Soy formula and isoflavones and the developing intestine

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Soy protein-based infant formulas (SF) are commonly used to feed infants during their first year of life. SF contains isoflavones, which influence cell proliferation, but their actions in the developing intestine have received little attention. Herein, the impact of soy isoflavones and SF on intestinal development and rotavirus (RV) infectivity is described. The isoflavone genistein has been found to reduce intestinal cell proliferation in vitro and in vivo in piglets without affecting intestinal enzyme activity or nutrient transport. However, isoflavones possess antiviral activity. The mix of isoflavones at the concentrations in SF, or genistin alone, inhibited RV infectivity by 40–60%. Thus, soy isoflavones are bioactive within the neonatal intestine and may reduce the severity of RV infections.

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INTRODUCTION

The first 4–6 months of life are a critical phase of neonatal development in which infants receive sole-source nutrition comprised of breast milk or infant formula. Although human milk (HM) is the ideal form of nutrition for human infants, most infants in the United States have received some formula by the age of 2 months. Soy infant formula (SF) has been used in the United States for five decades. At some time during the first year of life, approximately 32% of infants in Israel, 36% in the United States, and 13% in New Zealand have received an SF. In France, Italy, and the United Kingdom, use of SFs is much lower, ranging from 2 to 7%. SFs are formulated to meet the nutrient requirements of the term infant and clinical studies demonstrated normal growth and development and bone mineralization of term infants fed SFs. Additional studies confirmed that SFs do not interfere with the normal immune cellularity or the response to oral immunization. One key difference between SF versus cow’s milk-based formulas (CMF) and HM is the presence of phytochemicals, including isoflavones, in SFs. SFs contain ~32–47 mg isoflavones/L, compared to 1.2–10 μg/L in HM. Genistein and genistin are the predominant isoflavones in SF, and circulating genistein concentrations in infants fed an SF are 600-fold higher than in breastfed infants. These high circulating concentrations have raised concerns over the short- and long-term impacts of isoflavone exposure in early life, since the consumption of pharmacologically active compounds in infancy and childhood has the potential to exert developmental effects. To date, no adverse effects of short- or long-term use of SFs have been observed through young adulthood. However, many pediatric societies and advisory groups support additional research and have advised a precautionary approach in the use of SFs, restricting their use to specific clinical indications. This review describes the composition of SFs, their recommended use, and recent laboratory work, of ourselves and others, highlighting the impact of soy formula and soy components (e.g., isoflavones, fiber) on neonatal intestinal development.

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RECOMMENDATIONS ON THE USE OF SOY INFANT FORMULA

In 1998 and again in 2008, the American Academy of Pediatrics (AAP) concluded that SFs provide adequate nutrition for normal growth and development in term infants; however, there were few indications for their use in place of CMF. These indications are for infants with galactosemia and hereditary lactase deficiency and in situations in which a vegetarian diet is preferred. The most common reasons cited for use of SF by infant care providers are for relief of perceived formula intolerance or symptoms of colic. Indeed, a telephone survey of 1803 Israeli mothers at 2, 4, 6, and 12 months postpartum demonstrated that the decision to use SF as opposed to CMF was made by the mother rather than a healthcare provider in the majority of instances. The mother’s decision was most often based on her personal preference rather than concerns for cow’s milk allergies or for other symptoms. Another common use of SF has been for the prevention or treatment of allergies to CMF. Soy is antigenic, but it does not appear to be highly allergenic, resulting in a primary allergic response in only 0.5% of infants. Thus, SFs have been used to treat infants with allergy or food intolerance. In a double-blind, placebo-controlled food-challenge study, Zeigler et al. demonstrated that 86% of infants with immunoglobulin E-associated cow’s milk allergy were tolerant to soy. However, the latest recommendations support the use of extensively hydrolyzed protein formulations rather than SF for infants with documented cow’s milk-protein allergy, because 10–14% of these infants will develop a soy-protein allergy.

