Microbial Diversity and Abundance of *Parabacteroides* Mediate the Associations Between Higher Intake of Flavonoid-Rich Foods and Lower Blood Pressure

Amy Jennings⃣, Manja Koch, Corinna Bang⃣, Andre Franke, Wolfgang Lieb⃣, Aedín Cassidy⃣

**ABSTRACT:** We assessed, for the first time, to what extent the composition of the gut microbiome might explain the cross-sectional association of habitual flavonoid and flavonoid-rich food intake with systolic and diastolic blood pressure (BP) in a community-based sample (N=904) from Northern Germany. Gut microbiome composition was sequenced from 16S ribosomal RNA genes. Higher total flavonoid intakes and specifically the polymer subclass were associated with lower systolic BP (SBP; β T3-T1: −2.9% [95% CI, −5.1 to −0.7], *P*=0.01 and −3.7% [95% CI, −5.4 to −1.0], *P*=0.01). In food-based analyses, a higher intake of berries (SBP; β Q4-Q1: −2.9% [95% CI, −5.2 to −0.6], *P*=0.01; pulse pressure, −5.5% [95% CI, −9.6 to −1.2], *P*=0.01) and red wine (SBP; β Q4-Q1: −2.6% [95% CI, −4.8 to −0.3], *P*=0.03; pulse pressure, −6.1% [95% CI, −10.1 to −2.0], *P*<0.01) were associated with lower SBP and pulse pressure. There were no associations with diastolic BP. In food-based analyses, higher intakes of anthocyanin-rich berries and red wine were associated with higher alpha diversity (β Q4-Q1: 0.03 [95% CI, 0.0–0.1], *P*=0.04 and 0.1 [95% CI, 0.03–0.1], *P*<0.01). Higher intakes of berries and apples/pears were associated with a lower abundance of *Parabacteroides* (β Q4-Q1: −0.2 [95% CI, −0.4 to −0.1], *P*<0.01, *Q*=0.07 and −0.3 [95% CI, −0.4 to −0.1], *P*<0.01, *Q*=0.04). Structural equation modeling of these novel data suggests that microbial factors explained 15.2% to the association between flavonoid-rich foods and clinically relevant lower SBP. Further research should focus on interindividual variability in the gut microbiome in mediating the cardiovascular effects of flavonoid-rich foods. ([Hypertension. 2021;78:1016–1026. DOI: 10.1161/HYPERTENSIONAHA.121.17441.](https://www.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.121.17441))

**Data Supplement**

**Key Words:** blood pressure ■ diet ■ flavonoids ■ fruits ■ microbiota

Dietary flavonoids, a large group of plant-derived polyphenolic compounds, are commonly found in fruits, vegetables, tea, chocolate, and red wine.¹ Flavonoids are categorized into 6 main subclasses based on their chemical structure (flavonols, flavones, flavanones, flavan-3-ols, anthocyanins, and flavonoid polymers [procyanidins and other polymers]) which differ in bioavailability and biological activity.² Following ingestion, the majority of flavonoids interact with the gut microbiome, resulting in modulation of both the gut microbiome and the flavonoid structures, which are catabolized into metabolites with increased bioactivity.³⁴ In both animal model and short-term human intervention studies, increased intake of anthocyanin-rich berries, for example, significantly increased abundance of *Bifidobacterium*.⁵⁶ Subsequently, high levels of *Bifidobacterium* have been associated with increased urinary concentrations of the anthocyanin metabolites syringic acid, p-coumaric acid, 4-hydroxybenzoic acid, and homovanillic acid.⁷ These metabolites have been shown to be more cardioprotective than the parent compounds, improving lipid profiles and upregulating heme oxygenase-1, an antioxidant-response...
protein associated with decreased blood pressure (BP) and vascular resistance.\textsuperscript{6,9} There is mounting evidence that the composition of the gut microbiome can partly explain the association between dietary flavonoids and cardiometabolic health. In a prior analysis in the present sample, we have shown that up to 18.5\% of the association between habitual intake of anthocyanin-rich foods and visceral abdominal fat could be explained by microbial diversity and abundance of specific taxa in the order Clostridiales.\textsuperscript{10} In a randomized controlled trial, consumption of a high cocoa flavan-3-ol drink for 4 weeks significantly increased abundance of \textit{Lactobacillus} in healthy volunteers, this change in bacterial abundance was directly correlated with the decrease in plasma c-reactive protein concentrations observed over the course of the intervention.\textsuperscript{11} Furthermore, the diversity and quality of plant-based diets have shown to be particularly predictable by the microbiome.\textsuperscript{12} Although not yet fully explored, interindividual variability in the gut microbiome might also explain part of the associations between habitual flavonoid intake and systolic and diastolic BP, important cardiovascular risk factors. Therefore, we aimed to explore for the first time, the potential mediating effect of the gut microbiome on the associations between dietary flavonoid intake, flavonoid-rich foods, and BP in a community-dwelling sample. Specifically, we examined the associations between intakes of different flavonoid subclasses (flavanones, anthocyanins, flavan-3-ols, flavonols, flavones, polymeric flavonoids [and proanthocyanidins separately]) and their main habitual food sources with (1) systolic and diastolic BP and (2) gut microbiome diversity and abundances of taxa. Second, we investigated how much variance the gut microbiome explains of the proposed associations between intake of flavonoid subclasses/foods and BP.

A priori we hypothesized significant associations with the anthocyanin subclass as we have previously shown that higher baseline intake of anthocyanins is associated with an 8\% reduced risk of hypertension over 14 years of follow-up,\textsuperscript{13} 3–4 mm Hg lower peripheral and central systolic BP (SBP),\textsuperscript{14} and higher microbial diversity and abundance of taxa in the Clostridiales order.\textsuperscript{15}

### MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### Study Population

We used data from the Population-Based Recruitment for Genetics Research (PopGen) control cohort, a cohort recruited and systematically followed by the PopGen biobank. In brief, the sample consists of 747 individuals collected from population registries\textsuperscript{16} and 569 blood donors from the University Hospital Schleswig-Holstein in Kiel. Initially, this sample served as reference sample for genetic case-control comparisons (\(N=1316\)), why it was named PopGen control cohort.\textsuperscript{17} Subsequently, regular follow-up examinations with clinical and molecular phenotyping were conducted. The first follow-up examination (2010–2012), attended by 929 participants, comprised biochemical, phenotypic, and dietary assessments. BP measurements were available for 927 participants of which we excluded those who were missing dietary (\(n=5\)), microbiome (\(n=14\)), or covariate (\(n=4\)) data (Figure S1 in the Data Supplement). All participants were unaware of the specific hypotheses being tested and were not selected for a particular disease or trait. The study was approved by the Ethical review board of the Medical Faculty of Kiel University and all subjects provided written informed consent. The study adhered to the principles of the Declaration of Helsinki and the Code of Ethics of the World Medical Association. A priori we hypothesized significant associations with the anthocyanin subclass as we have previously shown that higher baseline intake of anthocyanins is associated with an 8\% reduced risk of hypertension over 14 years of follow-up,\textsuperscript{13} 3–4 mm Hg lower peripheral and central systolic BP (SBP),\textsuperscript{14} and higher microbial diversity and abundance of taxa in the Clostridiales order.\textsuperscript{15}
Blood Pressure

SBP and diastolic BP was measured 3x after an initial 5-minute rest period on the right arm of seated individuals with a digital BP monitor using a cuff of appropriate size after an overnight fast. Measurements were separated by 3-minute intervals. The mean of the second and third measurements was used for the present analyses.

