Metabolic Phenotype of Isoflavones Differ among Female Rats, Pigs, Monkeys, and Women[1]

Liwei Gu,*† Suzanne E. House,* Ronald L. Prior,* Nianbai Fang,** * Martin J. J. Ronis,*‡ Thomas B. Clarkson,†† Mark E. Wilson,‡‡ and Thomas M. Badger*†[2]

*Arkansas Children's Nutrition Center, Little Rock, AR 722 02; Departments of †Physiology/Biophysics, **Pharmaceutical Sciences, and ‡Pharmacology/Toxicology, University of Arkansas for Medical Sciences, Little Rock, AR 72202; ††Comparative Medicine Clinical Research Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157; and ‡‡Yerkes National Primate Center and Emory University, Atlanta, GA

ABSTRACT Various physiologic effects of soy food consumption have been attributed to the estrogenic actions of isoflavones. The order of estrogen receptor binding potency of soy-derived isoflavone aglycones is equol > genistein > daidzein, and their conjugates are less potent. Because the metabolic profile may be an important determinant of bioactivity after soy intake, we studied the serum and urine isoflavone concentrations in 3 animal models and compared them with isoflavone profiles in women. Female Sprague-Dawley rats, Hampshire/Duroc Cross pigs, cynomolgus monkeys, and women were fed diets containing soy protein isolate. Isoflavones and their metabolites were measured by LC-MS or electrochemical detection. Equol represented ~77 and 52% (molar ratio) of summed serum isoflavones (isoflavones plus metabolites) in rats and cynomolgus monkeys, respectively. Equol was undetectable in pig serum and human plasma, but daidzein and genistein contributed >88% of summed circulating isoflavones. Monkey and rat urine contained high levels of aglycones (>85% and >32%, respectively), whereas pigs and women excreted isoflavone mainly in the form of glucuronides (>80%), with <10% as aglycones. Isoflavones in human plasma were predominantly glucuronides (75%) with 24% as sulfates and <1% as aglycones; in monkey serum, however, 64% of isoflavones were sulfates, 30% glucuronides, and 6% aglycones. Equol was also a major serum metabolite of 6-mo-old rhesus monkeys (80% of summed isoflavones). Thus, there were significant interspecies differences in isoflavone metabolism, and the overall metabolic profile of pigs was closer to that of women than that of rats or monkeys. J. Nutr. 136: 1215–1221, 2006.

KEY WORDS: • *isoflavone* • *daidzein* • *genistein* • *equol* • *interspecies difference*

There has been great interest in the potential beneficial and adverse effects of isoflavones from soy, as demonstrated by the thousands of scientific publications on this subject in the past decade. Soy consumption is implicated in alterations of many physiologic, endocrinologic, and metabolic processes that have potential consequences in human health (1). Most of these effects were attributed to specific isoflavones and/or their metabolites. Therefore, it is important to know the circulating isoflavone profile after consumption of soy foods. An isoflavone metabolite of daidzein, equol, which is considered one of the most potent soy isoflavone metabolites, has received much attention (2). Two isoflavone metabolic phenotypes in human subjects (referred to as equol or nonequol producers) were shown to be related to the intestinal microflora; ~35% of the adult human population are able to produce significant amounts of equol from daidzein (2). The health implications of having high circulating levels of equol are not clear, but equol-producing premenopausal women had lower circulating concentrations of estrone and dehydroepiandrosterone that nonequol producers (3). Furthermore, *O*-desmethylangolensin (*O*-DMA)[3] production in postmenopausal women is associated with lower mammographic density or higher blood follicle stimulating hormone, respectively (4,5). Equol was also reported to affect male hormones and specifically bind dihydrotestosterone (6), and equol producing animals such as monkeys and rats were reported to have lower serum testosterone concentrations (7,8).

Most published studies are based on animal models, with the majority using rodents and primates. Although isoflavone

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[2] To whom correspondence should be addressed. E-mail: badgerthomasm@uams.edu.

[3] Abbreviations used: COT, Committee on Toxicology; DHD, dihydrodaidzein; DHG, dihydrogenistein; *O*-DMA, *O*-desmethylangolensin; SPI, soy protein isolate; SULT, sulfotransferases; UGT, UDP-glucuronosyltransferase.

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metabolism has been described in humans and several other species (9–11), little attention has been given to the interspecies differences in the metabolism of isoflavones, which could be crucial in explaining discrepancies in physiologic effects of soy in different animal models compared with humans, such as those involving soy-induced changes in serum LDL,VDL, and HDL cholesterol reported in cynomolgus monkeys vs. human subjects (12,13).

