Bioavailability of Genistein and Its Glycoside Genistin As Measured in the Portal Vein of Freely Moving Unanesthetized Rats

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The present study describes an in vivo bioavailability experiment for genistein and its glycoside genistin, either as pure compounds or from a soy protein isolate extract, using freely moving unanesthetized rats with a cannulation in the portal vein. The results show that genistein is readily bioavailable, being observed in portal vein plasma at the first point of detection at 15 min after dosing. The AUC_{0-24h} values for total genistein and its conjugates were 54, 24, and 13 μM h for genistein, genistin, and an enriched protein soy extract, respectively. These results indicate that the bioavailability of genistein is higher for the aglycon than for its glycoside. Genistin is partly absorbed in its glycosidic form. It is concluded that bioavailability studies based on portal vein plasma levels contribute to insight into the role of the intestine and liver in deglycosylation and uptake characteristics of glycosylated flavonoids.

KEYWORDS: Genistein; genistin; soy; bioavailability; in vivo rat study; isoflavones; glycosides

INTRODUCTION

Several of the major diseases of Western populations are hormone dependent, and epidemiological data reveal that not only genetic factors but also lifestyle factors, such as diet, play an important role in disease etiology. It has been estimated that about one-third of all human cancers are related to the modern diet (*1*, *2*). Epidemiological and migration studies have shown that the low incidence of hormonally dependent cancers in Asian countries can be associated with a diet rich in soybean (*3−6*). Soybeans contain 1−3 mg/g of isoflavones. Asian people consume approximately 50 mg of isoflavones per day, whereas the average daily intake of isoflavones in Western countries has been estimated to be <1 mg per day (*7−9*).

In soybeans and soy-derived foods genistein is mainly present as a glycoside, genistin. In spite of this, most metabolism studies primarily focus on the metabolism of genistein in humans and animals, and there is limited information on the metabolism and bioavailability of the glycoside genistin (*10−12*). One of the questions raised in flavonoid effect studies relates to the

bioavailability of the glycoside forms and/or the need for deconjugation of the glycosides before absorption can actually occur. It is well-known that colonic bacteria convert the (iso)-flavonic glycosides into the corresponding aglycones. In addition, extra- and intracellular β-glucosidase activities in the small intestine of humans and animals provide an additional route for deglycosylation and lead to a delayed appearance of the aglycon and its metabolites in plasma within minutes of glycoside administration (*10*, *13*). Some studies have even reported that flavonoids may be absorbed in their glycosylated form (*14*), whereas in the case of genistin others have reported the lack of absorption of the isoflavonic glycosides in man (*10*, *15*, *16*). On the other hand, it is well-established that the aglycone genistein is absorbed from the intestine and conjugated with glucuronic acid during transport across the intestinal epithelial cells. After transport to the liver, the glucuronide may be excreted in the bile, whereafter it could re-enter the small intestine, allowing genistein to be deconjugated, absorbed, and metabolized for the second time. This enterohepatic circulation may have important implications, because it delays the excretion and increases the duration of exposure.

In vivo, a primary location of glucuronidation appeared to be the intestinal wall, because an infusion of the rat duodenum with labeled [^{14}C]genistein elucidated that the portal vein blood contains predominantly the 7-*O*-glucuronide of genistein (*17*).

The aim of the present study was to obtain detailed informa-

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tion on the transport and metabolism of genistein and especially of its glycoside genistin, either dosed as pure compounds or extracted from a soy protein matrix, in an in vivo model, with special emphasis on the features of uptake of the glycosylated form. To this end, rats with a permanent cannulation of the portal vein were therefore used as a model for studying the intestinal transport and metabolism. Permanent cannulation of the portal vein allowed for repeated blood sampling while the rats were unanesthetized and freely moving. Using this model time-dependent genistein- and genistin-derived portal vein plasma profiles could be obtained and compared to the metabolite pattern recovered in the small intestine, colon, and feces. This provided a detailed insight into the bioavailability of genistein and its glycoside genistin.

MATERIALS AND METHODS

Materials. Genistein, genistin, polyethylene glycol, β-glucuronidase (from *Escherichia coli*, 2000 units/mL), and sulfatase (from *Abalone entrails*, 250 units/mL) were obtained from Sigma Chemical Co. (St. Louis, MO). [4-^{14}C]Genistein (specific activity = 0.2 mCi/mL) was purchased from Moravek Biochemical (Brea, CA). All other reagents used were of analytical grade and obtained from Merck (Darmstadt, Germany). An isoflavone-enriched soy protein isolate containing 87% (w/w) protein was derived from Protein Technology International (St. Louis, MO).

