Urinary Excretion of Phenolic Acids in Rats Fed Cranberry[†]

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Dietary flavonoids can be converted into phenolic acids by colonic microflora. Phenolic acids can then be absorbed into the circulation and may contribute to the health-promoting effects of the parent compounds. Phenolic acids can be further metabolized in other tissues via methylation and conjugation with glucuronide or sulfate. The objectives of this study were to identify and quantify the urinary excretion of 19 phenolic acids and their conjugates in rats fed three levels of a concentrated cranberry powder (3.3, 6.6, and 33 mg/kg of diet). The basic diet used was AIN93G diet containing very low amounts of any polyphenolic compounds. Of the phenolic acids studied, the amounts excreted varied by 4 orders of magnitude, with hippuric acid being excreted in the highest quantities. Amounts of 4-hydroxyphenylacetic acid (4HPAA), 3-hydroxyphenylacetic acid (3HPAA), 3-hydroxyphenylpropionic acid (3HPPA), and 4-hydroxycinnamic acid (4HCA) excreted were in the range of 18−33 μg/mg creatinine in animals fed the highest level of cranberry powder, whereas phenylacetic acid (PAA), gallic acid (GA), 3,4-dihydroxyphenylacetic acid (34HPAA), 3,4-dihydroxybenzoic acid (34HBA), 3,4-dihydroxycinnamic acid (34HCA), and 4-hydroxy-3-methoxycinnamic acid (FA) were excreted in the urine in concentrations of 0.1−2 μg/mg creatinine. As the amount of cranberry in the diet was increased, the amount of 4HPAA excreted decreased but the percentage of conjugated 4HPAA excreted increased (from 57 to 91%). For other phenolic acids analyzed, the percentage excreted in the conjugated form was approximately constant across levels of cranberry in the diet and ranged from 65 to 100% for the individual phenolic acids. Studies of bioactivity and health effects need to consider more than just the compound(s) in the food, because they can be metabolized to other lower molecular weight compounds, which in turn may also be methylated or conjugated in some form that may affect the perceived health effects.

KEYWORDS: Phenolics; phenolic acid conjugates; cranberry; urine; excretion; hippuric acid

INTRODUCTION

Foods of plant origin contain a large number of phytochemicals that may positively affect human health. Many dietary flavonoids are poorly absorbed from the gastrointestinal tract, but colonic bacteria can convert flavonoids into simple phenolic acids (PAs), which can be absorbed into the circulation and may contribute to health-promoting effects. However, the extent of absorption of many of these PAs is not known. The PAs and other metabolites that are formed by colonic microflora and in host tissues are excreted in the urine (*1, 2*). The parent flavonoids in the diet are often deglycosylated before absorption and are often conjugated by methylation, glucuronidation, or sulfation during the absorption process (*3−8*). However, little is known about the process of PA conjugation. Although some studies have focused on the free PAs (*8*), some studies have analyzed total PA following in vitro incubation with glucuronidase and sulfatase, which releases the parent free PA (*5, 7, 9*). Only recently have studies begun to focus on possible conjugated PA metabolites (*4, 10*).

Various PAs have been shown to have health effects. Protocatechuic acid (3,4-dihydroxybenzoic acid, 34HBA) is a natural phenolic compound found in many edible and medicinal plants and is a major metabolite of cyanidin-3-glycosides (*11*), which has been shown to have numerous health effects (*11−15*). 3,4-Dihydroxyphenylacetic acid (34HPAA) also exhibits antiproliferative activity in prostate and colon cancer cells, whereas other PAs were ineffective (*16*). In any study of the mechanisms of action of PAs on cellular function, it is important to know the form of the PA that is presented to the cell. Cellular responses may differ greatly with a conjugated PA compared to the free PA.

The objectives of the current study were to identify and quantify urinary excretion of PAs and their conjugates in rats fed three levels of a concentrated cranberry powder (CP). The diet used was a purified AIN-93G-based diet, which contained very limited amounts of any polyphenolic compounds. By using three different levels of cranberry polyphenolics in the diet, PAs produced as a result of CP intake could be identified if excretion of the PA displays a dose−response relationship.

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Table 1. Composition (Grams) of Diets Fed to Rats

ingredient	control	high fructose (HF)	HF + low CP[a]	HF + medium CP[a]	HF + high CP[a]
casein, 80 mesh	200	200	200	200	200
L-cystine	3	3	3	3	3
corn starch	629	0	0	0	0
maltodextrin 10	0	99.5	96.2	92.9	67.5
fructose	0	530	530	530	530
cellulose, BW200	75	50	50	50	49
corn oil	70	70	70	70	70
t-BHQ	0.014	0.014	0.014	0.014	0.014
mineral mix AIN-93G-MX	35	35	35	35	35
vitamin mix AIN-93-VX	10	10	10	10	10
choline bitartrate	2.5	2.5	2.5	2.5	2.5
CP[a]	0	0	3.3	6.6	33

[a] CP is a concentrated dried powder of cranberry prepared by Decas Botanicals Inc. (Carver, MA).

Table 2. Phenolic Acids Analyzed in Urine with Common Name, Abbreviation, MRM Transition Ion Pair Used for Quantitation, and Retention Time on Chromatographic Column

phenolic acid	common name	abbreviation	MW	ion pair	t_R (min)
phenylacetic acid		PAA	136	135.0/74.9	2
4-hydroxybenzoic acid		4HBA	138	137.4/93.1	8
3-hydroxybenzoic acid		3HBA	138	137.4/93.1	9
3,4-dihydroxybenzoic acid	protocatechuic acid	34HBA	154	152.5/109.2	5.4
3,4,5-trihydroxybenzoic acid	gallic acid	GA	170	169.2/124.9	3
2,5-dihydroxybenzoic acid	gentisic acid	25HBA	154	153.3/107.9	8.5
3-methoxy-4-hydroxybenzoic acid	vanillic acid	VA	168	166.7/122.7	8.4
3,4-dihydroxyphenylacetic acid	homoprotocatechuic acid	34HPAA	168	166.7/122.7	5.3
1,3,4,5-tetrahydroxycyclohexanecarboxylic acid 3-(3,4-dihydroxycinnamate)	chlorogenic acid	CGA	354	353.0/191.0	7
3-methoxy-4-hydroxyphenylacetic acid	homovanillic acid	HVA	182	180.8/136.7	7.2
3-hydroxyphenylacetic acid		3HPAA	152	150.7/106.9	8.3
4-hydroxyphenylacetic acid		4HPAA	152	150.7/106.9	7.5
3,4-dihydroxycinnamic acid	caffeic acid	34HCA	180	179.0/134.7	8.5
3-hydroxyphenylpropionic acid	phloretic acid	3HPPA	166	164.8/120.9	9.7
4-hydroxyphenylpropionic acid	hydrocinnamic acid	4HPPA	166	164.8/120.9	10.6
4-hydroxycinnamic acid	*p*-coumaric acid	4HCA	164	162.7/118.8	9.9
3-hydroxycinnamic acid		3HCA	164	162.7/118.8	10.8
4-hydroxy-3-methoxycinnamic acid	ferulic acid	FA	194	192.8/133.8	10
benzoylaminoacetic acid	hippuric acid	HA	179	178.3/134.0	7.5

MATERIALS AND METHODS

Animals and Diet. The protocol was approved by the Animal Care and Use Committee of University of Arkansas for Medical Sciences. Male Sprague−Dawley rats (175 ± 8.4 g; Charles River Laboratories, Wilmington, MA) were fed an AIN-93G based diet containing 0, 3.3, 6.6, or 33 g/kg diet of concentrated CP for 50 days. There were 10 rats per treatment with 2 rats housed together per cage. Purified diets were prepared by Research Diets Inc. (New Brunswick, NJ). Diets were formulated according to **Table 1**. Urine was collected (24 h) from six rats per treatment between days 30 and 35 using individual metabolism cages. Rats were sacrificed on days 57 and 58 by decapitation after euthanization in a CO_2 chamber. Animals were randomized before urine collection and sacrifice such that treatments were evenly distributed across the time frame. All samples were stored at −70 °C prior to analyses.

