Daidzein and genistein concentrations in human milk after soy consumption

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Soy isoflavones were quantified from human milk by a fast, precise, and selective HPLC method after enzymatic hydrolysis of conjugated isoflavones and extraction with ethyl acetate. Isoflavone aglycones and their mammalian metabolites equol and O-desmethylandolensin were separated selectively and identified by absorbance patterns, fluorometric and electrochemical detection, gas chromatography-mass spectrometric analysis after trimethylsilylation, and with internal and external authentic standards. HPLC injections of 20 μL of human milk showed detection limits of 1–3 pmol for all analytes by using diode-array detection. The detection limit could be improved by as much as 1000-fold by extended concentration through partitioning with ethyl acetate, by using electrochemical detection, by increasing the injection volumes, or by combining these techniques. We used the proposed method to monitor isoflavone concentrations in human milk and in human urine after challenge with 5, 10, and 20 g of roasted soybeans in the diet. Implications of the results for the potential of isoflavones to prevent cancer in newborn infants exposed to these agents are discussed.

INDEXING TERMS: isoflavonoids • phytoestrogens • equol • O-desmethylandolensin • breast-feeding • cancer prevention • newborns

Isoflavonoid compounds, specifically genistein and daidzein, have been implicated in the prevention of cancers [1], possibly through multiple effects connected with the inhibition of carcinogenesis [1–3]. Recent reports about activities of isoflavonoids—radical scavenging, antioxidant [4], antiestrogenic [5–8], antimitogenic, antiproliferative [9], differentiation-inducing [10], and angiogenesis-inhibiting [11] activities—add to the growing list of anticancer effects of these agents. Most importantly, genistein linked to an antibody against B cells led to 100% long-term survival in leukemic mice by killing 99,999% of cancer cells [12]. This most striking anticancer potency of genistein was therefore suggested to be a promising approach for future clinical applications [12]. Additionally, breast cancer incidence and tumor numbers were decreased in newborn mice given only three genistein doses [13]. This suggests a potent anticancer activity of this agent at a very early and critical period in life through direct effects of the isoflavone on the target tissue or by imprinting mechanisms. Genistein and daidzein are also thought to play a major role in reducing cancer risk [1, 14] because populations with high isoflavone exposure through soy consumption have low cancer rates [15, 16].

Human exposure to dietary genistein and daidzein occurs mainly through intake of soy food [17]. Concentrations of these agents in soy average 2 g/kg (dry wt.) but vary greatly [18–21].

Traditionally, gas chromatography-mass spectrometry (GC-MS) has been applied to determine soy isoflavones and their metabolites in human biological fluids, including urine [8, 22, 23], plasma [24], and feces [25, 26].1 Recently, the introduction of HPLC to measure these analytes in human urine [27, 28] has allowed the measurement of a variety of phytoestrogens, including aglycones and conjugated analytes, in one run. Compared with GC-MS, HPLC required fewer steps for sample preparation and analysis and demanded less technician time and less expensive instrumentation. In independent studies of soy intervention, HPLC [27] proved to be as accurate as GC-MS [8, 23] for measuring urinary isoflavone concentrations, as evidenced by similar results achieved in these trials.

Although isoflavone metabolites such as equol and its derivatives have been identified in cow's milk [29, 30], to our knowledge the presence of dietary isoflavones in human milk has not been reported.

In support of future studies favoring noninvasive protocols and assessing the potential cancer protective role of a diet containing soya or isoflavones, we developed an HPLC procedure to determine, for the first time, concentrations of soy isoflavones in human milk.

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1 Nonstandard abbreviations: SIM, selected-ion monitoring; GC-MS, gas chromatography-mass spectrometry; and SPE, solid-phase extraction.
**Materials and Methods**

**Participants and Samples**
One Caucasian and one Chinese woman between ages 32 and 34 years, both in week 15 postpartum, participated in this study. Both were in good health, of normal height and weight (55 and 57 kg), nonsmoking, not on any medication (including hormones or dietary supplements), and without particular dietary patterns (e.g., not vegetarians). During the study, the participants did not consume any alcohol and maintained their usual diet except for the Caucasian woman’s additional intake of 5, 10, and 20 g of roasted soybeans at times 0, 24, and 72 h, respectively, during intervention. All procedures of the protocol followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983.

