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Urinary Excretion of (Epi)catechins in Rats Fed Different Berries or Berry Products

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(Epi)catechins are associated with many health benefits in humans. However, their bioavailability, excretory pattern, and extent of conjugation in animals fed different sources or levels in the diet are not well documented. Two experiments were conducted to investigate the urinary excretion of (epi)catechins after feeding of different types of berries or different levels of the same berry source to rats. Experiment 1 investigated the effects of feeding a commercially available concentrated cranberry powder (CCP) at three different levels, 3.3, 6.6, and 33 g/kg of diet, whereas experiment 2 investigated the effect of feeding freeze-dried whole cranberry (CB), blueberry (BB), or black raspberry (BRB) powder at 50 g/kg of diet. Both experiments had an AIN-93-based control and a high-fructose diet (53−65% of the diet) to which was added three levels of CCP in experiment 1 and CB, BB, and BRB in experiment 2. (Epi)catechins were excreted as free and conjugated in both intact and methylated forms. Excretion of conjugated (epi)catechins was as high as 60% of the total consumed in some cases. A majority of both catechins and epicatechins excreted in the urine was in a methylated form. Excretion of epicatechins, including their methylated forms, ranged from 30 to 47% of the ingested amount, whereas that of catechins, including their methylated forms, ranged from 9 to 31%. Urinary excretion of (epi)catechins was dose dependent and increased with the amount of (epi)catechins present in the diet. On the basis of the excretory pattern of (epi)catechins in the urine, data suggested that the bioavailability of epicatechins may be higher than that of catechins and that (epi)catechins may be more available from blueberries compared to cranberries.

KEYWORDS: Catechins; epicatechins; procyanidins; polyphenols; cranberry; blueberry; black raspberry; urinary excretion

INTRODUCTION

Flavan-3-ols and other polyphenols are present in many fruits, including berries and their products. More commonly known as procyanidins or condensed tannins, flavan-3-ols have attracted much attention over the past several years for their potential health benefits against many chronic diseases (*1*−*4*). Flavan-3-ols consist of monomeric, oligomeric, or polymeric catechins and/or epicatechins. They are the major flavonoids present in the human diet and the subject of extensive research in relation to the prevention of chronic diseases.

Procyanidins in their monomeric forms, known as catechin or epicatechin (**Figure 1**), are absorbed from the intestinal tract (*5*,*6*). In rats, lower oligomers, up to pentamers, have been found to be absorbed from the intestine and detected in plasma (*7*, *8*), and dimers and trimers have been detected in urine (*9*). No reports on absorption and transport are available for oligomers beyond pentamers or the polymers. Given the limited bioavailability of even the lower oligomeric procyanidins, increased importance on human health has been given to the monomeric forms compared to their oligomeric or polymeric counterparts. Moreover, lack of commercially available standards beyond dimers has precluded the study of disease prevention mechanisms associated with these important dietary compounds.

Upon absorption, monomeric procyanidins are metabolized into different metabolites, which are found in plasma and may affect the overall biological effects associated with the intake of their parent compounds. Catechin and epicatechin can be transformed into *O*-methylated derivatives, primarily at the 3′- and 4′-positions (**Figure 1**), and also into glucuronide and sulfate conjugates (*5*, *10*, *11*). Previous studies have indicated that at least some of these derivatives retain antioxidant activity and radical scavenging activity at physiological conditions (*12*, *13*), suggesting their potential importance in the prevention of diseases. The objectives of the current study were to identify and quantify urinary excretion of (epi)catechins and their conjugates in rats fed different berries or their products. The diet used was a purified AIN-93G-based diet that contained no known sources of polyphenolic compounds. By using different types of berries or different levels of the same berry product in the diet, differences in the excretion pattern due to source or level in the diet could be established.

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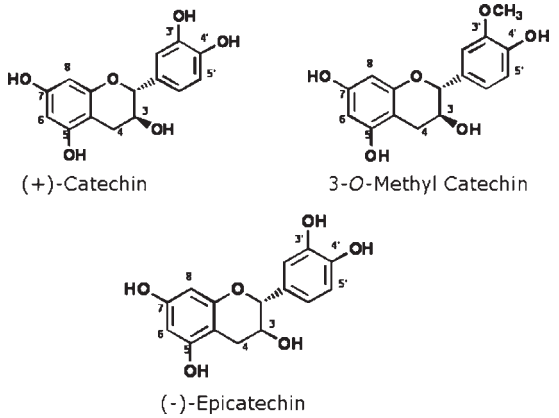

Figure 1. Structures of catechin, epicatechins, and 3′-*O*-methylcatechin.

MATERIALS AND METHODS

Chemicals. All chemicals used in the study were of HPLC grade or higher and were obtained either from Fisher Scientific (Hampton, NH), SigmaAldrich (St. Louis, MO), or SynerMed (Monterey Park, CA).

