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Bioavailability of Various Polyphenols from a Diet Containing Moderate Amounts of Berries†

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Berries are a rich source of various polyphenols. The objective of this study was to investigate the bioavailability of polyphenols from berries. Middle-aged subjects (n = 72) consumed moderate amounts of berry or control products for 8 weeks in a randomized, placebo-controlled dietary intervention trial. Average intake of berries was 160 g/day (bilberries, lingonberries, black currants, and chokeberries). Plasma and urine polyphenols were analyzed by GC-MS and HPLC and berry polyphenols by HPLC. The total intake of polyphenols was 837 mg/day. Plasma quercetin, *p*-coumaric acid, 3-hydroxyphenylacetic acid, caffeic acid, protocatechuic acid, vanillic acid, homovanillic acid, and 3-(3-hydroxyphenyl)propionic acid increased significantly from the baseline in the berry group compared to the control group ($p < 0.05$). The urinary excretion of quercetin, *p*-coumaric acid, and 3-hydroxyphenylacetic acid increased significantly in the berry group compared to the control group ($p < 0.05$). In conclusion, a number of polyphenols are bioavailable from a diet containing moderate amounts of blue and red berries.

KEYWORDS: Berries; polyphenols; bioavailability; human; dietary intervention

INTRODUCTION

Berries are an excellent source of various polyphenols (*1*). Quantitatively, the most important polyphenols in berries are the anthocyanidins (e.g., cyanidin), proanthocyanidins, ellagitannins, flavonols (e.g., quercetin), phenolic acids (e.g., caffeic acid), and flavan-3-ols (e.g., (+)-catechin).

A wide range of biological activities have been reported for polyphenols in vitro, including antioxidant, anticarcinogenic, and anti-inflammatory activities. Furthermore, many animal studies have indicated that polyphenols and polyphenol-rich foods may be beneficial (*2*−*4*). For instance, red wine attenuates hypertension in rats (*5*), and administration of wine and grape juice inhibits platelet aggregation in several species (*6*, *7*). The limited number of human studies performed so far indicate that some of these bioactivities may translate into health effects in humans (*8*−*12*). For instance, consumption of polyphenol-rich foods or supplements may induce beneficial changes in pathways related to cardiovascular health (*13*, *14*).

The health effects of polyphenols are largely dependent upon their bioavailability. This is still poorly known, although there have been significant advances in the field during the past 15 years. It is well established that the molecular forms reaching the peripheral circulation and tissues differ from those present in foods (*15*−*19*). The metabolites may account for some of the physiological effects observed for polyphenols.

The most important determinants of polyphenol bioavailability are the chemical structure of the aglycones and the sugar side chains, to which the aglycones are bound in plants (*20*, *21*). Biotransformation in the gastrointestinal tract plays an essential role in polyphenol bioavailability (*22*) For example, many polyphenols bound to glucose are hydrolyzed and conjugated in the small intestine and absorbed from there. Many polyphenols (e.g., quercetin-3-rutinoside) reach the colon, where the sugar side chains are cleaved by enzymes excreted by the microflora (*23*). These enzymes also cleave the ring structure of many polyphenols, which yields small molecular weight phenolic acids. In addition, many of the compounds are glucuronidated and sulfated in the gastrointestinal tract and liver, and some of them are methylated as well (*24*).

Few studies have investigated the bioavailability of flavonoids from berries in humans. In these studies focus has usually been on anthocyanins (*25*−*30*) and in some studies on quercetin (*31*, *32*). In this study, we investigated the bioavailability of different types of polyphenols during long-term consumption of a mixture of red- and blue-colored berries in middle-aged subjects.

MATERIALS AND METHODS

Subjects. Female (n = 46) and male (n = 26) volunteers, aged 45−70 years, were recruited through municipal health centers in Turku and by local newspaper advertisements. To be included in the study, the subjects were to have at least one of the following: mild hypertension

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(140−159 mmHg systolic pressure or 90−99 mmHg diastolic pressure), elevated plasma glucose (6.1−8.0 mmol/L), elevated serum total cholesterol (6.5−8.0 mmol/L) or triglycerides (2.0−3.9 mmol/L), or low HDL cholesterol (< 1 mmol/L). The exclusion criteria were smoking, intestinal disorder (not lactose malabsorption), any regular medication (except hormone replacement therapy) or dietary supplements, obesity (BMI > 35 kg/m^2), and vegetarianism. The majority of subjects drank coffee regularly, on average 3 cups per day, and only five were nonconsumers (four of them in the berry group).