Standards. Standards for PAs were products of Sigma Chemical Co. (St. Louis, MO). The names and abbreviations used for these PAs are listed in **Table 2**, and their structures are depicted in **Figure 1**.

Analysis of Proanthocyanidins and Anthocyanins. Procyanidins were determined using procyanidin A2 as a standard in the method using the aldehyde condensation of 4-dimethylaminocinnamaldehyde (DMAC) with procyanidins as described previously (*17*). Anthocyanins in the CP were determined as described previously (*18*).

Analysis of Phenolic Acids in Urine. For analysis of free PAs, urine samples 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. Recovery of PAs using the SPE method are presented in **Table 3** (see also **Figure 2**).

For analysis of total PAs, 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 110 U of 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 PAs; 200 μL of the enzyme-treated urine sample was added to the column in place of the 200 μL of urine.

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

Extraction of PAs from the CP. For analysis of the free forms of the PAs, 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
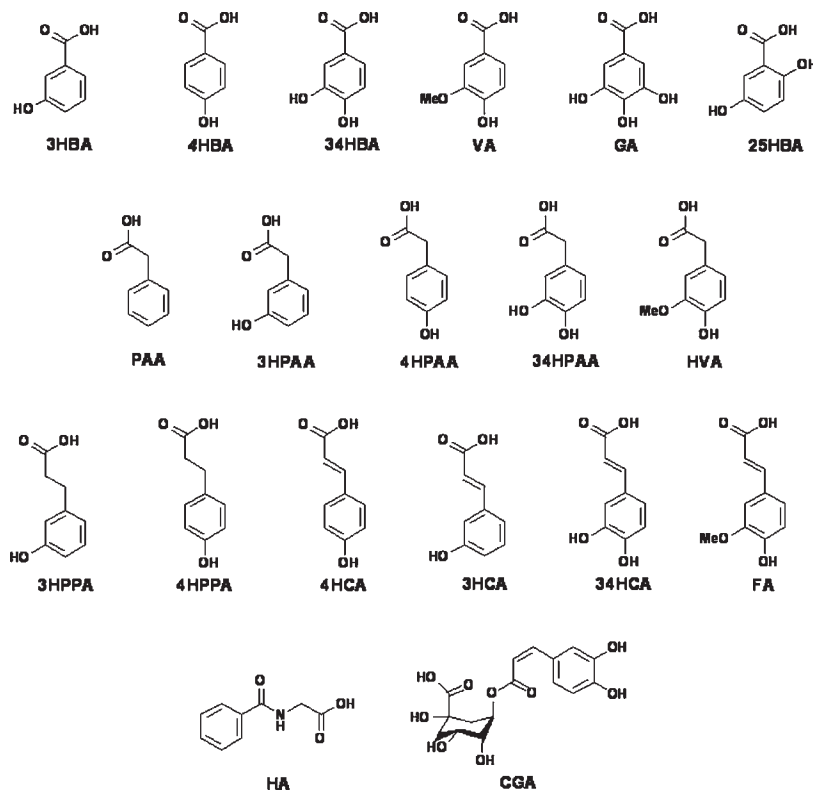
Figure 1. Chemical structures of catechins and phenolic acids. Names and symbols of all phenolic acids are listed in **Table 2**.

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 (PAs). The LC-MS/MS analyses were carried out using an Agilent 1100 HPLC system including an autosampler, a binary pump, and a diode array detector (Agilent Technologies, Palo Alto, CA), coupled with the 4000 Q TRAP mass spectrometer (Applied Biosystems, Forest City, CA). Separation was performed on a Phenom-enex 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 35 min linear gradient was as follows: 0−2−20−21−29−30−35 min, 20−20−35−40−60−70−20% of B, fol-lowed by 6 min of re-equilibration of the column before the next run. The mass spectrometer used an electrospray interface in negative ionization mode. Electrospray ionization tandem mass spectrometry (ESI-MS/MS) was conducted using a QTRAP quadrupole-linear ion trap (QLIT) instrument equipped with a Turbo Ion Spray (TISP) interface (Applied Biosystems/MDS Sciex, Concord, ON, Canada). The whole LC-MS system was controlled by Analyst software (v. 1.4.1, Applied Biosys-tems/MDS Sciex).

Multireaction monitoring (MRM) mode scan was performed. MRM uses the combination of a specific parent mass and a unique fragment ion to selectively monitor the compound to be quantified. The MRM transi-tion pairs used for quantification of 19 PAs are listed in **Table 2**. The standards for the calibration curve were prepared at concentrations of 50, 100, 200, 500, 1000, and 2000 ng/mL. Major parameters used for analysis on the mass spectrometer included 20 psi for curtain gas (CUR), −4500 V for the ion spray voltage (IS), 500 °C for source temperature, and 30 and 50 for ion source gas 1 (GS1) and ion source gas 2 (GS2), respectively. The declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) were optimized individually with each standard. The entrance potential (EP) was −10 for all standards. Peak areas from the chromatogram for each of the MRM pairs were determined and used for quantitation. PAs were monitored using their deprotonated ions and quantified against an external standard using quadratic curves. This method had a quantitation limit of 2 ng on column and an intra-assay variation of 7%.

Statistical Analysis. Data are presented as means ± standard error of means (SEM). Data were analyzed using one-way analysis of variance in

Table 3. Percentage Recovery of Phenolic Acids

phenolic acid	% recovery
3-hydroxyphenylacetic acid (3HPAA)	99.1 ± 2.3
4-hydroxyphenylacetic acid (4HPAA)	105.4 ± 14.7
3,4-hydroxyphenylacetic acid (34HPAA)	98.6 ± 3.7
3-hydroxybenzoic acid (3HBA)	104.8 ± 3.6
4-hydroxybenzoic acid (4HBA)	97.9 ± 3.5
3,4-dihydroxybenzoic acid (34HBA)	98.6 ± 3.7
2,5-dihydroxybenzoic acid (25HBA)	78.8 ± 4.5
chlorogenic acid (CGA)	78.9 ± 5.7
3-methoxy-4-hydroxybenzoic acid	116.6 ± 3.7
3-hydroxycinnamic acid (3HCA)	98.0 ± 2.9
4-hydroxycinnamic acid [*p*-coumaric acid] (4HCA)	96.1 ± 3.2
ferulic acid (FA)	103.4 ± 3.5
3,4-dihydroxycinnamic acid [caffeic acid] (24HCA)	87.3 ± 5.1
3-hydroxyphenylpropionic acid (3HPPA)	100.9 ± 2.6
4-hydroxyphenylpropionic acid (4HPPA)	72.4 ± 8.7
hippuric acid (HA)	99.5 ± 4.4

SigmaStat (Systat Software Inc., San Jose, CA). Linear regression techniques were used to calculate the regression coeffients to describe the relationships between dietary levels of CP and urinary excretion (μg/ mg of creatinine) of the individual PAs.