As with cow’s milk proteins, soy protein may cause severe protein-induced small-bowel enteropathy, which is a reversible celiac-like villus injury that can result in malabsorption and failure to thrive. Approximately 30–64% of infants with infantile food protein-induced enterocolitis caused by cow’s milk protein had concomitant soy-induced enterocolitis. Eosinophilic proctocolitis, a less severe form of enterocolitis, has also been reported in infants receiving soy-protein-based formula. The dietary-protein-induced enteropathy is immunologic in origin, but it is not mediated by immunoglobulin E (IgE), reflecting instead an age-dependent transient soy protein hypersensitivity. It is theorized that the intestinal mucosa damaged by CMF allows increased uptake and therefore increased immunologic response to the subsequent soy antigen. Because of the reported high frequency of sensitivity to both cow’s milk and soy antigens in infants, the use of extensively hydrolyzed formulas is recommended for the management of documented cow’s milk-protein-induced enteropathy or enterocolitis.

SFs have been used prophylactically to prevent the onset of CMF allergy or intolerance; however, the question of whether SFs were efficacious in infants without clinical evidence of allergy or food intolerance was controversial. A recent meta-analysis of five randomized or quasi-randomized studies conducted by the Cochrane Central Register of Controlled Trials found no significant effect of SF on the prevalence of childhood allergy, infant asthma, infant or childhood eczema, or infant or childhood rhinitis. Furthermore, no significant reduction in the incidence of childhood asthma, eczema, or rhinitis was observed with SF. The authors concluded that, based on these results, SF should not be recommended for prevention of allergy or food intolerance in infants at high risk of allergy or food intolerance. However, it is important to note that only three eligible studies enrolling high-risk infants with a history of allergy in a first-degree relative were included. Furthermore, no eligible study enrolled infants who were fed HM or studied the effect of early, short-term SF feeding. All compared prolonged feeding with SF versus CMF. Thus, further research is warranted to determine the role of SF in the prevention of allergy or food intolerance in infants who cannot be breastfed or who have a strong family history of allergy or cow’s milk-protein intolerance.

In the past, SFs have also been recommended following recovery from acute diarrheal diseases. Since SFs do not contain lactose, they appear to be beneficial in cases of lactase deficiency following infantile diarrhea. However, after rehydration, most infants with acute gastroenteritis can be managed with continued use of HM, standard dilutions of CMF, or a lactose-reduced CMF.

COMPOSITION OF SOY INFANT FORMULA: NUTRIENTS AND ISOFLAVONES

Nutrient composition

SFs were initially made with soy flour, which was nutritionally inferior to the soy protein isolate (SPI) currently used in SFs. SFs made from soy flour had a lower protein digestibility and contained a number of nonprotein components, including carbohydrates, fibers, phytates, and protease inhibitors that are largely absent from SPI. SPI is a purified, highly digestible soy protein that is the primary protein source in SF worldwide. Heat applied during the processing of soy protein removes 80–90% of protease inhibitor activity, which was present in the soy flour. SPI is comprised of at least 90% protein on a dry-weight basis and possesses a digestibility of at least 97% or higher, with a high concentration of balanced essential amino acids. The SPI is fortified with L-methionine, L-carnitine, and taurine, choline, inositol, and vitamins A, C, D, E, and K.
and minerals. L-methionine is added to improve the biological quality of the protein. Carnitine, which is needed for the oxidation of long-chain fatty acids, and taurine, the major conjugate of bile acids in infants, are added at concentrations found in HM. Calcium, phosphorus, iron, and zinc are added to SF at higher concentrations than those in CMF due to the presence of phytates that bind minerals and reduce their bioavailability. Vegetable oils, such as soy, palm, sunflower, olein, safflower, and coconut, constitute the major sources of fatty acids in SF. Corn starch, tapioca starch, and sucrose are used as carbohydrate sources.5,21 SF composition meets all AAP recommendations,5 the Infant Formula Act requirements for term infants (1980 and 1986),34 and the US FDA quality factors of supporting normal growth and having a high protein-efficiency ratio.35