Animals. The experimental protocol was approved by the Animal Welfare Committee of Wageningen University (Wageningen, The Netherlands). Male rats (Wistar, 200−250 g) were obtained at 7 weeks of age from Charles River and housed at the Laboratory Animals Centre (Wageningen University, The Netherlands). The rats were kept under standard conditions, temperature of 22 ± 1 °C, 12 h light/dark cycle, and humidity of 55% for 1 week before surgical operations and fed ad libitum with a standard diet (Hope Farms, Woerden, The Netherlands). After 1 week of acclimatization, the surgical operation leading to the cannulation of the portal vein was carried out according to the method of Van Dongen et al. (*18*). During the surgical operation, rats were anesthetized by inhalation of isoflurane using oxygen as carrier. Then the abdominal cavity was opened and a cannula was inserted into the portal vein toward the liver. The cannula was fixed and placed in the abdominal cavity and was anchored to the internal abdominal muscle near the xiphoid cartilage. Subsequently, the abdominal wall was closed and the cannula was tunneled subcutaneously and fixed together with an L-shaped adapter at the crown of the head. Twice a week the cannula was checked if blood appeared and closed with viscous PVP solution until the experiment started. Starting 1 day after the operation rats were fed with a commercially available soy-free semipurified diet [containing standard AIN-76 g/kg vitamin and mineral mixtures, 540 g/kg dextrose, 50 g/kg cellulose, 100 g/kg cornstarch, 200 g/kg casein and 50 g/kg corn oil (Hope Farms, Woerden, The Netherlands)]. The experiment started 1 week after the surgical operation.

Animal Experiment. Rats had limited access to soy-free-based food (10 g) 16 h before the experiment started. Throughout the experiment rats could freely move and were given free access to tap water. The experiments were initiated by oral administration per gavage of the test solutions. Test solutions of genistein and genistin were prepared in ethanol and were diluted in polyethylene glycol at a maximum of 0.1% (v/v) ethanol. Soy protein isolate was concentrated by dilution in ethanol (1 g/4 mL), shaken overnight, and sonicated for 30 min. Subsequently, the soy protein isolate was extracted three times by adding ethanol, vortexed, and centrifuged at 2000*g* for 10 min. The obtained supernatants were pooled and evaporated to dryness under a stream of nitrogen. The volume of the concentrate was adjusted to 1 mL with polyethylene glycol. At the start of the experiment, soy protein isolate was mixed with extracted soy protein isolate (1 g/4 mL), also called soy extract. The soy extract contained 22% protein and 15 mg of isoflavones/L. The specifications of the soy protein isolate were provided by the supplier (Protein Technology International, St. Louis, MO) and can also be found in the literature (*19*). **Table 1** gives an overview of the results of the analytical analysis of the isoflavone

Table 1. Isoflavone Composition of the Various Stock Solutions Administered to the Rats by Oral Gavage

composition analysis	stock solution (mM)		
	genistein	genistin	soy extract
genistein	13.8 ± 1.38		5.52 ± 2.36
genistin		9.36 ± 1.61	12.1 ± 1.62
daidzein			6.01 ± 2.48
daidzin			6.53 ± 2.15
glycetein			7.87 ± 1.07
glycetin			3.53 ± 1.19

composition of the stock solutions, used to prepare the various test solutions.

The test solutions were administered by oral gavage at a dose level of 15 mg of genistein [56 μmol/kg of body weight (bw)] or genistin/kg of bw (35 μmol/kg of bw) at a maximum volume per dosage of 1 mL/rat. The control rats received a solution of polyethylene glycol and 0.1% ethanol only. Before the experiment started, rats were weighed and divided by weight into four treatment groups: (1) a control group, (2) a genistein group, (3) a genistin group, and (4) a soy protein isolate group. Ten rats were used per experimental group. Aliquot samples of 0.3 mL of blood were taken from the portal vein via the cannula at 0, 0.25, 0.5, 1, 2.5, 4, 5, 6, and 7 h after dosing of the isoflavones. Twenty-four hours after administration of the various isoflavones, the animals were sacrificed by total (heparinized) blood collection via aortal puncture under isofluran anesthesia. Feces were collected during the whole duration of the experiment (24 h). Luminal contents were collected from five 10 cm parts of the intestine (i.e., 5 cm from the pyloric sphincter for duodenum, 5 cm from the flexura duodeno-jejunalis in the jejunum proximal part, 20 cm from the flexura duodeno-jejunalis in the jejunum distal part, 10 cm from the valvula ileocoecalis in the ileum, and 10 cm from the cecum to the rectum in the colon). All samples were stored at −80 °C until analysis of genistein, genistin, and their respective metabolites.

