Evidence for lack of absorption of soy isoflavone glycosides in humans, supporting the crucial role of intestinal metabolism for bioavailability[1–3]

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ABSTRACT

Background: The isoflavones daidzein and genistein occur naturally in most soyfoods, conjugated almost exclusively to sugars. Controversy exists regarding the extent of bioavailability of isoflavone glycosides, and the mechanism of intestinal absorption of isoflavones in humans is unclear. Evidence from intestinal perfusion and in vitro cell culture studies indicates that isoflavone glycosides are poorly absorbed, yet isoflavones are bioavailable and appear in high concentrations in plasma, irrespective of whether they are ingested as aglycones or glycoside conjugates.

Objective: The objective was to determine whether isoflavone glycosides are absorbed from the intestine intact and reach the peripheral circulation unchanged.

Design: Plasma was collected at timed intervals before and after healthy adults ingested 50 mg of one of the isoflavone β-glycosides (daidzin or genistin) or 250 mL soymilk containing mainly isoflavone glycosides. Electrospray ionization mass spectrometry was used to detect daidzin and genistin after solid-phase extraction of these conjugates from plasma. Bioavailability of isoflavones was confirmed by gas chromatography–mass spectrometry analysis.

Results: Specific and sensitive electrospray mass spectrometry failed to detect even traces of daidzin or genistin in plasma collected 1, 2, and 8 h after their ingestion as pure compounds or in a soyfood matrix. However, plasma was enriched in isoflavones that were hydrolyzable with a combined β-glucuronidase and sulfatase enzyme preparation.

Conclusion: Isoflavone glycosides are not absorbed intact across the enterocyte of healthy adults, and their bioavailability requires initial hydrolysis of the sugar moiety by intestinal β-glucosidases for uptake to the peripheral circulation.   *Am J Clin Nutr* 2002;76:447–53.

INTRODUCTION

The current revolution and nutritional interest in soyfoods is largely the result of the recognition that the soybean contains several key bioactive nonnutrients that research studies have shown contribute to the health attributes of this high-quality protein source. It is perhaps fair to say that most of the research interest in soy has focused on the role that its isoflavones play in disease prevention or treatment (1–3). The recent approval by the US Food and Drug Administration allowing the food industry to promote soy protein for heart health (4) led to an escalation in sales of soyfoods, and these foods are being promoted for their isoflavone content as well as for their protein content.

Isoflavones are a class of nonsteroidal estrogens that bear remarkable similarity in chemical structure and properties to estrogens (5). However, genistein, the most extensively researched soy isoflavone, shows conformational binding to the estrogen receptor that classifies it as a natural selective estrogen receptor modulator rather than an estrogen (6, 7). In addition to the ability of isoflavones to bind to estrogen receptors, hundreds of scientific publications attest to their wide range of nonhormonal properties, many of which have profound implications for cell function (5, 8–10). However, what is not often realized is that the demonstrable actions of isoflavones have been attributed to the aglycones genistein and daidzein, yet the soybean and most soyfoods have negligible amounts of these aglycones unless they have been fermented (11, 12). Isoflavones occur naturally in the soybean as various forms of β-glucosides, and this is also the case for most of the soyfoods on the market in the Western world (12).

Although the important role of intestinal bacteria in the metabolism of soy isoflavones was first recognized with the discovery of the urinary metabolite equol (1, 13, 14), little was known about the mechanisms involved in the intestinal absorption of isoflavones. Considerable debate focused on whether the glycoside conjugates of soy isoflavones could be absorbed intact from the intestinal tract. Similar controversy has plagued the field of flavonoids (15–19)—it has been suggested that flavonoid glycosides such as quercitin are absorbed by an active transport mechanism (20). Several studies suggest that quercitin-glycoside may,

on absorption, be rapidly hydrolyzed and conjugated within the enterocyte to glucuronic acid (18, 21). If this occurs in vivo in humans it may explain the difficulty in detecting flavonoid-glycosides in human plasma (22). However, there may be exceptions, because a recent study showed unequivocally that anthracyanin glycosides are absorbed intact by women (23).

