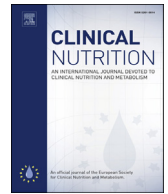




Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: <http://www.elsevier.com/locate/clnu>

Original article

High intake of orange juice and cola differently affects metabolic risk in healthy subjects

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ARTICLE INFO

Article history:

Received 21 December 2017

Accepted 19 February 2018

Keywords:

Orange juice

Cola

Uric acid

Insulin sensitivity

Insulin secretion

Microbiome

SUMMARY

Background: Higher consumption of sugar-containing beverages has been associated with an elevated risk of type 2 diabetes and gout. Whether this equally applies to cola with an unhealthy image and orange juice (OJ) having a healthy image remains unknown.

Methods: In order to investigate whether OJ and cola differently affect metabolic risk 26 healthy adults (24.7 ± 3.2 y; BMI 23.2 ± 3.3 kg/m²) participated in a 2 × 2-wk intervention and consumed either OJ or caffeine-free cola (20% Ereq as sugar from beverages) in-between 3 meals/d at *ad libitum* energy intake. Glycemic control, uric acid metabolism and gut microbiota were assessed as outcome parameters.

Results: Fecal microbiota, body weight, basal and OGTT-derived insulin sensitivity remained unchanged in both intervention periods. Levels of uric acid were normal at baseline and did not change with 2-wk cola consumption (−0.03 ± 0.67 mg/dL; *p* > 0.05), whereas they decreased with OJ intervention (−0.43 ± 0.56 mg/dL; *p* < 0.01) due to increased uric acid excretion (+130.2 ± 130.0 mg/d; *p* < 0.001). Compared to OJ, consumption of cola led to a higher daylong glycemia (ΔiAUC: 36.9 ± 83.2; *p* < 0.05), an increase in glucose variability (ΔMAGE-Index: 0.29 ± 0.44; *p* < 0.05), and a lower 24 h-insulin secretion (ΔC-peptide excretion: −31.76 ± 38.61 μg/d; *p* < 0.001), which may be explained by a decrease in serum potassium levels (−0.11 ± 0.24 mmol/L; *p* < 0.05).

Conclusion: Despite its sugar content, regular consumption of large amounts of OJ do not increase the risk of gout but may even contribute to lower uric acid levels. The etiology of impaired insulin secretion with cola consumption needs to be further investigated.

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1. Introduction

Sugar sweetened beverages (SSB) provide the primary source of added sugars in Western diets being most popular in adolescents and young adults in Germany [1–3]. Epidemiological evidence suggests that sugar-containing beverages contribute to type 2 diabetes [4–6] and gout [7–10] by mechanisms that are partly

independent of BMI or weight gain. As a proposed underlying mechanisms, a high dietary glycemic load may contribute to inflammation, insulin resistance, and impaired β-cell function [11–13]. On the other hand, fructose despite having a low-glycemic index, was shown to enhance degradation of purine nucleotides [14] and increase purine synthesis [15,16].

Sugar-containing beverages comprise a great spectrum of soft drinks, fruit drinks, sports drinks, energy and vitamin water drinks and squashes [6]. However, evidence for an impact of different types of beverages on metabolic risk remains limited and inconclusive. Although the perceived health image of orange juice is superior to that of cola, both beverages contain similar amounts of sugars. Daily consumption of one serving of SSB has been

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associated with around 18–20% higher prospective type 2 diabetes risk independent of BMI, whereas this association was found to be insignificant for fruit juices [17,18]. In addition, an increased risk of gestational diabetes has only been reported for cola consumption, whereas other SSBs had no effect [19]. Fruit juice or fructose-rich fruits (e.g. apples and oranges) have been associated with a higher risk of gout [7,20] with one glass of OJ per day increasing the risk by 41% [9], whereas others found no adverse effect of OJ consumption [21]. Associations from epidemiological studies do, however, not necessarily infer causality, and therefore, need to be confirmed by intervention studies. Recently it was recommended by experts that fruit juice shouldn't count to the five a day advice for a healthy fruit and vegetable consumption [22].

Despite their high genuine sugar content, fruit juices are rich in vitamin C that may increase clearance of uric acid [23,24]. In addition, the flavonoid hesperetin in OJ may decrease endogenous uric acid synthesis [25]. Citrus flavonoids such as naringin may exert anti-diabetic effects due to their antioxidant and anti-inflammatory properties [26] and glucose lowering and insulin sensitizing effects [27] e.g. by inhibition of α -glucosidase or α -amylase [28]. Lastly, SSBs and fruit juices may differently affect gut microbiota, and thus metabolic risk [29].

