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ORIGINAL ARTICLE

Soy protein supplement intake for 12 months has no effect on sexual maturation and may improve nutritional status in pre-pubertal children

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Aim: To evaluate the intake of a soy protein-based supplement (SPS) and its effects on the sexual maturation and nutritional status of prepubertal children who consumed it for a year.

Methods: Healthy children (n = 51) were recruited and randomly assigned to consume the lunch fruit juice with (n = 29) or without (n = 22) addition of 45 g of a commercial soy protein-based supplement (SPS) over 12 months. Nutritional assessment including anthropometry (bodyweight, height, triceps skinfold thickness, mid-upper arm circumference), body mass index (BMI), upper arm muscle area, arm muscle circumference, upper arm area, upper arm fat area data were derived from measures using usual procedures; age and gender-specific percentiles were used as reference. Sexual maturation was measured by Tanner stage. Isoflavones were quantified using liquid chromatography and tandem mass spectrometry.

Results: Height, BMI/age, weight/age and height/age were significantly different (P < 0.05) at 12 months between girls in the control and intervention groups. Statistically significant differences between groups by gender (P < 0.05) were found in boys in the control group for the triceps skinfold thickness and fat area. Nutritional status was adequate according to the World Health Organization parameters. On average, 0.130 mg/kg body weight/day of isoflavones were consumed by children, which did not show significant differences in their sexual maturation.

Conclusion: Consumption of SPS for 12 months did not affect sexual maturation or the onset of puberty in prepubertal boys and girls; however, it may have induced an increase in height, BMI/age, height/age and weight/age of the girls, associated with variations in fat-free mass.

Key words: isoflavones; nutritional status; prepubertal children; sexual maturation; soy protein isolate.

What is already known on this topic

1 Soy proteins are quite similar to that of meat and eggs, with a protein digestibility-corrected amino acid score reported in the range of 0.92–0.99 and a digestibility of 95–98%, similar to that of animal protein.
2 The isoflavones are non-covalently bound to soy proteins and co-precipitate during the process. A topic of concern regards isoflavones related to estrogenic activity, nutritional adequacy and sexual development.
3 Some studies suggest that soy protein formulas and supplements for both children and youth do not have health risks at the level of the endocrine system or bone metabolism due to the presence of isoflavones; however, some other studies on the estrogenic effect of isoflavones on children of prepubertal age who consume soy foods are controversial and not conclusive regarding the possible adverse effects on development and sexual maturation.

What this paper adds

1 Consumption of about 0.130 mg/kg bodyweight/day of isoflavones in a soy protein-based supplement for 12 months did not affect sexual maturation or the onset of puberty in prepubertal boys and girls.
2 The changes found in weight, height, body mass index/age, height/age and weight/age in girls could be associated with variations in fat-free mass, induced by the consumption of the nutritional supplement, as no significant changes were found in anthropometric arm indicators.

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Soy protein isolate is obtained from defatted soybean flour after an alkaline extraction of the protein and acid precipitation.[1] Soy isoflavones precipitate during the process and are non-covalently bound to the protein. Isoflavones are a class of phytoestrogens, a group[2] of plant compounds with non-steroidal structure, with an

aromatic ring in their chemical structure monosubstituted by a hydroxyl group.[3] The amount of isoflavones in soy protein isolate may vary according to the source of the soybean and factors related to the processing, such as pH, temperature and solvents.[4] Isoflavones are found in two forms. Glycosides are biologically inactive and attached to sugar unit, whereas aglycones are the correspondent free forms that are biologically active. The molecular weights and structural characteristics are similar to 17 β-estradiol. The estrogenic activity of the isoflavones is associated with the structural similarity that may enable them to bind to specific oestrogen receptors β and exert estrogenic or antiestrogenic actions.[3]