In this study we present for the first time convincing evidence that the quantitatively most important forms of isoflavones found in most soyfoods, namely, the glycosides daidzin and genistin are not absorbed intact into the peripheral circulation of healthy adults. These findings establish the crucial role that hydrolysis by intestinal β-glucosidases play in ensuring the bioavailability of isoflavones present in most of the soyfoods being consumed.

SUBJECTS AND METHODS

Human studies

To determine whether the 2 predominant isoflavones of most soyfoods, daidzin and genistin, are absorbed intact from the intestine and reach the peripheral circulation unchanged, we used a highly specific electrospray mass spectrometric method to measure them directly in plasma. The plasma was obtained from healthy women after they consumed either these pure β-glycosides or a soymilk that naturally contained mainly β-glycosides.

Twelve healthy premenopausal women aged ≥ 18 y were recruited. The studies were carried out at the General Clinical Research Center at Children's Hospital Medical Center in Cincinnati. Volunteers were excluded if they had preexisting chronic renal, liver, pulmonary, or cardiovascular disease; had taken antibiotics within the preceding 3 mo; or were taking oral contraceptives. The Human Investigations Review Board of the Children's Hospital Medical Center approved the study protocol and informed consent was obtained from each subject. The findings reported here for administration of pure isoflavone glycosides are an adjunct to a clinical study of the pharmacokinetics reported previously, and details of the study design are described elsewhere (24).

Briefly, after fasting overnight, healthy adults ingested 50 mg daidzin ($n = 4$), 50 mg genistin ($n = 3$), or 250 mL of a soymilk drink ($n = 5$). Blood samples (5 mL) were obtained by venipuncture before (baseline) and after 1, 2, and 8 h in subjects consuming the pure compounds and at baseline and after 2 and 8 h in those consuming soymilk. Blood was obtained via an indwelling catheter for the more frequent samplings and via evacuated tube or catheter for the samples taken at the later time, depending on the choice of the subjects. Blood samples were centrifuged at room temperature for 10 min at 3000 rpm in a benchtop centrifuge, and the plasma was separated and immediately frozen at −20 °C before being analyzed for isoflavone concentrations. Total and free (unconjugated) isoflavone concentrations were previously measured in the samples from these subjects with the use of gas chromatography–mass spectrometry (GC-MS), and these were reported elsewhere (24). These same plasma samples were used for the measurement of isoflavone glycosides.

Determination of isoflavone glycosides in plasma by electrospray ionization mass spectrometry

Plasma samples were analyzed by negative ion electrospray ionization MS (ESI-MS) for the intact β-glycosides daidzin and genistin (25). Isolation of isoflavone conjugates was carried out by solid-phase extraction after diluting the plasma (0.5 mL) with 10 vols 0.5 mol triethylamine sulfate/L (pH 5.0) and heating to 64 °C before passage through a wetted solid-phase C_{18}-Bond Elut cartridge (Varian Inc, Harbor City, CA; 24). The solid-phase cartridge was then washed with distilled water (5 mL) and isoflavones conjugates were recovered by elution with methanol (5 mL). The methanol extract was evaporated to dryness under nitrogen, and isoflavones were reconstituted in 0.4 mL of the HPLC mobile phase, 10 mmol ammonium acetate:acetonitrile (1:1, by vol)/L. As a positive control for the methodology, the baseline plasma sample for each subject was spiked with daidzin and genistin (200 ng/mL), extracted by the same procedure, and analyzed by ESI-MS in negative ion mode and with selected ion monitoring. ESI-MS was performed on a Micromass Quattro LC/MS (Manchester, United Kingdom). The desolvation temperature was 375 °C, and the source temperature was 100 °C. The sampling cone was held at 50 volts and the extractor at 2 volts. The specific fragment ions of mass-to-charge ratio (m/z) 253.05 for daidzin and m/z 269.05 for genistin were used to detect both glycosides. The individual isoflavones were quantified by comparing the peak area response in the specific ion channels at the correct retention time determined from authentic compounds, relative to the responses for known concentrations of the glycosides.