RESULTS

Concentrations of free PAs in CP are presented in **Table 4**. Quantities of 34HBA, 4HCA, HA, gallic acid (GA), and chloro-genic acid (CGA) were present in greatest quantities in the CP. The CP contained 1.51 mg of total anthocyanins/g and 56.2 mg of procyanidins/g, both of which were higher than the values supplied by the manufacturer. The relative distribution of pro-cyanidins has been presented in a separate publication (*22*). Dimers, trimers, tetramers, and polymers were the major pro-cyanidins present in CP, contributing approximately 88% of the reported procyanidins. The CP also contained 94.2 mg/g total phenolics (gallic acid equivalents) and 214 and 70.6 mg/g glucose
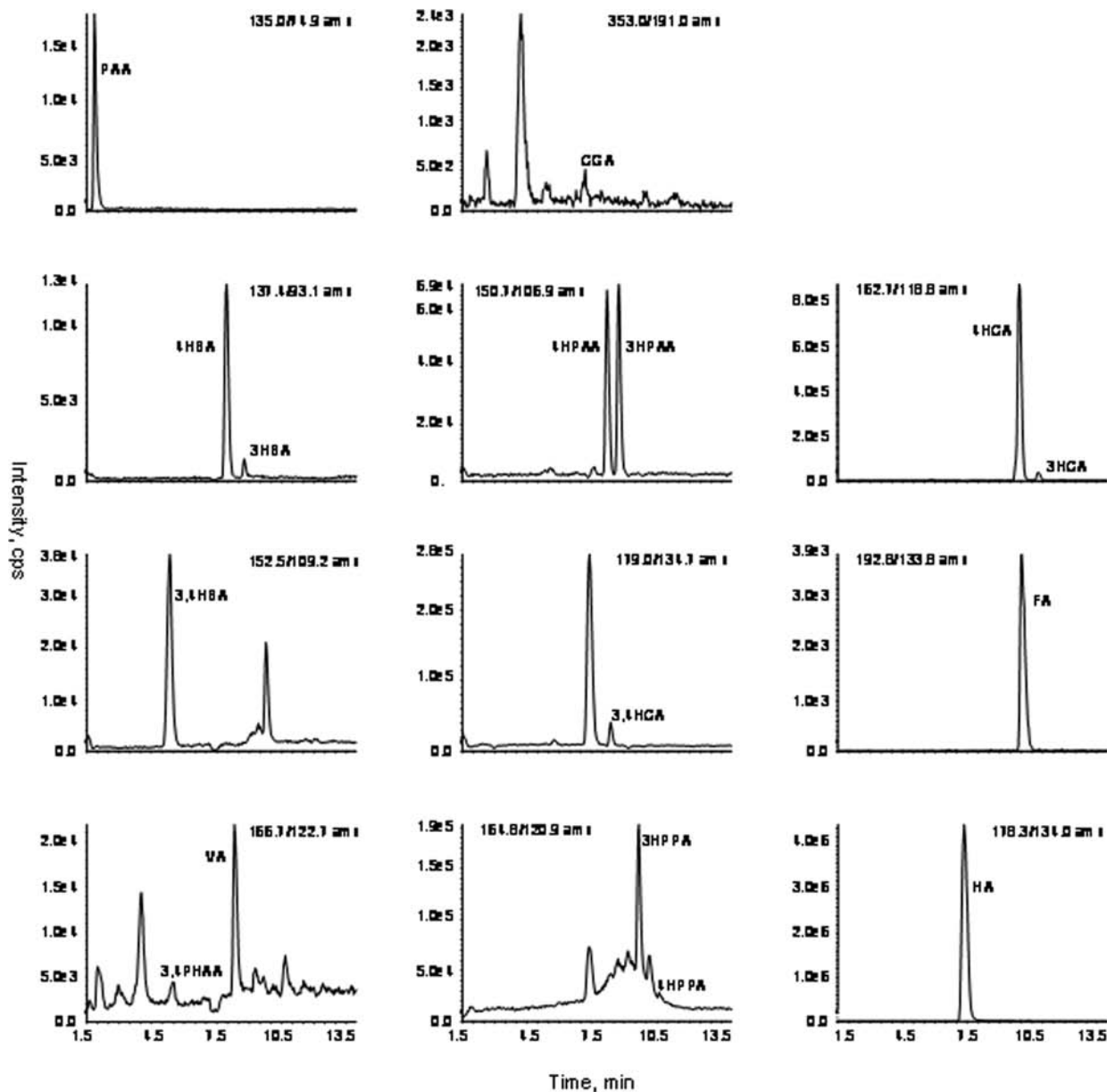
Figure 2. Typical chromatograms generated by HPLC-ESI-MS/MS in MRM mode of rat urine following consumption of a diet with 33 g/kg of cranberry powder. Abbreviations are as follows: PAA, phenylacetic acid; CGA, chlorogenic acid; 4HBA, 4-hydroxybenzoic acid; 3HBA, 3-hydroxybenzoic acid; 4HPAA, 4-hydroxyphenylacetic acid; 3HPPA, 3-hydroxyphenylacetic acid (phloretic acid); 4HCA, 4-hydroxycinnamic acid; 3HCA, 3-hydroxycinnamic acid; 34HBA, 3,4-dihydroxybenzoic acid (protocatechuic acid); 34HCA, 3,4-hydroxycinnamic acid (caffeic acid); FA, ferulic acid; 34HPAA, 3,4-dihydroxyphenylacetic acid; VA, 3-methoxy-4-hydroxybenzoic acid (vanillic acid); 3HPPA, 3-hydroxyphenylpropionic acid; 4HPPA, 4-hydroxyphenylpropionic acid (*p*-coumaric acid); HA, hippuric acid.

and fructose, respectively, on the basis of data provided by the manufacturer. The CP contains about 2.8 times more procyanidins than a whole cranberry freeze-dried powder, but much lower concentrations of anthocyanins.

Phenolic Acid Excretion. Urinary excretion of free, total, and conjugated PAs is presented in **Tables 5**, **6**, and **7**, respectively, and is expressed as micrograms of PA per milligram of urine creatinine. Of the PAs studied, the amounts excreted varied by 4 orders of magnitude, with hippuric acid (HA) being excreted in the highest quantities (331 μg/mg of creatinine) (**Table 6**). Total quantities of 4-hydroxyphenylacetic acid (4HPAA), 3-hydroxyphenylacetic acid (3HPAA), 3-hydroxyphenylpropionic acid (3HPPA), and 4HCA excreted were in the range of 10−40 μg/mg of creatinine in animals fed the highest level of CP. Amounts of phenylacetic acid (PAA), 34HPAA, 34HBA, 3,4-dihydroxy-

cinnamic acid (34HCA), and 4-hydroxy-3-methoxycinnamic acid (FA) were excreted in the urine in the concentration range of 0.1−2 μg/mg of creatinine. Urinary excretion of any form of GA and CGA was < 2 μg/mg of creatinine and was not significantly altered ($p > 0.10$) by the presence of CP in the diet (data not presented). Free PAA excretion (**Table 5**) was relatively low but was altered by diet ($p < 0.014$) and decreased with increased levels of CP in the diet. Essentially all of the PAA was in the free form.

4HPAA was present in the CP in the diet; however, the quantity excreted in the urine [free (**Table 5**) and total (**Table 6**)] decreased with increasing CP in the diet. Quantities excreted of the conjugated form of 4HPAA (**Table 7**) were not altered by diet.

The total quantity of 34HCA excreted was low compared to many of the other PAs (**Table 6**) even though 34HCA was present

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in appreciable quantities in the CP (84 μg/g). In all treatments, nearly all (95−100%) of the 34HCA was excreted in the conjugated form (**Table 9**).

Table 4. Concentration of Free Phenolic Acids in Cranberry Powder (CP)[a]

phenolic acid	free (μg/g)
phenylacetic acid (PAA)	<2
4-hydroxyphenylacetic acid (4HPAA)	32.1
3,4-dihydroxyphenylacetic acid (34HPAA)	<2
gallic acid (GA)	145.0
3-hydroxybenzoic acid (3HBA)	19.0
4-hydroxybenzoic acid (4HBA)	34.2
3,4-dihydroxybenzoic acid (34HBA)	512.0
2,5-dihydroxybenzoic acid (25HBA)	<10
chlorogenic acid	103.0
4-hydroxycinnamic acid (*p*-coumaric) (4HCA)	252.0
3-hydroxy-3-methoxycinnamic acid (ferulic, FA)	29.6
3,4-dihydroxycinnamic acid (caffeic acid) (34HCA)	84.2
3-hydroxyphenylpropionic acid (3HPPA)	15.3
hippuric acid (HA)	222.0

[a] CP is a concentrated dried powder of cranberry prepared by Decas Botanicals Inc. (Carver, MA).

