ABSTRACT
Soy formula (SF) has been fed to millions of infants worldwide. It has been shown to promote growth and development as well as milk-based formula (MF). Controversy has developed over the adequacy and safety of SF. Most concerns are based on in vivo and in vitro data that raise the possibility of estrogenic effects of isoflavones contained in SF. There are few studies of children who were fed SF, and thus insufficient data are available to judge if SF feeding results in clinically significant developmental effects and if there are any long-term health consequences (adverse or beneficial). However, the Arkansas Children’s Nutrition Center is conducting a prospective longitudinal study comparing growth, development, and health of breastfed children with formula-fed (SF and MF) children from birth through age 6 y. After 5 y of study, children in all 3 groups (n > 300) are growing and developing within normal limits, and there are no indications of adverse effects in the soy-fed children. Neonatal pig studies comparing SF, MF, and breast milk (BM) have shown diet-specific gene expression profiles in various target tissues. Therefore, although SF differed significantly from BM, MF also differed from BM, and SF differed from MF. Nonetheless, these animals grew and developed normally, and SF piglets had several health benefits (eg, increased bone quality) and no observable adverse effects. Thus, to date, our results suggest that SF supports normal growth and may have advantages in promoting bone development. Am J Clin Nutr 2009;89(suppl):1668S–72S.

INTRODUCTION
For years, the American Academy of Pediatrics (AAP) recommended breastfeeding for infants, and in cases in which breastfeeding was not possible, it recommended milk-based formula (MF) as a second choice and soy formula (SF) as a third choice (1). Recently, the AAP has refined their recommendation and established indications and contraindications for the use of SF; however, no position on whether soy foods are appropriate for older children has been established (2). However, commissions and expert panels in France, Israel, and the United Kingdom have recommended limited use of SF and soy foods for infants and children (discussed in reference 2). Furthermore, on the recommendation of an external review panel commissioned by the National Institute of Environmental Health Studies (NIEHS) (3, 4) to evaluate reproductive effects of genistein (a major isoflavone metabolite of soy foods) and SF, the NIEHS has proposed conducting clinical studies to investigate growth, body composition, reproductive development, and cognitive function of children fed SF (from birth through age 2 y). The current report discusses some of the many complex issues related to SF and the health implications of its use.

DISCUSSION
For reasons not well understood, over the past 3.5 decades Americans have fed SF to a large percentage of newborns. According to the AAP, SF accounts for ~20–25% of the formula market in the United States (2), which suggests that >20 million American infants have been fed SF since the 1970s, when modern SF began to be marketed. Because of rigorous industry and government standards for formulation of current infant formulas, no discernable or significant differences in growth, development, or health has been reported in infants fed SF compared with infants fed MF during the first year of life (5, 6). A longitudinal, prospective study (The Beginnings Study) is underway at the Arkansas Children’s Nutrition Center to compare growth, development, and health of breastfed or formula-fed (SF and MF) children. Preliminary data from The Beginnings Study support earlier published data and demonstrate that formula-fed children grow and develop within normal limits and that SF-fed infants essentially are indistinguishable from infants fed MF (7–9). However, in the past decade, questions have been raised by several physicians, scientists, and laypersons regarding potential long-term adverse health effects among adults who consumed SF as infants. These concerns have not been based on well-designed clinical studies in children but stem mainly from in vitro experiments or from animals injected or fed soy-associated phytochemicals rather than SF.

The primary issue of concern is the isoflavone content of the soy protein isolate (SPI) used as the sole protein source in infant formula sold in the United States. Isoflavones are only one class of >100 phytochemicals associated with soy protein, many of which are reported to have bioactivity under certain conditions (10, 11). A major theoretical concern about use of soy foods in infants and children is the potential for adverse effects on
development of the reproductive system and other hormone-sensitive tissues (3, 4). Thus, questions have been raised about potential adverse health effects on pubertal development, adult fertility, hormonally sensitive cancers (eg, breast, uterine, and endometrial cancers), and bone development (12–15).

At the heart of this concern is that soy isoflavones have been shown to bind and activate estrogen receptors (eg, ERα and ERβ) in vitro (16). The potency of isoflavones has been reported to be between 4 and 400,000 times lower than endogenous estrogens (eg, 17β-estradiol) depending on the biological system studied. The wide range of potencies is observed because the experimental conditions used to obtain these data vary enormously. In our opinion, a potency range of 1000–10,000 times less potency is considered to be the best estimate by most investigators familiar with soy isoflavone actions in cell culture and animal models and the cellular hormonal conditions in children, men, and women (17, 18).

