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Cranberry-lingonberry juice affects the gut and urinary microbiome in children - a randomized controlled trial

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The mechanism by which cranberry-lingonberry juice (CLJ) prevents urinary tract infections (UTI) in children remains unknown. We hypothesized that it alters the composition of the gut or urinary microbiome. Altogether, 113 children with UTIs were randomly allocated to drink either CLJ or a placebo juice for 6 months. We collected urinary samples at 3 months and fecal samples at 3, 6 and 12 months and used next-generation sequencing of the bacterial 16S gene. The children who consumed CLJ had a lower abundance of Proteobacteria ($p = 0.03$) and a higher abundance of Firmicutes phylum ($p = 0.04$) in their urinary microbiome at 3 months than did those in the placebo group. The abundance of *Escherichia coli* in the urinary microbiome was 6% in the CLJ group and 13% in the placebo group ($p = 0.42$). In the gut microbiome the abundance of Actinobacteria at 3 and 12 months was higher in the children receiving CLJ. The diversity of the urinary and gut microbiome did not differ between the groups. The children drinking CLJ had a different urinary and gut microbiome from those receiving a placebo juice. A healthy urinary microbiome may be important in preventing UTIs in children.

Key words: Children; cranberry; infectious disease; lingonberry; microbiome; urinary tract infection.

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INTRODUCTION

Urinary tract infections (UTI) are common febrile infections in infants and children [1,2]. Recurrences after the first UTI occur in 12% to 32% children within 1-year of follow up [3–6] and the same bacterial strain was isolated during the first UTI episode causes the next recurrent infection in 60–70% of cases [7].

The pathogens causing UTIs originate from the patient's own gut [8]. One interesting study in adult patients found that the abundance of *E. coli* in the gut microbiome was associated with *Escherichia* bacteriuria [9], while we have shown in a previous controlled study that the gut microbiome composition was associated with the risk of UTI in children hospitalized for pyelonephritis [10]. The role of the recently discovered urinary microbiome in the pathogenesis of UTIs remains unclear, however, as research on this subject is still scarce [11], but one recent study has shown that alpha diversity of the urinary microbiome, reflecting diversity found within one sample, was found to be lower in children with UTI [12]. Drinking cranberry-lingonberry juice (CLJ) has been shown to prevent UTI recurrences in adult women [13] and by us in a previous randomized study to prevent recurrent UTIs in children as well [6]. In animal models and human gut

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simulators, cranberry products have been shown to induce changes in the gut microbiome composition [14–17], although their effects on the gut microbiome in adults have varied [18,19].

Although cranberry-lingonberry juice (CLJ) is known to contain proanthocyanidin flavonoids, the exact mechanism by which it prevents UTIs remains unknown. We hypothesized that CLJ alters the composition of the gut or urinary microbiome, which may explain its efficacy. We, therefore, set out here to test the effects of cranberry-lingonberry juice on the gut and urinary microbiome in children in a randomized placebo-controlled trial.

METHODS

Study design and supervision

The children recruited for this randomized clinical trial had all been diagnosed with UTI in the Department of Pediatrics and Adolescent Medicine, Oulu University Hospital, Finland, the Health Care Center run by the city of Oulu, Finland, or the Pediatric Emergency Department of Oulu University Hospital between July 12 2013 and February 27 2018. The trial was designed to compare the gut and urinary microbiome in children who received cranberry-lingonberry juice for 6 months with those who received a placebo juice. We collected fecal samples at 3, 6 and 12 months after entry and urine samples at 3 months. A secondary outcome was the occurrence of UTI during the study.

The children recruited met the following inclusion criteria: age between 1 year and 16 years and a confirmed UTI within 7 days of entry. UTI at entry was defined as fever and/or a local urinary tract symptom, the presence of pyuria or nitrite and a positive urine culture, defined as the growth of $>10^5$ colony-forming units (CFU) per milliliter of the same pathogen in clean-voided urine or a urine collection pad sample. If the child had two urine samples available, growth of $>10^5$ CFU of the same pathogen in one sample and at least $10^{4–5}$ in the other were required. The exclusion criteria were continuous antimicrobial prophylaxis, or a severe congenital kidney or urinary tract anomaly as seen in ultrasound.

This was an investigator-driven academic clinical study, the CLJ and placebo juice products for which were donated by the manufacturer. The manufacturer did not, however, participate in the study design, analysis, or writing of the report, nor fund the research in any other way. The Regional Ethics Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland, approved the study protocol (Decision number EETTMK: 74/2012). All relevant national and local guidelines for clinical research were followed. All the parents of the children, and the children themselves when over 6 years of age, gave written informed consent before the children entered the study. The study was registered at ClinicalTrials.gov with the identifier code NCT01861353.

A parallel study design was used in which the children were assigned after recruitment to either the CLJ group or the placebo group (1:1) using a block randomization procedure with a block size of four. After obtaining written informed consent, the nurse or physician opened the next available sequentially numbered opaque envelope that contained the group designation, either juice A or juice B. The study was a double-blinded one, in that both the families and the nurses, and also the authors, were unaware which product had been given to a particular child. The juices both looked and tasted similar and were supplied in identical white, coded 200 mL cartons. The unblinding took place only after the microbiome analysis and other data had been recorded and were ready for the final analysis.

Intervention

On entry, the parents completed a questionnaire concerning background, clinical characteristics and their child's dietary habits, and the intervention was started within 7 days of obtaining a confirmed UTI diagnosis. All the UTI episodes were treated with antibiotics according to national and local guidelines, but the parents were asked not to give their children any other lingonberry or cranberry products during the trial.

The product that the CLJ group received contained 12.8% (weight/volume) cranberry juice, 12.4% (w/v) lingonberry juice and 10 g/dL added sugars, and the intervention lasted for 6 months with a daily dose of 5 mL/kg to a daily maximum of 300 mL. The juice was manufactured and donated by Eckes-Granini, Turku, Finland.

