Exposure to Soy-Based Formula in Infancy and Endocrinological and Reproductive Outcomes in Young Adulthood

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Context A large body of evidence documents the role of phytoestrogens in influencing hormone-dependent states. Infants fed soy formula receive high levels of phytoestrogens, in the form of soy isoflavones, during a stage of development at which permanent effects are theoretically possible. However, a paucity of data exists on the long-term effects of infant soy formulas.

Objective To examine the association between infant exposure to soy formula and health in young adulthood, with an emphasis on reproductive health.

Design, Setting, and Participants Retrospective cohort study conducted from March to August 1999 among adults aged 20 to 34 years who, as infants, participated during 1965-1978 in controlled feeding studies conducted at the University of Iowa, Iowa City (248 were fed soy formula and 563 were fed cow milk formula during infancy).

Main Outcome Measures Self-reported pubertal maturation, menstrual and reproductive history, height and usual weight, and current health, compared based on type of formula exposure during infancy.

Results No statistically significant differences were observed between groups in either women or men for more than 30 outcomes. However, women who had been fed soy formula reported slightly longer duration of menstrual bleeding (adjusted mean difference, 0.37 days; 95% confidence interval [CI], 0.06-0.68), with no difference in severity of menstrual flow. They also reported greater discomfort with menstruation (unadjusted relative risk for extreme discomfort vs no or mild pain, 1.77; 95% CI, 1.04-3.00).

Conclusions Exposure to soy formula does not appear to lead to different general health or reproductive outcomes than exposure to cow milk formula. Although the few positive findings should be explored in future studies, our findings are reassuring about the safety of infant soy formula.

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SOY ISOFLAVONES, A SUBCATegory of phytoestrogens, are naturally occurring plant compounds. Phytoestrogens have been shown to bind to estrogen receptors in the adult[1-3] and to act either as estrogens[4] or as antiestrogens.[5,6] For example, phytoestrogens can block the action of endogenous estrogens on the uterus.[5-7]

A large body of evidence documents the role of phytoestrogens in influencing hormone-dependent states.[4,8-10] Dietary phytoestrogens during adulthood have been suggested in numerous epidemiological studies to be protective against cancer of the prostate, colon, rectum, stomach, breast, and lung and to exert similarly protective effects against chronic conditions such as atherosclerosis and osteoporosis.[11-15] Evidence in some animal species of a contraceptive or sexual development effect from dietary phytoestrogens[16-26] has led some to suggest that dietary habits should be investigated in women with differences in menstrual cycle length.[27] Lowered sperm counts have also been suggested as possibly associated with phytoestrogens, but more recent work has not confirmed this pattern.[28]

Infants fed soy formula receive relatively high doses (per unit of body

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weight) of phytoestrogens during a developmental stage at which permanent changes are theoretically possible.[29-31] Delayed effects of soy-based infant formula on subsequent child or adult health have thereby been postulated,[30,31] generating substantial controversy in the lay and medical press. However, effects on human pubertal and reproductive development of phytoestrogen exposure in infancy have not been systematically investigated.[13] Given the paucity of data on the long-term effects of soy formulas, their widespread use, and expressed concerns about their safety,[30,31] we undertook a study to examine the association between exposure to soy formula in infancy and any subsequent possible effects on adult health, focusing on outcomes that could be estrogen related.

METHODS

Study Design

This was a retrospective cohort study of young adults, 20 to 34 years of age, who as infants had participated in multiple controlled but nonrandomized feeding studies conducted at the University of Iowa during the years 1965 to 1978. A telephone interview was conducted between March and August 1999 with eligible subjects who could be located and who agreed to be interviewed. The study was approved by the University of Pennsylvania Committee on Studies Involving Human Subjects.

The Cohort

Methods and procedures used in the original feeding studies have been previously described.[32,33] Briefly, participants were healthy term infants with birth weights of more than 2500 g whose mothers elected not to breastfeed. With few exceptions, they were white, reflecting the population in and around Iowa City. Infants were enrolled before the age of 9 days and were studied through 16 weeks of age. Assignment to study formulas was performed in rotation, in that all available infants were assigned to whatever formula was being studied at the time. Once enrollment of the predetermined number of infants was completed, all available infants were assigned to the next study formula. Infant characteristics or parental preferences were not taken into account. The same formula was fed for the entire study period. Selected solid foods were received by some infants. The study protocols involved periodic measurements of weight and length, measurement of formula consumption, and determination of selected serum chemical indexes. The actual phytoestrogen content of the infant formulas was not measured.

Criteria for inclusion in the present follow-up study were that the formula fed be classifiable as "milk based" or "soy based," ie, containing protein derived from cow milk or isolated soy protein or, in the case of one formula (12 subjects in follow-up), from soy flour. Individuals who received formulas with both soy and cow milk protein were excluded. In addition, subjects had to have completed the original feeding study as planned (about 85% of enrolled infants). Those who were adopted were excluded from follow-up. Also excluded were individuals who were profoundly disabled who would not have been able to be interviewed, deceased individuals, and individuals from countries other than the United States, because of difficulty in tracking and differences in lifestyle and language barriers.