The presence of isoflavones in soy protein isolates has been controversial. The National Toxicology Program and The Center for the Evaluation of Risks to Human Reproduction found that 'The overall evidence was considered insufficient to reach a conclusion on whether the use of soy infant formula produces or does not produce developmental toxicity with infant exposure in girls at recommended intake levels (10–47 mg/L of formula)'.[5] In addition, the American Academy of Pediatrics stated that, 'There is no conclusive evidence in animal, adult human or infant populations that soy isoflavones in the diet may adversely affect human development, reproduction or endocrine function'.[6] However, in the last decade, prepubertal girls who consumed soy foods and showed high levels of isoflavones in blood or urine have been associated with the risk of central precocious puberty [7] or an increased risk of menarche specifically in early adolescence.[1] On the contrary, it is also reported that girls with higher prepubertal isoflavone intake appear to enter puberty at a later age.[8] Some other studies suggest that long-term feeding with SPF in early life does not seem to produce oestrogen-like hormonal effects[9] and supports normal growth and may have advantages in promoting bone development.[2]

Some reviews weigh the evidence for and against the health benefits and adverse effects of phytoestrogens[10] and soy-based infant formulas.[11] Relatively few studies have explored the possibility that soy protein-based supplement (SPS) consumption in children may affect reproductive development, and given the conflicting evidence regarding exposure to soy protein supplements in infancy as well as the possible effects on the onset of puberty, it is in our interest to understand the effects of SPS in prepubertal children. The aim of the study was to evaluate the effects of the daily consumption of a commercial SPS for a year on the nutritional status and sexual maturation of children aged 7–9 years.

Methods

The population is a subsample (51 children aged 7–9 years) of an experimental study among children aged 2–9 years from public schools and participating in community dining rooms in Bogota, Colombia. Details of the study design were previously published.[12] Briefly, based on a sample size calculation, a randomised controlled trial was designed, and 150 children, aged 2–9 years, attending public schools and participating in three community dining rooms in Bogota were enrolled. All participants were healthy prepubertal children (Tanner 1) according to normal standards established by the World Health Organization (WHO), born at term (>37 weeks) and with normal weight at birth. Informed consent was obtained from them as well as from children 7 years or older. Paediatric and nutritional assessments were then performed and children screened for eligibility according to inclusion criteria, mentioned in the paragraph above, to classify nutritional status, and WHO growth reference standards were followed.[13] Participants were randomly assigned using a computer-generated random number sequence to either to an intervention group that received the lunch fruit juice with addition of a commercial preparation of SPS during 12 months or to the control group that consumed the lunch juice and a drink based on whole milk. A staff member in each dining room involved in the intervention was aware of group assignments and prepared, delivered and registered supplement record adherence daily.

Intervention

A total of 45 g of the soy protein supplement, which provides 230 kcal (flavours: strawberry, chocolate and vanilla), were dissolved in 190 cm^3 of the menu fruit juice, administered from Monday to Saturday for 12 months. The control group received the menu fruit juice and a drink based on whole milk. On Sundays, the parents or caretakers were given the supplement powder to give to the children in a similar way. Nutritionists performed home visits randomly on Sundays to verify supplement consumption, adherence and perception of participants and to give dietary advice to parents and caregivers (for supplement composition, see Table S1 (Supporting Information)).

Anthropometric measures were obtained by a trained nutritionist at 0, 6 and 12 months of the study using standardised methods, which refer to routine anthropometric protocol followed by the trained nutritionist according to the International Society for the Advancement of Kinanthropometry. Child height was measured to the nearest 0.1 cm using a Seca 213 mobile stadiometer (Seca, Hamburg, Germany). Weight was measured to the nearest 0.1 kg on a Tanita UM-051 scale (Tanita Corporation of America, Inc., Arlington Heights, IL, USA) with a tare function and the children wearing only a pair of shorts and a t-shirt. Weight (kg) and height (cm) were used to construct the anthropometric indices, body mass index for age (BMI/A), height for age (H/A) and weight for age (W/A). Height, weight and BMI *Z*-scores were calculated using WHO Anthroplus software (version 1.0.4; WHO, Geneva, Switzerland). The triceps skinfold thickness (T) was measured using a Harpenden Skinfold Caliper (HaB International Ltd., Southam, UK). Upper arm circumference (C) was measured using a Lufkin W606PM Anthropometric Tape (Champion Tape, Sturtevant, WI, USA). Upper arm muscle area (M), arm muscle circumference (CM), upper arm area (A) and upper arm fat area (F) were derived from measures using usual procedures. All estimates were calculated for each subject; age and gender-specific percentiles were used as reference for each of the variables.[14]

Evaluation of Tanner stage

Radiography of the hand and wrist was taken at the beginning (0 months) and at the end of the study (12 months), read by one paediatric endocrinologist and one radiologist independently, and bone age was calculated using the method of Greuchlich and

