Enterohepatic Circulation and Pharmacokinetics of Genistin and Genistein in Rats

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Supporting Information

ABSTRACT: Genistin and its aglycone genistein of isoflavone are naturally occurring in plants. The aim of this study is to develop an experimental animal model of enterohepatic circulation to investigate the metabolic biotransformation of genistin and genistein in rats. A paired-rat model was developed in which the drug was administered intravenously to the donor rat whose bile duct was cannulated into the duodenum of the untreated recipient rat. The blood sample was collected from the jugular vein of the donor and recipient rats after genistin administration. The results demonstrate that genistein was detected in both the donor and recipient rats after genistein administration (50 mg/kg, iv) in the donor rat, which suggested that the enterohepatic circulation of genistein occurred. The same phenomenon happened again in the biotransformation after genistin administration (50 mg/kg, iv) in the donor rat. Genistein was detected in the recipient rat’s blood sample after treatment with β-glucuronidase, which suggested that enzymatic hydrolysis occurred in the transformation of genistin into genistein. In conclusion, the research revealed the metabolic pathway of the glucuronidation of genistin into genistein.

1. INTRODUCTION

Genistein (4′,5,7-trihydroxyisoflavone), one of the major isoflavones in soybeans, Sophora japonica seeds, and botanical herbs such as Pueraria montana Radix. Genistin (genistein-7-D-glucoside) is a glycoside form of genistein found in soy-derived foods. These isoflavones have been proven to be beneficial in the treatment of osteoporosis,† diabetes,‡ and menopausal symptoms.§

The biotransformation mechanism of isoflavones has been previously described in animal experiments and human subjects, such as a previous study, demonstrating that genistin mainly underwent phase II biotransformation of methylation, glucosylation, glucuronidation, sulfonation, and acetylation in rats. The multiple complex metabolites of genistin-7-glucuronide, 4′-glucuronide, 7-sulfate, 4′-sulfate, 4′,7-diglucuronide, 7-glucuronide-4′-sulfate, and genistein have been observed in human plasma after ingestion of genistin and its glucoside in healthy young women. The malonylglucoside conjugation pathway is common for the isoflavones. Malonylgenistin is a metabolite of genistin in which the hydroxy hydrogen is replaced by a malonyl group. However, the malonylglucoside conjugates may not be the major metabolic product for the isoflavones. Not only in the phase II biotransformation of genistein but also in its biliary excretion, it is suggested that genistein and genistin may possibly go through the pathway of enterohepatic circulation. A potential metabolic pathway of genistin and genistein is shown in Figure 1.

Enterohepatic circulation represents the circulation that is utilized when a substance is absorbed through the portal vein into the liver. The substance is then excreted with biliary acids, bilirubin, or other substances from the liver as bile into the duodenum. Afterward, these substances are moved into the small intestine and absorbed by enterocytes and transported...
back to the liver. Enterohepatic circulation may be significantly affected by the chemical and physical characteristics of the substance itself. The formation of metabolites no matter whether phase I or phase II during absorption is also affected by enterocytes and hepatocytes during their circulation through the biliary system and intestinal tract. A previous report demonstrated that a double peak phenomenon was found in genistein, which suggests an enterohepatic circulation phenomenon. In addition, our previous report indicates that genistein has been detected in bile juice, and the distribution ratio of bile-to-blood (AUCbile/AUCblood) is 1.85 ± 0.42, which suggests the biliary excretion of genistein.

After treatment with the P-glycoprotein inhibitor cyclosporine, the bile-to-blood distribution ratio decreased, which suggests that the biliary excretion of genistein may be related to the P-glycoprotein transport system. An additional survey with the keywords "enterohepatic circulation and genistein" in PubMed found nine articles. However, no article was found referring to the enterohepatic circulation of genistin and its aglycone.

Based on the above survey, we hypothesized that genistin and genistein may undergo enterohepatic circulation through biotransformation. To investigate this hypothesis and the potential mechanism of the enterohepatic circulation of genistin and genistein, a paired-rat experimental design utilizing donor and recipient rats was used. The aim of this study was to develop a validated analytical system to monitor the analytes of genistin and genistein from the donor and recipient rats. Based on these analytical data, the pharmacokinetics and enterohepatic circulation of genistin and genistein were evaluated.