Isoflavones

The key difference between SF and CMF, which has led to most of the concerns regarding the safety of SF, is the presence of numerous phytochemicals, including the isoflavones. SFs contain ~32–47 mg isoflavones/L relative to 1.2–10 μg/L in HM.14,16,36 The predominant isoflavones in SF are genistein, daidzein, and glycitein, which occur primarily as β-glycoside conjugates, genistin, daidzin, and glycitin21 (Figure 1). Genistein (all forms) comprises approximately 60% of the total isoflavone content in SF.

Biological activities of isoflavones

After ingestion, glycoside forms of isoflavones are hydrolyzed to their aglycone forms by lactase phlorizin hydrolase, which is abundant in the neonatal small intestine.37 Infants can absorb genistein and daidzein as efficiently as adults46 and circulating genistein concentrations in SF-fed infants are 100- to 600-fold higher than in breastfed infants.14,16,38 In breastfeeding mothers consuming a soy protein beverage for 2–4 days, isoflavone concentrations significantly increased from baseline in maternal urine (18–135 nmol/mg creatinine), HM (5.1–70.7 nmol/L), and infant urine (29.8–111.6 nmol/mg creatinine).58 Although isoflavones were present in the milk of mothers consuming soy foods, the mean isoflavone concentration in the plasma of their breastfed infants was 19.7 ± 13.2 nmol/L, which is >100-fold lower than the 2–7 mmol/L (0.55–1.8 mg/mL) reported in infants fed SF.54 Interestingly, urinary isoflavone excretion per hour adjusted for dose per body weight was 81% lower for the infants than for their mothers after eating soy,58 suggesting a longer potential circulating half-life for isoflavones in the neonate.

Isoflavones affect cellular functions through diverse receptors and enzymes including acting as selective estrogen receptor modulator (SERM), inhibiting protein tyrosine kinases, inhibiting the activity of topoisomerase II, and other mechanisms.39–43 Genistein is structurally similar to 17 β-estradiol, but it preferentially binds to estrogen receptor-β (ERβ), whereas classic estrogens exert their effects via both ERα and ERβ.17 Accordingly, genistein may act as a natural SERM, but it exhibits a potency that is at least 1000-fold lower than estrogen.17,39,40 Accordingly, the estrogenic or antiestrogenic property of genistein depends upon the dosage, circulating endogenous estrogen concentration, and the target tissue.39

Genistein is also a protein tyrosine kinase inhibitor, which acts by either competing with adenosine triphosphate (ATP) at the tyrosine kinase ATP binding site of the epidermal growth factor receptor41,43 or by inhibiting c-src,44 a protein tyrosine kinase involved in the mitogen-activated protein kinase (MAPK) or p38 MAPK activation via the TGFβ receptor.45 Isoflavone-mediated inhibition of protein tyrosine kinases has also been demonstrated in studies investigating tumor necrosis factor, Toll-like receptors, and growth factor signaling cas-

Figure 1  Isoflavone content and composition of soy infant formulas. Concentrations (mg/L) of isoflavones in powdered soy infant formulas were determined by high-performance liquid chromatography. Total isoflavone content is shown at the top of each bar. Mean values for each isoflavone are shown in the mean bar.
Adapted from data presented in Andres et al. (2007).66
ER-positive cancer cells. Consequently, the physiological concentrations of genistein stimulated proliferation of cancer cells, whereas low concentrations of genistein stimulated proliferation of ER-positive cancer cells. Consequently, the physiological effects of genistein on cell dynamics are concentration-dependent.

