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S-equol glucuronidation in liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice

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ABSTRACT

S-equol, an active metabolite of the soy isoflavone daidzein, is mainly metabolized into glucuronide(s) by UDP-glucuronosyltransferase (UGT) enzymes in mammals. In the present study, *S*-equol glucuronidation was examined in the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice using a kinetic analysis. CL_{int} values for 7- and 4′-glucuronidation by liver microsomes were higher than those by intestinal microsomes in all species. CL_{int} values for total glucuronidation (sum of 7- and 4′-glucuronidation) were rats (7.6) > monkeys (5.8) > mice (4.9) > dogs (2.8) > humans (1.0) for liver microsomes, and rats (9.6) > mice (2.8) > dogs (1.3) ≥ monkeys (1.2) > humans (1.0) for intestinal microsomes, respectively. Regarding regioselective glucuronidation by liver and intestinal microsomes, CL_{int} values were 7-glucuronidation > 4′-glucuronidation for humans, monkeys, dogs, and mice, and 4′-glucuronidation > 7-glucuronidation for rats. These results suggest that the metabolic abilities of UGT enzymes toward *S*-equol in the liver and intestines markedly differ among humans, monkeys, dogs, rats, and mice.

1. Introduction

S-equol (7-hydroxy-3-(4′-hydroxyphenyl)-chroman, Fig. 1) is an active metabolite of the soy isoflavone daidzein, which is formed by intestinal bacteria (Setchell et al., 2002; Atkinson et al., 2005; Yuan et al., 2007; Setchell and Clerici, 2010a). It is not produced in all adults who consume soy foods; the frequency of equol-producers is approximately 30–50% (Lampe et al., 1998; Bolca et al., 2007; Yuan et al., 2007; Setchell and Clerici, 2010a). Previous studies reported that the frequency of equol-producers was significantly higher among Asians (25–30%), such as Japanese and Koreans, than among Caucasians (50–60%) (Morton et al., 2002; Akaza et al., 2004; Atkinson et al., 2005; Setchell and Clerici, 2010a). Although the reasons for these differences currently remain unclear, dietary factors, including soy, meat, vegetable, and carbohydrate intakes, and host genetics have been suggested to partly contribute to the phenotype of equol-producers (Setchell et al., 2002; Hedlund et al., 2005; Frankenfeld, 2011; Hong et al., 2012).

S-equol is a selective estrogen receptor-β agonist with weaker activity for estrogen receptor-α and stronger estrogenic effects than its precursor daidzein (Muthyala et al., 2004; Carreau et al., 2009; Setchell and Clerici, 2010b; Shinkaruk et al., 2010). *S*-equol also has potential as an antagonist of androgen receptors and may prevent androgen-mediated pathologies, such as prostate cancer and skin conditions (Hedlund et al., 2003; Lund et al., 2004, 2011; Setchell and Clerici, 2010b). In addition to its modulating effects on sex hormones, the antioxidant and free radical scavenging activities of *S*-equol are stronger than those of daidzein (Rimbach et al., 2003; Setchell and Clerici, 2010b; Liang et al., 2010; Mahmoud et al., 2013). Epidemiological studies have suggested that equol-producers exhibit more favorable responses to soy isoflavone-containing diets (Niculescu et al., 2007; Lampe, 2009; Setchell and Clerici, 2010a; Hong et al., 2012), indicating that the metabolite *S*-equol has greater biological potency than daidzein.

S-equol undergoes first-pass metabolism in mammals, including humans, save phase II metabolism by conjugation to glucuronic acid and, to a minor extent, sulfuric acid. *S*-equol circulates in plasma and is excreted in urine as 7- and/or 4′-glucuronides (Fig. 1) formed by UDP-glucuronosyltransferase (UGT) (Setchell and Clerici, 2010b; Schwen et al., 2012; Legette et al., 2014; Redmon et al., 2016). In this respect, its metabolism is similar to that of daidzein and genistein (Thomas et al., 2001; Shelnutt et al., 2002; Hosoda et al., 2011; Soukup et al., 2016).

Since *S*-equol is produced from daidzein by intestinal bacteria in mammals, glucuronidation in the intestines as well as liver has been suggested to contribute to the beneficial effects of *S*-equol. Although the

Abbreviations: UGT, UDP-glucuronosyltransferase

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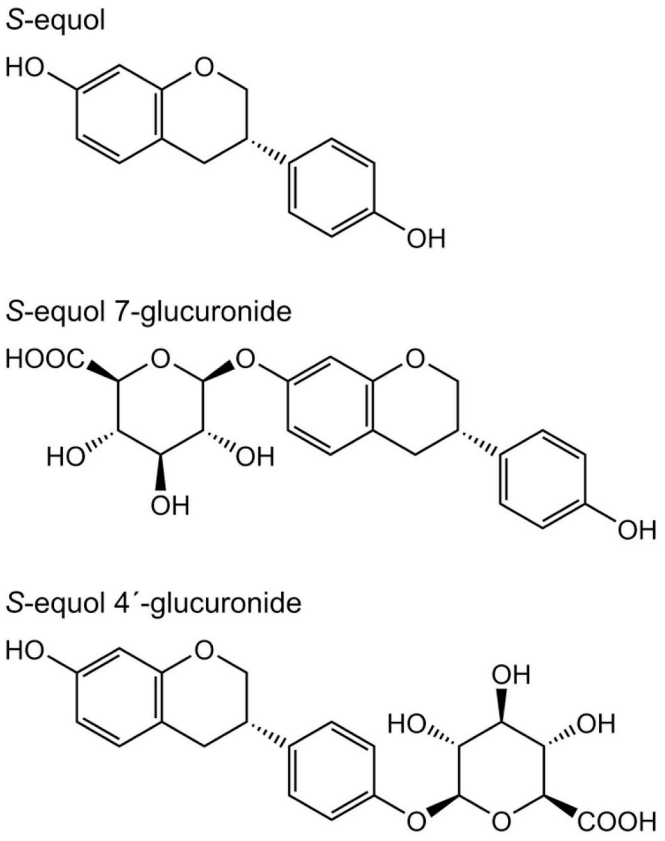

Fig. 1. Chemical structures of *S*-equol and its glucuronides.

activities of *S*-equol glucuronidation in the liver microsomes of humans and laboratory animals, such as monkeys, dogs, rabbits, and rodents, were previously examined by Redmon et al. (2016), limited information is currently available on species differences in the regioselective and tissue-dependent glucuronidation of *S*-equol. In the present study, the hepatic and intestinal glucuronidation of *S*-equol at the 7- and 4′-hydroxy groups by microsomal fractions of humans, monkeys, dogs, rats, and mice were investigated in order to predict the relationship between the metabolism and biological effects of *S*-equol.

