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Original Contribution

Human fecal water content of phenolics: The extent of colonic exposure to aromatic compounds

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Abstract

Phenolic compounds are not completely absorbed in the small intestine and so enter the colon, where they might exert physiological effects. To identify phenolics that are present in normal human colon, fecal water was prepared from 5 free-living volunteers with no dietary restrictions and analyzed by gas chromatography-mass spectrometry. Daily measurements were also performed on a single individual to examine the variation more closely. Levels of polyphenols were variable between individuals. Naringenin and quercetin had mean concentrations of 1.20 and 0.63 μM. All other flavonoids examined were present ≤0.17 μM. Simple phenolic and other aromatic acids were present at much higher concentrations. The major components were phenylacetic acid, 479 μM; 3-phenylpropionic acid, 166 μM; 3-(4-hydroxy)-phenylpropionic acid, 68 μM; 3,4-dihydroxycinnamic acid, 52 μM; benzoic acid, 51 μM; 3-hydroxyphenylacetic acid, 46 μM; and 4–hydroxyphenylacetic acid, 19 μM. Other phenolic acids ranged from 0.04 to 7 μM. Decreased dietary phenolic intake caused a decrease in polyphenol and monophenolic acid concentration in fecal water 24 h later. This study is the first to measure the range of aromatic compounds in human fecal water and demonstrates that phenolic acid concentrations are high. The biological effects of phenolics may play an important role in colon function.

Keywords: Colon; Fecal water; Polyphenol; Phenolic acid; Flavonoid; Gut bacteria; Metabolism; Gas chromatography—mass spectrometry; Free redicals

Introduction

Polyphenolic compounds such as flavonoids are synthesized by plants and often occur as glycosylated derivatives. They are widespread in human food, especially in fruits and vegetables, seeds, nuts, grains, and spices as well as beverages, such as wine and tea. Dietary polyphenol intake has been estimated to range between 0.15 and 1 g/day [1,2], and up to 1 g/day of total phenolics. Flavonoids in vitro have powerful antioxidant activities [3], being able to scavenge a wide range of reactive oxygen, nitrogen, and chlorine species and they may possess other biological activities [4], including an ability to affect signal transduction pathways and to inhibit various enzymes such as cycloxygenases and lipoxygenases, xanthine oxidase, metalloproteinases, drug metabolism enzymes, and telomerase. Epidemiological studies associate foods and beverages rich in flavonoids with decreased risk of age-related diseases [4]. Thus, considerable attention has been paid to dietary flavonoids and their potential role against human disease.

However, although flavonoids and their glycosides can be absorbed through the gastrointestinal tract (GIT) [1,2,5], their uptake is incomplete and circulating levels are low [4–9]. Maximum plasma levels of individual flavonoids achieved rarely exceed 1 μM after consumption of 10–100 mg of a single compound [2] and they undergo rapid phase II metabolism in different tissues, generating methylated, glucuronidated, and sulfated metabolites that have diminished antioxidant activity [5,9]. Hence it is debatable whether plasma levels of flavonoids in vivo are sufficient to exert significant antioxidant effects [4]. Nevertheless it has been argued that the stomach, intestinal lumen, and colon

Abbreviations: OH, hydroxy; MeO, methoxy; Me, methyl; GIT, gastrointestinal tract; GC-MS, gas chromatography-mass spectrometry; EI, electron ionization; BMI, Body Mass Index; COX, cyclooxygenases; BSTFA, bis(trimethylsilyl)trifluoracetamide; TMCS, trimethylchlorosilane.

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may contain substantial levels of unabsorbed phenolics, so that they and their metabolites may play a key role in the antioxidant defense of the GIT [4,10]. This is consistent with epidemiological evidence suggesting that diets rich in fruits and vegetables are associated with decreased risks of gastric, colon, and rectal cancer. Indeed, dietary phenolic intake in healthy individuals has already been associated with an increase in fecal antioxidant capacity [11]. In recent years, many studies have shown that components of the aqueous phase of human feces (fecal water) are able to alter the growth characteristics of colonocytes more effectively than components of the solid phase [12,13]. It is generally considered that fecal water interacts much more with the colonic epithelium than the solid phase and has more influence on the development of colon disease.

In order to begin to assess the biological feasibility of many of the suggested biological effects of phenolics [3,4], it is important to know the concentrations actually present in vivo. We have therefore measured the concentrations of a wide range of phenolics in human fecal water using gas chromatography-mass spectrometry (GC-MS) procedures. We examined not only flavonoids, but also monophenolic and other aromatic acids that have previously been identified during flavonoid metabolism by human intestinal microflora, from either in vitro [14–23] or gnotobiotic animal [24,25] studies. We studied a group of free-living volunteers with no dietary restrictions in order to estimate the widest possible range of phenolics that may be present in normal human colon. To examine the variation more closely, daily measurements were performed on a single individual.