Milk was collected from these women during breastfeeding from the breast not used in the feeding, starting 12 h before the first soybean intake. Milk samples were stored in plastic vials between 6 °C and −4 °C. Isoflavones in milk were found to be stable for at least 5 days when kept in this temperature range (data not shown). Six overnight urine samples and two additional samples, 12 h before and 87 h after first soybean intake, were stored in disposable bottles containing 0.2 g of boric acid and 0.1 g of sodium ascorbate to control for bacterial contamination and degradation of analytes. After mixing and determining the volume of each urine sample, 50 mL was transferred into disposable plastic tubes and stored between −4 °C and −20 °C.

All collection times, including times of previous urine voids, were recorded for adjustment purposes.

**Apparatus**
HPLC analyses were carried out on a “system Gold” chromatograph with a Model 507 autosampler and a Model 168 dual-channel diode-array detector (all from Beckman, Fullerton, CA), a Model FD100 fluorescence detector (GTI/SpectroVision, Concord, MA), and a Coulchem II-5200 electrochemical detector (ESA, Bedford, MA) with a 5011 coulometric cell. Absorbance readings were obtained with a DU-62 spectrophotometer (Beckman). Samples were evaporated with a Savant AS 160 Speed-Vac (Savant, Farmingdale, NY) at room temperature. GC-MS analysis was carried out with a Hewlett-Packard (Palo Alto, CA) Model 5890 gas chromatograph using a Model 5971A mass-selective detector and electron impact ionization at 70 eV.

**Reagents**
Methanol, hydrochloric acid, acetic acid, 96% ethanol, dimethyl sulfoxide, ethyl acetate, and all solvents used for HPLC and absorbance readings were analytical-grade or HPLC-grade from Fisher Scientific (Fair Lawn, NJ). Butylated hydroxytoluene, sodium acetate, genistin, and glucuronidase/sulfatase (isolated from _Helix pomatia_ type HP-25) were purchased from Sigma Chemical Co. (St. Louis, MO). Daidzein and genistein were obtained from ICN (Costa Mesa, CA), flavone from Aldrich (Milwaukee, WI), coumestrol from Serva (New York, NY), and _β_-glucuronidase isolated from _Escherichia coli_ (200 kU/L) and arylsulfatase isolated from _H. pomatia_ (1–5 kU/L) were purchased from Boehringer Mannheim (Indianapolis, IN). Equol and _O_-desmethyllangolensin were purchased from K. Wåhåla, University of Helsinki, Finland.

**Procedures**
**Extraction and acid hydrolysis of isoflavones from soybeans.** Roasted soybeans were extracted as described previously [17]. In brief, 1 g of soybeans was homogenized and extracted with simultaneous hydrolysis by refluxing for 1 h in a mixture of 10 mL of conc. hydrochloric acid and 40 mL of 96% aqueous ethanol containing 20 µg/L flavone (internal standard) and 0.25 g/L butylated hydroxytoluene.

**Enzymatic hydrolysis and extraction of isoflavones from human milk.** We mixed 2–4 mL of human milk equilibrated to room temperature with 25 µL of flavone solution (120 µg/L in 96% ethanol), 50 µL of _β_-glucuronidase reagent (200 kU/L), and 50 µL of arylsulfatase reagent (5 kU/L) and then stirred this for 1 h at 37 °C. This sample was extracted three times with 2 mL of ethyl acetate (ACS-certified) and the organic phases were combined after centrifugation; the combined phases were then dried under nitrogen. The dry extract was redissolved in 150 µL of methanol by vortex-mixing, after which 50 µL of 0.2 mol/L acetate buffer (pH 4) was added. After centrifugation, 150 µL of clear sample was put in an insert of an amber vial for automated HPLC injections of 20-µL samples.

In a parallel experiment, we mixed 25 µL of flavone (120 µg/L in 96% ethanol) with 130 µL of methanol and 45 µL of 0.2 mol/L acetate buffer (pH 4) in the same batch for calculating the recovery of the internal standard.

**Extraction and enzymatic hydrolysis of urinary isoflavones.** Urine was extracted as described previously [27]. In brief, 20 mL of clear urine was mixed with 5.0 mL of 0.2 mol/L acetate buffer (pH 4) and 200 µL of flavone internal standard (60 µg/L in 96% ethanol) and filtered through a preconditioned C18 reversed-phase solid-phase extraction (SPE) column (PGC Scientific, Gaithersburg, MD). We then washed the column with 2 mL of acetate buffer and eluted the analytes with 100% methanol.