The aim of this study was to compare the metabolic effects of high consumption of cola and OJ in healthy young adults. We hypothesized (i) that high sugar intake from cola would increase uric acid levels, in contrast to OJ consumption and (ii) that cola consumption would result in higher daylong glycemia and impaired insulin sensitivity when compared with OJ.

2. Methods

2.1. Study protocol

The present data are part of a larger quasi-randomized controlled cross-over intervention study that comprised three 2-week intervention periods: OJ consumption with meals, OJ consumption between meals and cola consumption between meals, whereby one cohort started with OJ consumption with meals and the other cohort with between meals and cola consumption represented the last intervention in both rounds [30]. The present evaluation only compared 2-wk cola vs. 2-wk OJ consumption between meals. The study was carried out at the Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany, from April 2016 to December 2016. Participants were recruited at the University of Hohenheim and on social networks, and divided into two cohorts. The first cohort ($n = 12$) was examined between February and March 2016, and the second cohort ($n = 14$) between January and April 2017. Inclusion criteria were: age between 20 and 45 years and a habitual three-meals-per-day structure. Exclusion criteria for enrollment included daily consumption of SSB or fruit juice, regular intake of medication and supplements, chronic disease, smoking, fructose intolerance and special diets (e.g., vegetarian).

All participants had to complete a physical activity interview and a three-day food record before the start of the study. They were requested not to change their physical activity and eating habits throughout the study. During a one week run-in period, consumption of OJ, citrus fruits and SSBs were avoided to facilitate equal baseline conditions. During the 2-wk intervention periods, participants consumed 20% of the individual energy requirements as sugar from orange juice or caffeine-free cola three times a day ≥ 2 h after the meals. All participants followed a three-meals-per-day structure without any in-between snacks. Both intervention

periods were separated by one- to two-weeks washout phase. During the intervention periods, consumption of alcohol and additional SSBs and fruit juices was not allowed. For the duration of the entire study, participants were asked to abstain from consumption of citrus fruits and citrus juices.

The present study was designed as a free-living study with 4 obligatory visits at the institute – one at the beginning (day 1, T_0) and the end (day 15, T_1) of each intervention period. On each of the visiting days, body weight was measured, an oral glucose tolerance test was performed, and blood samples were collected. Weight was assessed with a Tanita scale coupled to the BodPod™ system (COSMED, Rom, Italy). Height was measured with a stadiometer (seca 274, seca, Hamburg, Germany).

The study was registered at clinicaltrials.gov as NCT02974478 and the study protocol was approved by the ethics committee of the Medical Council of Baden-Württemberg, Germany. All participants provided informed written consent before participation.

2.2. Intervention with OJ and cola

Beverages used for intervention were 100% OJ with pulp of industrial production, containing 43 kcal/100 mL and 8.8 g sugar/100 mL according to the manufacturer (33% sucrose, 33% glucose and 34% fructose according to own analysis) and a commercial caffeine-free cola containing 44 kcal/100 mL and 11.1 g sugar/100 mL according to the manufacturer (20% sucrose, 41% glucose and 39% fructose according to own analysis). Sugar composition was analyzed according to Pöhl et al. [31] using anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) on an ICS-3000 ion chromatography system (Dionex, Sunnyvale, CA, USA). The OJ contained 185.91 mg/L hesperidin (3.4 ± 0.3 mg/kg body weight) and 38.87 mg/L narirutin resulting in a mean intake of 237.6 ± 41.5 mg/d hesperidin and 49.7 ± 8.7 mg/d narirutin. On average, OJ has been reported to contain between 200 and 600 mg hesperidin/L and 15–85 mg narirutin/L [32].

The sugar consumed with both beverages was 20% of daily energy requirement. Individual energy requirement was assessed by multiplying resting energy expenditure (REE predicted according to Harris & Benedict [33]) by physical activity level (PAL). Based on their reported daily physical activity individual PAL values ranged between 1.4 (low physical activity) and 1.8 (high physical activity) (estimated according to Brooks et al., 2004 [34]). The study-beverages were weighted in bottles and the filling line was marked. To determine the individual rations, subjects refilled the bottles up to the marking three times a day. Due to a higher sugar content of cola compared to OJ, the amount of liquids required to ensure identical sugar intake was calculated based on their sugar content, thus participants had to drink relatively less cola than OJ. All beverages were provided by the Institute of Nutritional Medicine.

