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US assessment of estrogen-responsive organ growth among healthy term infants: piloting methods for assessing estrogenic activity

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Abstract

Background—A mother's circulating estrogen increases over the third trimester, producing physiological effects on her newborn that wane postnatally. Estrogenization might be prolonged in newborns exposed to exogenous estrogens, such as isoflavones in soy formula.

Objective—We evaluated ultrasonography for monitoring growth of multiple estrogen-responsive organs in healthy infants and developed organ-growth trajectories.

Materials and methods—We studied 38 boys (61 visits) from birth to age 6 months and 41 girls (96 visits) from birth to age 1 year using a partly cross-sectional, partly longitudinal design. We measured uterus and ovaries in girls, testes and prostate in boys, and kidneys, breasts, thymus, and thyroid in all children. We imaged all organs from the body surface in one session of < 1 h.

Results—Uterine volume decreased from birth ($P<0.0001$), whereas ovarian volume increased sharply until age 2 months and then decreased ($P<0.001$). Testicular volume increased with age ($P<0.0001$), but prostatic volume showed minimal age trend. Breast bud diameter showed no age trend in girls but declined from birth in boys ($P=0.03$).

Conclusion—US examination of multiple estrogen-responsive organs in infants in a single session is feasible and yields volume estimates useful for assessing potential endocrine disruptor effects on organ growth.

Keywords

US; Infant; Organ growth; Soy formula; Ultrasonography

Introduction

The endocrine-disrupter hypothesis states, among other things, that environmental chemicals can alter hormone function in humans at levels encountered by the general population [1]. Toxicological studies have provided substantial evidence that environmental estrogens can influence animal development [2, 3], but direct studies in humans, especially children, have used methods and endpoints that are not sufficiently sensitive or specific and have been inconsistent in their results [4]. Term newborns recently exposed to the very high levels of estrogen circulating in the mother typically present with prominent breast buds and estrogenized vaginal wall cells [5]. Soon after birth, with residual maternal estrogen levels diminishing or absent, these physical characteristics generally begin to wane. If infants are exposed to an exogenous source of estrogen, however, evidence of estrogen exposure can continue or become more pronounced. Thus, infants provide a special opportunity to study the effects of post-natal exposure to estrogenic compounds.

Although difficult to measure exactly, estrogen levels are low in cow milk-based infant formula and human milk [6]. Soy-based formula, however, is a major component of many infants' diets [7, 8], and the isoflavones present in soy formula, [9] although weakly estrogenic [10], can prolong maternal estrogenization ex utero, thereby altering the growth pattern of estrogen-responsive organs. Subtle changes in the growth and development of an infant's organs that could be attributable to dietary differences would likely be overlooked unless such effects were a specific focus of study. For example, previous US studies have shown that breast-fed infants at age 3 months had smaller kidney size [11] but at 4 months had larger thymus size [12] than infants fed formula (unspecified as to soy or cow's milk). Arguably, a well-designed longitudinal US study would clarify the possible effects of soy formula on organ development.

Determining sample size for such a study requires baseline information about organ growth in healthy infants and executing it requires developing procedures acceptable to both healthy infants and their parents. US studies of specific organs, such as the thymus, have often involved either infants who were ill [13] or whose mothers were ill [14]. Though some US studies of organ growth in healthy children are available [15–23], we did not find sufficient data for planning a prospective longitudinal US study to assess dietary influences on the growth of multiple infant organs. To calculate the sample size required for a subsequent, larger study of dietary influences, we needed more detailed quantitative information than was available in the literature about growth trajectories for various organs early in the first year of life when the waning influence of maternal estrogen should be most evident. Consequently, we undertook the pilot study reported here. This pilot also allowed us to see whether we could find children meeting our stringent feeding group definitions.

We assessed neonatal organs whose growth trajectory might respond to waning levels of maternal estrogen, i.e. organs that are relatively large at birth and then diminish in size. These organs included the uterus, ovaries, breasts, testes and possibly prostate. Although we hypothesized that soy formula might prolong the waning phase and specifically recruited children fed soy formula, cow-milk formula or breast milk, this pilot study was not large

enough to detect any but implausibly large differences among feeding groups. Since there are also reported differences in growth [24] and immune function [25] by feeding group, we measured the thyroid and thymus, which contribute to regulation of these processes. Renal growth was not expected to vary in response to estrogen but could be readily measured and provided an indication of how well our methods could track organ size in the first months of life [26].