The placebo group received a juice containing natural cranberry flavor, red anthocyanin color, 5.5 g/L citric acid and 10 g/dL added sugars, also for 6 months and with a similar daily dose of 5 mL/kg to a daily maximum of 300 mL. This placebo juice was also manufactured by Eckes-Granini, Turku, Finland.

Samples and clinical follow up

Fecal samples were collected at 3 months, 6 months, and 12 months and urine samples at 3 months, and the parents kept a daily record of the consumption of the two juice products and any symptoms indicating UTI for a 12-month follow-up period. The symptom diaries were to be returned monthly, and if the parents did not comply, they were contacted either by email or by phone. All original medical records, including microbiological samples, were retrieved, and reviewed if the children had a UTI or suspected UTI episode during the 12-month follow up.

Primary and secondary outcomes

The primary outcome was the comparison of the composition of the gut or urinary microbiome between the children receiving CLJ and those receiving the placebo juice. The secondary outcomes were the occurrence of urinary tract infections and the time elapsing before the first recurrence. The diagnostic criteria for UTI were the same as at entry to the study. UTI episodes were recorded as separate events if there were at least 10 days between the first symptomatic day and the onset of new symptoms. UTI diagnosed during the study was treated with a standard antimicrobial therapy regimen, and the child continued drinking the juice.

Microbiome analysis and bioinformatics

We used the QIAamp DNA stool kit (Qiagen, Germany) to extract DNA according to the manufacturer's procedure and the QIAamp manual to extract the fecal and urine samples. To improve the DNA yield, the final DNA elution volume was reduced to 50 uL. A NanoDrop 1000 Spectrophotometer was used to quantify the concentration of DNA (Thermo Fisher Scientific).

We sequenced the V4–V5 region of the 16 S rRNA gene using primer 519F with unique barcodes and primer 926R and used the Phusion Flash High-Fidelity enzyme (Thermo Fisher Scientific) according to the manufacturer's protocol, together with an Applied BiosystemsTM Veriti 96-Well Thermal Cycler (Thermo Fisher Scientific), for PCR. A negative control (sterile water, HyCloneTM HyPure, Thermo Fisher Scientific) was included on each PCR plate, and the initialization program was run for 3 min at 98°C, followed by 35 cycles of reaction commencing at 98°C for 10 s followed by 30 s at an annealing temperature of 56°C and elongation for 30 s at 72°C. The final elongation time was 5 min at 72°C.

AMPure XP was used to purify the samples after they had been pooled (Beckman Coulter, CA, USA). The purified pool was passed over a 1% agarose gel, and the 16 S product was extracted from the gel and purified using the MinElute Gel Extraction Kit (Qiagen). The final product was purified using AMPure XP, evaluated with a Bioanalyzer and the pool concentration determined using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific). IonTorrent PGM was used for the sequencing (Thermo Fisher Scientific).

Quantitative Insights Into Microbial Ecology 2 (QIIME2; version 2019.10) was used for the analysis [20]. After excluding DNA readings <200 bp from the dataset, DADA2 was used to demultiplex and denoise the readings [21]. The denoised readings were then clipped at 15 and truncated at 260, after which chimeric readings were removed, yielding a total of 2,758,106 processed readings available for further analysis. After eliminating low-quality samples, the material included 116 fecal and urine samples for Principal Coordinate Analysis (PCoA) using Bray–Curtis dissimilarity, and also taxonomic analysis at the phylum and genus levels. The relative frequencies of the taxonomic compositions in each group of samples were computed. The HOMD (version 15.1) was employed for the taxonomic analysis (http://www.homd.org/index.php) and Krona for drawing the bacterial taxonomy pie charts [22]. We have deposited the Ion Torrent bacterial raw data in the Sequence Read Archive (SRA) with accession number PRJNA804368.

Sample size and statistical analysis

Since a meaningful analysis of the gut microbiome and the risk of UTI had been reported earlier with a group size of 30–40, this was estimated to be a sufficient group size for the present purposes [10]. Microbiome analyses were performed for all the randomized children with fecal or urine samples available. As the main interest lay in the mechanism of action of CLJ, we performed the analyses separately for all the children with samples available and for those with over 80% compliance in juice consumption, defined as the amount of juice consumed at 3 months (mL) divided by the target amount of juice to be consumed (mL). The clinical outcome, that is, the occurrence of UTI, was analyzed in all the children recruited, including those without samples available.

For the microbiome analysis, the number of OTUs, that is the number of different bacterial taxa with a >97% similarity threshold, diversity indices and the microbiome composition, including the relative abundances of the main bacterial phyla and genera of interest, were compared between the intervention groups with the Mann—Whitney *U* test. All comparisons of the microbiome composition regarding the relative abundance of the main bacterial phyla and the genus *E. coli* were performed to evaluate the pre-test hypothesis. All the other tests were exploratory and were controlled for a false discovery rate by Bonferroni correction. For the clinical outcomes, the proportion of children who had at least one recurrent UTI was compared by means of the standard normal deviate test (SND test) in the StatsDirect statistical program and the total number of UTIs between groups with the Mann—Whitney *U* test in the SPSS statistical program. The time to the first UTI recurrence was analyzed by the Kaplan—Meier method and tested between the groups by the Log Rank test with the SPSS program.

RESULTS

A total of 525 children were assessed for eligibility (Fig. 1), and 113 of the 248 eligible children were recruited. There were 56 children in CLJ group and 57 children in placebo group. The trial ended February 28, 2019 after the sample size was completed and every participant had finished their follow-up period. Altogether 184 fecal and urine samples were analyzed and there were 41 children who gave fecal or urine samples at 3 months and had >80% compliance with the juice consumption instructions. In total, 18 of these children were placed in CLJ group and 23 in placebo group. The number of children who stopped consuming the juice during the trial for some reason or gave no data on juice consumption was 29 in the CLJ group and 21 in the placebo group. No harmful side effects were detected during the trial. Most of the participants were girls (Table 1). *E. coli* was the most common uropathogen in the urine samples from both the CLJ and placebo group on entry. The most common antibiotics used to treat UTIs at study entry were cefalexin, cefuroxime, trimethoprime, and sulfadiazine+trimethoprime in both study groups.