To investigate whether genistein modulated intestinal cell proliferation, Caco-2BBe human intestinal cells were exposed to 0, 1, and 30 mg/L pure genistein for 24–48 h in vitro. The 30 mg/L genistein concentration was found to represent the total genistein concentration produced in the lumen of the intestine from free genistein and the conversion of genistin and acetylgenistin to genistein. We hypothesized that the high dose of genistein would inhibit, whereas a low dose would stimulate proliferation of intestinal cells in vitro. Indeed, the 1.0 mg/L genistein dose significantly increased intestinal cell proliferation. The genistein-induced proliferation was negated by the ER antagonist ICI 182,780, indicating that genistein stimulated proliferation in Caco-2BBe cells through the ER.

In contrast, the 30 mg/L genistein concentration significantly reduced the cell number, cellular proliferation, and caspase-3 activity compared to the control (0 genistein). Cell cycle analysis after 48 h exposure to 30 mg/L genistein, similar to those produced in the intestine of infants fed SF, revealed accumulation of cells in G2/M, indicating cell cycle arrest. Thus, in vitro data suggested that the proliferation of intestinal cells of soy formula-fed infants could be adversely impacted by isoflavones.

Given the limitation of extrapolating in vitro findings to the infant, we sought to determine how ingested genistein influenced intestinal structure and function in vivo using the piglet as the animal model for the human infant. The piglet and the human infant have a similar intestinal anatomy and digestive physiology, and the piglet is considered the best preclinical animal model for human intestinal development. Our hypothesis was that genistein at the concentration that could be produced from genistin at the level currently contained in soy infant formula would adversely impact intestinal structure and function.

**Effects of soy isoflavones on intestinal development**

The intestine of the SF-fed infant is nearly continuously exposed to isoflavones. Following digestion and absorption into the enterocyte, some genistein remains in the cell in its glucuronidated and aglycone forms, which can accumulate to sufficient levels to affect cell-cycle dynamics. ERs are present on rapidly proliferating intestinal stem cells, but very little attention has been focused on the impact of SF or its components on the intestinal development of SF-fed infants. Previous studies have shown that high concentrations of genistein inhibited proliferation of cancer cells, whereas low concentrations of genistein stimulated proliferation of ER-positive cancer cells. Consequently, the physiological effects of genistein on cell dynamics are concentration-dependent.

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To test this hypothesis, piglets (n = 8/group) were fed CMF alone, CMF + 1 mg/L genistein (LG), or CMF + 14 mg/L genistein (HG) for 10 days. Formula intake and piglet growth were similar in all groups. Importantly, the mean serum genistein concentration in the HG group was similar to that reported in SF-fed infants, indicating that piglets absorb and metabolize genistein in a similar manner as human infants. Total intestinal weight and length as well as jejunal and ileal villus height were not significantly affected by HG. To investigate the impact on intestinal cell dynamics, enterocyte proliferation, migration, and apoptosis were determined. No significant effects of genistein intake on enterocyte apoptosis were observed; however, the percentage of jejunal crypt cells that were positive for proliferating nuclear cell antigen (PCNA) in the HG piglets was 50% lower than CMF or LG (P = 0.001). These data demonstrated that genistein was bioactive within the neonatal intestine and that it reduced intestinal stem cell proliferation. This effect likely did not translate into significantly lower villus heights due to the short duration of the study (10 days). We previously showed that the enterocyte turnover rate in the piglet is 7–10 days. To investigate the effects of genistein on intestinal digestive function, disaccharidase activity was measured and intestinal electrophysiology was assessed in modified Ussing chambers. No effect of either dose of genistein on lactase or sucrase activities, barrier function, sodium-coupled nutrient transport, or chloride secretion was observed in jejunum or ileum. In summary, the lack of effect of genistein on nutrient transport and enzyme activity suggests that genistein preferentially impacts proliferating versus differentiated intestinal cells, and it supports the normal growth and nutritional status of infants fed SF.