Experimental Procedure. Data were the result of two separate experiments investigating the positive health effects of feeding berries or their products associated with high-fructose feeding in growing Sprague−Dawley rats. Details about these experiments and most of the other results have been described elsewhere (*14*, *15*). Experiment 1 investigated the effects of feeding a concentrated cranberry powder (Cransulin, Decas Botanical Synergies, Carver, MA) at three different levels in the diet, 3.3, 6.6, and 33 g/kg of diet, whereas experiment 2 investigated three different whole berry powders (blueberry, black raspberry, and cranberry) fed at 50 g/kg of diet. Both experiments used an AIN-93-based control diet and a high-fructose diet (53−65% of the diet) to which was added the respective berries or berry products. Control and high-fructose diets in both experiments had no known source of (epi)catechins or any other polyphenols. Diets were isocaloric and isonitrogenous in all of the experiments, as were the fat and fiber contents. Purified diets were prepared by Research Diets Inc. (New Brunswick, NJ). Male Sprague−Dawley rats (Charles River Laboratories Intl. Inc., Wilmington, MA) born on the same day were balanced for their initial body weight across treatments in each experiment and assigned at random to their respective treatments. Animals were housed either two (experiment 1) per cage or individually (experiment 2) under controlled conditions of temperature and a 12 h day/night cycle. They were provided with ad libitum access to food and water, both of which were provided fresh on a weekly basis. Cages were changed weekly.

Whole cranberry (CB) and concentrated cranberry powders (CCP) were provided by Decas Botanical Synergies; whole blueberry and black raspberry freeze-dried powders were provided by the Wild Blueberry Association of North America (WBANA) and the Oregon Raspberry and Blackberry Commission (Corvallis, OR), respectively. The experimental animal protocols were approved by the Animal Care and Use Committee of the University of Arkansas for Medical Sciences, Little Rock, AR.

Standards. (+)-Catechin and (−)-epicatechin were purchased from Sigma Chemical Co. (St. Louis, MO). 3′-*O*-Methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin were synthesized by methylating (+)-catechin or (−)-epicatechin with methyl iodine according to a published method (*16*). They were purified on preparative HPLC, and the methylation site was confirmed with tandem mass spectrometry (*17*, *18*). Typical chromatograms generated by HPLC-ESI-MS-MS in multiple reaction monitoring (MRM) mode after 400 μg/mL addition of (epi)catechin standards are presented in **Figure 2**.

Analysis of (Epi)catechins in Urine. For analysis of free (epi)catechins, urine samples (200 μL) were purified by solid phase extraction (SPE) using a Waters Sep-Pak Vac RC (500 mg) C_{18} cartridge. The column was washed with 3 mL of 100% MeOH and then equilibrated with 3 mL of 0.2% formic acid in H_2O. Thawed urine (200 μL) was loaded onto the column and then washed with 3 mL of 0.2% formic acid in H_2O. The sample was eluted with 2 mL of 0.2% formic acid in MeOH, and the solvents were evaporated with nitrogen to < 1 mL. Samples were adjusted to 1 mL in volumetric flasks with 0.2% formic acid in MeOH. Samples were extracted in duplicate.

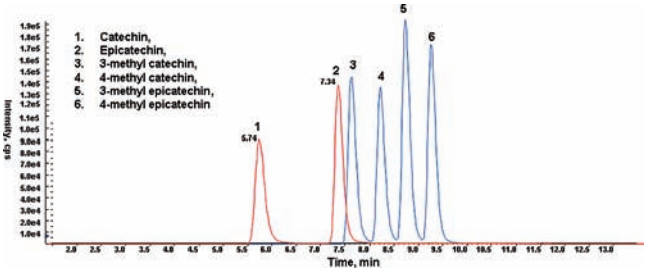

Figure 2. Typical chromatograms generated by HPLC-ESI-MS-MS in MRM mode after the injection of 400 μg/mL of (epi)catechin standards.

For analysis of total (epi)catechins, 200 μL of thawed urine was added to 1.8 mL of enzyme solution containing 1 M ammonium acetate, 0.5 mM ascorbic acid, and 110U sulfatase (*Helix pomatia*; Sigma S9626) in a screw-top glass tube. After incubation for 2 h at 37 °C, 100 μL of acetic acid was added to each tube. The same SPE procedure mentioned above was used for purifying total (epi)catechins; 200 μL of the enzyme-treated urine sample was added to the column in place of the 200 μL of urine.

(Epi)catechins in the urine were adjusted for urine dilution by determining creatinine concentration with a commercially available kit (Synermed, Westfield, IN). The (epi)catechin concentrations were divided by the analyzed creatinine concentration (expressed as mg/mL), and the final data were expressed as micrograms of (epi)catechins per milligram of creatinine. Although measurement of total urine volumes would have been desirable, we did not obtain a complete accurate set of volume data in experiment 1, so we chose this optional method for consistency in both experiments. It seems to be appropriate and useful, unless changes in dietary protein are involved in the study (*19*−*21*), which was not the case in our studies.

Extraction of (Epi)catechins from the Diet. For analysis of free forms, approximately 250−400 mg of sample was weighed directly into a glass culture tube with a threaded end. Eight milliliters of methanol/water (50:50; v/v, MeOH/H_2O) was added to each sample. Tubes were capped tightly and incubated for 2 h at 90 °C with vortexing every 30 min. Samples were cooled overnight at −20 °C and centrifuged at 4 °C for 15 min at 3600*g*. The supernatant was adjusted to 10 mL in a volumetric flask with 100% MeOH and aliquoted to HPLC vials after syringe filtration with a 0.22 μm filter. Samples were stored at −20 °C until analysis.

