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Effect of Sweetened Dried Cranberry Consumption on Urinary Proteome and Fecal Microbiome in Healthy Human Subjects

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Abstract

The relationship among diet, human health, and disease is an area of growing interest in biomarker research. Previous studies suggest that the consumption of cranberries (*Vaccinium macrocarpon*) could beneficially influence urinary and digestive health. The present study sought to determine if daily consumption of sweetened dried cranberries (SDC) changes the urinary proteome and fecal microbiome, as determined in a prospective sample of 10 healthy individuals. Baseline urine and fecal samples were collected from the subjects in the fasted (8–12 h) state. The subjects then consumed one serving (42 g) of SDC daily with lunch for 2 weeks. Urine and fecal samples were collected again the day after 2 weeks of SDC consumption. Orbitrap Q-Exactive mass spectrometry of urinary proteins showed that consumption of SDC resulted in changes to 22 urinary proteins. Multiplex sequencing of 16S ribosomal RNA genes in fecal samples indicated changes in relative abundance of several bacterial taxonomic units after consumption of SDC. There was a shift in the Firmicutes:Bacteroidetes ratio, increases in commensal bacteria, and decreases or the absence of bacteria associated with negative health effects. A decrease in uromodulin in all subjects and an increase in *Akkermansia* bacteria in most subjects were observed and warrant further investigation. Future larger clinical studies with multiomics and multitissue sampling designs are required to determine the effects of SDC consumption on nutrition and health.

Keywords: microbiome science, multiomics, nutrigenomics, proteomics, system diagnostics

Introduction

Integrated studies of proteome and microbiome in response to diet are of importance to move omics data to tangible clinical applications. Previously, cranberry polyphenol chemistry has been related to health outcomes (Burleigh et al., 2013; Feliciano et al., 2015; Krueger et al., 2013a; Pierre et al., 2013, 2014). In light of these past studies and the increase in consumption of sweetened dried cranberries (SDC), we determined if the consumption of SDC by healthy human subjects results in detectable changes to the urinary proteome and the fecal microbiome.

Cranberries contain proanthocyanidins (PACs) which improve gut barrier function in mouse models (Anhe et al., 2015; Pierre et al., 2013, 2014). Therefore, discovery and identification of signature shifts in endogenous urinary proteins or the fecal microbiome could inform the development of new biomarkers of gut barrier function. Urine and feces, in particular, have value as diagnostic specimens for tracking dietary interventions for health outcomes because collection is noninvasive. In typical human urine, the excretion of proteins is relatively low, but the sample volume can be large. Urine fluctuates in response to stimulants and contains potential biomarkers (Chen and Kim, 2016; Thomas et al., 2016). Diet may reversibly alter the human fecal microbiome at the species and genera level, although the microbiome is stable at the phylum level in long-term studies (Martinez et al., 2013).

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Table 1. Sweetened Dried Cranberry, Nutritional Data

Nutrient	*Unit*	*Per serving*
Water	G	6.32
Energy	Kcal	123
Protein	G	0.07
Total lipid (fat)	G	0.44
Carbohydrate, by difference	G	33.12
Fiber, total dietary	G	2.1
Sugars, total	G	29.02
Calcium	Mg	4
Iron	Mg	0.16
Magnesium	Mg	2
Phosphorus	Mg	3
Potassium	Mg	20
Sodium	Mg	2
Zinc	Mg	0.04
Vitamin C, total ascorbic acid	Mg	0.1
Thiamin	Mg	0.005
Riboflavin	Mg	0.011
Niacin	Mg	0.219
Vitamin B-6	Mg	0.015
Vitamin A, RAE	μg	1
Vitamin A, IU	IU	18
Vitamin E (alpha-tocopherol)	mg	0.84
Vitamin K (phylloquinone)	μg	3
Fatty acids, total saturated	g	0.035
Fatty acids, total monounsaturated	g	0.119
Fatty acids, total polyunsaturated	g	0.073
Fatty acids, total trans	g	0.001

USDA (2016).

The aims of this prospective study were to determine if daily consumption of SDC alters the urinary proteome and fecal microbiome in 2 weeks and evaluate if these changes might be related to urinary and digestive health.

Materials and Methods

Sweetened dried cranberries

SDC were a blend from two commercial sources (Ocean Spray Cranberries and Mariani) mixed at a 1:1 (w/w) ratio. The blended SDC were repackaged into 42 g individual servings (USDA, 2016) in a food grade processing facility. SDC composition is provided in Table 1 (USDA, 2016). The 4-(dimethylamino)cinnamaldehyde (DMAC) assay was used to quantify PAC content (Feliciano et al., 2012).