Subject Follow-up

A search was conducted to locate the 952 subjects of the original cohort. The search used a variety of approaches, including the use of national telephone and address directories. Records of the parents' and often grandparents' names and addresses were also available from the original study. Efforts to locate subjects were aided by the fact that many of the individuals born from 1966 through 1971 had been located when they were 8 years old as part of a previous follow-up study.[34]

Study Outcomes

Information on outcomes and a large number of potential confounding factors was obtained by trained interviewers using a structured, standardized telephone interview that took 30 to 60 minutes to complete. Reliability checks were done on 5% of questionnaires. Outcome variables were selected for investigation a priori based on 3 criteria. They were expected to be (1) potentially related to estrogenic effects, (2) clinically important, and (3) likely to have a sufficient sample size to detect a clinically relevant effect, based on a priori standard type I and type II error levels. Because potential estrogenic effects may be different in men and women, and many reproductive and sexual outcomes are sex-specific, these outcomes were selected and analyzed separately by sex.

The outcomes chosen for primary analysis in women were adult height, usual weight since the age of 18 years, usual body mass index, pubertal maturation (age at menarche, age when breasts developed enough to start wearing a bra), number of days between periods (during times when not using birth control pills, shots, or implants), number of days requiring pads or tampons, regularity of menstrual period, menstrual flow, pain with menstrual period (none, mild, severe), physical symptoms of pain (eg, dysmenorrhea, headaches), breast tenderness during menstrual cycle, premenstrual symptoms, breast size (bra cup size), reproductive outcomes (number of pregnancies, deliveries, abortions, and other complications), and education level attained as a proxy measure for intelligence.

In men, in addition to adult height, usual weight, and education level, outcomes investigated in the primary analyses included pubertal maturation (age at first ejaculation, age when voice changed, age when hair began to grow on chest, face, or pubic area) and pregnancy outcomes in sexual partners impregnated by the male study subjects. Other outcomes, such as congenital malformations in the offspring of study subjects, hormonal disorders, testicular cancer in men, and homosexual orientation, were included as secondary outcomes but were not expected to provide definitive results because these events were expected to occur too infrequently.

Confirmation was sought in the medical records of past and current physicians and hospitals of reports by the subjects of primary or secondary outcomes.

Data Analysis

The subjects were classified into exposure groups based on the original infant formula consumed. Descriptive statistics were performed on all the variables under investigation by sex and exposure group.

Discrete or ordinal variables (such as dysmenorrhea, menstrual flow, education level attained) were characterized by proportions, and the rates between groups (ie, soy formula vs cow milk formula) were compared by χ^2 or Fisher exact tests.[35] Next, calculations of unadjusted and adjusted relative risks (RRs) and associated 95% confidence intervals (CIs) were computed for all dichotomous outcomes using generalized linear models with a log link and a binomial error distribution,[36] operationalized with PROC GENMOD in SAS statistical software (SAS Institute Inc, Cary, NC).

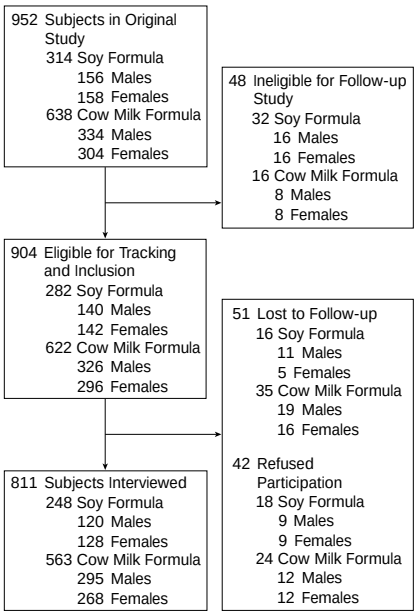
To compute the adjusted RR estimates, a single set of potential confounding variables was used: birth weight (obtained from records available from the infancy studies), current age, usual body mass, parents' usual weight (slim, average, somewhat overweight, extremely overweight) and height, presence of any hormone disorders (thyroid disease, treatment, use of steroids or growth hormone), duration of cigarette smoking, average monthly alcohol consumption, duration of use of soy foods as a major source of protein in diet (after infancy, defined as "ever use" at least once per week), duration of vegetarian diet, duration of dietary herbal supplement use, duration of use of mood-enhancing drugs, average number of hours spent per week in strenuous sports or vigorous work, average number of hours spent per day in sedentary activities, and sexually transmitted diseases. For women, adjustment was made also for "ever use" of birth control pills or progesterone injections or implants.

Distributions of continuous variables (such as body mass index, age at menarche, age when voice changed) were characterized by means and SDs, and differences between group means on these variables were initially compared by the *t* test or Wilcoxon rank sum test, as appropriate.[37] Hypotheses related to continuous outcomes were examined using linear regression[38] to estimate group mean differences and 95% CIs while adjusting for the same list of potential confounders.

All reproductive outcomes were evaluated, taking into account the correlation among pregnancies for each woman.[39] Live births, miscarriages, and abortions were evaluated as a proportion of total number of pregnancies. Full-term, preterm, and stillbirth outcomes were evaluated as a proportion of total pregnancies, excluding miscarriages, abortions, and ectopic pregnancies, since early losses in pregnancy were not eligible for late pregnancy outcomes. Multiple births were evaluated as a proportion of total live births. Unadjusted and adjusted RR estimates for all reproductive outcomes were computed using log linear models with binomial error[39] using PROC GENMOD in SAS statistical software, where adjustments for multiple pregnancies (or correct denominator) were made by specifying the appropriate offset for each subject. Adjustment was made for the same list of potential confounders as for discrete variables plus the presence of any reproductive organ disorder (polycystic ovarian syndrome, endometriosis, blocked fallopian tubes, pelvic inflammatory disease, or other problems with ovaries, uterus, or vagina), marital status, and education level attained.