2. RESULTS AND DISCUSSION

2.1. Method Validation. 2.1.1. Linearity and Limit of Detection. The calibration curves of genistein and genistin in rat plasma were obtained through a validated chromatographic system over the concentration range of 0.05–50 μg/mL. The concentrations of genistein and genistin were linearly related to the peak area in the chromatogram (r² > 0.995).

2.1.2. Recovery. The extraction recovery of genistein and genistin in rat plasma was 97–99 and 89–98%, respectively (Supporting Information Table S1). The results demonstrated that the values were all within the acceptable range and the method was accurate and precise.

2.1.3. Stability. Stability data of genistein and genistin in rat plasma are summarized in Supporting Information Table S2. The relative standard deviation (RSD) of genistein and genistin were within ±20%, and the results indicated that the three analytes in plasma were stable for 4 h at room temperature and for 12 h in the autosampler condition after preparation.

2.1.4. Precision and Accuracy. In interday analyses, the precision and accuracy of genistein ranged from 2.27 to 12.1 and −6.21–15%, respectively, and the precision and accuracy of genistin ranged from 2.37 to 18.2 and −9.80–14.1%, respectively. In intraday analyses, the precision and accuracy of genistein ranged from 5.61 to 11.3 and −7.76–18.2%, respectively, and the precision and accuracy of genistin ranged from 4.88 to 14.0 and 4.98–17.1%, respectively (Supporting Information Tables S3 and S4). Thus, the intraday and interday accuracy and precision were found to be acceptable for genistein and genistin analyses in support of further pharmacokinetic studies. The data demonstrated excellent reproducibility.

2.2. Pharmacokinetics and Enterohepatic Circulation of Genistein. The analytical data demonstrated in Figure S1A shows the chromatogram of blank rat plasma. No discernible peak was observed. Figure S1B shows the chromatogram of rat plasma spiked with genistein (1 μg/mL) and an internal standard (7.5 μg/mL). Figure S1C shows the chromatogram of the plasma sample obtained 45 min after genistein (50 mg/kg, iv) administration in the donor rat. Figure S1D shows the chromatogram of the plasma sample obtained at 10 min in the recipient rat. The mean plasma concentration–time profiles of genistein administration (50 mg/kg, iv) are shown in Figure 2.
The pharmacokinetic parameters were calculated by the WinNonlin program, and the data are demonstrated in Table 1. The maximal concentration was 16.93 μg/mL and detected at the initial time point. Afterward, the concentration was gradually decreased, indicating a one-compartment model. The elimination half-life (t½) was lower than half an hour, indicating a rapid decrease in the systemic circulation, and the genistin concentration was lower than undetectable limit at approximately 2 h after genistin administration (50 mg/kg, iv).

To monitor the enterohepatic circulation of genistin, the genistin level was detected in the recipient rat at 10 min after genistin administration (50 mg/kg, iv). However, the highest concentration of genistin was approximately 0.2 μg/mL, and the level of genistin approached the detection limit at up to 30 min. The area under the concentration curve (AUC) of the donor and recipient rats was 460.5 ± 92.9 and 2.91 ± 1.26 min μg/mL, respectively. The enterohepatic circulation of genistin is defined as the AUC ratio of recipient to donor (AUCRecipient/AUCDonor). Based on the experimental model, the enterohepatic circulation of genistin was 2.91/460.5 = 0.63% (Table 1).