The volunteers completed a questionnaire concerning diet, physical exercise, and health. On the basis of the questionnaire, eligible subjects were invited to participate in the screening, and 72 applicable participants were accepted in the clinical intervention.

Study Design. The participants were randomly divided into treatment and control groups. The randomization was stratified by gender. All volunteers completed the 8 week study.

In addition to the screening, blood samples were collected at baseline and at 2, 5, and 8 weeks. The laboratory staff was blinded for the treatments and the study groups. During the experimental period the subjects were advised to adhere to their normal dietary habits and lifestyle. They were asked to cease any habitually consumed berry products during the intervention period. The participants were also asked to refrain from any dietary supplements for 1 month prior to and during the study.

The subjects kept a food diary in which they recorded consumption of the study products daily. They also kept records on matters concerning physical activity, alcohol consumption, illness, gastrointestinal symptoms, and possible use of medication. The subjects were asked to record any deviations from instructions regarding diet or berry consumption. Compliance was emphasized, and each subject was asked about it separately when they came in for the blood samplings.

Diets. The berry group consumed two portions of berry products daily (the recommendation was one portion after lunch and one portion after dinner). Every other day, whole bilberries (100 g) and a nectar containing 50 g of crushed lingonberries were consumed. On the alternate days, black currant−strawberry purée (100 g, containing 80% black currant) and cold-pressed chokeberry−raspberry juice (70 mL of juice, containing 80% chokeberry) were consumed. The control group consumed one of four different control products each day: 200 mL of sugar−water, 100 g of sweet semolina or rice porridge, or 40 g of jelly sweets. The aim was to control for the increased energy intake in the berry group. The subjects were advised not to substitute any food with study products. All products were packed in serving size packages and kept frozen at −20 °C for 1−4 months before they were consumed. The participants received written instructions for melting and consuming the products. All products consumed were recorded in study diaries.

Composition of Berry Products. Total phenolic acids in the berry products were determined by HPLC after alkaline and acid hydrolysis (*33*). Soluble flavan-3-ols (catechins and procyanidins, i.e., condensed tannins) were quantified by HPLC according to the degree of polymerization (*34*). Flavonols and flavanones were quantified as aglycones (*35*) and ellagitannins as ellagic acid (*36*) with HPLC after acid hydrolysis. Anthocyanins were quantified as cyanidin-3-glucoside using a modified method by Gao and Mazza (*37*).

Sample Collection. Blood samples were taken at baseline and at weeks 2, 5, and 8 after an overnight fast. The samples were taken by trained laboratory technicians from the antecubital vein with minimal stasis with 20 gauge needles into vacuum tubes containing either no anticoagulant, sodium citrate (3.2%), heparin, or K_3EDTA (0.18%). Serum and plasma were separated within 1 h of venipuncture and stored at −70 °C. Collection of 24 h urine was done twice during the study (the day before the baseline visit and the last day of the intervention).

Chemical Analyses. Plasma and urine quercetin was analyzed by HPLC with electrochemical detection after solid-phase extraction (*38*). Other plasma and urine polyphenols were analyzed by a modification of a previously published method, which is based on gas chomatographic−mass spectrometric detection after silylation of polyphenols with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) (*39*). Prior to analyses, polyphenol glucuronides and sulfates were hydrolyzed by enzymatic hydrolysis. Precision was acceptable for all polyphenols (within-run CV, 7−12%; between-run CV, 10−14%).