LC/ESI-MS Analysis. Analysis of (epi)catechins and their metabolites in urine was carried out on an Agilent 1100 HPLC system coupled with the 4000 Q TRAP mass spectrometer (Applied Biosystems, Foster City, CA). Separation was performed on a Phenomenex Synergi Max-RP column (150 × 3.00 mm, 4 μm) using a flow rate of 0.4 mL/min. The solvent consisted of (A) 0.2% (v/v) of formic acid in water and (B) methanol. The 12 min gradient was as follows: 0−3−6−10−12 min, 25−25−55−85−25% of B, followed by 6 min of re-equilibration of the column before the next run. A scan using MRM mode was performed. The mass spectrometer used an electrospray interface in positive ionization mode. Identification of individual components was based upon elution time and transition pairs, whereas quantification was based upon the peak areas of their specific transition pairs: m/z 291 > 139 for (+)-catechin and (−)-epicatechin; m/z 305 > 139 for 3′-*O*-methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin. The declustering potential (DP) was optimized individually with each standard. Other parameters for the mass spectrometer included the following: entrance potential (EP), 10 V; collision energy (CE), 23 V; collision cell exit potential (CXP), 10.0 V; curtain gas (CUR), 20 psi; source temperature, 450 °C; ion spray voltage (IS), 4500 V; 30 and 50 psi for ion source gas 1 (GS1) and ion source gas 2 (GS2), respectively. Catechins were quantified using their specific fragments: m/z 291.2 > 139.1 for catechin and epicatechin; m/z 305.2 > 139.1 for 3′-*O*-methylcatechin, 4′-*O*-methylcatechin, 3′-*O*-methylepicatechin, and 4′-*O*-methylepicatechin. Catechins were quantified against an external standard using quadratic curves. This method had a quantification limit of 0.15 ng injected on column and an intra-assay variation of 7%.

Table 1. (Epi)catechin Composition (Micrograms per Gram of Dry Weight) of Berries and Their Products Used in the Study

berry product	catechin	epicatechin	3-methylcatechin	4-methylcatechin	3-methylepicatechin	4-methylepicatechin
concentrated cranberry powder[a]	85.0	137.5	0.07	0.01	0.77	ND
cranberry powder[b]	36.7	77.6	0.24	ND	0.54	0.02
blueberry powder[b]	31.2	11.0	0.06	ND	0.13	0.02
black raspberry powder[b]	10.3[c]					

[a] Concentrated cranberry powder was included at 3.3, 6.6, and 33 g/kg of diet (experiment 1). Freeze-dried whole cranberry, blueberry, and black raspberry powder was included at 5% of the diet (experiment 2). [b] Total as monomeric procyanidins. No methylated forms were determined in black raspberry powder. [c] Represents total of catechin and epicatechin as HPLC conditions did not adequately separate catechin and epicatechin to quantitate separately.

Table 2. Catechin and Epicatechin Intake (Micrograms per Day) of Rats Fed Different Berries or Berry Products[a]

treatment	catechin	epicatechin	3-methylcatechin	4-methylcatechin	3-methylepicatechin	4-methylepicatechin
			Experiment 1			
HF + low CCP	6.0	9.7	0.05	traces	traces	
HF + medium CCP	11.7	18.9	0.11	0.001	0.01	
HF + high CCP	60.3	97.2	0.55	0.007	0.05	
			Experiment 2			
HF + CB	42.4	89.6	0.006		0.012	traces
HF + BB	37.4	13.2	0.001		0.003	traces
HF + BRB	13.3[b]					

[a] No intake values are provided for animals in control and high-fructose fed groups given no known source of (epi)catechins or other polyphenols in the diet. [b] Represents total of catechin and epicatechin as HPLC conditions did not adequately separate catechin and epicatechin to quantitate separately.

Statistical Analysis. Data are presented as mean ± standard error of means. Data were analyzed using one-way analysis of variance in SigmaPlot (Systat Software Inc., San Jose, CA).

RESULTS

(Epi)catechin Content in Berries and Berry Products Used in the Studies. Berries or their products contained catechin and epicatechin, known together as the monomeric procyanidins, primarily in their unmethylated forms (**Table 1**). Little or no methylated (epi)catechins were detected in the berries. The CCP contained the highest amounts of catechin and epicatechin, with epicatechin present in higher amounts than catechin. Whereas CCP or the CB powder contained higher amounts of epicatechin than catechin, BB contained higher amounts of catechin than epicatechin. The combined total amount of (epi)catechins present in BB was only slightly more than a third of that present in CB. Black raspberry contained a relatively small amount of monomeric procyanidins, which was about 25% of that present in BB (**Table 1**).

Catechin/Epicatechin Intake. Daily intakes of catechin and epicatechin by rats given different berries or berry products in both experiments are given in **Table 2**. Intakes of both compounds were consistent with their concentration in the diet with animals on a high CCP diet consuming the most in experiment 1. Of the three freeze-dried whole berry powders included in the diet in experiment 2, animals in the CB group had highest intakes of 42.4 and 89.6 μg/day of catechin and epicatechin, respectively. Given that BRB had small amounts of the two compounds, their intake in animals fed BRB was < 10% of that in animals fed CB in the diet and was close to that in animals fed the lowest level of CCP in the diet in experiment 1. Because methylated (epi)catechins were present in only small amounts in all of the diets, their intake was mostly in trace amounts in both experiments, with the exception of 3-methylcatechin in animals fed high CCP in experiment 1, which had an intake of 0.55 μg/day.