Furthermore, data from animal models were used as the basis of safety recommendations for human soy consumption. For example, rodent and monkey data were cited as an important factor for the recent recommendations of the Committee on Toxicology in the United Kingdom to limit soy formula intake in infants (14). In the present study, we characterized the metabolic phenotype of isoflavones in female rats, monkeys, pigs, and women after consumption of diets containing soy protein isolate (SPI) and discuss the relevance for using these animals as research models.

MATERIALS AND METHODS

The animal experiments described in this report were conducted at 3 institutions under protocols approved by the Institutional Animal Care and Use Committees of the respective institutions: rats and pigs at the University of Arkansas for Medical Sciences (Little Rock, AR); cynomolgus monkeys at the Comparative Medicine Clinical Research Center of Wake Forest University School of Medicine (Winston-Salem, NC); and rhesus monkeys at the Yerkes National Primate Center at Emory University (Atlanta, GA).

Rats. In Expt. 1, Sprague-Dawley female rats (~300 g; $n = 9$) were housed individually in metabolic cages. Rats were fed diet (15) produced by Harlan Teklad using the AIN-93G diet formulation, except SPI replaced casein as the sole source of protein, additional amino acids were supplemented to meet the levels of essential amino acids recommended by the NRC, and corn oil replaced soy oil as previously described (16). The daily intake of genistein, daidzein, and glycitein was estimated to be 13.0, 9.9, and 2.4 mg/kg body weight, respectively, expressed as aglycone equivalents. Urine (24 h) was collected in the presence of ascorbic acid and sodium azide (0.1% wt/volume) on d 3. Rats were decapitated midmorning of d 4 and trunk blood collected. Samples were stored at −70°C.

In Expt. 2, rats were fed the standard AIN-93G diet made with casein as the sole protein (15). Adult female rats (~240 g, $n = 4$) had 1 intragastric and 1 femoral cannula surgically implanted and were allowed to recover for 7 d. Rats were deprived of food overnight; the next morning they were administered an i.g. bolus of SPI (dissolved in water) that provided 1.0, 0.6, and 0.1 mg/kg body weight genistein, daidzein, and glycitein, respectively. Blood samples (0.8 mL) were collected through the femoral cannula at 4 h postdose.

Pigs. Female piglets (Hampshire × Duroc Cross, 4.4 kg, $n = 5$) were weaned at age 48 h to a liquid diet formulated to meet the nutrient requirements for infant pigs established by the NRC (17). The diet was based on a previously published formulation (18), except casein was replaced with SPI as the sole protein source, and cysteine, methionine, and phenylalanine were supplemented to meet the amino acid requirement of infant pigs. The daily intake of genistein, daidzein, and glycitein was calculated on the basis of food intake for the last 3 d to be 10.9, 8.6, and 1.4 mg/kg body weight, respectively, expressed as aglycone equivalents. Urine (24 h) was collected on d 29; the piglets were killed on d 30 and blood (15 mL) was collected by cardiopuncture.

Monkeys. Young adult female cynomolgus monkeys ($n = 15$) were fed a diet formulated with SPI to contain 140 mg isoflavone/7530 kJ. The composition of this diet was published (19). Monkeys were fed 502 kJ/kg body weight, which provided 4.8, 3.7, and 0.8 mg genistein, daidzein and glycitein, respectively, expressed as aglycone equivalents. At the end of wk 5, blood was collected 4 h after the morning meal (one third of the daily food allotment). Monkeys were sedated (ketamine HCl, 100g/L; Ketaset, Fort Dodge Animal Health) and a blood sample (3–5 mL) was collected from the femoral vein for serum. Spot urine samples were also collected, with no attempt to collect 24-h samples or to time collection to meal intake.

Blood was collected from 3 infant rhesus monkeys (age 2, 4, and 6 mo old) at Yerkes National Primate Research Center and the serum was analyzed for total isoflavones and metabolites. The infants were breast-fed and the mothers were fed a commercial diet (Purina Mills, Jumbo Monkey Diet 5037), which contained 15.5% protein, 5% fat, 4% fiber, and 41.2% starch. It had an unspecified, but "low" soy protein content. Although newborn rhesus infant monkeys receive essentially all of their nourishment from breast milk, at about age 3 mo they begin to experiment with very small amounts of the standard monkey feed as part of the gradual weaning process (20).

