Ultrasonographic Patterns of Reproductive Organs in Infants Fed Soy Formula: Comparisons to Infants Fed Breast Milk and Milk Formula

Janet M. Gilchrist, PhD, RD, Mary Beth Moore, MD, MS, Aline Andres, PhD, Judy A. Estroff, MD, and Thomas M. Badger, PhD

**Objective** To determine if differences exist in hormone-sensitive organ size between infants who were fed soy formula (SF), milk formula (MF), or breast milk (BF).

**Study design** Breast buds, uterus, ovaries, prostate, and testicular volumes were assessed by ultrasonography in 40 BF, 41 MF, and 39 SF infants at age 4 months.

**Results** There were no significant feeding group effects in anthropometric or body composition. Among girls, there were no feeding group differences in breast bud or uterine volume. MF infants had greater \( P < .05 \) mean ovarian volume and greater \( P < .01 \) numbers of ovarian cysts per ovary than did BF infants. Among boys, there were no feeding group differences in prostate or breast bud volumes. Mean testicular volume did not differ between SF and MF boys, but both formula-fed groups had lower volumes than BF infants.

**Conclusions** Our data do not support major diet-related differences in reproductive organ size as measured by ultrasound in infants at age 4 months, although there is some evidence that ovarian development may be advanced in MF-fed infants and that testicular development may be slower in both MF and SF infants as compared with BF. There was no evidence that feeding SF exerts any estrogenic effects on reproductive organs studied. (J Pediatr 2010;156:215-20).

See related articles, p 209 and p 221

Estimates suggest that soy-based infant formulas (SF) are fed to 20% to 25% of all formula-fed infants in the United States at some point during the first year of life,\(^1,2\) amounting to more than 1 million infants each year. Concerns have been raised about potential adverse consequences of SF related to estrogenic effects of isoflavones associated with the soy protein isolate used as their sole protein source.\(^2\) Similar reports have appeared in the lay press and on Internet web sites.

Although the American Academy of Pediatrics (AAP) recommends breast-feeding (BF) rather than formula feeding and cow’s milk formula (MF) over SF, it does not find any adverse effects of SF.\(^3\) The AAP position is essentially that milk formula, although not as good as human breast milk, has been in use for decades, has been reformulated several times to meet nutrient and safety requirements, and has a long track record of safety and efficacy. Therefore, the AAP does not see an advantage of SF over milk formula for general use and recommends SF only for a few medical indications (infants with galactosemia, hereditary lactase deficiency, and secondary lactose intolerance) as well as in situations in which a vegetarian diet is preferred. This stance is prudent in view of international controversy over SF, and it provides the middle ground of “error on the side of safety without appropriate data in children.” This is also the standpoint taken by Australia, Canada, France, Ireland, New Zealand, Switzerland, and the United Kingdom, where SF use is limited because of concerns about potential estrogenic effects of soy isoflavones.\(^3,4\) One clear message from the AAP is that breast feeding is the gold standard against which MF is compared, and MF is the standard against which SF is compared.

A recent report of an expert panel selected by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (NTP-CERHR) concluded that “There are insufficient human or experimental animal data available to permit a determination of the developmental or reproductive toxicity of soy infant formula.”\(^5\) Several studies in rodents have shown that purified isoflavones added to the diet will affect development of reproductive tissues, including the mammary gland and uterus.\(^5-9\) However, very few studies have evaluated the potential effects of SF on human reproduction, and data from those reports are difficult to interpret or extrapolate to human infants.\(^10-12\) Two recent studies in human infants that assessed breast development were based on physical examination by palpation with comparison to a standardized object (beads/coin) for assessing breast size.\(^11,12\) Both studies suggest minor SF effects on breast tissue, but there were multiple limitations, including examiners were not blinded to infant feeding.
Participants for this study were 4 months old and were drawn from an ongoing, longitudinal cohort, the Beginnings Study (www.clinicaltrials.gov, ID # NCT00616395). Mothers had a healthy pregnancy, with no diagnoses (eg, diabetes, pre-eclampsia) or medications (eg, SSRIs, thyroid replacement) thought to affect infant growth or development. All mothers were non-smokers and denied alcohol use during pregnancy. In addition, mothers reported no use of any soy products or other estrogenic compounds during pregnancy and/or lactation. Infants were term (>38 weeks), had an appropriate-for-gestational age birth weight (6 to 9 pounds), and had no medical diagnoses or medications thought to affect growth or development. Parents chose the feeding method for their child, that is, BF, MF, or SF. All BF infants were fed breast milk exclusively for 4 months. Forty-four percent of the MF infants were exclusively fed MF from birth, 41% switched from BF to MF within 4 weeks, and 5% switched between 4 and 8 weeks. Of the SF infants, 23% were exclusively fed SF from birth, 45% were switched to exclusive SF feeding within 4 weeks, and 32% switched between 4 and 8 weeks. Parents also chose the formula and used Enfamil Prosobee Lipil (soy formula) (Mead Johnson & Johnson and Co., Evansville, Indiana), Enfamil Advance (milk formula) (Mead Johnson & Johnson and Co.), Similac Isomil Advance (soy formula) (Ross Products Division, Abbott Laboratories, Columbus, Ohio), or Similac Isomil (milk formula) (Ross Products Division). Formulas were provided as concentrates, and the use of each brand name was approximately equal across respective groups. The study was approved by the Institutional Review Board of the University of Arkansas for Medical Sciences, and informed consent was obtained from parents.