Catechin/Epicatechin Excretion: Experiment 1. Urinary excretion of (epi)catechins is presented in **Table 3**. Amounts of catechin and epicatechin excreted in all forms were highest in rats consuming the high CCP diet. Excretion of epicatechin in all forms (including methylated forms) was 3.74 μg/mg of creatinine, whereas catechin excretion in all forms, excluding the traces detected for 4-methycatechin, was 1.18 μg/mg of creatinine in the highest intake of CCP. Considerable amounts of epicatechins were also excreted in conjugated forms, with conjugated 3′-methylepicatechin and 4′-methylepicatechin reaching 72 and 80%, respectively. However, methylated catechins in animals fed high CCP in the diet were either not detected in the urine or present in only small amounts. Similarly, conjugated catechin in its intact unmethylated form was not excreted in animals fed high CCP in the diet even though it was found in the urine of animals fed low or medium CCP in the diet. Excretion of (epi)catechins methylated at the 3′-position was higher than that of the 4′-position. Quantities of 4-methylcatechin were quite low in all forms (free, total, and conjugated), and in some cases the amounts excreted were close to zero or not detected at all. Although there were no significant differences ($p > 0.05$) in the excretory patterns of (epi)catechins between the low and medium levels of CCP in the diet, an overall and consistent increase in the excretion of (epi)catechins in the urine was observed for animals fed the medium CCP compared to the low CCP level in the diet. Moreover, when urinary excretion of (epi)catechins was plotted against their intake, a linear increase of catechin and epicatechin excretion in the urine was observed with increasing level of CCP included in the diet (**Figure 3**). The linear relationship was true for both the total (free + conjugated forms of catechin and epicatechin separately (**Figure 3A**,**B**) and the combined total of epicatechin + catechin in the free (**Figure 3C**) and total form (free + conjugated forms of epicatechin + catechin, including their methylated versions, **Figure 3D**). Although the two control diets, control and HF, had no known source of (epi)catechins or other polyphenols, excretion of both compounds in small amounts was observed in the urine of rats fed these diets. Furthermore, both free and conjugated forms, including most in their methylated forms, were excreted in the urine in both diet groups.

Catechin/Epicatechin Excretion: Experiment 2. No (epi)catechins or their methylated forms were excreted in the urine of animals fed

Table 3. Urinary Excretion of (Epi)catechin (Mean ± SEM; Micrograms per Milligram of Creatinine) and Their Metabolites from Rats Fed a Purified Diet Containing 0, 3.3, 6.6, or 33 g of Concentrated Cranberry Powder (CP)/kg of Diet (Experiment 1)[a]

analyte	control	control + HF	HF + low CCP	HF + medium CCP	HF + high CCP	*p* value
free						
catechin	0.015 ± 0.005 c	0.008 ± 0.014 c	0.048 ± 0.010 b	0.060 ± 0.007 b	0.382 ± 0.088 a	<0.001
epicatechin	0.020 ± 0.008 c	0.012 ± 0.025 c	0.076 ± 0.020 b	0.098 ± 0.017 b	0.266 ± 0.024 a	0.04
3-methylcatechin	0.017 ± 0.005 b	0.041 ± 0.026 b	0.070 ± 0.010 b	0.127 ± 0.022 b	0.592 ± 0.103 a	<0.001
4-methylcatechin	ND	ND	traces	traces	0.023	
3-methylepicatechin	0.019 ± 0.009 b	0.021 ± 0.034 b	0.240 ± 0.056 b	0.224 ± 0.068 b	0.783 ± 0.175 a	0.03
4-methylepicatechin	0.002 ± 0.001 b	0.009 ± 0.002 b	0.013 ± 0.003 ab	0.012 ± 0.004 ab	0.034 ± 0.010 a	0.041
total						
catechin	0.018 ± 0.007 c	0.036 ± 0.022 bc	0.069 ± 0.019 b	0.083 ± 0.008 b	0.244 ± 0.038 a	<0.001
epicatechin	0.026 ± 0.009 c	0.079 ± 0.034 c	0.139 ± 0.036 b	0.161 ± 0.041 b	0.749 ± 0.096 a	0.008
3-methylcatechin	0.042 ± 0.011 c	0.051 ± 0.042 c	0.165 ± 0.029 b	0.207 ± 0.038 b	0.799 ± 0.080 a	<0.001
4-methylcatechin	ND	ND	traces	traces	0.023	
3-methylepicatechin	0.037 ± 0.010 b	0.102 ± 0.036 b	0.410 ± 0.070 b	0.687 ± 0.082 b	2.810 ± 0.620 a	<0.001
4-methylepicatechin	0.003 ± 0.001 b	0.017 ± 0.004 b	0.028 ± 0.005 b	0.045 ± 0.005 b	0.178 ± 0.041 a	<0.001
conjugated						
catechin	0.003 ± 0.005 b	0.002 ± 0.01 b	0.022 ± 0.010 a	0.023 ± 0.009 a	ND c	<0.001
epicatechin	0.005 ± 0.007 c	0.003 ± 0.013 c	0.063 ± 0.018 b	0.062 ± 0.027 b	0.483 ± 0.193 a	0.005
3-methylcatechin	0.025 ± 0.007	0.031 ± 0.026	0.096 ± 0.022	0.081 ± 0.027	0.017 ± 0.103	NS
4-methylcatechin	ND	ND	ND	ND	ND	
3-methylepicatechin	0.021 ± 0.005 c	0.007 ± 0.012 c	0.171 ± 0.019 b	0.463 ± 0.036 b	2.030 ± 0.250 a	<0.001
4-methylepicatechin	0.002 ± 0.001 b	0.009 ± 0.002 b	0.015 ± 0.002 b	0.033 ± 0.003 b	0.143 ± 0.288 a	<0.001

[a] Data presented as mean ± SEM. Means without a common letter differ significantly, and the level of significance is present in the last column. ND, not detected. No total 3-methylepicatechin was detected even though its free form was detected. Therefore, free form is also presented as total. Conjugated forms were determined by subtracting free forms from their total.

