Compared with Feeding Infants Breast Milk or Cow-Milk Formula, Soy Formula Feeding Does Not Affect Subsequent Reproductive Organ Size at 5 Years of Age $^{1,2}$

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Abstract

Background: Literature reports suggest that phytochemicals, such as isoflavones found in soybeans, impair reproductive function in animals and raise the possibility that consuming soy infant formula could alter hormonally sensitive organ development in children.

Objective: This study compared reproductive organs volumes and structural characteristics in children at age 5 y who were enrolled in the Beginnings study long-term cohort.

Methods: Breast bud, uterus, ovaries, prostate, and testes volumes and characteristics were assessed by ultrasonography in 101 children (50 boys and 51 girls) aged 5 y who were breastfed ($n=35$) or fed cow-milk formula ($n=32$) or soy formula ($n=34$) as infants. Analyses were adjusted for race, gestational age, and birth weight.

Results: Among girls, no significant differences were found in breast bud, ovarian, or uterine volumes; counts of ovaries with cysts; ovarian cysts numbers; ovarian cyst size; and uterine shape between the diet groups. Among boys, no significant differences were found in breast bud, testes, or prostate volumes or structural characteristics between the diet groups.

Conclusions: In this cohort, no early infant feeding effects were found on reproductive organs volumes and structural characteristics in children age 5 y. The follow-up of these children through puberty is planned and should help delineate potential early infant feeding effect on reproductive function later in life. This trial was registered at clinicaltrials.gov as NCT00616395.


Keywords: soy, infant formula, reproductive organs, breastfeeding, infant feeding, breast bud, uterus, ovaries, prostate, testes

Introduction

The American Academy of Pediatrics recommends breastfeeding as the ideal source of nutrition for infants (1). Although 76.5% of US infants were breastfed as newborns in 2009, this percentage dropped sharply in the first week, and only 16.2% were exclusively breastfed until age 6 mo (2). Rates of exclusive breastfeeding until age 6 mo were even lower in Arkansas (11.8%, 2007) (2). Alternatives to breastfeeding include the use of cow-milk–based formulas (MFs) as a first choice and soy-based formulas (SFs) as a second choice (1). Approximately 20% of formula-fed (FF) infants in the United States are fed SF during their first year of life (1, 3). Understanding the potential benefits or adverse effects of these early diets is important to optimize nutritional status, promote health, and prevent diseases later in life. Growth and development of SF-fed infants are shown to be similar to MF-fed infants. Several studies have suggested beneficial effects of isoflavones in the prevention and/or treatment of hormone-dependent diseases, including cancer, osteoporosis, and cardiovascular disease (4, 5). Nevertheless, concerns have been raised about the isoflavone content of SFs (6). Infants fed SF consume higher concentrations of isoflavones (6–11 mg/kg per day) compared with negligible concentrations in breastfed infants (<0.01 mg/kg per day), resulting in serum and urinary isoflavone concentrations in the range of 0.4–1.5 μmol/L (4, 7–9). These isoflavones can bind and activate estrogen receptors $\alpha$ and $\beta$, raising the possibility of potential estrogenic effects (10). In its 2011 report, the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction Expert Panel raised the concern level on the developmental toxicity of soy infant
formula from negligible to minimal concern for adverse developmental effects in infants fed SF (11). Numerous studies have indeed evaluated the effects of SF on growth (weight, length, head circumference) compared with MF or breastfeeding (12–19), but few studies have investigated the potential effects of early infant feeding on reproductive organs development during childhood in the clinical population. Results from clinical studies with menarche as an outcome variable are mixed, with reports of precocious or late menarche (20, 21). A prospective cohort of 33,501 US and Puerto Rican women (Sister Study) identified that SF feeding was associated with both very early (10 y) and late menarche (14 and 15 y) (20). Another longitudinal study of 2920 girls (ALSPAC; Avon Longitudinal Study of Parents and Children) demonstrated earlier menarche in girls fed SF as infants (12.4 y compared with 12.8 y in the study sample) (21). In a cross-sectional study, researchers found no effect of SF on breast adipose tissue, breast bud, and testes volume and observed breast and genital development or vaginal discharge (22). The single reported effect was a trend (P = 0.07) toward higher maturation index in vaginal wall cells of girls in the SF group. We have previously reported no effect of SF on reproductive organ volumes and structural characteristics at age 4 mo in the Beginnings study long-term cohort (19). To our knowledge, no reports of specific developmental landmarks in reproductive organ development between ages 4 mo and the onset of puberty are found. However, it is clear that early exposure to bioactive exogenous and endogenous compounds (such as estrogens) can affect the course of reproductive organ development, and this may take years to become apparent. Thus, the objective of the present study was to compare breast bud, ovaries, uterus, testes, and prostate volumes and structural characteristics at age 5 y in children who were breastfed or fed MF or SF as infants. Age 5 y was chosen as a midpoint between age 4 mo and the onset of puberty, because very early puberty onset was identified as age 10 or younger.