Thus, it appeared that the overall effects of genistein on the intestine of healthy piglets were minor. Formula-fed infants are at higher risk for gastrointestinal infections than breast-fed infants, although there are indications that SF ingredients may reduce the severity and duration of infantile diarrhea.

**EFFECT OF SOY FORMULA COMPONENTS ON DIARRHEAL DISEASES**

Many infants experience secondary or transient lactase deficiency following acute infantile diarrhea. SFs are lactose-free and a number of studies have shown that SFs improve recovery from infantile diarrhea. Approximately twice as many (22%) infants fed lactose-containing formulas experienced persistent diarrhea compared to 12% of infants fed SFs. Several studies also reported that the duration of diarrhea was shorter in infants receiving an...
SF\textsuperscript{57,58} while another study comparing human milk, CMF, and SF did not find any differences in the rate of recovery from rotavirus (RV) or non-RV diarrhea.\textsuperscript{33} As lactose-free and reduced-lactose CMFs are now available, the AAP recommends that the use of SF for this indication should be restricted.\textsuperscript{4} After rehydration, most infants with acute gastroenteritis can be managed with HM, standard dilutions of CMF, or a lactose-reduced CMF.\textsuperscript{5,32}

**Soy fiber**

Clinical studies have demonstrated that the addition of soy polysaccharide (SPS) added to SF was beneficial for the dietary management of diarrhea in infants and toddlers with acute infectious diarrhea\textsuperscript{59,60} or diarrhea due to antibiotic use.\textsuperscript{61} A study conducted in Peru demonstrated that the median duration of loose, watery stools was reduced from 163 h to 43 h in infants fed SF with added SPS, compared to SF alone.\textsuperscript{59} A subsequent outpatient study of U.S. infants and toddlers aged 6–24 months with acute diarrhea showed that the median duration of loose stools was 9.7 h in infants fed SF+SPS versus 32.1 h in infants fed standard SF. Burks et al.\textsuperscript{61} conducted a masked, randomized parallel study of older infants (n = 45; mean age 10.5 months) who were fed SF with or without added SPS for 10 days on occurrence of diarrhea during the administration of antibiotics. Total median fiber intake of the SF+SPS group was 6.53 g/day. The mean duration of diarrhea was reduced by half (25.1 ± 5.2 h vs. 51.6 ± 10.7 h) for children fed SF with added fiber compared to those fed the regular SF (P = 0.0013). We have also demonstrated that the addition of SPS to a CMF reduced the duration and severity of diarrhea in piglets infected with *Salmonella typhimurium*.\textsuperscript{62} In summary, soy fiber is efficacious for reducing the duration of diarrhea in infants and children and the severity of *Salmonella*-induced diarrhea in piglets. Currently, SPS is not added to commercial SF, but it should be considered for its therapeutic and potential prebiotic potential.

**Isoflavones**

Isoflavones and their related compounds within the flavonoid class exert antiviral properties in vitro and in vivo against a wide range of viruses.\textsuperscript{63} Genistein is, by far, the most studied isoflavone in this regard and has been shown to inhibit the infectivity of enveloped or non-enveloped viruses as well as single- or double-stranded RNA or DNA viruses. Flavonoids have been shown to reduce the infectivity of many viruses affecting humans and animals, including adenovirus, bovine viral diarrhea virus, herpes simplex virus, human immunodeficiency virus, and porcine reproductive and respiratory syndrome virus (PRRS) at concentrations ranging from physiological to supra-physiological (3.7 \(\mu\) M to 370 \(\mu\) M).\textsuperscript{61} Indeed, previous studies in pigs infected with PRRS demonstrated that dietary genistein reduced the serum concentrations of PRRS virus, interferon-\(\gamma\), and increased the concentration of \(\alpha1\)-acylglycoprotein in serum and spleen weight.\textsuperscript{64} The latter two observations suggested enhanced B-cell production in pigs fed genistein. In these virally challenged pigs, genistein acted as an orally active immune modulator. In contrast, in the same animal model, dietary daidzein did not affect the serum viral load, was less effective than genistein at reducing circulating interferon-\(\gamma\) concentrations, and was only a weak enhancer of body growth in PRRS-infected pigs.\textsuperscript{65} A key difference between the compounds is the ability of genistein, but not daizein, to inhibit tyrosine kinase activity, suggesting that this may be an underlying mechanism of the antiviral effects of genistein.\textsuperscript{63}