2. Materials and methods

2.1. Materials

S-equol (≥99%) was purchased from Daicel (Tokyo, Japan). *S*-equol 7-glucuronide (≥98%) and 4′-glucuronides (≥98%) were purchased from TopuBio Research (Toyama, Japan). Alamethicin and daidzein were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). UDP-glucuronic acid was obtained from Nacalai Tesque (Kyoto, Japan). Pooled microsomes of human livers (race, Caucasian and Hispanic; age, 20–78 years old), human intestines (race, Caucasian and Hispanic; age, 18–55 years old), monkey livers (strain, cynomolgus; age, 3–8 years old), monkey intestines (strain, cynomolgus; age, 2–5 years old), dog livers (strain, beagle; age, 0.5–3 years old), dog intestines (strain, beagle; age, > 6 months old), rat livers and intestines (strain, Sprague-Dawley; age, > 8 weeks old), and mouse livers and intestines (strain, CD1; age, > 11 weeks old) were obtained from Sekisui XenoTech (Lenexa, KS, USA). All other chemicals and reagents used were of the highest quality commercially available.

Table 1
S-equol glucuronidation activities in liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice.

	7-Glucuronidation	4′-Glucuronidation	7-/4′-Glucuronidation
	(nmol/mim/mg protein)		
Liver microsomes			
Human	4.13 ± 0.10	0.54 ± 0.02	7.62 ± 0.11
Monkey	18.8 ± 1.2	1.35 ± 0.17	13.9 ± 1.1
Dog	7.56 ± 0.40	1.09 ± 0.09	6.96 ± 0.09
Rat	9.28 ± 0.88	22.6 ± 1.5	0.41 ± 0.03
Mouse	20.4 ± 1.5	5.05 ± 0.20	4.05 ± 0.05
Intestinal microsomes			
Human	1.35 ± 0.07	0.25 ± 0.01	5.49 ± 0.02
Monkey	3.19 ± 0.18	0.19 ± 0.02	16.7 ± 0.23
Dog	0.61 ± 0.03	0.29 ± 0.02	2.12 ± 0.01
Rat	3.26 ± 0.27	19.1 ± 1.6	0.17 ± 0.00
Mouse	4.46 ± 0.22	1.69 ± 0.11	2.64 ± 0.04

The substrate concentration used was 20 μM. Each value represents the mean ± SD of three separate experiments.

2.2. Assay for S-equol glucuronidation activity

S-equol glucuronidation activities in the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice were assessed by measuring the formation of *S*-equol 7- and 4′-glucuronides using HPLC. The incubation mixture contained *S*-equol (0.2–200 μM), liver or intestinal microsomes, alamethicin (10 μg/mL), 10 mM $MgCl_2$, and 2000 μM UDP-glucuronic acid in a final volume of 200 μL of 50 mM Tris-HCl buffer (pH 7.4). The protein concentrations of liver microsomes were 50 μg protein/mL for humans and dogs, and 25 μg protein/mL for monkeys, rats, and mice. The protein concentrations of intestinal microsomes were 50 μg protein/mL for humans, monkeys, dogs, and

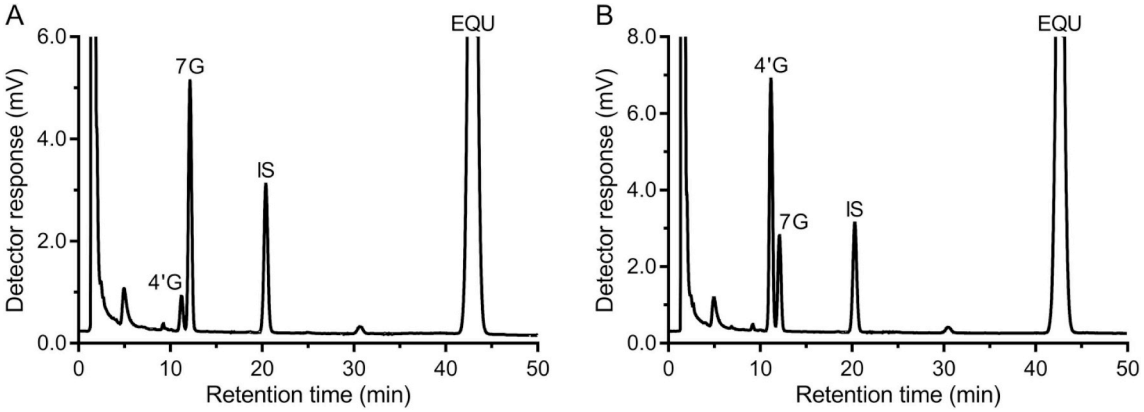

Fig. 2. HPLC analysis of *S*-equol glucuronidation activity in human and rat liver microsomes. Substrate concentration was 20 μM. Panels: (A) human liver microsomes; (B) rat liver microsomes. Peaks: 7G, *S*-equol 7-glucuronide; 4′G, *S*-equol 4′-glucuronide; IS, internal standard (daidzein); EQU, *S*-equol.