Clinical study design

Healthy human subjects ($n = 10$, 2 men and 8 women) between the ages of 20 and 41 (mean = 27.5 years ±9.95 standard deviation [SD]) with a body mass index of 20.5–28.7 (mean = 24.07 years ±2.31 SD) were recruited and screened. Volunteers with a medical history of immune-compromising diseases, urinary tract infection (UTI) within the past 6 months, chronic inflammatory bowel disease (IBD), digestive diseases, diabetes, or cranberry allergy were excluded. Subjects who already regularly consumed cranberry products were excluded. Volunteers disclosed prescription and over-the-counter medications, as well as supplements, and these were taken into consideration when we selected the study subjects. One subject maintained a vegetarian diet during the trial period. None of the subjects had notable dietary changes during the trial.

Those selected were enrolled under a protocol approved by the University of Wisconsin Health Sciences Institutional Review Board (IRB 2015-0317). A written and informed consent was obtained from all subjects.

Subjects were directed to complete a daily diet journal for 7 days before the baseline samples. Subjects continued to record dietary intake (including time of consumption) during the 2-week intervention period. Exercise or other daily activity was not recorded.

Baseline urine and fecal samples were collected from the subjects in the fasted (8–12 h) state. The subjects then consumed one serving (42 g) of SDC daily with lunch for 2 weeks. Urine and fecal samples were collected again the day after the 2-week SDC consumption period, as was done previously. A total of 10 paired pre- and post-consumption samples were collected in the present study. Samples were kept in a cooler with frozen gel packs from the time of collection until the time of submission to the laboratory. Urine samples were immediately frozen at −80°C from the time of submission (July and August 2015) until they were defrosted and aliquoted (4–5 mL) for analysis (December 2015). Fecal samples were immediately aliquoted (150 mg) and frozen at −80°C from the time of submission (July and August 2015) until they were defrosted for analysis (December 2015).

Analysis of urinary proteome

Preparation and proteome analysis of the urine samples were done by the UW School of Pharmacy Analytical Instrumentation Center Mass Spectrometry Facility (Fig. 1A). Using a label-free approach (Jerebtsova and Nekhai, 2014), we used mass spectrometry for characterization of the urinary proteome. The Orbitrap Q-Exactive Mass Spectrometry system combines quadrupole precursor ion selection with high-resolution accurate mass Orbitrap detection to deliver very high resolving power (up to 140,000 full width at half-maximum) to identify, quantify, and confirm compounds in complex samples, which facilitates proteomics, metabolomics, lipidomics, and metabolomics through detection of low-abundance components in complex samples (Michalski et al., 2011).

Each subject had a pre- and post-treatment urine sample, and a technical replicate for each sample was run through the Orbitrap Q-Exactive mass spectrometer. All centrifuge steps were spun at 4°C, 20,000 *g*. Four microliters urine of each sample was concentrated to 500 μL using Amicon Ultra 3 kda molecular weight (MW) spin filters pre-wet with optima liquid chromatograph mass spectrometry (LC-MS) grade water. Samples were buffer exchanged with 3x volume of 50 mM ammonia bicarbonate before protein quantification and digestion. Micro BCA of all 20 samples (pre- and post-) was performed. Urinary proteins were normalized with total ion current. Twenty micrograms protein (determined by Micro BCA) of each sample was digested as per standard digestion protocol.

Digested proteins were cleaned on C18 Zip Tips as per manufacturer's protocol. One microgram of each sample was injected in duplicate on a 115 min increasing acetonitrile (ACN) gradient. Pre- and post-Enolase QC runs to assess mass spec performance were performed, and a 60 min blank run between injections to clean the column and prevent carryover was undertaken. Resulting raw files were searched