This study had 80% statistical power to detect clinically relevant differences between the groups. For example, the study had sufficient power to detect height differences of 0.9 and 0.8 in (2.3 and 2.1 cm) and weight differences of 9.5 and 10.3 lb (4.3 and 4.6 kg) in men and women, respectively; a 1-day difference in menstrual cycle length; and a difference of 0.45 years in age at menarche. For dichotomous outcomes, we could detect statistically significant relative risks (RRs) of 2.8 or greater for outcomes with an incidence of approximately 5%, 2.1 for outcomes with an incidence of 10%, or 1.5 for outcomes with an incidence of 30%.

Figure. Tracking of Subjects in the Original Iowa Nutrition Studies in Infancy



RESULTS

Of the 952 subjects in the infant cohort, 48 were ineligible because they had used formula with both soy and cow milk (n=26), were from countries other than the United States (n=7), were adopted (n=2), were disabled (n=3), or were deceased (n=10), with causes of death accidental except for 2 lymphoma/leukemia cases in the cow milk group. Of the 904 who were eligible, 811 were interviewed: 248 (87.9%) of 282 fed soy formula and 563 (90.5%) of 622 fed cow milk formula (FIGURE). The groups differed in age ($P=.001$), with the soy formula group having more individuals in the youngest and the oldest groups compared with the cow milk formula group, whose age clustered in the 25- to 29-year range (TABLE 1). The only other differences between the study groups were in use of asthma or allergy drugs (soy greater than cow milk, $P=.08$ for men but

P=.047 for women) and a tendency for sedentary activities (P=.77 for men but P=.05 for women).

No statistically significant differences were noted for either women or men for adult height, usual weight, usual body mass index, or any of the indexes of pubertal maturation (TABLE 2, TABLE 3, and TABLE 4).

No statistically significant differences were observed in multiple measures of menstrual history, with 2 exceptions. Duration of menstruation requiring pads or tampons was slightly longer in the participants fed soy formula (adjusted mean difference, 0.37 days; 95% CI, 0.06-0.68) (P=.02), although without heavier bleeding (Table 3). Discomfort with menstrual period was also borderline significantly more common among subjects fed soy formula (unadjusted RR, 1.77; 95% CI, 1.04-3.00 for extreme vs none or mild discomfort) (P=.04), but cramps with menses was not significantly different (Table 3). Subjects fed soy formula were no more likely to seek medical attention to evaluate symptoms of pain associated with menstrual periods (P=.30).

Regarding pregnancy history, 54 (42.2%) of 128 women fed soy formula as infants reported 1 or more pregnancies vs 128 (47.8%) of 268 women fed cow milk formula as infants (P=.43). A total of 366 pregnancies (117 in the soy formula group and 249 in the cow milk formula group) were reported by these 182 women. No differences were seen in pregnancy outcomes (Table 3).

Evaluation of a large number of other outcomes selected a priori for secondary analyses (eg, cancer, reproductive organ disorders, hormonal disorders, libido dysfunction, sexual orientation, and birth defects in the offspring) also did not show statistically significant differences in unadjusted analyses between the 2 formula groups, in either women or men, although the sample sizes for these analyses were too small to be definitive (data available from the

Table 1. Selected Demographic and Other Characteristics of Study Participants*

Characteristics	Men, No./Total (%)		Women, No./Total (%)	
	Soy Formula	Cow Milk Formula	Soy Formula	Cow Milk Formula
Age group, y				
20-24	42/120 (35.0)	69/295 (23.4)	62/128 (48.4)	64/268 (23.9)
25-29	22/120 (18.3)	164/295 (55.6)	25/128 (19.5)	147/268 (54.8)
30-34	56/120 (46.7)	62/295 (21.0)	41/128 (32.0)	57/268 (21.3)
Marital status				
Always single	68/119 (57.1)	158/294 (53.8)	72/128 (56.2)	136/268 (50.8)
Ever married	51/119 (42.9)	136/294 (46.2)	56/128 (43.8)	132/268 (49.2)
Cigarette smoking				
Ever smoked >100 cigarettes	51/120 (42.5)	118/298 (39.6)	54/128 (42.2)	104/268 (38.8)
Years smoked, mean (SD), No.	7.1 (4.8)	7.0 (4.5)	6.3 (3.4)	6.6 (4.3)
Usual alcoholic drinks per month, mean (SD), No.	28.7 (28.7)	25.3 (23.7)	16.13 (17.2)	16.08 (16.7)
Ever use soy or tofu products as a major source of protein in diet (≥once per week)				
As primary source	6/120 (5.0)	17/294 (5.8)	5/127 (3.9)	17/268 (6.3)
Not as primary source	10/120 (8.3)	27/294 (9.2)	9/127 (7.1)	19/268 (7.1)
Not at all	104/120 (86.7)	250/294 (85.0)	113/127 (89.0)	232/268 (86.6)
Vegetarian eating practices†				
Lacto-ovo	6/120 (5.0)	13/294 (4.4)	20/127 (15.7)	28/265 (10.6)
Lacto	0/120 (0.0)	5/294 (1.7)	1/127 (0.8)	5/265 (1.9)
Vegan	0/120 (0.0)	4/294 (1.4)	0/127 (0.0)	2/265 (0.7)
Nonvegetarian	114/120 (95.0)	272/294 (92.5)	106/127 (83.5)	230/265 (86.8)
Use of asthma or allergy drugs				
Irregularly	10/120 (8.3)	44/295 (14.9)	12/128 (9.4)	23/269 (8.6)
Regularly	19/120 (15.8)	30/295 (10.2)	24/128 (18.8)	27/268 (10.1)
Use of recreational/mood-enhancement drugs				
Irregularly	49/120 (40.8)	123/293 (42.0)	48/128 (37.5)	100/268 (37.3)
Regularly	19/120 (15.8)	50/293 (17.1)	17/128 (13.3)	29/268 (10.8)
Ever use of birth control pills, injections, or implants	Not applicable	Not applicable	110/128 (85.9)	235/268 (87.7)
Physical activity, mean (SD), h/wk in past year				
Strenuous sports	6.0 (6.4)	4.8 (3.9)	3.6 (2.5)	3.9 (3.2)
Vigorous work	14.4 (18.1)	11.5 (15.5)	7.8 (12.1)	7.4 (12.8)
Sedentary activities	9.9 (3.4)	9.8 (3.5)	8.9 (3.4)	9.6 (3.5)