Extraction of Genistein, Genistin, and Metabolites from Plasma. Aliquots of 0.3 mL of portal venous blood samples were collected in tubes pretreated with heparin 0, 0.25, 0.5, 1, 2.5, 4, 5, 6, and 7 h after dosing. Plasma was prepared by centrifugation of the blood samples for 10 min at 2000*g* (4 °C) and stored at −80 °C until analysis.

The isoflavones were extracted by passage of the plasma over an activated Oasis HLB-30 cartridge (Waters, Etten-Leur, The Netherlands), pre-equilibrated with 3 mL of methanol and 3 mL of double-distilled water. The cartridge was washed with 3 × 3 mL of 10 mM ammonium acetate (pH 6.5) and 3 mL of 10% methanol in 10 mM ammonium acetate (pH 6.5), and isoflavones were eluted with 4 mL of methanol. The methanol fraction was evaporated to dryness under a stream of nitrogen at 37 °C. Residues were sonicated for 10 min in 0.1 mL of acetonitrile/10 mM ammonium acetate (pH 6.5) (1:1, v/v) and stored at −20 °C until analyzed by HPLC.

Extraction of Genistein and Genistin Metabolites from Feces. The feces were lyophilized and powdered using a pestle and mortar. Samples of 0.05 g were taken in duplicate, and 1 mL of ice-cold methanol was added. The samples were shaken for 1 h and sonicated for 30 min. Subsequently, samples were extracted three times by adding 1 mL of ice-cold methanol, vortexing, and centrifuging at 2000*g* for 10 min. The three methanol supernatants thus obtained were pooled, and the volume was adjusted to 10 mL with 10 mM ammonium acetate (pH 6.5). The fecal isoflavones were further extracted by passage over an activated Oasis HLB-30 cartridge (Waters), as described above.

Extraction of Genistein, Genistin, and Metabolites from Luminal Contents. The various lyophilized intestinal contents were extracted three times by adding 1 mL of ice-cold methanol, vortexing, and centrifuging at 2000*g* for 10 min. The three supernatants obtained were pooled, and the volume was adjusted to 5 mL with 10 mM ammonium acetate (pH 6.5). The isoflavones in the intestinal contents were further extracted by passage over an activated Oasis HLB-30 cartridge (Waters), as described above.

Detection of Genistein, Genistin, and Their Metabolites. The extracted plasma, fecal, or intestinal samples were analyzed by HPLC

equipped with an autosampler (Waters), a UV photodiode array detector (Waters), and an electrochemical detector (Decade, Antec Leyden BV, Leiden, The Netherlands). Separation was performed on a Supelcosil LC-ABZ column (25 cm × 4.6 mm i.d., 5 μm particles) from Supelco (Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands), maintained at 30 °C (Merck Hitachi column heater). The column was eluted at a flow rate of 1.0 mL/min employing a gradient of two eluents. The solvents for the gradient elution were 10 mM ammonium acetate (pH 6.5) with 2.5% (v/v) acetonitrile (eluent A) and acetonitrile (eluent B). The following gradient was used: 0−5 min, 10% eluent B; 5−10 min, 10−32% eluent B; 10−25 min, 32% eluent B; 25−35 min, 32−40% eluent B; 35−40 min, 40−50% eluent B; 40−45 min, 50% eluent B; 45−50 min, 50−10% eluent B; and 50−60 min, 10% eluent B. The eluent was monitored both at 260 nm and at 76 mV.

