shown by in vitro experiments and animal models to inhibit carbohydrate digestion and glucose absorption in the intestine, stimulate insulin secretion from β cells in the pancreas, regulate glucose release from the liver, and activate insulin receptors and glucose uptake in insulin-sensitive tissues (83–85). Emerging clinical evidence suggests that dietary modification to increase polyphenol intakes from whole-food sources can lead to improved glycemic control in T2D (86, 87). While exploring the antidiabetic effects of a cranberry extract in high-fat, high-sugar–fed mice, Anhê et al. (41) found a decrease in glucose-induced hyperinsulinemia and improved insulin sensitivity along with a reduction in weight gain and visceral obesity. As noted above, along with these improvements, the cranberry extract also altered the gut microbiome by increasing mucin-degrading bacteria. In light of the evolving link between the gut microbiota and diabetes, these findings provide an important connection between the studies documenting the effects of cranberry on gut barrier function and the potential to reverse the dysbiosis and metabolic inflammation underlying diabetes (88).

Human studies testing low-calorie cranberry juice and unsweetened dried cranberries were shown to produce favorable acute postprandial glycemic responses in adults with T2D (70, 71). However, the limited number of longer-term studies in patients with T2D generated discordant outcomes. Shidfar et al. (60) reported that daily cranberry juice consumed for 12 wk by 58 male patients with T2D induced significant decreases in fasting blood glucose when compared with the placebo group. In contrast, in a trial of 30 patients with T2D, Lee et al. (59) found no impact of daily supplementation with cranberry extracts for 12 wk on fasting blood glucose or glycated hemoglobin. In a diet therapy intervention in 27 adults with T2D, Chambers and Camire (89) found no significant effect of treatment with cranberry extract on measures of glycemia. However, in 12 healthy volunteers in a randomized crossover trial, Törrönen et al. (90) found that a berry puree containing cranberries was able to delay the postprandial plasma response to sucrose. Clinical trials in patients with metabolic syndrome have suggested some benefit associated with cranberry intervention

TABLE 2 Summary of open-label trials on the effects of cranberry on cardiometabolic markers[1]

Study, year (ref)	Intervention	Population	Age, y	*n*	Study design	Duration	Dose/d	Polyphenol content	Outcomes
Ruel et al., 2006 (66)	LC CJ, 27%	Healthy sedentary men	51 ± 10[2]	30	Crossover	4 wk/dose	125, 250, 500 mL	100 mg TPs, 5 mg ACNs, 74 mg PACs/125 mL	↓: TC:HDL-C, antioxidant capacity, NOx; ↑: HDL-C; NC: TC, LDL-C, TGs, apo A-I, apoB
Ruel et al., 2008 (67)	LC CJ, 27%	Healthy sedentary men	51 ± 10	30	Crossover	4 wk/dose	125, 250, 500 mL	100 mg TPs, 5 mg ACNs, 74 mg PACs/125 mL	↓: SBP, HDL-C, oxLDL-C, ICAM-1, VCAM-1; NC: DBP, heart rate, TC, LDL-C, TGs, apoB, E-selectin
Ruel et al., 2009 (68)	LC CJ, 27%	Healthy sedentary men	51 ± 10	30	Crossover	4 wk/dose	125, 250, 500 mL	100 mg TPs, 5 mg ACNs, 74 mg PACs/125 mL	↓: SBP, MMP-9, NOx; NC: DBP, mean arterial BP
Ruel et al., 2005 (69)	LC CJ, 27%	Healthy men	38 ± 8	21	Single-arm intervention; no control group	14 d	7 mL/kg body weight	Not reported	↓: oxLDL-C; ↑: antioxidant capacity; NC: SBP, DBP, TC, LDL-C, HDL-C, TC:HDL-C, TGs, apoB, LDL-C particle size
Wilson et al., 2008 (70)	CJ, 27%; LC CJ, 27%	Adults with T2D and obesity	65 ± 2	12	Crossover	Single dose	Not reported	Not reported for dosage given	CJ vs. LC CJ—↑: glucose at 30 and 60 min, insulin at 60 min; NC: glucose, insulin at 120 min
Wilson et al., 2010 (71)	SDC, LC SDC, RC, WB	Adults with T2D	62 ± 2	13	Crossover	30, 60, and 120 min/dose	40 g each	Phenolic and PAC profiles reported	WB, SDC, LC SDC vs. RC—↑: glucose at 30, 60, 120 min; insulin at 30, 60 min; glucose AUC; insulin AUC
Simão et al., 2013 (72)	LC CJ	Adults with metabolic syndrome	48.5 (control), 51.0 (cranberry)[3]	56	Parallel; no-intervention control	60 d	700 mL	364 mg TPs, 231 mg PACs	↑: Adiponectin; ↓: lipoperoxidation, protein oxidation; NC: CRP, IL-1, IL-6, TNF-α, folic acid, homocysteine

[1] ACN, anthocyanin; BP, blood pressure; CJ, cranberry juice; CRP, C-reactive protein; DBP, diastolic blood pressure; HDL-C, HDL cholesterol; ICAM, intercellular adhesion molecule; LC, low-calorie; LDL-C, LDL cholesterol; MMP, metalloproteinase; NC, no significant change; NOx, nitrites/nitrates; oxLDL-C, oxidized LDL cholesterol; PAC, proanthocyanidin; RC, raw cranberries; ref, reference; SBP, systolic blood pressure; SDC, sweetened dried cranberries; TC, total cholesterol; TP, total polyphenol; T2D, type 2 diabetes; VCAM, vascular cell adhesion molecule; WB, white bread; ↓, significantly different decrease from baseline, between groups, and/or across increasing doses; ↑, significantly different increase from baseline, between groups, and/or across increasing doses.