Free HA excretion (**Table 5**) was significantly higher ($p < 0.001$) in rats consuming the high-CP diet compared to the two lower levels of CP intake, which were higher than controls. However, dietary effects were not significant ($p > 0.05$) for total (**Table 6**) and conjugated (**Table 7**) HA.

Excretion of free 3-methoxy-4-hydroxybenzoic acid (vanillic acid, VA) (**Table 5**) was highest in the urine of rats consuming the highest intake of CP, but excretion of total (**Table 6**) and conjugated (**Table 7**) forms was not significantly altered by diet. At the highest level of CP intake, about 80% was in the conjugated form, but in other diet treatments, essentially all of the VA was excreted in the conjugated form (**Tables 6** and **7**).

For several of the PAs, the amounts of PAs excreted in the urine were linearly related to the intake of CP (**Table 8**). Regression equations and correlation coefficients were calculated ($Y = a + bX$), where Y = urinary excretion of PA (μg/mg of creatinine) and X = level of CP in the diet. When the regression equation was significant ($p < 0.001$) (see **Table 8**), the constant "a" provided an estimate of the PA excreted in the absence of CP in the diet. The coefficient "b" in turn provided an estimate of the incremental amount of PA excreted in each particular form with increasing CP in the diet. For several of the PAs such as 34HBA, 3HPAA,

Table 5. Free Phenolic Acids in Urine (Means ± SEM; Micrograms per Milligram of Creatinine) of Rats Fed a Purified Diet Containing 0, 3.3, 6.6, or 33 g of Concentrated Cranberry Powder (CP)/kg of Diet[a]

free phenolic acid	control	control + HF	HF + low CP	HF + medium CP	HF + high CP	p value
phenylacetic acid (PAA)	0.060 ± 0.007[A]	0.285 ± 0.092[B]	0.117 ± 0.010[AB]	0.132 ± 0.007[AB†]	0.094 ± 0.025[A]	0.014
3-hydroxyphenylacetic acid (3HPAA)	1.680 ± 0.170[B]	2.030 ± 0.160[B†]	2.600 ± 0.130[B]	3.030 ± 0.310[B†]	8.470 ± 0.560[A†]	<0.001
4-hydroxyphenylacetic acid (4HPAA)	25.600 ± 4.700[B]	23.500 ± 3.200[B]	15.500 ± 2.700[AB]	7.180 ± 2.220[A†]	4.080 ± 1.860[A†]	<0.001
3,4-dihydroxyphenylacetic acid (34HPAA)	0.550 ± 0.122	0.918 ± 0.160	0.916 ± 0.224	0.721 ± 0.165	0.491 ± 0.128	NS
3-hydroxybenzoic acid (3HBA)	0.065 ± 0.014[A]	0.079 ± 0.010[A†]	0.223 ± 0.021[B†]	0.222 ± 0.028[B†]	0.517 ± 0.056[C†]	<0.001
4 -hydroxybenzoic acid (4HBA)	2.200 ± 0.440	3.620 ± 0.280	2.630 ± 0.210[†]	1.980 ± 0.250[†]	2.270 ± 0.490	0.032
3,4-dihydroxybenzoic acid (3,4HBA)	0.039 ± 0.006[B†]	0.061 ± 0.010[B]	0.250 ± 0.021[B]	0.268 ± 0.058[AB]	0.743 ± 0.243[A]	0.002
2,5-dihydroxybenzoic acid (25HBA)	0.021 ± 0.003[C]	0.019 ± 0.005[BC†]	0.064 ± 0.008[BC]	0.092 ± 0.012[B]	0.254 ± 0.064[A]	<0.001
3-methoxy-4-hydroxybenzoic acid (VA)	0.090 ± 0.020[A]	0.049 ± 0.022[A]	0.046 ± 0.013[A†]	0.111 ± 0.040[A]	0.605 ± 0.215[B]	0.003
3-hydroxycinnamic acid (3HCA)	0.042 ± 0.004[A]	0.070 ± 0.011[AB†]	0.083 ± 0.009[AB]	0.142 ± 0.019[B]	0.542 ± 0.050[C†]	<0.001
4-hydroxycinnamic acid (4HCA)	0.365 ± 0.048[A]	0.125 ± 0.037[A†]	0.939 ± 0.072[A]	1.260 ± 0.120[A†]	3.960 ± 0.890[B]	<0.001
ferulic acid (FA)	0.087 ± 0.008[A†]	0.042 ± 0.015[A]	0.085 ± 0.007[A]	0.113 ± 0.027[A]	0.283 ± 0.065[B]	<0.001
3,4-dihydroxycinnamic acid (34HCA)	0.019 ± 0.004[†]	0.019 ± 0.006	0.056 ± 0.013[†]	0.048 ± 0.016	0.067 ± 0.031[†]	NS
3-hydroxyphenylpropionic acid (3HPPA)	0.856 ± 0.113[A]	0.970 ± 0.142[A†]	1.130 ± 0.070[AB]	1.810 ± 0.250[B†]	2.790 ± 0.380[C†]	<0.001
hippuric acid (HA)	35.000 ± 8.600[A]	52.800 ± 7.800[A]	108 ± 9.00[B]	115 ± 7.00[B†]	198 ± 14.0[C†]	<0.001

[a] HF, high fructose (53% fructose); low CP = 3.3 g of CP/kg of diet; medium CP = 6.6 g/kg; high CP = 33 g/kg. Means represent six observations per diet group except where noted ([†] = five observations; [‡] = four observations). Means within rows without a common superscript differ ($p < 0.05$). Data for gallic acid and chlorogenic acid are not presented as these were excreted in low quantities and were not affected by diet.

Table 6. Total Phenolic Acids in Urine (Means ± SEM; Micrograms per Milligram of Creatinine) of Rats Fed a Purified Diet Containing 0, 3.3, 6.6, or 33 g of Concentrated Cranberry Powder (CP)/kg of Diet[a]