Humans. The protocol was approved by the institutional Human Research Advisory Committee of the University of Arkansas for Medical Sciences (Little Rock, AR), and all 10 women gave their written consent. Four women reported consuming soy products daily; the others reported consumption of a typical Western diet with few soy products. None of the women were taking oral contraceptives at the time of the study, were pregnant, or had taken antibiotics in the past 4 mo. All were considered to be generally healthy. They ranged in age from 35 to 49 y and had a mean (± SEM) weight of 60.2 ± 0.8 kg. On d 1 of the study, subjects fasted overnight and then consumed a soy beverage (SPI and a banana blended with pineapple and orange juices) which contained 1.0, 0.6, and 0.1 mg/kg body weight genistein, daidzein, and glycitein, respectively, expressed as aglycone equivalents. Subjects were presented with a nutritious, balanced meal program provided by the dietitians of the Arkansas Children's Nutrition Center. Blood samples (10 mL) were collected in heparinized tubes 4 h after administration of the soy beverage through a catheter in a forearm. Urine (24 h) produced after soy ingestion was collected in ascorbic acid and sodium azide (0.1% wt:volume). Samples were stored at −70°C.

Isoflavone analyses. Aglycones and conjugated urinary and serum isoflavones were extracted and analyzed as previously described (21,22). Briefly, aglycones were measured directly by LC-MS, and conjugated isoflavones were enzymatically deconjugated; the resultant aglycones were measured by LC-MS. Deconjugation was accomplished by incubating urine or serum (100 μL) with a mixture of sulfatase and glucuronidase (100 U Sulfatase H-5, Sigma) for 3 h to obtain the total isoflavone concentrations. Samples were also incubated with β-glucuronidase (1000 U, B-1, Sigma) to obtain the sum of glucuronides and the aglycones. Isoflavone sulfates were calculated by subtracting the glucuronides and aglycones from the total concentration.

The intra- and interassay CV were 5.8 and 10.8%, respectively, calculated on the basis of repeated analysis of a control sample ($n = 6$). The detection limit was 0.8 pmol of each isoflavone injected on the column. Equol was quantified on an electrochemical detection system (ESA). A Synergy MAX-RP column (25 × 4.6 mm, 4 μm, Phenomenex) was used. The binary solvent system contained A (20% methanol containing 50 mmol/L sodium acetate, pH 4.8) and B [water:methanol:acetonitrile 40:40:20 (by vol), with 50 mmol/L sodium acetate, pH 4.8] and the flow rate was 0.8 mL/min. The gradient consisted of an initial condition of 35% B for 5 min, and a linear increase to 65% B in 5 min. The gradient then increased to 100% B from 65% B in 15 min which was held at 100% B for 10 min, and then returned to initial conditions in 5 min. The detector settings were 450, 580, 630, and 780 mV. Equol was monitored at 580 mV, the channel with the highest signal. The intra- and interassay CV were 3.5 and 9.0%, respectively, based on repeated analysis of a control sample ($n = 6$). The detection limit was 0.4 pmol injected on the column.

Urinary isoflavones were expressed on the basis of creatinine which was quantified spectrophotometrically using a kit from Synermed on a Chemistry Analyzer (model 9006, Precision systems) according to the manufacturer's instruction.

It should be noted that the purpose of this study was to compare the isoflavone profiles of serum and urine samples of different species, rather than focus on absolute isoflavone concentrations. This is because the dietary intake of soy protein (and isoflavones), the duration of soy intake, and age differed among experiments, making a direct comparison of serum or urinary isoflavone concentrations impractical, except for the rat Expt. 2 in which we matched the dose, duration, and

postprandial time course of SPI of young adult female rats to those for young adult women.

Statistical analyses. SigmaStat (V3.1, Jandal Scientific) was used for statistical analyses, and data are presented as means ± SEM. Data from multiple species were analyzed using 1-way ANOVA followed by Tukey's test. When there were only 2 groups, data were assessed by Student's *t* test. A paired *t* test was applied to compare values from different time points and to compare daidzein and genistein within species. No adjustment was made for multiple comparisons because the comparisons were exploratory. A Mann-Whitney Rank Sum test or Wilcoxon Signed Rank test was performed in place of Student's *t* test or paired *t* test, respectively, if the normality test failed. A difference of $P \leq 0.05$ was considered significant.

RESULTS

Striking species differences were observed in the profiles of total isoflavones and their metabolites in serum and plasma (**Fig. 1**). Dihydrodaidzein (DHD), *O*-DMA, and equol are the metabolites of daidzein produced by intestinal microflora. DHG is a metabolite produced by bacteria from genistein in the gut. Rat and monkey serum had high equol concentrations, which contributed 77 and 52% of summed isoflavones (isoflavones plus metabolites), respectively, whereas human plasma and pig serum had undetectable levels of equol. Thus, little daidzein was converted to equol in women and pigs, whereas rats and monkeys converted much of their ingested daidzein to equol. The serum daidzein:equol ratio was 1:19 and 1:3 in rats and monkeys, respectively, whereas daidzein and genistein contributed to the majority of the summed isoflavones in female pigs and women (88 and 91%, respectively). Genistein concentrations were higher than those of daidzein in rat serum and human plasma.