[2] Mean ± SD (all such values).

[3] Median ages in control and treatment groups.

but not specifically on outcomes of glycemic control (62, 72, 91).

Biomarkers of inflammation. In their analysis of observational data collected from NHANES, Duffey and Sutherland (92, 93) found inverse associations of popular polyphenol-containing beverages, such as cranberry juice, with obesity and inflammation. In their 8-wk randomized clinical trial, Novotny et al. (61) showed a reduction in C-reactive protein (CRP) after daily consumption of cranberry juice. In a clinical trial of 56 subjects with metabolic syndrome, Simão et al. (72) reported that daily intake of low-calorie cranberry juice for 8 wk had no significant effect on proinflammatory cytokines IL-1, IL-6, and TNF-α but did reduce biomarkers of lipid peroxidation and advanced oxidation protein products. Similarly, in an 8-wk study of 36 patients with metabolic syndrome, Basu et al. (62) observed increases in plasma biomarkers of antioxidant capacity and decreases in lipid peroxidation after the daily consumption of low-calorie cranberry juice. These results are consistent with in vitro experiments showing that cranberry polyphenols decreased the generation of reactive oxygen species and lipid peroxides and increased glutathione peroxidase activity and phospho-c-Jun N-terminal kinase (94).

Cranberries and Health: Knowledge Gaps

The recent growing body of research on cranberries and health is a part of the emerging evidence from in vitro, animal model, and human studies of plant polyphenols as protective dietary agents that act both directly and indirectly via their metabolites and/or interactions with the gut microbiota. Improvements in these research approaches, particularly in analytical methods as diverse as MS and gene-sequencing methods for microbial communities, are making important contributions to our understanding of polyphenol mechanisms and functions.

However, an important need in cranberry health research is the consistent use of a fully characterized SRM to help promote the generation of more readily comparable and replicable research protocols. SRMs have been available for some other polyphenol-rich foods, including highbush blueberries (95) and California table grapes (96), for in vitro, animal, and human studies. Although SRMs for cranberries have been developed by the National Institute of Standards and Technology and the Office of Dietary Supplements of the NIH, these materials are intended for the validation of analytical methods and quality assurance for in-house control materials. Furthermore, these SRMs are not accompanied by matching placebos for use in research studies. Wide availability of cranberry SRMs in sufficient quantities to conduct in vivo human health research remained lacking until recently. Because concerns for study accuracy and quality were raised because of the diversity of cranberry products available commercially and in research protocols (97), The Cranberry Institute undertook the development of a cranberry reference material in 2014 to ensure the

TABLE 3 Summary of human trials investigating the bioavailability and pharmacokinetic variables of cranberry[1]

Study, year (ref)	Population	*n*	Cranberry product and dose	Compounds studied	Variables studied	Timing of measurements
Feliciano et al., 2016 (78)	Healthy young men	10	450 mL CJ	Phenolic metabolites	Plasma AUC, Cmax, Tmax, % urinary recovery	Plasma: 1, 2, 4, 6, 8, and 24 h; urine: 0–8 h, 8–24 h
Zhang and Zuo, 2004 (110)	Healthy adults	1	1800 mL, 27% CJ	Flavonoids, phenolic acids, benzoic acids	Plasma and urine concentrations	0, 45, and 270 min
Milbury et al., 2010 (111)	Adults aged 62 ± 8 y with CAD	15	480 mL 54% CJ (835 mg TPs, 94.47 mg ACNs)	ACNs	Plasma AUC, Cmax, Tmax, T1/2 % urinary recovery	0–4 h
Iswaldi et al., 2013 (112)	Adults aged 25–40 y	4	0.6 mL/kg cranberry syrup	Polyphenols, phase I and II phenolic metabolites	Urine concentration	Urine: 0, 2, 4, and 6 h
McKay et al., 2015 (113)	Adults aged ≥50 y	10	54% CJ	Flavonoids, phenolic acids, PACs	Plasma AUC, Cmax, Tmax, antioxidant capacity, urine concentration	Plasma: 0.25, 0.5, 1–6, and 10 h; urine: 12, 14, 16, 18, and 20 h
Walsh et al., 2016 (114)	Healthy women aged 20–30 y	5	237 mL cranberry beverage (140 mg PACs); weekly for 7 wk	PACs	Urine concentration	24 h

[1] ACN, anthocyanin; CAD, coronary artery disease; CJ, cranberry juice; Cmax, maximal plasma concentration; PAC, proanthocyanidin; ref, reference; T1/2, biological half-life; Tmax, time to maximal plasma concentration.

authenticity and consistency of cranberry products used in research on human health.

The first question to be answered was whether the SRM would be developed from whole fruit or one of the many processed forms in which cranberry is consumed. The impact of processing, particularly juicing, on the phytochemical content and profile of fresh cranberries has been characterized (98, 99). Grace et al. (100) compared fresh and freeze-dried cranberries to cranberry-containing commercial products including juices (from concentrate and not from concentrate), sweetened dried cranberries, and cranberry sauces (homemade and commercially canned). Cranberry skins and flesh were cross-compared for anthocyanin and proanthocyanidin content. Proanthocyanidins were typically higher in skins than in flesh with the exception of the proanthocyanidin A-2 dimer. Anthocyanin and proanthocyanidin concentrations were lower in juice reconstituted from concentrate. In general, the retention of proanthocyanidins in processed cranberries was found to be robust, whereas anthocyanins were sensitive to degradation. Grace et al. (101) explored ways to better concentrate and stabilize cranberry bioactive compounds via complexing concentrated juices with proteins isolated from soy, hemp, peanuts, and peas for formulating both beverage and solid-food products. By using an in vitro model to simulate digestion, Ribnicky et al. (102) were able to show that protein complexes with blueberry polyphenols remained more intact and bioaccessible than the free bioactive compounds.