2.3. Assessment of fasting and postprandial glucose metabolism

Subjects came to the Institute of Nutritional Medicine between 6:30 and 8:00 am after an overnight fast ≥ 10 h. An oral glucose tolerance test (OGTT, intake of 75 g glucose, Accu-Chek® Dextrose O.G-T., Roche Diagnostics, Mannheim, Germany) was performed. Blood samples were collected before and 30, 60 and 120 min after drinking the glucose-solution. Area under the curve (AUC) was calculated as incremental AUC (iAUC) for 2 h from glucose and insulin OGTT-results using trapezoidal rule [35]. Matsuda Insulin Sensitivity Index was used to evaluate postprandial insulin sensitivity:

$$\text{Matsuda}_{\text{ISI}} = 10000 / \sqrt{(\text{fasting glucose [mg/dL]} \times \text{fasting insulin [\mu U/mL]}) \times (\text{mean glucose [mg/dL]} \times \text{mean insulin during OGTT [\mu U/mL]})}$$

[36]. Homeostatic Model Assessment-insulin resistance was used to calculate fasting insulin sensitivity: $\text{HOMA}_{\text{IR}} = \text{fasting glucose [mg/dL]} \times \text{fasting insulin [\mu U/mL]} / 405$ [37].

Interstitial glycemia was measured by continuous glucose monitoring (CGM, Dexcom G4 Platinum, Nintamed, Mainz, Germany) for 7 days during both interventions. A small sensor was placed at the back of the upper arm in the subcutaneous tissue to monitor interstitial glucose concentrations. Sensor readings were recorded in intervals of 5 min. The CGM-device was calibrated twice a day against fasting capillary blood samples. Area under the curve (AUC) was calculated as incremental AUC (iAUC) for 18 h (6:00 am–00:00 am) from 3 to 5 valid daylong CGM-data using trapezoidal rule [35].

Glucose variability was determined by mean amplitude of glycemic excursions (MAGE):

$\sum \lambda / \chi$ with $\lambda > \gamma$, where λ is the difference from peak to nadir, χ is the number of valid observations, and γ is 1 SD of mean glucose in a 24-h period [38,39] using a published macro [40]. Daylong insulin secretion was assessed by 24-h urinary C-peptide excretion at the end of each intervention.

2.4. Blood sampling and analytical methods

Blood sampling was performed by vein cannula. Plasma glucose was determined using hexokinase method (OSR6121, Beckman Coulter, Brea, CA, USA). Serum insulin (Elecsys® Insulin 06923321990, Roche Cobas e801) and urinary C-peptide excretion were measured by luminescence immunoassay (Elecsys® C-Peptide 06923330990, Roche Cobas e801). Levels of uric acid were measured by photometry in fasting blood samples (OSR6098, Beckman Coulter, Brea, CA, USA). Serum potassium level was determined potentiometrically using a Beckman Coulter instrument (AUH1011-1018, Brea, CA, USA).

2.5. Physical activity

Physical activity was continuously monitored using the triaxial activity monitor (ActivPAL™, PALTechnologies, Glasgow, UK). The device was fixed with waterproof patches on the upper thigh mid-way between the hip and the knee in the correct orientation according to the manufacturer's instruction and worn during both intervention periods.

2.6. Statistical analyses

Data are expressed as mean \pm SD. Statistical analyses were conducted using SPSS version 22.0 (SPSS, Chicago, IL, USA). Normal distribution was checked by Kolmogorov–Smirnov-Test. HOMA_{IR} did not meet the criteria of normal distribution. Differences between pre and post intervention (T_0 vs. T_1) of both interventions were analyzed by mixed-model ANOVA with repeated measures followed by Bonferroni correction. Differences after both interventions ($\Delta T_1 - T_0_{\text{OJ}}$ vs. $\Delta T_1 - T_0_{\text{cola}}$) were analyzed by paired t-test; Wilcoxon-test was used if data were not distributed normally. Associations between changes in uric acid ($\Delta T_1 - T_0_{\text{OJ}}$)

versus baseline uric acid concentration or between serum levels of potassium versus daylong insulin secretion were tested using Pearson's correlation. p values < 0.05 were considered as statistically significant. The box-and-whiskers-plot was used to display the distribution of serum potassium levels by quartiles. The lower and upper limits represent the 25- and 75%-quartile of the observed data. The bar within the boxplot represents the median and the whiskers indicate the range of the data. Whiskers maximal length is only allowed to be 1.5 times the interquartile range, outliers are drawn as a single dot.