Pyle.[15] Growth velocity in height (cm/year) was calculated from data obtained at 0, 6 and 12 months. Physical examinations of the children was evaluated by a paediatric endocrinologist and pubertal stage established by a Tanner scale.[16]

The identification and quantification of isoflavones (aglycones and glycones) in the nutritional supplement were performed by liquid chromatography–mass spectrometry (MS)/MS using the method of Clarke *et al*.[17] with some modifications, and a standard addition method was implemented for the quantitative analysis. The isoflavone standards biochanin A, genistin, genistein, daidzin, daidzein, glycytin, glycitein, formanonetin and ononine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Separation by liquid chromatography was performed using Waters' Acquity Ultra Performance Liquid Chromatography system equipment with a hypersil GOLD C-18 column (100 × 2.1 mm 3 μm particle size); sample ionisation and detection was performed by electrospray (ESI/MS) using a Waters Xevo TQD System in the negative ion mode.

Statistical analysis

The nutritional supplement intake was determined from the average consumption of 7–9-year-old children in g/day ± standard deviation; consumption of isoflavones was determined based on that value and the supplement concentration determined.

A linear model was used for the final measure, which considered the gender and group factors for the variables measured at two instances (0 and 12 months). For the variables weight (kg), height (cm) and the indicators expressed in *Z*-scores measured in three moments (0, 6, 12 months), a mixed model of measures repeated over time, we considered the factors of gender, group, time and the interaction between group and time. Comparisons of means between groups, time and gender were made using the *t*-test for the variables triceps skinfold thickness (T), upper arm circumference (C), upper arm muscle area (M), arm muscle circumference (CM), upper arm area (A), upper arm fat area (F), and data were categorised in percentiles and evaluated using frequency measurements; a χ^2 test to compare the groups at the measurement times. The measurement at time 0 was used as a covariable in all models to correct initial differences between groups, and a P value <0.05 was considered significant. All analyses were performed using the R software (R Foundation for Statistical Computing, Vienna, Austria)

Ethics approval

The study followed local and international ethical guidelines and was approved by the ethical committee (CIE-122-2014) of the Faculty of Sciences at the Pontificia Universidad Javeriana, Bogota, Colombia.

Results

Anthropometry

Characteristics of the participants are shown in Table 1. The increase in weight was expected, without reaching statistically significant differences at 6 or 12 months between the control and intervention groups ($P > 0.05$); however, for height, BMI/A, W/A and H/A, statistically significant differences ($P < 0.05$) at 12 months were found between the study groups (Table 2). When comparisons were made between groups by gender, it was found that these differences were statistically significant in girls from the intervention group (Table 3). Despite these differences,

Table 1 General characteristics of the participants in the study

	Intervention (n = 29)		Control (n = 22)	
	Boys (n = 13)	Girls (n = 16)	Boys (n = 11)	Girls (n = 11)
Age, years				
Mean ± SD	8.8 ± 0.7	8.3 ± 0.8	8.4 ± 0.8	8.2 ± 0.9
Median	9	8.1	8.2	8
Range	7.1–9.8	7.2–9.5	7.0–9.6	7.0–10.0
Body weight, kg				
Mean ± SD	28.3 ± 3.7	25.5 ± 4.1	25.3 ± 3.9	24.5 ± 3.7
Median	27.4	25.1	26.4	22.6
Range	22.8–35.6	20.2–29.2	17.0–28.6	21.6–32.0
W/A *Z*-score	0.057 ± 0.6	0.03 ± 1.0	0.01 ± 0.6	–0.31 ± 0.5
Height, m				
Mean ± SD	1.30 ± 0.05	1.25 ± 0.07	1.24 ± 0.04	1.22 ± 0.06
Median	1.31	1.24	1.24	1.2
Range	1.21–1.36	1.16–1.35	1.18–1.32	1.16–1.36
H/A *Z*-score	–0.26 ± 0.6	–0.34 ± 0.9	–0.81 ± 0.5	–0.67 ± 0.6
Body mass index				
Mean ± SD	16.6 ± 1.2	16.2 ± 1.2	17.4 ± 1.5	16.4 ± 1.3
Median	16.4	16.1	17.2	15.9
Range	14.9–19.5	14.5–18.1	15.0–19.8	14.9–19.2
BMI/A *Z*-score	0.32 ± 0.6	0.29 ± 0.9	0.72 ± 0.8	0.19 ± 0.6

BMI/A, body mass index for age; H/A, height for age; SD, standard deviation; W/A, weight for age.

