JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Digestion and Absorption of Ferulic Acid Sugar Esters in Rat Gastrointestinal Tract

ZHAOHUI ZHAO,* YUKARI EGASHIRA, AND HIROO SANADA

Laboratory of Food and Nutrition, Graduate School of Science and Technology, Chiba University, 648 Matsudo, Matsudo, Chiba 271-8510, Japan

We estimated the absorption site and absorptivity of ferulic acid (FA) and its sugar esters, namely 5-*O*-feruloyl-L-arabinofuranose (FAA) and feruloyl−arabinoxylan (FAXn), in rats on the basis of their recovery in intestinal content and feces by comparing the values with those of a nonabsorbable marker, poly R-478. The results indicated that free FA was absorbed almost completely before reaching cecum. About 40% of dietary FAA was absorbed in rat foregut and 57% disappeared in the cecum. In contrast, about 67% of the FA moiety in FAXn was released and then disappeared predominantly in the hindgut. These results suggested that the existing form of FA in diets affects its absorptivity, its absorption site, and its ensuing fate in the gastrointestinal tract. Those ingested FAs esterified with saccharides; especially, polysaccharides have to transit the hindgut where FA might be released and then absorbed and/or degraded by microflora in lumen. Such microbial degradation may be an important factor affecting the bioavailability of dietary FA.

KEYWORDS: Absorption; bioavailability; ferulic acid ester; poly R-478; rat; antioxidant

INTRODUCTION

A common understanding for most researchers that study the bioavailability of dietary FA is necessary because some in vitro and epidemiological studies (*1*−*4*) indicate that dietary FA may be important in the prevention of chronic disease. Many in vivo studies have estimated the bioavailability of free FA or FA in foodstuffs on the basis of urinary data (*5*−*8*) or both urine and plasma data (*9*−*12*). The bioavailability of FA and its sugar esters, namely FAA and FAXn, appears dependent on the absence or presence of the saccharide moiety and, in the later case, its structure (*12*). Comparing the fecal and urinary recovery of FA in wheat (wheat bran and whole wheat flour) with that of free FA shows that the cereal matrix appears to severely limit FA bioavailability (*9*). Nevertheless, the mechanism of effect of the food matrix on the bioavailability of dietary FA, specifically, how the structural complexity affects the absorptivity of dietary FA, is not completely clear as yet. According to the analysis of metabolites in urine and the metabolic studies in ruminants, Chesson et al. (*13*, a review article) presumed that the form of the phenolic acid and its location in the plant affect its fate following ingestion by mammals and bound acids are released only after extensive microbial attack at the modified forestomach of ruminants and the hindgut of nonruminant species. Studies (*14*−*16*) show that a more complex substrate with a high level cross-linking through diferulic bridges and ester-linked FA may inhibit the binding of the necessary enzymes, which contribute to the release of FA. Adam et al. (*9*), accordingly, ascribed the lower bioavailability of FA in wheat foods than that of free FA to the reduced accessibility of enzymes caused by the food matrix. Such an explanation, however, cannot answer the question that FA in wheat foods is so poorly recovered in rat urine (3.6−3.9%) despite the results indicated that most of them (62−79%) disappeared in rat gut in the same study (*9*). Furthermore, FAA, a typical FA sugar ester in cereals (*16*−*21*), also shows a lower bioavailability in rats than FA (*12*) although its structure does not appear so complex.

Analysis of urinary metabolites of hydroxycinnamates suggests that the degradation of FA by microflora seems to occur in the hindgut (*13*). An in vitro study (*22*) also shows that the released FA from wheat bran may be either utilized by microorganisms or transformed to other phenolic forms in the human colon. These studies imply that the metabolism of FA by microflora in the hindgut may be another important factor that affects the bioavailability of dietary FA. To prove this point, the information on the absorption site and the absorptivity of dietary FA are necessary. Concerning these points, however, little has been reported.

Furthermore, FAXn, FAA, and feruloyl−arabinofuranosyl−xylopyranosyl−xylose (a feruloylated oligosaccharide) show the same or even stronger antioxidative activities than FA in the low density lipoprotein (LDL) oxidation system (*18*) or in the microsomal lipid peroxidation system (*23*). Our pervious study (*12*) has shown that administered FA and its sugar esters are recovered in rat plasma and urine mainly as FA−sulfoglucuronide. As natural antioxidants, the fate of these FA sugar esters in the gastrointestinal tract needs to be investigated in detail.

By comparing a compound's concentrations along the gastrointestinal tract alone, reliable quantitative information about

* To whom correspondence should be addressed. Tel: 81-47-308-8861. Fax: 81-47-308-8859. E-mail: zhaohuizhao@hotmail.com.