Materials and methods

Study recruitment

The Study of Estrogen Activity and Development (SEAD) US pilot study was conducted at Children's Hospital Boston (CHB) and the Brigham and Women's Hospital (BWH) in Boston. The institutional review boards at the CHB and BWH, and the National Institute of Environmental Health Sciences approved the protocol.

We deemed infants eligible if they were born at 37 to 41 weeks' gestation, with birth weight between 2,500 and 4,500 g, and free of any major illness or known structural or endocrinological defect that might interfere with feeding, growth or development. In addition, boys had to have palpable testes. All participants must also have been in one of three mutually exclusive feeding categories: breast milk only, cow-milk formula only or soy formula only (Table 1). We excluded otherwise eligible children whose feeding histories fit none of our categories. Children categorized in this way showed much higher soy isoflavone concentrations in urine in the soy-formula group than either of the other two feeding groups [27]. Our consent documents were in English and Spanish, and we used interpreters as needed with Spanish-speaking parents.

Most newborns and infants were recruited from the postpartum floors and well-newborn nurseries at the BWH and during well-child visits to a large, general pediatric clinic at CHB. Additional patients were recruited in waiting rooms throughout CHB and through study advertisements in the *Boston Parent's Paper* (<http://boston.parenthood.com>). On the postpartum floor, nurseries and clinic, study staff flagged eligible infants by leaving a contact sheet on the patient's medical chart. The mother's or infant's healthcare provider then introduced the study to the parent and sought permission for our study staff to contact the parent. If, after discussion with study staff, at least one parent provided informed consent, a maternal and infant dietary questionnaire was obtained to confirm feeding group, and a study US examination was scheduled. For any child with multiple visits, feeding group assignment was re-confirmed by questioning the mother at each visit. We compensated families for meals, travel and parking expenses and provided gift cards that could be used at local grocery, toy or clothing stores.

Study design

Data on infant organ growth would ideally be collected longitudinally, by following each child over the entire period of interest. Because ours was a pilot study, we opted for a less precise but far less time-consuming, partly cross-sectional design that allowed at most four visits per child. Our design called for examinations during 10 age intervals for boys (0–6 months) and 16 age intervals for girls (0–12 months) (Table 2). We chose different maximal ages for each sex because boys' most estrogen-responsive organ (breast) has regressed by 6 months, whereas the age at which the girls' breasts regress was less well-characterized [5]. The width of age intervals was tighter at the younger ages and progressively relaxed at older ages. Our design called for examining two boys and two girls from each of the three feeding categories within each specified age interval. Thus, our design called for 60 examinations of boys up to age 6 months and 96 examinations of girls up to age 1 year.

US examination

US examinations were carried out by sonologists trained for this study. Infants' organs were examined in a specific sequence, reflecting a bottom-to-top ordering: first, uterus and ovaries, or prostate and testes, followed by kidneys, breast, thymus and finally thyroid. This sequence aided in keeping children calm during the examination, as many infants showed irritability during the thyroid measurement. Sedation was never used and a caregiver was always present during the exam.

A sonologist recorded three sets of images for each organ, but one of us (JAE) read every image. Each set of images provided measurements of the anterior-posterior (AP), sagittal and transverse dimensions. Thus, we had triplicate measurements for each dimension of each organ except kidney. For the kidneys, we recorded only the triplicate measurement in the sagittal dimension. For bilaterally paired organs (ovaries, testes, breasts and kidneys) and for the thyroid gland, which had two bilaterally distinct lobes, we measured each side separately. We prospectively noted the presence and number of ovarian follicles and recorded those greater than 1 cm in diameter. We assessed uterine morphology and documented the presence and characteristics of the central endometrial echo complex.

High-frequency, small-footprint linear-array US probes (Sequoia 15L8; Siemens Medical Solutions, Mountain View, CA, USA) were used to measure breasts, thyroid and testes; neonatal vector or curved-array probes (Sequoia 8V5 or 8C5; Siemens Medical Solutions) were used for kidneys and thymus. Either type of array could be used for prostate, uterus and ovaries.