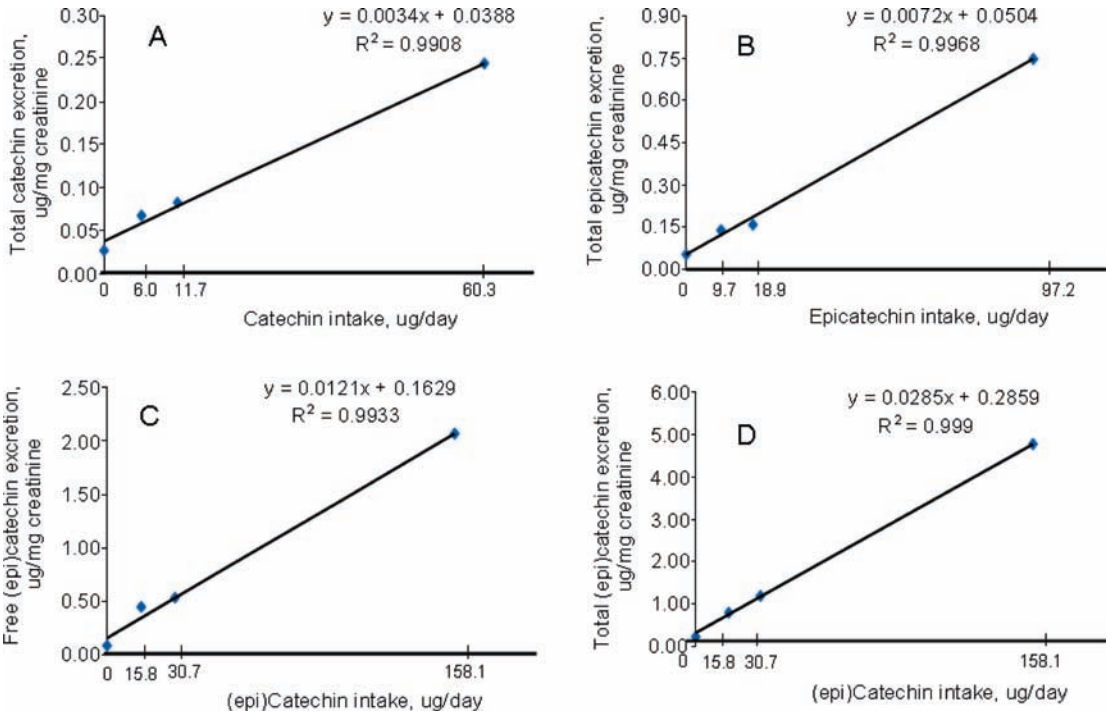

Figure 3. Correlation of catechin and epicatechin excretion with their dietary intake (experiment 1). Excretion at 0 levels of CCP in the diet was the average of the two treatments that had no known source of (epi)catechins or other polyphenols. The top two panels provide the relationship for total (free plus conjugated) catechin (**A**) and epicatechin (**B**) in intact unmethylated forms, whereas the bottom two panels provide the relationship for the combined total (epicatechin + catechin) of free and conjugated (**C**) and total (conjugated plus free) epicatechin + catechin (**D**), including the methylated forms.

the two polyphenol-free diets (**Table 4**), which was different from what was observed in experiment 1. In animals fed the three berries, there were significant differences ($p < 0.05$) caused by the berries in the excretion of methylated or unmethylated (epi)catechins in any form, except differences were not significant for epicatechin and 3′- or 4′-methylcatechin in conjugated form. Overall, excretion of free and total forms was highest in the urine of those fed CB, whereas the least was excreted in animals fed BRB with BB in between. Methylated (epi)catechins were more prevalent than their unmethylated versions irrespective of the type of berries included in the diet. Whereas total epicatechin methylated at the 3- position was excreted in greater amounts than its catechin counterparts in animals fed CB and BRB, total 3′-methylcatechin was excreted in the highest amounts when BB was included in the diet. When excretion of (epi)catechins as percent of the amount ingested through the diet was calculated (**Table 5**), catechins were excreted