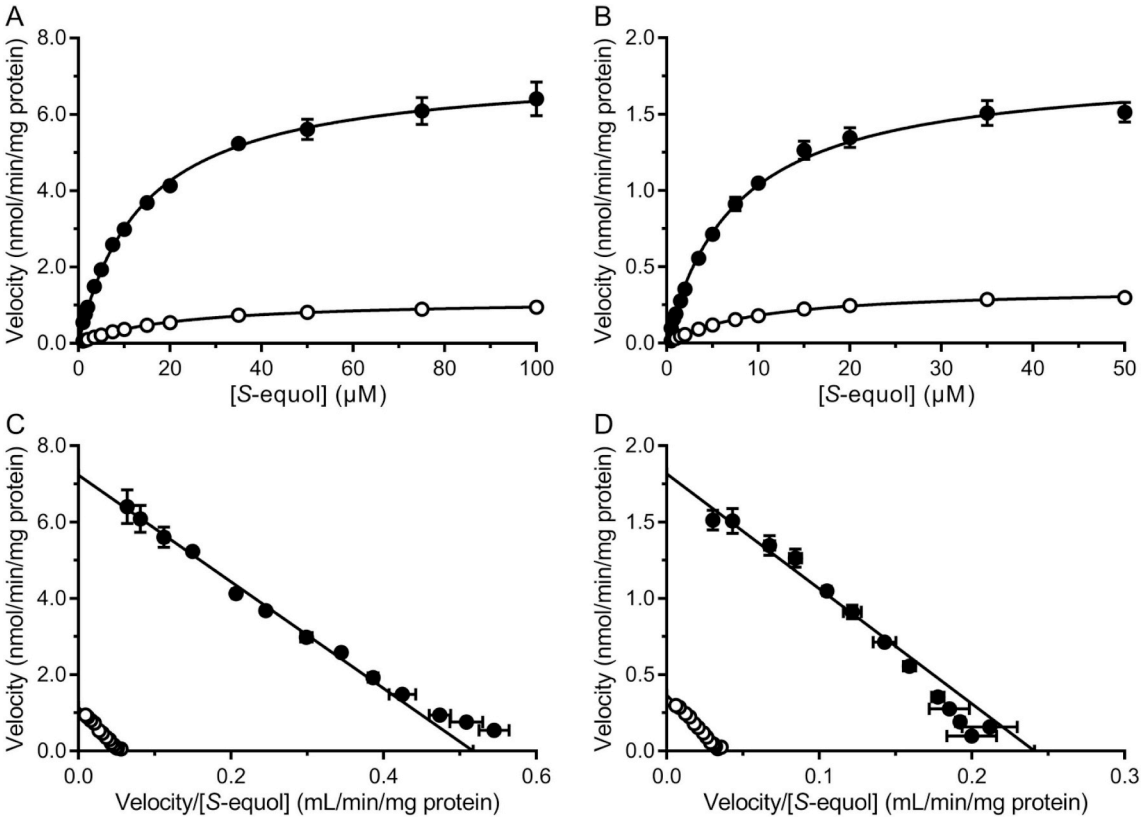

Fig. 3. Kinetics for *S*-equol glucuronidation in human liver and intestinal microsomes. *S*-equol concentrations were 1.0–100 μM for liver microsomes and 0.5–500 μM for intestinal microsomes. Each point represents the mean ± SD of three separate experiments. Panels: (A) *v* versus [*S*] plots for liver microsomes; (B) *v* versus [*S*] plots for intestinal microsomes; (C) *v* versus *V*/[*S*] plots for liver microsomes; (D) *v* versus *V*/[*S*] plots for intestinal microsomes. ●, 7-glucuronidation; ◯, 4′-glucuronidation.

mice, and 25 μg protein/mL for rats. *S*-equol was dissolved in methanol (final concentration in the incubation mixture, 1% v/v). After a pre-incubation at 37 °C for 2 min, the reaction was initiated by the addition of UDP-glucuronic acid. An incubation was performed at 37 °C. The incubation times of liver microsomes were 10 min for humans and 5 min for monkeys, dogs, rats, and mice. The incubation times of intestinal microsomes were 20 min for humans and dogs, 10 min for monkeys and mice, and 5 min for rats. The reaction was terminated by the addition of 200 μL of acetonitrile, spiked with daidzein (1000 pmol) as an internal standard, and then vortexed. Samples were centrifuged at 12000 × *g* at 4 °C for 10 min. The supernatant was filtered with a polytetrafluoroethylene membrane filter (0.45 μm), and 10 μL of the filtrate was subjected to HPLC. Standard curve samples spiked with *S*-equol 7- and 4′-glucuronides were prepared in the same manner as incubation samples.

An Inertsil ODS-SP column (5 μm, 3.0 mm i.d. × 150 mm; GL Sciences, Tokyo, Japan) was used in the examination of *S*-equol 7- and 4′-glucuronides. The column was maintained at 40 °C. *S*-equol 7- and 4′-glucuronides as well as the substrate and internal standard were isocratically eluted with 0.1% phosphoric/acetonitrile (82:18, v/v) at a flow rate of 0.6 mL/min. UV detection was performed at 200 nm. Under these conditions, the retention times of *S*-equol 7- and 4′-glucuronides, *S*-equol, and the internal standard were 12.1, 11.2, 42.8, and 19.7 min, respectively. Typical chromatograms of the assay for *S*-equol glucuronidation activity at a substrate concentration of 20 μM in human and rat liver microsomes is shown in Fig. 2.

2.3. Data analysis

Kinetic parameters (K_m and V_{max}) for *S*-equol glucuronidation by the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice were calculated by constructing *v* versus *V*/[*S*] plots (Eadie–Hofstee plots) using SigmaPlot v14.0 software (Systat Software, San Jose, CA, USA). The kinetic profile was estimated from the respective coefficient of determination and/or Akaike's information criterion values for the Michaelis–Menten, isoenzyme, substrate inhibition, and Hill equations. *In vitro* clearance (CL_{int}) values were obtained as the ratio of V_{max}/K_m. All values are expressed as the mean ± SD of three separate experiments.

3. Results

3.1. S-equol glucuronidation activities in liver and intestine microsomes

S-equol 7- and 4′-glucuronidation activities in the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice were initially examined at a substrate concentration of 20 μM (Table 1). The activities of 7- and 4′-glucuronidation in human liver microsomes were 4.13 and 0.54 nmol/min/mg protein, respectively. 7-Glucuronidation activities in the liver microsomes of monkeys, dogs, rats, and mice were 4.6-, 1.8-, 2.2-, and 4.9-fold, respectively, those in human liver microsomes, while 4′-glucuronidation activities were 2.5-, 2.0-, 42-, and 9.3-fold, respectively. The activity ratio of 7-/4′-glucuronidation in human liver microsomes was 7.62. Activity ratios in the liver microsomes of monkeys, dogs, rats, and mice were 1.8-, 0.9-, 0.05-, 0.1-, and 0.5-fold, respectively, that in human liver microsomes.

