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Soy Protein Formulas in Children: No Hormonal Effects in Long-term Feeding

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ABSTRACT

Recently, the finding of high plasma concentration of phyto-oestrogens in soy protein formula (SPF) fed children has focused scientific attention on the phyto-oestrogens (isoflavones genistein, daidzein, and their glycosides) contained in SPFs. The aim of this study was to evaluate some hormonal and metabolic effects of long-term (more than 6 months) SPF feeding. We enrolled 48 children, mean age 37 months (range 7-96 months), 27 males and 21 females. All children underwent physical examination. Bone age, urinary markers of bone metabolism, serum levels of bone alkaline phosphatase, osteocalcin, 17β-oestradiol, and intact parathyroid hormone were measured. Eighteen healthy children represented the control group. No abnormalities were observed in auxological parameters; none of the enrolled girls showed signs/symptoms of precocious puberty and none of the boys presented gynecomastia; bone age was within the normal range. The serum level of bone alkaline phosphatase, osteocalcin, 17β-ocstradiol, and intact parathyroid hormone, and the urinary levels of the markers of bone metabolism were all within normal values. We conclude that long-term feeding with SPFs in early life does not seem to produce oestrogen-like hormonal effects.

KEY WORDS

soy protein formulas, phyto-oestrogens

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INTRODUCTION

Soy beans (like other legumes) contain high concentrations of phyto-oestrogens. These are represented by isoflavones, mainly genistein and daidzein, which are easily absorbed by the gastrointestinal tract and metabolised to equol, demethylangolensin, and paraethylphenol[1]. Isoflavones interact with oestrogen receptors and may mimic hormonal activity[2,3]. The presence of phyto-oestrogens with their potential hormonal influence on development has raised some concern about the safety of soy protein formulas (SPFs). There is a lack of data of the biological effects from exposure to phyto-oestrogens. Despite the use of these feedings for more than 30 years, there is no clear evidence to support negative effects from exposure to phyto-oestrogens.

Setchell *et al.*[7] analysed the isoflavone levels in various SPFs and compared plasma levels of genistein and daidzein in infants fed with different SPFs, a cow's milk formula (CMF) and breast milk alone. The levels of isoflavones were similar in the various SPFs, and plasma levels of genistein and daidzein were significantly higher in SPF-fed infants than in those fed with CMF or breast milk.

At the present time, there is no scientific evidence that intake of isoflavones, at levels present in SPFs, causes negative effects. Epidemiological data from Asian countries[4], where soy consumption is common from an early age even in breast-fed infants, have demonstrated positive effects of phyto-oestrogens on many illnesses, for example protection against heart disease[5-8], hormone-dependent cancers[9-12] and osteoporosis[13].

The aim of the present retrospective study was to evaluate the hormonal effects of long-term SPF feeding in a group of 48 children who were

continuously fed with SPFs for a maximum of 82 months.

METHODS

Forty-eight children (27 boys and 21 girls), age range 7-96 months (37.2 ± 24.3 months [mean ± SD]) were enrolled in the study within the span of a year (December 1997-December 1998). All children had been fed exclusively with SPFs for at least 6 months (range 6-82 months, median 12 months), mainly as prevention or treatment of cow's milk allergy (18/48 for positive family history and 30/48 for documented cow's milk allergy). The amount of soy administered was calculated based on the child's weight (150 kcal/kg). Children older than 6 months obviously had different kinds of food present in the diet. Information was collected by a direct and in-depth interview with the parents, including specific questions regarding the child's diet.

At the time of the study, all children below 24 months of age (study group A, age 7-24 months, n = 20) were still taking SPFs regularly. Most (16/20) of the children between 25 and 72 months of age (study group B, n = 20) had discontinued SPF feeding; all children between 73 and 96 months (study group C, n = 8) had completely discontinued the daily intake of SPFs.

Eighteen healthy children (14 boys and 4 girls), age range 12-96 months (46.5 ± 23.7 months [mean ± SD]), who had not been fed SPFs in the first months of life, were enrolled in the study as a control group (group D: D_1, age 7-24 months, n = 4; D_2, age 25-72 months, n = 10; D_3, age >72 months, n = 4), with the same clinical and laboratory follow-up. The differences in age and male/female ratio between the SPF-fed children of the study groups and the control group were not statistically significant.

During follow-up, signs and symptoms of precocious puberty in girls, defined as the appearance of secondary isosexual characteristics before the age of 8 years, and the appearance of gynecomastia in boys were specifically sought.

All children in the study were evaluated for the following clinical parameters: height, weight, presence/absence of breast bud, testicular size, presence/absence of pubic/axillary hair. Percentiles for height and weight were derived from national anthropometric data[14].