Table 2 Assessment of nutritional status among study groups (intervention and control) over time (0, 6 and 12 months)

	Intervention (n = 29)			Control (n = 22)			P value		
	0	6	12	0	6	12	0	6	12
Weight, kg	26.5 ± 3.8	28.9 ± 4.5	31.2 ± 5.3	24.9 ± 3.8	26.9 ± 3.9	28.4 ± 4.4	0.705	0.355	0.064
Height, m	1.27 ± 0.1	1.30 ± 0.1	1.34 ± 0.1	1.24 ± 0.1	1.27 ± 0.1	1.30 ± 0.1	0.871	0.459	0.005
Body mass index (*Z*-score)	0.32 ± 0.7	0.35 ± 0.7	0.37 ± 0.9	0.52 ± 0.7	0.23 ± 0.6	0.15 ± 0.8	0.857	0.075	0.008
Weight/Age (*Z*-score)	0.04 ± 0.7	0.2 ± 0.7	0.3 ± 0.9	−0.15 ± 0.6	−0.17 ± 0.5	−0.39 ± 0.5	0.710	0.057	0.003
Height/Age (*Z*-score)	−0.35 ± 0.7	−0.21 ± 0.7	−0.15 ± 0.7	−0.77± 0.5	−0.67 ± 0.5	−0.80 ± 0.5	0.450	0.275	0.003

Table 3 Assessment of nutritional status among study groups (intervention and control) by sex (boys and girls) through time (0, 6 and 12 months)

	Intervention (n = 29)			Control (n = 22)			P value		
	0	6	12	0	6	12	0	6	12
Boys									
Weight	27.9 ± 3.8	29.9 ± 4.3	32.1 ± 5.2	25.3 ± 3.9	28.1 ± 3.8	30.1 ± 4.4	0.984	0.993	0.998
Height	1.29 ± 0.1	1.33 ± 0.1	1.36 ± 0.1	1.24 ± 0.1	1.28 ± 0.1	1.30 ± 0.1	0.997	0.999	0.793
Body mass index (*Z*-score)	0.29 ± 0.6	0.29 ± 0.6	0.40 ± 0.9	0.64 ± 0.7	0.24 ± 0.7	0.32 ± 0.8	0.957	0.700	0.881
Weight/Age (*Z*-score)	0.01 ± 0.6	0.15 ± 0.6	0.22 ± 0.8	0.02 ± 0.6	−0.03 ± 0.5	−0.07 ± 0.6	1.00	0.621	0.644
Height/Age (*Z*-score)	−0.33 ± 0.6	−0.21 ± 0.6	−0.19 ± 0.6	−0.86 ± 0.4	−0.70 ± 0.4	−0.80 ± 0.5	0.919	0.999	0.595
Girls									
Weight	25.6 ± 3.6	28.1 ± 4.6	30.6 ± 5.5	24.5 ± 3.7	25.6 ± 3.7	26.6 ± 3.9	0.999	0.429	0.035
Height	1.25 ± 0.1	1.28 ± 0.1	1.33 ± 0.1	1.22 ± 0.1	1.26 ± 0.1	1.29 ± 0.1	0.999	0.777	0.016
Body mass index (*Z*-score)	0.35 ± 0.8	0.40 ± 0.8	0.36 ± 0.9	0.39 ± 0.6	0.21 ± 0.6	−0.01 ± 0.7	0.997	0.52	0.018
Weight/Age (*Z*-score)	0.10 ± 0.9	0.19 ± 0.9	0.30 ± 0.9	−0.31 ± 0.5	−0.33 ± 0.4	−0.68 ± 0.4	0.953	0.505	0.026
Height/Age (*Z*-score)	−0.36 ± 0.8	−0.21 ± 0.7	−0.11 ± 0.7	−0.67± 0.6	−0.67 ± 0.5	−0.80 ± 0.6	0.977	0.476	0.016

the nutritional status of the children who were part of the study was adequate according to the parameters of the WHO.