To retain and encourage the study of the complete phytochemical profile of cranberry, the SRM is a freeze-dried whole-cranberry powder (FWCP). It is produced from a blend of cranberry varieties grown in Wisconsin and approximating the proportion available in the marketplace (i.e., 56% Stevens plus 11% each of Ben Lear, Grygleski, Pilgrim, and HyRed varieties for the first batch produced in 2015). The berries are individually frozen after harvest, freeze-dried, and ground into powder form. Silicon dioxide (3% total volume of powder) is added as an anticaking agent. The production process is fully documented from harvest to storage. Each 50 g (0.5 cup) of whole cranberries produces ~4.5 g FWCP.

Complete specifications for each nutrient and phytochemical ingredient were prepared by using a series of assays, including matrix-assisted laser desorption/ionization time-of-flight MS for authentication of proanthocyanidins (103, 104), 4-(dimethylamino)cinnamaldehyde assay for quantification of soluble proanthocyanidins (57, 103, 105), and butanol-hydrochloric acid for quantification of insoluble proanthocyanidins as well as characterization of efficacy via an established in vitro antiadhesion assay and microbiological testing.

Accurate quantification of proanthocyanidins for health research is essential but also problematic because proanthocyanidins are complex polydispersed hetero-oligomers (57). Previously, the procyanidin A2 (ProA2) dimer was recommended as the standard of choice for proanthocyanidin

analysis in the 4-(dimethylamino)cinnamaldehyde assay because cranberry proanthocyanidins contain ≥1 "A-type" interflavan bonds (106). However, current evidence shows that the use of the ProA2 dimer as a standard for quantification of complex proanthocyanidin oligomers results in a serious underestimation of proanthocyanidins (107). To address this problem, a cranberry proanthocyanidin standard (c-PAC), reflective of the structural heterogeneity of proanthocyanidins found in fresh cranberry (i.e., DP, hydroxylation pattern, and ratio of A- to B-type interflavan bonds), was developed. The use of the c-PAC to quantify proanthocyanidin content in FWCP resulted in values that were 3.6 times those determined by ProA2. Thus, adoption of this c-PAC standard reflects an improvement over the use of ProA2 for the accurate quantification of cranberry proanthocyanidins (105). Because these findings were only recently published, the soluble proanthocyanidin content of the FWCP is reported as both c-PAC and ProA2 equivalents, allowing researchers time to adopt the new methodology. The c-PAC was also used to quantify the FWCP insoluble proanthocyanidins by the butanol-hydrochloric acid method.

The polyphenol content of the FWCP includes the following: 28.35 mg total polyphenols (gallic acid equivalents)/g, 31.20 mg total soluble proanthocyanidins (c-PACs)/g, 8.77 mg soluble proanthocyanidins (ProA2)/g, 10.38 mg insoluble pro anthocyanidins (c-PACs)/g, 5.98 mg anthocyanins (cyanidin-3-galactoside equivalents)/g, 9.01 mg flavonols (quercetin-3-rhamnoside equivalents)/g, and 1.81-mg hydroxycinnamic acids (caffeic acid equivalents)/g (108). The FWCP processing and packaging facilities are compliant with FDA regulations. A suitable placebo was created from a blend of maltodextrin, citric acid, artificial flavoring, fructose, and food-grade coloring agents (109). Calcium silicate is added to the FWCP and placebo as a flow agent.

The use of the FWCP should help overcome some of the critical limitations associated with past studies that used uncharacterized or only partly characterized cranberry foods or extracts. Recipes for the administration of FWCP and placebo in human studies have been developed and are made freely available to researchers. Like other studies of whole foods, it is recommended that protocols that use the FWCP not apply this material directly to target tissues, with some possible exceptions such as oral and gastrointestinal cells. In vitro and ex vivo research approaches should consider the use of metabolite(s) on the basis of their likely bioavailability to these tissues, an approach not often followed in early studies of polyphenol-rich foods and extracts. The design of clinical trials that use the FWCP should also be informed by human bioavailability data generated from studies of other cranberry foods and extracts (**Table 3**) (110–114), although some consideration should be directed to results from animal models (115). However, because the product matrices and pharmacokinetic characteristics of these other products will undoubtedly differ, new studies on the absorption, metabolism, and elimination of the bioactive compounds in the FWCP must be undertaken. Although the availability of the FWCP as an SRM for clinical research may help ensure the consistency and full characterization of the cranberry intervention, the need to perform reasonable dose-response and time-course studies for each health-related outcome remains an important priority, as does the need to develop biomarkers of compliance to the intervention.

Summary

Cranberry juice, dried cranberries, and various cranberry extracts have been shown via in vitro, animal model, and human studies to possess an array of biochemical and physiologic activities mediated by their phytochemical constituents. Although the greatest research focus has been reasonably placed on their rich content of polyphenols, emerging evidence of their actions on the gut microbiota and cardiometabolic functions suggests that attention is also warranted on their synergy with cranberry phenolic acids, isoprenoids, and oligosaccharides. Acting in high concentrations within the gastrointestinal lumen, these cranberry compounds may act to quench reactive oxygen species, modulate inflammatory pathways, adhere to carbohydrates and proteins on bacterial surfaces, exert prebiotic effects, and alter the dynamic cross-talk between intestinal epithelial cells and the gut microbiota. These actions may underlie not only the antimicrobial effects of cranberries but their role in the complex pathogenesis of UTIs and inflammatory bowel diseases. The importance of these relations beyond the gastrointestinal tract has grown substantially with the recognition of the broad role that the gut microbiota plays in regulating energy homeostasis, glucose and lipid metabolism, and systemic inflammation, all factors associated with the maintenance of cardiometabolic health.