2.7. Fecal sample DNA isolation, amplification and sequencing

Stool samples were self-collected by patients at the first and last day (days 1 and 15) of the OJ and cola interventions, respectively and used for DNA extraction by mechanical and enzymatic lysis as described before [41]. Hypervariable region V4 of the bacterial 16S rRNA gene was amplified with Golay-barcoded primers 515F and 806R (Sigma Aldrich, St. Louis, USA) and Phusion High-Fidelity PCR Master Mix (Thermo Scientific, Lithuania) See [Supplementary Table S1](#) for primer sequences. PCRs were as follows: 2 min at 98 °C, followed by 30 cycles of 10 s at 98 °C, 15 s at 52 °C, and 10 s at 72 °C and a final extension of 5 min at 72 °C. Equimolar amounts of the resulting PCR amplicons, as determined with the SequalPrep Normalization Plate (Thermo Scientific, Waltham, USA), were combined, concentrated with the DNA Clean & Concentrator-5 kit (Zymo Research, Irvine, USA), adjusted to 100 ng per 60 μL and prepared for sequencing (Illumina MiSeq Regent Kit v3, 2 \times 300 bp) with the TruSeq Nano DNA LT Library Prep Kit (Low sample option, Single Indexing). Sequence data are available from the European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under study accession number ERP105156.

2.8. Sequence processing and microbiota analysis

Sequence reads were processed with QIIME [42]. Sample-specific barcodes were extracted with `extract_barcode.py`, read pairs merged with `join_paired.ends.py`, followed by demultiplexing with `split_libraries.py` (default parameters, except 'barcode_type "22"'). Primer sequences and spacers between barcodes and primers were removed with `cutadapt` [43]. Reads were clustered into operational taxonomic units (OTUs) based on a similarity threshold of 95% using `pick_open_reference_otus.py` (QIIME) with default settings.

Microbial diversity (α -diversity), based on the Shannon index, and overall taxonomic microbiota similarities (β -diversity), based on Bray–Curtis Dissimilarity, principal component analysis, analysis of similarities (ANOSIM) and other statistical calculations were calculated and visualized in R (www.r-project.org), using the RStudio environment (www.rstudio.com). Differentially abundant bacterial taxa were identified with LefSe [44].

A list of all QIIME and R commands used for sequence processing and analysis is provided as part of the supplement ([Supplementary Table S2](#)).

Table 1
Comparison of changes in body weight and glucose metabolism between interventions with orange juice and cola (n = 26).

	Orange juice intervention			Cola intervention			Comparison of changes during both interventions
	T ₀	T ₁	ΔT ₁ -T ₀	T ₀	T ₁	ΔT ₁ -T ₀	
Body weight [kg]	70.1 ± 14.5	70.3 ± 14.5	0.2 ± 0.9	70.3 ± 14.4	70.1 ± 14.5	-0.2 ± 0.8	p = 0.108
Basal glucose metabolism							
Glucose [mg/dL]	87.4 ± 5.8	88.0 ± 6.0	0.6 ± 6.2	89.7 ± 7.1	87.0 ± 7.3	-2.7 ± 7.0	p = 0.078
Insulin [μU/mL]	7.4 ± 3.6	7.5 ± 2.8	0.1 ± 3.0	7.3 ± 3.8	8.1 ± 3.8	0.9 ± 3.3	p = 0.398
HOMA Index	1.6 ± 0.8	1.6 ± 0.6	0.01 ± 0.67	1.6 ± 0.9	1.8 ± 0.9	0.12 ± 0.77	p = 0.929
OGTT							
Matsuda Index	7.1 ± 2.9	6.2 ± 2.3	-0.9 ± 2.3	7.1 ± 3.4	6.4 ± 2.8	-0.8 ± 2.4	p = 0.703
iAUC _{glucose} [mg/dL × 2 h]	39.0 ± 42.8	46.4 ± 30.0	7.4 ± 44.2	46.2 ± 45.2	39.5 ± 37.0	-6.7 ± 37.8	p = 0.171
iAUC _{insulin} [μU/mL × 2 h]	81.1 ± 42.0	97.1 ± 48.3	16.0 ± 42.8	93.5 ± 62.1	95.0 ± 40.1	1.4 ± 38.4	p = 0.469

Values are means ± SDs. paired t-test; Wilcoxon test; T₀, first day of study intervention; T₁, last day of study intervention.