Assessment of Dietary Energy and Nutrients. The subjects completed a 3-day food diary in which consumption of all foods was recorded twice during the study, 1 week before the experimental period and at 8 weeks (2 working days and 1 weekend day). In the 3-day food diaries the subjects reported the estimated weights of food consumed. They used a booklet with color photographs showing different portions sizes of foods (*40*). Household measures and standard units were also used to describe amounts of foods consumed. The subjects received detailed instructions on how to fill out the diaries, and the nurses performed an interview to check the diaries. Food and nutrient intakes were calculated by using DIET 32 software (version 1.4.4; Aivo Finland Oy, Turku, Finland), which is based on Fineli, the Finnish food composition database (2005; National Public Health Institute, Helsinki, Finland).

Ethical Appraisal. The study protocol was approved by the Ethics Committee of the Hospital District of Southwest Finland, and all volunteers gave their written informed consent.

Statistical Analysis. Statistical analyses were performed by using SPSS for Windows (version 15.0; SPSS Inc., Chicago, IL). Normal distributions were tested with the Kolmogorow−Smirnov's test. Some of the variables deviated from normality, and they were therefore logarithmically transformed for statistical analyses. Data are presented as medians and 95% CIs; $p < 0.05$ was considered to be significant. Differences between treatment groups at baseline were tested with Student's *t* test for independent samples. One-factor analysis of covariance (ANCOVA) with the 8 week value as dependent variable or repeated-measures ANCOVA with time (weeks 2, 5, 8) as repeating factor, and baseline value as covariate in both cases, was used to compare the significance of the effects of berry products and of the control products. Urinary excretion was calculated by the multiplication of urinary polyphenol concentration and volume excreted in the 24 h sample.

RESULTS

Baseline Characteristics. The berry (n = 35) and control (n = 36) groups were similar with respect to baseline characteristics: age (57.5 vs 58.4 years), sex (M/F 23/12 vs 23/13), BMI (26.0 vs 26.4 kg/m^2 in the berry and control groups, respectively), as well as blood pressure and plasma lipid profile.

Diet. According to the 3-day food records, the baseline diet did not differ between the groups (*10*). The intervention had no effect on the calculated intake of energy, carbohydrates, protein, saturated and unsaturated fats, soluble fiber, folate, α-tocopherol, carotenoids, calcium, sodium, and potassium.

Compliance with the experimental diets was excellent according to the diaries (data not shown) and plasma vitamin C (*10*). The weekly consumption of the study products remained unchanged during the study period.

Intake of Berry Polyphenols. According to the chemical analyses of the berry products, the mean total daily intake of polyphenols from berries was 837 mg (**Table 1**). The mean intake of phenolic acids was 62.5 mg/day and that of quercetin, 4.9 mg/day.

Plasma Polyphenols. The plasma concentrations did not differ significantly between groups ($p > 0.05$) at the baseline for any polyphenols (**Figure 1**). The plasma concentrations of polyphenols increased significantly more in the berry group than in the control group for most of the analyzed polyphenols.

The median (95% CI) plasma quercetin concentrations in the berry and in the control groups were 18.6 (16.9, 37.4) and 27.7 (25.0, 48.2) nmol/L at baseline, respectively (**Figure 1A**). Plasma quercetin concentrations increased in the berry group compared to the control group ($p < 0.001$, ANCOVA with repeated measures). Within the berry group, the concentrations were 51−84% higher compared to the baseline at the different time points.

Plasma caffeic acid concentration in the berry group was 64.3 (62.3, 95.2) nmol/L and in the control group 74.7 (70.4, 117) nmol/L at baseline (**Figure 1B**). It increased significantly in the berry group

Table 1. Daily Intake of Polyphenols from the Berry Products Consumed during the Intervention[a]

	day 1		day 2		
berry product	bilberries	lingonberries	black currant−strawberry purée	chokeberry−raspberry juice	av intake[b]
consumption/day (g)	100	50	100	70	160
total polyphenols (mg)	716	276	266	416	837
flavonols[c] (mg)	6.4	4.4	4.4	0.74	8.0
quercetin	4.6	2.2	2.2	0.72	4.9
flavanones[c] (mg)	0	0	0	0.36	0.18
anthocyanins[d] (mg)	550	48	192	240	515
procyanidins[e] (mg)	109	214	40	117	240
ellagitannins[f] (mg)	0	0	12.2	10.6	11.4
phenolic acids[c] (mg)	50.4	10	17.6	47	62.5
caffeic acid	11.9	2.5	3.4	35	26.4
protocatechuic acid	9.1	2.3	2.5	6.9	10.4
p-coumaric acid	7.9	0.77	5.5	1.8	5.5
vanillic acid	5.3	0.42	0.45	0.55	3.4
ferulic acid	0.95	0.77	0.94	0.80	1.7
gallic acid	2.6	0	2.5	1.3	3.2