Enterohepatic circulation occurs through biliary excretion and intestinal reabsorption, which may involve phase II metabolic conjugation and deconjugation. Compared to resveratrol, an aglycone of flavonoids, the enterohepatic recirculation was assessed using the ratio of AUC recipient to AUC donor of the bile-recipient rat relative to the bile-donor rat. The data demonstrated that the enterohepatic recirculation of resveratrol and resveratrol glucuronide were calculated to be 24.7 ± 15.1 and 24.0 ± 8.5%, respectively. The enterohepatic circulation of resveratrol was higher than that of genistin. A potential explanation is the different experimental model used to measure the aglycone resveratrol reflected hepatobiliary excretion from the bile duct but not the systemic circulation. Another explanation of the poor enterohepatic circulation may be that the enterohepatic recirculation contributes to the overall systemic circulation and complex metabolism. The third explanation is that the motility of the gastrointestinal tract may be markedly reduced during anesthesia and consequently the drug absorption is also reduced. The phenomena of poor bioavailability and poor enterohepatic circulation agree with the terpene lactones of the leaf extracts of Ginkgo biloba.

### 2.3. Pharmacokinetics and Enterohepatic Circulation of Genistin

Genistin preserves its hydrophilic characteristics more than genistin does because of the presence of a glycoside, which may shorten the retention time in the chromatogram. The analytical data demonstrated in Figure S2A show the chromatogram of blank rat plasma. No discernible peak was observed. Figure S2B shows the chromatogram of rat plasma spiked with genistin (1 μg/mL), an internal standard (7.5 μg/mL), and genistin (1 μg/mL). Figure S2C shows the chromatogram of the plasma sample obtained 45 min after genistin (50 mg/kg, iv) administration in the donor rat. Both genistin and genistein were detected in the donor rat. However, both genistin and genistein were not observed in the recipient rat (Figure S2D), which suggests that genistin underwent deglucosidation and was conjugated to produce genistein glucuronide. The chromatogram of Figure S2E shows the recipient plasma sample collected at 45 min after genistin administration (50 mg/kg, iv), and the plasma sample was incubated 1 h with β-glucuronidase. These data suggested that genistin first underwent deglucosidation, followed by conjugation with glucuronide. To monitor the total levels of genistin and genistein and their glucuronide products, glucuronidase was used for enzymatic hydrolysis. These data demonstrated that both genistin and genistein were detected after treatment with β-glucuronidase.

The pharmacokinetic curve of genistin in the donor rat after genistin administration (50 mg/kg, iv) showed that the concentration of genistin gradually decreased, which suggests that genistin is initially distributed into a central compartment (Figure 3). The elimination half-life was approximately 30 min, and the concentration of genistin in the donor rat decreased at the initial time point. Afterward, the concentration was gradually decreased, indicating a one-compartment model. The elimination half-life (t½) was lower than half an hour, indicating a rapid decrease in the systemic circulation, and the genistein concentration was lower than undetectable limit at approximately 2 h after genistein administration (50 mg/kg, iv).

To monitor the enterohepatic circulation of genistein, the genistein level was detected in the recipient rat at 10 min after genistein administration (50 mg/kg, iv). However, the highest concentration of genistein was approximately 0.2 μg/mL, and the level of genistein approached the detection limit at up to 30 min. The area under the concentration curve (AUC) of the donor and recipient rats was 460.5 ± 92.9 and 2.91 ± 1.26 min μg/mL, respectively. The enterohepatic circulation of genistein is defined as the AUC ratio of recipient to donor (AUCRecipient/AUCDonor). Based on the experimental model, the enterohepatic circulation of genistein was 2.91/460.5 = 0.63% (Table 1).
a regular elimination rate (Table 2). This pharmacokinetic behavior can be explained by a one-compartment model with rapid equilibrium with the tissue compartment. The elimination phenomenon of genistin in the donor rat of the enterohepatic circulation experimental model is in agreement with the metabolic disposition of chloramphenicol. However, another experiment with colchicine exhibited slow equilibration with peripheral tissues, which describes a two-compartment model in the donor rat.