Routinely 50 μL of extracted plasma, fecal, or luminal samples was injected onto the HPLC column, and genistein, genistin, and their metabolites were identified by comparison of retention times and UV spectra of the respective peaks to those of the known standards of isoflavones. The relative standard deviation of photodiode array and electrochemical analyses of genistein and genistin were 1.99% ($n =$ 13) and 0.97% ($n = 13$), respectively. Unknown metabolites were identified on the basis of spiking with [4-^{14}C]genistein. Thereto, two rats per time point were orally administered 15 mg of [4-^{14}C]genistein (0.375 kBq)/kg of bw and sacrificed at 0.5, 2, 4, and 24 h by total (heparinized) blood collection via aortal puncture under isofluran anesthesia. Plasma, fecal, and intestinal samples were collected and extracted according to the method described above. The distribution of radioactivity recovered in the chromatograms was analyzed on a Waters HPLC system connected to both a photodiode array detector (Waters) and an on-line radioactivity detector (LB506C, Berthold, Wildbad, Germany) according to a method described elsewhere (*20*). The chromatograms showed several labeled peaks of unchanged genistein and its (unknown) metabolites, which might be conjugated. The amount of genistein glucuronides and sulfates was calculated from the increase in the amount of genistein upon treatment of samples with β-glucuronidase and/or sulfatase, respectively. To this end, aliquots of extracted plasma were incubated with either β-glucuronidase (2000 units/mL) in 100 mM potassium phosphate (pH 7.0) or sulfatase (250 units/mL) with 17 mM D-saccharic acid 1,4-lactone in 0.5 M sodium acetate (pH 5.0) in a dilution of 2:1 (plasma/enzyme buffer by volume). After overnight incubation at 37 °C, the isoflavones were measured as described above. The UV and electrochemically established chromatograms were compared to the radiochemical chromatograms to verify and identify retention times of the conjugates: genistein glucuronide and sulfate metabolites.

Recovery of genistein extraction from plasma, feces, and intestinal contents was 67−94% of the dose applied, and the recovery of genistin extraction from plasma, feces, and intestinal contents was 82−87%.

Statistics. Results are presented as means $\pm$ SEM. Statistical evaluation of data was performed by one-way ANOVA ($p < 0.05$), and the comparison of values between the treated groups and the control group was based upon Tukey's honestly significant difference test ($p < 0.05$).

RESULTS

Genistein, Genistin, and Their Metabolites in Portal Vein Plasma. Figure 1 presents the time-dependent concentration of genistein [free (**Figure 1A**) and conjugated genistein (**Figure 1B**)] in the portal vein plasma of the rats administered either genistein, genistin, or the soy extract isoflavones. The amount of conjugated genistein represents here total glucuronides and sulfate metabolites of genistein minus free genistein.

The pharmacokinetic data are summarized in **Table 2**. For all test solutions a significant peak in the portal vein plasma concentration of genistein and its conjugates was detected 15 min after administration. Portal vein plasma genistein levels were highest in rats receiving genistein, amounting to a peak level (C_{max}) that was ∼6 times higher than the peak level
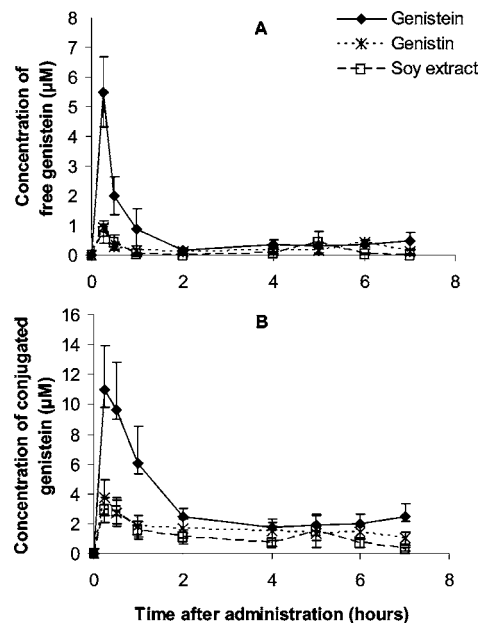

Figure 1. Concentration of (**A**) unconjugated (free) genistein and (**B**) conjugated genistein (i.e., glucuronides and sulfates) in portal vein plasma of cannulated rats. The rats were administered genistein, genistin, or soy extract. The dose of genistein and/or genistin was 15 mg/kg of bw. Data are expressed as mean $\pm$ SEM for $n = 10$ rats for genistein and genistin and $n = 9$ for soy extract.