Dietary Assessment

Dietary intake over the previous year was calculated using a self-administered 112-item food frequency questionnaire (FFQ), originally designed and validated for use in the German European Prospective Investigation into Nutrition and Cancer study.18 Flavonoid content values were assigned to each of the foods listed in the FFQ using data from the US Department of Agriculture as the primary data source.19,20 For foods assessed in the FFQ without values in the US Department of Agriculture database, we searched phenol explorer (www.phenol-explorer.eu) to ensure all available high-quality data on flavonoid values were included. Intakes were derived for the main subclasses of flavonoids habitually consumed: flavanones (eriodictyol, hesperetin, and naringenin), anthocyanins (cyanin-din, delphinidin, malvidin, pelargonidin, petunidin, and peonidin), flavan-3-ols (catechins and epicatechins), flavonols (quercetin, kaempferol, myricetin, and isorhamnetin), flavones (luteolin and apigenin), polymers (including proanthocyanidins [excluding monomers], theaflavins, and thearubigins), and proanthocy-anidins separately (dimers, trimers, 4–6mers, 7–10mers, poly-mers, and monomers). Total flavonoid intakes were calculated by summing the 6 component subclasses (flavanones, antho-cyanins, flavan-3-ols, flavonols, flavones, and polymers). Intakes of energy and other nutrients were determined using values from the German Food Code and Nutrient Database (version II.3).21

Gut Microbiome Composition Analysis

Fecal bacterial DNA was extracted using the QIAamp DNA Stool Mini kit from Qiagen on a QiAcube system. After extraction, the V1-V2 region of the 16S ribosomal RNA gene was sequenced on the MiSeq platform, using the 27F–338R primer pair and dual MID indexing (8 nucleotides each on the forward and reverse primers) with the MiSeq Reagent Kit v3, as previously described.22 After sequencing, MiSeq fastq files were derived from base calls for read 1 and 2 (R1 and R2), as well as both indices (11 and I2), using the Bcl2fastq module in CASAVA 1.8.2 with no mismatches in either index sequence allowed. Forward and reverse reads were merged with FLASH software (v1.2)23 and high-quality data were derived (sequences with <5% nucleotides with quality score >30 performed with fastx toolkit). After removing chimeras in sequences using UCHIME (v6.0) 10,000 reads for each sample were randomly selected.24 Sequences were clustered at each taxonomical level using the Ribosomal Database Project classifier with the latest reference database (version 14).25 Classifications with low confidence at the genus level (<0.8) were organized in an arbitrary taxon of unclassified family. Genus-level Operational Taxonomic Units (97% similarity) were created using the UPARSE routine.26

Covariate Assessment

Data on participant characteristics, including sex, age, smoking status, medication use, and physical activity, were collected by self-administered questionnaire. For physical activity, participants were asked to report the time spent walking, cycling, engaging in sports and gardening (average of summer and winter seasons), household work, and do-it-yourself activities per week over the past year and the number of flights of stairs climbed per day. The duration of each physical activity was multiplied by the corresponding metabolic equivalent intensity level and summed for all activities.27 Weight and height were measured with subjects dressed in light clothing without shoes; 2 kg were subtracted to account for clothing.

Statistical Analysis

We compared dietary and lifestyle characteristics between men and women using independent t tests (for continuous traits) or χ² tests (for categorical data). Participants were ranked into sex-specific tertiles of estimated intake for the flavonoid subclasses and associations with BP variables (SBP, diastolic BP, and pulse pressure [PP]) were assessed using ANCOVA. If a subclass was found to be significantly associated with any of the outcomes, we also examined the associations with foods known to contribute considerably to habitual intake of the respective subclass. Participants were categorized into quartiles of flavonoid-rich food intake (quartiles rather than tertiles of intake were used to ensure equal numbers of participants in each of the categories). We checked for effect modification by including multiplicative interaction terms for sex/menopausal status (male, premenopausal females, postmenopausal females) and quantities of flavonoid/flavonoid-rich food intake in the models.

We then examined the association between intake of the flavonoid subclasses and flavonoid-rich foods and tax relative abundance and microbial diversity using ANCOVA. Microbial diversity was defined using the Shannon index and the Bray-Curtis dissimilarity measure, for which we considered the first three principal components from principal coordinate analysis. We focused on those flavonoid subclasses and foods that had provided evidence for association with BP in the first analysis. To reduce random error in low abundance taxa, we focused our analysis on highly reproducible taxa (r²>0.97), which in this dataset was determined as taxa with >40 reads per replicate in 10,000 reads (21 taxa at genus level).28 If we observed significant associations of flavonoid subclasses with relative genus-abundance or diversity measure, we then assessed the relationship between these microbial factors and BP using ANCOVA. To detect genus with significantly different abundances between quantities of flavonoid subclasses and flavonoid-rich foods, Linear Discriminant Analysis Effect Size analysis was used without adjustment for covariates (https://huttenhower.sph.harvard.edu/galaxy/).29 All models were adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week), body mass index (kg/m²), use of BP medication...
Finally, we used structural equation modeling to quantify the amount of variation in the association between flavonoid/flavo-noid-rich food intake and BP that was explained by the microbiome. We considered any genus-level taxa or diversity measures that were significantly associated with both intake and BP in the models. We also combined these variables using principal component analysis to assess the combined function of the microbiome. We presented the results as a ratio of the indirect association—the association between flavonoid intake and BP mediated by the microbiome, to the total association—association between flavonoid intake and the microbiome on BP. This represented the proportion of the variance explained by the microbiome variable.

Two-sided \( P<0.05 \) were considered statistically significant for all analyses with exception of the microbial taxa abundances where a multiple testing correction was applied using the Benjamini-Hochberg method for false discovery rate where a \( Q<0.10 \) was considered statistically significant. BP readings were transformed using the natural log and data are presented as geometric means (95% CI) or where the difference between extreme quantiles are reported the percentage change in the dependent variable per unit change in the independent variable \((100\times[\exp(\beta)-1])\). Statistical analyses were performed with Stata statistical software version 15 (StataCorp, TX).

### RESULTS

The demographic characteristics and dietary intakes of the 904 participants aged 25 to 82 years are shown in Table 1 and Table S1. Mean total habitual flavonoid intake was 695 mg/d (SD, 651; median, 514 mg/d; interquartile range, 279–917) in men and 792 mg/d (SD, 630; median, 605 mg/d; interquartile range, 340–1060) in women.

#### Association of Flavonoid Intake With BP Traits

Higher intakes of total flavonoids, anthocyanins, polymers, and specifically of the proanthocyanidin component of the polymer class were associated with statistically significantly lower SBP and PP (Table 2). Higher intakes of flavan-3-ols were associated with lower SBP and higher intakes of flavonols and flavones were associated with lower PP (Table 2).