Prominent species differences also occurred in the urinary profiles of total isoflavones and their metabolites, similar to that in serum and plasma (**Fig. 2**). There were very low levels of equol in the urine of 3 pigs; they represented only 2% of summed isoflavones and were 15 times lower than daidzein. Equol was not detected in the urine of the women, whereas it represented

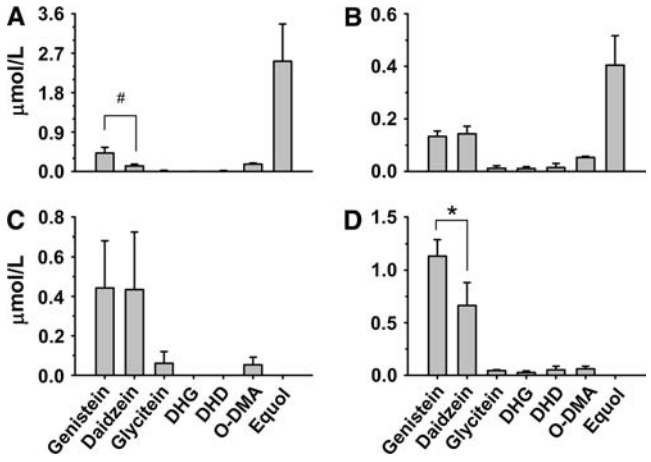

FIGURE 1 Total isoflavones and metabolites in the sera of female rats (*A*, $n = 9$, rat Expt. 1), cynomolgus monkeys (*B*, $n = 15$), and pigs (*C*, $n = 5$), and in the plasma of women (*D*, $n = 10$) after consumption of diets containing SPI. Data are means ± SEM. Daily genistein, daidzein, and glycitein intake (mg/kg body weight) was estimated to be 13.0, 9.9, and 2.4, respectively, for rats; 4.8, 3.7, and 0.8, respectively, for monkeys; 10.9, 8.6, and 1.4, respectively, for pigs; and 1.0, 0.6, and 0.1, respectively, for human subjects. $^{\#}P \leq 0.05$ by Wilcoxon Signed Rank test; $^{*}P \leq 0.05$ by paired *t* test.

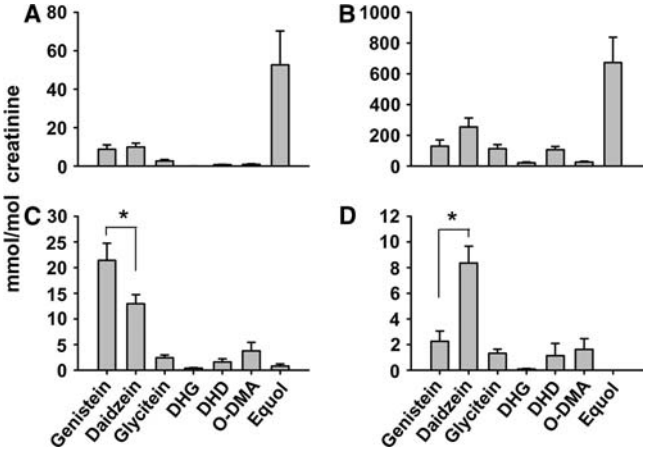

FIGURE 2 Total isoflavones and metabolites in the urine of female rats (*A*, $n = 9$, rat Expt. 1), cynomolgus monkeys (*B*, $n = 12$), pigs (*C*, $n = 5$), and women (*D*, $n = 6$) after consumption of diets containing SPI. Data are means ± SEM. Daily genistein, daidzein, and glycitein intake (mg/kg body weight) was estimated to be: 13.0, 9.9, and 2.4, respectively, for rats; 4.8, 3.7, and 0.8, respectively, for monkeys; 10.9, 8.6, and 1.4, respectively, for pigs; and 1.0, 0.6, and 0.1, respectively, for human subjects. $^{*}P \leq 0.05$ by paired *t* test.

69 and 51% of summed urinary isoflavones in rats and monkeys. Daidzein and genistein contributed 28, 38, 86, and 81% of summed isoflavones in the urine of rats, monkeys, pigs, and women, respectively, paralleling those from serum or plasma. Pigs excreted higher amounts of genistein than daidzein in urine, whereas excretion of daidzein in women was 4-fold that of genistein.

