Phytoestrogens in Breast Milk—Another Advantage of Breast-Feeding?

Phytoestrogens in foods and their metabolism have become a hot research topic as more research is published on the protective properties of the isoflavones genistein and daidzein. Plant isoflavonoids originate in soybean products and other legumes and are converted by intestinal bacteria to hormone-like compounds with weak estrogenic and antioxidative activity *[1]*. Besides their effects on sex hormone metabolism, they have been found to influence intracellular enzymes, protein synthesis, growth factors, malignant cell proliferation, differentiation, and angiogenesis, making them strong candidates to be natural cancer protective compounds. Epidemiological studies support the isoflavonoid hypothesis, with excretion of the greatest concentrations of these compounds found in countries with low cancer incidence *[2]*. Recent publication in *Science* of a study of biotherapy of B-cell precursor leukemia by targeting genistein to CD19-associated tyrosine kinases has further intensified interest in isoflavones *[3]*.

Despite the promise of these exciting compounds, much work needs to be completed before consumers get the green light to increase consumption of foods high in isoflavones. First, more background is needed on what foods are good sources of these phytochemicals. Isoflavonoids are found primarily in legumes (Leguminosae family) and occur in appreciable amounts in legumes commonly consumed by humans and animals. Foods such as soy, lentils, beans, and chickpeas are good sources of isoflavones. Foods do not lose substantial amounts of isoflavones in processing except for soy sauce, alcohol-extracted soy protein concentrate, soy protein isolates, and soy fiber *[4]*. In common foods, most isoflavones are present as glucosides *[5]*, as measured by solid-phase extraction and gas chromatography.

Unlike many researchers, Franke and Custer *[6]* analyzed the foods they were feeding to determine intake levels of isoflavones. Although this may seem like an obvious first step in a feeding study, the difficulty in measuring isoflavones in foods tempts many researchers to just feed soy and assume that isoflavones are being delivered. Franke and Custer *[6]* provide valuable information on extraction and analysis of isoflavones in soy products and breast milk that will be helpful to other investigators in this field. A previous report by this group *[7]* provides useful analytical details of the HPLC method they developed for measuring isoflavones in foods and biological samples.

In a recent study using gas chromatographic–mass spectrometric analysis of isoflavones, tofu contained the highest amounts of isoflavones among the products tested; moreover, there was some variability among different brands of tofu *[8]*. The soy drink contained lesser amounts of isoflavones, and soy-based infant formulas were devoid of isoflavones. This finding suggests that infants who consume soy formulas do not receive isoflavones and that the only way they can receive isoflavones is through breast milk. Thus, many soy protein products currently available may have low or no isoflavone content, and consumers who may think they are consuming these protective phytochemicals are not.

Information on the metabolism of isoflavones in humans is also lacking. Isoflavones are members of the isoflavonoid family, which is part of the much larger flavonoid family. The flavonoids, diphenolic plant compounds, include isoflavones, coumestans, and the flavones, flavonols, and flavanones (referred to as bioflavonoids). Various compounds within the isoflavonoid family have been identified *[9]*. The isoflavones formononetin, daidzein, and genistein and the isoflavonoid metabolites equol, dihydrodaidzein, *O*-desmethylangolensin (O-DMA), and methylequol have been identified in human urine *[10]*. Rat studies show equol is formed by intestinal bacteria; germ-free rats failed to excrete equol when fed commercial pellet food *[11]*. In addition, enzymes used in the conversion of daidzein to equol have been identified in human intestinal microflora *[11]*, and human fecal flora has been shown to produce equol from soya-rich broth *[12]*.

Daidzein, equol, O-DMA, and genistein have been identified in human plasma *[13]*; isoflavonoids have been found in human and animal urine *[14, 15]*; and daidzein, equol, and methylequol have been identified in cow's milk *[16]*. Most research on the metabolism of isoflavones in human subjects has measured isoflavones in urine because of analytical ease resulting from their high concentrations in urine and fewer interfering substances. Even low intakes of soy compounds greatly increase urinary excretion of isoflavones, although there are large individual differences in metabolism of these compounds. Adlercreutz et al. *[13]* found that urinary concentrations of equol increased as much as 1000-fold after soy consumption; however, they noted large variations in equol excretion among subjects. Some people produced very little or undetectable amounts of equol. Setchell et al. found two of six subjects to be "nonresponders" after consuming 40 g of textured soy protein for 5 days. They suggested that the rate of equol formation might depend on the composition of intestinal microflora, intestinal transit time, and variability in the redox state of the large intestine. Others report an inverse relationship between equol and O-DMA excretion after a soy challenge, suggesting individual variability in the preferred metabolic pathways of dietary isoflavones *[17]*.

Our group has reported that consumption of fermented soy products such as tempeh instead of tofu increased the urinary isoflavonoid recovery; this suggests that fermentation of a food product increases the availability of any isoflavones in soy *[18]*. In a similar feeding study, chick peas were also found to be a significant source of urinary isoflavonoids *[19]*. In a study of the bioavailability of soybean isoflavones *[20]*, seven women were fed different doses of isoflavones. Two women showed fecal isoflavone recovery 10–20 times that in the other five women. The average plasma concentration of genistein 24 h after the isoflavone dose in subjects excreting large amounts of fecal isoflavones was 2.5 times greater than that in subjects who excreted small amounts of fecal isoflavones. Subjects with low fecal excretion of isoflavones had high urinary concentrations. The authors concluded that human isoflavone bioavailability depends on the relative ability of gut microflora to metabolize these compounds. Franke and Custer *[6]* add another important piece to these findings, that isoflavones are also present in human milk.

A review by Whitten et al. *[21]* suggests that phytoestrogens have a broad range of actions and variability in potency across endpoints. This variability argues for the importance of fully characterizing each phytoestrogen in terms of its sites of action, balance of agonistic and antagonistic properties, natural potency, and short-term and long-term effects. Franke and Custer

[6] contribute significant data on the metabolism of these compounds in lactating women.

Another missing piece is information on the metabolism of isoflavonoids in infants, especially breast-fed infants, since soy formula appears to be lacking in isoflavones. The microflora of a breast-fed infant are thought to vary greatly from those of a formula-fed infant, the former having higher concentrations of bifidobacteria. The metabolic fate of isoflavones ingested from breast milk in infants cannot be determined from existing research studies and should be studied further.

Countries with high soy intakes have lower risks of cancers *[2]*, but soy, of course, is just one dietary variable. Animal studies provide intriguing data suggesting that early exposure to isoflavones modifies biological effect; for example, breast cancer incidence and tumor numbers were significantly decreased when three genistein doses were given to newborn mice *[22]*. Whitten et al. *[23]*, finding that a phytoestrogen diet induced a premature anovulatory syndrome in lactationally exposed female rats, concluded that the female offspring of mothers fed a diet containing low concentrations of phytoestrogen during lactation manifested early and nearly universal disruption of cyclicity of the persistent-estrus type. Levy et al. *[24]* also suggested that the effects of genistein are different from other estrogens, and timing of exposure during development appears to be an important factor in the effect of genistein on hormonal factors.

The Franke and Custer study *[6]* provides important evidence that breast-fed babies are consuming isoflavones if their mothers consume soy products. That these isoflavones may be protective is only speculative at present. A pressing issue is whether isoflavones have detrimental effects on breast-fed infants. This question becomes more critical if isoflavones are isolated and sold as dietary supplements, or if the concentrations of isoflavones in foods are enhanced by plant breeding or selected processing techniques.

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