Diversity of the gut microbiome

The diversity of the gut microbiome as evaluated by calculating the mean number of operative taxonomic units (OTU) and using the Shannon, Faith and Pielou diversity indices, did not differ between the children receiving CLJ and those receiving the

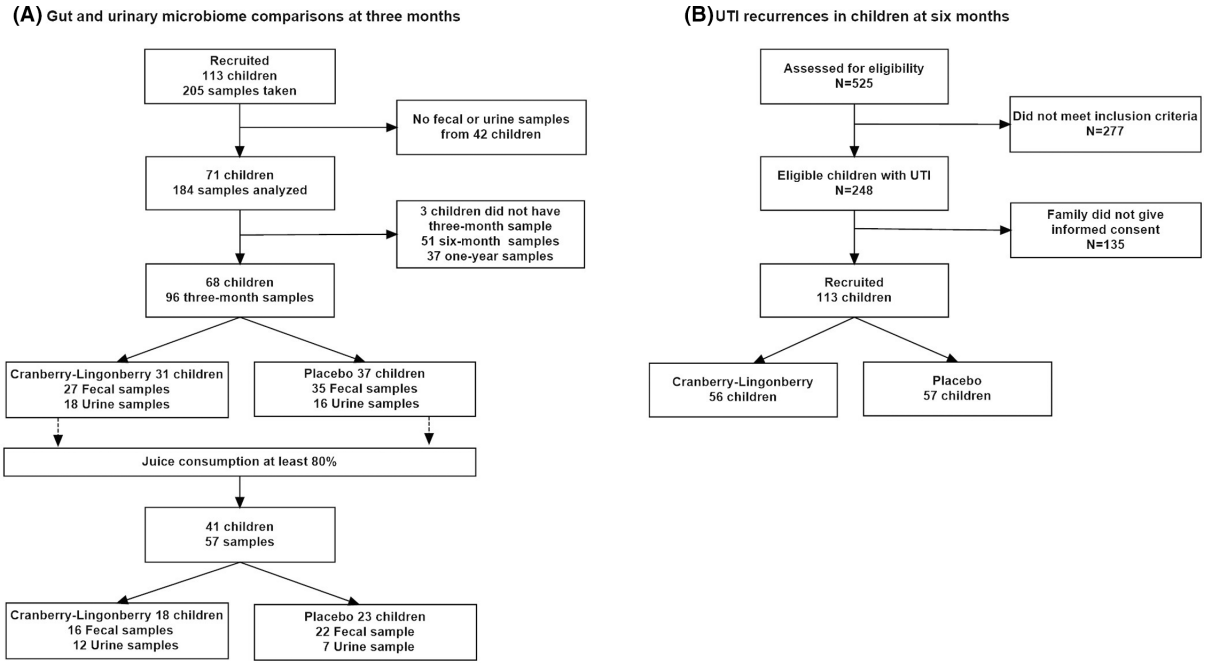

Fig. 1. Study design. (A) Gut and urinary microbiome. (B) Recurrences of urinary tract infection (UTI).

placebo juice at 3 months (Table 2), and there were no statistically significant differences between the groups in any of the diversity markers at 6 and 12 months, either (Table S1). Bray—Curtis dissimilarity analysis showed no clear clustering of the gut microbiome in the fecal samples at 3 months according to the treatment groups (Fig. 2).

Diversity of the urinary microbiome

We then analyzed the diversity of the urinary microbiome at 3 months using the same diversity variables (Table 2). There was no difference between the groups, nor was there any clear clustering of the urine samples by treatment groups at 3 months in the Bray—Curtis dissimilarity analysis of the urinary microbiome (Fig. 2).

Gut microbiome composition

We first compared the relative abundances of the main bacterial phyla in the gut microbiome. Bacteroidetes was the most abundant phylum in the gut microbiome in all the children with fecal samples available in both the CLJ and Placebo groups, comprising 63% (SD 18) and 65% (SD 17) of the gut microbiome, respectively (Table 3). The relative abundance of Actinobacteria in the CLJ group (mean 0.42%, SD 0.56) was higher than that in the placebo group (mean 0.28%, SD 0.97; p-value 0.02) at 3 months (Fig. 3), but at 6 months there was no statistically significant difference in the relative abundances of any phyla (Table S2), while at 12 months the relative abundance of Actinobacteria was higher in the CLJ group (mean 0.67%, SD 0.96) than in the placebo group (mean 0.17%, SD 0.21; p-value 0.04).

The overall gut microbiome composition of the children with >80% compliance in the CLJ and placebo groups is presented in Fig. 4. Bacteroidetes was the most abundant phylum in gut microbiome in both groups of children with fecal samples available and >80% compliance in juice consumption, with means of 59% (SD 17) in the CLJ group and 62% (SD 18) in the placebo group (Fig. 3), while the relative abundance of Actinobacteria was higher in the CLJ group (mean 0.64%, SD 0.64) than in the placebo group (mean 0.40%, SD 1.2; p-value 0.01) at 3 months. At 6 months, however, there were no statistically significant differences in the relative abundances of any phyla, although by 12 months the higher relative abundance of Actinobacteria in the CLJ group (mean 0.60%, SD 0.59) than in the placebo group (mean 0.21%, SD 0.22; p-value 0.05) reoccurred.