The eluate was dried with the Speed-Vac at room temperature, redissolved in 1.0 mL of 0.2 mol/L phosphate buffer (pH 7.0), mixed thoroughly with 50 µL of _β_-glucuronidase [31] and 50 µL of arylsulfatase, and incubated for 1 h at 37 °C. Subsequently, the enzymes of the hydrolyzed samples were inactivated by addition of 0.9 mL of 100% methanol. Samples were analyzed immediately or stored at −20 °C and analyzed by HPLC (after equilibration to room temperature, vortex-mixing, and centrifugation at 850 g for 5 min). The samples could be concentrated further by partitioning the isoflavones from the hydrolyzed sample into ethyl acetate. The combined organic phases were dried under nitrogen and redissolved in 150 µL of mobile phase plus 50 µL of 0.2 mol/L acetate buffer (pH 4); 20 µL of this was injected into the HPLC system.

In a parallel experiment, we mixed 200 µL of flavone (60
μg/L in 96% ethanol) with 0.9 mL of buffer and 0.9 mL of methanol in the same batch to calculate recovery of the internal standard.

**Trimethylsilylation.** Dry milk extracts or crystalline authentic standards were dissolved in 0.1 mL of 20 g/L N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA, Sigma) in imidazole and incubated for 15 min at 60 °C before GC-MS analysis.

**Chromatography.** All HPLC analyses were carried out on a 150 × 3.9 mm (i.d.) NovaPak C18 (4-μm particles) reversed-phase column (Waters, Milford, MA) coupled to a 10 × 4.6 mm (i.d.) Adsorbosphere C18 (5-μm particles) direct-connect guard column (Alltech; Deerfield, IL). Elution was performed at a flow rate of 0.8 mL/min with the following linear gradient of solvent B in solvent A [B = methanol/acetonitrile/dichloromethane (10/5/1 by vol), A = acetic acid/water (10/90 by vol)]: 5% for 5 min, from 5% to 45% in 20 min, from 45% to 70% in 6 min, and from 70% to 5% in 3 min, with equilibration for 15 min before subsequent injection. Analytes were monitored with the dual-channel diode-array detector at 260 and 280 nm and simultaneously with the coulometric detector at +500 mV during the entire HPLC run. Observed peaks were then scanned between 190 and 400 nm.

Gas chromatography was performed by injecting 3 μL of trimethylsilylated sample onto a 17 m × 0.2 mm (i.d.) Hewlett-Packard Ultra-1 capillary column (film thickness 0.11 μm) and using helium as the carrier gas at a flow rate of 1.0 mL/min with a 1:10 split. We used the following temperature program: initial temperature 180 °C, increasing at 10 °C/min until reaching a final temperature of 320 °C. Signals were registered in the selected-ion monitoring (SIM) mode at the following m/z ratios (determined after analysis of standards): daidzein = m/z 398, 383; genistein = m/z 471, 399, 228; equol = m/z 386, 192; O-desmethylangolensin = m/z 459, 281.

**CALIBRATOR SOLUTIONS AND HPLC CALIBRATION CURVES**
Stock solutions of calibrators were prepared by dissolving 1–3 mg of the crystalline compound in 20 μL of dimethyl sulfoxide and then adding methanol to give concentrations of 2–5 mol/L. Compounds with <95% purity as determined by HPLC analysis were discarded. The concentration of each stock solution was determined by absorbance readings as reported earlier [27]. Calibration curves, obtained by plotting concentration (0–150 μmol/L) as a function of peak area units when monitored at 260 nm, showed slopes of 1.81, 0.85, and 5.21 for daidzein, genistein, and desmethylangolensin, respectively. Equol, monitored at 280 nm, showed a slope of 5.63. For all analytes we observed calibration curves of very high linearity, as evidenced by coefficients of determination >0.996.

Recovery of analytes from milk and urine was calculated from peak areas obtained after HPLC analyses according to the slopes of the calibration curves obtained from serial dilutions of the stock calibration solutions. Milk concentrations were expressed as nanomoles per liter after adjustment for recovery of the internal standard. Urine excretion was expressed as nanomoles per hour after adjusting for the time between urine collection and previous void (hours), urine volume (milliliters), and recovery of the internal standard (%).