The activities of 7- and 4′-glucuronidation in human intestinal microsomes were 1.35 and 0.25 nmol/min/mg protein, respectively. 7-Glucuronidation activities in the intestinal microsomes of monkeys, dogs, rats, and mice were 2.4-, 0.5-, 2.4-, and 3.3-fold, respectively, that in human intestinal microsomes, while 4′-glucuronidation activities were 0.8-, 1.2-, 76-, and 6.8-fold, respectively. The activity ratio of 7-/

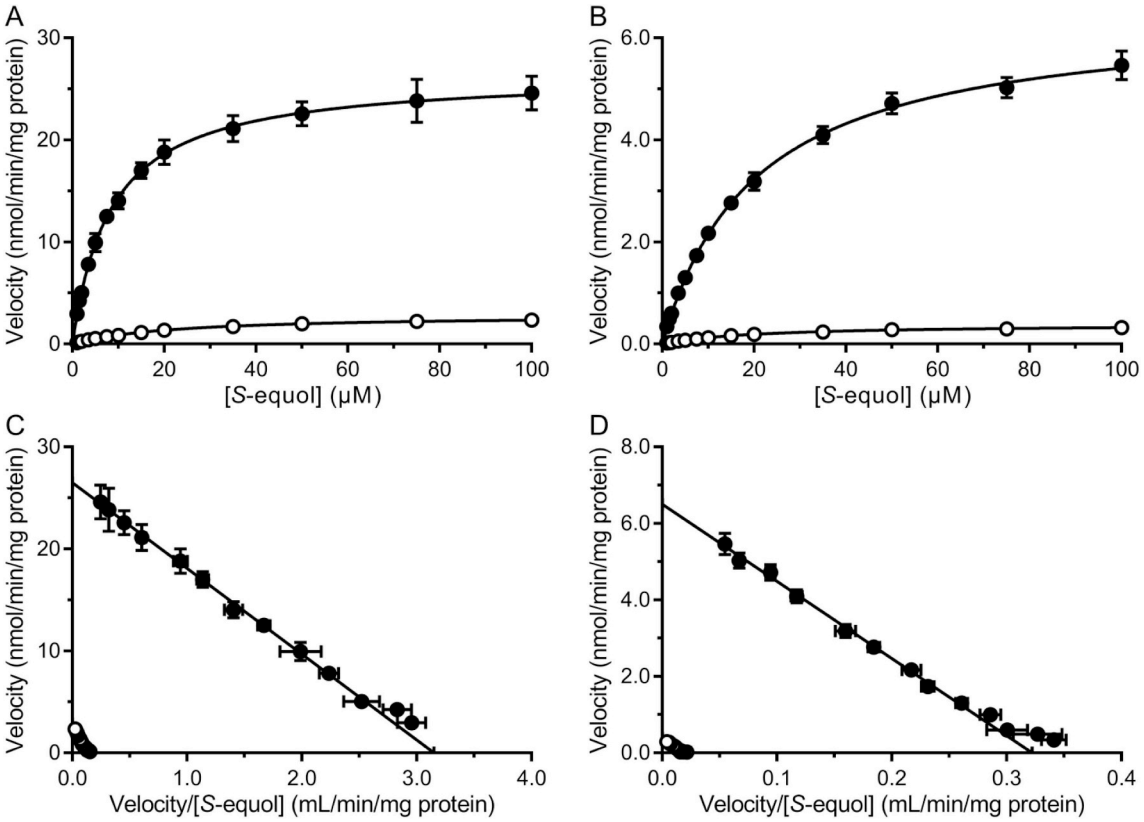

Fig. 4. Kinetics for *S*-equol glucuronidation in monkey liver and intestinal microsomes. *S*-equol concentrations were 1.0–100 μM. Each point represents the mean ± SD of three separate experiments. Panels: (A) *v* versus [*S*] plots for liver microsomes; (B) *v* versus [*S*] plots for intestinal microsomes; (C) *v* versus *V*/[*S*] plots for liver microsomes; (D) *v* versus *V*/[*S*] plots for intestinal microsomes. ●, 7-glucuronidation; ◯, 4′-glucuronidation.

4′-glucuronidation in human intestinal microsomes was 5.49. The activity ratios in the intestinal microsomes of monkeys, dogs, rats, and mice were 3.0-, 0.4-, 0.03-, and 0.5-fold, respectively, that in human intestinal microsomes.

3.2. Kinetics for S-equol glucuronidation by liver and intestinal microsomes

Kinetic analyses of *S*-equol glucuronidation by the liver and intestinal microsomes of humans, dogs, monkeys, rats, and mice were then performed at substrate concentrations of 0.2–200 μM (13 points in each enzyme source). The plots (*v* versus [*S*] and *v* versus *V*/[*S*]) for 7- and 4′-glucuronidation are shown in Figs. 3–7 for the liver and intestinal microsomes of humans, dogs, monkeys, rats, and mice. The calculated kinetic parameters are summarized in Table 2 for liver microsomes and Table 3 for intestinal microsomes.

Kinetics for the 7- and 4′-glucuronidation of *S*-equol by human liver microsomes followed the Michaelis–Menten model. K_m, V_{max}, and CL_{int} values were 14.0 μM, 7.24 nmol/min/mg protein, and 0.52 mL/min/mg protein for 7-glucuronidation, and 21.5 μM, 1.15 nmol/min/mg protein, and 0.05 mL/min/mg protein for 4′-glucuronidation, respectively. The kinetics for 7- and 4′-glucuronidation by the liver microsomes of monkeys and rats also followed the Michaelis–Menten model. K_m, V_{max}, and CL_{int} values for 7-glucuronidation to those by human liver microsomes were 0.6-, 3.7-, and 6.1-fold for monkey liver microsomes, and 0.5-, 1.7-, and 3.1-fold for rat liver microsomes, respectively. The K_m, V_{max}, and CL_{int} values for 4′-glucuronidation to those by human liver microsomes were 1.1-, 2.5-, and 2.6-fold for monkey liver microsomes, and 0.6, 33-, and 55-fold for rat liver microsomes, respectively. The kinetics for 7- and 4′-glucuronidation by dog liver microsomes fit the biphasic model. K_m, V_{max}, and CL_{int} values for 7-glucuronidation in the high- and low-affinity phases were 0.03-, 0.05-, and 1.6-fold, and 3.8-, 3.5-, and 0.9-fold, respectively, those by human liver microsomes. K_m, V_{max}, and CL_{int} values for 4′-glucuronidation in the high- and low-affinity phases were 0.03-, 0.1-, and 4.4-fold, and 2.6-, 3.1-, and 1.4-fold, respectively, those by human liver microsomes. The kinetics for 7- and 4′-glucuronidation by mouse liver microsomes fit the biphasic and Michaelis–Menten models, respectively. K_m, V_{max}, and CL_{int} values for 7-glucuronidation to those by human liver microsomes were 1.2-, 5.3-, and 4.3-fold, respectively. K_m, V_{max}, and CL_{int} values for 4′-glucuronidation in the high- and low-affinity phases were 0.5-, 3.0-, and 7.2-fold, and 5.6-, 17-, and 3.2-fold, respectively, those by human liver microsomes.