Data analysis

We followed the commonly accepted procedure of approximating organ volume from the measured linear dimensions [11, 16, 18–22, 28]. We approximated the volume of each organ as though each were a simple geometric solid, either a cylinder with an elliptical base for breast buds and the uterus, or an ellipsoid for all other organs. Volumes are represented by the formulas $2\pi abc$ and $4/3 \pi abc$ for cylinder and ellipsoid, respectively, where a , b , and c , formally axis half-lengths, were taken as half the geometric means of the triplicate AP, sagittal and transverse measured dimensions, respectively. We also considered breast bud diameter, approximated as the geometric mean of the triplicate sagittal and transverse measured dimensions. For bilaterally symmetrical organs, we analyzed the geometric means of the two individually calculated volumes (or diameters for breast buds). In a sub-sample of 10 people of various ages, we compared our ellipsoidal volume approximation for thymus to the thymic index [17], calculated as the product of a transverse linear dimension with a measured area in the longitudinal plane.

Growth is not linear in age, even in this restricted age range. To describe typical or average trajectories of organ size across age, we used mixed-model regression techniques, fitting separate models for each organ. Mixed-model regression techniques are extensions of usual regression methods that are applicable to situations where some subjects provide more than one observation [29]. In our data, each infant provides information about a piece of the average trajectory (sometimes one point, sometimes several). Heuristically, the purpose of our mixed-model analysis was to amalgamate these pieces into a single average trajectory. Our analysis assumed a common trajectory shape for every child but let each child, in effect, have his or her own intercept—acknowledging that some children are larger or smaller at birth and accounting for the fact that measurements from the same child are likely more closely related to one another than to measurements from different children. We modeled the base 2 logarithm of organ size (volume, length or diameter) using quadratic polynomials in the square root of age, a model that permits curved trajectories like those we anticipated.

We considered a sequence of models, one that used separate polynomials for each feeding group, a second that used separate intercepts for each feeding group but a common function of age, and a third that used a common polynomial for each feeding group. We examined linear trends across age for categorical responses (e.g., presence/absence of ovarian follicles) using generalized linear mixed-model regression that accounted for between- and within-infant variation. Reported *P* values are for each statistical test individually and were not adjusted for the multiplicity of tests across organs.

Results

We had a total of 157 completed research study visits; 96 visits by 41 infant girls and 61 visits by 38 infant boys (Table 3). For boys, 59 of 61 visits (97%) provided size measures for every organ (two visits lacked prostate volume); for girls, 92 of 96 visits (96%) provided size measures for every organ (one visit lacked thymus volume, another lacked ovary volume, a third lacked both breast-bud diameter and thyroid volume).

Triplicate assessments of linear dimensions from separate images of a particular organ generally agreed well. Considering separate measurements for bilateral organs, we measured 28 linear dimensions for each sex. Intra-class correlation coefficients for 19 of 28 dimensions each for girls and boys were greater than 0.80, with minima of 0.55 (AP right-lobe thyroid) for girls and 0.57 (AP thymus) for boys. Replicate measurements for thyroid dimensions were generally the least consistent among all organs, a feature that reflects either the infants' generally higher irritability during thyroid measurements or the organ's irregular shape. Thymus, uterus, and prostate measurements showed somewhat lower reliability among replicate measurements than did kidney, breast buds, ovaries and testes (data not shown). Kidney and breast measurements were the most consistent, with intra-class correlation coefficients typically exceeding 0.90. In addition, based on a subsample of 10 children, our approximation of thymus volume was highly correlated with the thymic index of Hasselbalch et al. [17] (Spearman rank correlation coefficient 0.79), suggesting that use of either measure would yield comparable results.

None of the organs studied showed statistically significant differences in growth trajectories among feeding groups. Kidney length and thyroid volume exhibited growth trajectories that did not seem influenced by withdrawal of maternal estrogen. Kidney length increased with age for both boys and girls ($P < 0.0001$ for each sex) following trajectories consistent with growth in body size (Fig. 1). Thyroid volume increased with age in girls ($P = 0.004$) but an increasing trend was not statistically significant in boys ($P = 0.38$) (Fig. 2). The higher measurement error in thyroid compared to other organs, along with the shorter age range studied in boys compared to girls, might have impaired our ability to detect a volume increase in boys.