**Results and Discussion**
HPLC CONDITIONS, DETECTION LIMITS, AND CALIBRATION HPLC separations of isoflavone aglycones and conjugates (Fig. 1) were improved from our previous report [27] by including methanol and dichloromethane as additional organic modifiers in the mobile phase and by using a less-steep linear gradient. Better selectivity was achieved with the present system because it provided baseline separation of the soy isoflavones daidzein, glycine, and genistein and of their acetyl and malonyl esters present in soy foods (not shown). In addition, the mammalian isoflavone metabolites equol and O-desmethylangolensin (Fig. 2) as well as other dietary phytoestrogens such as formononetin, biochanin-A, and coumestrol (Fig. 2E) were selectively separated.

However, the present system led to later retention times and thereby, to lower peak heights. Consequently, we calculated the detection limits for the analytes (Table 1) by using peak heights and found these to be slightly greater than those in the HPLC system used in our earlier report [27]. However, detection limits of analytes isolated from human milk and urine were 10-fold lower than those given in Table 1 because of the concentration step during extraction (see Materials and Methods). Also, using higher volumes of starting material or increasing the injection volume could further lower the detection limits. More importantly, coulometric detection at +500 mV improved (lowered)

![Molecular structure of analytes and internal standard (flavone).](image-url)
the detection limits by more than fivefold relative to those obtained by monitoring absorbance at the absorbance maximum for each analyte (Table 1).

Although retention times were altered, the peak areas obtained by monitoring at the respective absorbance maxima of the analytes remained unchanged. Therefore, the present HPLC system showed sensitivity equal to that of the previously applied system [27], as evidenced by the nearly identical slopes for the calibration curves. Sensitivity was further improved by using electrochemical detection instead of monitoring ultraviolet absorbance (Table 1).

The present system also produced calibration curves having extremely high linearity in the concentration range of interest for all analytes included in this assay (see Materials and Methods).

### Table 1. HPLC detection limit and sensitivity.

|             | UV detection limit, nmol/L | ECD detection limit, nmol/L | Decrease of detection limit | Increase of sensitivity
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>54.3</td>
<td>15.8</td>
<td>3.43</td>
<td>3.44</td>
</tr>
<tr>
<td>Genistein</td>
<td>26.6</td>
<td>13.9</td>
<td>1.91</td>
<td>1.94</td>
</tr>
<tr>
<td>Equol</td>
<td>164.2</td>
<td>29.7</td>
<td>5.53</td>
<td>7.90</td>
</tr>
<tr>
<td>O-Desmethylflavonolensin</td>
<td>50.2</td>
<td>85.2</td>
<td>0.59</td>
<td>1.01</td>
</tr>
<tr>
<td>Coumestrol</td>
<td>67.4</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

* Ultraviolet absorbance at 260 nm.
* Electrochemical values obtained by coulometric detection at 500 mV.
* Determined by peak height with a 20-μL HPLC injection at signal-to-noise ratio = 5.
* Coulometric values relative to absorbance values.
* Ultraviolet values obtained by absorbance at 280 nm.
* nd = not determined.

**ISOFLAVONE CONCENTRATIONS**

*In soybeans.* Total concentrations of daidzein, genistein, and glycitein in roasted soybeans obtained after extraction and simultaneous acid hydrolysis were 830, 913, and 174 mg/kg, respectively—values in good agreement with those reported earlier [17]. Consequently, the dosage applied during dietary intervention in this study amounted to 0.08, 0.15, and 0.30 mg/kg for daidzein and 0.08, 0.17, and 0.33 mg/kg for genistein—amounts found to exhibit biological effects [3], especially when given in combination [32].

**Extracted from human urine.** Because isoflavonoids occur in human urine mainly as sulfates and glucuronides [22, 33], hydrolysis is recommended before HPLC analysis so that a less-complex chromatogram is obtained [27]. The sequence of enzymatic hydrolysis and SPE did not change the final results, which suggests a lack of enzyme inhibitors in urine. Also, phase separation with ethyl acetate gave similar yields relative to SPE. However, performing SPE first was found to be the fastest and most convenient method and therefore, was utilized in this study, as previously described [27]. Hydrolysis with a glucuronidase/sulfatase mixture prepared from *H. pomatia* showed interfering peaks with isoflavone signals in the HPLC chromatogram of human milk extracts; therefore, we used in all assays glucuronidase from *E. coli*, which lacked these interfering compounds.