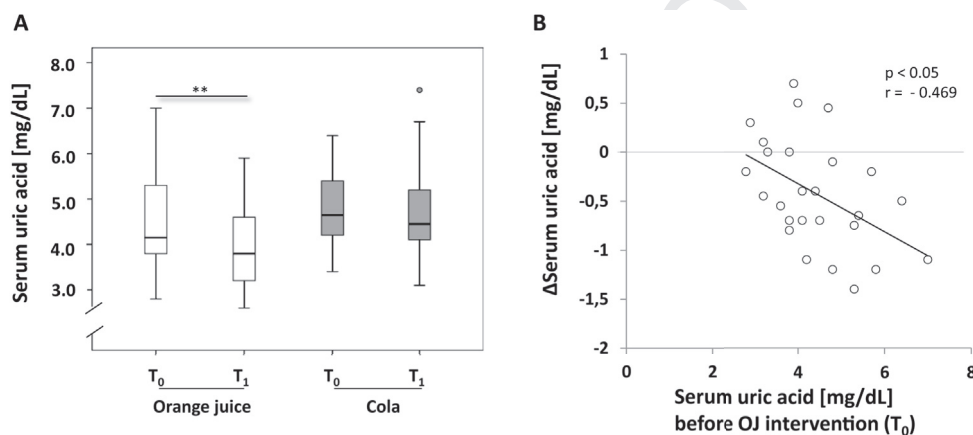


Fig. 1. (A) Comparison of serum uric acid levels at the beginning (T₀) and end (T₁) of both intervention periods; **p < 0.01; mixed-model ANOVA with repeated measures and Bonferroni correction; (B) relationship between serum uric acid levels before orange juice intervention (T₀) and changes during orange juice intervention (Δ); *p < 0.05; r, Pearson's correlation coefficient; (n = 26).

3. Results

Baseline characteristics of the study population are shown in Table 1. Thirteen women and thirteen men of ages between 20 and 33 years were included. BMI ranged between 19.1 and 33.9 kg/m². According to WHO criteria, 20 subjects were normal weight, five overweight and one obese.

Mean REE and physical activity did not differ between both interventions (OJ vs. cola: 1664 ± 274 and 1666 ± 273 kcal/d; 10,528 ± 3438 and 9917 ± 3871 steps/d; both p > 0.05). The sugar

intake with OJ (112.5 ± 19.7 g/d) and cola (113.7 ± 19.7 g/d) was identical by design, whereas the volume and caloric value of consumed beverages was slightly higher in OJ (1278 ± 223 mL/d and 549 ± 96 kcal/d) vs. cola (1024 ± 177 mL/d and 451 ± 78 kcal/d; both p < 0.001). OJ contained 185.9 mg/L of hesperidin and 38.9 mg/L narirutin and 350 mg/L vitamin C (daily intake during orange juice intervention for hesperidin: 237.6 ± 41.5 mg/d, narirutin: 49.7 ± 8.7 mg/d and vitamin C: 447.3 ± 78.2 mg/d).

Body weight as well as parameters of basal and post OGTT glucose metabolism did not change with both intervention periods

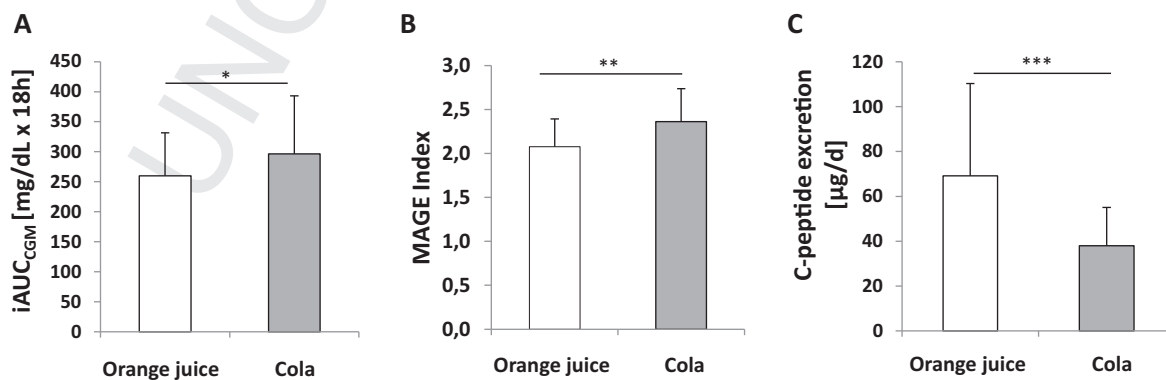


Fig. 2. Comparison of iAUCs for daylong glycemia (A; average of 3–5 days), MAGE-Index (B; average of 3–5 days) and C-peptide excretion (C; one day at the end of intervention) between orange juice and cola intervention. Values are means ± SDs; *p < 0.05, **p < 0.01, ***p < 0.001; paired t-test; (n = 26).