Based on these observations, we tested the hypothesis that soy isoflavones at the concentrations present in SF would inhibit rotavirus (RV) infectivity.\textsuperscript{66} RV infection was selected for study because it is a major cause of acute gastroenteritis in infants and children worldwide.\textsuperscript{67} Virus infectivity was assessed in MA-104 cells using a focus forming unit assay.\textsuperscript{68} All isoflavones at the mean concentrations present in SF (Figure 1) were tested individually and as the complete mixture (MIX). Genistin and the MIX significantly reduced RV infectivity by 33–62% and 66–74%, respectively, compared with the control (no isoflavone treatment). Genistin and the MIX were effective across a 16-fold range of RV concentrations, suggesting they would be biologically relevant at different severities of the infection. Based on previous studies,\textsuperscript{65} we hypothesized that genistin was the isoflavone with antiRV bioactivity. Indeed, when genistin was removed from the MIX, no antiRV activity was observed for the other isoflavones. In addition, in a dose-response assay, genistin significantly reduced RV infectivity at a concentration as low as 8 mg/L,\textsuperscript{66} which is lower than that present in SF.\textsuperscript{14} When investigating possible mechanisms of action, genistin or the MIX were found to decrease RV infectivity by modulating virion attachment to epithelial cells, but did not alter RV triple-layered structure. It was also clear that isoflavones inhibited RV infectivity at a postbinding step; however, genistin did not appear to act through inhibition of protein tyrosine kinases and topoisomerase II or by acting as a SERM.\textsuperscript{66} A recent RV-infection study conducted in the piglet model utilized soy-based diets, however, they were used as the control in comparison to soy + plasma protein, which has significant therapeutic value against RV infection.\textsuperscript{69} There was no CMF-fed group, therefore, it is unknown whether the SF was more or less efficacious than CMF at reducing the severity and/or duration of diarrhea following RV infection.\textsuperscript{67} In summary, modulation of SF isoflavone composition and
concentration may represent a novel nutritional approach to reduce the severity of RV infection in infants. However, future research is needed in this regard.

**EFFECT OF SOY FORMULA ON THE INTESTINAL MICROBIOTA**

Establishment of the mammalian gastrointestinal microbiota begins during the birth process and involves gradual changes building from low to high diversity until dense, stable populations colonize the gut. Microbial colonization is critical for adequate development of gut physiology and immune functions that protect against allergies and inflammatory or autoimmune disorders later in life. The acquisition of a microbiota is influenced by both host and environmental factors. One of the key environmental factors influencing the microbiota is the mode and type of feeding. Even though differences between the microbiota of breastfed and formula-fed infants have been known for years, there are conflicting reports in the literature regarding the composition of the neonatal gastrointestinal microbiota and the factors that shape it. The general dogma was that *Bifidobacterium* dominated the microbiota of breastfed infants, whereas formula-fed infants harbored a more diverse, adult-like microbiota. Some studies have found a lower abundance of *Bifidobacterium* and a higher abundance of aerobic bacteria in the feces of formula-fed infants relative to breastfed infants, yet other reports have found no such difference between the two feeding-type groups. A recent study by Palmer et al., using a microbial SSU rDNA microarray designed to give nearly comprehensive coverage of known SSU rDNA species, demonstrated relatively low frequency and abundance of *Bifidobacteria*. In these predominantly breastfed infants, *Bifidobacteria* were rarely the major constituents of the fecal microbiota and they did not appear until several months after birth; thereafter, they persisted as a minority population. Based on these findings, the authors concluded that the emphasis on *Bifidobacteria* in the infant GI microbiota may be out of proportion to its prevalence, abundance, and relevance to health.