detected in portal vein plasma of rats exposed to similar amounts of genistin or the soy extract isoflavones (**Figure 1A**; **Table 2**). The maximum concentrations (C_{max}) for genistein observed 15 min after dosing were 5.49, 0.91, and 0.78 μM for the genistein, genistin, and soy extract exposed rats, respectively (**Figure 1A**; **Table 2**), whereas the area under the curve (AUC_{0-24h}) values for genistein were 9.76, 2.65, and 1.14 μM h for the genistein, genistin, and soy extract administered rats, respectively. Portal vein plasma genistein patterns of genistin and soy extract exposed rats appeared to be similar. For rats exposed to genistin and soy extract a second genistein portal vein plasma peak was observed at, respectively, 6 and 5 h, amounting to about 50 and 55% of the plasma peak concentration at 15 min after dosing. In our hands the administration of genistein did not show a clear second peak at later time points.

The HPLC chromatograms of plasma samples from the rats that were administered with genistein showed several other peaks. Treatment of these samples with β-glucuronidase and sulfatase resulted in the loss of five peaks, with a concomitant formation of genistein. **Figure 1B** shows the time-dependent plasma concentration of conjugated genistein as measured, upon deconjugation with sulfatase and glucuronidase, after dosing with genistein, genistin, or the soy extract. This figure shows a similar trend as presented in **Figure 1A** for unconjugated genistein, with the concentration of conjugated genistein being highest in the plasma of rats administered genistein and amounting to a C_{max} of ∼11 μM at 15 min after administration. The C_{max} values of conjugated genistein in the portal vein plasma of rats dosed with genistin and the soy extract were 3.76 and 2.93 μM, respectively.

As observed in the portal vein plasma for genistein (**Figure 1A**), for genistin and the soy extract isoflavones exposed rats the time-dependent portal vein plasma profile of conjugated genistein also revealed a second peak at time points similar to those observed in **Figure 1A**, that is, at 5 h for soy-extracted isoflavones and at 6 h for pure genistin administration.

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Table 2. Plasma Pharmacokinetic Variables of Genistein, Conjugated Genistein, and Genistin of Rats That Received a Single Oral Dose of Genistein, Genistin, or Soy Extract

compound administered	genistein			conjugated genistein			genistin		
	AUC[a] (μmol h/L)	C_{max} (μmol/L)	t_{max} (h)	AUC (μmol h/L)	C_{max} (μmol/L)	t_{max} (h)	AUC (μmol h/L)	C_{max} (μmol/L)	t_{max} (h)
genistein	9.76	5.49	0.25	44.6	11.0	0.25			
genistin	2.65	0.91	0.25	20.9	3.79	0.25	0.85	0.15	2
soy extract	1.14	0.78	0.25	10.5	2.93	0.25	1.42	0.52	5

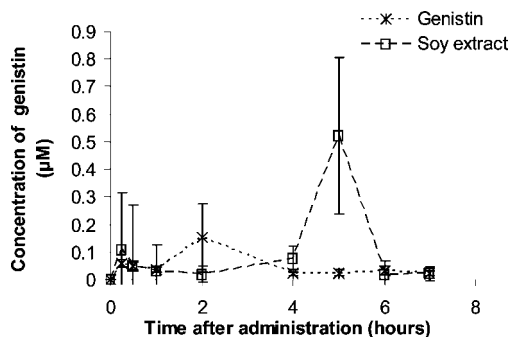[a] AUC was calculated over a time period of 0–24 h after dosing.

Figure 2. Concentration of genistin in portal vein plasma of cannulated rats. The rats were administered genistin or soy extract. The dose of genistin was 15 mg/kg of bw. Data are expressed as mean ± SEM for n = 10 rats for genistin and n = 9 for soy extract.

Figure 2 shows the portal vein plasma concentration of genistin after administration of genistin or the soy-extracted isoflavones. The C_{max} values for genistin appeared at 2 and 5 h having a level of 0.15 and 0.52 μM after genistin or soy extract dosing, respectively, and the AUC_{0-24h} values were 0.85 and 1.42 μmol h/L for genistin and soy extract administration, respectively (**Table 2**). From these data it follows that the portal vein plasma levels of genistin amounted to a level that is generally 10−25 times lower than those of deconjugated genistein including its metabolites. This points to an efficient deconjugation of the glycosylated form before absorption occurs but also indicates intestinal uptake of the glycoside genistin.