total phenolic acid (free + conjugated forms)	control	control + HF	HF + low CP	HF + medium CP	HF + high CP	p value
phenylacetic acid (PAA)	0.105 ± 0.021	0.21 ± 0.05	0.13 ± 0.01[†]	0.14 ± 0.04	0.147 ± 0.019	NS
3-hydroxyphenylacetic acid (3HPAA)	3.74 ± 0.31[A]	3.48 ± 0.09[A†]	5.49 ± 0.59[A]	8.00 ± 0.62[B]	20.40 ± 1.30[C†]	<0.001
4-hydroxyphenylacetic acid (4HPAA)	53.60 ± 4.20[A]	46.20 ± 6.50[AB]	36.70 ± 2.80[BC]	31.80 ± 1.70[BC]	24.80 ± 3.30[C]	<0.001
3,4-dihydroxyphenylacetic acid (34HPAA)	1.65 ± 0.23	1.05 ± 0.20	1.61 ± 0.33	1.32 ± 0.21	1.07 ± 0.13[†]	NS
3-hydroxybenzoic acid (3HBA)	6.26 ± 4.07	0.92 ± 0.51[†]	1.11 ± 0.18	1.60 ± 0.27	3.18 ± 0.61[†]	NS
4-hydroxybenzoic acid (4HBA)	6.55 ± 1.08[B]	7.51 ± 0.36[B]	7.87 ± 0.62[B]	7.20 ± 1.00[B]	11.90 ± 1.20[A]	0.003
3,4-dihydroxybenzoic acid (34HBA)	0.26 ± 0.02[A†]	0.25 ± 0.01[A]	0.80 ± 0.04[AB]	1.03 ± 0.13[B]	4.63 ± 0.39[C†]	<0.001
2,5-dihydroxybenzoic acid (25HBA)	0.01 ± 0.01[C†]	0.05 ± 0.01[C†]	0.13 ± 0.04[BC]	0.15 ± 0.05[BC]	0.25 ± 0.08[C†]	0.008
3-methoxy-4-hydroxybenzoic acid (VA)	3.34 ± 2.01[†]	1.83 ± 0.76[†]	2.03 ± 0.42[†]	1.84 ± 0.35[†]	3.16 ± 1.54[†]	NS
3-hydroxycinnamic acid (3HCA)	0.22 ± 0.04[A]	0.34 ± 0.06[A†]	0.51 ± 0.07[A]	0.89 ± 0.11[A]	3.38 ± 0.44[B]	<0.001
4-hydroxycinnamic acid (4HCA)	2.40 ± 0.07[A†]	0.99 ± 0.22[A†]	5.77 ± 0.49[A]	8.76 ± 0.64[A]	33.40 ± 6.40[B]	<0.001
ferulic acid (FA)	3.53 ± 0.29[A]	0.89 ± 0.29[B]	1.41 ± 0.12[B]	2.40 ± 0.28[AB]	6.67 ± 1.02[C]	<0.001
3,4-dihydroxycinnamic acid (34HCA)	0.72 ± 0.15[A]	1.09 ± 0.18[AB]	1.00 ± 0.20[AB]	0.89 ± 0.19[AB]	1.67 ± 0.29[B]	0.040
3-hydroxyphenylpropionic acid (3HPPA)	1.80 ± 0.26[A]	2.50 ± 0.33[A†]	3.54 ± 0.42[A]	6.37 ± 0.57[A†]	18.20 ± 3.70[C]	<0.001
hippuric acid (HA)	646 ± 249	171 ± 56[†]	142 ± 31[†]	218 ± 64[†]	331 ± 142	NS

[a] HF, high fructose (53% fructose); low CP = 3.3 g of CP/kg of diet; medium CP = 6.6 g/kg; high CP = 33 g/kg. Means represent six observations per diet group except where noted ([†] = five observations; [‡] = four observations). Means without a common superscript differ ($p < 0.05$). Data for gallic acid and chlorogenic acid are not presented as these were excreted in low quantities and were not affected by diet.

Table 7. Conjugated Phenolic Acids in Urine (Means ± SEM; Micrograms per Milligram of Creatinine) of Rats Fed a Purified Diet Containing 0, 3.3, 6.6, or 33 g of Concentrated Cranberry Powder (CP)/kg of Diet[a]

conjugated phenolic acid	control	control + HF	HF + low CP	HF + medium CP	HF + high CP	p value
phenylacetic acid (PAA)	0.04 ± 0.02[AB]	−0.07 ± 0.05[B]	0.02 ± 0.01[AB] †	−0.02 ± 0.03[AB†]	0.05 ± 0.01[A]	0.028
3-hydroxyphenylacetic acid (3HPAA)	2.06 ± 0.20[A]	1.42 ± 0.21[A‡]	2.89 ± 0.59[AB]	4.73 ± 0.58[B†]	11.90 ± 1.30[C‡]	<0.001
4-hydroxyphenylacetic acid (4HPAA)	28.00 ± 4.90	22.69 ± 7.29	21.10 ± 4.70	24.20 ± 1.50[†]	22.50 ± 3.50[†]	NS
3,4-dihydroxyphenylacetic acid (34HPAA)	1.10 ± 0.28	0.13 ± 0.17	0.70 ± 0.23	0.60 ± 0.32	0.51 ± 0.23[†]	NS
3-hydroxybenzoic acid (3HBA)	6.19 ± 4.06	0.93 ± 0.64[‡]	0.86 ± 0.22[†]	1.23 ± 0.26[†]	2.14 ± 1.11[†]	NS
4-hydroxybenzoic acid (4HBA)	4.35 ± 1.11[AB]	3.90 ± 0.20[A]	4.97 ± 0.85[AB] †	5.22 ± 1.16[AB] †	9.64 ± 1.70[B]	0.009
3,4-dihydroxybenzoic acid (34HBA)	0.22 ± 0.0[A‡]	0.19 ± 0.02[A]	0.55 ± 0.03[A]	0.76 ± 0.09[A]	4.10 ± 0.50[B†]	<0.001
2,5-dihydroxybenzoic acid (25HBA)	−0.01 ± 0.01[†]	0.04 ± 0.01[‡]	0.07 ± 0.03	0.06 ± 0.03	0.00 ± 0.15[†]	NS
3-methoxy-4-hydroxybenzoic acid (VA)	3.26 ± 2.02[†]	1.77 ± 0.77[†]	2.13 ± 0.50	1.75 ± 0.36[†]	2.53 ± 1.74[†]	NS
3-hydroxycinnamic acid (3HCA)	0.18 ± 0.04[A]	0.29 ± 0.07[A‡]	0.43 ± 0.08[A]	0.75 ± 0.11[A]	3.04 ± 0.52[B†]	<0.001
4-hydroxycinnamic acid (4HCA)	2.07 ± 0.11[A†]	0.91 ± 0.30[A‡]	4.83 ± 0.51[A]	7.31 ± 0.71[A†]	29.40 ± 7.00[B]	<0.001
ferulic acid (FA)	3.27 ± 0.27[A†]	0.85 ± 0.30[B]	1.33 ± 0.12[AB]	2.29 ± 0.27[AB]	6.38 ± 1.08[C]	<0.001
3,4-dihydroxycinnamic acid (34HCA)	0.81 ± 0.13[A†]	1.07 ± 0.18[AB]	0.81 ± 0.18[A†]	0.84 ± 0.19[A]	1.77 ± 0.32[B†]	0.017
3-hydroxyphenylpropionic acid (3HPPA)	0.94 ± 0.26[A]	1.52 ± 0.49[A‡]	2.41 ± 0.42[A]	4.17 ± 0.38[A‡]	15.30 ± 4.50[B†]	<0.001
hippuric acid (HA)	611 ± 250	118 ± 61[†]	34.7 ± 32.4[†]	109 ± 76[‡]	157 ± 168[†]	NS