On the basis of previously published data, we hypothesized that these organ volumes and structural characteristics would not differ between feeding groups.

Methods

Participants. Participants were infants enrolled in the ongoing longitudinal cohort, the Beginnings study (registered at clinicaltrials.gov as NCT00616395) who were recruited from the Central Arkansas region between ages 1 and 2 mo. Pregnancies were reported uncomplicated with no medical diagnoses or medications known to affect fetal or infant growth and development. All mothers were nonsmokers, who denied alcohol use during pregnancy. Infants were full term (>37 wk), weighed between 2.7 kg (6 pounds) and 4.1 kg (9 pounds) at birth, had no medical diagnoses, or had not been administered medications known to affect growth or development. Gestational length, race, birth weight, and birth length were self-reported. During the study period (2010–2014), 150 participants were eligible to complete a sonographic assessment, and 101 (67%) parents agreed to the additional assessment. The study was approved by the institutional review board of the University of Arkansas for Medical Sciences, and written consent was obtained from each participant’s legal guardian.

Infant Diet. Parents made decisions about which diet to feed their infant before enrolling in the study. All FF infants remained on their selected formula until age 12 mo; that is, FF participants did not change feeding group during the study period. FF infants were either on MF or SF. MF-fed infants started their formula at approximately age 2.2 ± 0.7 wk; whereas SF-fed infants started their formula at approximately age 3.2 ± 2.7 wk. Among the breastfed participants included in this analyses, 89% (n = 31) were exclusively breastfed until age 9 mo and 57% (n = 20) were still exclusively breastfed by age 12 mo.

Anthropometrics. Anthropometric measures were obtained at age 5 y ± 2 wk using standardized methods. Child height was measured to the nearest 0.1 cm using a stadiometer. Child weight was measured to the nearest 0.1 kg on a Tanita BWB-800 scale (Tanita Corporation of America, Inc.).

Sonograms. Sonogram measurements were performed at the Radiology Department of the Arkansas Children’s Hospital by registered diagnostic medical sonographers. Sonograms were obtained with either an Acuson Sequoia unit (Siemens Medical Solutions USA, Inc.) with 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 or a Philips IU22 unit with an 8- to 5-MHz curved array for prostate, a 5-MHz curved array for ovaries and uterus, a 9- to 3-MHz linear array for breast, and linear 17–5 MHz for testicles. Images were stored in the hospital’s picture archiving and communication system (DR Systems, Inc.) and were reviewed and measured by 2 board-certified radiologists (MBM and LEL). The sonographers and radiologists were blinded to participant treatment group. Children were placed on a padded examination table in the supine position. All organs were measured in triplicate and in 3 orthogonal planes: sagittal, transverse, and anteroposterior. For paired organs (i.e., breast buds, ovaries, and testes), measurements were obtained in triplicate on each side. An average of the 3 values was calculated for each of the 3 views. Uterus length (sagittal) was measured to include the fundus and cervix; the anteroposterior diameter was measured within the fundus or midportio. 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. During measurement of the testes, the position of each testicle was noted as scrotal or undescended. Parents whose child presented with undescended testes at the sonographic assessment were recommended to follow up with their primary care physician. The final diagnosis and any history of testicular surgical procedures were recorded. Breast volume was calculated as sagittal × transverse × anteroposterior. Volumes of prostate, uterus, and ovaries were calculated using the following formula for a prolate ellipse: 0.523 × sagittal × transverse × anteroposterior. Testes volume was calculated with the following empiric formula of Lambert (23): sagittal × transverse × anteroposterior × 0.71. Ovarian and testes volumes were calculated as the mean values for the right and left organ. All organ volumes were standardized by body weight and expressed as mm³/kg.