The kinetics for 7- and 4′-glucuronidation of *S*-equol by human intestinal microsomes followed the Michaelis–Menten model. K_m, V_{max}, and CL_{int} values were 7.54 μM, 1.82 nmol/min/mg protein, and 0.24 mL/min/mg protein, and 10.2 μM, 0.37 nmol/min/mg protein, and 0.04 mL/min/mg protein for 4′-glucuronidation, respectively. The kinetics for 7- and 4′-glucuronidation by the intestinal microsomes of monkeys and rats and mice also followed the Michaelis–Menten model. K_m, V_{max}, and CL_{int} values for 7-glucuronidation to those by human intestinal microsomes were 2.7-, 3.6-, and 1.3-fold for monkey intestinal microsomes, 0.5-, 0.4-, and 0.9-fold for dog intestinal microsomes, and 1.3-, 2.7-, and 2.0-fold for rat intestinal microsomes, respectively. K_m, V_{max}, and CL_{int} values for 4′-glucuronidation to those by human intestinal microsomes were 2.1-, 1.1-, and 0.5-fold for monkey intestinal microsomes, 0.2-, 0.9-, and 3.8-fold for dog intestinal microsomes, and 1.5-, 89-, and 55-fold for rat intestinal microsomes, respectively. The kinetics for 7- and 4′-glucuronidation by mouse intestinal microsomes fit the biphasic model. K_m, V_{max}, and CL_{int} values for 7-glucuronidation in the high- and low-affinity phases were 1.2-, 2.7-, and 2.3-fold, and 28-, 7.4-, and 0.3-fold, respectively, those by human intestinal microsomes. K_m, V_{max}, and CL_{int} values for 4′-glucuronidation in the high- and low-affinity phases were 0.9-, 2.5- and 2.5-fold, and 19-, 32-, and 1.5-fold, respectively, those by human liver microsomes.

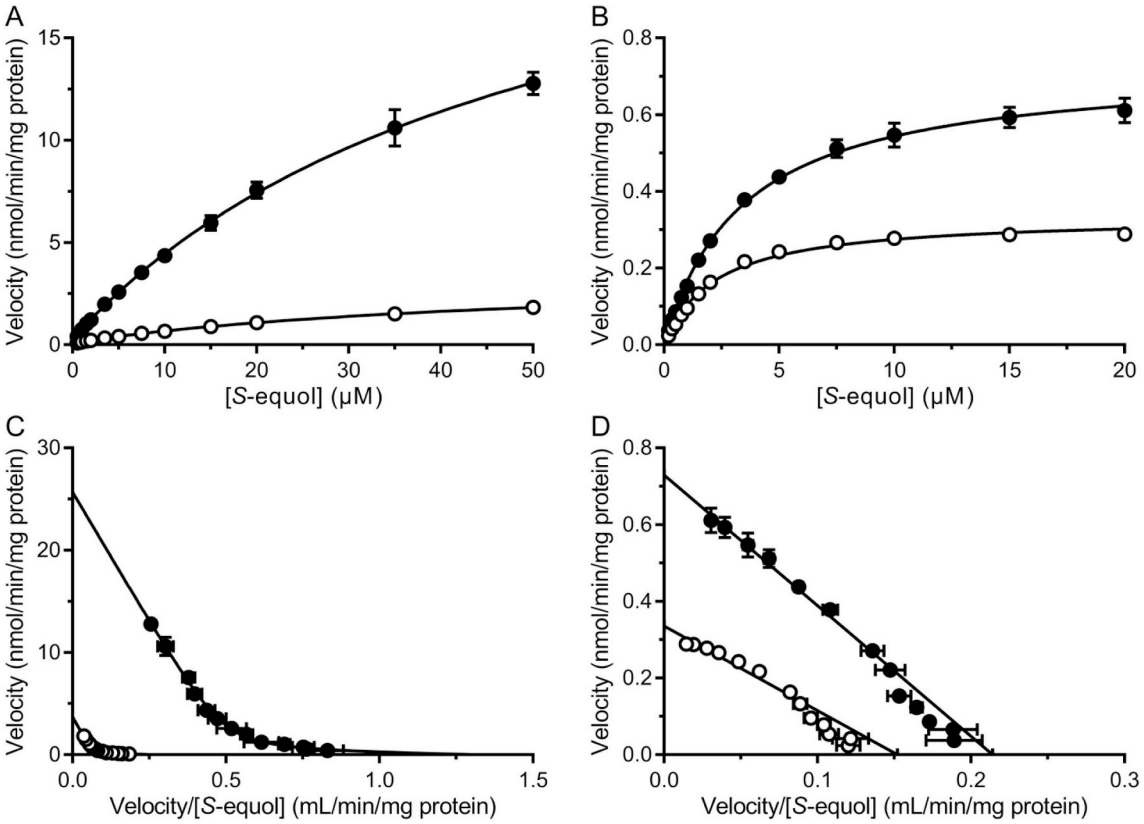

Fig. 5. Kinetics for *S*-equol glucuronidation in dog liver and intestinal microsomes. *S*-equol concentrations were 0.5–50 μM for liver microsomes and 0.2–20 μM for intestinal microsomes. Each point represents the mean ± SD of three separate experiments. Panels: (A) *v* versus [*S*] plots for liver microsomes; (B) *v* versus [*S*] plots for intestinal microsomes; (C) *v* versus *V*/[*S*] plots for liver microsomes; (D) *v* versus *V*/[*S*] plots for intestinal microsomes. ●, 7-glucuronidation; ◯, 4′-glucuronidation.