Both the serum profiles and the concentrations of isoflavones differed between rats and women (**Fig. 3**) even when the same dose of SPI and isoflavones (1.0, 0.6, and 0.1 mg/kg body weight genistein, daidzein, and glycitein, respectively) was administered and the blood samples were collected during the same 4-h postconsumption time period. The plasma genistein and daidzein concentrations in women were 11- and 4-fold those of female rats, respectively. Moreover, a single administration of SPI resulted in high serum equol concentrations in rats, whereas equol was not detected in women.

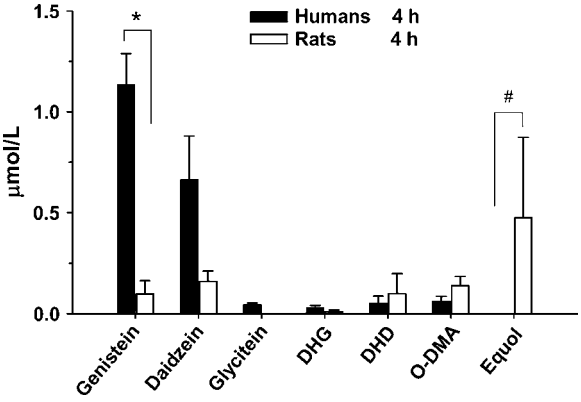

FIGURE 3 Total isoflavones and metabolites in the plasma of women ($n = 10$) and rat serum (rat Expt. 2, $n = 4$) 4 h after a matched single dose of SPI. Data are means ± SEM. Daily genistein, daidzein, and glycitein intake (mg/kg body weight) was 1.0, 0.6, and 0.1 for both rats and women. $^{*}P \leq 0.05$ by Student's *t* test; $^{\#}P = 0.07$ by Student's *t* test.

The proportions of aglycones and conjugates of major isoflavones in blood and urine are expressed as a percentage of the total concentrations for comparison purposes (**Table 1**). Equol aglycone represented a relatively high percentage (6%) of the total serum equol in cynomolgus monkeys, and the metabolites were present mainly as sulfates (64%), with only 30% as glucuronides. On the other hand, rats had a low proportion of total serum equol as aglycones (0.7%) or sulfates (7%), but 93% were glucuronide conjugates. Monkeys also had a high percentage of serum daidzein sulfates and high genistein sulfates, whereas rats and humans had a very low proportion of each.

There was a conspicuous difference between monkeys and rats in isoflavone excretion compared with women and pigs (Table 1). Rats and monkeys excreted high percentages of isoflavone as aglycones (33–47 and 90–97%, respectively), whereas pigs and women excreted very low levels (0.1–0.3 and 4–6%, respectively). Women and pigs excreted the majority of soy isoflavones as glucuronide (82–87%) and ~10–14% as sulfates. The fractional excretion of genistein in 24-h urine in pigs was higher than that in rats and humans. Rats excreted much less ingested glycitein than did pigs and women (**Table 2**).

Genistein, daidzein, and equol were quantified in the serum of infant rhesus monkeys (**Figure 4**). Other minor metabolites were below the detection limits. The concentrations of total genistein and daidzein were very low at 2, 4, and 6 mo, reflecting both the low level of isoflavones in breast milk and the very low consumption of standard monkey feed by infants. Serum concentrations of equol accounted for 80% of summed isoflavones at 6 mo, suggesting that infant monkeys were beginning to eat small amounts of the feed consumed by the adults in the colony. These data demonstrate that infant rhesus monkeys are capable of converting daidzein to equol very early in life.

DISCUSSION

In this study, we compared the isoflavone metabolic phenotype of women after the consumption of SPI with that of animals most widely used as experimental models, i.e., monkeys, rats, and pigs. The most striking findings of this study were that female monkeys had a serum profile more closely resembling that of laboratory rats than women, and the concentrations of the isoflavone equol were high in monkeys and undetectable in women. Equol is formed when daidzein is metabolized by gut bacteria to DHD, which is subsequently transformed into *O*-DMA or equol (2). Thus, any species differences in equol production are likely to be the result of intestinal bacterial composition. Rats and monkeys have gut bacteria that favor equol biosynthesis, whereas this capacity in women and pigs appears to be limited. Equol was reported to account for 71-90% of the summed isoflavones in rat serum (11), similar to the proportion in our study. The total content of isoflavones and metabolites (0.81 ± 0.15 μmol/L) and the proportion of equol (52%) in cynomolgus monkey serum in our study were consistent with a previous report (10) in which 59% of summed isoflavones were equol. Chimpanzees and mice were reported to be similar to cynomolgus monkeys and rats in metabolizing large amounts of daidzein into equol (11,23).