Further substantiating the actions and mechanisms of cranberry constituents can best be accomplished by taking advantage of recent advances in cranberry research. For example, efforts to identify biomarkers of compliance to clinical protocols, as well as their relation to physiologic and health outcomes, may evolve from improved understanding of cranberry constituents (e.g., the specific nature of proanthocyanidin interflavan bonds and DP, as well as a more robust phytochemical profile) and the numerous bioactive catabolites arising from the biotransformation of cranberry constituents by the gut microbiota and phase I, II, and III metabolism pathways. Furthermore, a greater degree of accuracy, consistency, and quality of new studies has become possible with the availability of a fully characterized FWCP and matched placebo as SRMs.

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References

1. US Department of Health and Human Services; US Department of Agriculture. Scientific report of the 2015 Dietary Guidelines Advisory Committee [Internet]. US Department of Health and Human Services; 2015 [cited 2016 Jan 23]. Available from: http://health.gov/dietaryguidelines/2015-scientific-report/.
2. Blumberg JB, Camesano TA, Cassidy A, Kris-Etherton P, Howell A, Manach C, Ostertag LM, Sies H, Skulas-Ray A, Vita JA. Cranberries and their bioactive constituents in human health. Adv Nutr 2013;4:618–32.
3. Kaspar KL, Howell AB, Khoo C. A randomized, double-blind, placebo-controlled trial to assess the bacterial anti-adhesion effects of cranberry extract beverages. Food Funct 2015;6:1212–7.
4. Shmuely H, Yahav J, Samra Z, Chodick G, Koren R, Niv Y, Ofek I. Effect of cranberry juice on eradication of Helicobacter pylori in patients treated with antibiotics and a proton pump inhibitor. Mol Nutr Food Res 2007;51:746–51.
5. Zhang L, Ma J, Pan K, Go VLW, Chen J, You W. Efficacy of cranberry juice on Helicobacter pylori infection: a double-blind, randomized placebo-controlled trial. Helicobacter 2005;10:139–45.
6. Shmuely H, Ofek I, Weiss EI, Rones Z, Houri-Haddad Y. Cranberry components for the therapy of infectious disease. Curr Opin Biotechnol 2012;23:148–52.
7. Bodet C, Grenier D, Chandad F, Ofek I, Steinberg D, Weiss EI. Potential oral health benefits of cranberry. Crit Rev Food Sci Nutr 2008;48:672–80.
8. Feldman M, Grenier D. Cranberry proanthocyanidins act in synergy with licochalcone A to reduce Porphyromonas gingivalis growth and virulence properties, and to suppress cytokine secretion by macrophages: Synergy between proanthocyanidins and licochalcone A. J Appl Microbiol 2012;113:438–47.
9. LaPlante KL, Sarkisian SA, Woodmansee S, Rowley DC, Seeram NP. Effects of cranberry extracts on growth and biofilm production of Escherichia coli and Staphylococcus species. Phytother Res 2012;26:1371–4.
10. Ulrey RK, Barksdale SM, Zhou W, van Hoek ML. Cranberry proanthocyanidins have anti-biofilm properties against Pseudomonas aeruginosa. BMC Complement Altern Med 2014;14:499–510.
11. Weiss EI, Houri-Haddad Y, Greenbaum E, Hochman N, Ofek I, Zakay-Rones Z. Cranberry juice constituents affect influenza virus adhesion and infectivity. Antiviral Res 2005;66:9–12.
12. Patel KD, Scarano FJ, Kondo M, Hurta RAR, Neto CC. Proanthocyanidin-rich extracts from cranberry fruit (Vaccinium macrocarpon Ait.) aelectively inhibit the growth of human pathogenic fungi Candida spp. and Cryptococcus neoformans. J Agric Food Chem 2011;59:12864–73.
13. Rane HS, Bernardo SM, Howell AB, Lee SA. Cranberry-derived proanthocyanidins prevent formation of Candida albicans biofilms in artificial urine through biofilm- and adherence-specific mechanisms. J Antimicrob Chemother 2014;69:428–36.
14. Feliciano RP, Krueger CG, Reed JD. Methods to determine effects of cranberry proanthocyanidins on extraintestinal infections: relevance for urinary tract health. Mol Nutr Food Res 2015;59:1292–306.
15. Feliciano RP, Meudt JJ, Shanmuganayagam D, Krueger CG, Reed JD. Ratio of "A-type" to "B-type" proanthocyanidin interflavan bonds affects extra-intestinal pathogenic *Escherichia coli* invasion of gut epithelial cells. J Agric Food Chem 2014;62:3919–25.