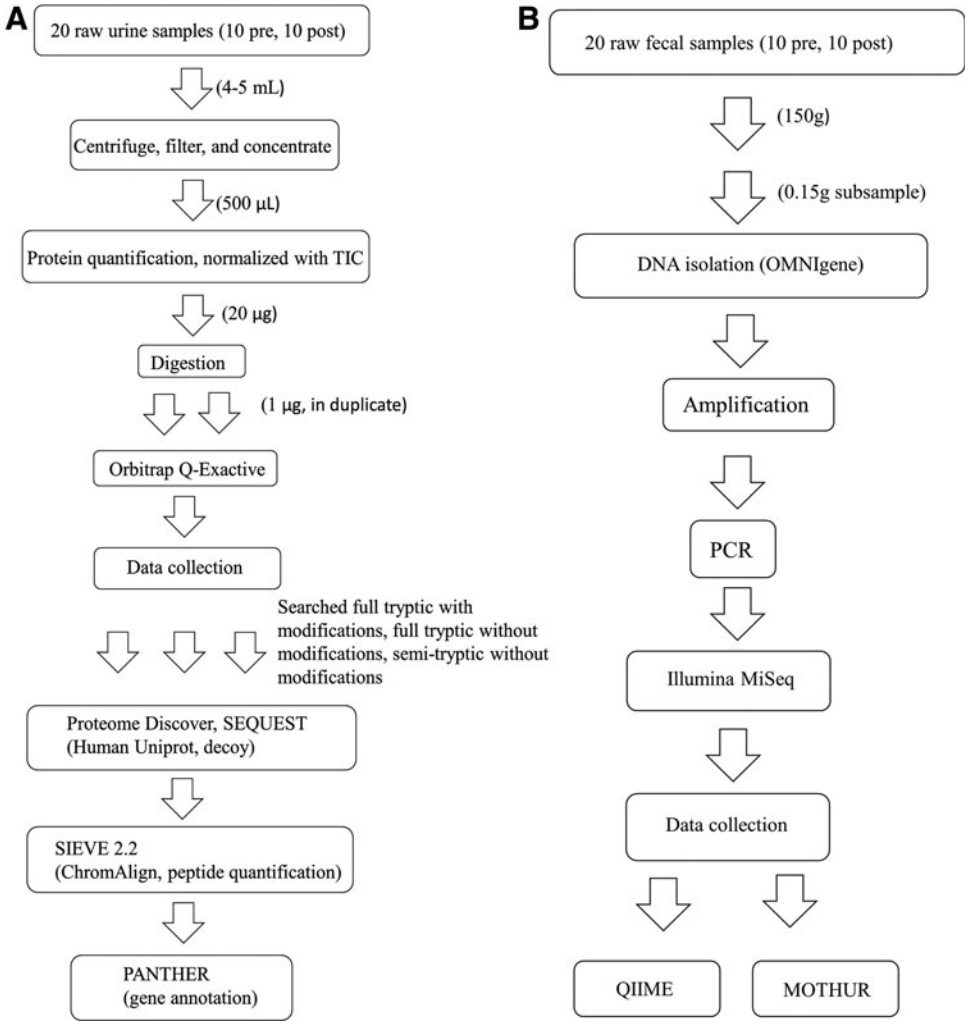

FIG. 1. Procedures for *proteomic* **(A)** analyses. Procedures for *microbiome* **(B)** analyses. TIC, total ion current.

thrice each (one full tryptic with modifications, one full tryptic without modifications, and one semitryptic without modifications) using Proteome Discoverer 1.4.1.14, SEQUEST HT (Thermo Fisher) against the human UniProt database, including a decoy.

ChromAlign and peptide quantification was performed using SIEVE 2.2 (Thermo Fisher). SIEVE data output includes protein identification for each subject, including the number of peptides, frames, hits, and the pre/post protein ratio calculation. Since our method used a label-free technique, results are relative. Further analysis was conducted with the data from the semitryptic approach using proteins composed of two or more peptides.

For proteins found to be significantly different, we conducted a batch analysis of gene annotation information through PANTHER version 10: expanded protein families and functions and analysis tools (Mi et al., 2016) and the PANTHER Classification system (Mi et al., 2013).

Analysis of fecal microbiome

The fecal microbiome analysis was performed by the University of Wisconsin-Madison Biotechnology Center (Fig. 1B). DNA was isolated from 0.15 g of fecal matter using the OMNIgene adapted MO BIO PowerFecal DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). DNA concentration was verified using the Qubit® dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA). Samples were prepared as described in the 16S Metagenomic Sequencing Library Preparation Protocol, Part No. 15044223 Rev. B (Illumina, Inc., San Diego, CA, USA) with the following modifications: The 16S ribosomal RNA gene V3/V4 variable region was amplified with nested primers:

forward primer: 5′-ACACTCTTTCCCTACACGACGC TCTTCCGATCTCCTACGGGNGGCWGCAG-3′

reverse primer: 5′-TGACTGGAGTTCAGACGTGTGCT CTTCCGATCTGACTACHVGGGTATCTAATCC-3′

Region specific primers were previously described in Klindworth et al., 2013, and were modified to add Illumina adapter overhang nucleotide sequences to the gene-specific sequences. Following initial amplification, library size was verified on an Agilent DNA1000 chip and cleaned using a 1x volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences, Union City, CA, USA). Illumina dual indexes and sequencing adapters were added using the following primers:

forward primer: 5′-AATGATACGGCGACCACCGAGA TCTACAC[55555555]ACACTCTTTCCCTACACGACGC TCTTCCGATCT-3′

reverse primer: 5′ CAAGCAGAAGACGGCATACGAG AT[77777777]GTGACTGGAGTTCAGACGTGTGCTCTT CCGATCT-3′

Bracketed sequences are equivalent to the Illumina Dual Index adapters D501–D508 and D701–D712. Following PCR, samples were cleaned using a 1x volume of AxyPrep Mag PCR clean-up beads (Axygen Biosciences). Quality and quantity of the finished libraries were assessed using an Agilent DNA1000 chip and Qubit dsDNA HS Assay Kit, respectively. Libraries were standardized to 2 μM and pooled before sequencing. Paired-end 250 bp sequencing was performed using the Illumina MiSeq Sequencer and a MiSeq 600 bp (v3) sequencing cartridge. A technical replicate was run for each sample.

Raw data were analyzed using the standard Illumina Pipeline, version 1.8.2. OTU assignments and diversity plots were created using QIIME analysis pipeline (Caporaso et al., 2010). Additional analysis was done using the program MOTHUR (Schloss et al., 2009).

Statistical analysis

We used a two-tailed binomial test with a p-value of 0.05 to determine if the number of individuals with common proteins or bacteria changing in the same direction was significant. Because there were only 10 subjects, we considered results with a p-value between 0.05 and 0.10 as trending toward significance with a view to potential leads to inform future hypothesis-driven studies with more subjects.

Results

Sweetened dried cranberries

We tested the SDC for composition. By the DMAC assay, we found 0.62 mg/g (A2 equivalents) of soluble PAC and 2.22 mg/g of cranberry PAC (cPAC) equivalents in the SDC product.

Urinary proteome

Technical replicate injections had a 71% overlap at the protein level and 68% overlap at the peptide level, which are in line with published results (Addona et al., 2009). We detected 767 proteins with two or more peptides (Supplementary Table S1). To the best of our knowledge, 464 of the 767 proteins have not been previously described in the human urinary proteome (Adachi et al., 2006; Li et al., 2010; Marimuthu et al., 2011; Santucci et al., 2015; Zerefos et al., 2012; Fig. 2). The unique proteins we identified in this study are listed in Supplementary Table S2. Individual variation in both the number of proteins identified and the composition of the proteome was high. Although 64 proteins were found in at