Under assay conditions with a detection limit of 8 nmol/L in serum or urine, equol was not detected in any of the women in this study; this is comparable to limits used by other researchers (24). Previous reports suggested that 25–35% of the Western population is capable of producing equol after a soy challenge (25), but the majority of the people are nonproducers (5). Our data on women were consistent with a recent report in which the molar ratios of daidzein, *O*-DMA, and equol in 25 nonequol producing human subjects were 100:35:0.3 in urine and 100:2.3:0.6 in plasma (26). The proportions of daidzein, *O*-DMA, and equol in the urine and plasma of 24 human subjects, including 36% equol producers, were 100:24:24 and 100:25:22, respectively (25). We conclude that human subjects in general are nonequol producers or poor equol producers compared with rats and monkeys. Pigs were similar to humans in terms of metabolizing minute amounts of daidzein into equol, and this compares favorably with the only study (to our knowledge) that measured equol levels in pigs fed soy-containing diets (27).

Equol was reported to be 4 times as estrogenic as daidzein (2). Experimental and epidemiological evidence suggests an important role of equol for clinical effectiveness in certain subpopulations of human subjects. Premenopausal equol producers have lower plasma estrone concentrations, but higher

TABLE 1

Proportion of isoflavone conjugates in urine and serum of women and female rats, monkeys, and pigs after consumption of diets containing SPI[1]

	Animal serum or human plasma			Urine		
	Aglycone	Glucuronide	Sulfate	Aglycones	Glucuronide	Sulfate
			%			
Daidzein						
Rats	7.3 ± 1.5^{a}	68.2 ± 4.9^{a}	24.5 ± 3.6^{b}	40.8 ± 7.5^{b}	47.3 ± 6.1^{b}	11.9 ± 4.5
Monkeys	0.6 ± 0.4^{b}	34.5 ± 2.5^{b}	64.9 ± 3.1^{a}	90.9 ± 5.0^{a}	6.4 ± 1.1^{c}	2.7 ± 1.7
Pigs	5.0 ± 2.9^{ab}	73.3 ± 7.5^{a}	21.6 ± 5.5^{b}	4.3 ± 1.8^{c}	85.6 ± 6.6^{a}	10.0 ± 5.2
Women	1.4 ± 0.4^{b}	75.1 ± 1.7^{a}	23.5 ± 4.8^{b}	0.3 ± 0.1^{c}	86.1 ± 1.3^{a}	13.6 ± 3.7
Genistein						
Rats	3.6 ± 0.9	50.4 ± 8.8^{ab}	45.9 ± 8.4^{b}	46.9 ± 9.2^{b}	41.6 ± 8.1^{b}	11.5 ± 2.6
Monkeys	3.5 ± 1.3	23.8 ± 7.0^{b}	72.8 ± 5.7^{a}	89.2 ± 0.7^{a}	5.6 ± 3.5^{c}	5.2 ± 4.2
Pigs	2.2 ± 0.7	48.7 ± 9.3^{ab}	49.0 ± 9.0^{ab}	6.0 ± 2.0^{c}	81.6 ± 10.0^{a}	12.4 ± 8.5
Women	1.2 ± 0.3	78.4 ± 2.0^{a}	20.4 ± 1.2^{b}	0.1 ± 0.1^{c}	86.7 ± 3.8^{a}	13.3 ± 3.8
Equol						
Rats	0.7 ± 0.3	92.6 ± 1.0^{a}	6.7 ± 1.4^{b}	32.8 ± 8.8^{b}	65.5 ± 9.4^{a}	1.8 ± 1.3
Monkeys	6.1 ± 3.8	29.6 ± 4.7^{b}	64.2 ± 1.2^{a}	96.3 ± 3.7^{a}	3.7 ± 2.5^{b}	0.0 ± 0.0

[1] Data are means ± SEM, $n = 7$–9 for rats, 6–8 for monkeys, 5 for pigs, and 6 for women. Data were analyzed by 1-way ANOVA with Tukey's test (daidzein and genistein) or Student's *t* test (equol). Means in a column with superscripts without a common letter differ, $P \leq 0.05$.

TABLE 2

Percentage of daily isoflavone dose excreted in the 24-h urine from women and female rats, monkeys, and pigs after consumption of diets containing SPI[1]