[a] HF, high fructose (53% fructose); low CP = 3.3 g CP/kg of diet; medium CP = 6.6 g/kg; high CP = 33 g/kg. Means represent six observations per diet group except where noted ([†] = five observations; [‡] = four observations). Means without a common superscript differ ($p < 0.05$). Data for gallic acid and chlorogenic acid are not presented as these were excreted in low quantities and were not affected by diet.
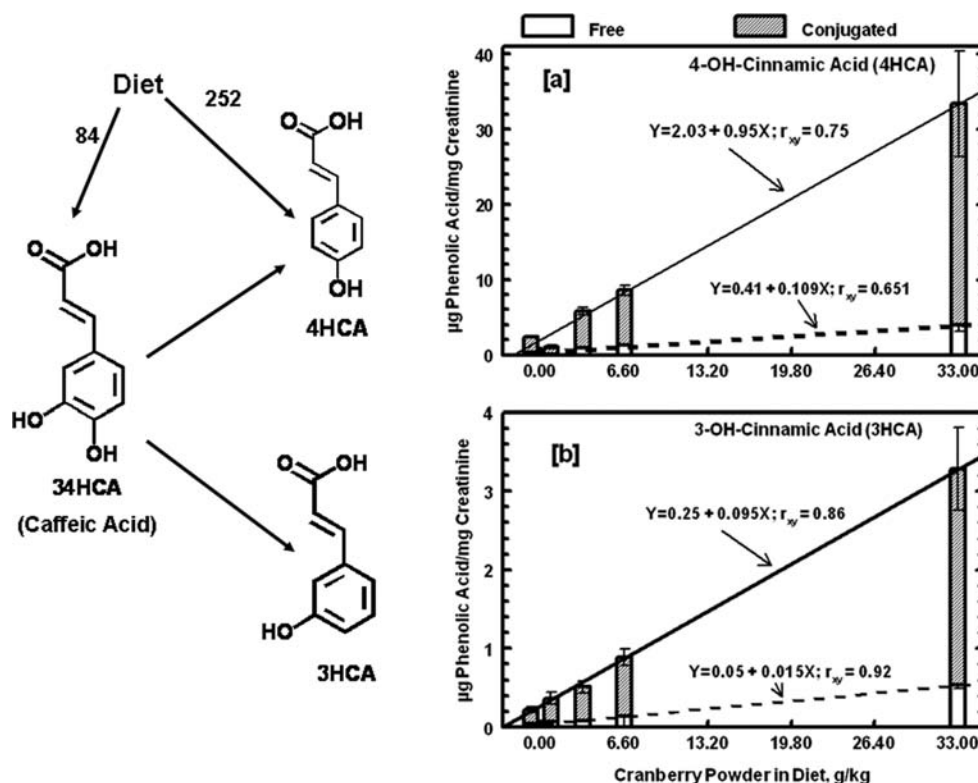
Figure 3. Urinary excretion of free, conjugated, and total 4-hydroxycinnamic acid (*p*-coumaric acid; 4HCA) and 3-hydroxycinnamic acid (3HCA) (g/mg of creatinine) in rats fed diets containing 0, 3.3, 6.6, or 33 g/kg of cranberry powder (CP). The two bars at 0 intake of cranberry represent a diet with starch (first) or fructose (second) as the major carbohydrate source. Possible interconversion pathways and relationships with dietary sources are presented to the left of the graphs. Numbers on arrows indicate the amount of phenolic acid present in the cranberry powder (µg/g). Abbreviations: 34HCA, 3,4-hydroxycinnamic acid, caffeic acid; 4HCA, 4-hydroxycinnamic acid; 3HCA, 3-hydroxycinnamic acid.

3HPPA, 4HCA, 3HCA, and FA, the coefficients from the total PA equation minus the free PA coefficient were similar to the coefficient calculated for the conjugated PA excreted in the urine. This would be expected if the regression equations were significant, the correlation coefficients were relatively high, and the differences in our analytical data between the total and free forms accurately predicted the amount of conjugated PA. The regression equation coefficients that were statistically significant are presented in **Table 8**. The percentages of each PA excreted in the conjugated form for 6 PAs were proportional to intake and were

calculated as the ratio of the "*b*" coefficient of the conjugated PA regression coefficient to the "*b*" coefficient for the total PA regression coefficient. For 34HBA, 3HPAA, 3HPPA, 4HCA, 3HCA, and FA the total PA excreted in the conjugated form was 90.2, 60.1, 88.7, 88.6, 90.5, and 96.0% (**Table 9**). For the other conjugated PAs present in **Table 9**, the relative percentage of the total excreted either varied with diet (4HBA, 3HBA, VA, 4HPAA, HA) or was constant across diets with all of the PA excreted in the conjugated form (34HCA) or none of the PA was excreted in the conjugated form (25HBA).

Table 8. Regression Coefficients ($Y = a + bX$) of Cranberry Powder (CP) in Diet (X) and Urinary Excretion of Phenolic Acid (Micrograms per Milligram of Creatinine) (Y)[a]

	regression coefficients ($Y = a + bX$)		
	a	b	R^2
free urine phenolic acid			
4-hydroxybenzoic acid (4HBA)			NS
3-hydroxybenzoic acid (3HBA)	0.111 ± 0.018	0.013 ± 0.001	0.817
3,4-dihydroxybenzoic acid (34HBA)	0.124 ± 0.084	0.019 ± 0.005	0.425
3-methoxy-4-hydroxybenzoic acid (VA)	0.037 ± 0.053	0.017 ± 0.003	0.467
3-hydroxyphenylacetic acid (3HPAA)	1.838 ± 0.149	0.201 ± 0.010	0.939
4-hydroxyphenylacetic acid (4HPAA)	20.180 ± 1.952	-0.555 ± 0.136	0.389
3,4-dihydroxycinnamic acid (caffeic acid, 34HCA)			NS
3-hydroxyphenylpropionic acid (3HPPA)	1.023 ± 0.114	0.056 ± 0.008	0.670
4-hydroxycinnamic acid (*p*-coumaric) (4HCA)	0.413 ± 0.313	0.109 ± 0.018	0.651
3-hydroxycinnamic acid (3HCA)	0.050 ± 0.016	0.015 ± 0.001	0.918
ferulic acid[b] (FA)	0.056 ± 0.022	0.007 ± 0.001	0.557
hippuric acid (HA)	64.699 ± 7.030	4.283 ± 0.491	0.745
conjugated urine phenolic acids			
4-hydroxybenzoic acid[b] (4HBA)	4.116 ± 0.708	0.168 ± 0.040	0.465
3-hydroxybenzoic acid (3HBA)			NS
3,4-dihydroxybenzoic acid (34HBA)	0.135 ± 0.108	0.119 ± 0.007	0.912
3-methoxy-4-hydroxybenzoic acid (VA)			NS
3-hydroxyphenylacetic acid (3HPAA)	2.019 ± 0.326	0.303 ± 0.024	0.875
4-hydroxyphenylacetic acid (4HPAA)			NS
3,4-dihydroxycinnamic acid (caffeic acid, 34HCA)	0.831 ± 0.110	0.027 ± 0.008	0.337
3-hydroxyphenylpropionic acid (3HPPA)	1.145 ± 1.036	0.430 ± 0.069	0.630
4-hydroxycinnamic acid[b] (*p*-coumaric) (4HCA)	1.611 ± 2.572	0.845 ± 0.143	0.648
3-hydroxycinnamic acid[b] (3HCA)	0.199 ± 0.161	0.086 ± 0.010	0.803
ferulic acid[b] (FA)	0.925 ± 0.359	0.167 ± 0.021	0.736
hippuric acid (HA)			NS
total urine phenolic acids			
4-hydroxybenzoic acid (4HBA)	6.942 ± 0.478	0.147 ± 0.032	0.437
3-hydroxybenzoic acid (3HBA)			NS
3,4-dihydroxybenzoic acid (34HBA)	0.260 ± 0.085	0.132 ± 0.006	0.950
3-methoxy-4-hydroxybenzoic acid (VA)			NS
3-hydroxyphenylacetic acid (3HPAA)	3.909 ± 0.357	0.504 ± 0.025	0.941
4-hydroxyphenylacetic acid (4HPAA)	44.199 ± 2.506	-0.653 ± 0.166	0.357
3,4-dihydroxycinnamic acid[b] (caffeic acid, 34HCA)[†]	0.870 ± 0.112	0.024 ± 0.007	0.264
3-hydroxyphenylpropionic acid (3HPPA)	2.275 ± 0.948	0.485 ± 0.061	0.710
4-hydroxycinnamic acid (*p*-coumaric) (4HCA)	2.034 ± 2.104	0.954 ± 0.122	0.745
3-hydroxycinnamic acid (3HCA)	0.254 ± 0.112	0.095 ± 0.007	0.862
ferulic acid[b] (FA)	0.980 ± 0.342	0.174 ± 0.020	0.770
hippuric acid (HA)			NS

[a] Values for intercept (a) and slope (b) are mean $\pm$ SEM. All regressions are significant ($p \leq 0.001$) except where indicated with [†], in which case the regression is significant at $p \leq 0.01$. [b] Excretion of the phenolic acid in the control group with no fructose was significantly different ($p < 0.05$) from the high-fructose control group. In this case, only the high-fructose group was included in the regression analysis, and the low-fructose control group was omitted.