### Table 1. Characteristics and Dietary Intakes of PopGen Participants by Sex (N=904)

| Characteristic                  | Male (n=516) | Female (N=388) | P value |
|--------------------------------|--------------|----------------|---------|
| Age, y                         | 60.8 (12.0)  | 60.6 (13.2)    | 0.79    |
| Physical activity (metabolic equivalents/wk) | 99.3 (65.4)  | 113 (60.9)     | <0.01   |
| Current smoker (yes)           | 77 (14.9%)   | 57 (14.7%)     | <0.01   |
| BMI, kg/m²                     | 27.7 (4.0)   | 27.0 (5.5)     | 0.05    |
| Use of blood pressure medication (yes) | 187 (38.2%)  | 141 (36.3%)    | <0.01   |
| Family history of coronary heart disease (yes) | 63 (12.2%)   | 63 (16.2%)     | 0.02    |
| Systolic blood pressure, mm Hg | 142 (18.1)   | 137 (18.4)     | <0.01   |
| Diastolic blood pressure, mm Hg | 85.8 (8.8)   | 83.6 (9.2)     | <0.01   |
| Pulse pressure, mm Hg          | 56.3 (13.4)  | 53.2 (13.6)    | <0.01   |
| Total flavonoids, mg/d         | 695 (651)    | 792 (630)      | 0.02    |
| Flavanones, mg/d               | 23.5 (29.6)  | 19.9 (21.9)    | 0.05    |
| Anthocyanins, mg/d             | 37.5 (39.0)  | 43.5 (43.7)    | 0.03    |
| Flavan-3-ols, mg/d             | 198 (301)    | 236 (301)      | 0.06    |
| Flavonols, mg/d                | 30.5 (24.4)  | 31.0 (22.6)    | 0.73    |
| Flavones, mg/d                 | 4.5 (3.3)    | 4.3 (3.3)      | 0.37    |
| Polymers, mg/d                 | 401 (339)    | 458 (323)      | 0.01    |
| Proanthocyanidins, mg/d*       | 506 (430)    | 584 (425)      | 0.01    |
| Apple/pears, portions/d        | 0.6 (0.6)    | 0.9 (0.7)      | <0.01   |
| Berries, portions/d            | 0.1 (0.3)    | 0.2 (0.4)      | <0.01   |
| Strawberries, portions/d       | 0.4 (0.4)    | 0.6 (0.6)      | <0.01   |
| Grapes, portions/d             | 0.2 (0.4)    | 0.4 (0.5)      | <0.01   |
| Red wine, portions/d           | 0.1 (0.2)    | 0.1 (0.2)      | 0.02    |
| Tea, portions/d                | 0.7 (1.3)    | 0.7 (1.3)      | 0.47    |
| Peppers, portions/d            | 0.1 (0.1)    | 0.2 (0.2)      | <0.01   |
| Energy, kcal/d                 | 2544 (813)   | 1928 (509)     | <0.01   |
| Fiber, g/d                     | 23.1 (7.6)   | 21.2 (6.3)     | <0.01   |

Values are mean (SD) or N (%). P values are for the differences between males and females calculated from independent t tests for continuous data and \( \chi^2 \) test for categorical data. BMI indicates body mass index; and PopGen, Population-Based Recruitment for Genetics Research.

*Proanthocyanidins are also included in the polymer subclass.
In food-based analyses, focused on the top foods that contributed to intakes of flavonoid subclasses that were associated with lower BP measures, we also observed lower BP/PP; berries and red wine were associated with significantly lower SBP and PP (Table 3). Higher intakes of apples/pears and peppers were also associated with lower PP. We observed no association of intakes of grapes or tea and SBP or PP, and associations between all flavonoid subclasses and flavonoid-rich foods with diastolic blood pressure were nonsignificant.

**Flavonoid Intake and the Gut Microbiome**

Higher intakes of berries (Q4-Q1=0.03 [95% CI, 0.0–0.1], \(P=0.04\)) and red wine (Q4-Q1=0.1 [95% CI, 0.03–0.1], \(P<0.01\)) were associated with higher microbial alpha diversity (Figure S2) and differences in principal components of the Bray-Curtis measure of beta diversity (Berries and principal coordinate analysis 3, \(P=0.02\); red wine and principal coordinate analysis 1, \(P=0.03\); and red wine and principal coordinate analysis 2, \(P=0.01\), Figures S3 and S4). Higher intakes of apples/pears (Q4-Q1=−0.3 [95% CI, −0.4 to −0.1], \(P<0.01\), \(Q=0.04\)) and berries (Q4-Q1=−0.2 [95% CI, −0.4 to −0.1], \(P<0.01\), \(Q=0.07\)) were associated with lower relative abundance of the *Parabacteroides* genus (Figure 1 and Table S2). Findings that higher intakes of berries were associated with lower relative abundance of *Clostridium XIVa* (Q4-Q1=−0.1 [95% CI, −0.2 to −0.01], \(P=0.03\), \(Q=0.21\)) and higher relative abundance of *Roseburia* (Q4-Q1=0.1 [95% CI, 0.03 to 0.3], \(P=0.02\), \(Q=0.18\)) did not reach thresholds of statistical significance after adjusting for multiple testing. Higher intakes of red

**Table 2. Measures of Blood Pressure by Sex-Specific Tertiles of Flavonoid Subclass Intake in 904 Men and Women From the PopGen Cohort**