A previous report demonstrated that the bioavailability of genistin was higher for aglycone than that for its glycoside. However, genistin was undetectable in the recipient rat, which may be explained by the level of genistin being lower than the detection limit in this analytical system. Another explanation may be that genistin has been metabolized via phase II conjugation to produce its metabolite. To investigate the possible mechanism of phase II metabolism, the plasma concentration of genistin was determined by enzymatic hydrolysis. Both donor and recipient plasma samples were treated with β-glucuronidase and incubated in a 37 °C water bath for deconjugation. The results demonstrated that genistin was detected in the plasma of both donor and recipient rats when treated with β-glucuronidase (Figure 3). These data suggested that the level of phase II genistin glucuronide was higher than that of genistin itself, which suggested the high efficacy of enzymatic glucuronidation. The UDP-glucuronosyltransferases serve as versatile and important conjugation enzymes in the phase II biotransformation of xenobiotics. The results agree with the previous report in which liquid chromatography coupled to a single-quadrupole mass spectrometry was used to detect genistin and its metabolites. Genistin glucuronide was the predominant metabolite, and only small amounts of sulfate conjugate and aglycone were observed. These data reflect the previous report that the total genistin was composed of mixed conjugates of glucuronide and sulfate conjugation. Genistin deconjugation may occur in multiple targets, such as genistein-7-β-d-glucuronide and genistein-4′-β-d-glucuronide.

The manner of hepatobiliary excretion for the flavonoids suggests that these phytochemicals potentially undergo enterohepatic circulation. The experimental results indicated that genistin (Figure 2) was detected in the recipient and donor rats following genistin administration (50 mg/kg, iv), which is consistent with the previous reports of other flavonoids, such as fisetin, genistein, biochanin A, apigenin, and chrysin.23,24

### 3. Material and Methods

#### 3.1. Chemicals and Reagents

Genistein, genistin, and β-glucuronidase (EC 3.2.1.31, type H-1, 330,000 units/g solid also containing sulfatase activity) from *Helix pomatia* and ornidazole as internal standards were purchased from Sigma-Aldrich (St. Louis, MO). The solvents and reagents for chromatography were purchased from Spectrum (NJ) and Macron (Hamilton, PA). The standard solutions of genistein and genistin were stored in methanol at 20 °C. Triple deionized water from Millipore (Bedford, MA) was used for all preparations.

#### 3.2. Liquid Chromatography

The high-performance liquid chromatography (HPLC) system consisted of a chromatographic pump (LC-20AT; Shimadzu, Kyoto, Japan), an on-line injector (SIL-20C) equipped with a 10 μL sample loop to inject the sample and a photodiode array detector (SPD-M20A). Genistein, genistin, and an internal standard were separated with an Agilent ZORBAX SB-phenyl column (150 × 4.6 mm id particle size 5 μm). The mobile phase for the genistin group was water and acetonitrile (61:39, v/v) at a flow rate of 0.8 mL/min. The mobile phase for the genistin group was also water and acetonitrile (68:32, v/v) at a flow rate of 0.8 mL/min. The optimal photodiode-array detection for genistin and genistin was set at a wavelength of 254 nm.

#### 3.3. Method Validation

##### 3.3.1. Calibration Curve

All calibration curves were required to have a correlation value of at least 0.995. Calibration standards of plasma samples were prepared by adding known amounts of genistein and genistin (10 μL) into the blank rat plasma (40 μL) to give a range of 0.05–50 μg/mL. These mixtures were supplemented with 150 μL of internal standard solution (10 μg/mL).

##### 3.3.2. Precision and Accuracy

The intraday and interday variabilities for genistein and genistin were determined by quantitating six replicates at concentrations of 0.05, 0.1, 0.5, 1, 5, and 20 μg/mL using the HPLC method described above on the same day and six consecutive days, respectively. The accuracy (% bias) was calculated from the nominal concentrations (Cnom) and the mean value of observed concentrations (Cobs) as follows: accuracy (% bias) = [(Cnom − Cobs)/Cnom] × 100. The precision (relative standard deviation: RSD) was calculated from the observed concentrations as follows: precision (% RSD) = [standard deviation (SD)/Cobs] × 100. The same data were used to determine both accuracy and precision.

##### 3.3.3. Recovery

Recovery was assessed at three different concentrations (0.05, 5, and 50 μg/mL) by comparing the peak area of postextraction spiked samples with that of a standard solution.