[a] Intake calculations for polyphenols based on chemical analyses of berry products. [b] Average intake of polyphenols from two alternative days. [c] As aglycones. [d] Anthocyanins as cyanidin-3-glucoside. [e] Total procyanidins. [f] Ellagitannins as ellagic acid.

compared to the control group ($p < 0.001$) and nearly doubled compared to the baseline (the average was 112.2 nmol/L between weeks 2 and 8) during berry consumption.

Plasma protocatechuic, *p*-coumaric, and vanillic acid concentrations increased in the berry group compared to the control group during berry consumption ($p < 0.001$, $p < 0.001$, and $p = 0.005$, respectively) (**Figure 1C−E**). The average increases were 21% for protocatechuic acid, 40% for *p*-coumaric acid, and 31% for vanillic acid.

Berry consumption also affected the plasma concentrations of other polyphenols. In the berry group 3-(3-hydroxyphenyl)-propionic acid (33HPPA) and 3-hydroxyphenylacetic acid (3-HPAA) increased compared to the control group ($p = 0.033$ and $p = 0.009$, respectively) (**Figure 1F,G**). At baseline, the median (95% CIs) concentrations of 33HPPA were 370 (289, 1886) nmol/L in the berry group and 648 (641, 2613) nmol/L in the control group. The median (95% CIs) concentrations of 3-HPAA were 180 (157, 288) and 235 (198, 353) nmol/L in the berry and control groups, respectively, at baseline. In the berry group the increase in 3-HPAA concentration was 22−31% depending on the time point.

Plasma 3-hydroxy-4-methoxyphenylacetic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) increased significantly more in the berry group compared to the control group ($p < 0.001$ and $p = 0.015$, respectively) (**Figure 1H,I**). In the berry group, the average increases were 7% for both homovanillic acid and 3,4-dihydroxyphenylacetic acid from the baseline values of 91 (79, 102) and 144 (131, 157) nmol/L, respectively.

The baseline concentrations of plasma ferulic acid and isoferulic acid were 38.0 (34.5, 85.7) and 80.6 (62.6, 92.6) nmol/L in the berry group and 61.0 (50.9, 76.6) and 70.4 (69.1, 110.7) nmol/L in the control group, respectively. Their concentrations did not change during the intervention. The same applied to the 3-(3,4-hydroxyphenyl)propionic acid (dihydrocaffeic acid) (data not shown).

Urinary Polyphenols. The 24 h urinary excretions did not differ significantly between groups for any polyphenol ($p > 0.05$ for all compounds) at baseline, except 3-(3-hydroxyphenyl)propionic acid ($p = 0.036$). The 24 h urinary excretion of quercetin increased significantly more in the berry group compared to the control group ($p = 0.004$) (**Table 2**). It doubled compared to the baseline in the berry group, whereas in the control group quercetin excretion appeared to decrease. In addition, the excretion of *p*-coumaric acid and 3-hydroxyphenylacetic acid increased in the berry group compared to the control group ($p = 0.001$ and $p < 0.000$, respectively). The increases were 18% for *p*-coumaric acid and 87% for 3-hydroxyphenylacetic acid in the berry group.

The median 24 h urinary excretions (all subjects) (95% CIs) of caffeic, protocatechuic, vanillic, and homovanillic acid were 43.3 (37.9, 51.9), 14.2 (6.6, 22.9), 59.8 (33.2, 87.3), and 32.9 (29.2, 36.1) μmol/day, respectively, at baseline. Berry consumption did not affect the urinary excretions of these compounds. The same applied for 3-(3-hydroxyphenyl)propionic acid, 3,4-dihydroxyphenylacetic acid, ferulic acid, and dihydrocaffeic acid.