4. Discussion

S-equol is a metabolite of daidzein with stronger estrogenic and antioxidant activities than its precursor (Muthyala et al., 2004; Setchell and Clerici, 2010b; Shinkaruk et al., 2010 Mahmoud et al., 2013). *S*-equol circulates in plasma and is excreted in urine predominantly as glucuronide conjugates in humans, monkeys, and rats (Setchell and Clerici, 2010b; Hosoda et al., 2011; Schwen et al., 2012; Soukup et al., 2016). Since glucuronidation is important for the detoxification and elimination of a large number of xenobiotics in mammals (Dutton, 1980; Miners and Mackenzie, 1991), biotransformation is considered to reduce the biological effects of *S*-equol. However, species differences in the regioselective and tissue-dependent glucuronidation of *S*-equol among humans and laboratory animals have not yet been investigated. In the present study, the hepatic and intestinal glucuronidation of *S*-equol in humans, monkeys, dogs, rats, and mice were examined by an *in vitro* system with microsomal fractions.

UGT activities toward *S*-equol at the 7- and 4′-hydroxyl groups in the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice were initially assessed at a single substrate concentration (20 μM) in order to obtain a clearer understanding of the general aspect of species differences in *S*-equol glucuronidation. 7-Glucuronidation activities in liver microsomes were higher in monkeys and mice, and were the lowest in humans. 4′-Glucuronidation activity was lower than 7-glucuronidation activity in the liver microsomes of humans, monkeys, dogs, and mice, but was higher in those of rats. Consequently, the activity ratio of 7-/4′-glucuronidation in the liver microsomes of humans, monkeys, dogs, rats, and mice varied, suggesting that the regioselectivity of *S*-equol glucuronidation by liver microsomes markedly differs among species.

Redmon et al. (2016) recently reported the 7-glucuronidation activities of *S*-equol in the liver microsomes of humans and some laboratory animals at a single substrate concentration (35 μM), whereas 4′-glucuronidation activities have not been examined in any species. Among the species examined in the present study, 7-glucuronidation activity in liver microsomes was the highest in monkeys and the lowest in humans (monkeys > rats ≥ mice ≥ dogs » humans), and the difference in activity between humans and monkeys was approximately five-fold. The profile and ranking order of 7-glucuronidation activity in the liver microsomes of humans, monkeys, dogs, rats, and mice in the present study was slightly different from those reported by Redmon et al. (2016).

The intestinal glucuronidation of *S*-equol in *in vivo* and/or *in vitro* systems has not yet been investigated. The present study showed that *S*-equol was glucuronidated at both the 7-hydroxy and 4′-hydroxy groups by the intestinal as well as liver microsomes of all species examined. Species differences in the activities of *S*-equol 7- and 4′- glucuronidation were approximately seven-fold (mice > rats ≥ monkeys > humans > dogs) and one hundred-fold (rats » mice > dogs ≥ humans ≥ monkeys), respectively. Accordingly, the activity ratio of 7-/4′-glucuronidation in intestinal microsomes varied by approximately one hundred-fold (monkeys » humans > mice ≥ dogs > rats). Thus, the regioselectivity of *S*-equol glucuronidation in intestinal microsomes markedly differed among species (humans, monkeys, dogs, and mice, 7-glucuronidation > 4′-glucuronidation; rats, 4′-glucuronidation > 7-glucuronidation), similar to liver microsomes. Many UGT isoforms have been reported to be expressed in the hepatic and/or extrahepatic tissues of mammals, including humans and laboratory animals, and their enzymatic functions have been analyzed (Mackenzie et al., 2005; Harbourt et al., 2012; Sato et al., 2014; Kutsukake et al., 2019). Based on the UGT properties characterized, species and regioselective differences in *S*-equol glucuronidation appear to be attributed to the