Table 4. Urinary Excretion of (Epi)catechin (Mean ± SEM; Micrograms per Milligram of Creatinine) and Their Metabolites from Rats Fed a Control Diet, High-Fructose Diet (HF), and High-Fructose Diets with Cranberry (HF + CB), Blueberry (HF + BB), and Black Raspberry (HF + BRB) (Experiment 2)[a]

analyte	control	HF	HF + CB	HF + BB	HF + BRB	*p* value
free						
catechin	ND	ND	0.415 ± 0.181 a	0.157 ± 0.031 ab	0.042 ± 0.017 b	0.007
epicatechin	ND	ND	0.233 ± 0.102 a	0.047 ± 0.018 b	0.070 ± 0.042 ab	0.017
3-methylcatechin	ND	ND	1.12 ± 0.50 a	0.722 ± 0.145 ab	0.128 ± 0.038 b	0.006
4-methylcatechin	ND	ND	0.057 ± 0.020 a	0.017 ± 0.004 b	0.011 ± 0.002 b	<0.001
3-methylepicatechin	ND	ND	2.04 ± 0.80 a	0.506 ± 0.190 b	0.491 ± 0.254 b	0.005
4-methylepicatechin	ND	ND	0.067 ± 0.026	0.021 ± 0.008	0.028 ± 0.016	NS
total						
catechin	ND	ND	0.088 ± 0.019 b	0.213 ± 0.047 a	0.019 ± 0.005 b	<0.001
epicatechin	ND	ND	0.311 ± 0.080 a	0.082 ± 0.030 b	0.096 ± 0.043 b	<0.001
3-methylcatechin	ND	ND	0.356 ± 0.060 b	1.16 ± 0.20 a	0.085 ± 0.011 b	<0.001
4-methylcatechin	ND	ND	0.011 ± 0.003 a	0.008 ± 0.001 ab	0.003 ± 0.001 b	<0.001
3-methylepicatechin	ND	ND	2.85 ± 0.45 a	0.728 ± 0.144 b	0.650 ± 0.205 b	<0.001
4-methylepicatechin	ND	ND	0.110 ± 0.017 a	0.037 ± 0.008 b	0.050 ± 0.017 b	<0.001
conjugated						
catechin	ND	ND	ND b	0.056 ± 0.025 a	ND b	0.010
epicatechin	ND	ND	0.078 ± 0.097	0.034 ± 0.030	0.026 ± 0.012	NS
3-methylcatechin	ND	ND	ND b	0.439 ± 0.111 a	ND b	0.009
4-methylcatechin	ND	ND	ND	ND	ND	
3-methylepicatechin	ND	ND	0.802 ± 0.724	0.223 ± 0.162	0.159 ± 0.067	NS
4-methylepicatechin	ND	ND	0.042 ± 0.021	0.015 ± 0.006	0.022 ± 0.003	NS

[a] HF, high fructose (60% fructose); ND, not detected. Diets contained 5% whole cranberry, blueberry, and black raspberry powder on dry weight basis. Means without a common letter differ ($p < 0.05$) and represent six observations per treatment. Conjugated forms were determined by subtracting free forms from their total.

Table 5. Excretion of Total (Epi)catechins, Including Their Methylated Versions, in the Urine of Rats as Percent Ingested through the Diet[a]

	experiment 1[b]			experiment 2		
analyte	HF + low CCP	HF + medium CCP	HF + high CCP	CB	BB	BRB
catechin	31.3	21.0	16.8	9.01	28.0	
epicatechin	46.2	36.7	29.9	29.0	46.8	48.1[c]

[a] Values include 3- and 4-methyl(epi)catechins excreted in the urine. [b] Estimates were based on the average urine volume and creatinine excretion in experiment 2 because accurate urine volumes were not available in experiment 1. [c] Includes both catechins and epicatechins.

in smaller amounts in animals fed CB and BB at 9 and 28.0%, respectively. However, epicatechins were excreted in higher amounts in animals fed BB than in animals fed CB. In both cases, animals fed BB excreted a larger proportion of the amount ingested than the animals fed CB. With BRB, a combined total of 48% of the amount ingested was excreted through the urine (**Table 5**). Depending on the type of berries included in the diet, a considerable amount of some of the (epi)catechins were also found to be excreted in the conjugated form. For instance, 25−40% of the total unmethylated epicatechin excreted in the urine was in its conjugated form. Excretion of conjugated 3′- and 4′-methylated epicatechins was also in a similar range of 28−44% of the total. However, excretion of conjugated intact or methylated catechins was detected only in animals fed BB in the diet, but not with either CB or BRB in the diet. When the excretion of sum of the amounts of all total and free forms of (epi)catechins, including their methylated versions, was plotted against the total (epi)catechin content in the berries, a linear relationship was observed for both free and total forms (**Figure 4**). The correlation coefficient was similar in both cases.

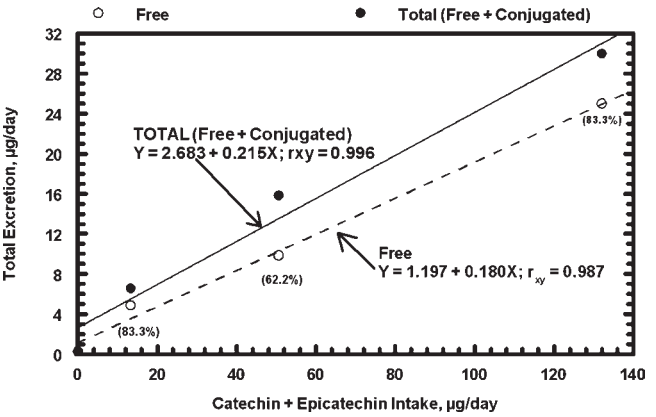

Figure 4. Correlation of free and total (epicatechin + catechin) excretion with the level of total epicatechin + catechin intake from the freeze-dried whole berry powders (experiment 2). Both free and total (epi)catechin excretions included their methylated forms. The three levels of total intake of epicatechin + catechin of 13.0, 50.6, and 132.1 μg/day correspond to their intake from black raspberry, blueberry, and cranberry freeze-dried powders, respectively. Numbers in parentheses represent the percentage of the free form (unconjugated) to the total (free + conjugated forms).