Statistical Analysis. Children’s characteristics measured in the interval scale are summarized as means ± SDs and were compared across feeding groups using the Kruskal-Wallis rank test. Children’s characteristics measured in the nominal scale are summarized as counts and percentages and were compared across feeding groups using Fisher’s exact test. All models that compared outcomes across feeding groups were adjusted for race, gestational age, and birth weight. Breast, ovarian, testicle, uterine, and prostate volumes and uterine length were compared across feeding groups using median regression. The numbers of ovaries with cysts were summarized as counts and percentages and were compared across feeding groups using negative binomial regression with robust variance estimators to account for the repeated measurements within participants. The number of cysts per ovary and ovarian cyst size are summarized as medians and IQRs and were compared across feeding groups using cluster median regression with robust variance estimators to account for the repeated measurements within participants. The IQR, also called the midspread, is a measure of statistical dispersion. It is the difference between the 3rd quartile (75%) and the 1st quartile (25%) of the observed values. Outcomes measured in the nominal scale (uterus shape, presence of endometrial stripes, and testes position) are summarized as counts and percentages. Uterus shape

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was compared across feeding groups using multinomial logistic regression; whereas the presence of endometrial stripes and testes position were compared across feeding groups using logistic regression. A priori, statistical significance was set at the 5% level. Statistical analyses were performed with the Stata statistical package (version 13.0; Stata Corporation).

**Results**

Among the 101 participants, 50 boys (17 breastfed, 14 fed MF, and 19 fed SF) and 51 girls (18 breastfed, 18 fed MF, and fed 15 SF) underwent a sonographic assessment at age 5 y. The majority of children were Caucasian (89.1%; Table 1). A significant difference was found in the race distribution. The numbers of African Americans in the SF group was higher (23.5%) than in the MF group (3.1%) and breastfed group (0%). No significant group differences were found in birth weight, birth length, or gestational age. Participants did not differ in age at examination and had similar weight and height at age 5 y.

Three data points were missing from the sonographic assessments. The transverse image quality of the right ovary for a girl participant was poor, so the right ovary measurement was excluded for the analyses. One boy participant refused the scrotal assessment; thus the testes and prostate were not imaged. Finally, one child (a boy) had invalid body weight data due to a cast on his leg at the time of the assessment, so the adjusted organ volumes were not computed.

Breast bud volumes were not significantly different between groups in girls ($P = 0.79$; Figure 1A) or boys ($P = 0.55$; Figure 1B). A total of 101 ovaries were imaged. No significant group differences were found in ovarian volumes ($P = 0.75$; Figure 1C), counts of ovaries with cysts ($P = 0.75$), ovarian cyst numbers ($P = 0.63$), or ovarian cyst size ($P = 0.42$; Table 2). Fifty-one uteri were imaged. No significant group differences were found in uterine volume ($P = 0.64$; Figure 1E), uterine length ($P = 0.52$), the presence of a visible endometrial stripe ($P = 0.45$), or uterine shape (heart vs. cylindrical vs. pear; $P = 0.57$; Table 2). No significant differences were found in testes volumes ($P = 0.11$; Figure 1D) or testicle position (scrotal or undescended; $P = 0.81$; Table 2). Finally, no significant differences were found in prostate volumes ($P = 0.57$; Figure 1F). Because of the significant difference in race distribution across groups, secondary analyses were performed on Caucasian children only. The secondary analyses yielded similar results with no differences observed in organ volumes or structural characteristics across groups.

**Discussion**

This study compared breast bud, ovaries, uterus, testes, and prostate volumes and structural characteristics in 5-y-old children who were breastfed or fed MF or SF as infants. Volumes and shapes of primary and secondary sex organs that are known to be hormone sensitive did not differ between the diet groups. Uterine and breast tissues are especially sensitive to estrogens; in fact, uterine weight has long been used in the pharmaceutical industry to identify compounds with estrogenic properties. Several research groups have suggested that high concentrations of xenoestrogens, such as isoflavones present in soy infant formula, can disrupt female and/or male reproductive development (24). Indeed, estrogens have the potential to affect the function or development of any tissue or organ with estrogen receptors. In this study, infants consumed the highest formula volumes at age 4 mo, and it was previously demonstrated at that age that the volumes and shapes of uterus, breast buds, ovaries, testes, or prostates in infants fed SF did not differ from infants who were breastfed or fed MF. The present study was aimed at determining whether there were effects in the same organs later in life but before puberty. On the basis of previously published data at age 4 mo (19), we postulated similar results would be found at age 5 y.