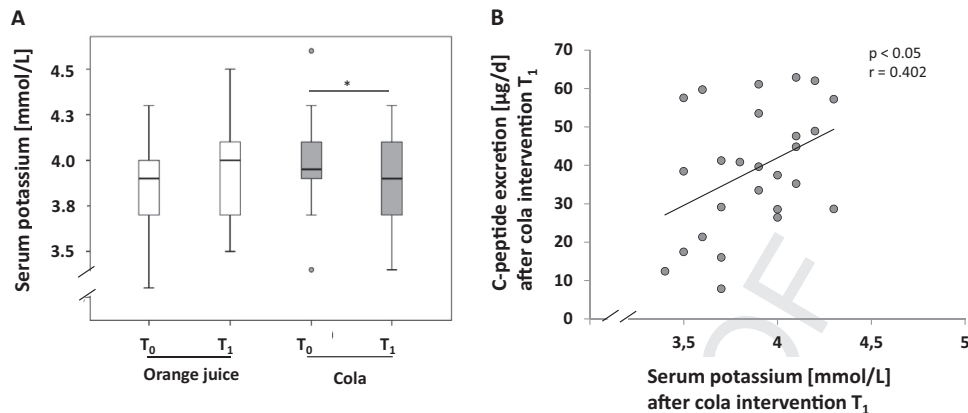


Fig. 3. (A) Comparison of serum potassium levels between the beginning (T₀) and end (T₁) of both intervention periods; *p < 0.05; mixed-model ANOVA with repeated measures and Bonferroni correction; (B) relationship between C-peptide excretion and serum potassium levels after cola intervention; *p < 0.05; r, Pearson's correlation coefficient; (n = 26).

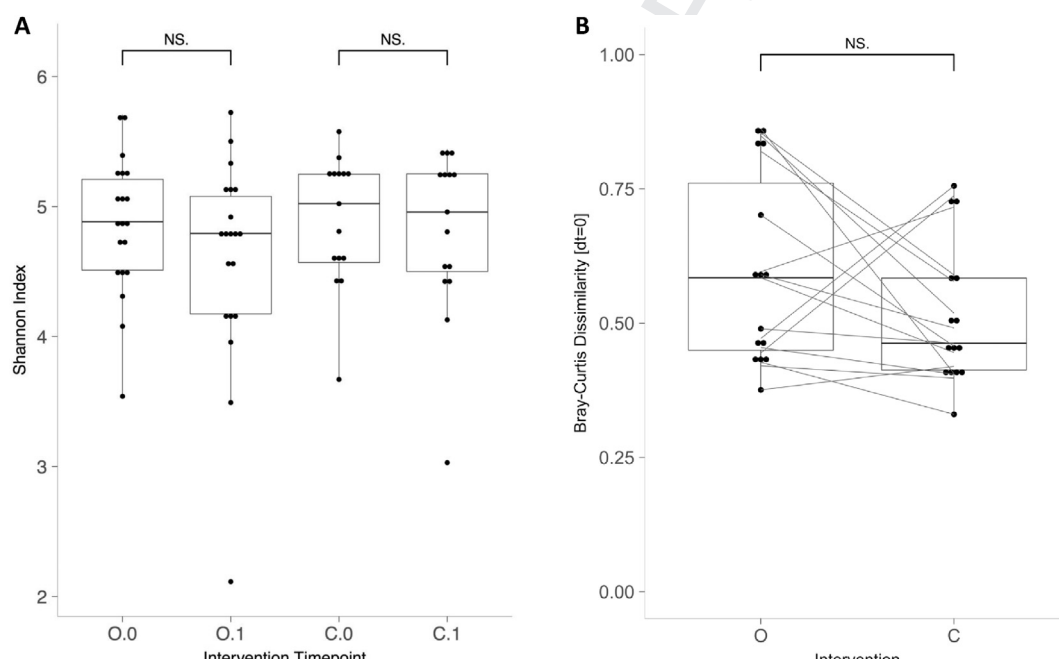


Fig. 4. (A) Microbial diversity of fecal samples collected at the beginning (O.0, C.0) and end (O.1, C.1) of both intervention periods, based on the Shannon diversity index; paired Wilcoxon signed rank test (n = 20 for OJ (O) and n = 15 for cola (C), respectively); NS, not significant; (B) Comparison of changes in taxonomic microbiota compositions between intervention periods, based on Bray–Curtis dissimilarity calculated between the beginning and end of each intervention; paired Wilcoxon signed rank test (n = 15).

(Table 1). Levels of uric acid were all within the normal range at baseline and did not change with 2-wk cola consumption, whereas they decreased with OJ intervention (-0.43 ± 0.56 mg/dL, $p < 0.01$; Fig. 1A). The decrease in uric acid levels was more pronounced at higher baseline levels ($r = -0.47$; $p < 0.05$; Fig. 1B). Daylong excretion of uric acid increased after two weeks of intervention with OJ ($\Delta T_1 - T_0$: 130.2 ± 130.0 mg/d; $p < 0.001$), whereas no change was observed with cola consumption ($\Delta T_1 - T_0$: -22.7 ± 214.4 mg/d; $p > 0.05$).