Thymus volume increased with age in both girls and boys ($P = 0.003$ for each sex). In boys, the increase appeared approximately linear from birth until age 6 months; in girls, the increase in thymus volume appeared to level off after about 4 months, with a possible decline thereafter (Fig. 3).

Breast bud diameter decreased with age in boys ($P = 0.03$), consistent with withdrawal of maternal estrogen; but breast bud diameter was essentially constant in girls ($P = 0.76$) (Fig. 4).

Uterine volume decreased with age ($P < 0.0001$) and appeared to be strongly influenced by the withdrawal of maternal estrogen (Fig. 5). The initial decline from birth until about 2 months was precipitous; from 2 months through the end of the study, uterine volume leveled off and remained smaller on average than at birth. After about 4 months of age, soy-fed

infant girls tended to have slightly greater uterine volumes than the average trajectory whereas volumes of breast- and cow-formula-fed infants straddled that trajectory.

We initially interpreted a highly reflective mid-line endometrial stripe in the uterus as evidence of estrogen-influenced proliferation of endometrial tissue. We observed this stripe in most girls on most visits (83/88); it was absent only in infant girls > 5 months of age. Nevertheless, we became concerned that this mid-line stripe might simply represent the interface of the opposing interior walls of the uterus and not endometrial proliferation per se. Consequently, we looked at the number of distinct layers visible within the midline endometrial halo, interpreting the presence of multiple layers as suggesting endometrial proliferation. Among 83 girls with a halo present, multiple layers were more common than a single layer (1 infant with 3 layers, 68 infants with two, and 14 infants with one). Of the 14 infants exhibiting only a single layer, however, 11 were > 5 months of age ($P=0.13$). These results are consistent with a waning influence of maternal estrogen on the complexity of the midline signal as girls aged.

Ovarian volume exhibited strong age dependence ($P<0.001$), increasing sharply in girls from birth to age 1–2 months and then gradually decreasing up to 12 months (Fig. 6). Of the 96 total infant girl visits, ovarian follicles were assessed in 94 visits (98%) and detected in 78 of those (83%); the total number of follicles detected (both ovaries combined) ranged from 0 to 11. The age trajectory for the number of follicles roughly tracked that of ovarian volume, peaking at 1–3 months of age and declining thereafter. Thus, ovarian volume was positively associated with the number of follicles detected ($P<0.0001$), though the number of follicles was not the sole determinant of ovarian size. We interpret the sharp early increase and subsequent decline in ovarian size as a response to follicle stimulating hormone, which would be suppressed by maternal estrogen during fetal life but then briefly secreted by the infant [30].

Prostatic volume was more variable across repeated visits for the same boy than were other organ volumes. Nevertheless, $\log_2$ -transformed prostatic volume showed a mild but statistically significant curvilinear age trajectory ($P=0.02$), increasing during the first 30–60 days and then leveling off or falling slightly (Fig. 7).

Testicular volume increased from birth through age 6 months ($P<0.0001$), with a larger increase during the first 2 months than during the subsequent 4 (Fig. 8). Neither prostatic nor testicular volume exhibited growth trajectories that could be unambiguously attributed to withdrawal of maternal estrogen.

Discussion

We consider as estrogen-responsive those organs that exhibit maximal size at, or shortly after, birth and then recede or decrease in size thereafter until subsequent growth-mediated increases take over. The uterus showed the clearest evidence of this loss of the trophic effect of estrogen, despite the relatively greater variability we encountered in measuring it. In addition, the midline echo complex in the uterus might indicate an estrogenized endometrium. Boys' breast buds also showed some regression in size with increasing age. Ovaries had a complex growth curve, with an initial increase in size followed by a decrease after 2–4 months. This pattern is likely an indirect effect of estrogen caused by the suppression of fetal gonadotropins by maternal estrogen, followed by a post-natal surge in follicle stimulating hormone. Post-natal estrogen might blunt that surge, and thereby reduce ovarian volume, or at least the component attributable to follicles. We found that infant girls in our study had detectable ovarian follicles in 83% of visits. Our results for ovarian volume as well as for the presence, initial appearance and subsequent decrease in size of ovarian

follicles are consistent with other reports [16, 28]. These findings indicate that the uterus, ovaries, breast buds in boys, and perhaps breast buds in girls, would likely be the most relevant organs for a future targeted study of possible estrogen influences on organ size in infants.