| Subclass, mg/d                  | T1                          | T2                          | T3                          | \(P\) value |
|--------------------------------|-----------------------------|-----------------------------|-----------------------------|-------------|
|                                | Systolic blood pressure, mmHg | Diastolic blood pressure, mmHg | Pulse pressure, mmHg        |             |
| Total flavonoids [Ref]         | −3.2 (−5.2 to −1.2)         | −2.9 (−5.1 to −0.7)         | −3.2 (−5.4 to −1.0)         | 0.01        |
| Flavanones [Ref]               | 0.2 (−1.7 to 2.2)           | 0.3 (−1.8 to 2.4)           | 0.0 (−2.0 to 2.1)           | 0.79        |
| Anthocyanins [Ref]             | −1.0 (−3.0 to 1.1)          | −3.4 (−5.6 to −1.3)         | −3.7 (−5.9 to −1.5)         | <0.01       |
| Flavan-3-ols [Ref]             | −2.3 (−4.3 to −0.3)         | −2.2 (−4.3 to −0.1)         | −2.5 (−4.5 to −0.5)         | 0.04        |
| Flavonols [Ref]                | −3.5 (−5.5 to −1.4)         | −2.4 (−4.7 to −0.1)         | −3.2 (−5.4 to −1.0)         | 0.01        |
| Polymers [Ref]                 | −2.7 (−4.7 to −0.7)         | −3.7 (−5.9 to −1.5)         | −2.5 (−4.5 to −0.5)         | 0.01        |
| Proanthocyanidins* [Ref]       | −2.5 (−4.5 to −0.5)         | −3.2 (−5.4 to −1.0)         | −2.5 (−4.5 to −0.5)         | 0.01        |
|                                | Flavanones [Ref]            | 0.6 (−1.0 to 2.3)           | 1.3 (−0.5 to 3.0)           | 0.15        |
| Anthocyanins [Ref]             | 0.4 (−1.4 to 2.1)           | 0.2 (−1.6 to 2.2)           | −1.1 (−2.8 to 0.7)          | 0.34        |
| Flavan-3-ols [Ref]             | −0.4 (−2.1 to 1.4)          | −1.2 (−2.9 to 0.6)          | −1.0 (−2.8 to 0.7)          | 0.15        |
| Flavonols [Ref]                | −0.8 (−2.6 to 1.0)          | −0.3 (−2.2 to 1.7)          | −1.1 (−2.8 to 0.7)          | 0.22        |
| Flavones [Ref]                 | 2.3 (0.5 to 4.1)            | 1.4 (−0.5 to 3.3)           | −1.1 (−2.8 to 0.7)          | 0.22        |
| Polymers [Ref]                 | −1.4 (−3.1 to 0.3)          | −1.5 (−3.4 to 0.4)          | −1.1 (−2.8 to 0.7)          | 0.22        |
| Proanthocyanidins* [Ref]       | −1.1 (−2.8 to 0.7)          | −1.2 (−3.1 to 0.7)          | −1.1 (−2.8 to 0.7)          | 0.22        |
|                                | Total flavonoids [Ref]       | −6.2 (−9.8 to −2.5)         | −5.8 (−9.7 to −1.7)         | 0.01        |
| Flavanones [Ref]               | −0.7 (−4.3 to 3.1)          | −1.2 (−5.0 to 2.6)          | −6.2 (−9.8 to −2.5)         | 0.52        |
| Anthocyanins [Ref]             | −2.8 (−6.5 to 1.0)          | −8.7 (−12.4 to −4.8)        | −6.2 (−9.8 to −2.5)         | <0.01       |
| Flavan-3-ols [Ref]             | −4.9 (−8.5 to −1.1)         | −3.8 (−7.5 to 0.1)          | −2.8 (−6.5 to 1.0)          | 0.07        |
| Flavonols [Ref]                | −7.5 (−11.1 to −3.7)        | −5.7 (−9.8 to −1.5)         | −4.9 (−8.5 to −1.1)         | 0.01        |
| flavanes [Ref]                 | −3.1 (−6.8 to 0.7)          | −4.1 (−8.0 to −0.2)         | −7.5 (−11.1 to −3.7)        | 0.04        |
| Polymers [Ref]                 | −4.3 (−7.9 to −0.5)         | −7.0 (−11.0 to −2.9)        | −3.1 (−6.8 to 0.7)          | <0.01       |
| Proanthocyanidins* [Ref]       | −4.3 (−7.9 to −0.6)         | −6.3 (−10.2 to −2.2)        | −4.3 (−7.9 to −0.6)         | <0.01       |

Values are mean difference from T1 (95% CI). Model adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week in tertiles), BMI (kg/m², in tertiles), use of blood pressure medication (y/n), family history of coronary heart disease (y/n) and daily intakes of fiber (g) and energy (kcal). \(P\) denotes \(P\) trend calculated using ANCOVA. Participant numbers for the subclasses were as follows: Q1=302, Q2=301, Q3=301, except for the flavanone, anthocyanin, and flavones subclasses where Q1=303, Q2=300, Q3=301. BMI indicates body mass index; and PopGen, Population-Based Recruitment for Genetics Research.

\*Proanthocyanidins are also included in the polymer subclass.
wine were significantly associated with higher abundance of Oscillibacter (Q4-Q1=0.3 [95% CI, 0.1–0.6], P=0.01, Q=0.05) and unclassified Ruminococcaceae (Q4-Q1=0.9 [95% CI, 0.3–1.5], P<0.01, Q=0.05). Intakes of flavonoid subclasses were not associated with measures of alpha (Shannon index) or beta (Bray-Curtis index) diversity and associations with genus-level taxa were all nonsignificant after adjustment for multiple testing (data not shown).

Linear Discriminant Analysis Effect Size analysis confirmed enriched abundance of Oscillibacter and Ruminococcus genus in the highest consumers of red wine compared to the lower consumers (Linear Discriminant Analysis>3.5; P<0.05, Figure S5) and in the Parabacteroides genus in the lowest consumers of berries (Linear Discriminant Analysis >3.5; P<0.05, Figure S6) but not apples/pear (Figure S7).

There was no significant interaction between sex/ menopausal status and intake of flavonoid subclasses in the BP models but the association between a higher red wine intake and lower SBP was greater in premenopausal (β=−0.02, P<0.01) and postmenopausal women (β=−0.01, P=0.01) compared with men (β=0.00, P=0.99). Interactions between sex/ menopausal status and red wine intake in the microbiome models were not significant so further stratified analyses were not conducted.

**Table 3. Measures of Blood Pressure by Sex-Specific Quartiles of Foods Rich in Flavonoids in 904 Men and Women From the PopGen Cohort**

| Food, portion/d | Q1 | Q2 | Q3 | Q4 | P value |
|-----------------|----|----|----|----|---------|
| Systolic blood pressure, mmHg | | | | | |
| Apple/pears [Ref] | −0.3 (−2.8 to 2.3) | −2.4 (−4.7 to −0.1) | −1.9 (−4.4 to 0.6) | | 0.05 |
| Berries [Ref] | −3.3 (−5.5 to −1.1) | −3.5 (−5.7 to −1.3) | −2.9 (−5.2 to −0.6) | | 0.01 |
| Grapes [Ref] | −1.3 (−3.5 to 1.0) | −2.2 (−4.3 to −0.1) | −1.6 (−3.8 to 0.7) | | 0.10 |
| Red wine [Ref] | −1.9 (−4.0 to 0.3) | −2.4 (−4.8 to 0.1) | −2.6 (−4.8 to −0.3) | | 0.03 |
| Tea [Ref] | 0.0 (−2.4 to 2.5) | −1.3 (−3.6 to 1.0) | 0.2 (−2.0 to 2.5) | | 0.93 |
| Peppers [Ref] | −1.5 (−4.2 to 1.3) | −2.4 (−5.1 to 0.4) | −2.2 (−4.8 to 0.6) | | 0.13 |
| Diastolic blood pressure, mmHg | | | | | |
| Apple/pears [Ref] | 2.0 (−0.2 to 4.3) | 0.1 (−1.8 to 2.1) | 0.8 (−1.4 to 3.0) | | 0.94 |
| Berries [Ref] | −1.6 (−3.5 to 0.4) | −1.2 (−3.1 to 0.7) | −0.9 (−2.9 to 1.1) | | 0.40 |
| Grapes [Ref] | −0.7 (−2.6 to 1.2) | −0.5 (−2.4 to 1.3) | 0.2 (−1.8 to 2.1) | | 0.92 |
| Red wine [Ref] | −1.8 (−3.6 to 0.0) | −0.8 (−2.9 to 1.3) | −0.2 (−2.1 to 1.7) | | 0.78 |
| Tea [Ref] | 0.3 (−1.8 to 2.4) | −0.6 (−2.5 to 1.4) | −0.2 (−2.0 to 1.8) | | 0.70 |
| Peppers [Ref] | −0.4 (−2.8 to 1.9) | −0.2 (−2.5 to 2.2) | −0.2 (−2.5 to 2.1) | | 0.99 |
| Pulse pressure, mmHg | | | | | |
| Apple/pears [Ref] | −3.7 (−8.2 to 1.1) | −6.2 (−10.2 to −1.9) | −5.7 (−10.1 to −1.1) | | 0.01 |
| Berries [Ref] | −5.6 (−9.6 to −1.4) | −6.5 (−10.5 to −2.5) | −5.5 (−9.6 to −1.2) | | 0.01 |
| Grapes [Ref] | −1.9 (−6.1 to 2.4) | −4.0 (−7.8 to 0.0) | −3.6 (−7.7 to 0.7) | | 0.05 |
| Red wine [Ref] | −1.8 (−5.8 to 2.3) | −4.4 (−8.7 to 0.2) | −6.1 (−10.1 to −2.0) | <0.01 |
| Tea [Ref] | −0.6 (−5.0 to 4.1) | −2.5 (−6.6 to 1.8) | 0.5 (−3.6 to 4.9) | | 0.97 |
| Peppers [Ref] | −2.7 (−7.7 to 2.5) | −5.7 (−10.6 to −0.7) | −5.3 (−10.0 to −0.3) | | 0.03 |