Correlation between Plasma Concentration and Urinary Excretion of Polyphenols. There was a correlation between 8 week plasma concentration and urinary excretion for the three compounds, for which an increase was observed in both urine and plasma after berry consumption. The correlation was statistically significant for quercetin ($r = 0.41$, $p = 0.016$), *p*-coumaric acid ($r = 0.40$, $p = 0.018$), and 3-hydroxyphenylacetic acid ($r = 0.50$, $p = 0.003$). There was no correlation between plasma concentration and urinary excretion for the other polyphenols analyzed.

DISCUSSION

Our study shows that several polyphenols are bioavailable from a diet containing various wild and cultivated berries. Plasma concentrations and urinary excretion of several polyphenols increased significantly during the 8 week intervention.

During the intervention, the subjects in the berry group consumed products prepared from bilberries, lingonberries, black currants, and chokeberries and small amounts of red raspberries and strawberries. The other group received control products. The study products were consumed in addition to the subjects' habitual diets. Compliance was excellent, possibly because the amount of study products consumed daily was moderate and because the berry products were considered to be flavorful. The berry products were prepared from the most commonly consumed berries in the Nordic countries (except for chokeberries, which are more commonly consumed in some eastern European countries). The rationale for using a combination of different berries was to ensure a high intake of various polyphenols and to get good compliance. As seen in **Table 1**, the berries provided daily altogether 837 mg of various types of polyphenols. According to Ovaskainen et al. (*41*), the mean total intake of polyphenols