No statistically significant differences were found in triceps skinfold thickness (T), upper arm circumference (C), upper arm muscle area (M), arm muscle circumference (CM) and upper arm fat area (F) between the control and intervention groups ($P > 0.05$) (Table 4). However, statistically significant differences between groups by gender ($P < 0.05$) were found in boys in the control group for the triceps skinfold thickness (T) and upper arm fat area (F) (Table 5), with a decrease in the percentage of children between the percentiles 25 and 75 and an increase in the 75–90 percentiles (Figures S1 and S2, Supporting Information).

For the upper arm muscle area (M) and arm muscle circumference (CM), an increase in the percentage of children in the control and intervention groups and in girls in the control group was found in the 25–75 percentiles; however, it was only significant for the children in the control group (Figures S3 and S4, Supporting Information) but did not become statistically significant with respect to the children in the intervention group ($P > 0.05$) (Table 5). In addition, there was a slight increase in the percentage of boys and girls in the intervention group in the 75–90 percentiles (Figures S3 and S4, Supporting Information). The increase in the percentage of children prone to excess in triceps skinfold thickness (T) and upper arm fat area (F) who did not consume the nutritional supplement probably indicates that prepubertal boys tend to have a greater reserve of fatty tissue in these areas than girls, as has been reported in other studies in India.

An increase in the percentage of children in the control group was observed between the 25 and 75 percentiles for upper arm circumference (C), while in the boys and girls in the intervention group, the same trend was maintained throughout the study (Figure S5, Supporting Information).

Sexual maturation

Growth velocity measured at 6 and 12 months showed no statistically significant differences between the control and intervention groups ($P > 0.05$) or by gender between groups ($P > 0.05$) (Table 6). Likewise, no significant differences were found for bone age ($P > 0.05$). All participants were in Tanner 1 at the beginning and remained in this state until the end of the study.

Isoflavones quantification and nutritional supplement intake

The glycones genistin and daidzin demonstrated higher concentrations, 73.5 and 26.5%, respectively, followed by their corresponding aglycones genistein (65.7%) and daidzein (28.2%); glycitein was found in lower concentration (6.01%) (Table 7).

Table 4 Assessment of body composition among groups (intervention and control) through time (0, 6 and 12 months)

	Intervention									Control									*P* value		
	Percentage of children at 0 months			Percentage of children at 6 months			Percentage of children at 12 months			Percentage of children at 0 months			Percentage of children at 6 months			Percentage of children at 12 months			0	6	12
	P < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75			
T	6.9	61.8	31.7	2.9	76. 3	20.8	13.8	69.0	17.2	17.2	65.6	17.2	22.7	54.6	22.7	22.7	45.5	31.8	0.885	0.622	0.090
F	20.7	62.1	17.2	10.3	72.4	17.2	17.2	65.5	17.2	31.8	63.6	4.55	31.8	59.1	9.1	31.8	45.5	22.7	0.602	0.210	0.180
M	51.7	37.9	10.4	41.4	58.6	0.0	24.1	62.1	13.8	72.7	27.3	0.0	59.1	40.9	0.0	36.4	63.6	0.0	0.277	0.586	0.234
C	34.5	51.7	13.8	24.1	72.4	3.4	27.6	55.2	17.2	59.1	40.9	0.0	45.5	54.5	0.0	40.9	59.1	0.0	0.265	0.616	0.737
MC	55.2	37.9	6.9	41.4	58.6	0.0	27.6	55.2	17.2	72.7	27.3	0.0	59.1	40.9	0.0	40.9	59.1	0.0	0.499	0.907	0.655

P < 25: Depletion; *P* = 25–75: Normal; *P* > 75: Excess. C, arm circumference; F, fat area; M, muscle area; MC, arm muscle circumference; T, triceps skinfold thickness.