10.1021/jf034455u CCC: $25.00 © 2003 American Chemical Society
Published on Web 08/01/2003

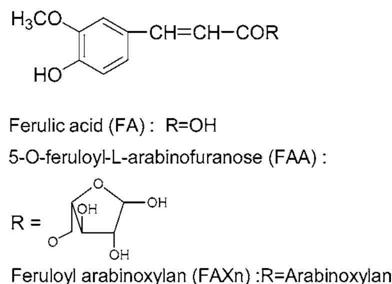


Figure 1. Chemical structure of FA and its sugar esters prepared form corn bran cell wall.

the change of these compounds in the intestine cannot be provided. Thus, nonabsorbable reference markers are usually required in many intestinal absorption studies, as changes in concentration of absorbate relative to that of marker permit quantitation of absorbate movement (*24*). Poly R-478, a stable polymeric food dye, has been proven to be an effective and easily measured nonabsorbable marker for use in both animal and human intestinal absorption studies (*24*−*26*). In this study, we estimated the amount of dietary FA that remained in intestinal content and feces with the aid of poly R-478 after FA, FAA, and FAXn were fed to rats. The purpose was to determine the absorptivity and absorption site of the dietary FA in rats and to provide in vivo data for explaining the mechanism of the effect of food matrix on the bioavailability of dietary FA. The results showed that the structural complexity affects the absorptivity, the absorption site, and the ensuing fate of dietary FA in the gastrointestinal tract. The degradation of FA in the food matrix by microflora in the hindgut may be an important factor affecting the bioavailability of dietary FA.

MATERIALS AND METHODS

Chemicals. Poly R-478 (68550-77-6) and β-glucuronidase (EC3.2.1.3 1) type H-2 were from Sigma Chemical Co. (St. Louis, MO). FA, salicylic acid, and other chemicals were analytical or high-performance liquid chromatography (HPLC) grade (Wako Pure Chemical Industries, Osaka, Japan).

Preparation of FA Sugar Esters. FAA and FAXn (**Figure 1**) were prepared from refined corn bran as described previously (*18*, *23*). Briefly, the hydrolysate of refined corn bran (Nihon shokuhin Kakou Co., Ltd.) was fractionated on Sephadex LH-20 after it was hydrolyzed in 30 mmol/L oxalic acid solution (121 °C, 60 min). FAA and FAXn were collected from the low or the high molecular weight fractions, respectively, according to the thin-layer chromatography and the UV absorbance (320 nm) of each fraction. FAA was condensed under vacuum. FAXn was condensed, lyophilized, and ground to powder.

Animals and Diets. Twenty 5 week old Wistar male rats purchased from CLEA Japan (Tokyo, Japan) were housed in an air-conditioned room (22 ± 2 °C) with a dark period from 19:00 to 7:00. They were fed with a commercial diet (type CE-2, CLEA Japan) for 3 days and then with the blank diet (**Table 1**) for 11 days. Whereafter, the rats were divided into five groups and fed designated diets (**Table 1**) for 9 days. All rats were fed by the method of "meal feeding" (two meals per day, during 7:00−8:00 and 19:00−20:00) and allowed free access to water during the entire experimental period. Feces were collected in the last 2 days of the experiment. At 10:00 o'clock on the last day, blood was drawn from their heart with a heparinized syringe under pentobarbital anesthesia. Then, the hind ileum (20 cm long proximal to cecum) and the cecum were removed from the rats. After they were weighed, their contents were drained into tubes. Plasma was prepared by centrifuging the blood at 2000*g* for 15 min at 4 °C. Feces and contents were lyophilized and ground to powder. All of the samples were stored frozen at −30 °C until required for analysis. The care and treatment of the rats were carried out according to the guidelines prescribed by the Faculty of Horticulture, Chiba University, and the National Institutes of Health Guide for the care and use of laboratory animals (*27*).

Table 1. Composition of Diets (g/kg)

constituents	blank	control	FAXn	FAA	FA
casein	200	200	200	200	200
corn oil	50	50	50	50	50
vitamin mixture[a]	10	10	10	10	10
salt mixture[a]	35	35	35	35	35
choline bitartrate	2	2	2	2	2
DL-methionine	3	3	3	3	3
cellulose	50	49.5	0	47	48
sucrose	500	500	500	500	500
corn starch	150	150	140	150	150
test substance[b]			59.5	2.5	1.5
poly R-478[c]		0.5	0.5	0.5	0.5

[a] AIN-76. [b] A diet of FAXn, FAA, and FA contained equimolar amounts of total FA (7.72 mmol FA/kg diets). FAA was added to the diet as an aqueous solution. [c] Nonabsorbable marker.

Determination of Total FA in Plasma. A 50 μL amount of plasma was acidified (to pH 5.0) with acetic acid, to which was added 10 μL of 2 mmol/L salicylic acid aqueous solution as internal standard (IS). To this sample solution, 5 μL of β-glucuronidase type H-2 solution (with 500 units of β-glucuronidase and 25 units of sulfatase) was then added. After the solution was saturated with nitrogen gas and stoppered, the mixture solution was incubated at 37 °C for 2.5 h in a shaking water bath. The FA in the incubated solution was extracted with 0.43 mL of 0.05 mol/L HCl−ethanol by the following procedure: vortexed for 40 s, sonicated for 60 s, again vortexed for 40 s, and then centrifuged at 10 000*g* for 10 min at 4 °C. A 10 μL (20 μL if necessary) amount of the supernatant was applied for HPLC analysis.