*Data are No./Total (%) except where indicated otherwise. No statistically significant differences were found between soy formula and cow milk formula groups except for age group (P = .001 for both men and women); use of asthma or allergy drugs (soy greater than cow milk: for men, P = .08, for women, P = .047); and sedentary activities (for men, P = .77, for women, P = .05).

†"Lacto-ovo" refers to avoiding meat, poultry, and fish but eating eggs and dairy products; "lacto," avoiding all of the above plus eggs; and "vegan," avoiding all animal products and eating only vegetables, fruits, nuts, grains, etc.

Table 2. Summary of Results for Continuous Outcomes Selected for Primary Analyses: Women*

Outcome	Soy Formula (n = 128)		Cow Milk Formula (n = 268)		Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)
	No. of Subjects	Group Mean (SD)	No. of Subjects	Group Mean (SD)		
Adult height, in	128	65.3 (2.3)	268	65.5 (2.5)	−0.25 (−0.79 to 0.30)	−0.31 (−0.73 to 0.10)
Usual weight since age 18 y, lb	127	138.0 (21.4)	261	139.6 (24.7)	−1.95 (−7.20 to 3.29)	−1.64 (−6.47 to 3.19)†
Usual body mass index, kg/m^2	127	22.8 (3.3)	261	22.9 (3.7)	−0.13 (−0.93 to 0.67)	−0.16 (−0.95 to 0.62)‡
Pubertal maturation						
Age at menarche, y	128	12.6 (1.4)	267	12.7 (1.3)	−0.02 (−0.31 to 0.28)	−0.03 (−0.32 to 0.26)
Age when breasts developed enough to start wearing a bra, y	127	12.3 (1.2)	268	12.3 (1.6)	−0.01 (−0.33 to 0.32)	−0.02 (−0.33 to 0.29)
Menstrual history						
Cycle length, No. of days between periods	122	28.1 (5.9)	257	29.0 (10.1)	−0.58 (−2.49 to 1.33)	−0.58 (−2.54 to 1.38)
Duration of menstrual bleeding, No. of days requiring pads or tampons	127	5.0 (1.4)	267	4.7 (1.3)	0.34 (0.04 to 0.63)	0.37 (0.06 to 0.68)

*CI indicates confidence interval. Variables included in the adjusted analyses are listed in the "Data Analysis" section.
†Also adjusted for total number of pregnancies, the result was −1.26 (−6.06 to 3.55)
‡Also adjusted for total number of pregnancies, the result was −0.11 (−0.89 to 0.67).

Table 3. Summary Results for Categorical Outcomes Selected for Primary Analysis: Women*