Figure 3 presents the time-dependent portal vein plasma profile of different conjugated genistein metabolites (i.e., glucuronidated and sulfated forms of genistein and an unknown genistein-like metabolite). From these profiles it is concluded that independent of the form in which genistein is administered, namely, in its unconjugated form, in its glycosylated form as genistin or as a soy extract in which genistein is glycosylated to a large extent, genistein glucuronides appear to be the major metabolites. Although a C_{max} for the glucuronidated metabolites occurred at the same time, that is, 15 min for all administrations, the levels of C_{max} were lower when the isoflavone was administered as genistin or the soy extract, pointing to a less efficient formation of these metabolites.

Genistein, Genistin, and Their Metabolites in Feces. **Figure 4** presents the genistein metabolite patterns detected in 24 h feces of rats exposed to genistein, genistin, or the soy extract. In addition to the detection of genistein and genistin themselves, the feces appeared to contain several additional genistein-derived metabolites, including equol and *O*-desmethylangolensin (ODMA) as well as four unidentified metabolites. However, several of these latter metabolites could be identified as genistein glucuronides and sulfates because following treatment with β-glucuronidase or sulfatase some of these peaks disappeared, accompanied by an increase in the intensity of the peak at the position of genistein. The amounts of equol
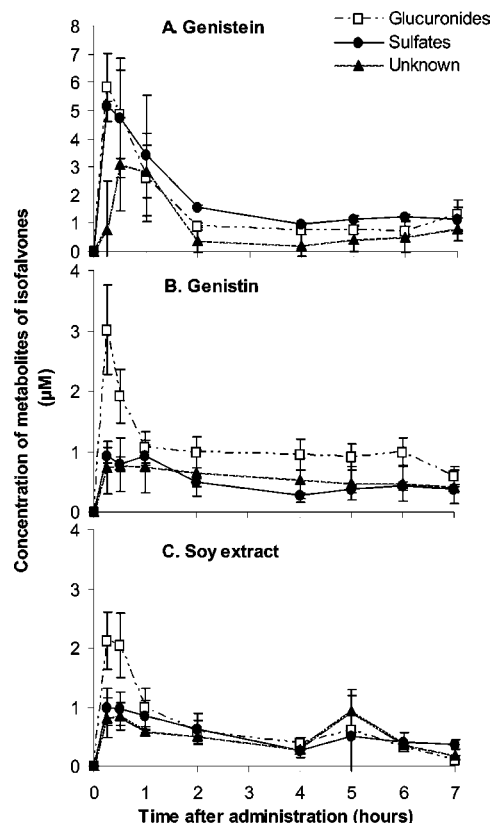

Figure 3. Metabolite profiles in portal vein plasma of cannulated rats. The rats were administered (**A**) genistein, (**B**) genistin, or (**C**) soy extract. The dose of genistein and/or genistin was 15 mg/kg of bw. Data are expressed as mean ± SEM for n = 10 rats for genistein and genistin and n = 9 for soy extract.

and ODMA detected in the 24 h feces of rats receiving the soy extract were approximately 11 and 1 μmol, respectively. This appeared to be significantly higher if compared to the amounts detected in 24 h feces of rats administered genistein and genistin, where the amounts of equol and ODMA were approximately 1.0 and 0.2 μmol for genistein administration and 1.2 μmol and 0.5 μmol for genistin administration, respectively. The fecal amount of genistein was highest in rats administered genistein, as approximately 1.8 μmol of genistein was recovered in those feces. Amounts of approximately 0.4 and 0.2 μmol of genistein were detected in feces of rats administered genistin or the soy extract, respectively. The total recovery of genistein, genistin, and their metabolites in the feces was 29, 36, and 35% of the dose administered of genistein, genistin, or the soy extract, respectively.