However, 2 particularly complicating issues in the literature relate to scientists equating 1) isoflavones with endogenous estrogens and 2) the actions of extracted isoflavones with the actions of soy foods that contain isoflavones. Even though soy isoflavones can bind and activate ERα and ERβ, they do not behave like typical estrogen agonists but rather as selective ER modulators and, in addition, have many other actions that are ER independent, eg, tyrosine kinase inhibition (10, 12, 17, 18). It is unfortunate that soy isoflavones have been called “phytoestrogens,” because they are not estrogens and are not truly estrogenic at nutritionally relevant concentrations. The weak isoflavone potency for activating the ERs combined with competition with endogenous estrogens for the ERs make isoflavone-related ER activity minimal when fed in amounts similar to those found in SF, even when fed during early development (17–19). Moreover, although some studies have shown similar gene expression profiles for genistein (the major soy isoflavone) and estradiol in some tissues in vitro and in vivo (20–23), ingestion of soy foods results in a complex mixture containing hundreds of phytochemicals and peptides being introduced to the gastrointestinal tract, many of which are absorbed and have biological actions.

This situation is not unlike the mixture of phytochemicals found in a typical meal containing a mixed salad and vegetables. Gene expression profiles of uterus and mammary epithelial cells of female rats fed SPI, the sole protein source in American infant formulas, or fed an equivalent concentration of pure genistein differ significantly (24, 25). In addition, when comparing SPI feeding with estradiol treatment in ovariectomized female rats, only 4% of estradiol-regulated genes in liver also were affected by SPI feeding (19). Thus, SF consumption does not equate to estrogen exposure or being exposed to a single compound, eg, a purified isoflavone. Unfortunately, much of the literature that describes the effects of “soy” is not about soy foods or SF but details the effects of a single purified soy isoflavone, genistein. The biological effects of purified isoflavones are not the same as those of SF or other soy foods.

Nonetheless, there are 2 main and potentially problematic issues that are well documented in infants who are fed SF. The first is the high circulating total isoflavone concentrations of infants who are fed SF. These have been reported to be ≈ 1 μg/mL, which is 10 times greater than the mean serum concentrations reported for Japanese adult soy consumers and 1000 times greater than circulating estradiol levels of pregnant American women (26). However, >99% of circulating isoflavones exist as biologically inactive conjugated glucuronides and sulfates as the results of first-pass metabolism during absorption from the gastrointestinal tract and hepatic metabolism (27, 28). Relative to endogenous estrogens, the concentrations of bioactive isoflavone aglycones probably are only ≈ 10–50 times greater than adult concentrations of total estradiol. When the lower potency of isoflavones is considered relative to estradiol, the increased potential for estrogenic exposure is low (29). However, even taking into account the lower potency of soy isoflavones and the low percentage of bioactive circulating isoflavones, infants are exposed to relatively high isoflavone concentrations at a period of life when female estrogen exposure typically is low. Furthermore, male infants normally are exposed to even lower concentrations of estrogens, raising the possibility of adverse effects of isoflavones on estrogen-sensitive systems. Thus, concerns about potential estrogenic exposure during a particularly vulnerable period of human development have been raised.

The second issue relates to the lifetime isoflavone exposure profile. Soy foods, thus soy isoflavones, have been in the diet of some populations for centuries, and soy foods generally are accepted as being healthy. But the introduction of SF to infants is a relatively new phenomenon (<50 y), and little is known about its long-term health consequences. Populations that consume significant amounts of soy foods usually do so for their entire lives, except for the brief neonatal period of breastfeeding. This exposure pattern means that all or most cells within the body are likely to have at least some concentration of isoflavones or their metabolites. Thus, at conception, the eggs and sperm are likely to contain isoflavones, and because women continue to eat soy foods during pregnancy, the developing fetus is exposed to isoflavones. Therefore, children of women who are typical soy consumers are exposed to soy from conception through birth. Because the concentration of isoflavones is extremely low in breast milk (BM), isoflavone exposure of breastfed infants of soy-consuming mothers is low until the introduction of solid foods at age ≈ 5–6 mo. Because children of soy-consuming populations are exposed to soy foods, children ages ≈ 1 y and older have relatively high soy isoflavone exposure thereafter. Thus, the pattern of soy isoflavone exposure profile is well defined in these populations.