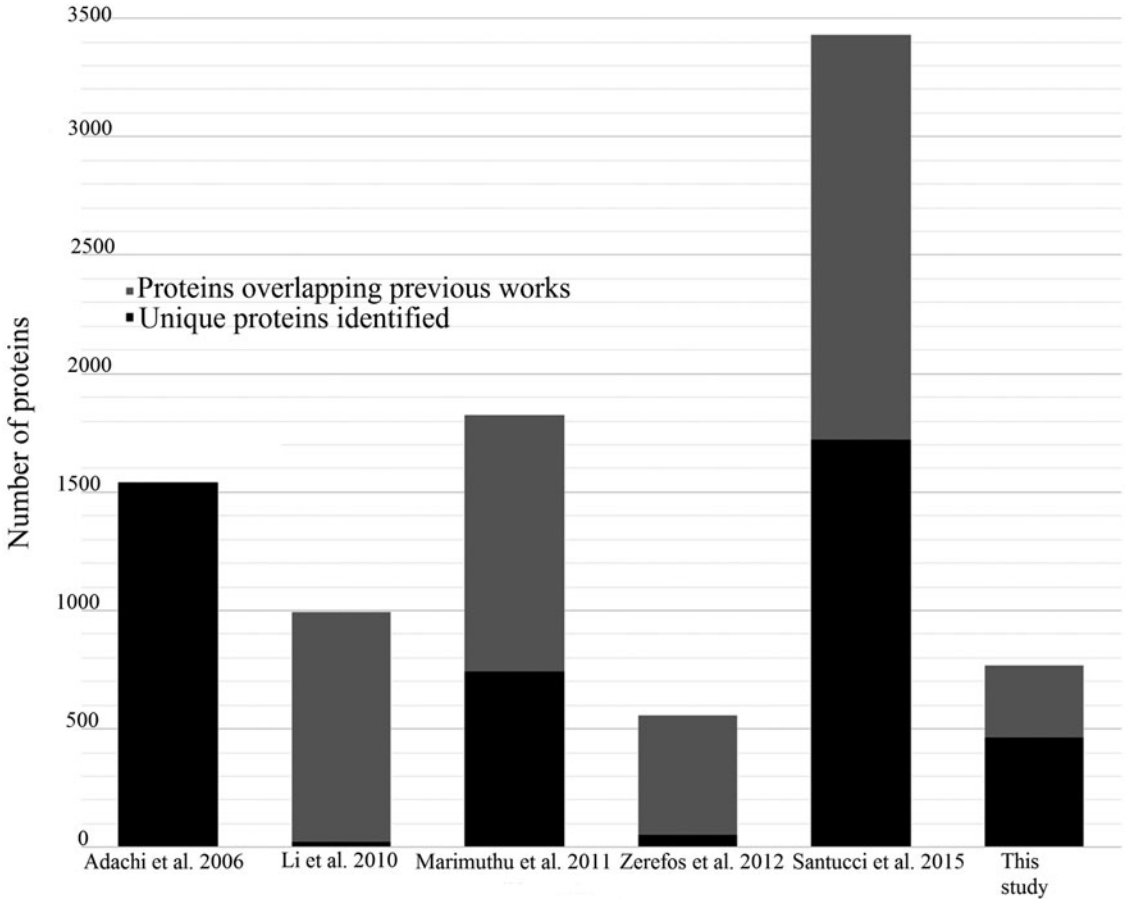

FIG. 2. Comparison with previous large urinary proteomic publications. Each bar is the total number of proteins identified in the study; the *black* portion of each bar represents proteins not previously identified, while the *gray* portion represents proteins identified in one or more of the previous works.

Table 2. Notable Differences in Pre- Versus Post-trial Proteins in Our Study Sample ($N=10$ Subjects)

UniProt KB	*Description*	*Change*
P02768	ALBU Serum albumin	9/10 decreased
P10909	CLUS Clusterin	9/10 decreased
P39059	COFA1 Collagen alpha_1 (XV) chain	9/10 decreased
P01133	EGF Pro_epidermal growth factor	9/10 decreased
Q16270	IBP7 Insulin_like growth factor_binding protein 7	9/10 decreased
P01834	IGKC Ig kappa chain C region	9/10 decreased
P05154	IPSP Plasma serine protease inhibitor	9/10 decreased
P01042	KNG1 Kininogen_1	9/10 decreased
P10253	LYAG Lysosomal alpha_glucosidase	9/10 decreased
P10451	OSTP Osteopontin	9/10 decreased
P98160	PGBM Basement membrane_specific heparan sulfate proteoglycan core protein	9/10 decreased
Q12907	LMAN2 Vesicular integral_membrane protein	10/10 decreased
Q96FE7	P3IP1 Phosphoinositide_3_kinase_interacting protein 1	10/10 decreased
P07911	UROM Uromodulin	10/10 decreased
P01009	A1AT Alpha_1_antitrypsin	8/9 decreased
P05090	APOD Apolipoprotein D	8/9 decreased
O60494	CUBN Cubilin	8/9 decreased
P05155	IC1 Plasma protease C1 inhibitor	8/9 decreased
Q6GTX8	LAIR1 Leukocyte_associated immunoglobulin_like receptor 1	8/9 decreased
P35555	FBN1 Fibrillin_1	8/9 increased
P55290	CAD13 Cadherin_13	8/8 decreased
Q7Z5L0	VMO1 Vitelline membrane outer layer protein 1 homolog	8/8 decreased

least 80% of our subjects, a high percentage of proteins were only identified in individual subjects (420/767, 54.76%).

Twenty-two proteins were found to significantly change ($p<0.05$) between the pre- and postsamples (Table 2). Collectively, they belong to the gene ontology (GO) biological process categories of platelet degranulation (53.96-fold enrichment, $p=9.75\times10^{-6}$) and single multicellular organism process (3.13-fold enrichment, $p=6.41\times10^{-4}$). Seven of these proteins are mapped to GO pathways (Table 3).

Of the 25 proteins found in all subjects (Table 4), three proteins, namely the vesicular integral membrane protein, phosphoinositide 3-kinase interacting protein 1, and uromodulin, were observed to significantly decrease in all subjects after the consumption of SDC ($p=0.000977$). Eleven proteins were observed to decrease in 9 of 10 subjects (p=0.010742) after SDC consumption: serum albumin, clusterin, collagen alpha 1 (XV) chain, pro-epidermal growth factor, insulin-like growth factor binding protein, Ig kappa chain C region, plasma serine protease inhibitor, kininogen 1, lysosomal alpha glucosidase, osteopontin, and basement membrane specific heparan sulfate proteoglycan core protein. Fibrillin-1 increased in 8/9 subjects ($p=0.019531$) after SDC consumption. Cadherin 13 and vitelline membrane outer layer protein 1 homolog were found to decrease after SDC consumption in the 8 subjects in which it was detected ($p=0.003906$).