Radiological signs and biochemical markers of increased bone metabolism were also studied. These included bone age according to Tanner and Whitehouse (TW2) and urinary markers of bone metabolism: urinary deoxypyridoline (chemiluminescence), calciuria, creatinuria, phosphaturia (colorimetry), as well as serum levels of bone alkaline phosphatase (immuno-enzymatic assay), osteocalcin, oestradiol (radioimmunoassay) and intact parathyroid hormone (chemiluminescence).

Statistical analysis

Univariate comparisons were carried out using Fisher's Exact test and chi-squared test for discrete variables, and the Mann-Whitney test for continuous non-parametric variables. p-values were considered significant below 0.05.

RESULTS

None of the female participants in the study demonstrated clinical signs of precocious puberty and none of the male participants showed gynecomastia. Their height (Fig. 1) and weight, as BMI, were within the normal range compared with those of children of the same age, sex and race. Bone age corresponded to the chronological normal range (Fig. 2).

Serum levels of bone alkaline phosphatase, osteocalcin, oestradiol, and intact parathyroid hormone were similar to those of the control group. The levels of urinary markers of bone metabolism (deoxypyridoline, calciuria, phosphaturia and creatinuria) were all similar to those of the control group. Oestradiol was under the lowest detectable level for our RIA method (20 pg/ml) in all the children in both the SPF-fed group and the control group.

Univariate analysis showed only a significantly lower value of calciuria (1.84 ± 1.5 vs 2.4 ± 1.8, U 226.5, Z 2.08, p = 0.037) in SPF-fed infants versus the control group and a significantly higher value of phosphaturia only in SPF-fed infants aged 7-24 months (26.97 ± 15.17 vs 12.05 ± 0.12, U 6.5, Z

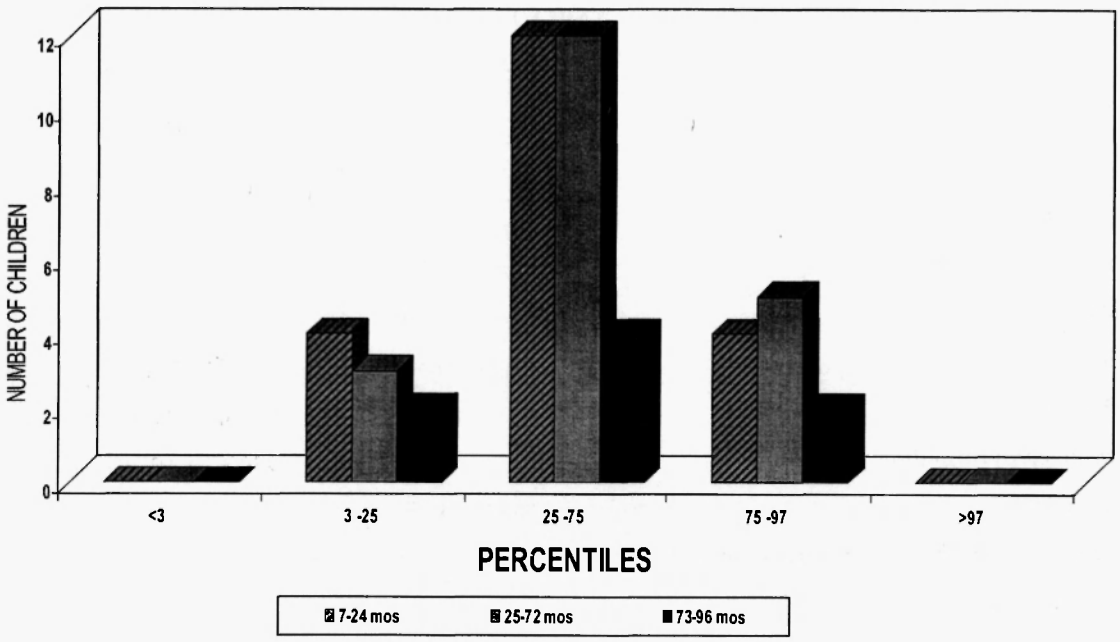

Fig. 1: Height of the children of the study groups A (7-24 months), B (25-72 months) and C (73-96 months) divided into percentile classes according to national anthropometric data.