16. Sánchez-Patán F, Barroso E, van de Wiele T, Jiménez-Girón A, Martín-Alvarez PJ, Moreno-Arribas MV, Martínez-Cuesta MC, Peláez C, Requena T, Bartolomé B. Comparative in vitro fermentations of cranberry and grape seed polyphenols with colonic microbiota. Food Chem 2015;183:273–82.
17. Ou K, Sarnoski P, Schneider KR, Song K, Khoo C, Gu L. Microbial catabolism of procyanidins by human gut microbiota. Mol Nutr Food Res 2014;58:2196–205.
18. Polak D, Naddaf R, Shapira L, Weiss EI, Houri-Haddad Y. Protective potential of non-dialyzable material fraction of cranberry juice on the virulence of *P. gingivalis* and *F. nucleatum* mixed infection. J Periodontol 2013;84:1019–25.
19. Kim D, Hwang G, Liu Y, Wang Y, Singh AP, Vorsa N, Koo H. Cranberry flavonoids modulate cariogenic properties of mixed-species biofilm through exopolysaccharides-matrix disruption. PLOS ONE 2015;10:e0145844.
20. Pinzón-Arango P, Holguin K, Camesano T. Impact of cranberry juice and proanthocyanidins on the ability of Escherichia coli to form biofilms. Food Sci Biotechnol 2011;20:1315–21.
21. Sun J, Marais J, Khoo C, LaPlante K, Vejbory R, Givskov M, Tolker-Nielsen T, Seeram N, Rowley D. Cranberry (Vaccinium macrocarpon) oligosaccharides decrease biofilm formation by uropathogenic Escherichia coli. J Funct Foods 2015;17:235–42.
22. Hotchkiss AT, Nuñez A, Strahan GD, Chau HK, White AK, Marais JPJ, Hom K, Vakkalanka MS, Di R, Yam KL, et al. Cranberry xyloglucan structure and inhibition of *Escherichia coli* adhesion to epithelial cells. J Agric Food Chem 2015;63:5622–33.
23. Zafriri D, Ofek I, Adar R, Pocino M, Sharon N. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. Antimicrob Agents Chemother 1989;33:92–8.
24. Pinzón-Arango PA, Liu Y, Camesano TA. Role of cranberry on bacterial adhesion forces and implications for *Escherichia coli*–uroepithelial cell attachment. J Med Food 2009;12:259–70.
25. Signoretto C, Canepari P, Stauder M, Vezzulli L, Pruzzo C. Functional foods and strategies contrasting bacterial adhesion. Curr Opin Biotechnol 2012;23:160–7.
26. Liu Y, Black MA, Caron L, Camesano TA. Role of cranberry juice on molecular-scale surface characteristics and adhesion behavior of Escherichia coli. Biotechnol Bioeng 2006;93:297–305.
27. de Llano DG, Esteban-Fernández A, Sánchez-Patán F, Martínlvarez P, Moreno-Arribas M, Bartolomé B. Anti-adhesive activity of cranberry phenolic compounds and their microbial-derived metabolites against uropathogenic Escherichia coli in bladder epithelial cell cultures. Int J Mol Sci 2015;16:12119–30.
28. Pastorelli L, De Salvo C, Mercado JR, Vecchi M, Pizarro TT. Central role of the gut epithelial barrier in the pathogenesis of chronic intestinal inflammation: lessons learned from animal models and human genetics. Front Immunol [Internet] 2013 [cited 2016 Jan 26]. Available from: http://journal.frontiersin.org/article/10.3389/fimmu.2013.00280/abstract.
29. Wong X, Carrasco-Pozo C, Escobar E, Navarrete P, Blachier F, Andriamihaja M, Lan A, Tomé D, Cires MJ, Pastene E, et al. Deleterious effect of p-Cresol on human colonic epithelial cells prevented by proanthocyanidin-containing polyphenol extracts from fruits and proanthocyanidin bacterial metabolites. J Agric Food Chem 2016;64:3574–83.
30. Johansson MEV, Ambort D, Pelaseyed T, Schütte A, Gustafsson JK, Ermund A, Subramani DB, Holmén-Larsson JM, Thomsson KA, Bergström JH, et al. Composition and functional role of the mucus layers in the intestine. Cell Mol Life Sci 2011;68:3635–41.
31. Yusuf S, Nok AJ, Ameh DA, Adelaiye AB, Balogun EO. Quantitative changes in gastric mucosal glycoproteins: effect of cholinergic agonist and vagal nerve stimulation in the rat. Neurogastroenterol Motil 2004;16:613–9.
32. Fallon PG, Jolin HE, Smith P, Emson CL, Townsend MJ, Fallon R, Smith P, McKenzie ANJ. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. Immunity 2002;17:7–17.
33. Finkelman FD, Shea-Donohue T, Goldhill J, Sullivan CA, Morris SC, Madden KB, Gause WC, Urban JF. Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. Annu Rev Immunol 1997;15:505–33.