Values are mean difference from T1 (95% CI). Model adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week in tertiles), BMI (kg/m2, in tertiles), use of blood pressure medication (y/n), family history of Coronary Heart Disease (y/n) and daily intakes of fiber (g) and energy (kcal). P denotes P trend calculated using ANCOVA. Participant numbers for the flavonoid-rich foods were as follows: apples/pears Q1=186, Q2=168, Q3=282, Q4=268; berries Q1=267, Q2=198, Q3=224, Q4=215; grapes Q1=256, Q2=196, Q3=244, Q4=208; red wine Q1=219, Q2=272, Q3=167, Q4=246; Tea Q1=204, Q2=183, Q3=244, Q4=273. BMI indicates body mass index; and PopGen, Population-Based Recruitment for Genetics Research.

**Interrelation of Flavonoid Intake, BP Traits, and Gut Microbiome Composition**

Of the taxa significantly associated with intakes of flavonoid subclasses or flavonoid-rich foods, higher abundance of Parabacteroides was associated with significantly higher SBP and higher abundance of unclassified Ruminococcaceae with lower SBP and PP (Table 4). Higher alpha diversity was associated with lower SBP and beta diversity was associated with both SBP and PP.

The proportion of the association between intake of berries and SBP that could be explained by the gut microbiome was 7.9% for Parabacteroides, 2.8% for beta diversity, 5.0% for alpha diversity (Figure 2). A linear combination of these gut microbiome variables (first principal component: 43% of the variance) explained 11.6% of the association between intake of berries and SBP. For red wine and SBP, alpha diversity explained 13.0% of the association and unclassified Ruminococcaceae 9.6%, a linear combination of these gut microbiome
variables (first principal component: 75% of the variance) explained 15.2% of the association. The proportion of the association between flavonoid-rich foods and PP that could be explained by \textit{Parabacteroides} was 8.2% for apples/pears and 7.3% for berries (data not shown).

**DISCUSSION**

To our knowledge, to date, no study has directly investigated the gut microbiome that explains the associations between flavonoid subclass intakes, flavonoid-rich foods, and BP in a community-based sample. Our data suggest that up to 15.2% of the association between flavonoid-rich foods, including berries, red wine, and apples/pears, and SBP could be explained by the gut microbiome. Specifically, higher intake of berries (equivalent to 1.6 portions per day, containing an estimated 112 mg of anthocyanins) was associated with 4.1 mm Hg lower SBP, 11.6% of this association could be explained by higher alpha and beta diversity and lower relative abundance of \textit{Parabacteroides}. For red wine, intake of 2.8 glasses (250 mL) per week was associated with 3.7 mm Hg lower SBP of which 15.2% could be explained by a combination of alpha diversity and higher relative abundance of unclassified \textit{Ruminococcaceae}.

**Figure 1.** Relative abundance of genera by quantile of the intake of flavonoid-rich foods in 904 men and women from the PopGen cohort.

Bars represent beta coefficient and error bars represent 95% CI. Values adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week in tertiles), body mass index (kg/m², in tertiles), use of blood pressure medication (y/n), family history of coronary heart disease (y/n) and daily intakes of fiber (g) and energy (kcal). *P < 0.05 and Q value (false discovery rate adjusted) < 0.10, *P < 0.05 and Q value nonsignificant, calculated using ANCOVA. Participant numbers for the flavonoid-rich foods were as follows: apples/pears Q1=186, Q2=168, Q3=282, Q4=268; berries Q1=267, Q2=198, Q3=224, Q4=215; Red wine Q1=219, Q2=272, Q3=167, Q4=246. PopGen indicates Population-Based Recruitment for Genetics Research.
The magnitude of the association between anthocyanin intake and SBP (−4.8 mm Hg) was similar to our previous observations in a female cohort. A reduction in SBP of this scale is likely to have clinical benefits. Hypertension is a major risk factor for cardiovascular disease and the cost of treatment is substantial. In the UK, the national cost of treating hypertension was estimated to be £4.8 billion in 2014/15 (GSK, 2015).\(^\text{30}\) Furthermore, a recent study estimated that reducing the population average SBP by 5 mm Hg could reduce 45 000 quality-adjusted life years and save £850 million related health and social care costs.\(^\text{31}\)

Similar trends can be observed in animal studies. For example, feeding rats blueberry powder for four weeks was associated with reductions in BP and concurrent increases in gut microbiome, such as \textit{Parabacteroides}, and the development of hypertension.\(^\text{39}\) However, in a recent study, although \textit{Parabacteroides distasonis} was shown to generate succinate in the gut, this was associated with reduced weight gain and hyperglycemia in mice.\(^\text{40}\) Further, in vitro studies have shown that apple intake increases abundance of \textit{Parabacteroides}, and findings from mice models suggest that higher intake of lingonberries increase relative abundance of \textit{Parabacteroides} by up to 15\%.\(^\text{41}\) Feeding rats blueberry powder for four weeks was associated with reductions in BP and concurrent increases in \textit{Parabacteroides}-like species.\(^\text{42}\)

Our research also showed that gut bacterial alpha diversity mediated the associations between intakes of apples/pears and berries on BP.\(^\text{37,38}\) A proposed mechanism underlying these associations is the production of succinate, as a direct correlation has been made between succinate-producing gut microbiome, such as \textit{Parabacteroides}, and the development of hypertension.\(^\text{39}\) However, in a recent study, although \textit{Parabacteroides distasonis} was shown to generate succinate in the gut, this was associated with reduced weight gain and hyperglycemia in mice.\(^\text{40}\) Furthermore, in vitro studies have shown that apple intake increases abundance of \textit{Parabacteroides}, and findings from mice models suggest that higher intake of lingonberries increase relative abundance of \textit{Parabacteroides} by up to 15\%.\(^\text{41}\) Feeding rats blueberry powder for four weeks was associated with reductions in BP and concurrent increases in \textit{Parabacteroides}-like species.\(^\text{42}\)