DISCUSSION

(Epi)catechins are monomeric procyanidins that are often linked with many of the positive health effects associated with the consumption of green tea, red wine, cocoa products, berry fruits, and many other plant products that are rich in polyphenols. After absorption from the intestine, they can be excreted through the urine either intact or in methylated or conjugated forms. In these studies we report the effect of feeding different berry powders, cranberry, blueberry, and black raspberry, or different levels of a commercially available concentrated cranberry powder that has relatively high levels of procyanidins, including (epi)catechins, on the urinary excretion pattern and conjugation of (epi)catechins in growing male Sprague−Dawley rats. This is part of a series of studies investigating the health effects, metabolism, and extent of conjugation of many plant polyphenols, including procyanidins, anthocyanins, phenolic acids, (epi)catechins, and their metabolites. These experiments were conducted in rats fed diets containing high levels of fructose. We would not expect this diet to greatly alter the absorption and metabolism of catechins that we studied compared to a diet with starch in place of fructose, but we cannot conclude

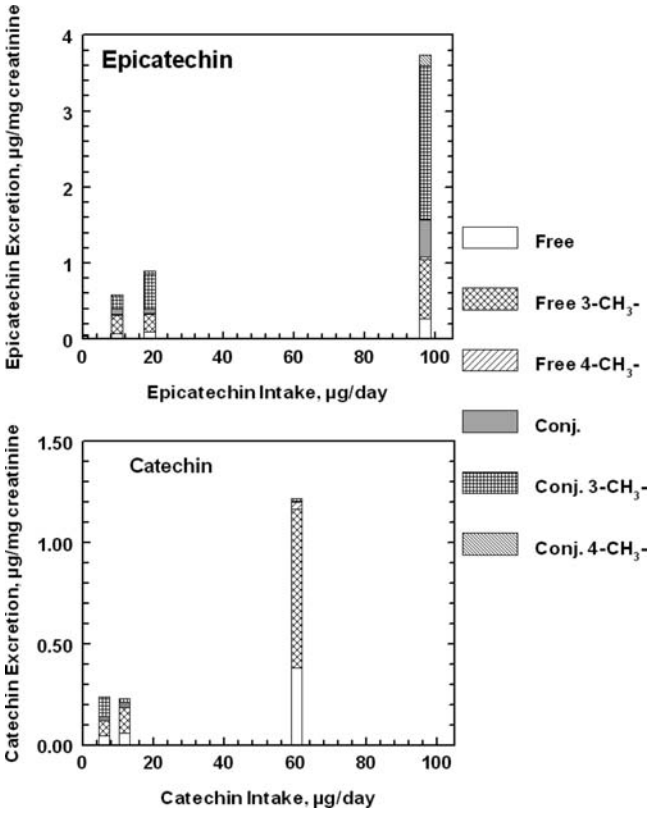

Figure 5. Epicatechin and catechin excretion as free and conjugated forms and as methylated forms in the 3′- or 4′-position at different dietary intakes (experiment 1).

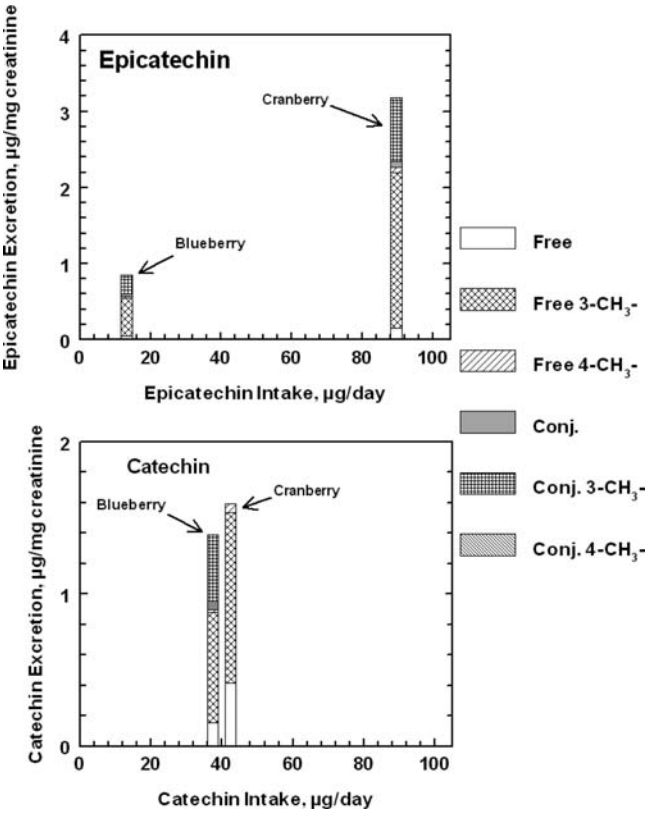

Figure 6. Epicatechin and catechin excretion as free and conjugated forms and as methylated forms in the 3′- or 4′-position in rats fed blueberry or cranberry powder (experiment 2). Data on black raspberry are not presented as separate estimates of intake were not obtained for epicatechin and catechin.

this for certain. We did not observe major changes in the control diets in the metabolism of phenolic acids (*15*).