Hydrolysis of isoflavone β-glycosides by *Helix pomatia* digestive juice

Helix pomatia digestive juice is commercially marketed as a β-glucuronidase and sulfatase preparation and has for 30 y been the enzyme preparation of choice for hydrolysis of steroid and isoflavone conjugates (26). It is not recognized as a β-glucosidase, but our early studies indicated that it also contains significant β-glycosidase activity and therefore any absorbed isoflavone β-glycosides would be hydrolyzed in vitro during the methodological workup of plasma samples and analyzed as their respective aglycones. To better understand the efficiency of *H. pomatia* digestive juice in hydrolyzing isoflavone glycosides, we incubated in vitro 100 mg each of daidzin and genistin with 0.1 mL *H. pomatia* digestive juice suspended in 10 mL 0.05 mol sodium acetate buffer/L (pH 4.5) at 37 °C. Before the enzyme-buffer mixture was added, it was passed through a solid-phase C_{18} Bond Elut cartridge to remove residual amounts of isoflavones that we previously found to occur naturally in this enzyme preparation. The concentrations of daidzin and genistin remaining, and daidzein and genistein formed during incubation, were determined by HPLC on aliquots of the mixture removed at timed intervals over the next 24 h.

RESULTS

Validation of methodology for detection of isoflavone glycosides in plasma

The negative ion electrospray mass spectra of daidzin and genistin, published here for the first time, are characterized by their molecular ions ([M-H]$^-$) at m/z 415 and m/z 431, respectively (**Figure 1**). Under the ionization conditions, the base peak in the mass spectrum of both compounds was at m/z 253 and m/z 269, respectively, and these ions arose from fragmentation and cleavage of the glycoside moiety (25). Because they were the most abundant ions in the spectra, these were chosen for high-sensitivity selected ion monitoring detection of daidzin and genistin in plasma samples.

When equimolar amounts of daidzin and genistin were ionized, the ion abundance from genistin was approximately twice that of daidzin under the ionization conditions used. The limit of detection of
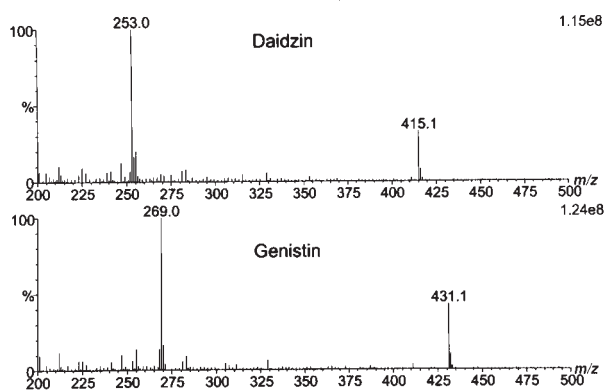
FIGURE 1. Negative ion electrospray mass spectrum of the isoflavone β-glycosides daidzin and genistin. *m/z*, mass-to-charge ratio.

genistin and daidzin by selected ion monitoring of these ions was found to be 2.5 and 5 ng/mL, respectively, with a signal-to-noise ratio of 3:1 and well below the physiologic range of plasma isoflavone concentrations after soyfood ingestion. The recovery of daidzin and genistin when added to human plasma samples was 70% and 75%, respectively. When baseline plasma samples were spiked with 200 ng daidzin and genistin/mL, both isoflavone glycosides were readily detected by ESI-MS (**Figure 2**) from peaks coincident with the HPLC retention times of the pure standards. Under the conditions analyzed, daidzin consistently eluted from the column with an absolute retention time of 10.58 min and was detected from its specific ion at *m/z* 253. The corresponding absolute retention time of genistin was 11.27 min, and the 2 glycosides were completely separated under the chromatographic conditions used.