For the 24 months starting in November 2005, anthropometric measures (weight, length, and head circumference) were obtained from 4-month-old participants using standardized methods, and body composition was assessed by air displacement phlethysmography (ADP, PeaPod, Life Measurement, Inc., Concord, California). Participants who consented to an ultrasound assessment were studied in the approximate order as they were recruited, and participants entered each diet group at approximately the same rate. This resulted in 18 to 23 boys and girls per diet group who were evaluated in the Radiology Department of the Arkansas Children’s Hospital by registered diagnostic medical sonographers using a sonograms protocol established during a pilot study of infant feeding.13,14 Sonograms were obtained using an ACUSON Sequoia unit (Siemens Medical Solutions USA, Inc., Malvern, Pennsylvania) and the following transducers: high-frequency 8.0-MHz linear array for breast buds; 8.0-MHz vector for prostate, ovaries, and uterus; 15-MHz high-frequency linear array for testicles. Images were stored in the hospital’s PAACS system (DR Systems, Inc., San Diego, California) and were reviewed and measured by one Board Certified Radiologist (M.B.M.). The sonographers and the radiologist were blinded to participant treatment group. All organs were measured in triplicate and in 3 orthogonal planes: sagittal (SAG), transverse (TRV), and anteroposterior (AP). For paired organs (ie, breast buds, ovaries, and testes), measures were obtained in triplicate on each side. An average of the 3 values was calculated for each of the 3 views.13,14

Uterus length (SAG) was measured to include the fundus and cervix; the AP diameter was measured within the fundus or midportion. Uterine characterizations were “pear-shaped” (fundus smaller than cervix), “cylindrical” (fundus equal to cervix), or “heart-shaped” (fundus larger than cervix). The presence or absence of a visible endometrial stripe was noted. During measurement of the ovaries, all identified follicles/cysts were measured. Macrocysts were defined as cysts >1.0 cm. During measurement of the testes, the position of each testicle was noted as inguinal or scrotal.

Breast volume was calculated as SAG * TRV * AP. Volumes of prostate, uterus, and ovaries were calculated using the formula for a prolate ellipse: 0.523 * SAG * TRV * AP. Testicular volume was calculated using the empiric formula of Lambert15: SAG * TRV * AP * 0.71. Ovarian and testicular volumes were calculated as the mean values for the right and left organs.

All values in the Tables and Figure are given as means ± SEM unless otherwise stated. Ultrasonographic data are plotted against body weight, and the Student t test was used to test for differences between MF-fed and BF-fed infants and between SF-fed and MF-fed infants to test the 2 hypotheses established before conducting the study. It should also be noted that 2-way ANOVA using feeding group and sex as the groups was also performed with SigmaStat (version 3.5, Systat Software, Inc., San Jose, California), and 2-way interactions were considered with comparisons of nominal category organ characteristics computed with a χ² statistic. A t test and ANOVA detected the same treatment effects on organs, but in view of the hypotheses being tested, we report here P values from t tests only.

No statistically significant group differences were observed for socioeconomic status, race, and maternal anthropometrics (data not shown). Although serum isoflavones were not obtained in the participants of this report, mean (± SD) genistein concentrations of 3-month-old infants collected during the same time period ranged from 0.59 ± 0.3 to
lished age-appropriate reference norms, however, minor feeding group differences were observed within these normal values. This raises questions about what magnitude of difference would be clinically significant or biologically relevant, especially in light of the relatively high interindividual variability observed. This cannot be answered by this small clinical study and will require large numbers of study participants and a longitudinal study to determine if any early observations reflect permanent or transient conditions and/or if they result in beneficial or adverse health effects.