Determination of Total FA in Intestinal Contents and Feces and FA Sugar Esters. The amount of total FA (all forms of FA) in intestinal contents and feces was determined as the free form by use of HPLC after the sample was hydrolyzed with NaOH aqueous solution according to the method of Shibuya (*28*) with modification. The sample (0.02 g) was extracted with 0.5 mol/L NaOH (2 mL) at 60 °C for 2.5 h under a stream of N_2. The collected extract was acidified to pH 3.5 with 2 mol/L HCl and then extracted with 2 mL of ethyl acetate three times. The extract was then evaporated to dryness at 30 °C with a stream of N_2. To the dry residues, 1 mL (10 mL if necessary) of mobile phase A (described below) was added. The solution was then filtered with a 0.45 μm membrane filter, and 10 μL of the filtrate was applied to HPLC. The same method was used for determining the concentration of total FA in FA sugar esters.

Determination of Free FA and FAA in Intestinal Contents and Feces. About 0.02 g of the lyophilized intestinal content or feces was extracted with 0.5 mL of acetate buffer (0.1 mol/L, pH 5.0, including 0.5 mmol/L of salicylic acid as IS). A 50 μL aliquot of the extract was then treated by the same procedure as that used for treating the extract prepared by the NaOH aqueous solution in the previous section except that 0.5 mL of ethyl acetate was used and 1 mL of mobile phase A was added to the dry residues. Then, 20 μL of the supernatant (10 μL if necessary) was applied for HPLC analysis after being filtered.

HPLC Analysis. The conditions for HPLC analyses were the same as that used previously (*12*) except the mixing program of mobile phases: solvent A (20% methanol in 5mmol/L HCl) and solvent B (acetonitrile) were mixed using a linear gradient apparatus by changing solvent B as follows: 0 (0 min) → 3 (5 min) → 15 (15 min) → 25 (22 min) → 0 (26 min) → 0% (30 min). Identifications of the compounds were confirmed by comparing retention times and absorption spectra to those of standard materials. Quantification was accomplished using calibration of the standards.

Theory for Evaluating the Fraction Absorbed by the Aid of Nonabsorbable Marker (Poly R-478). The fractional absorption of FA and its sugar esters was evaluated from the ratio of the recovery of dietary total FA in rat gastrointestinal tract to that of poly R-478 in the same site (the relative fraction of total FA remained). According to

the theoretical analysis of Yuasa et al. (29) on the evaluation of fractional absorption of D-xylose, the FR in the gastrointestinal site can be defined as follows:

$$FR = (C_x/C_d)/(P_x/P_d) \times 100\% \quad (1)$$

where C_x and C_d are the concentrations of total FA in the content of the X site in gastrointestinal tract and in the diet, respectively, and P_x and P_d are the concentrations of poly R-478 in the content of the X site and in the diet, respectively. Consequently, the FD in the gastrointestinal tract before the bolus reaching the X site can be given as follows:

$$FD = 100 - FR \quad (2)$$

Here, when the dietary FA is stable in the gastrointestinal tract, FD can be referred to as the absorptivity of the dietary FA.

The amount of poly R-478 in intestinal content and feces was determined by the method of Dupas et al. (24) and Stahl et al. (25) with modification as follows: 0.02 g of lyophilized intestinal content sample was suspended in 2 mL of 0.9% NaCl solution and then was mixed and centrifuged (1200g, 15 min) to get the soluble supernatants. About 1.5 mL of supernatants was centrifuged again at 15 000g for 60 min and then applied to a spectrophotometer (UV-12000 Shimadzu, Japan) at 520 nm. All samples were read against blank solutions that were identically prepared from the intestinal content of the blank group rats. Quantification of poly R-478 was accomplished using calibration of the standards.

Statistical Analysis. Data are shown as means ± standard deviation (SD) ($n = 4$). Tukey's multiple range test was used when significant differences were obtained by one way analysis of variance. The significance was set at $P < 0.05$.

RESULTS

Effects of Feeds on Growth of Rats. Rats fed for 9 days with the diet containing FA or its sugar esters together with nonabsorbable marker (poly R-478) did not differ in food intake, body weight gain, or eviscerated carcass weight (data not shown) from those of the blank group rats, which were fed with standard diet based on AIN-76 (**Table 1**). However, the rat fed the FAXn diet appeared to have a slight symptom of diarrhea since day 7, and the weight of its cecum together with content was approximately 3.1 times that of other groups on day 9 (data not shown).

Plasma Concentration of Total FA. Total FA was not detected in the plasma from the rats of the blank or control groups. The plasma concentrations of total FA in rats fed with FA diet and FAA diet were 11 ± 3 and 12 ± 4 μmol/L ($n = 4$), respectively. They were about 10 times that in rats fed with the FAXn diet.