### Table 4. Measures of Blood Pressure by Tertile of Microbial Diversity and Relative Abundance of Genus in 904 Men and Women From the PopGen Cohort

| Gut microbial factor | T1          | T2          | T3          | P value |
|----------------------|-------------|-------------|-------------|---------|
| **Systolic blood pressure, mm Hg** |             |             |             |         |
| Oscillo bacter *     | [Ref]       | −1.5 (−3.4 to 0.5) | 0.7 (−1.3 to 2.7) | 0.48 |
| Parabacteroides      | [Ref]       | −0.9 (−2.8 to 1.0) | 2.1 (0.2 to 4.1) | 0.04 |
| Unclassified Ruminococcaceae | [Ref]       | −0.8 (−2.8 to 1.1) | −2.3 (−4.2 to −0.3) | 0.02 |
| Shannon Index        | [Ref]       | −0.9 (−2.8 to 1.1) | −2.4 (−4.3 to −0.4) | 0.02 |
| Bray-Curtis (PcoA-1) | [Ref]       | −0.7 (−2.6 to 1.3) | −1.8 (−3.7 to 0.1) | 0.06 |
| Bray-Curtis (PcoA-2) | 1.0 (−1.0 to 3.0) | 0.4 (−1.6 to 2.4) | 0.72 |
| Bray-Curtis (PcoA-3) | [Ref]       | −2.7 (−4.6 to −0.8) | −2.2 (−4.1 to −0.3) | 0.02 |
| **Pulse pressure, mm Hg** |             |             |             |         |
| Oscillo bacter *     | [Ref]       | −2.0 (−5.6 to 1.7) | 2.3 (−1.5 to 6.3) | 0.22 |
| Parabacteroides      | [Ref]       | −0.8 (−4.4 to 2.9) | 3.9 (0.2 to 7.8) | 0.04 |
| Unclassified Ruminococcaceae | [Ref]       | −0.7 (−4.3 to 3.1) | −4.3 (−7.8 to −0.6) | 0.02 |
| Shannon Index        | [Ref]       | −0.3 (−3.9 to 3.4) | −3.3 (−6.8 to 0.3) | 0.08 |
| Bray-Curtis (PcoA-1) | [Ref]       | −2.4 (−5.9 to 1.3) | −4.3 (−7.7 to −0.7) | 0.02 |
| Bray-Curtis (PcoA-2) | 3.3 (−0.4 to 7.2) | 0.7 (−3.0 to 4.6) | 0.70 |
| Bray-Curtis (PcoA-3) | [Ref]       | −3.5 (−7.0 to 0.1) | −2.6 (−6.2 to 1.0) | 0.15 |

Values are mean difference from T1 (95% CI). Model adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week in tertiles), BMI (kg/m²), in tertiles), use of blood pressure medication (y/n), family history of coronary heart disease (y/n) and daily intakes of fiber (g) and energy (kcal). P denotes trend calculated using ANCOVA. Participant numbers for the quantiles of microbial diversity and relative abundance were as follows: Oscillo bacter Q1=304, Q2=299, Q3=301; Parabacteroides Q1=303, Q2=300, Q3=301; Roseburia Q1=305, Q2=298, Q3=301; Unclassified Porphyromonadaceae Q1=313, Q2=290, Q3=301; Unclassified Ruminococcaceae Q1=302, Q2=301, Q3=301; Bray-Curtis (PcoA-1) Q1=302, Q2=301, Q3=301; Bray-Curtis (PcoA-2) Q1=302, Q2=301, Q3=301; Shannon Index Q1=302, Q2=301, Q3=301. BMI indicates body mass index; and PcoA, principal coordinate analysis; and PopGen, Population-Based Recruitment for Genetics Research.
microbial communities seen in hypertension, although it is not clear if these physiological changes are a cause or a consequence of hypertension.

As in our previous research, our results differed for intakes of flavonoid subclasses and flavonoid-rich foods, not surprising given their wide differences in both bioavailability and bioactivity.\textsuperscript{15} Intakes of flavonoid subclasses were not associated with measures of microbial diversity and associations with genus-level taxa were all nonsignificant after adjustment for multiple testing. Our results showed that major food contributors to the same subclass can have differential associations with the microbiome. Berries and red wine, for example, were both significant contributors to intakes of the anthocyanin subclass but were found to be associated with different taxa. These foods have different anthocyanin profiles with red wine typically rich in malvidin and berries in cyanidin.\textsuperscript{19} Discrepancies have been shown between the biological activities of delphinidin/malvidin-versus cyanidin-type anthocyanins that are explained by differences in their structure and metabolism in the gut.\textsuperscript{46}

In conclusion, we have shown, for the first time, that participants with the highest intakes of flavonoid-rich foods, including berries (equivalent to 1.6 portions per day), red wine (equivalent to 2.8 glasses [250 mL] per week), and apples/pears (equivalent to 1.6 portions per day), had up to 4 mm Hg lower SBP and PP, as well as greater microbial diversity, lower abundance of \textit{Parabacteroides} and higher unassigned genera belonging to the \textit{Ruminococcaceae} family, compared with those with the lowest intakes. These data highlight the key role microbial diversity and abundance of these taxa play in the observed associations between flavonoid-rich foods-gut microbiome and BP with up to 15\% of the association between flavonoid-rich foods and BP explained by the gut microbiome.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Percentage variation explained by microbial factors in the associations between flavonoid-rich foods and systolic blood pressure in N=904 men and women from the PopGen cohort.}
\end{figure}

Values are the path coefficients, calculated using structural equation modeling, for the ratio of the indirect to total association between intake of flavonoid-rich foods, microbial factors, and blood pressure. The total association is the association between intake and blood pressure controlling for microbial factors and the indirect association is the association between intake and blood pressure explained by microbial factors. Factor 1 is the first principal component from analysis of all the microbial factors associated with intake of berries (43\% of variation) and red wine (49\% of variation) and blood pressure. Models adjusted for sex (male, premenopausal women, postmenopausal women), age (years), smoking status (never, former, current), physical activity (metabolic equivalents per week in tertiles), body mass index (kg/m\textsuperscript{2}, in tertiles), use of blood pressure medication (y/n), family history of coronary heart disease (y/n) and daily intakes of fiber (g), and energy (kcal). PCA indicates principal component analysis; PcoA, principal coordinate analysis; and PopGen, Population-Based Recruitment for Genetics Research.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|}
\hline
Factor 1 (PCA) & 0.56 \pm 0.12 \% \\
Shannon Index & 0.50 \pm 0.13 \% \\
Unclassified ruminococcaceae & 0.35 \pm 0.14 \% \\
Factor 1 (PCA) & 0.41 \pm 0.15 \% \\
Bacillus-Curtis (PcoA 3) & 0.23 \pm 0.16 \% \\
Shannon Index & 0.22 \pm 0.17 \% \\
Parabacteroides & 0.18 \pm 0.18 \% \\
\hline
\end{tabular}
\caption{Percentage variation explained by microbial factors in the associations between flavonoid-rich foods and systolic blood pressure in N=904 men and women from the PopGen cohort.}
\end{table}