34. McKenzie GJ, Bancroft A, Grencis RK, McKenzie AN. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. Curr Biol 1998;8:339–42.
35. Birchenough GMH, Johansson ME, Gustafsson JK, Bergström JH, Hansson GC. New developments in goblet cell mucus secretion and function. Mucosal Immunol 2015;8:712–9.
36. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. Gastroenterology 2012;142: 1100–1.e2.
37. Martinez-Medina M, Denizot J, Dreux N, Robin F, Billard E, Bonnet R, Darfeuille-Michaud A, Barnich N. Western diet induces dysbiosis with increased E coli in CEABAC10 mice, alters host barrier function favouring AIEC colonisation. Gut 2014;63:116–24.
38. Piya MK, Harte AL, McTernan PG. Metabolic endotoxaemia: is it more than just a gut feeling? Curr Opin Lipidol 2013;24:78–85.
39. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–81.
40. Martinez-Medina M, Garcia-Gil LJ. Escherichia coli in chronic inflammatory bowel diseases: an update on adherent invasive Escherichia coli pathogenicity. World J Gastrointest Pathophysiol 2014;5: 213–27.
41. Anhê FF, Roy D, Pilon G, Dudonné S, Matamoros S, Varin T, Garofalo C, Moine Q, Desjardins Y, Levy E, et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. Gut 2015;64:872–83.
42. Denis M-C, Desjardins Y, Furtos A, Marcil V, Dudonné S, Montoudis A, Garofalo C, Delvin E, Marette A, Levy E. Prevention of oxidative stress, inflammation and mitochondrial dysfunction in the intestine by different cranberry phenolic fractions. Clin Sci 2015;128:197–212.
43. Pierre JF, Heneghan AF, Feliciano RP, Shanmuganayagam D, Krueger CG, Reed JD, Kudsk KA. Cranberry proanthocyanidins improve intestinal sIgA during elemental enteral nutrition. JPEN J Parenter Enteral Nutr 2014;38:107–14.
44. Pierre JF, Heneghan AF, Feliciano RP, Shanmuganayagam D, Roenneburg DA, Krueger CG, Reed JD, Kudsk KA. Cranberry proanthocyanidins improve the gut mucous layer morphology and function in mice receiving elemental enteral nutrition. JPEN Parenter Enteral Nutr 2013;37:401–9.
45. Hermsen JL, Gomez FE, Sano Y, Kang W, Maeshima Y, Kudsk KA. Parenteral feeding depletes pulmonary lymphocyte populations. JPEN Parenter Enteral Nutr 2009;33:535–40.
46. Kudsk KA, Gomez FE, Kang W, Ueno C. Enteral feeding of a chemically defined diet preserves pulmonary immunity but not intestinal immunity: the role of lymphotoxin beta receptor. JPEN J Parenter Enteral Nutr 2007;31:477–81.
47. Mosenthal AC, Xu D, Deitch EA. Elemental and intravenous total parenteral nutrition diet-induced gut barrier failure is intestinal site specific and can be prevented by feeding nonfermentable fiber. Crit Care Med 2002;30:396–402.
48. Wu Y, Kudsk KA, DeWitt RC, Tolley EA, Li J. Route and type of nutrition influence IgA-mediating intestinal cytokines. Ann Surg 1999; 229:662–7; discussion: 667–8.
49. Nantz MP, Rowe CA, Muller C, Creasy R, Colee J, Khoo C, Percival SS. Consumption of cranberry polyphenols enhances human γδ-T cell proliferation and reduces the number of symptoms associated with colds and influenza: a randomized, placebo-controlled intervention study. Nutr J 2013;12:161–9.
50. Diard M, Garry L, Selva M, Mosser T, Denamur E, Matic I. Pathogenicity-associated islands in extraintestinal pathogenic Escherichia coli are fitness elements involved in intestinal colonization. J Bacteriol 2010;192: 4885–93.
51. Johnson JR, Russo TA. Molecular epidemiology of extraintestinal pathogenic (uropathogenic) Escherichia coli. Int J Med Microbiol 2005;295:383–404.
52. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol 2010;7:653–60.
53. Pitout JDD. Extraintestinal pathogenic Escherichia coli: a combination of virulence with antibiotic resistance. Front Microbiol [Internet] 2012 [cited 2016 Jan 26]. Available from: http://journal.frontiersin.org/article/10.3389/fmicb.2012.00009/abstract.
54. Mathison BD, Kimble LL, Kaspar KL, Khoo C, Chew BP. Consumption of cranberry beverage improved endogenous antioxidant status and protected against bacteria adhesion in healthy humans: a randomized controlled trial. Nutr Res 2014;34:420–7.
55. Takahashi S, Takahashi S, Hamasuna R, Yasuda M, Arakawa S, Tanaka K, Ishikawa K, Hayami H, Yamamoto S, Kubo T, et al. A randomized clinical trial to evaluate the preventive effect of cranberry juice (UR65) for patients with recurrent urinary tract infection. J Infect Chemother 2013;19:112–7.
56. Foxman B, Cronenwett AEW, Spino C, Berger MB, Morgan DM. Cranberry juice capsules and urinary tract infection after surgery: results of a randomized trial. Am J Obstet Gynecol 2015;213:194. e1–8.
57. Krueger CG, Reed JD, Feliciano RP, Howell AB. Quantifying and characterizing proanthocyanidins in cranberries in relation to urinary tract health. Anal Bioanal Chem 2013;405:4385–95.
58. Rodriguez-Mateos A, Vauzour D, Krueger CG, Shanmuganayagam D, Reed J, Calani L, Mena P, Del Rio D, Crozier A. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Arch Toxicol 2014;88:1803–53.
59. Lee IT, Chan YC, Lin CW, Lee WJ, Sheu WH-H. Effect of cranberry extracts on lipid profiles in subjects with type 2 diabetes. Diabet Med 2008;25:1473–7.
60. Shidfar F, Heydari I, Hajimiresmaiel SJ, Hosseini S, Shidfar S, Amiri F. The effects of cranberry juice on serum glucose, apoB, apoA-I, Lp(a), and Paraoxonase-1 activity in type 2 diabetic male patients. J Res Med Sci 2012;17:355–60.
61. Novotny JA, Baer DJ, Khoo C, Gebauer SK, Charron CS. Cranberry juice consumption lowers markers of cardiometabolic risk, including blood pressure and circulating C-reactive protein, triglyceride, and glucose concentrations in adults. J Nutr 2015;145:1185–93.
62. Basu A, Betts NM, Ortiz J, Simmons B, Wu M, Lyons TJ. Low-energy cranberry juice decreases lipid oxidation and increases plasma antioxidant capacity in women with metabolic syndrome. Nutr Res 2011;31: 190–6.
63. Dohadwala MM, Holbrook M, Hamburg NM, Shenouda SM, Chung WB, Titas M, Kluge MA, Wang N, Palmisano J, Milbury PE, et al. Effects of cranberry juice consumption on vascular function in patients with coronary artery disease. Am J Clin Nutr 2011;93:934–40.
64. Flammer AJ, Martin EA, Gössl M, Widmer RJ, Lennon RJ, Sexton JA, Loeffler D, Khosla S, Lerman LO, Lerman A. Polyphenol-rich cranberry juice has a neutral effect on endothelial function but decreases the fraction of osteocalcin-expressing endothelial progenitor cells. Eur J Nutr 2013;52:289–96.
65. Ruel G, Lapointe A, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Evidence that cranberry juice may improve augmentation index in overweight men. Nutr Res 2013;33:41–9.
66. Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Favourable impact of low-calorie cranberry juice consumption on plasma HDL-cholesterol concentrations in men. Br J Nutr 2006;96:357–64.
67. Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Low-calorie cranberry juice supplementation reduces plasma oxidized LDL and cell adhesion molecule concentrations in men. Br J Nutr 2008;99:352–9.
68. Ruel G, Pomerleau S, Couture P, Lemieux S, Lamarche B, Couillard C. Plasma matrix metalloproteinase (MMP)-9 levels are reduced following low-calorie cranberry juice supplementation in men. J Am Coll Nutr 2009;28:694–701.
69. Ruel G, Pomerleau S, Couture P, Lamarche B, Couillard C. Changes in plasma antioxidant capacity and oxidized low-density lipoprotein levels in men after short-term cranberry juice consumption. Metabolism 2005;54:856–61.