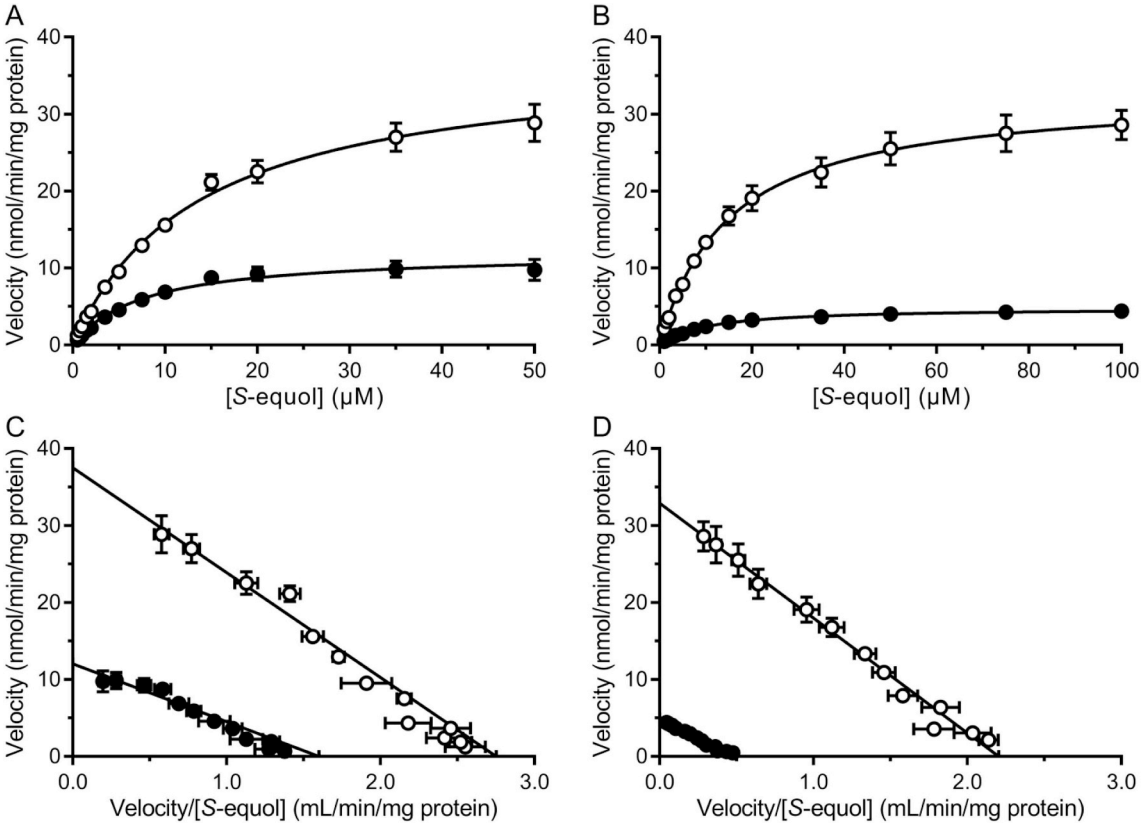

Fig. 6. Kinetics for *S*-equol glucuronidation in rat liver and intestinal microsomes. *S*-equol concentrations were 0.5–50 μM for liver microsomes and 1.0–100 μM for intestinal microsomes. Each point represents the mean ± SD of three separate experiments. Panels: (A) *v* versus [*S*] plots for liver microsomes; (B) *v* versus [*S*] plots for intestinal microsomes; (C) *v* versus *V*/[*S*] plots for liver microsomes; (D) *v* versus *V*/[*S*] plots for intestinal microsomes. ●, 7-glucuronidation; ◯, 4′-glucuronidation.

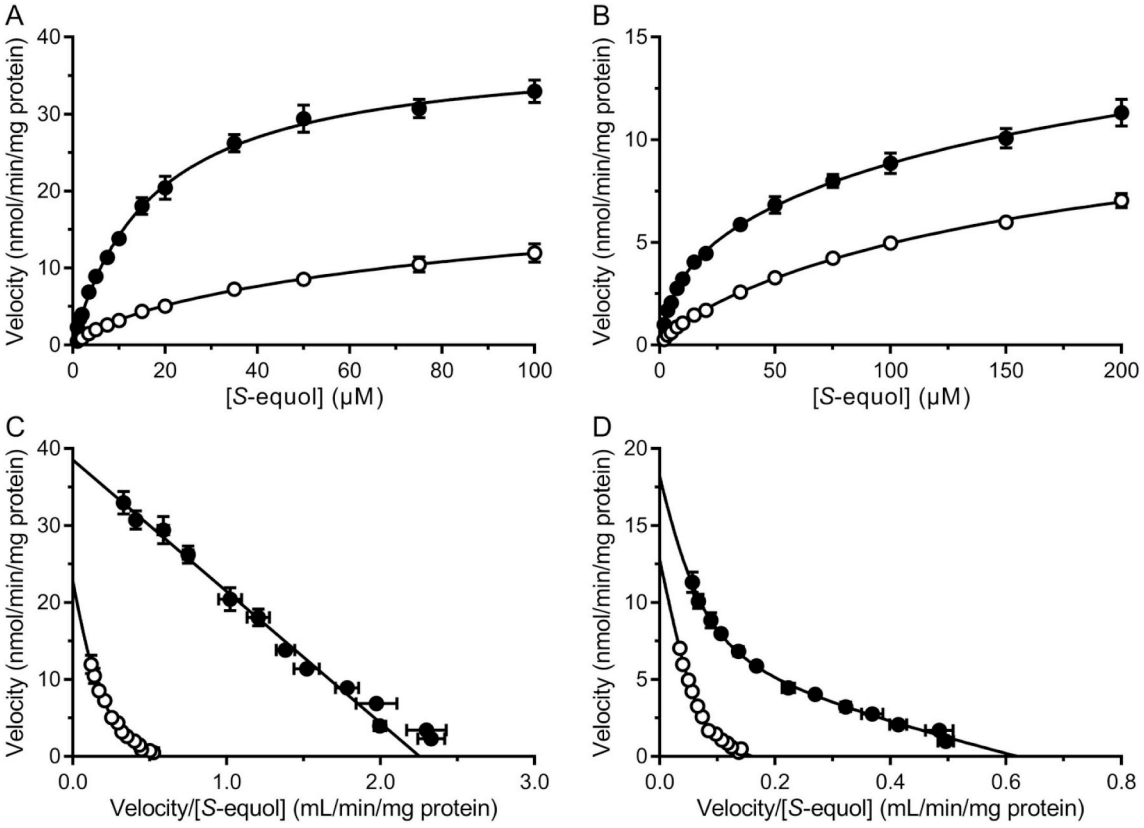

Fig. 7. Kinetics for *S*-equol glucuronidation in mouse liver and intestinal microsomes. *S*-equol concentrations were 1.0–100 μM for liver microsomes and 2.0–200 μM for intestinal microsomes. Each point represents the mean ± SD of three separate experiments. Panels: (A) *v* versus [*S*] plots for liver microsomes; (B) *v* versus [*S*] plots for intestinal microsomes; (C) *v* versus *V*/[*S*] plots for liver microsomes; (D) *v* versus *V*/[*S*] plots for intestinal microsomes. ●, 7-glucuronidation; ◯, 4′-glucuronidation.

Table 2
Kinetic parameters for *S*-equol glucuronidation by liver microsomes of humans, monkeys, dogs, rats, and mice.

	K_m (μM)	V_{max} (nmol/mim/mg protein)	CL_{int} (mL/min/mg protein)	Kinetic model
7-Glucuronidation				
Human	14.0 ± 1.7	7.24 ± 0.53	0.52 ± 0.02	Michaelis–Menten
Monkey	8.41 ± 0.78	26.5 ± 2.1	3.15 ± 0.05	Michaelis–Menten
Dog				Biphasic
High-affinity phase	0.46 ± 0.13	0.38 ± 0.08	0.84 ± 0.15	
Low-affinity phase	52.5 ± 6.6	25.5 ± 1.1	0.49 ± 0.04	
Rat	7.50 ± 0.86	12.1 ± 1.6	1.60 ± 0.04	Michaelis–Menten
Mouse	17.1 ± 0.88	38.6 ± 1.4	2.25 ± 0.15	Michaelis–Menten
4′-Glucuronidation				
Human	21.5 ± 3.8	1.15 ± 0.15	0.05 ± 0.00	Michaelis–Menten
Monkey	22.6 ± 4.4	2.88 ± 0.63	0.13 ± 0.01	Michaelis–Menten
Dog				Biphasic
High-affinity phase	0.62 ± 0.14	0.13 ± 0.01	0.22 ± 0.04	
Low-affinity phase	56.1 ± 13.2	3.61 ± 0.45	0.07 ± 0.01	
Rat	13.7 ± 1.9	37.6 ± 4.1	2.76 ± 0.11	Michaelis–Menten
Mouse				Biphasic
High-affinity phase	9.74 ± 1.41	3.50 ± 0.35	0.36 ± 0.03	
Low-affinity phase	120 ± 37	19.6 ± 6.2	0.16 ± 0.01	

Each value represents the mean ± SD of three separate experiments.

expression levels and enzymatic functions of each UGT isoform in the liver and intestines differing among humans, monkeys, rats, and mice.