##### 3.3.4. Stability

Stability tests were performed at three different concentrations: low (0.05 μg/mL), medium (5 μg/mL), and high (20 μg/mL). The room-temperature stability was conducted on analytes at room temperature for 4 h. The long-term stability was evaluated after storage of the samples at −80 °C for 2 weeks. The autosampler stability was evaluated by analyzing analytes in the sample injector at 8 °C for 12 h. The freeze–thaw stability of the analytes was assessed by a comparison of plasma samples after three freeze–thaw cycles with a frozen temperature (−80 °C) and a thawing temperature (25 °C) on consecutive days.

#### 3.4. Experimental Animals

Male Sprague–Dawley rats (220–280 g) were obtained from the Laboratory Animal Center at National Yang-Ming University. All experimental
protocols involving animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC number: 1080113) of the National Yang-Ming University. Laboratory rodent diet 5001 (PMI Feeds, Richmond, IN) was used for food. Rats were housed with a 12 h light/dark photoperiod cycle and given ad libitum access to food and water. The experimental animal model for enterohepatic circulation is shown in Figure 4. The bile duct of the donor animal was cannulated proximal to the liver with a 15 cm section of polyethylene tubing (Intramedic PE-10, ID 0.28 mm; OD 0.61 mm, Sparks, MD), the other end of which was inserted through the bile duct into the duodenum of the recipient rat. The cannulation demonstrated that a PE-10 at the common bile duct of the recipient rat cannulated to the duodenum of the donor rat. The cannulation of (b) demonstrated that a PE-10 at the common bile duct of the recipient rat cannulated to the duodenum of the donor rat.

Figure 4. Experimental animal model for enterohepatic circulation. The donor rat received the drug and the recipient rat did not receive any drug. The cannulation demonstrated that a PE-10 at the common bile duct of the donor rat cannulated to the duodenum of the recipient rat. To balance the fluid losses and gains in the donor and recipient rats, the bile duct of the recipient rat was also cannulated to channel bile back to the donor rat. The animals were divided into two groups, the genistein group (50 mg/kg, iv) and the genistin group (50 mg/kg, iv). Following a 1 h period of stabilization, the drug was administered to the donor rat through a femoral cannula. The blood samples (150 μL) were collected through the donor’s and recipient’s carotid cannulas at 5, 10, 15, 30, 45, 60, 75, 90, 120, 180, and 240 min.

3.5. Sample Preparation. Each collected blood sample was transferred to a heparinized microcentrifuge tube and centrifuged at 13 000 rpm for 10 min. The plasma (50 μL) was then mixed with 150 μL of internal standard solution (10 μg/mL). The denatured protein precipitate was separated by vortex for 20 s and finally centrifuged at 13 000 rpm for 10 min at 4 °C.

3.6. Deconjugation. For genistein deconjugation, aliquots (50 μL) of plasma sample were incubated with 10 μL of β-glucuronidase in a 37 °C water bath for 1 h. Following incubation, the internal standard solution was added to denature the protein precipitate.

3.7. Calculation and Statistical Analysis. The WinNonlin Standard Edition (version 1.1, Scientific Consulting Inc., Apex, NC) was used to calculate the pharmacokinetic data, and the results are presented as the mean ± standard deviation.

4. CONCLUSIONS

The present study demonstrated a paired-rat experimental model to reveal that genistein was detected in both the donor and the recipient rats after genistein administration (50 mg/kg, iv) in the donor rat. The AUC ratio represents the enterohepatic circulation of the analytes, and the data demonstrated that the recipient and donor AUC ratio (AUCrecipient/AUCdonor) was approximately 0.63%. Genistein was detected in the recipient rat’s blood sample after treatment with β-glucuronidase, which suggested that enzymatic hydrolysis occurred in the transformation of genistin into genistein. The research demonstrated that genistin was hydrolyzed by β-glucosidase into genistein and then went through phase II biotransformation by UDP-glucuronosyltransferase to produce genistein glucuronide.

Data are expressed as the mean ± SEM (n = 6). Significant differences were observed between the donor and recipient groups.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b02762.