The age 5 y was chosen as a midpoint between age 4 mo and the onset of puberty because very early puberty onset was previously identified as age 10 y or younger. Combined, the results obtained at ages 4 mo and 5 y suggest that consuming SF does not result in alterations in reproductive organ development (as assessed by volumes and structural characteristics); thus, no evidence was found for estrogenic actions of SF when consumed during infancy by age 5 y in this cohort.

We had previously found no significant differences between groups except for greater ovarian volume in MF-fed girls and greater testes volume in breastfed boys at age 4 mo (19). These differences were no longer present at age 5 y, suggesting that these effects are either transients or do not affect organ development at age 5 y. These results do not point to differences in organ development for SF-fed children at age 4 mo or 5 y. Numerous studies in experimental models suggest that phytoestrogens affect reproductive health [see review by Jefferson et al. (25)], but clinical studies are scarce and inconclusive. Clinical studies have mostly focused on interpreting associations between early infant feeding with age of menarche in girls. A recent study has demonstrated associations of SF with precocious (10 y) and late menarche (14–15 y) (20). The study found that

**TABLE 1** Cohort characteristics in 5-y-old children who were BF or fed MF or SF as infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=101)</th>
<th>BF (n=35)</th>
<th>MF (n=32)</th>
<th>SF (n=34)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Caucasian</td>
<td>90 (89.1)</td>
<td>35 (100.0)</td>
<td>29 (90.6)</td>
<td>26 (76.5)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>9 (8.9)</td>
<td>0 (0.0)</td>
<td>1 (3.1)</td>
<td>8 (23.5)</td>
<td></td>
</tr>
<tr>
<td>Other/unknown</td>
<td>2 (2.0)</td>
<td>0 (0.0)</td>
<td>2 (6.3)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>F</td>
<td>51 (50.5)</td>
<td>18 (51.4)</td>
<td>19 (58.3)</td>
<td>15 (44.1)</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>50 (49.5)</td>
<td>17 (48.6)</td>
<td>14 (41.7)</td>
<td>19 (55.9)</td>
<td></td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.3 ± 1.0</td>
<td>39.5 ± 0.9</td>
<td>39.1 ± 0.8</td>
<td>39.4 ± 1.2</td>
<td>0.21</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.5 ± 0.3</td>
<td>3.6 ± 0.3</td>
<td>3.5 ± 0.4</td>
<td>3.4 ± 0.3</td>
<td>0.16</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td>60.8 ± 26.0</td>
<td>58.6 ± 24.0</td>
<td>61.3 ± 26.2</td>
<td>62.6 ± 28.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Child's age, wk</td>
<td>261.7 ± 3.4</td>
<td>261.3 ± 2.6</td>
<td>261.7 ± 2.9</td>
<td>262.1 ± 4.5</td>
<td>0.79</td>
</tr>
<tr>
<td>Weight at 5 y, kg</td>
<td>19.4 ± 2.8</td>
<td>18.9 ± 2.2</td>
<td>19.2 ± 2.8</td>
<td>20.1 ± 3.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Length at 5 y, cm</td>
<td>110.5 ± 3.8</td>
<td>110.2 ± 3.5</td>
<td>110.0 ± 3.5</td>
<td>111.2 ± 4.3</td>
<td>0.63</td>
</tr>
</tbody>
</table>

1 Values are means ± SDs or percentages. BF, breastfed; MF, cow-milk formula; SF, soy-based formula.
children who were fed SF as infants had a 25% increased risk of menarche before age 12 y compared with girls fed other types of infant formula. However, the study was limited because 2% of the subjects had early soy exposure, and loss to follow-up was high. Previous epidemiologic studies are in agreement with these findings (21), whereas others did not find evidence that SF feeding is associated with the age of menarche (26). Studies have also investigated the link between higher serum isoflavones concentrations and age of menarche. One study on 200 Korean girls demonstrated an association between higher serum isoflavone concentrations and precocious puberty (27); whereas, 2 other observational studies found an association of higher urinary isoflavone concentrations with later onset of breast development (28, 29), suggesting that isoflavones may have differential effects. Interpretation of these conflicting results is still being debated. Some suggest that phytoestrogen exposure early in the prepubertal period when endogenous estradiol concentrations are low could predispose girls to central puber-

cious puberty; whereas, later phytoestrogen exposure could serve to interfere with endogenous estrogen-mediated stimulation of breast development because of weak estrogen receptor agonist activity (25). A cross-sectional study on estrogenic activity of 37 boys and 35 girls who were exclusively fed breast milk (for the first 3 mo of life) or fed exclusively MF or SF during the first 6 mo of life found no early feeding effects on breast adipose tissue, breast buds (7 girls and 7 boys), and testes volume (7 boys); breast and genital development; or vaginal discharge (22). There was, however, a trend toward higher maturation index of vaginal wall cells from SF-fed girls (P = 0.07). The sample size of that study was limited.