Most studies of thymic volume are conducted in conjunction with immune function assessment. The patterns we observed for thymic growth in both girls and boys in our study mirrored those reported by Hasselbalch et al. [12, 31] in a longitudinal study. They observed an initial increase in median thymic index (both sexes combined) from birth to 4 months, little change to 8 months, followed by a decrease to 12 months. Among girls, we observed an increase until about 4 months, and then a plateau or gradual decline, whereas we saw a steady increase in thymic volume during the first 6 months in boys. The truncated length of observation for boys precluded observing a later decline in volume. Thymic volume, though calculated differently, was highly correlated with the Hasselbalch thymic index in a subset of our infants. At age 6 months, boys had, on average, a larger thymus than girls, a pattern observed in other studies [15].

Our results for testicular volumes up to age 6 months support a recent report by Kuijper et al. [20] that boys had a peak testicular volume of 0.44 cm³ at 5 months of age; subsequently, volume declined until the end of observation at 12 months of age. In our sample of boys, testicular volume steadily increased until the study period ended at 6 months. Prostate volume was more variable across repeated visits by the same boy than other organs. We measured the prostate transperineally, and visualization of the margins was influenced by the degree of bladder filling. Visit-to-visit variation in bladder filling could contribute to observed variability in prostate volumes.

This pilot study has several limitations. First, the number of subjects is too small to provide statistical evidence regarding dietary differences in organ growth trajectories. It is, however, sufficient to provide a general picture of the shape of growth trajectories that can inform the design of future studies. Another consequence of few subjects is that our data are insufficient to provide reliable normative growth curves for these organs; a much larger study would be required, preferably one that tracked each individual at many points throughout the age range. Lacking such comprehensive measurements on individuals precluded our ability to track the growth of any individual infant's organs; however, the partly cross-sectional, partly longitudinal nature of our study meant that we could complete it more quickly. More data on each individual would also allow us to further assess whether the functional form of our regression models was adequate. In addition, we caution that our approach to calculating organ volume based on simple geometric solids, though commonly used, is at best an approximation to the actual volume of the organ.

Recently the same sonographic methods that we used were employed in study of 120 4-month-old infants in three feeding groups: soy-formula, cow-milk formula, breast milk (defined differently from ours) [32]. That study did not show consistent estrogenic effects on organ volume in the soy-fed children; however, only 25% of soy-fed children were fed soy from birth, lowering that group's overall exposure to soy isoflavones.

Conclusion

Our estimates of growth trajectories of several potentially estrogen-responsive organs in infants, from birth to age 6 months in boys and from birth to age 12 months in girls, suggest that the uterus and ovaries in girls and the breast buds in boys are the most predictive organs for assessing the possible influence of exogenous estrogen early in life.