Daylong glycemia measured by iAUC glucose and glucose variability assessed by MAGE index were higher during cola consumption compared to OJ intervention (Fig. 2A and B). When compared with OJ consumption, 24 h-C-peptide excretion was markedly reduced with cola intervention (OJ: 70.6 ± 41.8 µg/d vs. cola: 38.8 ± 16.3 µg/d; $p < 0.001$; Fig. 2C). Figure 3A shows a decrease in serum potassium after the cola intervention

(-0.11 ± 0.24 mmol/l; $p < 0.05$), whereas potassium levels remained unchanged with OJ consumption ($+0.11 \pm 0.35$ mmol/l; $p > 0.05$). Lower levels of potassium after cola intervention were associated with lower C-peptide excretion ($r = 0.402$; $p < 0.05$; Fig. 3B).

Neither the OJ nor the cola intervention was associated with significant changes in fecal microbiota diversity (Shannon diversity index; $p > 0.05$; Fig. 4A) or taxonomic composition (Bray–Curtis dissimilarity; $p > 0.05$; Fig. 4B). Analysis of similarity (ANOSIM; [45]) identified significant differences between fecal samples collected from different individuals but not at different time points, i.e. before and after OJ or cola intervention (Supplementary Fig. S1). Bacterial taxa did not show differential relative abundance between the beginning and end points of either the OJ or cola intervention (phylum to genus level; linear discriminant analysis (LDA) effect size < 2). A search for associations between the relative abundances

of bacterial taxa and levels of serum uric acid, C-peptide excretion and serum potassium did not identify significant correlations ($p > 0.05$).

4. Discussion

In line with our hypotheses, two weeks of 20% energy intake as genuine sugar from OJ led to a more favorable metabolic profile compared to the ingestion of same amount of sugar from a caffeine-free cola beverage. Serum uric acid levels significantly decreased following OJ consumption. This result does not support the assumption of an increased risk of gout associated with OJ consumption, as proposed from prospective data of the Health professionals' follow-up study [7] and the Nurses' Health Study [9] using food-frequency questionnaires and the American College of Rheumatology survey criteria for gout. Quite the contrary, our data suggest that regular consumption of larger amounts of OJ may prevent hyperuricemia and gout in healthy individuals. The conflicting results from epidemiological data may be due to confounding: Although the consumption of fruit/vegetable juice has been shown to closely align with a prudent dietary pattern [46], consumption of 100% juice was shown to decline sharply with age [47] and higher age increases the risk of gout [8]. In addition, a lower education level was associated with higher SSB consumption [48] and a lower socioeconomic status may also increase the risk of gout [3,49].

Lower serum uric acid levels after two weeks of OJ consumption as observed in our study could partly be explained by an increased uric acid excretion (see results). The latter may be due to an uricosuric effect of vitamin C. In a randomized controlled trial, serum uric acid levels were inversely correlated with changes in serum ascorbic acid and supplementation with 250–500 mg/d of vitamin C once or for two months was shown to increase glomerular filtration rate, thus reducing serum uric acid levels [23,50]. Similarly, lower levels of uric acid have been observed after four weeks of 500 mL/d OJ consumption [51]. Although in our study the mean intake of vitamin C from OJ was 447 ± 78 mg/d (range 308–580 mg/d), we cannot rule out an additional uric acid lowering effect by a flavonoid-dependent inhibition of xanthine oxidase. Several *in vitro* studies have shown a competitive inhibition of flavonoids such as hesperetin, naringenin and naringin on xanthine oxidoreductase activity [52–54]. In hyperuricemic rats, administration of 5 mg/kg body weight hesperetin partly explained the decrease in uric acid levels observed with OJ [25].

The gut microbiota may play a role in the bioavailability of bioactive flavonoids. Prior to absorption, glycosylated forms of flavanones must be hydrolyzed by the glycosidase activities of the colonic microbiota [55–57]. However, the efficiency of absorption may also be affected by the microbiota because microorganisms can degrade the flavonoid aglycones they produce [58].

Although fructose has immediate uric acid raising effects [14] mediated by an increased ATP degradation to the uric acid precursor AMP, 20% energy intake as sucrose from cola beverages did not raise serum uric acid levels in our healthy subjects. The urate-raising effect of fructose has, however, been shown to be most pronounced in patients suffering from gout and hyperuricemia [14,59–61]. Therefore, prevention of hyperuricemia caused by heavy OJ consumption in healthy adults may not be transferable to treatment, because adverse effects of fructose in these patients may antagonize the urate lowering effects of vitamin C or flavonoids from OJ. Compared to the effect of OJ, pharmacological inhibition of xanthine oxidase by allopurinol and febuxostat can cause severe adverse effects such as hepatitis, nephropathy or allergic reactions [62–64]. Therefore, the efficacy of OJ in the treatment of hyperuricemia in gout patients deserves further study.