Method was fully validated in terms of linearity, extraction recovery, stability, precision, and accuracy according to US Food and Drug Administration guidance; all calibration curves were required to have a correlation value of at least 0.995; recovery experiment is to compare the peak area of postextraction spiked samples with that of the standard solution; short- and long-term stabilities of analytes in biological samples were evaluated to determine the stability of genistein and genistin in plasma samples under different conditions, including room-temperature stability, autosampler stability, and freeze–thaw stability; and intraday and interday variabilities for genistein and genistin were determined by quantitating six replicates concentrations on the same day and 6 consecutive days, respectively.

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Author Contributions
The authors contributed to this work as follows: Y.-Y.Y. performed the study, analyzed the data, and prepared the manuscript. T.-H.T. designed the experiments, edited the paper, and secured research funding.

Notes
The authors declare no competing financial interest.

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■ REFERENCES

(1) Abdallah, H. M.; Al-Abd, A. M.; Asaad, G. F.; Abdel-Naim, A. B.; El-halaway, A. M. Isolation of antosteoporotic compounds from seeds of Sophora japonica. PLoS One 2014, 9, No. e98559.
(2) Feng, T.; Liu, F.; Sun, L.; Huo, H.; Ren, X.; Wang, M. Associated-Extraction Efficiency of Six Cyclodextrins on Various Flavonoids in *Pueraiae Lobatae* Radix. *Molecules* 2019, 24, 93.

(3) Wong, K. H.; Razmovski-Naumovski, V.; Li, K. M.; Li, G. Q.; Chan, K. Comparing morphological, chemical and anti-diabetic characteristics of *Pueraiae Lobatae* Radix and *Pueraiae Thomsonii* Radix. *J. Ethnopharmacol.* 2015, 164, 53–65.

(4) van der Velpen, V.; Geelen, A.; Hollman, P. C.; Schouten, E. G.; van’t Veer, P.; Afman, L. A. Isoflavone supplement composition and equal producer status affect gene expression in adipose tissue: a double-blind, randomized, placebo-controlled crossover trial in postmenopausal women. *Am. J. Clin. Nutr.* 2014, 100, 1269–1277.

(5) Liang, Y.; Zhao, W.; Wang, C.; Wang, Z.; Wang, Z.; Zhang, J. A Comprehensive Screening and Identification of Genistin Metabolites in Rats Based on Multiple Metabolite Templates Combined with UHPLC-HRMS Analysis. *Molecules* 2018, 23, No. 1862.

(6) Yuan, B.; Zhen, H.; Jin, Y.; Xu, L.; Jiang, X.; Sun, S.; Li, C.; Xu, H. Absorption and plasma disposition of genistin differ from those of genistein in healthy women. *J. Agric. Food Chem.* 2012, 60, 1428–1436.

(7) Yerramsetty, V.; Gallaher, D. I.; Ismail, B. Malonylglucoside conjugates of isoflavones are much less bioavailable compared with unconjugated β-glucosidic forms in rats. *J. Nutr.* 2014, 144, 631–637.

(8) Sfakianos, J.; Coward, L.; Kirk, M.; Barnes, S. Intestinal uptake and biliary excretion of the isoflavone genistein in rats. *J. Nutr.* 1997, 127, 1260–1268.

(9) Roberts, M. S.; Magnusson, B. M.; Burczynski, F. J.; Weiss, M. Enterohepatic circulation. *Clin. Pharmacokinet.* 2002, 41, 751–790.

(10) Chang, L.; Ren, Y.; Cao, L.; Sun, Y.; Sun, Q.; Sheng, N.; Yuan, L.; Zhi, X.; Zhang, L. Simultaneous determination and pharmacokinetic study of six flavonoids from Fructus Sophorae extract in rat plasma by LC–MS/MS. *J. Chromatogr. B: Biomed. Sci. Appl.* 2012, 904, 59–64.

(11) Tsai, T.-H. Concurrent measurement of unbound genistein in the blood, brain and bile of anesthetized rats using microdialysis and its pharmacokinetic application. *J. Chromatogr. A* 2005, 1073, 317–322.