Isoflavone concentrations in human plasma samples after oral administration of the pure isoflavones daidzin and genistin and after soymilk consumption

Typical plasma total and free (unconjugated) isoflavone concentrations of daidzein and genistein measured by GC-MS in timed samples collected after healthy adults consumed 50 mg pure daidzin or genistin or 250 mL of a soymilk drink are shown in **Figure 3**. The total plasma isoflavone concentrations were

obtained after the plasma was hydrolyzed by *H. pomatia* digestive juice, and they serve to establish that administered isoflavone glycosides, either as pure compounds or naturally occurring in a soyfood, are bioavailable. These studies, however, do not establish the type of conjugates circulating in plasma, although they do show that the proportion of unconjugated aglycones circulating is relatively low. The possibility that the high circulating concentrations of daidzein and genistein measured in plasma are the result of direct absorption of β-glycosides into plasma, rather than of the presence of glucuronide or sulfate conjugates, could not be ruled out by this protocol because we established that *H. pomatia* digestive juice efficiently hydrolyzes in vitro daidzin and genistin to the respective aglycones, daidzein and genistein.

The time course for the hydrolysis of daidzin and genistin by *H. pomatia*, as measured by HPLC from the proportion of glycosides to aglycones remaining in the incubation mixture, is shown in **Figure 4**. These in vitro studies show that under the analytic conditions used, *H. pomatia* completely hydrolyzes daidzin and genistin within 15 min, and this enzyme preparation, in addition to having β-glucuronidase and sulfatase activity, is also a useful source of β-glucosidases.

Failure to detect the isoflavone glycosides daidzin and genistin in human plasma

Typical selected ion current chromatograms obtained from the plasma samples collected 1 and 2 h after the oral administration of 50 mg daidzin or genistin to healthy adults are shown in Figure 2. No detectable peaks were observed in the fragment ion channels for daidzin (*m/z* 253) and genistin (*m/z* 269) at the retention times corresponding to the β-glycosides. Similarly, we also did not detect β-glycosides in plasma collected 8 h after oral administration of the glycosides (data not shown). The findings were the same in the subjects who consumed 250 mL of a soymilk drink. Isoflavone glycosides could not be detected in samples collected 2 or 8 h after ingestion of soymilk (**Figure 5**). By contrast, when baseline plasma samples were spiked with daidzin and genistin, then extracted and analyzed in an identical manner, both isoflavones were detected with intense peaks. These results clearly confirm the lack of isoflavone glycosides in human plasma after ingestion of either the pure compounds or the β-glycosides naturally present in most soyfoods.
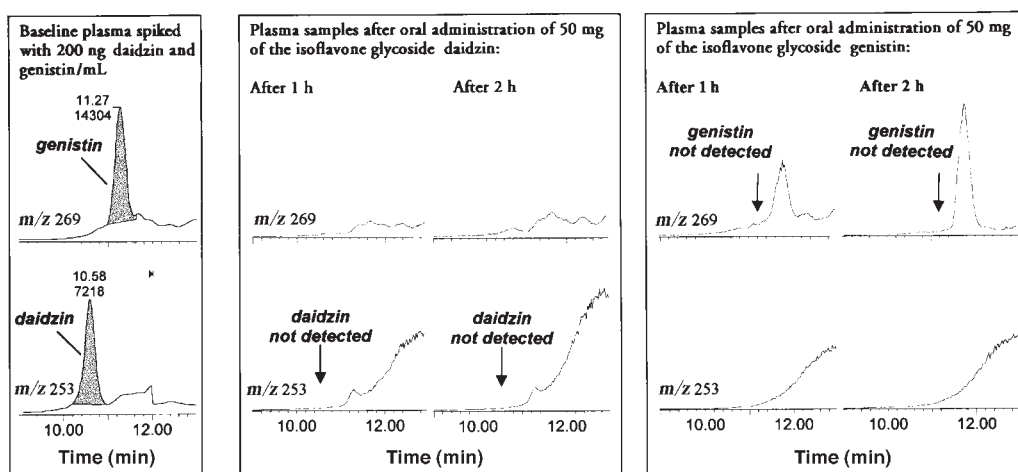
FIGURE 2. Typical selected ion current chromatograms from negative ion electrospray mass spectrometric analysis of plasma spiked with the pure compounds and plasma collected from healthy adults 1 and 2 h after oral administration of daidzin and genistin. *m/z*, mass-to-charge ratio.
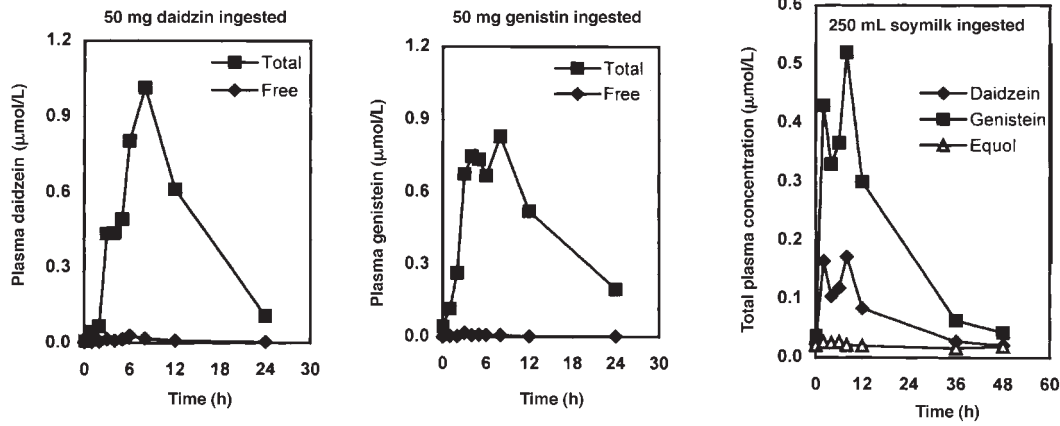
FIGURE 3. Typical plasma concentrations of total conjugated and unconjugated forms of daidzein and genistein in 2 healthy adults after oral administration of 50 mg of the β-glycosides daidzin and genistin.