Genistein and Its Metabolites in Luminal Contents of the Intestines. Figure 5 shows the luminal amount of unconjugated genistein detected for rats of the three dose groups. For comparison, amounts detected in the feces are also included.
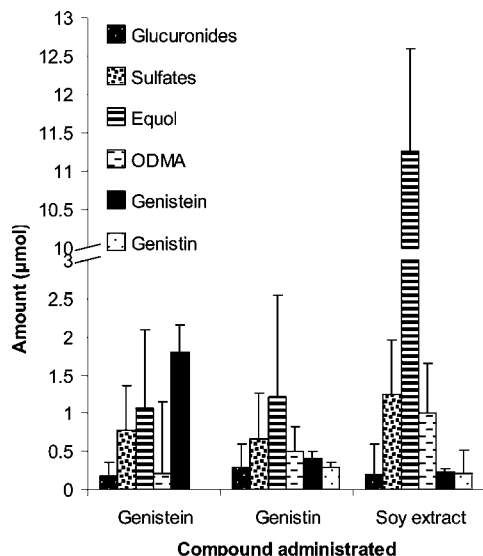
Figure 4. Metabolite amount in 24 h feces of cannulated rats. The rats were administered genistein, genistin, or soy extract. The dose of genistein and/or genistin was 15 mg/kg of bw. Data are expressed as mean ± SEM for $n = 10$ rats for genistein and genistin and $n = 9$ for soy extract.
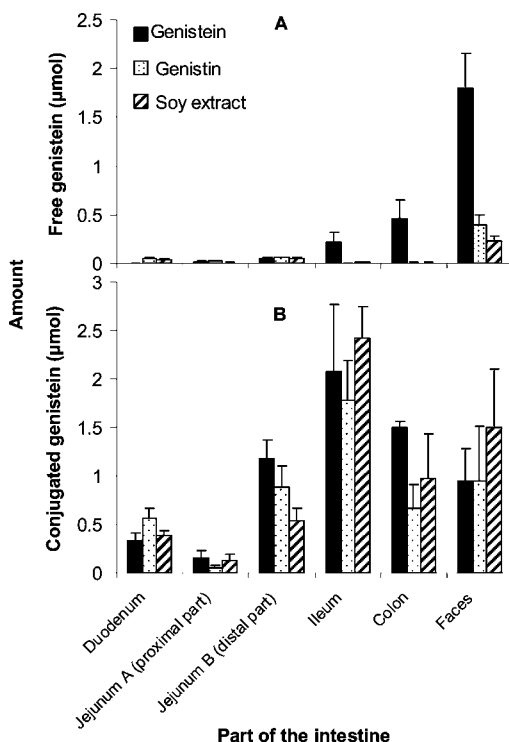

Figure 5. Amount of free and conjugated genistein in contents of the intestines and 24 h feces of cannulated rats. The rats were administered genistein, genistin, or soy extract. The dose of genistein and/or genistin was 15 mg/kg of bw. Data are expressed as mean ± SEM for $n = 10$ rats for genistein and genistin and $n = 9$ for soy extract.

As expected, the genistein amount was highest in rats dosed with this compound. The luminal contents of rats administered genistein contained genistein at a level higher by a factor of almost 3 than the intestinal contents of rats dosed with genistin or the soy extract (**Figure 5A**). Furthermore, the amount of unconjugated genistein in all parts of the intestine was more or less identical in rats administered genistin or the soy extract. The highest amount of conjugated genistein metabolites recovered in the luminal contents was in the ileum part of the intestine for all of the compounds administered (**Figure 5B**).

DISCUSSION

This study focuses on the in vivo intestinal transport and metabolism of the isoflavone genistein in both its aglyconic and glycosidic forms, when dosed either as pure compounds or extracted from a soy matrix (i.e., enriched protein isolate). To this end, rats were used that were permanently cannulated in their portal vein, but could freely move. Our experiments show that the time (t_{max}) at which the maximum plasma concentration (C_{max}) appeared was identical among all three test groups and observed at the first sampling point (i.e., 15 min). In contrast, most studies with human volunteers reported a first sampling point after approximately 1 or 2 h (*10*, *11*, *21*−*24*) or even later with a mean first sampling time point of 4−6.5 h after dosing (*12*). Coldham et al. (*25*, *26*) reported an in vivo bioavailability study of genistein in rats with a first sample point and a C_{max} at 30 min after an oral administration.

The observations of the present study point to a swift absorption of free and conjugated genistein within the first 15 min after administration. However, the exact C_{max} and t_{max} values of genistein or genistin could not be estimated due to experimental limitations, because this model did not allow an earlier first sampling point. Therefore, these parameters remain to be studied in more detail. Nevertheless, the present study demonstrates in all three test groups a C_{max} at 15 min after dosing. It might therefore be concluded that the initial hydrolysis of genistin to its aglycon genistein is relatively fast and not a major rate-limiting step for the absorption of genistin. The differences in C_{max} between humans and rats may be due to anatomical and physiological differences between these mammalian species.