FR in the Different Sites along the Gastrointestinal Tract. About 0.01−0.03 mg total FA/g dry content was detected in intestinal content and feces from the rats of the control group. For the FA diet group, 0.1 ± 0.1 mg total FA/g dry content ($n = 4$) was detected in the ileum but the total FA concentration in the cecal content or in the feces was as low as that detected in the control group. By comparing the recovery of dietary FA with that of nonabsorbable marker (poly R-478), we got the FR in different sites of intestine and in feces (**Figure 2**). It appeared that only 1.6% of FA in the FA diet remained in the content of the ileum. In contrast, 60% of total FA in the FAA diet could reach the ileum but only 3% remained after going through the cecum. The amount of total FA in the FAXn diet appeared to have almost no change during passage to the rat ileum. Its relative fraction that remained in cecal content was 56% and that in feces was about 33%.

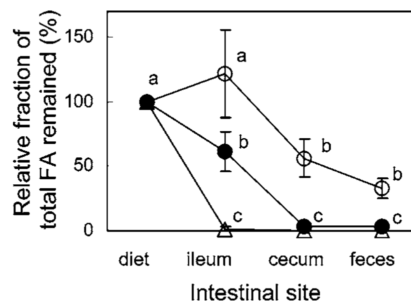


Figure 2. FR in the intestinal tract and feces of rats fed diets containing FAXn (○), FAA (●), or FA (△) with equimolar amounts of total FA (7.72 m mol FA/kg diets). FR was the ratio of the recovery of dietary total FA (all forms of FA) to that of dietary nonabsorbable marker (poly R-478) along intestinal tract (see eq 1 in the Materials and Methods). Values are means ± SD, $n = 4$. Means in each group without a common letter differ significantly, $P < 0.05$. See **Table 1** for the composition of diets. Ileums were sampled the posterior part (20 cm long proximal to cecum).

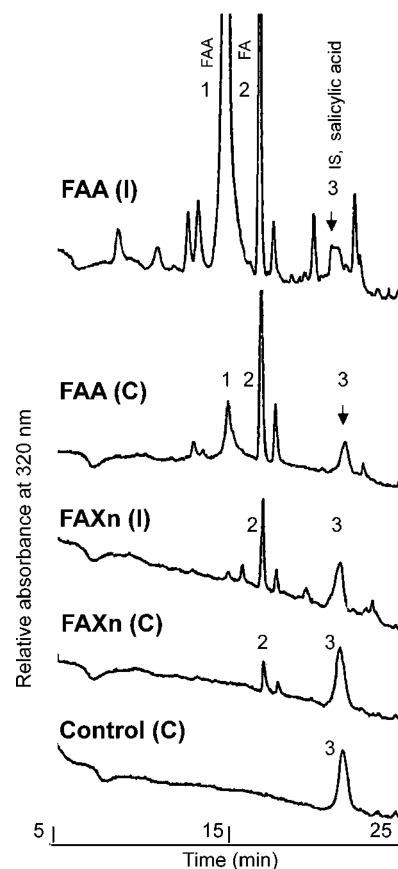


Figure 3. Representative HPLC–UV (320 nm) chromatograms of acetate buffer extracts from the ileal (I) or cecal (C) contents of rats fed with FAA diet, FAXn diet, or control diet (control). See **Table 1** for the composition of diets. A 10 μL amount of injection samples (FAA diet group) and 20 μL of injection samples (FAXn and control groups) were applied to HPLC.

Concentration of Free FA and FAA in the Intestinal Content and Feces of Rats Fed with FAA or FAXn Diet. To know whether and where the ingested FA sugar esters are digested in the rat gastrointestinal tract, free FA in the contents and feces was determined (**Figure 3**). For the FAA diet group, about 6 and 11% of total FA was detected as free FA in the extractable fraction of ileal and cecal content, respectively (**Table 2**). In the ileal content from the rats of the FAXn diet group, only 0.3% of total FA was detected as free FA. This

Table 2. Concentrations of Free FA and FAA in the Acetate Buffer Extractable Fraction of Intestinal Content and Feces of Rats Fed with Diets Containing FAA or FAXn[a]

	mg FA/g dry intestinal content or feces		
	free FA	free FAA	total FA
		FAA	
ileum[b]	0.84 ± 0.04 (6%)[c]	2.04 ± 0.17 (15%)	14.06 ± 0.33 (100%)
cecum	0.20 ± 0.03 (11%)	0.12 ± 0.06 (6%)	1.81 ± 0.31 (100%)
		FAXn	
ileum[b]	0.07 ± 0.01 (0.3%)	ND[d]	25.81 ± 4.82 (100%)
cecum	0.11 ± 0.03 (0.7%)	ND	15.70 ± 3.64 (100%)
feces	0.13 ± 0.05 (2%)	ND	6.55 ± 2.38 (100%)

[a] See **Table 1** for composition of diets. Values are means ± SD, $n = 4$. [b] The posterior part of the ileum, 20 cm long proximal to cecum. [c] %, the percentage of the concentration of free FA or FAA to that of total FA in the sample. [d] ND, not detected (<0.02 mg/g dry intestinal content or feces).