Table 3. Gene Ontology Pathway Analyses for Proteins Observed as Being Different Between Pre- Versus Posttrial

Pathway accession	*Mapped ID*	*Pathway name*
P00057	P55290 CAD13	Wnt signaling pathway
P06664	P01133 EGF	Gonadotropin releasing hormone receptor pathway
P00011	P01042 KNG1 P01009 A1AT	Blood coagulation
P00034	P39059 COFA1 P98160 PGBM	Interin signaling pathway
P00012	P55290 CAD13	Cadherin signaling pathway
P06959	P10909 CLUS	CCKR signaling map
P00018	P01133 EGF	EGF receptor signaling pathway

Fecal microbiome

A principal coordinate analysis plot of beta-diversity is shown in Figure 3. The pre- versus post-treatment samples did not group together. When we compared the microbial phyla present in the initial samples versus the final samples, the differences were not significant. Six of 10 subjects increased the representation of *Bacteroidetes* and 7 of 10 subjects decreased the representation of *Firmicutes* in the total bacteria present. Taken together, 7 of 10 subjects had a decreased *Firmicutes:Bacteroidetes* ratio. Although not significant, 7 of 10 subjects had increased species diversity in the post sample, and one subject had no change in the total number of species present. Therefore, 8 of 10 subjects had increased or unchanged species diversity, approaching statistical significance ($p=0.0546875$).

Nine subjects had measurable levels of *Akkermansia*, and 7 of 9 had a higher relative abundance of *Akkermansia* in the total bacterial population in the postsample, with a p-value of 0.08984375, trending toward significance (Fig. 4). *Akkermansia* was not detected in the fecal microbiome of one subject, while another subject had a relatively large decrease. In contrast, five subjects had greater than a one log increase in relative abundance of *Akkermansia*.

Discussion

Integrated omics studies in multiple tissues and samples offer a system scale perspective on host–environment interactions,

Table 4. Urinary Proteins Identified in All Subjects

UniProt KB	*Description*
P02768	ALBU_HUMAN Serum albumin
P10909	CLUS_HUMAN Clusterin
P39059	COFA1_HUMAN Collagen alpha_1 (XV) chain
P01133	EGF_HUMAN Pro_epidermal growth factor
Q16270	IBP7_HUMAN Insulin_like growth factor_ binding protein 7
P01834	IGKC_HUMAN Ig kappa chain C region
P05154	IPSP_HUMAN Plasma serine protease inhibitor
P01042	KNG1_HUMAN Kininogen_1
P10253	LYAG_HUMAN Lysosomal alpha_glucosidase
P10451	OSTP_HUMAN Osteopontin
P98160	PGBM_HUMAN Basement membrane_ specific heparan sulfate proteoglycan core protein
Q12907	LMAN2_HUMAN Vesicular integral_ membrane protein VIP36
Q96FE7	P3IP1_HUMAN Phosphoinositide_3_kinase_ interacting protein 1
P07911	UROM_HUMAN Uromodulin
P14209	CD99_HUMAN CD99 antigen
Q14624	ITIH4_HUMAN Inter_alpha_trypsin inhibitor heavy chain H4
P10153	RNAS2_HUMAN Non_secretory ribonuclease
P30530	UFO_HUMAN Tyrosine_protein kinase receptor UFO
P12109	CO6A1_HUMAN Collagen alpha_1 (VI) chain
Q6EMK4	VASN_HUMAN Vasorin
P02461	CO3A1_HUMAN Collagen alpha_1 (III) chain
P41222	PTGDS_HUMAN Prostaglandin_H2 D_isomerase
Q9HCU0	CD248_HUMAN Endosialin
P02671	FIBA_HUMAN Fibrinogen alpha chain
P02751	FINC_HUMAN Fibronectin

VIP36, vesicular integral membrane protein.

including those with food and nutrient intake. In this context, urinary proteomics has been noted as an emergent field of omics science and diagnostic medicine over the past decade (Adachi et al., 2006). Seminal articles in this field (Adachi et al., 2006; Li et al., 2010; Marimuthu et al., 2011; Santucci et al., 2015; Zerefos et al., 2012) have described roughly 4100 urinary proteins.