Materials and methods

Subjects

Five male volunteers (4 Asian, 1 Caucasian) from our laboratory had no history of gastrointestinal disease and had not taken antibiotics 6 months prior to the study. Volunteers were all omnivorous nonsmokers, aged between 23 and 37 years with a BMI range of 20.6–22.2 kg/m^2. Dietary questionnaires revealed that all volunteers consumed a midday and evening meal of rice or noodles with meat and vegetables. Breakfast was generally light consisting of bread and coffee/tea or milk and 2 subjects ate no breakfast. Average daily fruit/vegetable consumption was 3.4 portions/day (range 1–6) and all subjects consumed at least one portion. In order to monitor the influence of daily changes in an omnivorous diet, fecal water samples were prepared from feces collected in four 24-h periods (starting on Wednesday 9 AM) from a male, Caucasian 37-year-old nonsmoker. The volunteer had regular bowel movements approximately every 24 h throughout the study and an approximate transit time of 19 h was calculated based on the appearance of a nondigestable marker dye (carmine red) in the stool. The

Table 1
Retention times and characteristic ions used for selected-ion monitoring of trimethylsilylated derivatives

Phenolic compound	Retention time (min)	Base ion (*m/z*)	Qualifier ion (*m/z*)
4-Ethyl phenol	3.40	179	194
Benzoic acid	3.50	179	105
Phenylacetic acid	4.10	164	193
catechol (1,4-diOH benzene)	4.35	254	239
3,4,5-triMeO benzene	5.35	239	254
1,3,5-triMeO benzene	5.41	168	125
3-Phenylpropionic acid	5.50	104	222
4-Ethyl benzoic acid	6.05	163	133
2-OH benzoic acid	7.02	267	135
4-MeO benzoic acid	7.17	135	165
4-OH,3-Me benzaldehyde (vanillin)	7.42	194	224
t-Cinnamic acid	7.55	205	161
1,2,4 or 1,2,3-triOH benzene	7.75	239	342
3-OH benzoic acid	8.02	267	282
3-OH phenylacetic acid	8.80	296	191
3,4-diOH benzaldehyde	8.95	267	282
4-OH benzoic acid	9.09	267	223
3-(3-MeO phenyl)-propionic acid	9.30	134	237
4-OH phenylacetic acid	9.40	179	296
1,3,5-triOH benzene (phloroglucinol)	9.66	342	327
2-OH,5-MeO benzoic acid (Internal Standard)	10.59	297	223
3,4-diMeO phenylacetic acid	11.00	209	268
2,3-diOH benzoic acid	11.67	355	193
3-(4-OH-phenyl)-propionic acid	12.10	192	310
4-OH,3-MeO benzoic acid (vanillic acid)	12.25	297	312
3-OH,4-MeO benzoic acid (isovanillic acid)	12.45	297	312
4-OH,3-MeO phenylacetic acid (homovanillic acid)	12.53	326	311
2,5-diOH benzoic acid	12.63	355	267
3,5-diOH benzoic acid	13.75	355	370
3,4-diOH benzoic acid	13.85	370	193
3,4-diOH phenylacetic acid	14.02	384	267
3,4-O-diMe gallic acid	14.46	342	312
4-O-Me gallic acid	14.90	370	400
4-OH,3,5-diMeO benzoic acid	15.05	327	342
2,3,4-triOH benzoic acid	15.34	443	355
4-OH cinnamic acid (p-coumaric acid)	15.43	293	308
3-(3,4-diOH phenyl)-propionic acid	15.61	398	267
3-O-Me gallic acid	15.69	400	385
3-(3,4,5-triMeO phenyl)-propionic acid	15.80	312	297
3,4,5-triMeO cinnamic acid	15.89	310	295
3,4,5-triOH benzoic acid (gallic acid)	15.97	458	443
4-OH,3-MeO cinnamic acid (ferulic acid)	16.84	338	308
3-OH,4-MeO cinnamic acid (isoferulic acid)	16.96	338	323
3,4-diOH cinnamic acid (caffeic acid)	17.33	396	381
2,4,5-triMeO cinnamic acid (Internal Standard)	17.75	279	310
3,5-diMeO, 4-OH cinnamic acid	18.00	368	338
6-methylflavone (Internal Standard)	18.50	236	134
Resveratrol	20.56	444	429