Previous studies have shown the excretion of (epi)catechins in both free and conjugated forms in urine as well as bile (*11*,*18*,*22*,*23*). In the current study, although some (epi)catechins were excreted only in their free forms (e.g., catechins when fed from CB or BRB in the diet) (**Figures 5** and **6**), >60% of the total amount was excreted in conjugated forms in certain cases (e.g., intact epicatechin in animals fed high CCP in experiment 1). Epicatechin was shown to be partially methylated and 100% conjugated during absorption in an in situ perfusion model of small intestinal absorption in the rat (*23*). Furthermore, methylated versions of (epi)catechins were excreted in higher amounts than intact (epi)catechins (**Figure 5** and **6**). Such changes may account for some of the differences in their biological activity or health effects observed with the consumption of various amounts or sources of (epi)catechins as well as the possibility of a different mode of action if these methylated (epi)catechins were to retain their biological activity, as has been suggested in the literature (*12*, *13*, *24*). However, contrasting influences of in vivo glucuronidation and methylation on the bioactivity of epicatechin have also been observed (*25*).

Of the amounts ingested through the diet, epicatechins, including their methylated forms, were excreted in the urine in higher proportions than catechins irrespective of the type of berry or level of CCP included in the diet (**Table 5**). In experiment 1, epicatechins, including the methylated ones, were excreted in larger amounts per milligram of creatinine than catechins and their methylated forms as a percent of intake. Previous results with cocoa have demonstrated that absorption of catechin is much less bioavailable than epicatechin when both are consumed together in a cocoa beverage (*26*) and (−)-catechin bioavailability is significantly reduced compared to (+)-catechin (*27*). In other foods besides cocoa, catechin bioavailability is also lower than that of epicatechin but not of the magnitude observed with cocoa consumption (*28*, *29*).

On the basis of average urine volumes and creatinine excretion in experiment 2, urinary excretion as a percent of intake was 46.2, 36.7, and 29.9% for epicatechins and 31.3, 21.0, and 16.8% for catechins in animals fed low, medium, and high CCP, respectively (**Table 5**). Similarly, in experiment 2, excretion of catechins and epicatechins was proportionate to intake and was lower at 9 and 29% for animals fed CB than those fed BB at 28 and 47%, respectively. These results indicated that epicatechins were more bioavailable than catechins from CB and BB in agreement with previous data (*28*, *29*). Also, earlier data suggested that procyanidin dimers B2 and B5 were cleaved into constituent monomeric units while transferring across the small intestine and that epicatechin was the primary bioavailable molecule (*30*). Moreover, CB contains considerably higher amounts of procyanidin dimers that may have been broken down into constituent monomeric epicatechin units similar to what has been observed with cocoa powder (*31*), thus contributing to the increased epicatechin excretion relative to dietary intake of monomeric forms.

Urinary excretion of (epi)catechins appears to vary considerably. For instance, epicatechin excretion was 55% of the total amount administered orally, whereas that of catechin was 30% (*28*) in rats. Similarly, excretion of catechin and epicatechin after 24 h of consumption of grape seed extracts was 27 and 36%, respectively (*9*). However, in two separate studies in humans with or without ileostomy, a combined total of only 27−28% of the (epi)catechins ingested in the form of green tea was excreted in the

urine (*32*, *33*). In another study, excretion of catechins in humans after red wine consumption was <10% of the amount ingested (*34*). A combined total of 48% of (epi)catechin ingested was excreted in the urine of rats fed BRB in the current study. Although data on (epi)catechin content of BRB are lacking in the literature and we did not analyze them separately in the current study, their excretion of >48% of the amount ingested suggested that BRB may be high in epicatechin content. Urinary excretion of catechins in the current study was within the lower range that has been observed previously.

As shown in **Figures 3−5**, urinary excretion of (epi)catechins was dependent on the amount ingested through the diet, with a stronger correlation of the free than total form excreted in the urine. Moreover, the relationship was linear irrespective of the type of the berry included in the diet. These results may indicate that the bioavailability of (epi)catechins is not affected by the source. Indeed, the bioavailability of epicatechins was similar in rats given cocoa powder or purified epicatechins (*35*). An important aspect of the current study was that the amounts of berries or berry products included in the diet and, thereby, the ingested amounts of (epi)catechins were within normal and practical ranges of human consumption, particularly with the two lowest doses of CCP, which if extrapolated on a metabolic body weight basis ($wt^{0.75}$) from the rat to the human would equate to ∼5−12 g/day.

In conclusion, the proportion of ingested epicatechins excreted in the urine was higher than catechins irrespective of the type of berries or level of berry product included in the diet. A majority of both catechins and epicatechins excreted in the urine was in methylated forms, primarily at the 3′-position. The extent of conjugation of cranberry epicatechin appeared to be greater when the concentrated cranberry powder was fed (67.6% of total) compared to the whole cranberry powder (28.2% of total) (**Figure 5** vs **6**). Urinary excretion of (epi)catechins was dose dependent and increased with the amount present in the diet. Given that epicatechins were excreted in higher amounts than catechins in the urine, their bioavailability may be higher than that of catechins.

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