(12) Marier, J.-F.; Vachon, P.; Gritsas, A.; Zhang, J.; Moreau, J.-P.; Ducharme, M. P. Metabolism and disposition of resveratrol in rats: extent of absorption, glucuronidation, and enterohepatic recirculation evidenced by a linked-rat model. *J. Pharmacol. Exp. Ther.* 2002, 302, 369–373.

(13) Ogilvy, A.; Smith, G. The gastrointestinal tract after anaesthesia. *Eur. J. Anaesthesiol. Suppl.* 1995, 10, 35–42.

(14) Chen, F.; Li, L.; Xu, F.; Sun, Y.; Du, F.; Ma, X.; Zhong, C.; Li, X.; Wang, F.; Zhang, N.; et al. Systemic and cerebral exposure to and pharmacokinetics of flavonoids and terpene lactones after dosing standardized *Ginkgo biloba* leaf extracts to rats via different routes of administration. *Br. J. Pharmacol.* 2013, 170, 440–457.

(15) Tsai, T.; Shum, A. Y.; Chen, C. Enterohepatic circulation of chloramphenicol and its glucuronide in the rat by microdialysis using a hepato-duodenal shunt. *Life Sci.* 1999, 66, 363–370.

(16) Chen, Y.-J.; Huang, S.-M.; Liu, C.-Y.; Yeh, P.-H.; Tsai, T.-H. Hepatobiliary excretion and enterohepatic circulation of colchicine in rats. *Int. J. Pharm.* 2008, 350, 230–239.

(17) Steensma, A.; Faassen-Peters, M. A.; Noteborn, H. P.; Rietjens, I. M. Bioavailability of genistein and its glycoside genistin as measured in the portal vein of freely moving unanesthetized rats. *J. Agric. Food Chem.* 2006, 54, 8006–8012.

(18) Turgeon, D.; Carrier, J.-S.; Chouinard, S.; Bélanger, A. Glucuronidation activity of the UGTT2B17 enzyme toward xeno- biotics. *Drug Metab. Dispos.* 2003, 31, 670–676.

(19) Rahikainen, T.; Häkkinnen, M. R.; Finel, M.; Pasanen, M.; Juvenon, R. O. A high throughput assay for the glucuronidation of 7-hydroxy-4-trifluoromethylcoumarin by recombinant human UDP-glucuronosyltransferases and liver microsomes. *Xenobiotica* 2013, 43, 853–861.

(20) Holder, C. L.; Churchwell, M. I.; Doerge, D. R. Quantification of soy isoflavones, genistein and daidzein, and conjugates in rat blood using LC/ES-MS. *J. Agric. Food Chem.* 1999, 47, 3764–3770.

(21) Shelnutt, S. R.; Cimino, C. O.; Wiggins, P. A.; Ronis, M. J.; Badger, T. M. Pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein in men and women after consumption of a soy beverage. *Am. J. Clin. Nutr.* 2002, 76, 588–594.

(22) Zhang, J.; Guo, Q.; Wei, M.; Bai, J.; Huang, J.; Liu, Y.; Su, Z.; Qiu, X. Metabolite Identification and Pharmacokinetic Profiling of Isoflavones from Black Soybean in Rats Using Ultra-high-Performance Liquid Chromatography with Linear-Ion-Trap–Orbitrap and Triple-Quadrupole Tandem Mass Spectrometry. *J. Agric. Food Chem.* 2018, 66, 12941–12952.

(23) Huang, M.-C.; Hsieh, T. Y.; Cheng, Y.-Y.; Lin, L.-C.; Tsai, T.-H. Pharmacokinetics and Biliary Excretion of Fisetin in Rats. *J. Agric. Food Chem.* 2018, 66, 6300–6307.

(24) Zeng, M.; Sun, R.; Basu, S.; Ma, Y.; Ge, S.; Yin, T.; Gao, S.; Zhang, J.; Hu, M. Disposition of flavonoids via recycling: Direct biliary excretion of enterically or extrahepatically derived flavonoid glucuronides. *Mol. Nutr. Food Res.* 2016, 60, 1006–1019.