Species	Genistein dose excreted[2]	Daidzein dose excreted[3]	Glycitein dose excreted[4]	Genistein dose excreted as total genistein	Genistein dose excreted as DHG	Daidzein dose excreted as total daidzein	Daidzein dose excreted as DHD	Daidzein dose excreted as *O*-DMA	Daidzein dose excreted as equol
					%				
Rats	2.6 ± 0.7[b]	21.2 ± 5.4[b]	4.7 ± 1.3[b]	2.58 ± 0.7[b]	0.02 ± 0.01	3.3 ± 0.7[b]	0.2 ± 0.1	0.3 ± 0.1	17.3 ± 4.5[a]
Monkeys	ND[5]	ND	ND	ND	ND	ND	ND	ND	ND
Pigs	44.9 ± 7.1[a]	46.0 ± 10.7[a]	17.0 ± 3.8[a]	44.2 ± 7.1[a]	0.7 ± 0.3	31.2 ± 7.9[a]	3.8 ± 2.6	9.0 ± 3.3	1.9 ± 0.7[a]
Women	11.8 ± 5.2[b]	55.0 ± 2.3[a]	18.1 ± 1.6[a]	11.4 ± 5.1[b]	0.4 ± 0.2	41.4 ± 3.1[a]	5.6 ± 2.5	8.0 ± 3.9	0[b]

[1] Values are means ± SEM, $n = 9$ for rats, 3 for pigs, and 6 for women. One-way ANOVA with Tukey's test was used to analyze species differences. Means in a column with superscripts without a common letter differ, $P \leq 0.05$.
[2] Total genistein + total DHG.
[3] Total daidzein + total DHD + total *O*-DMA + total equol.
[4] Total glycitein.
[5] Not determined 24-h urine samples were not collected.

concentrations of sex hormone–binding globulin and progesterone, indicative of reduced breast cancer risk (3). Postmenopausal women who are equol or *O*-DMA producers had lower mammographic density or higher blood follicle–stimulating hormone, respectively (4,5). Blair et al. (19) also suggested that the much higher proportion of equol in monkey serum than in human subjects may account for the different effects of soy protein on plasma lipoproteins; as discussed in more detail below, this may explain the reduced serum testosterone concentrations recently reported in young monkeys fed soy formula (7).

Our data suggest that equol production in rats is rapid and requires no noticeable induction period. Soy-naïve rats had high serum equol concentrations 4 h after a bolus of SPI administered through a gastric cannula. It is also interesting to note that when the same dose of SPI and isoflavones (mg isoflavone/kg body weight) was administered by i.g. infusion to female rats or consumed as a beverage by women, the total genistein and daidzein concentrations in human plasma were 11- and 4-fold those in rat serum.

During or after absorption, isoflavones are conjugated in the intestine and the liver by UDP-glucuronosyltransferase (UGT) and sulfotransferases (SULT) into glucuronides and sulfates, respectively. Unconjugated isoflavones (aglycones) are more estrogenic than their conjugates in vitro, and it is thought that they are also the more bioactive form in vivo (28). On the other hand, sulfate conjugates of endogenous gonadal steroids are thought to be more potent than steroid glucuronides and to be an important source of free cellular steroids upon sulfatase hydrolysis (29,30). It is possible, therefore, that sulfated isoflavones are a primary source of aglycones after enzymatic hydrolysis in target tissues. The in vivo bioactivity of isoflavone conjugates is largely unknown. However, isoflavone sulfoconjugates were reported to have weak in vitro bioactivity. For example, daidzein sulfoconjugates inhibited sterol sulfatase (30), and genistein sulfoconjugates were estrogen receptor ligands, antioxidants, and inhibitors of platelet aggregation, inflammation, cell adhesion, and chemotaxis (31,32).

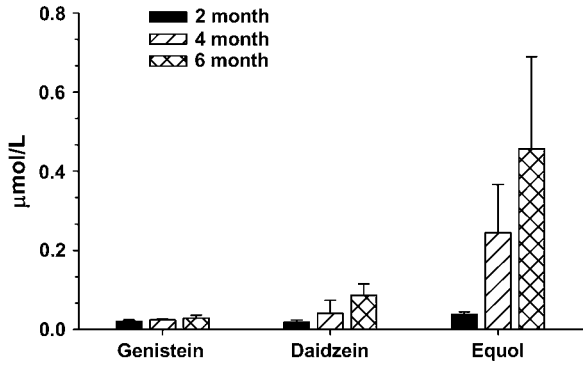

FIGURE 4 Total genistein, daidzein, and equol in the serum of breast-fed infant rhesus monkeys ($n = 3$). Mothers were fed Jumbo Monkey Diet #5037 (Purina Mills). DHD, DHG, glycitein, and O-DMA were not detected. Data are means ± SEM.

The serum (and target tissue) metabolic profile of equol aglycone and metabolites could be an important factor in determining the overall biological activity of soy isoflavones. Monkeys in the current study had high percentages of unconjugated and sulfated conjugates and lower percentages of glucuronides, whereas rats have very low aglycone equol, lower sulfates and high percentages of glucuronides. Women in this study had no detectable equol and lower percentages of aglycones, greater percentages of glucuronides, and lower sulfates than monkeys or rats. This suggests that the isoflavone metabolic phenotype of cynomolgus monkeys favors more estrogenic bioactivity than the rat profile, and both rats and monkeys have a more "estrogenic isoflavone phenotype" than women in this study.