This issue of what constitutes an appropriate control for formulas is important. In the present report, we presented...
comparisons for all 3 diet groups. However, based on the AAP statement of May 2008 that “human milk is the ideal source of nutrition for infant feeding” and the AAP recommendations that breast-feeding be considered the standard for infant feeding followed by milk-based infant formula, the most appropriate comparisons may be between BF and MF and between MF and SF. MF formula varied \( (P < 0.05) \) from BF on 3 measures: ovarian volume, numbers of cysts per ovary, and testicular size. Thus, although MF has a long track record as safe and efficacious, it may have developmental effects that differ from breast milk. On the other hand, the organ volumes of SF infants were the same as BF and/or MF. In no case did we observe a difference between infants fed BF and SF that was not also reflected in MF infants. This suggests that SF tracks with either breast milk or milk formula with respect to all the effects presented in this study: growth, development, body composition, and ultrasonically determined volumes of reproductive organs under the conditions of this study. Although we conducted this study in infants at age 4 months, the period of greatest isoflavone exposure for SF infants and an age at which it has been hypothesized that isoflavones would exert biological activity in infants, no evidence of estrogenic effects of SF on reproductive organs was found.

Our results differ from 2 previous reports in human infants that used a physical examination to evaluate the effects of SF on breast development. Even though we observed a sex difference in the volume of breast buds, with girls having larger volumes than boys, we do not have an explanation for this difference. This is in contrast to Bernbaum et al, who found no sex difference in diameter of breast buds of infants by comparing the physical feel of breast buds with a custom set of beads. Their technique would be comparable with the clinical use of Prader orchidometer beads for assessment of testicular size, which has been shown to be significantly less accurate than ultrasound assessment when compared with volume measurement of excised organs. Using similar methods, female SF infants were reported to have a higher prevalence of breast buds at age 2 years than female infants who were fed BF or MF. In that study, breast buds were designated to be present if a mass had a diameter \( > 1.5 \) cm. Investigators were not blinded to treatment groups in either of these studies. Although we were able to assess the presence of breast buds of each child in our study by using ultrasound, none of the infants in our study had a breast bud diameter \( > 1.5 \) cm, and no feeding group differences were detected in either boys or girls.

The organs examined in the current study have been demonstrated to be sensitive to estrogen exposure. Normative ultrasound data of uterine volumes conducted among prepubertal girls from birth to age 15 years suggest that exposure to maternal/placental estrogen in utero results in uterine enlargement at birth, which quickly resolves over the first month of life and remains low until near the onset of puberty. However, Trotter et al demonstrated that uterine width, depth, and length could be increased postnatally during a double-blinded, placebo-controlled study of estrogen and progesterone replacement among premature female infants. Furthermore, these treatment effects persisted after the therapy was discontinued. Estrogen exposure also results in a “heart-shaped” uterus, in which the fundus is larger than the cervix and in the presence of an endometrial echo, which can be observed on sonography. We observed no feeding group differences in uterine length, volume, shape, or prevalence of an endometrial stripe. All of our measurements were within published reference values for healthy normal infants.

Two groups have previously reported that although ovarian size increases with pubertal stage, the uterus begins to increase in size sooner, suggesting that it may be a more sensitive indicator of hormonal exposure. Similar to previous reports, we observed a high prevalence of ovarian follicles per cyst (average 86%) among our sample of healthy neonates. This did not differ by feeding group, nor did the average size of follicles per cyst or presence of macrocysts \( > 1 \) cm. However, we did observe larger ovarian volume and more cysts per ovary among female MF infants compared with BF infants. Similar to what we have observed in our animal experiments (unpublished results), there were no statistical differences in ovarian volume between the two formula groups (MF vs SF) or between SF and BF groups. The reason for the difference between MF and BF is unclear but probably does not represent an estrogenic effect.

Among boys, we observed no feeding group differences in prostate volume, which is androgen-dependent. All of our volumes were within normative data for boys from 0 to 10 years of age. This is similar to what has previously been reported in animal models of soy exposure, in which no effect of SF has been observed on prostate weight. Although there were no differences in testicular volume between SF and MF boys, both formula groups were significantly lower than BF and were within published normative ranges. These results are in direct contradiction to what was observed.

<table>
<thead>
<tr>
<th>Table II. Uterine characteristics by feeding group</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="https://example.com/tableII.png" alt="image" /></td>
</tr>
</tbody>
</table>

*Uterine images from 4 MF and 1 SF infants were not used because of artifacts.*

<table>
<thead>
<tr>
<th>Table III. Characteristics of ovarian cysts by feeding group</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="https://example.com/tableIII.png" alt="image" /></td>
</tr>
</tbody>
</table>