Outcome	No./Total of Subjects (%)		Unadjusted Relative Risk (95% CI)	Adjusted Relative Risk (95% CI)
	Soy Formula (n = 128)	Cow Milk Formula (n = 268)		
Regular menstrual periods				
Always/usually irregular	26/128 (20.3)	54/268 (20.1)	0.96 (0.62-1.50)	0.91 (0.58-1.44)
Always/usually regular	102/128 (79.7)	214/268 (79.9)		
Menstrual flow				
Heavy/extremely heavy, clots	35/128 (27.3)	67/268 (25.0)	1.04 (0.71-1.51)	0.98 (0.67-1.44)
Extremely light/light/average, normal	93/128 (72.7)	201/268 (75.0)		
Missed menstrual periods (for ≥3 mo, except during pregnancy)	27/128 (21.1)	66/266 (24.8)	0.83 (0.55-1.25)	0.91 (0.62-1.33)
Spotting in middle of menstrual period	41/128 (32.0)	65/268 (24.2)	1.26 (0.89-1.78)	1.18 (0.88-1.58)
Discomfort with menstrual period				
Extremely painful	23/128 (18.0)	30/268 (11.2)	1.77 (1.04-3.00)	Not calculable
Mildly painful/not painful	105/128 (82.0)	238/268 (88.8)		
Cramps during menstrual period	91/128 (71.1)	180/268 (67.2)	1.06 (0.92-1.23)	1.05 (0.94-1.18)
Breast tenderness	12/128 (9.4)	22/268 (8.2)	1.34 (0.67-2.69)	Not calculable
Premenstrual syndrome	69/127 (54.3)	158/266 (59.4)	0.91 (0.75-1.11)	0.99 (0.92-1.06)
Bra cup size				
Size D or higher	23/128 (18.0)	54/266 (20.3)	0.87 (0.55-1.38)	Not calculable
Size A, B, or C	105/128 (82.0)	212/266 (79.7)		
Education level attained				
College/trade school or >college	117/128 (91.4)	235/268 (87.7)	1.06 (0.99-1.13)	1.02 (0.81-1.27)
≤High school	11/128 (8.6)	33/268 (12.3)		
Ever pregnant	54/128 (42.2)	128/268 (47.8)	0.92 (0.82-1.02)	0.94 (0.85-1.04)
Pregnancy outcomes				
Live births	76/117 (65.0)	148/249 (59.4)	1.13 (0.84-1.52)	1.10 (0.80-1.51)
Elective abortions	19/117 (16.2)	54/249 (21.7)	0.87 (0.50-1.52)	1.01 (0.54-1.91)
Medical abortions	3/117 (2.6)	6/249 (2.4)	1.41 (0.34-5.90)	Not calculable
Miscarriages	15/117 (12.8)	38/249 (15.3)	0.56 (0.26-1.21)	0.65 (0.28-1.48)
Ectopic deliveries	1/117 (0.8)	3/249 (1.2)	0.78 (0.08-7.53)	Not calculable
Molar deliveries	0/117 (0.0)	0/249 (0.0)	Not applicable	Not applicable
Full-term deliveries†	66/79 (83.5)	136/148 (91.9)	0.87 (0.63-1.20)	0.84 (0.60-1.19)
Preterm deliveries†	10/79 (12.7)	12/148 (8.1)	2.11 (0.84-5.31)	Not calculable
Stillborn deliveries†	3/79 (3.8)	0/148 (0.0)	Not calculable	Not calculable
Multiple births (twins)‡	4/76 (5.3)	4/148 (2.7)	4.42 (0.81-24.09)	Not calculable
Attempting pregnancy without success	4/74 (5.4)	5/140 (3.6)	1.61 (0.44-5.87)	Not calculable

*CI indicates confidence interval. Variables included in the adjusted analyses are listed in the "Data Analysis" section.
†Denominator is total pregnancies minus abortions, miscarriages, and ectopic pregnancies.
‡Denominator is total live births.

authors on request). Only regular use of weight control medications (ie, daily, for at least 3 months) was borderline significantly higher in women fed soy formula as infants (unadjusted RR, 1.70; 95% CI, 1.01-2.87).

A total of 228 subjects reported primary or secondary outcomes investigated in this study. Almost two thirds (n=146 [64.0%]) of these subjects granted consent to review their medical records. After allowing for incomplete and/or inaccurate physician addresses or unavailable records (n=24) and for conditions that could not be validated because they were self-diagnosed (n=7), the medical conditions of 115 subjects were potentially available for validation. Records were actually obtained for 81 subjects, for a physician response rate of 70.4% (81/115). The medical record review showed that of 106 medical conditions reported, most were confirmed by the information recorded in the medical records in both groups (33 [84.6%] of 39 in the soy formula group and 59 [88.1%] of 67 in the cow milk formula group).

COMMENT

Based on a 1998 infant feeding survey (Paul Harris, Ross Products Division, Abbott Laboratories, Columbus, Ohio, written communication, April 2001), 18% of infants are fed soy formula sometime during the first year of life. Given the 2000 US Census estimate of nearly 4 million US infants younger than 1 year,[40] an estimated 750 000 US infants are fed these formulas each year. Even if the adverse outcomes under consideration here were relatively uncommon, the potential for a major public health impact is large. Conversely, insupportable allegations of adverse effects can affect a large proportion of the population, denying them access to a useful type of infant feeding product. Accurate assessment of any risk associated with exposure to soy formula is important, and our study has yielded no systematic cause for concern.

Our findings are consistent with other studies that found no changes in weight and height or effects on puberty or fertility associated with the consumption of soy isoflavones.[10,11] However, we believe this to be the largest controlled study evaluating the long-term effects of exposure to soy formula in infancy. This study had sufficient statistical power (>80%) to detect clinically significant differences between the groups in most outcomes, as is reflected in the relatively narrow CIs presented. The study results were unequivocally negative across a large number of outcomes that potentially may be influenced by the estrogenic or antiestrogenic activity of phytoestrogens. This study found mostly neither positive nor negative effects in subjects exposed in infancy to soy formula when compared with those exposed to cow milk formula. From among the many different factors studied, significant findings were seen only for slightly longer duration of monthly menstruation and for greater discomfort with menstruation. The prolongation of menstrual bleeding was small and was not accompanied by heavier bleeding. Both findings were borderline positive and were 2 of many that were tested. To place this in perspective, if we were to consider a Bonferroni adjustment for the number of hypotheses investigated in this article, neither of these 2 findings would be considered even close to statistically significant at the resulting stricter level of 0.05/30=0.0017.[41] Furthermore, the clinical significance of these findings is not known.