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References

1. Rogan WJ, Ragan NB. Some evidence of effects of environmental chemicals on the endocrine system in children. *Int J Hyg Environ Health*. 2007; 210:659–667. [PubMed: 17870664]
2. Rozman KK, Bhatia J, Calafat AM, et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. *Birth Defects Res B Dev Reprod Toxicol*. 2006; 77:485–638. [PubMed: 17186522]
3. Rozman KK, Bhatia J, Calafat AM, et al. NTP-CERHR expert panel report on the reproductive and developmental toxicity of soy formula. *Birth Defects Res B Dev Reprod Toxicol*. 2006; 77:280–397. [PubMed: 16998908]
4. Arbuckle TE, Hauser R, Swan SH, et al. Meeting report: measuring endocrine-sensitive endpoints within the first years of life. *Environ Health Perspect*. 2008; 116:948–951. [PubMed: 18629319]
5. Bernbaum JC, Umbach DM, Ragan NB, et al. Pilot studies of estrogen-related physical findings in infants. *Environ Health Perspect*. 2008; 116:416–420. [PubMed: 18335112]
6. Borgert CJ, LaKind JS, Witorsch RJ. A critical review of methods for comparing estrogenic activity of endogenous and exogenous chemicals in human milk and infant formula. *Environ Health Perspect*. 2003; 111:1020–1036. [PubMed: 12826475]
7. American Academy of Pediatrics Committee on Nutrition. Soy protein-based formulas: recommendations for use in infant feeding. *Pediatrics*. 1998; 101:148–153. [PubMed: 11345979]
8. Setchell KD, Zimmer-Nechemias L, Cai J, et al. Exposure of infants to phytoestrogens from soy-based infant formula. *Lancet*. 1997; 350:23–27. [PubMed: 9217716]
9. Setchell KD, Zimmer-Nechemias L, Cai J, et al. Isoflavone content of infant formulas and the metabolic fate of these phytoestrogens in early life. *Am J Clin Nutr*. 1998; 68:1453S–1461S. [PubMed: 9848516]
10. Barnes S. Soy isoflavones—phytoestrogens and what else? *J Nutr*. 2004; 134:1225S–1228S. [PubMed: 15113976]
11. Schmidt IM, Damgaard IN, Boisen KA, et al. Increased kidney growth in formula-fed versus breast-fed healthy infants. *Pediatr Nephrol*. 2004; 19:1137–1144. [PubMed: 15309602]
12. Hasselbalch H, Jeppesen DL, Engelmann MD, et al. Decreased thymus size in formula-fed infants compared with breastfed infants. *Acta Paediatr*. 1996; 85:1029–1032. [PubMed: 8888912]
13. Jeppesen DL, Hasselbalch H, Nielsen SD, et al. Thymic size in preterm neonates: a sonographic study. *Acta Paediatr*. 2003; 92:817–822. [PubMed: 12892161]
14. Jeppesen D, Hasselbalch H, Ersboll AK, et al. Thymic size in uninfected infants born to HIV-positive mothers and fed with pasteurized human milk. *Acta Paediatr*. 2003; 92:679–683. [PubMed: 12856977]
15. Aaby P, Marx C, Trautner S, et al. Thymus size at birth is associated with infant mortality: a community study from Guinea-Bissau. *Acta Paediatr*. 2002; 91:698–703. [PubMed: 12162605]
16. Cohen HL, Shapiro MA, Mandel FS, et al. Normal ovaries in neonates and infants: a sonographic study of 77 patients 1 day to 24 months old. *AJR*. 1993; 160:583–586. [PubMed: 8430559]
17. Hasselbalch H, Nielsen MB, Jeppesen D, et al. Sonographic measurement of the thymus in infants. *Eur Radiol*. 1996; 6:700–703. [PubMed: 8934137]
18. Ingram S, Hollman AS, Azmy AF. Ultrasound evaluation of the paediatric prostate. *Br J Urol*. 1994; 74:601–603. [PubMed: 7827814]
19. Khadilkar VV, Khadilkar AV, Kinare AS, et al. Ovarian and uterine ultrasonography in healthy girls between birth to 18 years. *Indian Pediatr*. 2006; 43:625–630. [PubMed: 16891683]