It is postulated that the appearance of two subsequent plasma peaks might be in line with the general speculation that upon first passage through the intestinal wall, the isoflavones are readily conjugated with glucuronic acid. After transport to the liver, the glucuronides are excreted in the bile, allowing this isoflavone after re-entering the small intestine to be absorbed and metabolized for a second time, yielding the second peak observed in plasma (*25*, *26*).

For the first time, it is shown that shortly after oral intake of the glycoside genistin, the compound is also present as an intact molecule in plasma of the portal vein, albeit at concentrations that are ~10−25 times lower if compared to the levels of the deglycosylated form genistein or its metabolites. These observations are in contrast with those of Sesink et al. (*27*) and Setchell et al. (*21*). Sesink et al., studying the intestinal metabolism of quercetin-3-glucoside, reported that no quercetin-3-glucoside was found in blood samples taken from the mesenteric vein, and Setchell et al. (*21*) communicated that there is a lack of absorption of the isoflavone glycosides in humans. In the latter study adults ingested 50 mg of genistin and blood samples were obtained by a vein puncture. In both of these studies, however, liver metabolism could significantly have contributed to the observed plasma metabolite patterns. In our study the blood samples were taken from the portal vein, eliminating as much as possible the contribution of liver metabolism. Therefore, our results suggest that limited amounts of the glycoside are able to enter the portal vein and are subsequently fully deglycosylated and metabolized upon first pass through the liver. This conclusion is in line with results of in vitro studies using an isolated rat small intestinal perfusion model or Caco-2 cell models (*28*−*30*). In these studies it was shown that a small amount of genistin indeed crosses the intestinal cellular monolayer. The difference in absorption between quercetin-3-glucoside and genistin may be due to differences in the specificity of the glycosidase.

Bioavailability of Genistein and Genistin

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In the present study the AUC$_{0-24h}$ values for total genistein were 54, 24, and 13 μmol h/L upon dosing of genistein, genistin, and the enriched protein soy extract, respectively. These results indicate that the bioavailability of genistein is higher for the aglycon if compared to the glycosidic form. This is in line with the study of Piskula et al. (*15*) and likely to represent the relative bioavailability expected in man (*31*). In addition, comparison of our genistein bioavailability data for genistin and soy extract exposed rats reveals that the bioavailability is not significantly influenced by the presence of other compounds present in the soy-enriched protein isolate as for other isoflavonic aglycones and/or glycosides such as daidzein and glycitein. Andlauer et al. (*32*) reported a higher genistein absorption in rats after dosing with tofu products if compared to genistin applied as a pure compound. In vitro and in situ studies demonstrated a possible interaction of genistein and its conjugates with apical intestinal efflux transporters, such as MRP2 (multidrug resistance protein 2) as shown by Walle et al. (*33*) and BCRP1/ABCG2 (breast cancer resistance protein) as reported by Imai et al. (*34*). These interactions may provide a possibility for higher genistein plasma concentrations upon inhibition of the MRP2- and BCRP-mediated efflux from intestinal cells back into the intestinal lumen. One could therefore hypothesize that tofu contains inhibitors of these efflux transporters. Obviously, in our extracted soy protein isolate these inhibitors may not be present. This statement is supported by the fact that daidzein, the other major isoflavone present in our soy extract, shows a low interaction with the BCRP1/ABCG2 transporter (*34*).

In the present study the major amounts of genistein and its metabolites are found in the luminal contents of the small intestine and feces up to total recoveries of approximately 73 and 80% of the genistein and genistin administered, respectively. The recovery of genistein and its metabolites in feces is in line with the recovery reported by Coldham et al. (*25*). These authors used radioactive labeled genistein in rats.

In conclusion, the present study, using freely moving rats with a permanent cannulation in the portal vein, reveals that measurement of the isoflavone metabolite profiles in portal vein plasma contributes to an insight into the role of (de)glycosylation during the uptake. It is demonstrated that the glycosylated form in which these bioactive food ingredients generally occur in our diet can be absorbed to a limited extent and also that intestinal deglycosylation is highly efficient.

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