A more chronic effect of fructose on uric acid levels may result from fructose-induced hyperinsulinemia [11,65] that may impair renal excretion of urate and correlates with higher serum uric acid levels [66]. Since insulin secretion as assessed by C-peptide excretion was markedly lower during cola intervention, this effect might have impeded an increase in uric acid levels in our study.

The unexpected finding of lower insulin secretion with cola consumption is in line with a higher daylong glycemia and glucose variability compared to OJ consumption (Fig. 2A). Remarkably, prepregnancy consumption of sugar-sweetened cola (≥ 5 vs. < 1 servings/week) was associated with 22% increase in gestational diabetes risk after controlling for potential confounders, whereas no significant association with gestational diabetes was observed for other SSBs [19]. Because the main factor of the pathogenesis of gestational diabetes is a relatively impaired insulin secretion coupled with pregnancy-induced insulin resistance [67], diminished β -cell function by other ingredients of cola beverages may be highly relevant. Caramel coloring in cola-type soft drinks contains advanced glycation end products that may be positively associated with insulin resistance and inflammation [68].

In our study, a slight decrease in serum potassium levels was observed after the intervention with cola (Fig. 3A). This could however be due to higher baseline levels of serum potassium after the OJ intervention. After the intervention with cola, lower daylong insulin secretion (by C-peptide excretion) was associated with lower serum potassium levels (Fig. 3B). Insulin secretion of β -cells is known to be impaired with low serum potassium levels because ATP-dependent K channels are more likely to open and release K ions, which inhibits membrane depolarization and calcium mediated release of secretory granules. Accumulating evidence gives rise to the assumption that a high consumption of cola may lower serum potassium levels [69] and can even cause severe symptoms of hypokalemia in case reports on heavy cola consumption (3–7 l per day; [70–72]). The underlying mechanism remains unclear, and three potential mechanisms of cola-induced hypokalemia have been proposed: (i) increased redistribution of potassium into cells by caffeine [73] or glucose-induced insulin secretion [74]; (ii) increased urinary potassium losses due to caffeine [73] or osmotic diuresis by high sugar intake [74] and (iii) increased potassium losses by osmotic diarrhea caused by the high fructose intake [74]. Since the cola beverage used in our study was non-caffeinated and the subjects had low insulin levels and did not report any incidence of diarrhea upon inquiry of the study team, all explanatory approaches are implausible. The possible link between heavy cola consumption, lower serum potassium levels and insulin secretion may impact recommendations for prevention of gestational diabetes and prevention of type 2 diabetes.

Despite the observed differences in the physiological reactions of study participants to the OJ and cola interventions, no consistent impact of either intervention on the fecal microbiota could be identified across the entire study population. While it is possible that none of these effects were microbiota-dependent, the gut microbiota response to dietary change has also previously been reported to significantly vary between individuals (reviewed by Sonnenburg & Bäckhed [75]) and functional redundancy between taxonomically distinct bacteria from different individuals appears to be a hallmark of the gastrointestinal microbiota [76]. In order to identify and differentiate the effects of OJ and cola consumption on the microbiota, larger study cohorts might therefore be needed with participants stratified based on the initial microbiota compositions.

In conclusion, health consequences of commonly consumed sugar-containing beverages cannot be solely refined to their sugar content because they differ profoundly due to secondary

constituents. In contrast to SSB, orange juice may protect against hyperuricemia.

Conflict of interest

RC received a donation from the Baumann–Gonser–Stiftung, Bonn, Germany. Research was partly financed by a grant from Baumann–Gonser–Stiftung. FB was awarded a prize by the Baumann–Gonser–Stiftung. Prior to completing the clinical study, one of the authors (JA) has taken up an R&D position at Döhler GmbH (Darmstadt, Germany). Prior to completing the clinical study, one of the authors (RS) has taken up an R&D position at DSM Nutritional Products (Kaiseraugst, Switzerland). None of the other authors reported a conflict of interest related to the study.

Acknowledgments

Authors contributions: Writing of the manuscript: ABW, FB, LVD, WFF; data acquisition: FB, FH, LVD, AF, ED, DP, AN, TP, RS; data analysis: FB, FH, LVD, AF; discussion of data and proof-reading of the manuscript: ABW, FB, FH, LVD, AF, ED, DP, WFF, JA, RS, RC; study design: ABW.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.02.028>.

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