DISCUSSION

Current interest in soy isoflavones is based on a vast literature reporting a wide range of biological properties for genistein and daidzein (5, 8–10) and on clinical studies supporting their potential health benefits (2, 3). However, these 2 aglycones are found only in trivial amounts in most of the soyfoods we consume (11, 12) because the soybean plant extensively conjugates isoflavones to a range of sugar molecules. Even with processing of the soybean, these conjugates, particularly the β-glycosides daidzin and genistin are extremely stable (27). Only soyfoods that have undergone fermentation with bacterial cultures, such as miso, natto, tempeh, etc, contain appreciable levels of the isoflavone aglycones (11, 12, 28). Therefore, the extent to which these isoflavone glycosides are bioavailable is pertinent to their efficacy. This is important to address in light of the recent promotion of isoflavone aglycones for their supposedly greater bioavailability than the corresponding glycoside conjugates (29, 30).

It was shown > 2 decades ago that soy isoflavones consumed in foods appear in very high concentrations relative to endogenous estrogens in the urine of healthy humans (1, 14), and the important role that intestinal bacterial metabolism plays in the metabolism of soy isoflavones was soon established (1). The mechanism of intestinal absorption of isoflavones and flavonoids has been controversial, and in recent years several animal (18, 31–34) and cell culture (17, 20, 35, 36) studies have examined the intestinal absorption of isoflavone glycosides. All of these studies used either intestinal cell culture or perfusion systems, and although useful data were obtained, the question of whether isoflavone glycosides are absorbed by humans remains to be definitively resolved. Our pharmacokinetic studies showed that when the pure β-glycosides daidzin and genistin were administered orally, their bioavailability as measured from the area under the curve of the plasma concentration curves was found to be significantly greater than that of the corresponding aglycones (24). Similar observations were noted for the flavonoids (15, 16). Paradoxically, the in vitro studies do not support direct uptake of isoflavone (35) or flavonoid glycosides (36), although recent data suggest that some uptake of quercitin 4′-β-glycoside might take place by a transporter (20) but that either rapid hydrolysis occurs within the enterocyte or efflux back across the apical membrane into the lumen occurs, facilitated by the multidrug-resistance protein MRP2 (36).