*Ovarian images from 1 SF and 4 MF infants were not used because of artifacts.  
† \( P < .01 \) compared with BF. **The organs examined in the current study have been demonstrated to be sensitive to estrogen exposure. Normative ultrasound data of uterine volumes conducted among prepubertal girls from birth to age 15 years suggest that exposure to maternal/placental estrogen in utero results in uterine enlargement at birth, which quickly resolves over the first month of life and remains low until near the onset of puberty.**
by Tan et al\textsuperscript{28} who reported an increase in testicular weight and elevated Sertoli and Leydig cell counts among SF-fed male marmoset monkeys compared with their MF-fed male twins. This disparity may be due to the high levels of equol in infant monkeys.\textsuperscript{30} Ultrasound-derived reference data for testicular volume among boys from 0 to 6 years suggest that testicular volume increases in the first 5 months of life and then reaches a prepubertal low at around age 9 months. The volume increase coincides with a period of “mini puberty” resulting from gonadotrophic hormones reaching a peak at age 3 to 4 months.\textsuperscript{18}

There are some limitations to the present study design. One limitation is that we conducted sonographic assessments at only one age. In light of observed feeding group differences, it would be beneficial to have measurements of the same children throughout the first 6 to 9 months of life to see if there is an actual difference in maximum testicular volume or simply a shift in the growth curve due to timing of the testosterone surge. Another limitation is the length of SF exposure, which varied between 2 and 4 months. However, this SF exposure reflects the exposure pattern of infants in the United States, as most infants start SF after having problems with BF or MF. Furthermore, the period between ages 2 and 4 months results in the highest circulating concentrations of the potential estrogenic isoflavones. Therefore, infants start complementary foods, and the isoflavone levels begin to decline and the potential effects would be expected to lessen. Thus, if SF effects were to occur, we would expect that the infant at age 4 months would have the most significant effects.

We tentatively conclude that anthropometric development and body composition do not differ between formula-fed and breast-fed infants at age 4 months. Our data do not support major diet-related differences in reproductive organ size as measured by ultrasound in infants at this age. However, there is some evidence that ovarian development may be advanced in MF-fed infants compared with BF and SF infants. Furthermore, there was some evidence that formula-fed infants may have smaller testes and that the numbers of ovarian cysts per ovary are greater than in BF infants. There is no evidence from the current study that feeding soy formula exerts any recognizable estrogenic effects on reproductive organs studied. Last, the numbers of infants per diet group were small and thus the data should be interpreted with caution.

References


50 Years Ago in THE JOURNAL OF PEDIATRICS

Anticipatory Guidance in Pediatric Practice
Hill LF. J Pediatr 1960;56:299-307

In 1960 pediatricians were first and foremost infectious disease specialists. *Haemophilus influenzae* meningitis and epiglottitis, *Streptococcus pneumoniae* meningitis and pneumonia, *Neisseria meningitides* disease, newborn sepsis, and other life- and limb-threatening infectious diseases were the battleground that were daily fought by our colleagues. The articles published in pediatric journals at that time were concerned with these infectious diseases, the treatment of premature infants, and trauma. Hill’s prescient discussion about anticipatory guidance is an early look at what has become the main focus of primary care pediatrics in the 21st century. The conquering of the deadly infectious diseases with the *Haemophilus influenzae* type B, pneumococcal, and meningococcal vaccines, as well as our older routine viral and bacterial immunizations, has altered the primary role of the pediatrician from an interventionist to a specialist in prevention. In his discussion, Hill reviews the state of what he, for the first time, labeled “anticipatory guidance.” From normal infantile crying, through development with stranger anxiety, body curiosity, eating patterns, temper tantrums, toilet training, and home safety, his article presages many of the anticipatory guidance issues that primary care providers currently discuss. The need for adolescent independence and discussions about puberty, sexual activity, and sexually transmitted diseases were all discussed 50 years ago in *The Journal*.

The American Academy of Pediatrics along with the Health Resources and Services Administration and other state and federal groups has developed and promoted the “Bright Futures” endeavor. Within its mission lie the core anticipatory guidance issues recommended for pediatric practitioners to discuss at routine visits. Hill’s issues will be found prominently within the pages, albeit in more detail. The list of discussion points within the program have progressed far beyond Hill’s dreams and include issues that were unknown at his time. From sleeping position and sudden infant death syndrome to sexual identity and HIV prevention, from physical punishment to domestic violence, from foreign adoption issues to gang activity, gun safety and conduct disorder, from seatbelts to car seats, pediatricians must discuss a myriad of topics that are critically important to the healthy outcomes of our children. Although the list is longer, although many of the topics are newer and perhaps less comfortable to discuss, although the focus of the pediatric visit may have changed, Hill accurately and presciently described the importance of leading our parents and children through the labyrinth of development toward a healthy adulthood.

Eugene R. Hershorin, MD
Division of General Pediatrics
Department of Pediatrics
University of Miami Miller School of Medicine
Miami, Florida
10.1016/j.jpeds.2009.09.038