Urinary microbiome composition

Since the general composition of the urinary microbiome in children is still unclear, we will first report

Table 1. Baseline characteristics of the subjects in the cranberry-lingonberry juice and placebo juice groups

Characteristic	All children		>80% juice compliance	
	CLJ (N = 56)	Placebo (N = 57)	CLJ (N = 18)	Placebo (N = 23)
No. of girls n (%)	55 (98)	56 (98)	17 (94)	22 (96)
Mean age, years (SD)	7.2 (4.0)	5.2 (2.9)	7.2 (4.1)	5.6 (3.1)
Previous UTI morbidity			n = 17	n = 21
Mean number of UTIs (SD)	1.2 (2.7)	1.6 (4.0)	1.9 (3.7)	2.2 (5.7)
Children with at least 1 previous UTI n (%)	20 (39)	18 (34)	9 (53)	6 (21)
Children with previous antibiotic prophylaxis n (%)	2 (3.7)	7 (12.3)	0 (0.0)	2 (8.7)
Children with current antibiotic prophylaxis n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Growth in the urine sample at entry (CFU) n (%)				
Escherichia coli $>10^5$	46 (84)	48 (86)	17 (94)	20 (91)
Escherichia coli 10^4–10^5	3 (5.5)	4 (7.1)	0 (0.0)	1 (4.2)
Escherichia coli $< 10^4$	1 (1.8)[1]	1 (1.8)[1]	0 (0.0)	0 (0.0)
Klebsiella $>10^5$	1 (1.8)	1 (1.8)	0 (0.0)	0 (0.0)
Pseudomonas $>10^5$	0 (0.0)	1 (1.8)	0 (0.0)	0 (0.0)
Staphylococcus saprophyticus $>10^5$	4 (7.3)	0 (0.0)	1 (5.6)	0 (0.0)
Enterococcus faecalis $>10^5$	0 (0.0)	1 (1.8)	0 (0.0)	1 (4.5)
Antibiotics used to treat UTI at study entry n (%)				
Cefalexin, cefuroxime or both	19 (34)	23 (40)	10 (56)	10 (43)
Trimethoprim	16 (29)	9 (16)	4 (22)	4 (17)
Sulfadiazine + Trimethoprim	13 (23)	15 (26)	2 (11)	5 (22)
Nitrofurantoin	0 (0.0)	2 (3.5)	0 (0.0)	1 (4.3)
Pivmecillinam	3 (5.4)	0 (0.0)	0 (0.0)	0 (0.0)
Amoxicillin-clavulanate	1 (1.8)	0 (0.0)	1 (5.6)	0 (0.0)
Other combinations	2 (3.6)	5 (8.8)	1 (5.6)	1 (4.3)
Unknown	2 (3.6)	3 (5.3)	0 (0.0)	2 (8.7)
Frequency of intake of berry or fruit juices n (%)				
More than twice a week	19 (35)	10 (18)	3 (17)	2 (8.7)
1–2 times a week	21 (38)	25 (45)	8 (44)	11 (48)
Less than once a week	15 (27)	21 (38)	7 (39)	10 (43)
Frequency of intake of berries n (%)				
More than twice a week	11 (20)	16 (29)	8 (44)	7 (32)
1–2 times a week	30 (55)	30 (54)	8 (44)	11 (50)
Less than once a week	14 (25)	10 (18)	2 (11)	4 (18)
Obstipation	4 (7.5)	2 (3.6)	2 (11)	0 (0.0)

N may vary at some points because of missing data.
CFU, colony forming unit; CLJ, cranberry-lingonberry juice; SD, Standard deviation; UTI, urinary tract infection.
[1]Suprapubic aspiration.

on the findings in children with samples available at 3 months regardless of the treatment group (N = 34). The mean number of OTUs in the urinary microbiome was 50, the highest relative abundances being in Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria in this order, the most common genera *Prevotella, Lactobacillus, Staphylococcus*, and *Peptoniphilus* and the most common species *E. coli*.

The most abundant phylum in the urinary microbiome of all the children in the CLJ group with urine samples available at 3 months was Firmicutes, with a mean relative abundance of 51% (SD 30) and that in the placebo group Bacteroidetes, with a mean relative abundance of 49% (SD 29). No statistically significant differences in the relative abundances of the phyla were found between the groups (Fig. 3).

The overall urinary microbiome composition of the children in the CLJ and placebo groups with >80% compliance is presented in Fig. 4. Here, the most abundant phylum found in urinary microbiome was Firmicutes with a higher mean relative abundance of 50% (SD 27) in the CLJ group at 3 months as compared with 27% for the placebo group (SD 21) (p-value 0.035) (Fig. 3), whereas the relative abundance of Proteobacteria was lower in the CLJ group (mean 6.6%, SD 21) than that in the placebo group (mean 16%, SD 33; p-value 0.034). The relative abundances of the other phyla did not show any statistically significant differences.

Escherichia coli in the gut and urinary microbiome

We analyzed the abundance of the most common uropathogen, *E. coli*, in both the gut and urinary

Table 2. Diversity of the gut and urinary microbiome at 3 months

All samples		CLJ (N = 27)	Placebo (N = 35)	Mann—Whitney
Gut	Diversity index	Mean (SD)	Mean (SD)	p-Value
	OTUs	66.63 (20.83)	71.41 (29.59)	0.77
	Shannon	4.30 (0.83)	4.29 (0.83)	0.99
	Faith	6.43 (1.74)	6.27 (2.39)	0.43
	Pielou	0.71 (0.09)	0.71 (0.11)	0.59
		CLJ (N = 18)	Placebo (N = 16)	Mann—Whitney
Urinary	Diversity index	Mean (SD)	Mean (SD)	p-Value
	OTUs	51.33 (40.26)	49.13 (18.93)	0.42
	Shannon	3.17 (1.53)	3.16 (1.31)	0.97
	Faith	5.44 (2.36)	5.60 (1.73)	0.58
	Pielou	0.56 (0.23)	0.56 (0.19)	0.76
>80% juice compliance		CLJ (N = 16)	Placebo (N = 22)	Mann—Whitney
Gut	Diversity index	Mean (SD)	Mean (SD)	p-Value
	OTUs	73 (24.86)	69.82 (23.35)	0.67
	Shannon	4.42 (0.72)	4.34 (0.92)	0.95
	Faith	6.54 (1.77)	6.66 (2.86)	0.64
	Pielou	0.72 (0.82)	0.71 (0.12)	0.62
		CLJ (N = 12)	Placebo (N = 7)	Mann—Whitney
Urinary	Diversity index	Mean (SD)	Mean (SD)	p-Value
	OTUs	57.83 (45.01)	53.43 (15.04)	0.40
	Shannon	3.42 (1.32)	3.23 (1.08)	0.55
	Faith	5.69 (2.30)	6.04 (1.61)	0.45
	Pielou	0.60 (0.19)	0.56 (0.16)	0.45