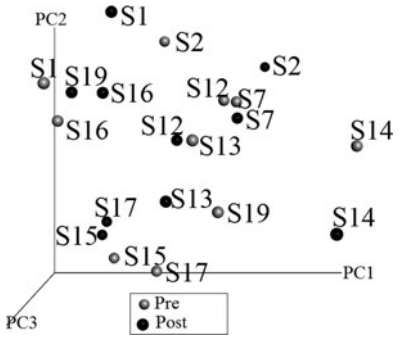

FIG. 3. A weighted principal coordinate analysis plot of fecal microbiome beta-diversity. Each *dot* represents a single subject; *gray dots* are pretreatment and *black dots* are posttreatment. A single subject tends to group with itself, rather than with time (pre- vs. posttreatment).

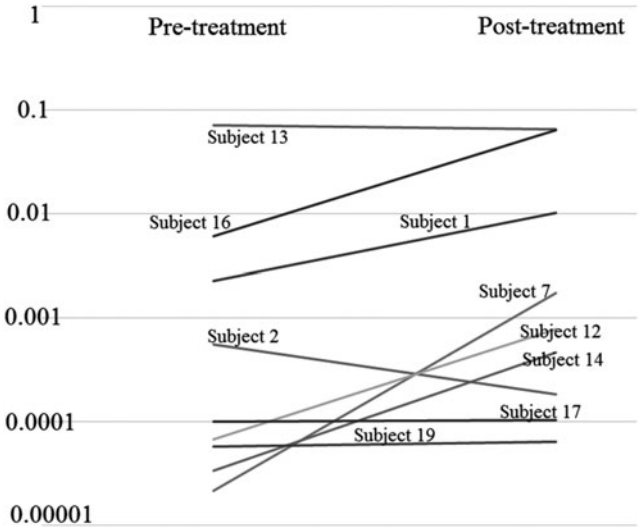

FIG. 4. Log-scale plot of relative pre- versus posttreatment *Akkermansia* to total microbial population values in individual subjects. Subject 15 did not have measurable levels of *Akkermansia*. Seven of nine subjects had relative increases in *Akkermansia*.

In the present study, we sought to prospectively determine whether the effects of daily consumption of SDC would lead to changes in the urinary proteome and fecal microbiome in 10 subjects in 2 weeks. Based on our own research (Krueger et al., 2013b) and of others (Liu et al., 2012; Nagaraj and Mann, 2011), we anticipated that the present sample size would offer new insights on proteome and microbiome changes in response to cranberry consumption. We observed 767 proteins in 10 subjects. To the best of our knowledge, 464 of those proteins have not been previously described in the human urinary proteome.

In contrast to a previous report (Nagaraj and Mann, 2011), we did not find a large list of proteins common to all subjects. We observed that 420 of 767 identified proteins were unique to a single subject.

Interestingly, of the 25 proteins found to be common in all 10 subjects in this study, LMAN2 was not previously reported in the publications described above. LMAN2 binds the sugar residues of glycoproteins and the sugar chain in bacteria (Shirakabe et al., 2011) and is involved in protein sorting and segregation (Fiedler et al., 1994). Two proteins, IC1 and LAIR1, found in 9 of 10 subjects, were also not a part of the common core lists. Complement inhibitor 1 is part of the classical primary complement pathway, and defects can result in a primary immune deficiency illness (Grumach and Kirschfink, 2014). Supplementation of complement inhibitor 1 slows the clotting process, binds gram-negative bacteria, and has anti-inflammatory effects (Landsem et al., 2016). LAIR1 is expressed on hematopoietic cells and carries immunoreceptor motifs on its tail (Cao et al., 2015). LAIR1 is considered an immunoinhibitory collagen receptor, and defects in LAIR1 can result in autoimmune disorders, viral illnesses, and cancer (Sun et al., 2014).

Taken together, our report of 464 urinary proteins that have not been previously reported in the literature and the addition

of the proteins above to the common core of proteins indicate that there is still much to learn about the urinary proteome.

Compared to a previous study using a dietary supplement consisting of cranberry powder (Krueger et al., 2013b), only one of the eight previously reported proteins was detected in the current study [Ig kappa chain V_III region CLL (P04207)]. In the current study, it was neither found to be common to all subjects nor was it found to be significantly different in the subjects in which it was found. The reasons for these findings are unclear. The previous study was shorter (6 days), and cranberry *powder* was administered, rather than SDC. Alternatively, with the small sample size of that study ($n=10$) and our study ($n=10$), the discrepancy may be attributable to individual differences.