To our knowledge, it is yet to be shown whether isoflavone glycosides can be directly absorbed intact in humans. However, it was shown in an isolated rat small intestinal perfusion model that only 1.4% of genistin crossed the intestinal wall after perfusion of the luminal compartment with pure genistin (32). Likewise, only 2.1% of genistin and 2.6% of daidzin appeared on the vascular side after luminal perfusion of each of these glycosides in the form of a
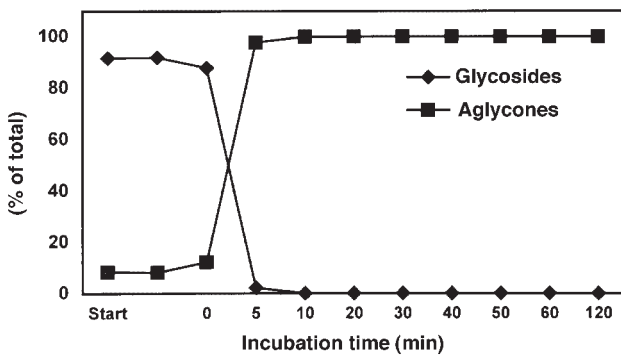

FIGURE 4. Confirmation of the hydrolytic activity of *Helix pomatia* digestive juice toward isoflavone glycosides typically present in a natural soy germ extract.
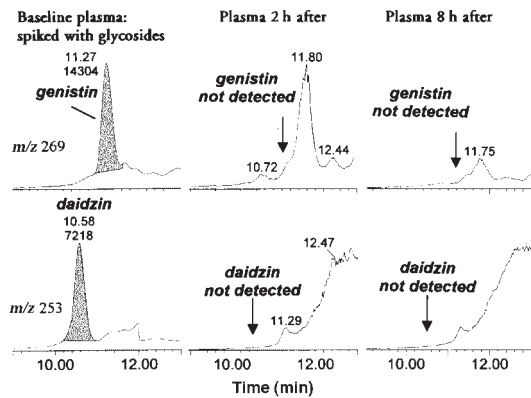

FIGURE 5. Typical selected ion chromatograms from negative ion electrospray mass spectrometric analysis of plasma obtained from healthy adults 2 and 8 h after they consumed 250 mL of a soymilk beverage. *m/z*, mass-to-charge ratio.

simulated digested tofu mixture (33). These are extremely small proportions, and the concentrations measured in these studies are close to the detection limits of the HPLC methods used. Notwithstanding this, it is evident that there is negligible uptake of glycosides across the apical membrane of the rat enterocyte. This was also shown to be the case in vitro with the use of monolayers of human Caco-2 cells (35), although intracellular efflux of genistin across the apical membrane to the luminal side was shown (35).

In our attempt to establish whether the β-glycosides could be absorbed and reach the peripheral circulation unchanged in healthy humans, we used sensitive ESI-MS techniques to detect and directly measure the concentration of daidzin and genistin in timed blood samples collected from 7 adults who each ingested 50 mg daidzin or genistin. The administered dose was within the usual dietary intakes of persons consuming modest amounts of soy and was consistent with the intakes of citizens of Asian countries where soy is regularly consumed (37–39). Plasma was also analyzed from 5 healthy adults who consumed a standard serving of a soymilk beverage containing a total of ≈25 mg isoflavones, mostly as β-glycosides. Overall, the bioavailability of these isoflavones was confirmed in all individuals by the appearance and disappearance of the free (unconjugated) and total daidzein and genistein concentrations in plasma, the latter measured by GC-MS after prior hydrolysis of the plasma extract with *H. pomatia* digestive juice (24). It is relevant to point out that we found that *H. pomatia* digestive juice, one of the common preparations used to hydrolyze steroid glucuronides and sulfates, hydrolyzes isoflavone glycosides very efficiently (Figure 4). Therefore, previous measurement of plasma isoflavone concentrations after hydrolysis with *H. pomatia* would have completely cleaved any glycosides that might have been absorbed as well as the glucuronide and sulfate conjugates formed. Detailed information on the mode of conjugation cannot therefore be obtained by this common analytic approach.