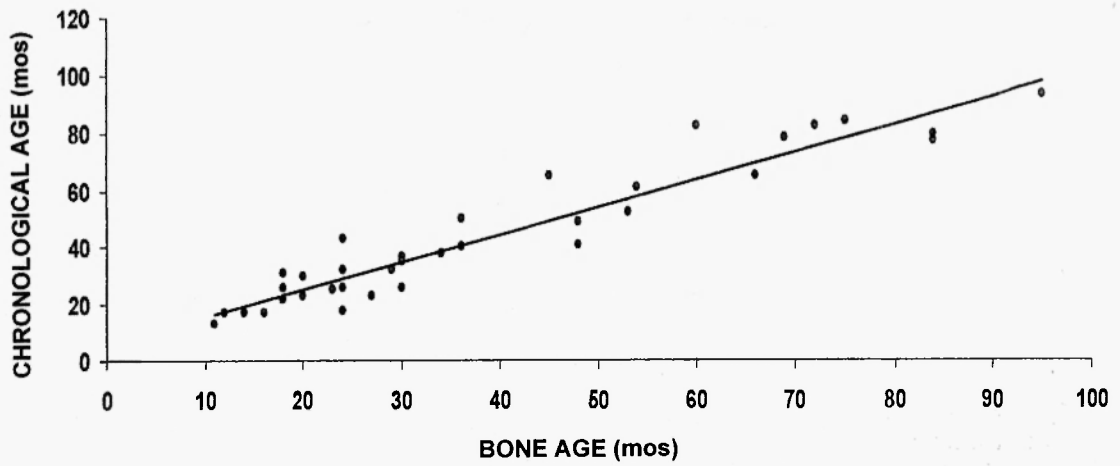

Fig. 2: Bone age and chronological age in children fed with soy protein formulas.

-2.3, p = 0.022) with respect to the age-related control group (Tables 1, 2).

DISCUSSION

It is well known that soy beans and foods derived from soy contain high concentrations of phyto-oestrogens. Few studies have examined the effects of phyto-oestrogens in infants, yet this has been a particularly controversial area. Setchell *et al.*[2] analysed isoflavone levels in five different SPFs, and studied plasma levels of genistein and daidzein in seven 4 month-old infants, compared to the levels in seven infants fed with CMF and seven

TABLE 1

Univariate analysis of laboratory data for study group versus control group irrespective of age

	Study group	Control group	p
Osteocalcin (ng/ml)	10.82 ± 5.45	10.57 ± 6.5	0.62
Parathyroid hormone (pg/ml)	27.58 ± 19.83	28.83 ± 23.35	0.73
Bone alkaline phosphatase (IU/l)	95.64 ± 30.22	99.94 ± 21.72	0.41
Calciuria (mmol/l)	1.84 ± 1.50	2.43 ± 1.84	**0.03**
Creatinuria (g/l)	0.64 ± 0.38	1.24 ± 2.18	0.28
Phosphaturia (mmol/l)	23.90 ± 15.34	19.48 ± 12.84	0.19
Urinary deoxypyridinoline (mmol/l)	201.50 ± 131.48	230.17 ± 107.07	0.29
Creatinine (mg/l)	5.60 ± 3.33	6.21 ± 3.11	0.44
Urinary deoxypyridinoline/creatinine (mmol/mg)	37.33 ± 12.41	38.43 ± 10.54	0.80

Data are expressed as means ± SD.

fed with breast milk. The isoflavone level was similar in all the five SPFs, and the plasma levels of genistein and daidzein were significantly higher in the SPF-fed infants than in those fed CMF or breast milk. The authors emphasized that the daily dose of phyto-oestrogens ingested by infants fed with SPF was 6-11 times the minimum dose per kilo which has been shown to have hormonal effects in adults who eat soy-based food. In infants fed with SPFs during the first months of life, the concentration of isoflavones was 13,000-22,000 times greater than the plasma concentration of oestradiol present in the first months of life in infants fed with SPFs, whereas the level was insignificant in infants fed with CMF or breast milk.

Absorption of isoflavones by infants was clearly demonstrated by the appearance of daidzein and genistein in the urine of 4 month-old SPF-fed infants, although there was not a high variability in the concentrations excreted; equol, a specific bacterial metabolite of daidzein, was not detected in urine. It was either not detectable or present only in trace amounts in the serum of 4 months-old SPF-fed infants, thus highlighting the requirement of a mature intestinal flora for this biotransformation[15]. Furthermore, Hugget *et al.* have postulated that most of the phyto-oestrogens (mainly genistein and daidzein) present in the plasma of SPF-fed infants are in a conjugated form, and therefore unable to cause hormonal effects[15]. Leiner[16] asserts that phyto-oestrogens have a low affinity for human oestrogen receptors, and therefore have only weak oestrogen-like effects.

The study of Irvine *et al.*[17] has shown that young infants are able to digest, absorb, and excrete genistein amd daidzein from SPFs as efficiently as adults consuming soy products.