Evidence in animals of reproductive disturbances associated with ingestion of feed rich in estrogenic substances includes a lower conception rate in sheep after prolonged grazing in clover pastures rich in isoflavones,[17-19] infertility in cattle after consuming feed containing coumestrol,[20] decreased fertility in captive cheetahs fed dietary estrogens,[21] hyperestrogenism in pigs fed diets containing zearalenone,[22] and uterotropic effects in mice fed soybean.[16,23-25] In contrast, a study of rhesus monkeys fed soy isolates for 6 months observed no adverse effects on the reproductive systems of either sex, as evaluated by reproductive hormone concentrations and organ weights at autopsy.[42] Ours is the only epidemiologic study, to our knowledge, that examines the possibility of infertility in young adults who were fed soy formula in infancy. No statistically

Table 4. Summary Results for Outcomes Selected for Primary Analyses: Men*

Outcome	Soy Formula (n = 120)		Cow Milk Formula (n = 295)		Unadjusted Mean Difference (95% CI)	Adjusted Mean Difference (95% CI)
	No. of Subjects	Group Mean (SD)	No. of Subjects	Group Mean (SD)		
Adult height, in	120	71.7 (2.5)	295	71.4 (2.6)	0.30 (−0.27 to 0.87)	0.09 (−0.36 to 0.53)
Usual weight since age 18 y, lb	119	186.8 (35.8)	295	179.8 (29.3)	6.32 (−0.66 to 13.30)	2.61 (−3.92 to 9.15)
Usual body mass index, kg/m^2	119	25.6 (4.6)	295	24.8 (3.6)	0.69 (−0.20 to 1.57)	0.36 (−0.54 to 1.26)
Pubertal maturation						
Age at first ejaculation, y	108	13.2 (1.2)	274	13.0 (1.4)	0.23 (−0.08 to 0.54)	0.17 (−0.16 to 0.50)
Age when voice changed, y	111	14.3 (1.7)	262	14.0 (1.5)	0.30 (−0.06 to 0.66)	0.29 (−0.09 to 0.67)
Age when hair appeared on chest/face/pubic area, y	115	13.9 (1.6)	286	13.7 (1.7)	0.22 (−0.15 to 0.59)	0.20 (−0.19 to 0.59)
Education level attained, No./total (%)						
College/trade school or >college	93/119	(78.2)	231/294	(78.6)	0.99 (0.88 to 1.11)	0.97 (0.89 to 1.11)
≤High school	26/119	(21.8)	63/294	(21.4)		

*CI indicates confidence interval. All data are mean (SD) except for education level. Variables included in the adjusted analyses are listed in the "Data Analysis" section.

significant effects were seen on fertility as measured by pregnancy or on miscarriage, medical abortion, or ectopic pregnancy rates.

In a population-based cohort study in the United Kingdom,[43] a vegetarian diet during pregnancy was associated with a 5-fold higher risk of hypospadias, although regular consumption of soy products was not significantly different between mothers with and without affected offspring. In our study, rates of neither genital nor urologic birth defects in the offspring of subjects fed soy formula in infancy were significantly different from those fed cow milk formula.

Isoflavones from soy formulas are well absorbed by infants, as evidenced by their presence in plasma[44] and their urinary excretion in amounts representing 13% to 38% of intakes. However, soy isoflavones are largely conjugated. Unconjugated isoflavones are postulated to be extensively metabolized by infants into glucuronide and sulfate conjugates that are likely to exert low or negligible biological activity.[45] In addition, it is unknown when an infant acquires the flora necessary to metabolize isoflavones. Transit time, which is reduced in infants, may result in less absorption.[10] It may well be that phytoestrogens are lightly bound to many of the proteins that bind estrogens and reduce their activity. Alternatively, the newborn period could be a time when exposure has little effect. This study does not provide data on the short-term activity or effects of phytoestrogens. However, these results are reassuring regarding the long-term effects of phytoestrogen exposure of this type.

Our study has several limitations. Isoflavone levels were not measured in the soy formulas fed to the subjects. However, manufacturing processes have changed very little since the study formulas were made. Isoflavone content of the study formulas can, therefore, be assumed to have been similar to that in currently marketed soy formulas, which has been reported to range from 32 to 47 mg/L.[44] At the usual intake volumes for infants during the first 16 weeks of age, isoflavone intake probably ranged from 4.2 to 9.4 mg/kg per day. Soy flour has at least twice the isoflavone content of soy protein isolate, so the isoflavone intake of the infants fed the soy flour formula may have been between 9 and 16 mg/kg per day.

Selection bias in the way infants were assigned to study formulas was unlikely. Although assignment to formula group was not randomized, it was systematic in a fashion not likely to be related to outcome. By achieving relatively complete ascertainment of the soy formula and cow milk formula cohorts, we limited the potential of selection bias due to loss to follow-up or participation refusals. Since phytoestrogens are found in many foods,[46-49] exposure in infancy and adulthood to a variety of foods could mask any specific postulated effects of soy formula. We controlled in multivariate analyses for total duration of using soy products as a major source of dietary protein, use of herbal supplements, relying on a vegetarian diet, and significant alcohol consumption. No biological effects of infant soy exposure were detectable.

An important strength of this study is that the ascertainment of exposure in infancy to soy formula or cow milk formula was not dependent on subject or parental recall, because the information on exposure was obtained from the research records from the initial clinical trials. In addition, we sought the medical records of subjects with outcomes to validate the information on medical outcomes obtained by interviews, with reassuring results.

Obtaining information on other exposure to soy during childhood and adulthood, as well as information on other exposures and on the outcome variables of interest, could be subject to recall bias because the respondents' recollections could be flawed or intentionally distorted. Since the study hypotheses regarding the outcomes were not known to the subjects and because the outcomes of interest to the initial clinical trials were not the same as the outcomes of interest to the follow-up cohort study, differential recall bias between the exposure groups is unlikely. Nevertheless, nondifferential recall can bias the results toward the null.

Interviewer bias should not be an issue in this study because we used structured interviews and the interviewers were blinded to the study hypotheses and to the formula group assignment of the subjects in the initial clinical trials. Because of the wide-ranging contents of the interview, the interviewers could not guess the association of interest to the study.