**Extracted from human milk.** Milk presents a unique challenge for isoflavone extraction because of the high content of proteins that easily form gel aggregates, leading to low recovery of analytes. Therefore, proteins have been removed from milk by precipitation [34, 35] or filtration [36], or analytes have been extracted directly without affecting proteins [37]. Tests with isoflavonoid-supplemented milk samples revealed that SPE did...
Table 2. Recovery of soy isoflavones and their main metabolites added to aqueous solution or to human milk.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Daidzein</th>
<th>Genistein</th>
<th>Equol</th>
<th>O-Desmethyl-angolensin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water + enzyme (n = 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount added, nmol</td>
<td>0.5–63.2</td>
<td>0.3–39.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>103.6</td>
<td>99.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV, %</td>
<td>9.4</td>
<td>3.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human milk (n = 2 each)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB-0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount added, nmol</td>
<td>63.2</td>
<td>39.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>96.0</td>
<td>93.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Su-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amount added, nmol (n = 2)</td>
<td>63.2</td>
<td>39.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>93.1</td>
<td>93.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BB6-3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial amount, pmol</td>
<td>16.7</td>
<td>20.3</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amount added, pmol</td>
<td>209.5</td>
<td>285.1</td>
<td>1491.5</td>
<td>698.3</td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>93.1</td>
<td>76.1</td>
<td>69.8</td>
<td>71.1</td>
</tr>
<tr>
<td>BB7-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial amount, pmol</td>
<td>11.4</td>
<td>19.1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amount added, pmol</td>
<td>209.5</td>
<td>285.1</td>
<td>1491.5</td>
<td>698.3</td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>82.3</td>
<td>82.7</td>
<td>81.5</td>
<td>85.2</td>
</tr>
<tr>
<td>BB9-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial amount, pmol</td>
<td>65.8</td>
<td>37.9</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amount added, pmol</td>
<td>1833.1</td>
<td>765.6</td>
<td>37354.4</td>
<td>9689.2</td>
</tr>
<tr>
<td>Mean recovery, %</td>
<td>127.9</td>
<td>93.8</td>
<td>108.8</td>
<td>111.5</td>
</tr>
<tr>
<td>Overall mean recovery in human milk, %</td>
<td>98.5</td>
<td>87.9</td>
<td>86.7</td>
<td>89.3</td>
</tr>
</tbody>
</table>

n = number of samples tested; nd = not detected; + = present, but below the limit of quantification.

Fig. 3. Absorbance scans of genistein (A) and daidzein (B).

Absorbance scans for authentic standards (two upper traces in A and two lower traces in B) are identical with those for material obtained in HPLC peaks 2 (lower trace in A) and 1 (upper trace in B) from human milk extracts.
IDENTIFICATION OF ISOFLAVONOIDS EXTRACTED FROM HUMAN MILK AND URINE

Analytes (Fig. 1) were routinely identified by retention times in various HPLC systems and by ultraviolet absorption patterns obtained by diode-array detection (Fig. 3) in comparison with authentic standards and with reported absorbance data [42, 43]. In addition, we used fluorometric [27, 41] and coulometric [44] detection to confirm the presence of these agents in human milk. Trimethylsilylation of representative milk extracts followed by GC-MS analysis in SIM mode again indicated the presence of daidzein and genistein in human milk because the GC retention times and the mass fragmentation patterns were identical to those of authentic standards (Fig. 4 and 5). These GC-MS values were also in excellent agreement with those reported previously [26]. None of the other soy isoflavones or metabolites shown in Fig. 1 was detected in human milk extracts with this GC-MS/SIM method, from which we deduce their absence in human milk after soy consumption.
**ISOFLAVONE CONCENTRATIONS IN HUMAN MILK AND URINE AFTER SOY CHALLENGE**

Urine analysis by this procedure revealed patterns (Fig. 6) identical to those of the conjugated soy isoflavones daidzein, genistein, and glycitein and of their main metabolites equol and O-desmethylangolensin, as reported previously [23, 27]. In contrast, analysis of human milk showed that only daidzein and genistein conjugates were above the HPLC detection limit, even after challenge with 25 g of roasted soybeans (data not shown). The exclusive occurrence of the major soy isoflavones in milk is not surprising; glycitein exposure through soy challenge is minor due to its low concentrations in soybeans [23, 45]. Preferential excretion of the metabolites over the parent isoflavones is suggested by the isoflavone:metabolite ratio, which is seen to be much higher in plasma than in urine or feces [24, 26]. Because milk is produced by secretory processes of blood [46], the low plasma concentrations of isoflavonoid metabolites in human blood might explain their absence in milk.