There is no scientific evidence that intake of isoflavones at levels present in SPFs causes negative effects. Nonetheless, given evidence suggesting that early exposure to soy and/or isoflavones might have long-term effects, further research following infants fed SPFs into adulthood is warranted[18]. Epidemiological data from Asian countries[4], where soy consumption is common from an early age even in breast-fed infants, has shown a low rate of breast and prostate cancer, cardiovascular disease and osteoporosis later in life[5-13]. Since all the mentioned studies were carried out in adult populations and the controversy aroused by the study of Setchell *et al.*[2] has not yet been resolved, we thought it appropriate to determine whether the high levels of phyto-oestrogens present in SPFs could have demonstrable hormonal effects and, as a result,

TABLE 2

Univariate analysis of laboratory data for study groups A, B and C versus their age-related control groups

		Study group (A / B / C)	Control group (D_1 / D_2 / D_3)	p
Osteocalcin (ng/ml)				
A-D_1	7-24 months	11.85 ± 6.04	9.37 ± 6.54	0.43
B-D_2	25-72 months	9.64 ± 4.60	9.14 ± 4.55	0.71
C-D_3	>72 months	11.67 ± 6.56	21.50 ± 9.61	0.16
Parathyroid hormone (pg/ml)				
A-D_1	7-24 months	33.22 ± 25.83	38.00 ± 22.49	0.47
B-D_2	25-72 months	22.22 ± 12.07	25.08 ± 25.43	0.70
C-D_3	>72 months	26.00 ± 4.54	33.00 ± 9.89	0.35
Bone alkaline phosphatase (IU/l)				
A-D_1	7-24 months	105.18 ± 31.79	119.00 ± 10.58	0.25
B-D_2	25-72 months	88.31 ± 27.35	92.75 ± 22.28	0.46
C-D_3	>72 months	83.50 ± 27.29	105.00 ± 9.89	0.35
Calciuria (mmol/l)				
A-D_1	7-24 months	1.13 ± 0.73	1.45 ± 0.12	0.07
B-D_2	25-72 months	2.37 ± 1.80	2.84 ± 2.20	0.33
C-D_3	>72 months	1.63 ± 0.40	2.13 ± 0.38	0.16
Creatinuria (g/l)				
A-D_1	7-24 months	0.60 ± 0.38	0.91 ± 0.15	0.05
B-D_2	25-72 months	0.64 ± 0.39	1.44 ± 2.72	0.60
C-D_3	>72 months	0.86 ± 0.31	0.80 ± 0.84	1
Phosphaturia (mmol/l)				
A-D_1	7-24 months	26.97 ± 15.17	12.05 ± 0.12	**0.02**
B-D_2	25-72 months	22.44 ± 16.21	22.77 ± 14.31	0.95
C-D_3	>72 months	21.15 ± 12.47	16.3 ± 15.1	0.64
Urinary deoxypyridinoline (mmol/l)				
A-D_1	7-24 months	219.35 ± 140.64	322.85 ± 17.23	0.19
B-D_2	25-72 months	186.61 ± 135.77	195.08 ± 96.88	0.53
C-D_3	>72 months	212.70 ± 78.90	237.80 ± 201.66	1
Creatinine (mg/l)				
A-D_1	7-24 months	5.31 ± 3.41	7.92 ± 1.22	0.07
B-D_2	25-72 months	5.42 ± 3.38	5.43 ± 2.74	0.79
C-D_3	>72 months	7.63 ± 2.76	7.12 ± 7.44	1
Urinary deoxypyridinoline /creatinine (mmol/mg)				
A-D_1	7-24 months	41.86 ± 12.74	40.75 ± 5.92	0.92
B-D_2	25-72 months	35.63 ± 11.99	38.27 ± 12.61	0.64
C-D_3	>72 months	29.25 ± 8.83	34.70 ± 5.65	0.64

Data are expressed as means ± SD.

negatively modify the physical development of infants fed with such formulas. No signs of oestrogenization were observed and the markers of bone metabolism were all normal even after 82 months (two patients in study group C) of soy milk ingestion.

This clinical study is a confirmation of Leiner's theory[16] that phyto-oestrogens have a low affinity for human oestrogen receptors and therefore have only weak effects in biological assays[17]. Furthermore, our findings are consistent with a recent controlled study of Strom *et al.*[19] who found neither positive nor negative effects in individuals exposed in infancy to SPFs when compared with those exposed to CMFs.

We believe that the lower values of calciuria that we found in SPF-fed infants versus the control group as well as the higher values of phosphaturia in SPF-fed infants aged 7-24 months compared to their age-related control group should be confirmed with a higher number of children before drawing conclusions from these findings.

We conclude from this study that long-term feeding with SPFs in early life does not seem to produce oestrogen-like hormonal effects.

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