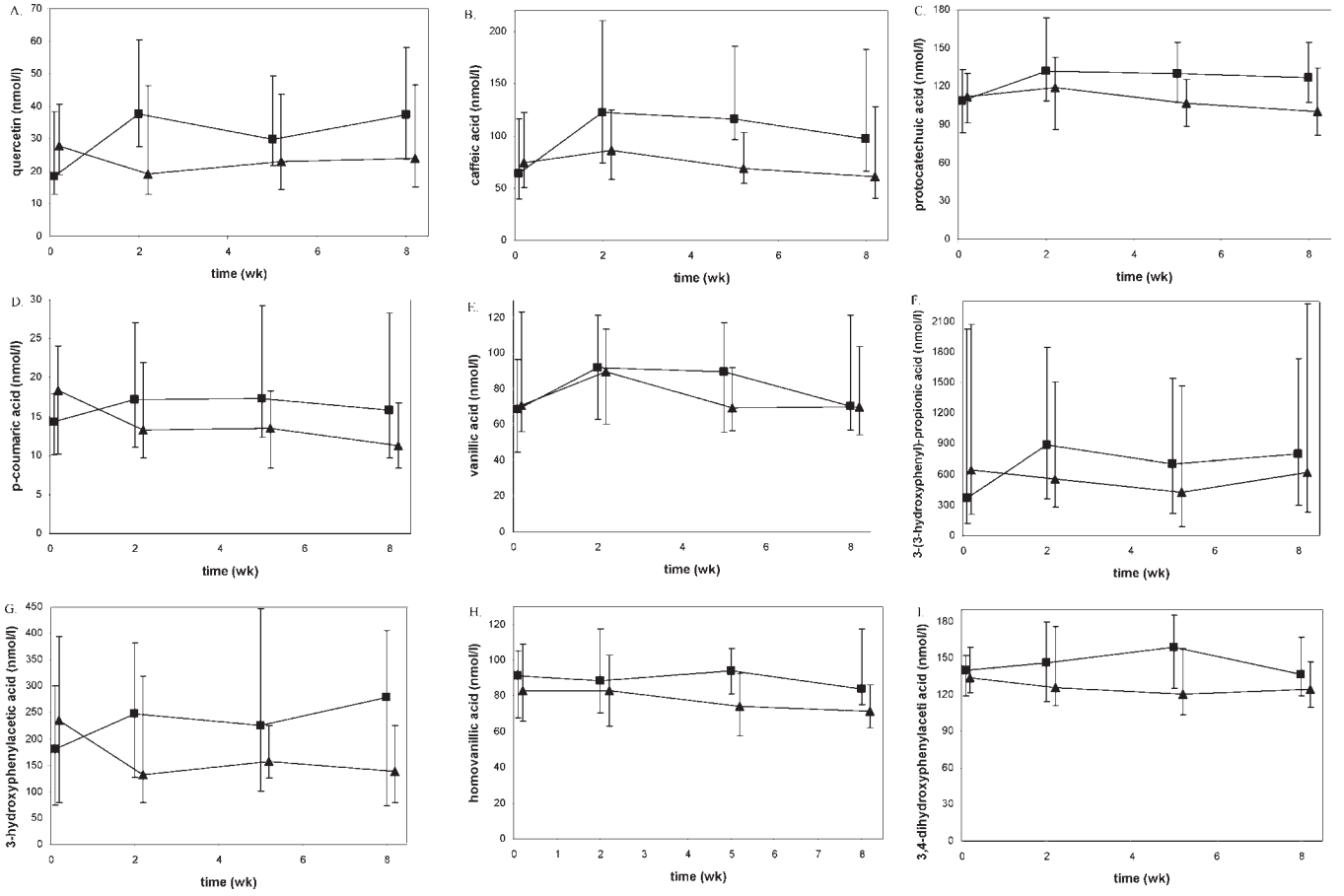

Figure 1. Plasma polyphenol concentrations (median ± 25th percentile) in middle-aged subjects consuming berries or control products as a part of their habitual diet for 8 weeks (■, berry group; ▲, control group): (**A**) quercetin; (**B**) caffeic acid; (**C**) protocatechuic acid; (**D**) *p*-coumaric acid; (**E**) vanillic acid; (**F**) 3-(3-hydroxyphenyl)propionic acid; (**G**) 3-hydroxyphenylacetic acid; (**H**) homovanillic acid (3-hydroxy-4-methoxyphenylacetic acid); (**I**) 3,4-dihydroxyphenylacetic acid (DOPAC).

in Finnish adults is 863 mg/day; thus, the intake of polyphenols was doubled in the berry group.

Daily berry consumption increased plasma quercetin concentrations and urinary excretions of quercetin in the berry group compared to the control group. The increase in plasma quercetin was similar to what was previously demonstrated by our group after consumption of berries (*31*). However, the increase was higher than what was previously reported after the consumption of black tea or red wine, but less than what was found when subjects consumed fried onions (*42*). Quercetin exists in berries as different glycosides (*1*), which are cleaved prior to absorption in different parts of the intestines. Part of the quercetin that reaches the colon may be metabolized to compounds such as 3,4-dihydroxyphenylacetic acid, 3-hydroxyphenylacetic acid (*23*), protocatechuic acid (*43*), or 3-(3-hydroxyphenyl)propionic acid (*44*). The plasma concentrations of all of these potential metabolites increased significantly in the berry group, as well as the urinary excretion of 3-hydroxyphenylacetic acid.

Plasma caffeic acid concentrations were clearly increased in the berry group compared to the control group. Previous studies have shown that this compound is bioavailable from coffee and fruits (*45*, *46*). Caffeic acid is mainly obtained as quinic acid esters of chlorogenic acids, but some caffeic acid glycosides are also present in the diet. The conjugates are cleaved by cytosolic esterases in the gut mucosa or by enzymes produced by the microflora during absorption. Caffeic acid can be metabolized to various compounds, including dihydrocaffeic acid, 3-(3-hydroxyphenyl)propionic acid (*44*), isoferulic acid, and ferulic acid (*47*). Of these potential caffeic acid metabolites, only plasma 3-(3-hydroxyphenyl)propionic acid increased significantly in this study. It is possible that coffee drinking may have confounded the results because of the high chlorogenic acid content of coffee.

p-Coumaric acid is usually found as glucose or coenzyme A esters in dietary plants. Protocatechuic acid is obtained from berries, but it is also formed by ring fission of quercetin and may be a metabolite of anthocyanins (*48*). It may be further converted to vanillic acid (*49*). Vanillic acid, on the other hand, may be formed from dihydroferulic acid or caffeic acid, as well as from anthocyanins. Homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid) has been shown to be metabolized from four different flavonoids (*49*). 3,4-Dihydroxyphenylacetic acid is an endogenous dopamine metabolite and may be formed from quercetin as well (*23*).