The isoflavone exposure pattern described above differs dramatically from that of typical Americans. Although it is true that soy food consumption is increasing in the United States, it still is low. At conception, it is highly unlikely that American men and women have measurable isoflavone concentrations in the sperm or eggs, respectively. In addition, few pregnant women in the United States consume soy foods in amounts that would expose their fetus to measurable isoflavone concentrations. However, infants fed SF are exposed to high isoflavone concentrations during the first year of life and once weaned to the typical American diet are not exposed further to soy isoflavones or, at the most, the exposure level is low. Thus, although there is a long health history of people who have the lifetime soy isoflavone exposure pattern typical of populations of high soy consumers (eg, some Southeast Asians), there is little information about the long-term health consequences of people who consume soy isoflavones with the lifetime pattern of typical American infants fed SF.

Even with the large differences in isoflavone exposure patterns and “dose” described above, there is no published research in children or adults that there are adverse health effects of feeding SF to infants. As far as we are aware, there are no peer-reviewed publications documenting adverse effects in infants fed SF or in...
older children or adults who were fed SF as infants. So, what is the scientific basis on which countries, eg, France, Israel, and the United Kingdom, have recommended restricting use of SF to infants or soy foods to older children? The evidence is based primarily on cell culture data and animal studies (17, 24, 29). Most of these data have little or no relevance to health effects of SF, because they were derived from experiments that do not model the human condition. These studies used purified isoflavones, rather than soy foods actually consumed by human infants, or used animal models, eg, rodents, that did not allow neonatal exposure to SF and in which it was not practical to feed SF to neonates. There was one study, however, that had a large impact on the European decisions to limit SF feeding to children. That study came from the laboratory of Richard Sharpe in which twin marmoset male monkeys were fed SF or MF. The monkeys fed SF had decreased serum testosterone concentrations and increased Leydig’s cell numbers at the end of formula feeding and had larger testes and lower serum testosterone concentrations as adults (30). However, these animals went through normal puberty and were fertile as adults (31). On the surface, this seems like a powerful study, because it is in a primate model and the infants were fed formulas similar to those consumed by human infants and during the neonatal period. This study overcame the major objections to previous animal studies that used purified isoflavones and did not study the appropriate neonatal period or did so by injecting the isoflavones rather than feeding SF. It was clear that France and the United Kingdom, with a lack of data in human subjects to suggest a health concern, relied heavily on this study to arrive at their recommendations to limit SF consumption in infants.

The NIEHS and the National Toxicology Program commissioned a panel to evaluate the available data on genistein and SF on human reproduction. Few data were presented on human subjects fed SF. The NIEHS relied on animal and in vitro data from poorly conceived experimental models to make their conclusions. They, like the European groups, relied heavily on the Sharpe study in marmosets. However, although this appeared to be a carefully conducted study, it has little applicability to human infants who consume SF. Monkeys differ from human subjects in many aspects, and infant marmosets metabolize isoflavones differently from human infants. Infant monkeys convert most of their ingested daidzein (1 of the 2 major soy isoflavones) to the highly estrogenic isoflavone metabolite equol (29). Furthermore, equol is shown to drive conversion of testosterone to dihydrotestosterone, which lowers serum testosterone concentrations (32). Human infants do not produce equol and there are no reports of enlarged testes in infants fed SF (29). Studies in the neonatal pig model human growth, development, metabolism, and endocrine systems; like human infants, piglets do not produce equol (29). When piglets were fed MF or SF or were fed by a sow from 48 h through 21 d, testicular weights did not differ between diet groups (Table 1). These data indicate that testicular development does not differ between soy-fed piglets and sow-fed (ie, breastfed) or MF-fed piglets. However, feeding SF to piglets resulted in tibia bone that contained greater trabecular bone mineral density, total mineral content, and cortical thickness (P < 0.05) than sow-fed piglets as measured by peripheral quantitative computerized tomography (Table 2). Together, these data suggest that an appropriate animal model for studies of SF cannot support health concerns previously raised by primate studies or by in vitro and in vivo experiments of purified soy isoflavones.