Misclassification bias[50] could have occurred in several ways in this study. Misclassification of initial exposure is unlikely, since this information was objectively recorded in the research records of the subjects during the initial clinical trials. Outcome misclassification is likely to vary according to the specific outcome of interest, since respondents are more likely to answer correctly when asked about the height or education level attained than when asked about abortions or sexual preference. We attempted to confirm some of this information by review of the current medical records.

Lack of generalizability of the conclusions might be a problem because most subjects were from the Midwest, had higher socioeconomic status, and were white. Although for reproductive outcomes it is difficult to imagine that the results could not be generalized to other populations, it is possible that for other outcomes, such as sexual preference and education level attained, the results may be less generalizable to other ethnic or geographic groups. On the other hand, the relative homogeneity of the cohort studied with respect to socioeconomic status is useful in ensuring the validity of the comparisons across the 2 exposure groups, since there is less risk of confounding by demographic or socioeconomic status.

Finally, we cannot, of course, extrapolate from these findings on short-term exposure to phytoestrogens in soy formulas the effects of long-term exposure to phytoestrogens, nor to outcomes that we did not study, including longer-term outcomes that would not have occurred yet.

In conclusion, for more than 30 primary hypotheses that were tested, the observed differences between subjects exposed in infancy to soy formula vs cow milk formula were small and few reached statistical significance. The results with regard to menstruation should be interpreted with caution, given that the clinical significance of slightly prolonged menstrual bleeding in the absence of greater menstrual flow is not known. Given the large number of comparisons evaluated in these analyses, the few marginally significant findings may be due to chance. Although perhaps these few marginal positive findings should be followed up in future studies, the findings of the current study are reassuring about the safety of soy infant formula.