Overall, there were large interindividual variations in the plasma concentrations of all compounds. The largest interindividual variation was seen in the 3-(3-hydroxyphenyl)propionic acid concentrations. The variation could be caused by differences in the intestinal microflora, which is affected by diet and genetic differences (*50*, *51*). As polyphenols are mostly excreted during the 24 h following their ingestion (*22*), the excretion in 24 h urine should be directly related to the amounts ingested during the same and the previous day.

One limitation of the study was that we used fasting blood samples. Some polyphenols have short half-lives, and their concentrations may therefore no longer be elevated in fasting samples. Furthermore, we did not attempt to analyze all polyphenols

Table 2. Excretion of Polyphenols in 24 h Urine (Micromoles per Day) in the Berry and Control Groups at Baseline and after 8 Weeks of Intervention

		berry group		control group		
polyphenol	urine sample (24 h)	median	95% CI	median	95% CI	p^a
quercetin	week 0	147	(127, 388)	217	(119, 297)	
	week 8	342	(239, 497)	174	(90.8, 289)	0.004
p-coumaric acid	week 0	5.58	(5.40, 10.4)	7.66	(5.75, 14.6)	
	week 8	7.92	(6.77, 12.53)	5.56	(3.11, 9.32)	0.001
3-hydroxyphenylacetic acid	week 0	38.3	(27.4, 113)	54.1	(47.6, 187)	
	week 8	81.3	(47.7, 123)	33.6	(26.4, 76.5)	<0.001
caffeic acid	week 0	33.7	(26.2, 57.2)	52.5	(31.1, 56.8)	
	week 8	45.3	(31.1, 82.6)	44.9	(24.4, 66.6)	ns
protocatechuic acid	week 0	12.1	(10.7, 30.5)	16.8	(13.1, 34.1)	
	week 8	18.5	(16.3, 30.7)	16.8	(15.5, 39.8)	ns
vanillic acid	week 0	46.4	(42.8, 121)	66.4	(64.2, 106)	
	week 8	65.7	(62.0, 121)	68.0	(61.9, 99.3)	ns
homovanillic acid	week 0	34.6	(29.4, 41.8)	29.3	(22.1, 47.5)	
	week 8	35.8	(29.2, 53.0)	30.3	(27.4, 46.5)	ns
3-(3-hydroxyphenyl)propionic acid	week 0	40.5	(23.2, 97.3)	68.5	(40.0, 87.3)	
	week 8	57.6	(30.4, 90.3)	58.2	(24.6, 70.9)	ns
3,4-dihydroxyphenylacetic acid	week 0	55.6	(51.1, 86.2)	46.4	(44.4, 64.6)	
	week 8	59.4	(56.6, 85.6)	54.5	(51.0, 75)	ns
ferulic acid	week 0	43.0	(40.6, 67.5)	63.8	(51.8, 95.5)	
	week 8	52.6	(41.9, 93.4)	59.5	(51.3, 94.5)	ns
dihydrocaffeic acid	week 0	32.5	(11.1, 100)	55.1	(26.8, 111)	
	week 8	36.2	(13.7, 126)	37.3	(18.0, 100)	ns

[a] One-way ANOVA, with baseline value as covariate.

possibly obtained from berries. We concentrated on compounds that in our opinion are likely to be absorbed after berry consumption and ignored compounds that are present in berries in very small amounts or appear to have poor bioavailability due to instability or large molecular weight or are quickly excreted (e.g., proanthocyanidins, anthocyanins). It is, however, important to note that such compounds may also contribute to the health effects of berries. Our study also did not estimate absolute bioavailability of berry polyphenols. Confounding by polyphenols obtained from the subjects' habitual diet, as well as incomplete knowledge about the origin of several of the compounds that were analyzed, precluded the assessment of absolute bioavailability.

In conclusion, polyphenols are bioavailable from berries in humans. Plasma concentrations and urinary excretions of several polyphenols increased significantly. However, due to metabolism and differences in the degree of uptake of different polyphenols, the polyphenol profile in plasma and urine differ greatly from the polyphenol profile in berries. The potential health effects of these compounds, particularly the metabolites, should be explored further.

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