While much work remains to be done in furthering our understanding of the development of the neonatal microbiota and its contributions to intestinal development, there is a particular dearth of information regarding how SFs impact the neonatal microbiota. In 2004, Hoey et al. reported on the urinary excretion of isoflavones, intestinal microbiota, and their metabolites in infants and children who had been fed a CMF or an SF in early infancy. Their goal was to determine at what age the metabolism of daidzein to equol and/or O-desmethyangelonensin (O-DMA) by the gut microbiota was established, and whether exposure to isoflavones in early infancy influenced their metabolism at a later stage of development. Infants and children (aged 4 months–7 years) who had been fed an SF (n = 30) or a CMF (n = 30) were divided into four age groups: 4–6 months (7 SF; 7 CMF), 7–12 months (7 SF; 9 CMF), 1–3 years (6 SF; 8 CMF), and 3–7 years (10 SF; 6 CMF). Infants (4–6 months) consumed an average of 600 mL of SF per day, resulting in a mean isoflavone exposure of 3.7 mg/kg/d. Urinary genistein, daidzein, and glycitein were detected in 100% and O-DMA in 75% of 4–6-month-old SF-fed infants, suggesting that infants acquire bacteria that are able to metabolize daidzein to O-DMA at a young age. In contrast, equol was detected in the urine of only 25% of the 4–6-month-old SF-fed infants. Urinary isoflavonoids were present at very low levels or not detected in infants fed a CMF.

To determine whether the type of formula influences the ability of infants to produce equol and/or O-DMA, a soy yogurt challenge (containing 4·8g soy protein and −22 mg total isoflavones) was given to SF- and CMF-fed infants (>6 months of age) and to children who had been fed with SF as infants but were no longer consuming soy. Equol excretion was detected in 19% of the SF infants and children but in only 5% of the CMF infants, whereas O-DMA excretion was similar in the two groups. By 3–7 years of age, the proportion of subjects excreting O-DMA and equol was similar in both groups. Therefore, the authors concluded that the bacterial organisms that confer the ability to convert daidzein to equol were not acquired until later childhood; however, there appeared to be no lasting effect of early-life isoflavone exposure on isoflavone metabolism.

In the same study, fecal bacteria numbers were detected by fluorescent in situ hybridization (FISH) with oligonucleotide probes. The numbers of bifidobacteria (*P < 0.001*), bacteroides, and clostridia (*P < 0.05*) were significantly lower for the SF group compared with the CMF group. However, there were no differences in fecal short-chain fatty acid concentrations, pH, NH₃ concentrations, or β-glucosidase or β-glucuronidase activities. Given the small number of subjects enrolled in this study, a larger scale investigation of the impact of SF on the fecal microbiota using metagenomic approaches is clearly needed.

**CONCLUSION**

Soy formulas are widely used around the world. Recently, pediatric societies have narrowed the indications for the use of SFs to the treatment of galactosemia or if the parent prefers a vegan diet for the infant. However, it appears that the decision to choose an SF rather than a CMF is often made by the parent rather than the healthcare provider. Thus, the level of SF use will likely con-
continue to exceed the clinical indications for it. SF contains ingredients that are not present in CMF, including isoflavones. The available literature does not support short- or long-term detrimental effects of early exposure to isoflavones.\textsuperscript{4,7–13,18,19,23} Emerging data suggest that soy fiber and soy isoflavones may have beneficial effects in the prevention and treatment of diarrheal diseases; however, more work is needed in this area. Lastly, given the appreciation of the role of early colonization in life-long health, there is a critical need to enhance our understanding of how SF influences development of the microbiota.

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