The relative excretion of 4HCA and 3HCA is presented in **Figure 3**. The proportions excreted in the conjugated form were 88.6 and 90.5, respectively (**Table 9**). Urinary excretion of total 4HCA was higher than any other PA measured with the exception of HA (**Table 6**) (**Figure 3a**). The total excretion of 4HCA, which was present in the diet in high concentrations (252 μg/g), was ~10-fold higher than that of 3HCA, which was not found in the CP. 3HCA likely represents a dehydroxylated metabolite of 34HCA, which was present in the diet (**Figure 3**).

34HBA was present in the CP in the highest abundance of any PA measured (512 μg/mg of creatinine), but its urinary excretion is relatively low (**Figure 4a**) compared to that of 4HCA, which was also present in the diet. At zero CP intake, 51% of the 34HBA was excreted in the conjugated form, and 90% of the 34HBA excreted with higher CP intakes was excreted in the conjugated form.

The percentage of 4HBA excreted in the conjugated form in rats receiving no CP was 62.3%, whereas at the high intake of CP, the percentage was 81.1% (**Tables 6** and **7**). The regression equations for total and conjugated, but not free 4HBA excreted relative to CP intake were statistically significant ($p < 0.001$), but

the correlation coefficients were low in both cases (0.44 and 0.47). For 3HBA and VA the amounts excreted in the conjugated form were essentially 100% in the control treatment but decreased to 67 and 80% in rats fed the medium- and high-CP treatments, respectively (**Table 9**).
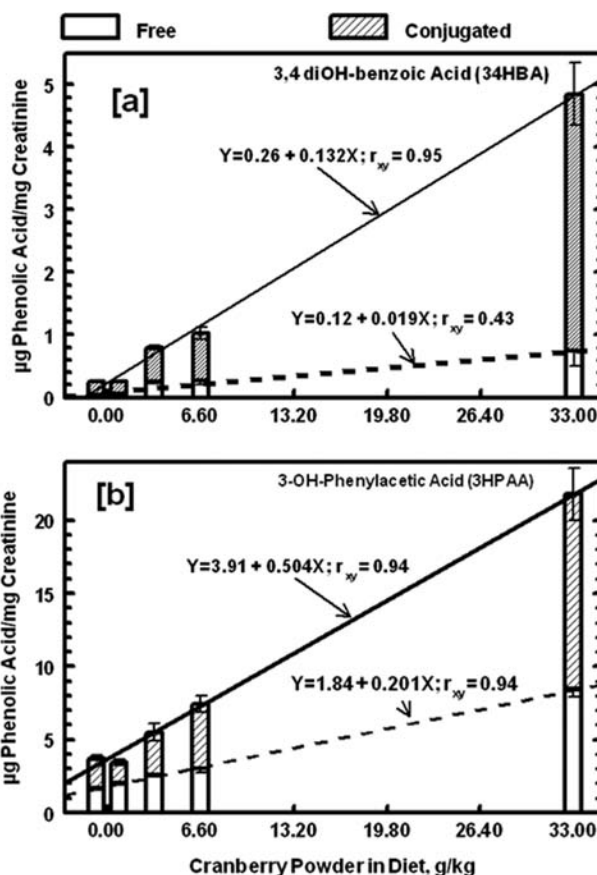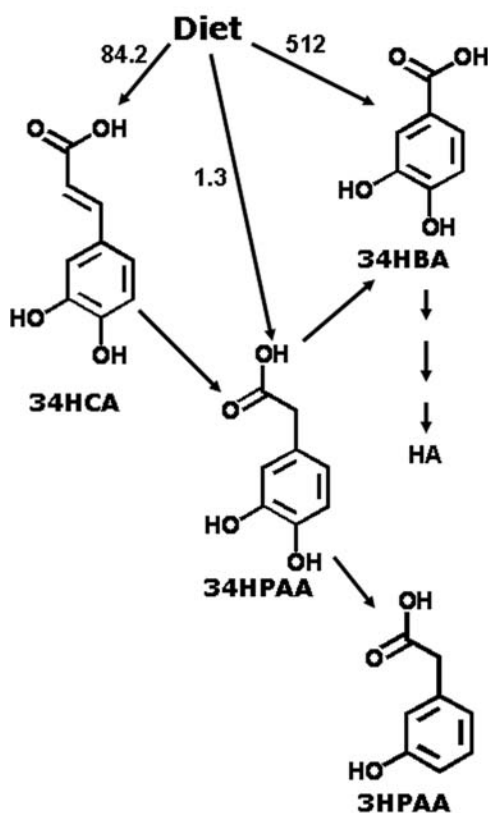
The relative amounts of the free, conjugated, and total forms of 3HPAA are presented in **Figure 4b**. 3HPAA was not found in CP, but its total excretion in the urine was fourth highest of the PAs measured and the amount excreted increased linearly with increasing CP intake. 3HPAA may be a metabolite of 34HPAA, which was present in low quantities in the CP. About 60% of the 34HPAA was excreted in the conjugated form. Total 4HPAA was excreted in about the same quantities as 3HPAA in rats fed the highest level of CP. However, 4HPAA excretion was highest in the control rats, and the amount of total (**Table 6**) and free (**Table 5**) forms excreted decreased with increasing amounts of CP in the diet. The amount of conjugated 4HPAA excreted (μg/mg of creatinine) was approximately constant (**Table 7**), but the percentage of the total that was excreted in the conjugated form increased with increased CP intake (from 52 to 83.5%) (**Table 9**).

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Urinary excretion of total 25HBA was quite low, but was significantly altered by diet ($p < 0.008$) (**Table 6**). At the lowest level of CP intake, ~55% of the 25HBA was excreted in the conjugated form, which decreased to 0 at the highest level of CP

Table 9. Estimated Percentage of Total Phenolic Acids Excreted in Urine That Are Present in the Conjugated Form

phenolic acid	% conjugated
4-hydroxybenzoic acid (4HBA)	51, 81
3-hydroxybenzoic acid[a] (3HBA)	~100, 67
3,4-dihydroxybenzoic acid (34HBA)	90.2
3-methoxy-4-hydroxybenzoic acid[a] (VA)	~98, 80
3-hydroxyphenylacetic acid (3HPAA)	60.1
4-hydroxyphenylacetic acid[a] (4HPAA)	52, 83.5
3,4-dihydroxycinnamic acid (caffeic acid, 34HCA)	~100
3-hydroxyphenylpropionic acid (3HPPA)	88.7
4-hydroxycinnamic acid (*p*-coumaric) (4HCA)	88.6
3-hydroxycinnamic acid (3HCA)	90.5
ferulic acid (FA)	96.0
hippuric acid[a] (HA)	94.6, 47.4
gentisic acid (25HBA)	~0

[a] Percentage of phenolic acid in the conjugated form varied depending upon level of cranberry in the diet. The first number indicates percentage of excreted phenolic acid that was excreted in the conjugated form in the diet with no cranberry, and the second number indicates percentage of excreted phenolic acid that was excreted in the conjugated form in the highest level of cranberry powder in the diet. With the other phenolic acids, the percentage of conjugated phenolic acid was constant across levels of cranberry powder in the diets.

intake when 25HBA was all in the free form (**Table 5**). The regression equations with level of CP in the diet were not significant.

Total urinary excretion of FA (6.7 μg/mg of creatinine was about double that of 3HCA (**Table 6**). At zero intake of CP, 88.1% of the FA was in the conjugated form. With increased excretion of FA at higher intakes of CP, essentially all of the increase was in the conjugated form, and at the highest level of CP intake, 95% of the FA was excreted in the conjugated form. High fructose in the diet seemed to decrease the conjugated and total FA (**Tables 6** and **7**). This may be explained by some background FA in the corn starch compared to the fructose source.