ESI-MS permits the direct analysis of intact isoflavone glycosides (25, 40), and we found this approach to have sensitivity as low as 10 nmol/L, more than sufficient to detect isoflavone glycosides reaching the peripheral circulation when ingested at nutritionally relevant levels. Analysis of the plasma collected 1, 2, and 8 h after oral administration of daidzin or genistin did not show even traces of the unchanged isoflavone glycosides in the 7 subjects studied (Figure 2). Similarly, the β-glycosides were not detected in the plasma of 5 healthy adults 2 or 8 h after they drank 250 mL of a soymilk beverage (Figure 5) despite total plasma isoflavone concentrations reaching the mmol/L range. These time points were selected to account for rapid proximal and possible delayed distal absorption of glycosides. In fact, we previously reported that the time to reach maximal plasma isoflavone concentrations for daidzein and genistein after oral intake of daidzin and genistin is 8–10 h in healthy adults (24). Plasma obtained at later time points, although collected, was not analyzed because isoflavone glycosides would not be expected to survive the extensive amount of bacterial β-glucosidase in the colon (41). Although our studies clearly show that intact isoflavone glycosides do not reach the peripheral circulation, the possibility remains, albeit unlikely, that they may be present in the portal vein and then metabolically processed by the liver. Obtaining portal vein blood would be unethical, but it was reported many years ago that rat portal vein blood contained mainly glucuronide conjugates of both isoflavones and lignans (42), and this is likely the case for humans.

Although daidzin and genistin could not be found in any of the plasma samples analyzed, the high circulating concentration of isoflavones measured after hydrolysis of plasma by *H. pomatia* (Figure 3) are accounted for by glucuronide and sulfate conjugates. Previous studies showed that the major circulating and urinary forms of isoflavones are glucuronides (13, 14, 43, 44), and it was always assumed that conjugation took place in the liver by the action of one or more UDP-glucuronyltransferase isozymes. Later studies with everted rat intestinal sacs provided the first direct evidence that isoflavones could be conjugated to glucuronic acid in the enterocyte (45). Curiously, portal vein blood from rats was shown in 1980 to have almost exclusively equol-glucuronide, as well as glucuronides of the lignans enterolactone and enterodiol (46), which indirectly suggested either that the intestine is capable of glucuronidation of isoflavones or that direct uptake occurs of isoflavone glucuronides secreted in bile during enterohepatic recycling (45, 47). The relative quantitative contribution of the intestine and liver to glucuronidation is difficult to assess in vivo, and it is probable that conjugation takes place in both organs. Several isozymes of UDP-glucuronyltransferase catalyze daidzein and genistein conjugation, and these appear to have tissue and substrate specificity. UGT-1A10 shows a high specificity toward genistein and is expressed with greatest activity in the colon (48); it is not expressed in rat liver (49). Glucuronidation of daidzein by several UGT isozymes, including UDP-1A1 and UDP-1A9, appears to preferentially occur in the rat liver (48).

Although the intestine plays a key role in conjugation of soy isoflavones, it has become evident that the enterocyte also possesses transport systems capable of differentially shunting glucuronides across the basolateral and apical membranes, favoring transport to the vascular side (31–33). Intestinal perfusion studies indicate that approximately one-third of the genistein glucuronide formed in the rat small intestine is returned to the lumen (31–33), where it would then be expected to be hydrolyzed more distally by bacterial glucuronidases abundant in the colon (41). Whether the glucuronides use a specific transporter for efflux from the enterocyte, such as MRP2, is not known, probably because commercial sources of isoflavone glucuronides have been unavailable for testing. MRP2 was shown to transport flavonoid glycosides (36), genistin (35), and estradiol-17 β-glucuronide (50) out of the cell across the apical membrane. Relatively small proportions of isoflavones are found in plasma as sulfates (43), and conjugation is presumed to occur in tissues other than the intestine, most probably the liver or kidneys.