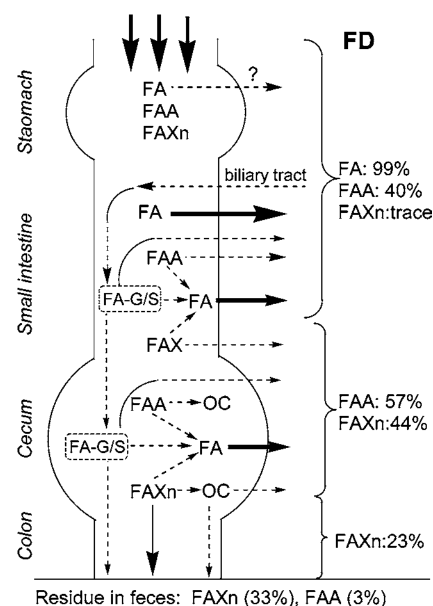
proportion increased to 0.7% in cecal content and to 2% in feces from the same group (**Table 2**).

DISCUSSION

Method Availability for Evaluating Fraction Absorbed. In this work, we evaluated the absorption of FA and its sugar esters along with the rat gastrointestinal tract by comparing the recovery of dietary total FA with that of nonabsorbable marker, i.e., poly R-478, in the intestinal content (**Figure 2**). Because FA might be metabolized into other compounds by intestinal flora in the hindgut (*13*), such an evaluation may not be able to accurately reflect FA absorptivity. Microbial population, however, is negligible (although low numbers are present in the ileum) in the rat foregut (*13*). Accordingly, the value of FD (eq 2 in the Materials and Methods) in the foregut can provide a direct evaluation of FA absorbed and that in the hindgut and feces can provide information on FA disappearance that would implicate its absorption potential together with microbial metabolic change. We did not consider the effect of the enterohepatic circulation on the absorptivity because we sampled the intestinal content from the cecum and the posterior of the ileum located far enough from the biliary entry. It should be noted that the color of the poly R-478 solution extracted from the cecal content and feces of the FAXn diet group was a slightly different color from that of standard solution; that effect on the determination of the dye concentration was not checked. This color change might be caused by the fermentation that occurred in the cecum of rats fed with the FAXn diet.

Absorption Pathway of FA Sugar Esters. We have presumed (*12*) that two metabolic pathways may exist for FAA metabolism in rats. One is that FAA is first hydrolyzed into FA and then undergoes the metabolic pathway of FA, and another is that FAA is absorbed directly and then hydrolyzed and metabolized in mucosal cells and/or in the circulation. Andreasen et al. have reported the presence of esterases with activity on FAA in cell free intestinal extracts (*30*). In the present study, free FA was detected in both ileal and cecal content from the rats of the FAA diet group (**Table 2**, **Figure 3**) indicating that FAA digestion may take place in lumenal content. These results support the presumption on the first metabolic pathway. The second one has been given an adequate discussion in our previous study. Accordingly, both of the two metabolic pathways might jointly work for FAA metabolism in rats, although further verifying studies in vivo on FAA metabolism are needed. It should be noted that only 17−21% of total FA in intestinal content was detected as free form of FA and FAA in the acetate

Scheme 1. Proposed Scheme of Possible Digestion and Absorption of FA Sugar Esters in Rat[a]



[a] Abbreviations: G, glucuronide moiety; S, sulfate moiety; OC, other compounds (not FA or FA conjugates). The arrows with dotted lines mean presumed fluxes. FD was calculated from FR in **Figure 2** (according to eq 2).

buffer extractable fraction in this study (**Table 2**). The rest, which might be in conjugated forms and/or in some forms in the particulate fraction of rat intestinal content, would be required for further investigation.

For the FAXn diet group, a trace of free FA was detected in ileal content. It may be from the hydrolysis of conjugated FA, which was excreted in bile. Adam et al. (*9*) have shown that about 6.2% of perfused FA in small intestine is excreted in bile in the form of conjugated FA, and we found in preliminary experiments that conjugated FA could be release as free FA when incubated (37 °C, 2.5 h) with the intestinal content. By contrast with that in ileal content, an appreciable amount of free FA was detected in cecal content and in feces (**Table 2**, **Figure 3**). This indicated that FAXn may first be digested to release free FA in cecum by microflora and consequently partly absorbed in the form of free FA. Nevertheless, the results in our previous study (*12*) show that FAXn absorption may also occur in the foregut, which was ascribed to the existence of the same metabolic mode of FAX in the FAXn mixture as that of FAA. In this study, however, the FR value in the ileum (**Figure 2**) did not suggest that any FAXn is absorbed in the foregut. This may be explained by the fact that FAX is not thought to be the main compound in the FAXn mixture, so that the amount of FAXn absorbed in the foregut might not be enough to affect the FR value.