Kinetic analyses on *S*-equol glucuronidation in the liver and intestinal microsomes of humans, monkeys, dogs, rats, and mice were subsequently performed at a broad range of substrate concentrations. The kinetics of 7-glucuronidation by the liver and intestinal microsomes of humans, monkeys, and rats fit the Michaelis–Menten model; however, the values for kinetic parameters markedly differed among species. In dogs, the kinetics of liver microsomes followed the biphasic model, whereas intestinal microsomes fit the Michaelis–Menten model. The kinetics for mice fit the Michaelis–Menten model for liver microsomes and the biphasic model for intestinal microsomes. Regarding 4′-glucuronidation by liver and intestinal microsomes, the kinetic models in each species were the same as those for 7-glucuronidation, with the exception of the biphasic model for mouse liver microsomes. Thus, the kinetic profiles of *S*-equol glucuronidation in liver and intestinal microsomes vary by species, suggesting that the roles of UGT isoform(s) in *S*-equol glucuronidation markedly differ among humans, monkeys, dogs, rats, and mice.

The V_{max} value (y-intercept) and CL_{int} value (x-intercept) based on v versus $V/[S]$ plots for the 7- and 4′-glucuronidation of *S*-equol by liver microsomes were higher than those by intestinal microsomes in all species. In total glucuronidation (sum of 7- and 4′-glucuronidation), V_{max} and CL_{int} values were mice (6.9) > rats (5.9) > dogs (3.5) ≈ monkeys (3.5) > humans (1.0), and rats (7.6) > monkeys (5.8) > mice (4.9) > dogs (2.8) > humans (1.0) for liver microsomes, and rats (17) > mice (14) » monkeys (3.2) > humans (1.0) > dogs (0.5), and rats (9.6) > mice (2.8) > dogs (1.3) ≥ monkeys (1.2) > humans (1.0) for intestinal microsomes, respectively. In addition, it is noteworthy that the extent of species differences in V_{max} and CL_{int} values for 4′-glucuronidation was markedly larger than that for 7-glucuronidation in both microsomal fractions. V_{max} and CL_{int} values were 7-glucuronidation > 4′-glucuronidation for humans, monkeys, dogs, and mice from the viewpoint of regioselective glucuronidation, while the opposite regioselectivity (4′-glucuronidation > 7-glucuronidation) was observed for rats in liver and intestinal microsomes. These results suggest that the metabolic abilities and regioselectivities of UGT enzymes toward *S*-equol in the liver and intestines markedly differ between humans and non-human primates (monkeys), medium-sized laboratory animals (dogs), or rodents (rats and mice), and also that the enzymatic functions and tissue distributions of UGT isoforms involved in *S*-equol glucuronidation markedly vary among species. Further studies are needed in order to clarify the roles of hepatic and intestinal UGT isoforms in the regioselective glucuronidation and bioactive mechanism of *S*-equol using the recombinant enzymes of each species.

Table 3
Kinetic parameters for *S*-equol glucuronidation by intestinal microsomes of humans, monkeys, dogs, rats, and mice.

	K_m (μM)	V_{max} (nmol/mim/mg protein)	CL_{int} (mL/min/mg protein)	Kinetic model
7-Glucuronidation				
Human	7.54 ± 0.13	1.82 ± 0.03	0.24 ± 0.01	Michaelis–Menten
Monkey	20.3 ± 2.0	6.51 ± 0.34	0.32 ± 0.02	Michaelis–Menten
Dog	3.41 ± 0.06	0.73 ± 0.03	0.21 ± 0.01	Michaelis–Menten
Rat	10.1 ± 0.7	4.86 ± 0.51	0.48 ± 0.02	Michaelis–Menten
Mouse				Biphasic
High-affinity phase	8.82 ± 0.40	4.92 ± 0.14	0.56 ± 0.01	
Low-affinity phase	211 ± 51	13.4 ± 2.4	0.07 ± 0.01	
4′-Glucuronidation				
Human	10.2 ± 0.2	0.37 ± 0.01	0.04 ± 0.00	Michaelis–Menten
Monkey	21.0 ± 3.2	0.39 ± 0.02	0.02 ± 0.00	Michaelis–Menten
Dog	2.21 ± 0.07	0.34 ± 0.01	0.15 ± 0.00	Michaelis–Menten
Rat	14.9 ± 0.6	32.9 ± 3.3	2.20 ± 0.13	Michaelis–Menten
Mouse				Biphasic
High-affinity phase	9.43 ± 1.59	0.93 ± 0.11	0.10 ± 0.01	
Low-affinity phase	193 ± 46	12.0 ± 1.8	0.06 ± 0.01	

Each value represents the mean ± SD of three separate experiments.

5. Conclusions

The hepatic and intestinal glucuronidation of *S*-equol in humans, monkeys, dogs, rats, and mice was examined in an *in vitro* system using microsomal fractions. CL_{int} values for 7- and 4′-glucuronidation were higher in liver microsomes than in intestinal microsomes in all species. CL_{int} values for total glucuronidation (sum of 7- and 4′-glucuronidation) were rats > monkeys > mice > dogs > humans for liver microsomes, and rats > mice > dogs ≥ monkeys > humans for intestinal microsomes. Regioselectivity based on CL_{int} values was 7-glucuronidation > 4′-glucuronidation for humans, monkeys, dogs, and mice, and 4′-glucuronidation > 7-glucuronidation for rats in liver and intestinal microsomes. These results suggest that the metabolic abilities and regioselectivities of UGT enzymes toward *S*-equol in the liver and intestines differ among humans, monkeys, rats, and mice.

Conflicts of interest

The authors declare no conflicts of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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