CLJ, cranberry-lingonberry juice; OTU, operational taxonomic unit; SD, standard deviation.

microbiome (Table 3; Table S2). Here no differences were found between the groups at any point in either population in the case of the gut microbiome (Fig. 3). Although the mean relative abundance of *E. coli* in the urine samples was 6% (SD 21) in CLJ group and 13% (SD 33) in placebo group, this difference was not statistically significant (p-value 0.42).

Other genera of interest

Additionally, we compared the relative abundances of the genera *Lactobacillus* and *Staphylococcus* between the groups (Table 3; Table S2) but found no significant differences in the mean relative abundance of either genus in the gut or urinary microbiome at any point in time.

Clinical outcomes

Neither the proportion of children with at least one recurrent UTI nor the total number of UTIs differed statistically significantly between the groups at either 3 or 6 months (Table 4). Likewise, the Kaplan—Meier analysis of the time to the first UTI episode, that is, the time from the day the patients started drinking the juice until the end of the 6-month period or until the follow up was stopped for some reason, showed no statistically significant difference (log-rank test p-value 0.21; Fig. 5).

DISCUSSION

This placebo-controlled trial showed that the children who had received cranberry-lingonberry juice had a different composition of their urinary and gut microbiome from those who had received the placebo juice but that the diversity of the gut and urinary microbiome was not affected. These results suggest that the mechanism of action of cranberry and lingonberry juice in preventing UTI in children may be related to changes in both the gut and urinary microbiome.

Cranberry and lingonberry contain many phenolic compounds with complex metabolism, so that at least 60 phenolic metabolites have been identified in plasma and urine after cranberry juice consumption [23,24]. The proanthocyanidin compounds (PACs) found in both lingonberries and cranberries are thought to be the main bioactive components of these berries [24] and have been shown to have an antimicrobial effect on many gram-positive pathogens and to inhibit hemagglutination of *E. coli in vitro* [24]. Small amounts of PACs and larger numbers of their intestinal metabolites have been