Relation between Absorption Site and Absorptivity of Free FA and Its Sugar Esters. That only 1.6% of FA in diet was recovered in ileum (**Figure 2**) indicated that ingested FA was almost absorbed in the gastrointestinal tract before the bolus reaching the cecum (**Figure 2**, **Scheme 1**), and thus, its absorptivity is equal to the FD (over 99%) according to the discussion on method availability (above). This result is consistent with that of an in vitro study (*31*), which shows that perfused FA is absorbed predominantly as free form and the absorptivity through rat jejunum is 8-fold of that through ileum. These results support the conclusion that free FA has a very high bioavailability, which was evaluated on the basis of the high urinary recovery of FA and the high total FA plasma

Table 3. Summary of the FD in Gastrointestinal Tract of Rats or in Model Human Gut System[a]

source of dietary FA	quantity of total FA ingested	FD (%)	recovery in urine or fermented solution[b] (%)	degradation rate[c] (%)	ref
		in vivo system (in rats): single oral administration to rats			
free FA	70 μmol/kg body	99.4	72.0	27.4	*12*
FAA	70 μmol/kg body	98.7	54.0	44.7	*12*
FAXn	70 μmol/kg body	80.0	20.0	60.0	*12*
		in vivo system (in rats): feed to rats in diet			
free FA	62.7 μmol/day	99.2	51.8	47.4	*9*
wheat bran	73.7 μmol/day	62.0	3.9	58.1	*9*
whole wheat flour	81.2 μmol/day	79.0	3.6	75.4	*9*
		in model human gut system: fermentation for 24 h			
coarse wheat bran	1.68 mg in 350 mg of feces[d]	81.0	<4	>77.0	*22*
fine wheat bran	1.87 mg in 350 mg of feces[d]	57.2	<3	>54.2	*22*

[a] FD was defined as the amount of unrecovery in feces (in vivo system) or in residue (in model human gut system). It was calculated from the data of literature. [b] Recovery in urine (in vivo system) or fermented solution (in model human gut system). [c] Degradation rate, the proportion of that being metabolized into other compounds (not FA or FA conjugates). The amount of recovery in urine or in fermented solution subtracted from that of FD was referred to as the amount of degradation rate. [d] Quantity of total FA incubated in model human gut system.

concentration (*12*). Whether ingestion with diet (*9*) or single oral administration (*12*), a considerable amount of ingested FA was not recovered in feces or in urine (see **Table 3**). Adam et al. (*9*) ascribed the disappeared FA to the degradation of FA by the microflora; our results here, however, implied that that might be mainly due to metabolizing in the circulation system but not in the lumen because 99% of ingested FA disappeared in the foregut where microflora are absent. It has been shown that absorbed FA could be metabolized into other phenolic compounds in human circulation (*32*).

The amount of total FA in the FAA diet decreased to about 60% after reaching the hind ileum and sequentially decreased to about 3% in the cecum (**Figure 2**). Such results indicate that about 40% of the total FA in ingested FAA might be absorbed in the foregut (**Scheme 1**), and the others reach cecum where most of them disappeared at last. For the FAXn diet group, the value of FR did not change almost in the foregut but decreased to 56% in cecum and further decreased to about 33% in the feces (**Figure 2**), suggesting that FAXn mainly disappears in the hindgut (**Scheme 1**). That the rat cecum together with its content of this group was significantly heavier than that of other groups suggested that the FAXn diet could induce intensive fermentation in rat cecum.