Studies that have examined the relation between phytoestrogen exposure and adult female reproductive tract function are also scarce. A retrospective study reported that exposure to SF does not appear to lead to different general health or reproductive outcomes than exposure to MF. The differential findings were longer menstrual bleeding (0.37 d) and more dysmenorrhea (RR: 1.77; 95% CI: 1.04, 3.00) in women who were exposed to SF compared with women who were not exposed to SF (26). Another epidemiologic study reported a slightly increased risk of early diagnosis of uterine fibroids linked with the use of SF during infancy (20).

A report by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction Expert Panel on the developmental toxicity of soy infant formula concluded that the use of soy infant formula in healthy full-term infants does not impair growth during infancy (11). However, they identified the lack of clarity on whether studies in experimental animals treated with genistein alone can be extrapolated to infants fed soy infant formula. The panel also identified gaps in research and needs for future studies. Among them, it was noted that larger (in terms of sample size) and longer (time span) longitudinal, prospective cohort studies are needed, for example, a longitudinal study that captures soy exposure from birth through puberty (11). The Beginnings study was designed to address this gap in research by studying infants who were breastfed or fed MF or SF. Infants were enrolled at age 2 mo and will be followed until puberty.

**TABLE 2** Ovarian cysts, uterine, and testes characteristics in 5-y-old children who were BF or fed MF or SF during infancy

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>BF</th>
<th>MF</th>
<th>SF</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaries imaged, n</td>
<td>35</td>
<td>36</td>
<td>30</td>
<td>0.75</td>
</tr>
<tr>
<td>Ovaries with cysts, n (%)</td>
<td>25 (69.4)</td>
<td>23 (63.9)</td>
<td>18 (60.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Cysts, n/ovary</td>
<td>1.5 (2.0)</td>
<td>2.0 (3.0)</td>
<td>1.0 (3.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Cysts size, cm</td>
<td>0.52 (0.09)</td>
<td>0.47 (0.15)</td>
<td>0.47 (0.19)</td>
<td>0.52</td>
</tr>
<tr>
<td>Uterus imaged, n</td>
<td>18</td>
<td>18</td>
<td>15</td>
<td>0.52</td>
</tr>
<tr>
<td>Uterus length, mm</td>
<td>24.9 (4.2)</td>
<td>26.6 (7.2)</td>
<td>27.6 (5.8)</td>
<td>0.52</td>
</tr>
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<td>0.52</td>
</tr>
</tbody>
</table>

*Data are counts (percentages) or medians (IQRs). BF, breastfed; MF, cow-milk formula; SF, soy formula. *Adjusted for race, gestational age, and birth weight.
The present study has several limitations, the greatest of which may be sample size. Although the sample size was fairly large for a detailed sonographic study of this type, it remains relatively small after dichotomizing by sex and feeding group. Second, this study is reporting data at age 5 y. Although this time point allows for the detection of potential prepubertal effects after SF feeding during infancy, there remains the possibility that structural effects will not be manifested until the onset of puberty or later, when primary and secondary sex organs become more active. Thus, the planned follow-up of this cohort through puberty will be critical.

This study has numerous strengths. First, this is a prospective, longitudinal study with careful documentation of infant feeding and careful assessments of growth and development for all breastfed, MF-fed, and SF-fed infants from age 2 mo through age 5 y. Second, 80% (n = 71) of children presented in this study were part of an earlier sonographic study at age 4 mo when circulating isoflavone concentrations were maximal, and similar results were reported. Third, the study measured organ volumes by sonography, and the images were evaluated by radiologists blinded to the children’s early infant feeding group.

In summary, no feeding group differences were found in reproductive organ volumes and structural characteristics in children aged 5 y who were breastfed or fed MF or SF as infants. The follow-up of these children through puberty is planned and should help delineate potential early infant feeding effect on reproductive function later in life.

Acknowledgments
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References