Uromodulin is the most abundant urinary protein produced in the kidney and is associated with an increase in innate immune responses in the kidney (Rampoldi et al., 2011). Mouse studies suggest that uromodulin may provide protection to the bladder in a UTI by binding with bacteria (Rampoldi et al., 2011). Uromodulin is also a damage associated molecular pattern that is associated with kidney injury (Anders and Schaefer, 2014) and stimulation of a proinflammatory response in the urinary tract (Darisipudi et al., 2012; Garimella and Sarnak, 2017; Rampoldi et al., 2011). Therefore, the function of uromodulin in the urinary tract remains unclear. Our subjects were prescreened for UTIs before beginning the trial. Since we assume that the subjects maintained a healthy urinary tract system throughout the trial, a decrease in all subjects is an important observation regarding the relationship between uromodulin and urinary tract health. The effect of SDC consumption on uromodulin requires more research.

Although it is not clear if microbial dysbiosis is the cause or effect of disease (Round and Mazmanian, 2009), characterization of the microbial community can highlight such dysbiosis. Bacterial diversity is decreased in microbial dysbiosis (Kim et al., 2016; Zhernakova et al., 2016). Seven of our subjects had a richer microbiota after the intervention, and one had no change. The trend we observed may suggest that SDC favor species richness in the gut, perhaps because SDC contain a diversity of microbial substrates such as fiber and polyphenols (Blumberg et al., 2016).

Another measure commonly addressed in discussions of digestive health is the *Firmicutes:Bacteroidetes* ratio (Ley et al., 2006; Mariat et al., 2009). A reduction in the ratio could indicate an increase in *Bacteroidetes*, a decrease in *Firmicutes*, or both. *Firmicutes* are associated with increased energy efficiency and absorption, a condition that could cause obesity. Seven of our subjects lowered their *Firmicutes:Bacteroidetes* ratio in the trial. Specifically, 6/10 subjects increased *Bacteroidetes* and 7/10 subjects decreased *Firmicutes*. An interesting anomaly occurred with the subject who maintained a vegetarian diet during the trial—the ratio increased. In that subject, *Prevotella* (a *Bacteroidetes*) was the largest contributor to the microbiome in the pretrial sample, and *Ruminococcus* (a *Firmicutes*) was the largest contributor to the microbiome in the post-intervention sample.

Falony et al. (2016) included only the genus *Bifidobacterium* of the phylum *Actinobacteria* as part of the global core bacteria. We found both the genera *Bifidobacterium* and *Eggerthella* in all our samples. *Actinobacteria* produce bioactive metabolites, including antibacterials, antifungals, antivirals, and immunomodifiers. Although it was not significant, we found an increased representation in the total bacterial community in both genera. Based on the functions of the phylum, SDC may be stimulating immune function in the gut.

Our previous work (Pierre et al., 2013) demonstrated that when cPACs are added to enteral nutrition in mice, mucin production in the gut increases. *Akkermansia* are mucin degrading bacteria. Increased mucin production could support an increased population of *Akkermansia*. The presence of *Akkermansia* mediates symptoms associated with metabolic syndrome (Anhe et al., 2015, 2016; Li et al., 2016; Roopchand et al., 2015; Schneeberger et al., 2015). Of our 10 subjects, 9 subjects had detectable levels of *Akkermansia* in both the pretrial state and post-intervention. Of those, seven had an increased relative abundance of *Akkermansia* in the total bacterial composition.

Conclusions

The addition of SDC to the diet of 10 subjects daily for 2 weeks influenced the composition of the urinary proteome and fecal microbiome. Twenty-two proteins were found to have differences between pre- and post-treatment, including uromodulin. With the function of uromodulin in the urinary system unclear, but understood to be implicated in urinary health, the decrease found in all subjects was intriguing. Utilizing a labeled isotope approach to target and quantify uromodulin in relation to SDC will be a focus in upcoming studies. The present study also added to the number of proteins found in healthy human urine by about 10%. Targeting the genera of interest, including *Akkermansia* to obtain more quantitative results, is also warranted in the future.

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Author Disclosure Statement

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Abbreviations Used

cPAC = cranberry PAC
DMAC = 4-(dimethylamino)cinnamaldehyde
GO = gene ontology
IBD = chronic inflammatory bowel disease
IRB = Institutional Review Board
PAC = proanthocyanidin
SD = standard deviation
SDC = Sweetened Dried Cranberries
UTI = urinary tract infection