20. Kuijper EA, van Kooten J, Verbeke JI, et al. Ultrasonographically measured testicular volumes in 0- to 6-year-old boys. *Hum Reprod.* 2008; 23:792–796. [PubMed: 18281246]
21. Perry RJ, Hollman AS, Wood AM, et al. Ultrasound of the thyroid gland in the newborn: normative data. *Arch Dis Child Fetal Neonatal Ed.* 2002; 87:F209–F211. [PubMed: 12390993]
22. Schmidt IM, Main KM, Damgaard IN, et al. Kidney growth in 717 healthy children aged 0–18 months: a longitudinal cohort study. *Pediatr Nephrol.* 2004; 19:992–1003. [PubMed: 15206038]
23. Soyupak SK, Narli N, Yapicioglu H, et al. Sonographic measurements of the liver, spleen and kidney dimensions in the healthy term and preterm newborns. *Eur J Radiol.* 2002; 43:73–78. [PubMed: 12065125]
24. Stettler N. Nature and strength of epidemiological evidence for origins of childhood and adulthood obesity in the first year of life. *Int J Obes (Lond).* 2007; 31:1035–1043. [PubMed: 17589539]
25. Ostrom KM, Cordle CT, Schaller JP, et al. Immune status of infants fed soy-based formulas with or without added nucleotides for 1 year. Part 1: vaccine responses, and morbidity. *J Pediatr Gastroenterol Nutr.* 2002; 34:137–144. [PubMed: 11840030]
26. Zerlin JM, Blane CE. Sonographic assessment of renal length in children: a reappraisal. *Pediatr Radiol.* 1994; 24:101–106. [PubMed: 8078709]
27. Cao Y, Calafat AM, Doerge DR, et al. Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula. *J Expo Sci Environ Epidemiol.* 2009; 19:223–234. [PubMed: 18665197]
28. Haber HP, Mayer EI. Ultrasound evaluation of uterine and ovarian size from birth to puberty. *Pediatr Radiol.* 1994; 24:11–13. [PubMed: 8008485]
29. Cnaan A, Laird NM, Slasor P. Using the general linear mixed model to analyse unbalanced repeated measures and longitudinal data. *Stat Med.* 1997; 16:2349–2380. [PubMed: 9351170]
30. Winter JS, Hughes IA, Reyes FI, et al. Pituitary-gonadal relations in infancy: 2. Patterns of serum gonadal steroid concentrations in man from birth to 2 years of age. *J Clin Endocrinol Metab.* 1976; 42:679–686. [PubMed: 1262442]
31. Hasselbalch H, Jeppesen DL, Ersboll AK, et al. Thymus size evaluated by sonography: a longitudinal study on infants during the first year of life. *Acta Radiol.* 1997; 38:222–227. [PubMed: 9093155]
32. Gilchrist JM, Moore MB, Andres A, et al. Ultrasonographic patterns of reproductive organs in infants fed soy formula: comparisons to infants fed breast milk and milk formula. *J Pediatr.* 2010; 156:215–220. [PubMed: 19846109]

Fig. 1.

US estimates of kidney sagittal length in infants versus age. Length at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories. Top panel, boys, $\log_2(\text{length}) = (2.1909) + (-0.0447) \cdot \text{age}^{0.5} + (-0.0010) \cdot \text{age}$; bottom panel, girls, $\log_2(\text{length}) = (2.1009) + (0.0447) \cdot \text{age}^{0.5} + (-0.0012) \cdot \text{age}$

Fig. 2. US estimates of thyroid volume in infants versus age. Volume at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories. Top panel, boys, $\log_2(\text{volume}) = (-1.4011) + (0.0682) \cdot \text{age}^{0.5} + (-0.0029) \cdot \text{age}$; bottom panel, girls, $\log_2(\text{volume}) = (-1.2666) + (0.0085) \cdot \text{age}^{0.5} + (0.0011) \cdot \text{age}$

Fig. 3.

US estimates of thymus volume in infants versus age. Volume at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories. Top panel, boys, $\log_2(\text{volume}) = (-0.1325) + (-0.0389) \cdot \text{age}^{0.5} + (-0.0010) \cdot \text{age}$; bottom panel, girls, $\log_2(\text{volume}) = (2.9498) + (0.1437) \cdot \text{age}^{0.5} + (-0.0068) \cdot \text{age}$

Fig. 4. US estimates of breast bud diameter in infants versus age. Diameter at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories. Top panel, boys, $\log_2(\text{diameter}) = (-0.1325) + (-0.0389) \cdot \text{age}^{0.5} + (-0.0010) \cdot \text{age}$; bottom panel, girls, $\log_2(\text{diameter}) = (-0.2301) + (-0.0031) \cdot \text{age}^{0.5} + (0.0007) \cdot \text{age}$

Fig. 5. US estimates of uterine volume in infant girls versus age. Volume at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories, $\log_2(\text{volume}) = (2.7759) + (-0.2298) \cdot \text{age}^{0.5} + (0.0080) \cdot \text{age}$

Fig. 6. US estimates of ovary volume in infant girls versus age. Volume at each visit by an infant is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories, $\log_2(\text{volume}) = (-3.7301) + (0.6023) \cdot \text{age}^{0.5} + (-0.0266) \cdot \text{age}$

Fig. 7. US estimates of prostatic volume in infant boys versus age. Volume at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curves represent fitted trajectories, $\log_2(\text{volume}) = (-1.2728) + (0.2479) \cdot \text{age}^{0.5} + (-0.0145) \cdot \text{age}$