Although about 57.2−81% of feruloyl groups were released from the wheat bran cell wall after 24 h of fermentation in a model human gut system (*22*, see **Table 3**), only <5% of that released was detected in the fermentation solution. Because it was tested in vitro, that free FA does not appear to bind noncovalently to wheat bran fiber; the FA released from the fiber is either rapidly utilized by gut microorganisms or transformed to other phenolic forms (*22*). In the present work, about 60% of total FA in ingested FAA reached the cecum where they may encounter the degradation by microorganisms. Thus, we presumed that part of those disappeared FAA in the cecum might share the same fate of those feruloyl groups released from wheat bran in the gut model fermentation. This might answer the question that bioavailability of oral administered FAA was only 56% of that of oral administered FA, despite the fact that they were administered to rats at an equimolar amount and their fecal recoveries were also equal (*12*, see **Table 3**). Because FAXn diet caused intensive fermentation in rat cecum and FAXn stayed longer in the hindgut than FAA did, FA moiety in FAXn may be metabolized into other compounds (not FA or FA conjugates) by gut microflora easier than that of FAA. This may explain that the degradation rate (unrecovered total FA either in feces or in urine) for the FAXn diet group was 15% larger than that for the FAA diet group (*12*, see **Table 3**). For those FA in whole wheat flour or wheat bran, the fact that they have to transit the whole gut after being ingested by rats may decide their same fate in the hindgut as that of FA in FAXn. Therefore, their plasma and urinary recovery in rat was very poor although the results showed that most of them (62−79%) disappeared in the gut (*9*, see **Table 3**). Taken together, the results shown here and elsewhere (*9*, *12*, *13*, *22*) have indicated the mechanism of effect of structural complexity on the absorptivity and ensuing bioavailability of dietary FA in rat. That is, free FA and a part of simple FA sugar esters (e.g., FAA) could absorb directly in the foregut causing a higher absorptivity; feruloylated polysaccharides together with the part of simple FA sugar esters not absorbed in the foregut have to transit the hindgut where FA is absorbed after being released by microflora while part of FA may be metabolized into other compounds (not FA and FA conjugates) by microflora. Whereafter, all of the absorbed feruloyl groups (wherever they are from) have to go through the first pass metabolism by both intestinal and hepatic enzymes before they enter the circulation. In one word, the effect of structural complexity on the fate of dietary FA in the gastrointestinal tract was induced by influencing their absorption sites: free FA and FA in simple bound forms may avoid the degradation by microflora for they may be absorbed in the foregut before encountering the microflora, but FA bound with polymers can not be absorbed in the foregut and thereby is possibly degraded by microflora in the hindgut. On this basis, microbial degradation of FA in the gut may be one of the important factors affecting the bioavailability of dietary FA.

ABBREVIATIONS USED

FA, ferulic acid; FAA, 5-*O*-feruloyl-L-arabinofuranose; FAX, feruloyl−arabinosyl−xylose; FAXn, feruloyl−arabinoxylan; total FA, all forms of FA, containing free FA and all kinds of its derivatives; FR, the relative fraction of total FA remained in gastrointestinal site; FD, the fraction of dietary total FA disappeared from rat gastrointestinal tract.

NOTE ADDED AFTER ASAP

Reference 12 in the original ASAP posting of August 1, 2003, contained an incorrect volume number, which has now been corrected.

LITERATURE CITED

(1) Slavin, J. L. Whole grains, refined grains and fortified refined grains: what is the difference? *Asia Pac. J. Clin. Nutr.* **2000**, *9*, S23−S27.

(2) Bravo, L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutr. Rev.* **1998**, *56*, 317−333.

(3) Kikuzaki, H.; Hisamoto, M.; Hirose, K.; Akiyama, K.; Taniguchi, H. Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.* **2002**, *50*, 2161−2168.

(4) Kawabata, K.; Yamamoto, T.; Hara, A.; Shimizu, M.; Yamada, Y.; Matsunaga, K.; Tanaka, T.; Mori, H. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. *Cancer Lett.* **2000**, *157*, 15−21.

(5) Choudhury, R.; Srai, S. K.; Debnam, E.; Rice-Evans, C. Urinary excretion of hydroxycinnamates and flavonoids after oral and intravenous administration. *Free Radical Biol. Med.* **1999**, *27*, 278−286.

(6) Bourne, L. C.; Rice-Evans, C. Bioavailability of ferulic acid. *Biochem. Biophys. Res. Commun.* **1998**, *253*, 222−227.

(7) Bourne, L.; Paganga, G.; Baxter, D.; Hughes, P.; Rice-Evans, C. Absorption of ferulic acid from low-alcohol beer. *Free Radical Res.* **2000**, *32*, 273−280.

(8) Virgili, F.; Pagana, G.; Bourne, L.; Rimbach, G.; Natella, F.; Rice-Evans, C.; Packer, L. Ferulic acid excretion as a marker of consumption of a French maritime pine (*pinus maritima*) bark extract. *Free Radical Biol. Med.* **2000**, *28*, 1249−1256.

(9) Adam, A.; Crespy, V.; Levrat-Verny, M. A.; Leenhardt, F.; Leuillet, M.; Demigné, C.; Rémésy, C. The bioavailability of ferulic acid is governed primarily by the food matrix rather than its metabolism in intestine and liver in rats. *J. Nutr.* **2002**, *132*, 1962−1968.

(10) Rechner, A. R.; Kuhnle, G.; Bremner, P.; Hubbard, G. P.; Moore, K. P.; Rice-Evans, C. The metabolic fate of dietary polyphenols in humans. *Free Radical Biol. Med.* **2002**, *33*, 220−235.

(11) Rondini, L.; Peyrat-Maillard, M. N.; Marsset-Baglieri, A.; Berset, C. Sulfated ferulic acid is the main in vivo metabolite found after short-term ingestion of free ferulic acid in rats. *J. Agric. Food Chem.* **2002**, *50*, 3037−3041.

(12) Zhao, Z.; Egashira, Y.; Sanada, H. Ferulic acid sugar esters are recovered in rat plasma and urine mainly as the sulfoglucuronide of ferulic acid. *J. Nutr*. **2003**, *133*, 1355−1361.