\textbf{Strengths and Limitations}

The strength of the current study was that we were able to examine associations and interrelationships between intake of flavonoid-rich foods, BP, and the composition of the gut microbiome concurrently. Our findings suggest that the direct association between intake of flavonoid-rich foods, such as apples/pears, berries, and red wine, and BP is modulated by relative abundance of \textit{Parabacteroides} and bacterial diversity. These data were collected from a large and well-characterized community-dwelling sample with an extensive array of possible confounding variables available. As with all observational studies, there are some potential sources of error. Residual or unmeasured confounding can lead to bias, and we are unable to infer causation from these findings. In addition, despite our detailed adjustment for a range of dietary and lifestyle variables, there is still the possibility of residual or unmeasured confounding from additional unmeasured factors. Although the value of self-reported dietary assessment has been questioned,\textsuperscript{47} it is well established that FFQs are a useful and valid tool to discriminate and rank individuals sufficiently to examine and predict relationships between food intake and health end points in large cohorts.\textsuperscript{48} The FFQ data allowed us to calculate intakes of all major flavonoid subclasses and accurately rank participants according to intakes of flavonoid-rich foods, although it may not have captured all food sources and measurement errors are inevitable,\textsuperscript{49} but these measurement errors are likely to attenuate true associations toward the null.\textsuperscript{47} Furthermore, we observed limited associations of flavonoid subclasses with the gut microbiome, and as flavonoid-rich foods, such as apples /pears, berries, and red wine are important sources of other phytochemicals, such as chlorogenic acid, ellagitannins, and resveratrol, it could be these constituents that also contribute to explaining the observed associations. Future work should consider measuring urinary flavonoid metabolite concentrations as potential biomarkers of intake.
ARTICLE INFORMATION
Received March 30, 2021; accepted June 16, 2021.

Affiliations
Institute for Global Food Security, Queen’s University Belfast, Northern Ireland (A.J., A.C.). Institute of Epidemiology and Biobank PopGen, University Hospital Schleswig Holstein, Campus Kiel and Kiel University, Kiel Germany (M.K., W.L.). Institute of Clinical Molecular Biology, Kiel University, Kiel, Germany (C.B., A.F.).

Acknowledgments
The authors’ responsibilities were as follows—A. Jennings, M. Koch, W. Lieb, and A. Cassidy designed the study; W. Lieb and M. Koch collected the data; C. Bang and A. Franke conducted the microbiome assessment; A. Jennings performed the statistical analysis; A. Jennings and A. Cassidy drafted the article; M. Koch and W. Lieb provided critical review of the article; and all authors read and approved the final article.

Sources of Funding
Supported in part by Deutsche Forschungsgemeinschaft (German Research Foundation) grant EXC 2216; 390884018 (to W. Lieb) under Germany’s Excellence Strategy, and German Federal Ministry of Education and Research grant 01GRO468. The PopGen 2.0 network is supported by German Federal Ministry of Education and Research grant 01EY103 (to W. Lieb) and the Medical Faculty of the University of Kiel.

Disclosures
A. Cassidy received funding from the US Highbush Blueberry Council (USHBC) with oversight from the United States Department of Agriculture and A. Cassidy acts as an advisor to the USHBC grant committee. The other authors report no conflicts.

REFERENCES
1. Manach C, Scalbert A, Morand C, Rémyes C, Jiménez L. Polyphenols: food sources and bioavailability. Am J Clin Nutr. 2004;79:727–747. doi: 10.1093/ajcn/79.5.727
2. Manach C, Williamson G, Morand C, Scalbert A, Rémyes C. Bioavailability and bioeffectiveness of polyphenols in humans. I. Review of 97 bioavailability studies. Am J Clin Nutr. 2005;81(1 Suppl):230S–242S. doi: 10.1093/ajcn/81.1.230S
3. Cassidy A, Minihane AM. The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. Am J Clin Nutr. 2017;105:10–22. doi: 10.1093/ajcn/116.3.3015
4. Edwards M, Czank C, Woodward GM, Cassidy A, Kay CD. Phenolic metabolites of anthocyanins modulate mechanisms of endothelial function. J Agric Food Chem. 2015;63:2425–2431. doi: 10.1021/jf5041993
5. Vendrame S, Guglielmeta S, Riso P, Arcidi S, Klimis-Zacas D, Porini M. Six-week consumption of a wild blueberry powder drink increases bifidobacteria in the human gut. J Agric Food Chem. 2011;59:12815–12820. doi: 10.1021/jf2028688
6. Petersen C, Wankhade UD, Bharat D, Wong K, Mueller JE, Chintapalli SV, Piccolo BD, Jalili A, Ta Z, Symons JD, et al. Dietary supplementation with strawberry induces marked changes in the composition and functional potential of the gut microbiome in diabetic mice. J Nutr Biochem. 2019;66:65–69. doi: 10.1016/j.jnutbio.2019.01.004
7. Boto-Ordóñez M, Upi-Sarda M, Queipo-Ortuño MI, Tulipani S, Tihanovs F, Andres-Lacueva C. High levels of Bifidobacteria are associated with increased levels of anthocyanin microbial metabolites: a randomized clinical trial. Food Funct. 2014;5:1932–1938. doi: 10.1039/c4fo00296c
8. Clemente-Postigo M, Queipo-Ortuño MI, Boto-Ordóñez M, Coin-Aragüez L, Roca-Rodriguez MM, Delgado-Lista J, Cardona F, Andres-Lacueva C, Tihanovs FJ. Effect of acute and chronic red wine consumption on lipopolysaccharide concentrations. Am J Clin Nutr. 2013;97:1053–1061. doi: 10.3945/ajcn.112.051158
9. Warner EF, Rodríguez-Ramiro I, O’Connell MA, Kay CD. Cardiovascular mechanisms of action of anthocyanins may be associated with the impact of microbial metabolites on heme oxygenase-1 in vascular smooth muscle cells. Molecules. 2018;23:E889. doi: 10.3390/molecules23040898
10. Jennings A, Koch M, Jensen MK, Bang C, Kassubek J, Müller HP, Nöttling U, Franke A, Lieb W, Cassidy A. The role of the gut microbiome in the association between habitual anthocyanin intake and visceral abdominal fat in population-level analysis. Am J Clin Nutr. 2020;111:340–350. doi: 10.1093/ajcn/nqz299
11. Touniex I, Rodriguez-Mateos A, Vulevic J, Gibson GR, Keik-Urbe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr. 2011;93:62–72. doi: 10.1093/ajcn/njr007
12. Asnicar F, Berry SE, Valdes AM, Nguyen LH, Piccinno G, Drew DA, Leeming E, Gibson R, Le Roy C, Khatib HA, et al. Microbiome connections with habitual metabolisms and habitual diet from 1,098 deeply phenotyped individuals. Nat Med. 2021;27:321–322. doi: 10.1038/s41591-020-01183-8
13. Cassidy A, O’Reilly EJ, Kay C, Sampson L, Franz M, Forman JP, Curban G, Rimm EB. Habitual intake of flavonoid subclasses and incident hypertension in adults. Am J Clin Nutr. 2011;93:338–347. doi: 10.3945/ajcn.110.006783
14. Jennings A, Welch AA, Fairweather-Tait SJ, Kay C, Minihane AM, Chowienycz P, Jiang B, Cecelja M, Spector T, Macgregor A, et al. Higher anthocyanin intake is associated with lower arterial stiffness and central blood pressure in women. Am J Clin Nutr. 2012;96:781–788. doi: 10.3945/ajcn.112.042036
15. Jennings A, Koch M, Jensen MK, Bang C, Kassubek J, Muller HP, Nöttling U, Franke A, Lieb W, Cassidy A. The role of the gut microbiome in the association between habitual anthocyanin intake and visceral abdominal fat in population-level analysis. Am J Clin Nutr. 2019;111:340–350. doi: 10.1093/ajcn/nqz299
16. Koch M, Borggrefe J, Barbasero J, Groth G, Jacobs G, Siegert S, Lieb W, Mueller MJ, Bosy-Westphal A, Heller M, et al. Dietary patterns associated with magnetic resonance imaging-determined liver fat content in a general population study. Am J Clin Nutr. 2011;94:359–377. doi: 10.3945/ajcn.110.13072019
17. Krawczak M, Nikolaus S, von Ebersfeit H, Croucher RI, El Mokhtari NE, Schreiber S, PopGen: population-based recruitment of patients and controls for the analysis of complex genotype-phenotype relationships. Community Genet. 2006;9:55–61. doi: 10.1159/000090694
18. Kroke A, Klipstein-Grobusch K, Voss S, Möseneder J, Thielecke F, Noack R, Boeing H. Validation of a self-administered food-frequency questionnaire administered in the European Prospective Investigation into Cancer and Nutrition (EPIC) Study: comparison of energy, protein, and macronutrient intakes estimated with the doubly labeled water, urinary nitrogen, and repeated 24-h dietary recall methods. Am J Clin Nutr. 2011;94:439–447. doi: 10.1093/ajcn/70.4.439
19. Bhagwat S, Haytowitz DB. USDA database for the flavonoid content of selected foods. Release 3.2 (November 2015). Nitrogen Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. https://doi.org/10.15482/USDA.ADC/1324665.
20. Bhagwat S, Haytowitz DB. USDA database for the proanthocyanidin content of selected foods. Release 2 (2015). Nitrogen Data Laboratory, Beltsville Human Nutrition Research Center, ARS, USDA. https://doi.org/10.15482/USDA.ADC/1324621.
21. Dehne LI, Klemm C, Henseler G, Herrmann-Kunze E. The German Food Code and Nutrient Data Base (BLS II.U). Eur J Epidemiol. 1999;15:355–359. doi: 10.1023/a:1007583427681
22. Koich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq illumina sequencing platform. *Appl Environ Microbiol.* 2013;79:5112–5120. doi: 10.1128/AEM.01043-13

23. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 2011;27:2957–2963. doi: 10.1093/bioinformatics/btr507

24. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics.* 2011;27:2194–2200. doi: 10.1093/bioinformatics/btr109

25. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences to the new bacterial taxonomy. *Appl Environ Microbiol.* 2007;73:5261–5267. doi: 10.1128/AEM.00062-07

26. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods.* 2013;10:996–998. doi: 10.1038/nmeth.2604

27. Ainsworth BE, Haskell WL, Herrmann SD, Meckes N, Bassett DR Jr, Tudor-Locke C, Greer JL, Vezina J, Whitt-Glover MC, Leon AS. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc.* 2011;43:1575–1581. doi: 10.1249/MSS.0b013e31821ece12

28. Wang J, Thingholm LB, Skieciwelc E, Rausch P, Kummén M, Hov JR, Degenhardt F, Heinsen FA, Rühlemann MC, Szymczak S, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet.* 2016;48:1396–1406. doi: 10.1038/ng.3695

29. Segata N, Izard J, Waldron L, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and exploration. *Genome Biol.* 2011;12:R60. doi: 10.1186/gb-2011-12-6-r60

30. Reboldi G, Gentile G, Angeli F, Ambrosio G, Mancia G, Verdecchia P. Effects of flavonoids in cardiovascular diseases. *J Hypertens.* 2011;29:1253–1269. doi: 10.1097/HJH.0b013e3283469976

31. Public Health England. 2014.

32. Reboldi G, Gentile G, Angeli F, Ambrosio G, Mancia G, Verdecchia P. Effects of flavonoids in cardiovascular diseases. *J Hypertens.* 2011;29:1253–1269. doi: 10.1097/HJH.0b013e3283469976

33. Jennings et al. Microbiome, Flavonoids, and Blood Pressure

34. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Le Roy CI, Wells PM, Si J, Raes J, Bell JT, Spector TD. Red wine consumption associated with increased gut microbiota composition and metabolic output using an in vitro coculture form. *Nutrients.* 2017;9. doi: 10.3390/nu9060533

35. Overall J, Bonney SA, Wilson M, Beeham A, Grace MH, Esposito D, Lila MA, Komarnytsky S. Metabolic effects of berries with structurally diverse anthocyanins. *J Nutr.* 2011;142:1575–1581. doi: 10.3945/jn.112.162743

36. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui G, Geng B, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome.* 2017;5:14. doi: 10.1186/s41396-018-0068-2

37. Willett W. *Nutritional Epidemiology.* Oxford University Press; 2012.

38. Carlsen MH, Karlsen A, Lillegaard IT, Gran JM, Drevon CA, Blomhoff R. Alterations of the gut microbiota in hypertension. *Front Cell Infect Microbiol.* 2017;7:381. doi: 10.3389/fcimb.2017.00381

39. Yugali T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 2011;27:2957–2963. doi: 10.1093/bioinformatics/btr507

40. Wang J, Thingholm LB, Skieciwelc E, Rausch P, Kummén M, Hov JR, Degenhardt F, Heinsen FA, Rühlemann MC, Szymczak S, et al. Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota. *Nat Genet.* 2016;48:1396–1406. doi: 10.1038/ng.3695

41. Segata N, Izard J, Waldron L, Miropolsky L, Garrett WS, Huttenhower C. Metagenomic biomarker discovery and exploration. *Genome Biol.* 2011;12:R60. doi: 10.1186/gb-2011-12-6-r60

42. Overall J, Bonney SA, Wilson M, Beeham A, Grace MH, Esposito D, Lila MA, Komarnytsky S. Metabolic effects of berries with structurally diverse anthocyanins. *J Nutr.* 2011;142:1575–1581. doi: 10.3945/jn.112.162743

43. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui G, Geng B, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome.* 2017;5:14. doi: 10.1186/s41396-018-0068-2

44. Le Roy CI, Wells PM, Si J, Raes J, Bell JT, Spector TD. Red wine consumption associated with increased gut microbiota composition and metabolic output using an in vitro coculture form. *Nutrients.* 2017;9. doi: 10.3390/nu9060533

45. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Le Roy CI, Wells PM, Si J, Raes J, Bell JT, Spector TD. Red wine consumption associated with increased gut microbiota composition and metabolic output using an in vitro coculture form. *Nutrients.* 2017;9. doi: 10.3390/nu9060533

46. Willett W. *Nutritional Epidemiology.* Oxford University Press; 2012.

47. Carlsen MH, Karlsen A, Lillegaard IT, Gran JM, Drevon CA, Blomhoff R. Alterations of the gut microbiota in hypertension. *Front Cell Infect Microbiol.* 2017;7:381. doi: 10.3389/fcimb.2017.00381

48. Yugali T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics.* 2011;27:2957–2963. doi: 10.1093/bioinformatics/btr507

49. Jennings et al. Microbiome, Flavonoids, and Blood Pressure

50. Willett W. *Nutritional Epidemiology.* Oxford University Press; 2012.