70. Wilson T, Meyers SL, Singh AP, Limburg PJ, Vorsa N. Favorable glycemic response of type 2 diabetics to low-calorie cranberry juice. J Food Sci 2008;73:H241–5.
71. Wilson T, Luebke JL, Morcomb EF, Carrell EJ, Leveranz MC, Kobs L, Schmidt TP, Limburg PJ, Vorsa N, Singh AP. Glycemic responses to sweetened dried and raw cranberries in humans with type 2 diabetes. J Food Sci 2010;75:H218–23.
72. Simão TNC, Lozovoy MAB, Simão ANC, Oliveira SR, Venturini D, Morimoto HK, Miglioranza LHS, Dichi I. Reduced-energy cranberry juice increases folic acid and adiponectin and reduces homocysteine and oxidative stress in patients with the metabolic syndrome. Br J Nutr 2013;110:1885–94.
73. Rodriguez-Mateos A, Heiss C, Borges G, Crozier A. Berry (poly)phenols and cardiovascular health. J Agric Food Chem 2014;62:3842–51.
74. Kim MJ, Ohn J, Kim JH, Kwak H-K. Effects of freeze-dried cranberry powder on serum lipids and inflammatory markers in lipopolysaccharide treated rats fed an atherogenic diet. Nutr Res Pract 2011;5:404–11.
75. Kim MJ, Chung J-Y, Kim JH, Kwak H-K. Effects of cranberry powder on biomarkers of oxidative stress and glucose control in *db/db* mice. Nutr Res Pract 2013;7:430–8.
76. Kim MJ, Kim JH, Kwak H-K. Antioxidant effects of cranberry powder in lipopolysaccharide treated hypercholesterolemic rats. Prev Nutr Food Sci 2014;19:75–81.
77. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257–65.
78. Feliciano RP, Boeres A, Massaccesi L, Istas G, Ventura R, Nunes dos Santos C, Heiss C, Rodriguez-Mateos A. Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols. Arch Biochem Biophys. In press.
79. Rodriguez-Mateos A, Feliciano RP, Boeres A, Weber T, Dos Santos CN, Ventura MR, Heiss C. Cranberry (poly)phenol metabolites correlate with improvements in vascular function: A double-blind, randomized, controlled, dose-response, crossover study. Mol Nutr Food Res 2016. (Epub ahead of print; DOI: 10.1002/mnfr.201600250).
80. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness. J Am Coll Cardiol 2010;55:1318–27.
81. Laurent S, Cockcroft J, Van Bortel L, Boutouyrie P, Giannattasio C, Hayoz D, Pannier B, Vlachopoulos C, Wilkinson I, Struijker-Boudier H, et al. Expert consensus document on arterial stiffness: methodological issues and clinical applications. Eur Heart J 2006;27:2588–605.
82. Nürnberger J, Keflioglu-Scheiber A, Opazo Saez AM, Wenzel RR, Philipp T, Schäfers RF. Augmentation index is associated with cardiovascular risk. J Hypertens 2002;20:2407–14.
83. McDougall GJ, Kulkarni NN, Stewart D. Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. Biofactors 2008;34:73–80.
84. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, Poutanen K. Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci 2010;11:1365–402.
85. Pinto M da S, Ghaedian R, Shinde R, Shetty K. Potential of cranberry powder for management of hyperglycemia using *in vitro* models. J Med Food 2010;13:1036–44.
86. Bozzetto L, Annuzzi G, Pacini G, Costabile G, Vetrani C, Vitale M, Griffo E, Giacco A, De Natale C, Cocozza S, et al. Polyphenol-rich diets improve glucose metabolism in people at high cardiometabolic risk: a controlled randomised intervention trial. Diabetologia 2015;58:1551–60.
87. Dragan S, Andrica F, Serban M-C, Timar R. Polyphenols-rich natural products for treatment of diabetes. Curr Med Chem 2015;22:14–22.
88. Hartstra AV, Bouter KEC, Bäckhed F, Nieuwdorp M. Insights into the role of the microbiome in obesity and type 2 diabetes. Diabetes Care 2015;38:159–65.
89. Chambers BK, Camire ME. Can cranberry supplementation benefit adults with type 2 diabetes? Diabetes Care 2003;26:2695–6.
90. Törrönen R, Sarkkinen E, Tapola N, Hautaniemi E, Kilpi K, Niskanen L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. Br J Nutr 2010;103:1094–7.
91. Basu A, Lyons TJ. Strawberries, blueberries, and cranberries in the metabolic syndrome: clinical perspectives. J Agric Food Chem 2012;60:5687–92.
92. Duffey KJ, Sutherland L. Adult cranberry beverage consumers have healthier macronutrient intakes and measures of body composition compared to non-consumers: National Health and Nutrition Examination Survey (NHANES) 2005–2008. Nutrients 2013;5:4938–49.
93. Duffey KJ, Sutherland LA. Adult consumers of cranberry juice cocktail have lower C-reactive protein levels compared with nonconsumers. Nutr Res 2015;35:118–26.
94. Martín MA, Ramos S, Mateos R, Marais JPJ, Bravo-Clemente L, Khoo C, Goya L. Chemical characterization and chemo-protective activity of cranberry phenolic powders in a model cell culture: response of the antioxidant defenses and regulation of signaling pathways. Food Res Int 2015;71:68–82.
95. US Highbush Blueberry Council. USHBC blueberry placebo and blueberry powder policy [Internet]. US Highbush Blueberry Council; 2010. [cited 2016 Feb 24]. Available from: http://www.blueberry.org/research/research-2010/research-policy-2010.pdf.
96. California Table Grape Commission. Guidelines for use of California grape powder in research studies [Internet]. California Table Grape Commission; 2015. [cited 2016 Feb 24]. Available from: http://www.grapesfromcalifornia.com/docs/Grape_Powder_Usage_Guidelines_10–21–15.pdf.
97. Jepson RG, Williams G, Craig JC. Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev [Internet] 2012 [cited 2016 Jan 26]. Available from: http://doi.wiley.com/10.1002/14651858.CD001321.pub5.
98. Pappas E, Schaich KM. Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. Crit Rev Food Sci Nutr 2009;49:741–81.
99. White BL, Howard LR, Prior RL. Impact of different stages of juice processing on the anthocyanin, flavonol, and procyanidin contents of cranberries. J Agric Food Chem 2011;59:4692–8.
100. Grace MH, Massey AR, Mbeunkui F, Yousef GG, Lila MA. Comparison of health-relevant flavonoids in commonly consumed cranberry products. J Food Sci 2012;77:H176–83.
101. Grace MH, Guzman I, Roopchand DE, Moskal K, Cheng DM, Pogrebnyak N, Raskin I, Howell A, Lila MA. Stable binding of alternative protein-enriched food matrices with concentrated cranberry bioflavonoids for functional food applications. J Agric Food Chem 2013;61:6856–64.
102. Ribnicky DM, Roopchand DE, Oren A, Grace M, Poulev A, Lila MA, Havenaar R, Raskin I. Effects of a high fat meal matrix and protein complexation on the bioaccessibility of blueberry anthocyanins using the TNO gastrointestinal model (TIM-1). Food Chem 2014;142:349–57.
103. Feliciano RP, Krueger CG, Shanmuganayagam D, Vestling MM, Reed JD. Deconvolution of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry isotope patterns to determine ratios of A-type to B-type interflavan bonds in cranberry proanthocyanidins. Food Chem 2012;135:1485–93.
104. Reed JD, Krueger CG, Vestling MM. MALDI-TOF mass spectrometry of oligomeric food polyphenols. Phytochemistry 2005;66:2248–63.
105. Krueger CG, Chesmore N, Chen X, Parker J, Khoo C, Marais JPJ, Shanmuganayagam D, Crump P, Reed JD. Critical reevaluation of the 4-(dimethylamino)cinnamaldehyde assay: cranberry proanthocyanidin standard is superior to procyanidin A2 dimer for accurate quantification of proanthocyanidins in cranberry products. J Funct Foods 2016;22:13–9.
106. Prior RL, Fan E, Ji H, Howell A, Nio C, Payne MJ, Reed J. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. J Sci Food Agric 2010;90:1473–8.