A schematic representing our current understanding of the mechanisms of intestinal handling of isoflavones is depicted in **Figure 6**. Data presented here clearly show that isoflavone
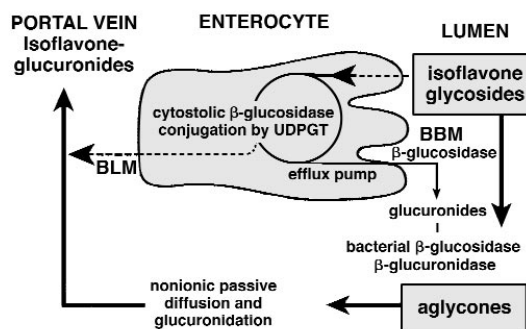

FIGURE 6. General scheme of the mechanism of intestinal absorption and metabolism of isoflavone glycosides. BLM, basolateral membrane; BBM, brush border membrane.

glycosides, which compose the bulk of the isoflavones in most soyfoods, are not absorbed across the enterocyte even though daidzein and genistein reach high concentrations in plasma. Some absorption of aglycones, but not β-glycosides, was shown to take place in the rat stomach (51), and if this is true for humans it would contribute to the faster absorption rates of aglycones than of β-glycosides (24, 30). Hydrolysis of the sugar moiety is therefore a prerequisite for the absorption of isoflavone glycosides. Bacterial β-glucosidase (EC 3.2.1.21) was isolated from human feces (41, 52). The plasma kinetics, notably the long $t$max (time to reach maximal blood concentration) of the isoflavone β-glycosides in healthy humans, suggest that most of the hydrolysis must occur distally and is most likely of bacterial action (1). If the behavior of soy isoflavones is similar to that of the flavonoid glucosides of quercitin (19), some hydrolysis of isoflavones must occur in the proximal intestine, which is consistent with the presence of several cytosolic and brush border membrane–bound β-glucosidases (53–56). The activity of these enzymes toward flavonoids is highly expressed in the jejunum, and in vitro intestinal perfusion studies attest to hydrolysis of isoflavone glucosides occurring on the luminal side (57). The role of intracellular β-glycosidase (54) is difficult to assess, and it could be speculated that it hydrolyzes any isoflavone glycosides that penetrate the apical membrane. The intestinal β-glucosidases also show a high affinity for isoflavones, especially when the glucose residue is at position 7 of the molecule, as is the case for most dietary isoflavones (57). Given the abundance of β-glucosidase activity along the entire length of the human intestine, isoflavone β-glycosides would have little opportunity to survive intact. Bacterial β-glucosidase activity shows a pattern of developmental expression (41), being present early in life with sufficient activity to facilitate the absorption of isoflavone glycosides contained in soy infant formula, thereby accounting for very high plasma isoflavone concentrations in infants (58, 59).

Because isoflavone aglycones are phenolic, their pK_a (negative logarithm of equilibrium constant for association) is favorable for nonionic passive diffusion from the jejunum, and we suggest that this is the most likely mechanism of absorption. Therefore, hydrolysis of the sugar moiety is an essential prerequisite for bioavailability of soy isoflavones, and the reduced bioavailability we observed with high dietary intakes of isoflavones (60) is best explained by the substrate concentration exceeding the intraluminal hydrolytic capacity in the intestine or by rate-limiting uptake of aglycones. The implication of these findings is that foods containing modest amounts of isoflavones consumed throughout the day are most likely to provide the highest steady state plasma isoflavone concentrations, thereby favoring greater biological efficacy. We present here the first direct evidence that isoflavone β-glycosides do not cross the intestine of healthy humans fed either the pure compounds or a soyfood in which β-glycoside conjugates predominate.

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