**TABLE 1**

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<thead>
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<th>BM</th>
<th>MF</th>
<th>SF</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
<td>6.18 ± 0.29</td>
<td>5.55 ± 0.41</td>
<td>5.86 ± 0.29</td>
</tr>
<tr>
<td>Testicular weight (g)</td>
<td>10.9 ± 1.3</td>
<td>9.0 ± 1.6</td>
<td>9.7 ± 1.7</td>
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\(^1\) Values are means ± SEMs. Four male piglets/group (age 21 d). There were no significant differences between the groups.

It also should be noted that in the only study to follow up adults who were fed SF as infants, no adverse reproductive effects were found and the authors concluded that there was no evidence for long-term adverse health outcomes (33). However, although this study was conducted carefully, the numbers of subjects were small and the participants were only in their mid-30s, so it was not possible to determine if there were any longer-term health effects. A study by Dolinoy et al in agouti mice fed the soy isoflavone, genistein, was cited by the panel (mentioned above) reviewing for the NIEHS (34). The results from that study demonstrated that purified genistein shifted global DNA methylation profile and resulted in phenotype changes believed to be caused by epigenetic regulation of the agouti locus. The authors suggested that this might occur in infants fed SF. This was used as documentation of a potentially persistent negative effect of SF, even though the study did not model the human condition. Although genistein is a metabolite of phytochemicals associated with the SPI used to make SF, the effects of purified genistein added to the diet are not equal to the effects of feeding soy foods or SF (24, 25). When a similar study in agouti mice was conducted using the same amount of genistein, except provided as SPI rather than purified genistein, these phenotype effects did not occur and there were no epigenetic effects (35). Although this result was ignored by the NIEHS panel, it is important and points out that just because soy foods have isoflavones does not mean that these isoflavones behave the same as isoflavones purified from soy foods.

**CONCLUSIONS**

Feeding SF to infants is shown to be efficacious for normal growth and development. However, feeding SF introduces the developing body to phytochemicals not present in BM or MF. Although many people are concerned about adverse effects of these phytochemicals, this concern is not supported by convincing data in children, adults, or appropriate animal models in which soy foods (including SF) were studied. The translational research from the

**TABLE 2**

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<tr>
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<th>BM</th>
<th>MF</th>
<th>SF</th>
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<tbody>
<tr>
<td>Total BMD (mg/cm²)</td>
<td>198.01 ± 10.0</td>
<td>209.8 ± 12.1</td>
<td>213.4 ± 9.05</td>
</tr>
<tr>
<td>Trabecular BMD (mg/cm²)</td>
<td>108.7 ± 3.3b</td>
<td>112.7 ± 7.5ab</td>
<td>116.7 ± 4.6b</td>
</tr>
<tr>
<td>Cortical BMD (mg/cm²)</td>
<td>413.6 ± 7.6</td>
<td>420.1 ± 13.5</td>
<td>412.5 ± 6.8</td>
</tr>
<tr>
<td>Total mineral content (mg)</td>
<td>91.1 ± 6.4</td>
<td>94.5 ± 5.5</td>
<td>97.1 ± 4.8</td>
</tr>
<tr>
<td>Cortical thickness (cm)</td>
<td>0.65 ± 0.10b</td>
<td>0.86 ± 0.26b</td>
<td>0.96 ± 0.18b</td>
</tr>
</tbody>
</table>

\(^1\) Values are means ± SEMs. Four male and 4 female piglets/group (age 21 d). BMD, bone mineral density. Values with different superscript letters differ significantly, P < 0.05.
Arkansas Children’s Nutrition Center supports the notion that early exposure to soy foods, including SF, actually may provide health benefits rather than adverse effects, eg, improved body and bone composition and prevention of breast cancer (8, 9, 36). However, our data from children in The Beginnings Study are considered preliminary, and definitive results await completion of that study. (Other articles in this supplement to the Journal include references 37–63.)

The authors’ responsibilities were as follows—TM: lead scientist for the study and contributed to the study design, data analyses, and writing of the manuscript; JMG: provided oversight and contributed to the data collection and writing of the manuscript; RTP, AA, KS, and JRC: contributed to the data collection and analysis and to writing the manuscript; and MRR: provided significant advice and contributed to the data analysis and to writing the manuscript. TMB is a member of the Scientific Advisory Board of the Soy Nutrition Institute. The authors had no conflicts of interest.

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