A recent report from the Committee on Toxicology (COT) in the United Kingdom recommended restricting soy infant formula to children ≥3 mo old (14). This was based in good part on lower mean serum testosterone concentrations of monkeys fed soy formula compared with twin controls fed milk-based formula (7). The COT report suggested that human infants fed soy formula might experience a similar reduction in testosterone. However, the findings of the current study demonstrate that women (at least those in this study) and female monkeys differ in their metabolism of isoflavones, such that far less of the most potent isoflavone, equol, is present in women than in monkeys. This difference in metabolism may be an important factor in the biological activity of isoflavones in infant monkeys and human infants fed soy formula. In recent unpublished preliminary studies of isoflavone pharmacokinetics of >50 infants fed soy formula at our nutrition center, no equol producers were observed. However, the present study demonstrates that infant monkeys can produce large amounts of equol. In fact, the concentrations of equol in infant monkeys

that consumed very small amounts of soy-containing diet were in the range of serum total isoflavone concentrations reported in Japanese adults who were regular consumers of soy foods (33). This reinforces the great differences between human subjects and monkeys with respect to isoflavone metabolism and phenotype. These data also provide a potentially important explanation for the recent effects reported by Sharpe et al. (7) on serum testosterone concentrations of infant monkeys fed soy formula.

Just as human subjects differ from nonhuman primates, however, there are also differences among monkey species (34,35). In the present study we analyzed serum from cynomolgus and rhesus monkeys, whereas marmoset monkeys were used in the study by Sharpe et al. (7). Thus, more complete studies are currently planned in our laboratory to carefully study isoflavone metabolism in the 3 monkey species.

The large differences in the relative ratios of isoflavone aglycones, glucuronide conjugates, and sulfate conjugates among species may be accounted for by the relative activities of UGT and SULT because considerable interspecies disparity in UGT and SULT activities toward xenobiotics and endogenous compounds in vitro was reported (35). Cynomolgus monkeys were shown to have higher UGT and SULT activities toward acetaminophen than humans (35). Substantial species dissimilarities in hepatic and intestinal glucuronidation and sulfation of tea flavonoids were also observed between rats and humans (36). Various isoforms of UGT and SULT have different activities and specificities toward isoflavones (37). Although pigs were reported to have a low sulfation capability (38), the comparable levels of sulfated and glucuronidated isoflavones in women and pigs of the present study could be related to similar expression of those specific conjugating enzymes involved in isoflavone metabolism.

The fractional excretion of isoflavones in our study was comparable to previously reported ranges (26). Plasma genistein concentrations were higher than daidzein in humans, yet more daidzein was excreted from urine. This may be due to faster elimination of daidzein conjugates (22) and higher volume of distribution of total daidzein than of genistein (9). The lower fractional excretion of isoflavones in rats suggests an intestinal microflora with greater isoflavone degradation potential compared with pigs and women. However, other factors such as renal isoflavone metabolism and transport of conjugated metabolites into bile and urine may be involved. The marked differences in the proportion of conjugates between serum and urine in the same species indicates an important role of kidney in the metabolism and/or transport of isoflavones. We observed previously that isoflavones in rat kidney (μmol/kg) were several fold of those in the serum (μmol/L) (39). Thus, the kidney concentrates isoflavones from blood and either conjugates/deconjugates them extensively before excretion, or transports isoflavones with differing efficiencies. The lower proportion of sulfates in urine than in circulation in all 4 species suggests that isoflavone sulfates may be selectively conserved in renal metabolism and excretion. It was an unexpected observation that monkeys excrete 90% of isoflavone as aglycones. Isoflavone glucuronides may be deconjugated by β-glucuronidase, a normal constituent of urine excreted by renal tubular epithelial cells (40). The activities of glucuronidase and sulfatase in human urine were found to be extremely low (40). The enzyme activities in other animals have not been reported, but are likely to be low. Probable explanations are that there are fundamental species differences in renal metabolism or transport of isoflavones.

In summary, our study demonstrated significant interspecies differences in isoflavone metabolism. Cynomolgus monkeys fed diets made with SPI had an isoflavone metabolic phenotype close to that of Sprague-Dawley rats fed similar diets, and yet there were also significant and perhaps biologically important differences between rats and monkeys. Both rat and monkey serum isoflavone profiles differed from those of women and pigs. Female pigs had an overall metabolic profile closer to women than to either the female cynomolgus monkeys or rats, and pigs may be a better animal model for studying the health effects of isoflavones in nonequol producers.

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