DISCUSSION

This paper represents one of the first studies to investigate the extent of conjugation of PAs and their excretion in the urine. Previously, most of the measurements have been on the free or total PAs following enzymatic digestion that are present in urine or blood. Because PAs can be produced by colonic microflora from a number of different phenolic compounds and also endogenously from amino acids such as tyrosine and phenylalanine, diet can make a major impact on some PAs excreted.

PAs are major metabolites of procyanidins as well as other polyphenols. Gu et al. (*5*) observed that 3-methoxy-4-hydroxyphenylacetic acid (VA) and 3-hydroxyphenylacetic acid (3HPAA) were the dominant PAs excreted in rats fed sorghum bran in diet.

Figure 4. Urinary excretion of free, conjugated, and total 3,4-dihydroxybenzoic acid (3,4HBA) and 3-hydroxyphenylacetic acid (3HPAA) (μg/mg of creatinine) in rats fed diets containing 0, 3.3, 6.6, or 33 g/kg of cranberry powder (CP). The two bars at 0 intake of cranberry represent a diet with starch (first) or fructose (second) as the major carbohydrate source. Possible interconversion pathways and relationships with dietary sources are presented to the left of the graphs. Numbers on arrows indicate the amount of phenolic acid present in the cranberry powder (μg/g). Abbreviations: 34HBA, 3,4-dihydroxybenzoic acid (protocatechuic acid); 34HPAA, 3-hydroxyphenylacetic acid (homoprotocatechuic acid); 3HPAA, 3-hydroxyphenylacetic acid; 34HCA, 3,4-hydroxycinnamic acid (caffeic acid); 34HCA, 3,4-hydroxycinnamic acid; HA, hippuric acid.

In the current study, 4HCA, 4HPAA, 3HPAA, and 3HPPA were the major PAs excreted in greatest quantity, other than HA, when CP was included in the diet. In this study, only small amounts of VA were excreted. 3HPPA was excreted in high amounts in rats fed 20 or 40% sorghum bran (*5*), which was also present in appreciable quantities in rats fed CP in the current study. High excretion rates of 3HPPA have been observed in previous studies with rats fed wine polyphenols or with humans who consumed grape seed extracts (*1, 7*). 3HPPA was also a predominant product when the polymeric procyanidins were incubated with human colon microflora (*23*). All of these cited dietary components (*1, 7, 23*) have appreciable amounts of proanthocyanidins, which may indicate that 3HPPA is a specific metabolite of proanthocyanidins.

34HBA was present in the cranberry in the highest concentration of any of the PAs measured (512 μg/g) (**Table 4**). Urinary excretion of 34HBA was increased when CP was included in the diet (> 18-fold), and there was a significant linear response with increasing CP in the diet (**Figure 4a**). This increased excretion of 34HBA in rats fed CP may result from the high 34HBA in the CP and/or the degradation of cyanidin-3-galactoside. 34HBA has been recently shown to be a major metabolite of the anthocyanin cyanidin-3-glucoside (*11*) in humans with serum and fecal 34HBA accounting for 44 and 28% of the cyanidin-3-glucoside ingested. Surprisingly, no 34HBA was detected in the urine of humans. Tsuda et al. (*24*) found high levels of 34HBA in the plasma and intestine of rats fed cyanidin-3-glucoside. Degradation products of cyanidin and cyanidin-3-glucoside have been identified as 34HBA (*25*) and phloroglucinaldehyde (*26*). In cultured cell media, the degradation products 34HBA and phloroglucinaldehyde were further metabolized to glucuronide and sulfate conjugates. These authors (*26*) suggested that a significant proportion of intestinal metabolites of anthocyanins are likely to be conjugates of their degradation products. Our in vivo study confirms this conclusion in that about 90% of the 34HBA excreted was in the conjugated form (**Figure 4a**; **Table 9**). The high degree of conjugation of 34HBA may be a factor that limits the extent of excretion of 34HBA in the urine if the rate of conjugation becomes limiting. 34HBA has also been shown to alter fatty acid oxidation in the heart of rats (*27*). 34HBA can also be methylated into 3-methoxy-4-hydroxybenzoic acid (VA) in the cell by catechol-*O*-methyltransferase (*28*). 34HBA and VA were present at high concentrations in the serum from rats fed sorghum bran (*5*); however, inclusion of CP in the diet in the present study did not significantly alter VA excretion.

4HPAA, 34PAA, 34HPPA, and 34HBA are primarily endogenous metabolites of amino acids (*5*). Excretion of 4HPAA in the urine is highest of any PA measured (> 8-fold) with the exception of HA in rats fed the control AIN-93 diet in this study (**Table 6**) and a previous study (*5*). As sources of polyphenolics from CP (**Table 6**) or sorghum (*5*) were added to the diet, the excretion of 4HPAA decreased by ~50%. PAs can originate from tyrosine or phenylalanine in the gut (*29*). On the other hand, both 3HPAA and 3HPPA are primarily exogenous metabolites of polyphenols (*5*). Urinary excretion of 3HPAA and 3HPPA were increased 5.9- and 7.3-fold, respectively, at the highest level of CP intake relative to urinary excretion in rats fed the control diet containing no CP (**Table 6**). 3HPPA but not 3HPAA was found in CP. Large increases in the excretion of both of these PAs were also observed in rats fed sorghum bran in the diet compared to diets containing no sorghum bran (*5*).

34HPAA, but not 3HPAA or 4HPAA, was shown to inhibit prostaglandin E$_2$ production by CCD-18 colon fibroblast cells stimulated with IL-1β and to inhibit inflammation in vivo in the writhing and paw pressure test in rodents (*30*). Excretion of both

3HPAA and 4HPAA was increased with feeding CP to rats, but 34HPAA excretion was not affected by dietary CP. 3,4-Dihydroxyphenylpropionic acid and 3-(4-hydroxy-3-methoxyphenyl)cinnamic acid, compounds that we did not measure, were also shown to have anti-inflammatory activity in vitro and in vivo (*30*).

In summary, a majority of the PAs analyzed in this study were present in the urine in the conjugated form as either glucuronide or sulfate conjugates. In most cases, the percentages of the total excreted in the conjugated form were > 50%, with many > 80%. Thus, measurement of just the free forms does not give a true representation of the amounts of PAs that are excreted. Studies of bioactivity and health effects need to consider more than just the parent compound(s) in the food. The compounds in the food may themselves be methylated or conjugated, but they may also be metabolized to other lower molecular weight compounds, which in turn may also be methylated or conjugated in some form. Very little is known about the effects of methylation or conjugation on the bioactivity of polyphenolics.

ABBREVIATIONS USED

ACNs, anthocyanins; CP, cranberry powder; PAA, phenylacetic acid; 4HBA, 4-hydroxybenzoic acid; 3HBA, 3-hydroxybenzoic acid; 34HBA, protocatechuic acid (3,4-dihydroxybenzoic acid); GA, gallic acid; 25HBA, gentisic acid (2,5-dihydroxybenzoic acid); VA, vanillic acid; 34HPAA, homoprotocatechuic acid; CGA, chlorogenic acid; HVA, homovanillic acid; 3HPAA, 3-hydroxyphenylacetic acid; 4HPAA, 4-hydroxyphenylacetic acid; 34HCA, caffeic acid (3,4-dihydroxycinnamic acid); 3HPPA, phloretic acid (3-hydroxyphenylpropionic acid); 4HPPA, 4-hydroxyphenylpropionic acid; 4HCA, 4-hydroxycinnamic acid; 3HCA, 3-hydroxycinnamic acid; FA, ferulic acid; HA, hippuric acid.

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