107. Feliciano RP, Shea MP, Shanmuganayagam D, Krueger CG, Howell AB, Reed JD. Comparison of isolated cranberry (Vaccinium macrocarpon Ait.) proanthocyanidins to catechin and procyanidins A2 and B2 for use as standards in the 4-(dimethylamino)cinnamaldehyde assay. J Agric Food Chem 2012;60:4578–85.
108. The Cranberry Institute. Freeze-dried whole cranberry powder [Internet]. The Cranberry Institute; 2015. [cited 2016 Feb 24]. Available from: http://www.cranberryinstitute.org/health_research/FWCP%20Specifications%20Sheet%2010%201%2015.pdf.
109. The Cranberry Institute. Placebo for freeze-dried whole cranberry powder [Internet]. The Cranberry Institute; 2015. [cited 2016 Feb 24]. Available from: http://www.cranberryinstitute.org/health_research/FWCP%20Placebo%20Specifications%20Sheet%2010%201%2015.pdf.
110. Zhang K, Zuo Y. GC-MS determination of flavonoids and phenolic and benzoic acids in human plasma after consumption of cranberry juice. J Agric Food Chem 2004;52:222–7.
111. Milbury PE, Vita JA, Blumberg JB. Anthocyanins are bioavailable in humans following an acute dose of cranberry juice. J Nutr 2010; 140:1099–104.
112. Iswaldi I, Arráez-Román D, Gómez-Caravaca AM, Contreras M del M, Uberos J, Segura-Carretero A, Fernández-Gutiérrez A. Identification of polyphenols and their metabolites in human urine after cranberry-syrup consumption. Food Chem Toxicol 2013;55:484–92.
113. McKay DL, Chen C-YO, Zampariello CA, Blumberg JB. Flavonoids and phenolic acids from cranberry juice are bioavailable and bioactive in healthy older adults. Food Chem 2015;168:233–40.
114. Walsh JM, Ren X, Zampariello C, Polasky DA, McKay DL, Blumberg JB, Chen C-YO. Liquid chromatography with tandem mass spectrometry quantification of urinary proanthocyanin A2 dimer and its potential use as a biomarker of cranberry intake: liquid chromatography. J Sep Sci 2016;39:342–9.
115. Liu H, Garrett TJ, Tayyari F, Gu L. Profiling the metabolome changes caused by cranberry procyanidins in plasma of female rats using (1) H NMR and UHPLC-Q-Orbitrap-HRMS global metabolomics approaches. Mol Nutr Food Res 2015;59:2107–18.