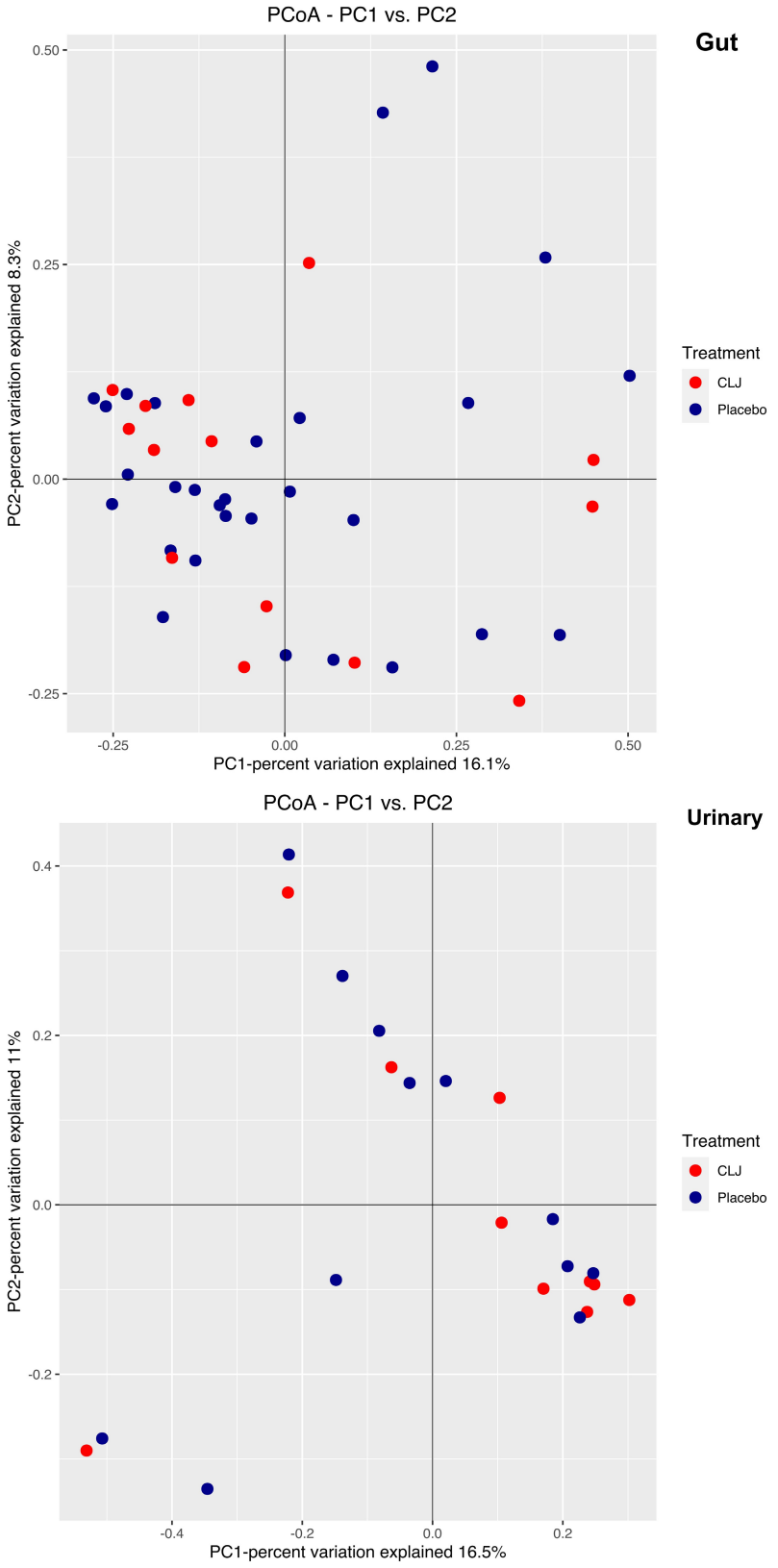

Fig. 2. Principal Coordinate Analysis (PCoA) of the gut and urinary microbiome in the children with over 80% juice drinking compliance at 3 months. Bray—Curtis dissimilarity was used to describe the compositional dissimilarities of the datasets.

Table 3. Relative abundance of taxa in the gut and urinary microbiome at 3 months in all children with available samples and in those with over 80% compliance of juice consumption. Asterix (*) indicates $p < 0.05$

		All samples			>80% juice compliance		
		CLJ (N = 27)	Placebo (N = 35)	Mann—Whitney	CLJ (N = 16)	Placebo (N = 22)	Mann—Whitney
Gut	Phyla	Mean % (SD)	Mean % (SD)	p-Value	Mean % (SD)	Mean % (SD)	p-Value
	Actinobacteria	0.42 (0.56)	0.28 (0.97)	0.02*	0.64 (0.64)	0.40 (1.2)	0.01*
	Bacteroidetes	63 (18)	65 (17)	0.54	59 (17)	62 (18)	0.41
	Firmicutes	30 (17)	30 (16)	0.98	36 (17)	33 (17)	0.57
	Proteobacteria	2.6 (3.1)	3.3 (3.9)	0.36	2.6 (1.8)	3.8 (4.6)	0.64
	Genera						
	Staphylococcus	0.05 (0.25)	0.03 (0.10)	0.30[1]	0.08 (0.32)	0.02 (0.08)	0.80[1]
	Lactobacillus	0.03 (0.09)	0.05 (0.17)	0.99[1]	0.03 (0.11)	0.07 (0.20)	0.61[1]
	Species						
	Escherichia coli	0.78 (2.5)	0.39 (0.80)	0.43	0.31 (0.37)	0.47 (0.85)	0.69
		CLJ (N = 18)	Placebo (N = 16)	Mann—Whitney	CLJ (N = 12)	Placebo (N = 7)	Mann—Whitney
Urinary	Phyla	Mean % (SD)	Mean % (SD)	p-value	Mean % (SD)	Mean % (SD)	p-value
	Actinobacteria	2.0 (2.6)	2.4 (2.5)	0.47	1.5 (1.6)	2.3 (2.3)	0.31
	Bacteroidetes	35 (27)	49 (29)	0.13	42 (24)	53 (30)	0.31
	Firmicutes	51 (30)	35 (25)	0.10	50 (27)	27 (21)	0.04*
	Proteobacteria	12 (26)	13 (23)	0.14	6.6 (21)	16 (33)	0.03*
	Genera						
	Staphylococcus	1.4 (3.6)	7.6 (16)	0.24[1]	1.3 (3.9)	8.1 (21)	0.55[1]
	Lactobacillus	5.4 (23)	6.0 (23)	0.92[1]	8.0 (28)	0.14 (0.38)	0.64[1]
	Species						
	Escherichia coli	11 (26)	11 (24)	0.77	6.5 (21)	13 (33)	0.42

CLJ, cranberry-lingonberry juice; SD standard deviation.
[1]Bonferroni correction used on p-values ≤0.05 as these genera were not involved in the hypothesis.

found in human urine after the consumption of cranberry products [25,26], and their consumption has been shown to reduce the adherence of uropathogenic *Escherichia coli* (UPEC) to the uroepithelium in a dose-dependent manner in a laboratory setting, possibly on account of the presence of PAC metabolites in the urine [25,27–30]. We show here that the consumption of cranberry-lingonberry juice appears to change the composition of both the gut and urinary microbiome.

We had hypothesized that the urinary microbiome might have a role in the pathogenesis of UTIs, since it is known that the urinary microbiome of adults with urological conditions such as neurogenic bladder dysfunction, interstitial cystitis, and urgency incontinence differs from that in healthy individuals [31]. Also, a recent study of asymptomatic children aged 6–10 years has shown that the urinary microbiomes of girls and boys differ significantly, with girls having increased OTU richness and a higher Shannon diversity index, and that there are significant differences in the abundances of most common genera between the genders [32]. Another study of children younger than 2 years showed that the urinary microbiome of children with UTI has significantly reduced alpha-diversity relative to those without UTI [12]. Our present results suggest that it may be possible to modify the urinary microbiome by dietary interventions such as cranberry-lingonberry juice, which might thereby play a role in UTI prevention.

The effect of cranberry and lingonberry products on the gut microbiome seems to be limited in humans by comparison with the changes found in animal models and simulators [14–19]. In the present material, the gut microbiome composition with regard to uropathogens was unaffected by CLJ consumption, which suggests that the effect of CLJ on UTIs might be caused by other mechanisms, or indirectly through changes in other bacteria found in the gut microbiome. The small increase found in the abundance of Actinobacteria in the CLJ group relative to the placebo group is interesting. While