Fig. 8. US estimates of testicular volume in infant boys versus age. Volume at each visit is plotted by a symbol indicating feeding group; dashed lines connect multiple visits by the same infant; solid curve represents fitted trajectory: $\log_2(\text{volume}) = (-2.6054) + (0.2388) \cdot \text{age}^{0.5} + (-0.0070) \cdot \text{age}$

Table 1

Definitions for the three feeding categories

Milk source	Age at exam	Note
Breast-milk category		
Breast milk exclusively	0–48 h	Restriction: Babies in the breast milk category are not allowed to have had any soy foods including soy formula and solid foods containing soy in their lifetime.
Breast milk exclusively	49 h–3 months	
Breast milk exclusively OR Breast milk and cow-milk formula	> 3 months	
Cow-milk-formula category		
Cow-milk formula exclusively	0–48 h	Exception: If a baby was breastfed in the nursery, the baby must have gone home on cow-milk formula and have been on cow-milk formula exclusively ever since. Such a child cannot participate until he or she has been fed exclusively cow-milk formula for at least 2 weeks.
Cow-milk formula exclusively	49 h–3 months	
Cow-milk formula exclusively	> 3 months	
Restriction: Babies in the cow-milk-formula category are not allowed to have had any soy foods including soy formula and solid foods containing soy in their lifetime.		
Soy-formula category		
Soy formula exclusively	0–48 h	Exception: If a baby was fed something other than soy formula in the nursery, the baby must have gone home on soy and been on soy exclusively ever since. Such a child cannot participate until he or she has been fed exclusively soy formula for at least 2 weeks.
Soy formula exclusively	49 h–3 months	
2/3 of lifetime on soy formula exclusively and continuously, and including the 2 weeks up to the exam	> 3 months	

Table 2

Scheduled age intervals and number of infant visits observed in each interval by feeding group and sex

Scheduled age	Acceptance range	Breast fed		Cow milk fed		Soy fed		Total
		Boy	Girl	Boy	Girl	Boy	Girl	
< 48 h (< 2 d)	< 2 d	2	2	2	2	2	2	12
1 week (7 d)	2 < age ≤ 10 d	2	3	2	2	2	2	13
2 week (14 d)	10 < age ≤ 17 d	2	2	2	2	2	2	12
3 week (21 d)	17 < age ≤ 24 d	2	2	2	2	2	2	12
4 week (28 d, 1 month)	24 < age ≤ 42 d	2	2	2	2	2	1	11
8 week (56 d, 2 month)	6 < age ≤ 10 week (42 < age ≤ 70 d)	2	2	2	2	2	1	11
12 week (84 d, 3 month)	10 < age ≤ 14 week (70 < age ≤ 98 d)	2	3	2	2	2	1	12
16 week (112 d, 4 month)	14 < age ≤ 18 week (98 < age ≤ 126 d)	2	2	2	2	2	2	12
20 week (140 d, 5 month)	18 < age ≤ 22 week (126 < age ≤ 154 d)	2	2	2	2	2	2	12
24 week (168 d, 6 month)	22 < age ≤ 26 week (154 < age ≤ 182 d)	3	2	2	2	2	2	13
28 week (196 d, 7 month)	26 < age ≤ 30 week (182 < age ≤ 210 d)	–	2	–	2	–	2	6
32 week (224 d, 8 month)	30 < age ≤ 34 week (210 < age ≤ 238 d)	–	2	–	2	–	2	6
36 week (252 d, 9 month)	34 < age ≤ 38 week (238 < age ≤ 266 d)	–	3	–	2	–	2	7
40 week (280 d, 10 month)	38 < age ≤ 42 week (266 < age ≤ 294 d)	–	2	–	2	–	2	6
44 week (308 d, 11 month)	42 < age ≤ 46 week (294 < age ≤ 322 d)	–	2	–	2	–	2	6
48 week (336 d, 12 month)	46 < age ≤ 54 week (322 < age ≤ 378 d)	–	2	–	2	–	2	6
Total		21	35	20	32	20	29	157

Table 3

Number of infants with single or multiple visits by sex

Number of visits per child	Boys	Girls
One	23	18
Two	10	5
Three	2	5
Four	3	12
Five	0	1
Total Infants	38	41
Total Visits	61	96