Soybean challenge led to a fast and dose-dependent response
in human milk (Fig. 6). Maximum milk concentrations were reached 10–14 h after soy intake, and baseline was reached 2–4 days later, depending on the dose. The isoflavone patterns in milk followed those in urine except with a slight delay (Fig. 6). This is in good agreement with many other polar micronutrients or drugs, which show a faster urinary excretion than secretion into milk [46]. Most importantly, however, the Caucasian woman showed higher concentrations of genistein conjugates than those of daidzein conjugates in plasma and, because of secretory processes, also in milk. This is in contrast to higher daidzein than genistein concentrations observed in breast milk of a Chinese woman (see below), a pattern generally reported from plasma [24], urine [22, 23, 27, 28], and feces [26], which suggests large interindividual variation.

Milk concentrations of daidzein and genistein conjugates increased rapidly after soy consumption; this was followed by their rapid decrease and, most interestingly, by a subsequent increase before reaching baseline values (Fig. 6). This biphasic elimination pattern has already been observed in animal plasma after treatment with the flavone hydroxyfarrerol [47] or the flavone baicalin [39] and, more importantly, in human plasma [33] and urine [27] after soy intervention. This biphasic phenomenon has been suggested to result from enterohepatic circulation [33], a process known to occur with flavonoids [48]. Enterohepatic circulation might also, therefore, explain the biphasic pattern observed in human milk.

Isoflavone concentrations in three milk and urine samples from a Chinese woman eating her usual diet (including tofu soup once a day for dinner) were analyzed by the proposed procedure. The concentrations of daidzein and genistein in her milk (80–110 and 30–50 nmol/L, respectively) were similar to those observed in the Caucasian woman’s milk after challenge with roasted soybeans. This is in good agreement considering the similar total isoflavone exposure from these two food items [17]. However, urinary excretion of these compounds was less by the Chinese woman (80–150 nmol/h daidzein and 8–33 nmol/h genistein) than by the Caucasian woman during soybean intervention. This difference may reflect interindividual variation in
excretion [23, 27] or the women's ingestion of different food items.

Although demonstrated exclusively in carcinogen- or cytokine-induced cell and animal models, the anticancer properties of the soy isoflavones genistein and daidzein are particularly intriguing when considered in light of these results. Breastfeeding is known to be beneficial not only to the mother, by limiting fertility and protecting against ovarian and breast cancer, but also particularly to the infant. Newborn babies are protected by mother's milk from a variety of diseases e.g., various types of infections, diabetes mellitus, and multiple sclerosis [49]. Additionally, lower incidence of sudden infant death syndrome [50] and better intellectual development [51] are attributed to breast-feeding. Our results add another important item to the growing list of benefits of breast-feeding, a decrease in cancer incidence and severity—both of which are significantly reduced when newborn animals are treated with only three single doses of genistein [13]. The data presented here suggest a cancer-preventive effect of breast-feeding to the offspring when mothers consume soy foods, in that such infants would be exposed to the known anticancer agent genistein and also daidzein. This effect, which would take place at a very early and most critical developmental period, might protect the individual throughout life. Also, our findings may provide the basis for an alternative explanation for the lower cancer rates observed in Asian populations with high consumption of soy. The lower cancer rates in these populations might not be the result of isoflavone exposure by soy consumption in adulthood or childhood [52] but rather of isoflavone exposure shortly after birth, in a critical period of life, through mother's milk containing these agents.

Conceivably, the isoflavone conjugates obtained from mother's milk are more bioavailable to the newborn child than are the conjugates from soy foods. Young infants might not be able to absorb isoflavones from soya because their gut flora are incompletely developed, preventing hydrolysis of the acylated and nonacylated isoflavone glucosides present in soy foods. Bio-transformation and bioavailability studies of soy isoflavones in infants are required to further explore the cancer-preventive effects of these compounds in humans.

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