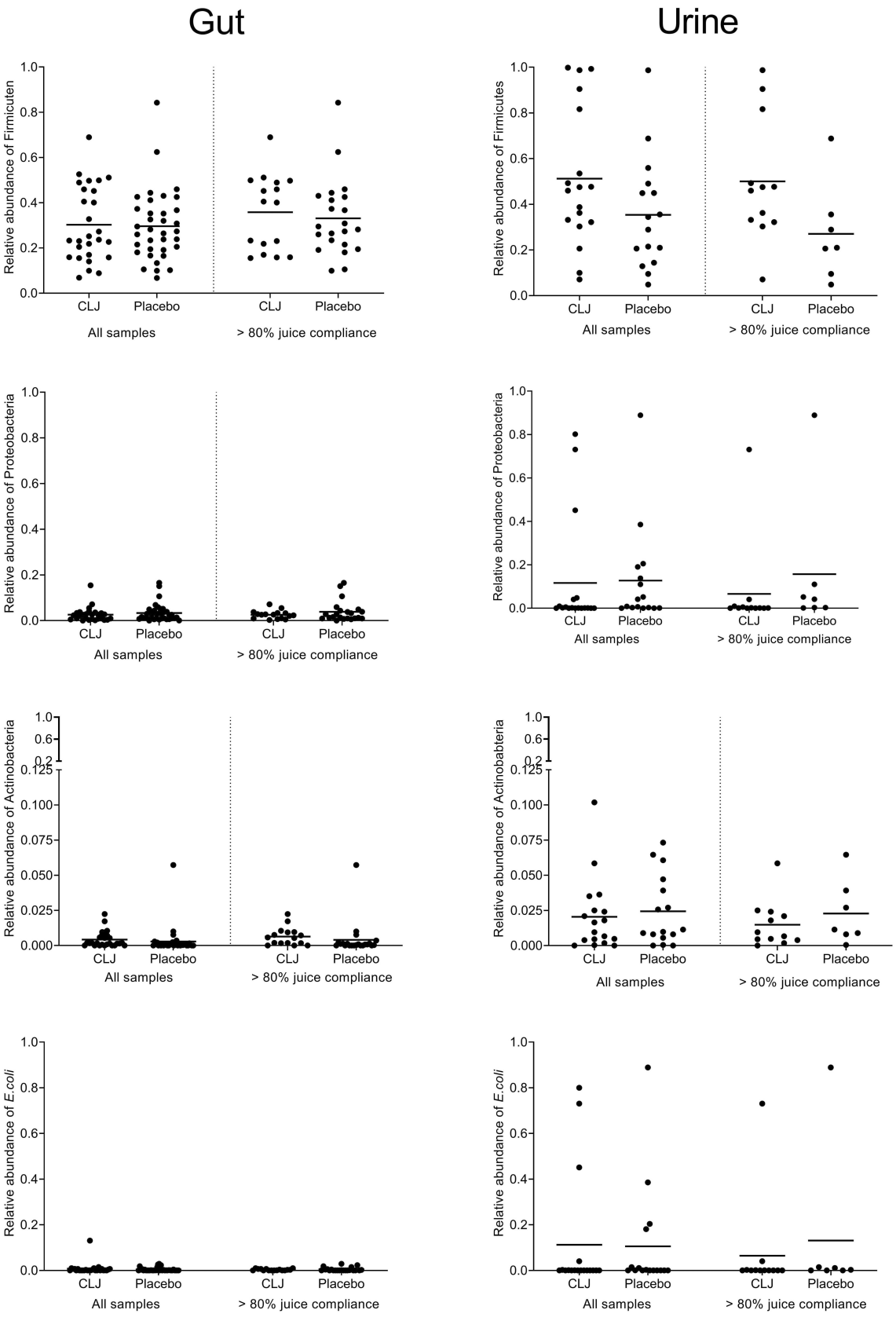

Fig. 3. Relative abundances of Firmicutes, Proteobacteria, Actinobacteria, and *E. coli* in the gut and urinary microbiome at 3 months in all the children with samples available and in those with over 80% juice drinking compliance. Each dot represents the result of one participant. Horizontal lines indicate means of the relative abundances in each group. Dot plot graph.