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REFERENCES

1. Shutt DA, Cox RI. Steroid and phytoestrogen binding to sheep uterine receptors in vitro. *J Endocrinol.* 1972;52:299-310.
2. Shemesh M, Lindner HR, Ayalon N. Affinity of rabbit uterine oestradiol receptor for phytol-oestrogens and its use in competitive protein-binding radioassay for plasma coumestrol. *J Reprod Fertil.* 1972;29:1-9.
3. Tang BY, Adams NR. Effect of equol on estrogen receptors and on synthesis of DNA and protein in the immature rat uterus. *J Endocrinol.* 1980;85:291-297.
4. Setchell KDR, Borriello SP, Hulme P, Kirk DN, Axelson M. Nonsteroidal estrogens of dietary origin: possible roles in hormone-dependent disease. *Am J Clin Nutr.* 1984;40:569-578.
5. Katzenellenbogen DS, Ferguson ER, Lan NC. Fundamental differences in the action of estrogens and antiestrogens on the uterus. *Endocrinology.* 1977; 100:1252-1259.
6. Folman Y, Pope GS. The interaction in the immature mouse of potent oestrogens with coumestrol, genistein and other utero-vaginotrophic compounds of low potency. *J Endocrinol.* 1966;34:215-225.
7. Sutherland RL, Murphy LC, Foo MS, Green MD, Whybourne AM, Krozowski ZS. High-affinity anti-oestrogen binding site distinct from the oestrogen receptor. *Nature.* 1980;288:273-275.
8. Setchell KD, Cassidy A. Dietary isoflavones: biological effects and relevance to human health. *J Nutr.* 1999;129:758S-767S.
9. Adlercreutz H. Phytoestrogens: epidemiology and a possible role in cancer protection. *Environ Health Perspect.* 1995;103(suppl 7):103-112.
10. Klein KO. Isoflavones, soy-based infant formulas, and relevance to endocrine function. *Nutr Rev.* 1998;56:193-204.
11. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol.* 1996;87: 897-904.
12. Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer. *Lancet.* 1997;350:990-994.
13. Humfrey CD. Phytoestrogens and human health effects. *Nat Toxins.* 1998;6:51-59.
14. Murkies AL, Wilcox G, Davis SR. Phytoestrogens. *J Clin Endocrinol Metab.* 1998;83:297-303.
15. Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of the effects of soy protein intake on serum lipids. *N Engl J Med.* 1995;333:276-282.
16. Drane HM, Patterson DSP, Roberts BA, Saba N. Oestrogenic activity of soy-bean products. *Food Cosmetics Toxicol*. 1980;18:425-427.
17. Bennetts HW, Underwood EG, Shier FL. A specific breeding problem of sheep on subterranean clover pastures in Western Australia. *Aust Vet J.* 1946;22:2-12.
18. Moule GR, Braden AWH, Lamond DR. The significance of oestrogens in pasture plants in relation to animal production. *Anim Breeding Abstracts.* 1963; 31:139-157.
19. Shutt DA. The effects of plant oestrogens on animal reproduction. *Endeavour.* 1976;35:110-113.
20. Adler JH, Trainin D. The apparent effect of alfalfa on the reproductive performance of dairy cattle. In: Proceedings of the Fourth International Congress on Animal Reproduction and Artificial Insemination; June 5-9, 1961; The Hague, the Netherlands.
21. Setchell KDR, Gosselin SJ, Welsh MB, et al. Dietary estrogens: a probable cause of infertility and liver disease in captive cheetahs. *Gastroenterology.* 1987; 93:225-233.
22. Mirocha CJ, Pathre SV, Christensen CM, Zearalenone. Mycotoxins in human and animal health. In: Rodricks JV, Hesseltine CW, Mehlman MA, eds. *Proceedings of a Conference on Mycotoxins in Human and Animal Health; October 4-8, 1976; College Park, Maryland.* Park Forest, Ill: Pathorox Publications Inc; 1977:345.
23. Carter MW, Smart WWG, Matrone G. Estimation of estrogenic activity of genistein obtained from soybean meal. *Exp Biol Med.* 1953;84:506-507.
24. Cheng E, Story CD, Yoder L, Hale WH, Burroughs W. Estrogenic activity of isoflavone derivatives extracted and prepared from soybean oil meal. *Science.* 1953;118:164-165.
25. Drane HM, Patterson DSP, Roberts BA, Saba N. The chance discovery of oestrogenic activity in laboratory rat cake. *Food Cosmetics Toxicol.* 1975;13:491-492.
26. Whitten PL, Lewis C, Russel E, Naftolin F. Phytoestrogen influences on the development of behavior and gonadotropin function. *Exp Biol Med.* 1995; 208:82-86.
27. Cassidy A, Bingham S, Setchell KDR. Biological effects of a diet of soy protein rich in isoflavones on the menstrual cycle of premenopausal women. *Am J Clin Nutr.* 1994;60:333-340.
28. Acacio B, Gottfried T, Israel R, Sokol R. Evaluation of a large cohort of men presenting for a screening semen analysis. *Fertil Steril.* 2000;73:595-597.
29. Sheehan DM. Herbal medicines, phytoestrogens and toxicity: risk:benefit considerations. *Proc Soc Exp Biol Med.* 1998;217:379-385.
30. Irvine C, Fitzpatrick M, Robertson L, Woodhams D. The potential adverse effects of soybean phytoestrogens in infant feeding [letter]. *N Z Med J.* 1995; 108:208-209.
31. Irvine CH, Fitzpatrick MG, Alexander SL. Phytoestrogens in soy-based infant foods: concentrations, daily intake, and possible biologic effects. *Exp Biol Med.* 1998;217:247-253.
32. Fomon SJ, Thomas LN, Filer LJ Jr, et al. Food consumption and growth of normal infants fed milk-based formulas. *Acta Paediatr Scand Suppl.* 1971; 223:1-36.
33. Fomon SJ, Ziegler EE, Nelson SE, Edwards BB. Requirements for sulfur-containing amino acids in infancy. *J Nutr.* 1986;116:1405-1422.
34. Fomon SJ, Rogers RR, Ziegler EE, Nelson SE, Thomas LN. Indices of fatness and serum cholesterol at age eight years in relation to feeding and growth during early infancy. *Pediatr Res.* 1984;18:1233-1238.
35. Mehta CR, Walsh SL. Comparison of exact, mid-p and Mantel-Haenszel confidence intervals for the common odds ratio across several 2×2 contingency tables. *Am Stat.* 1992;46:146-150.
36. Kleinbaum DG, Kupper LL, Morgenstern H. *Epidemiologic Research: Principles and Quantitative Methods.* Belmont, Calif: Lifetime Learning Publications; 1982.
37. Siegel S, Castellan NJ. *Nonparametric Statistics for the Behavioral Sciences*. 2nd ed. New York, NY: McGraw-Hill; 1988.
38. Kleinbaum DG, Kupper LL. *Applied Regression and Other Multivariate Methods.* Boston, Mass: Duxbury Press; 1978.
39. McCullagh P, Nelder JA. *Generalized Linear Models.* 2nd ed. New York, NY: Chapman & Hall; 1989.
40. US Census Bureau Web site. Available at: http://www.census.gov/population/estimates/nation/. Accessibility verified July 5, 2001.
41. Miller RG. *Simultaneous Statistical Inference*. New York, NY: Springer-Verlag; 1981.
42. Anthony MS, Clarkson TB, Hughes CL, et al. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J Nutr.* 1996;126:43-50.
43. North K, Golding J. A maternal vegetarian diet in pregnancy is associated with hypospadias. *Br J Urol Int.* 2000;85:107-113.
44. Setchell KDR, Zimmer-Nechemias L, Cai J, Heubi JE. Exposure of infants to phytoestrogens from soy-based infant formula. *Lancet.* 1997;350:23-27.
45. Huggett AC, Pridmore S, Malnoe A, Haschke F, Offord EA. Phyto-oestrogens in soy-based infant formula. *Lancet.* 1997;350:815-816.
46. Setchell KDR. Naturally occurring non-steroidal estrogens of dietary origin. In: McLahian J, ed. *Proceedings of the Symposium on Estrogens in the Environment: Influences on Development; 1985 April 10-12; Raleigh, North Carolina*. New York, NY: Elsevier; 1985:69-85.
47. Price KR, Fenwick GR. Naturally occurring food toxicants: estrogens. In: Rechcigl M, ed. *CRC Handbook of Naturally Occurring Food Toxicants*. Boca Raton, Fla: CRC Press; 1985:81-100.
48. Rosenblum ER, Campbell IM, Van Thiel DH, Gavaler JS. Isolation and identification of phytoestrogens from beer. *Alcohol Clin Exp Res.* 1992;16:843-845.
49. Gavaler JS, Rosenblum ER, Deal SR, Bowie BT. The phytoestrogen congeners of alcoholic beverages: current status. *Exp Biol Med.* 1995;208:98-102.
50. Greenland S. Statistical uncertainty due to misclassification. *J Clin Epidemiol.* 1988;41:1167-1174.