(13) Chesson, A.; Provan, G. J.; Russell, W. R.; Scobbie, L.; Richadson, A. J.; Stewart, C. Hydroxycinnamic acids in the digestive tract of livestock and humans. *J. Sci. Food Agric.* **1999**, *79*, 373−378.

(14) Grabber, J. H.; Hatfield, R. D.; Ralph, J. Diferulate cross-links impede the enzymatic degradation of nonlignified maize walls. *J Sci. Food Agric.* **1998**, *77*, 193−200.

(15) Bunzel, M.; Ralph, J.; Marita, J.; Hatfield, R. D.; Steinhart, H. Diferulates as structural components in soluble and insoluble cereal dietary fibre. *J. Sci. Food Agric.* **2001**, *81*, 653−660.

(16) Faulds, C. B.; Kroon, P. A.; Saulnier, L.; Thibault, J. F.; Williamson, G. Release of ferulic acid from maize bran and derived oligosaccharides by *Aspergillus niger* esterases. *Carbohydr. Polym.* **1995**, *27*, 187−190.

(17) Saulnier, L.; Vigouroux, J.; Thibault, J. F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res.* **1995**, *272*, 241−253.

(18) Ohta, T.; Semboku, N.; Kuchii, A.; Egashira, Y.; Sanada, H. Antioxidative activity of corn bran cell-wall fragments in theLDL oxidation system. *J. Agric. Food Chem.* **1997**, *45*, 1644−1648.

(19) Clifford, M. N. Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden. *J. Sci. Food Agric.* **1999**, *79*, 362−372.

(20) Kato, Y.; Nevins, D. J. Isolation and identification of *O*-(5-*O*-Feruloyl-α-L-arabinofuranosyl)-(1→3)-*O*-*β*-D-xylopyranosyl-(1→4)-D-xylopyranose as a component of *Zea* shoot cell walls. *Carbohydr. Res.* **1985**, *137*, 139−150.

(21) Saulnier, L.; Thibault, J. F. Ferulic acid and diferulic acids as components of sugar-beet pectins and maize bran heteroxylans. *J. Sci. Food Agric.* **1999**, *79*, 396−402.

(22) Kroon, P. A.; Faulds, C. B.; Ryden, P.; Robertson, J. A. Williamson, G. Release of Covalently bound ferulic acid from fiber in the human colon *J. Agric. Food Chem.* **1997**, *45*, 661−667.

(23) Ohta, T.; Yamasaki, S.; Egashira, Y.; Sanada, H. Antioxidative activity of corn bran hemicellulose fragments. *J. Agric. Food Chem.* **1994**, *42*, 653−656.

(24) Dupas, J. L.; Moreau, M.; Hofmann, A. F. Polymeric dyes: useful nonabsorbable reference markers for intestinal perfusion studies in animals. *J. Pharm. Sci.* **1985**, *74*, 328−330.

(25) Stahl, G. E.; Fayer, J. C.; Ling, S. C.; Watkins, J. B. Comparison of nonabsorbable markers poly R-478 and [^{14}C]PEG-4000 for use in developmental absorption studies. *J. Pediatr. Gastroenterol. Nutr.* **1991**, *12*, 485−493.

(26) Walson, P. D.; Carter, D. E.; Ryerson, B. A.; Clark, D. Intestinal absorption of two potential polymetric food additives in man. *Food Cosmet. Toxicol*. **1981**, *19*, 687−690.

(27) National Research Council. *Guide for the Care and Use of Laboratory Animals*; Publication No. 85-23; National Institutes of Health: Washington, DC, 1985.

(28) Shibuya, N. Phenolic acids and their carbohydrate esters in rice endosperm cell walls. *Phytochemistry* **1984**, *23*, 2233−2237.

(29) Yuasa, H.; Kuno, C.; Watanabe, J. Evaluation of the fractional absorption of D-xylose by analysis of gastrointestinal disposition after oral administration in rats. *Biol. Pharm. Bull.* **1996**, *19*, 604−607.

(30) Andreasen, M. F.; Kroon, P. A.; Williamson, G.; Garcia-Conesa, M. T. Esterase activity able to hydrolyze dietary antioxidant hydroxycinnamates is distributed along the intestine of mammals. *J. Agric. Food Chem.* **2001**, *49*, 5679−5684.

(31) Spencer, J. P. E.; Chowrimootoo, G.; Choudhury, R.; Debnam, E. S.; Srai, S. K.; Rice-Evans, C. The small intestine can both absorb and glucuronidate luminal flavonoids. *FEBS Lett.* **1999**, *458*, 224−230.

(32) Jacobson, E. A.; Newmark, H.; Baptista, J.; Bruce, W. R. A preliminary investigation of the metabolism of dietary phenolics in humans. *Nutr. Rep. Int.* **1983**, *28*, 1409−1417.

Received for review May 5, 2003. Revised manuscript received July 10, 2003. Accepted July 15, 2003.

JF034455U