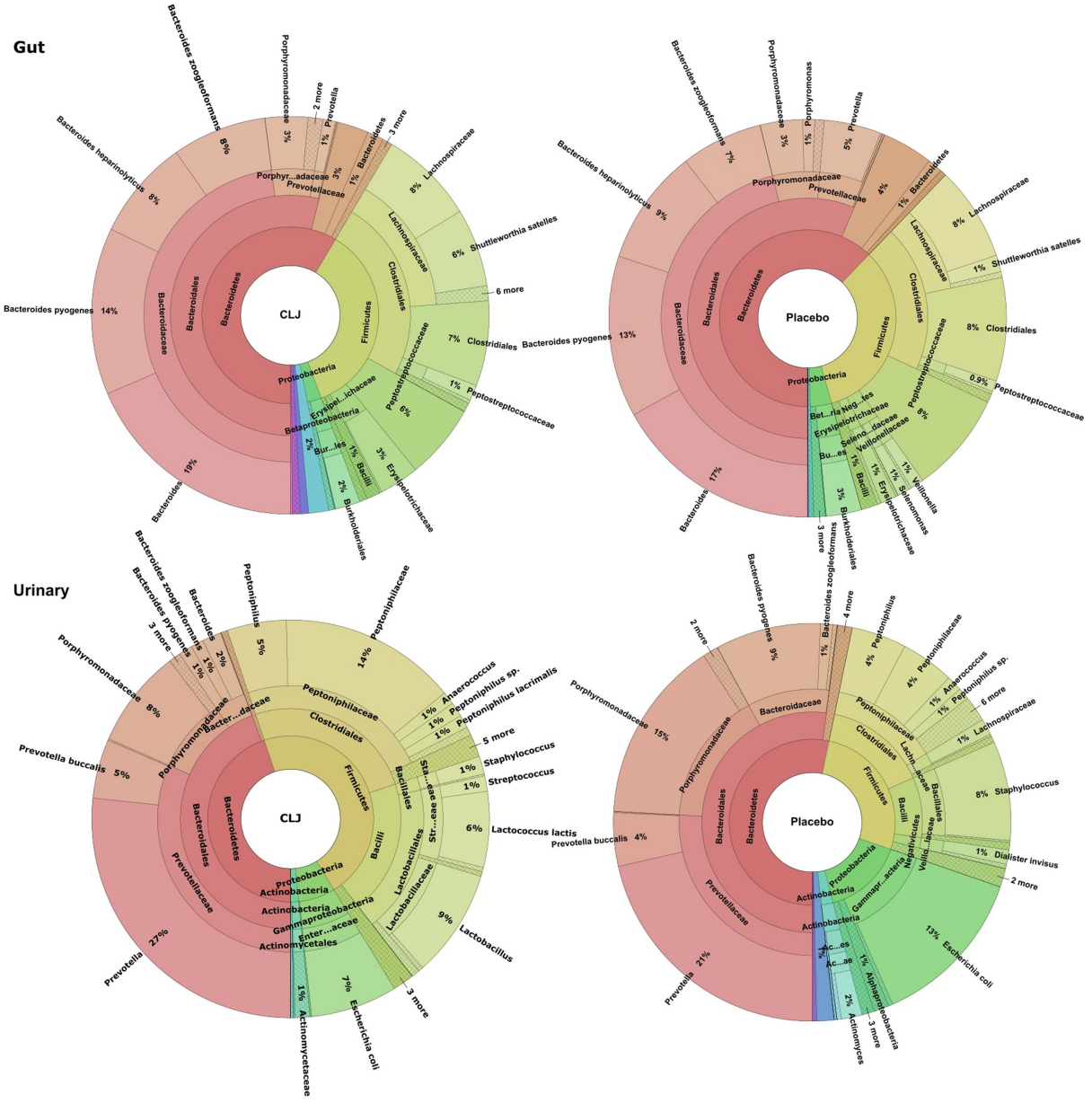

Fig. 4. Composition of the gut and urinary microbiome in the children with over 80% juice drinking compliance at 3 months.

no clear link has been established between the abundance of Actinobacteria and UTI, it has been shown that Actinobacteria have an important role in maintaining gut homeostasis and that an increase in their abundance might lead to other health benefits [33].

The main strength of this study lies in its placebo-controlled randomized clinical design and

Table 4. Urinary tract infection (UTI) recurrences in the subjects in the cranberry-lingonberry juice (CLJ) and placebo groups at 3 and 6 months

		CLJ (N = 56) n (%)	Placebo (N = 57) n (%)	Risk ratio	Proportion difference (95% CI)	p-Value
3 months	Children with at least one UTI	6 (11)	13 (23)	0.47	−12 (−26 to 1.9)	0.09
	Total number of UTI episodes	8	18			0.11
6 months	Children with at least one UTI	12 (21)	17 (30)	0.72	−8.4 (−24 to 7.9)	0.29
	Total number of UTI episodes	19	25			0.33

CLJ, Cranberry-lingonberry juice; CI, Confidence interval; UTI, Urinary tract infection.

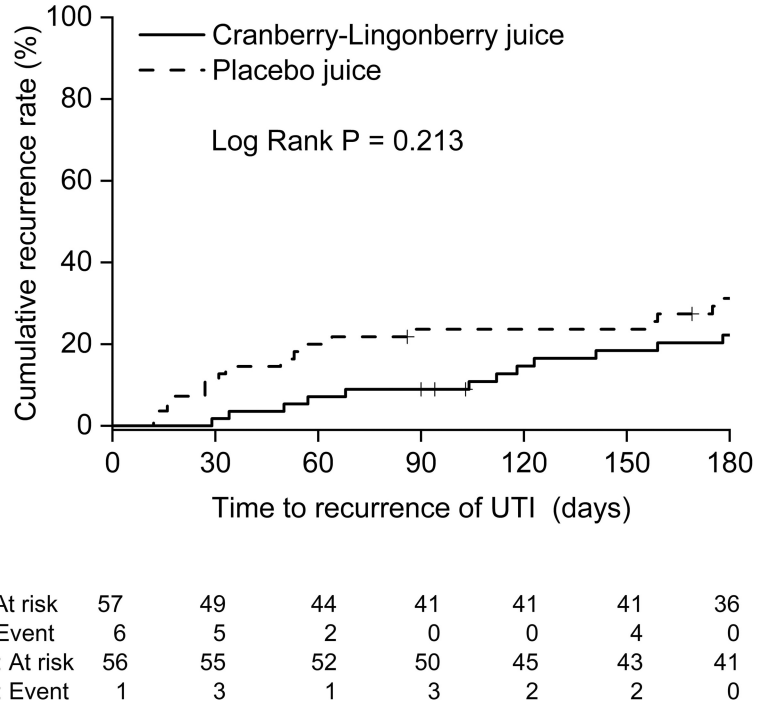

Fig. 5. Time to urinary tract infection (UTI) recurrences. The Kaplan—Meier method was used to estimate the survival function. The Log rank test was used for the statistical comparisons.

the analysis of both the gut and urinary microbiome. There are nevertheless some limitations as well. The number of children with good compliance and sufficient fecal and urine samples available was low. Also, the consumption of cranberry-lingonberry juice did not significantly reduce UTI recurrences in these children, most likely because the sample size was planned with the primary outcome of microbiome composition in mind rather than the clinical outcome. In our previous randomized cranberry juice trial [6] a reduction in UTI occurrence was evident at 3 months.

CONCLUSIONS

We found that children who consumed cranberry-lingonberry juice had different compositions of both the gut and urinary microbiome from those of children drinking the placebo juice. A healthy urinary microbiome could be important for preventing UTIs in children.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare. This was an investigator-driven academic clinical study. The cranberry-lingonberry juice was donated

by Eckes-Granini, Turku, Finland. Eckes-Granini did not participate in the study design, analysis, or writing of the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Diversity of the gut microbiome at 6 and 12 months.
Table S2. Relative abundances of taxa in the gut microbiome at 6 and 12 months in all the children with samples available and in those with over 80% juice drinking compliance.