Table 5 Assessment of body composition among groups (intervention and control) by sex (boys and girls) through time (0, 6 and 12 months)

	Intervention										Control									*P* value		
	n	Percentage of children at 0 months			Percentage of children at 6 months			Percentage of children at 12 months			*n*	Percentage of children at 0 months			Percentage of children at 6 months			Percentage of children at 12 months			0	6	12
		P < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75		*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75	*P* < 25	*P* = 25–75	*P* > 75			
Boys	13										11											
T		15.4	53.8	30.8	0.0	76.9	23.1	0.0	84.6	15.4		27.3	63.6	9.1	18.2	63.6	18.2	27.3	27.3	45.5	0.640	0.826	0.003
F		23.1	61.5	15.4	0.0	69.2	30.8	0.0	84.6	15.4		27.3	63.6	9.1	18.2	63.6	18.2	27.3	27.3	45.5	0.936	0.553	0.002
M		77.0	7.7	15.4	62.0	38.0	0.0	23.1	61.5	15.4		63.7	36.4	0.0	72.8	27.3	0.0	18.2	81.8	0.0	0.352	0.995	0.697
C		46.2	46.2	7.7	30.8	69.2	0.0	30.8	46.2	23.1		45.5	54.5	0.0	45.5	54.5	0.0	27.3	72.7	0.0	0.910	0.995	0.363
MC		84.6	7.7	7.7	61.6	38.5	0.0	30.8	46.2	23.1		63.7	36.4	0.0	72.8	27.3	0.0	27.3	72.7	0.0	0.426	0.999	0.475
Girls	16										11											
T		25.1	56.3	18.8	18.8	75.0	6.3	31.3	50.0	18.8		27.3	63.6	9.1	45.5	54.5	0.0	27.3	63.6	9.1	0.805	0.319	0.562
F		18.8	62.5	18.8	18.8	75.0	6.3	31.3	50.0	18.8		36.4	63.6	0.0	45.5	54.5	0.0	36.4	63.6	0.0	0.559	0.344	0.529
M		31.3	62.5	6.3	25.0	75.0	0.0	25.0	62.5	12.5		81.9	18.2	0.0	45.5	54.5	0.0	54.6	45.5	0.0	0.090	0.298	0.593
C		25.0	56.3	18.8	31.3	62.5	6.3	25.0	62.5	12.5		81.9	18.2	0.0	54.6	45.4	0.0	54.6	45.4	0.0	0.054	0.738	0.188
MC		31.3	62.5	6.3	25.0	75.0	0.0	25.0	62.5	12.5		81.9	18.2	0.0	54.6	45.4	0.0	54.6	45.4	0.0	0.090	0.298	0.271

P < 25: Depletion; *P* = 25–75: Normal; *P* > 75: Excess. C, arm circumference; F, fat area; M, muscle area; MC, arm muscle circumference; T, triceps skinfold thickness.

Table 6 Assessment of sexual maturation

	Grow velocity, cm/year		*P* value	Bone age		*P* value
	Beginning	End		Beginning	End	
Intervention (*n* = 29)	7.1 ± 2.3	6.8 ± 5.6	0.512	7.3 ± 1.2	8.6 ± 1.6	0.461
Control (*n* = 22)	6.6 ± 2.2	5.2 ± 1.8		7.1 ± 1.6	8.1 ± 1.4	
Boys intervention (*n* = 13)	6.3 ± 1.2	5.3 ± 1.5	0.976	7.0 ± 1.5	8.2 ± 1.6	1.00
Boys control (*n* = 11)	6.5 ± 1.6	5.0 ± 1.7		6.6 ± 1.5	7.9 ± 1.4	
Girls intervention (*n* = 16)	7.8 ± 2.8	8.0 ± 7.2	0.955	7.5 ± 0.9	8.7 ± 1.6	0.719
Girls control (*n* = 11)	6.6 ± 2.7	5.4 ± 1.9		7.6 ± 1.5	8.5 ± 1.4	

Table 7 Content of isoflavones in the nutritional supplement

Isoflavones	Content, μg/g	%
Glycones		
Genistin	116.2	73.50
Daidzin	41.9	26.50
Aglycones		
Genistein	74.4	65.72
Daidzein	32	28.27
Glicitein	6.8	6.01
Total isoflavones	271.3	
Glycones	158.1	58.27
Aglycones	113.2	41.72

Total isoflavones expressed as the sum of glycones and aglycones.

Table 8 Intake of nutritional supplement and isoflavones

Intake	Mg/day	Mg/kg body weight/day
Supplement, g	38.59 ± 2.5	
Total isoflavones	3.49 ± 0.2	0.130 ± 0.04
Aglycones		
Genistein	0.957 ± 0.06	0.036 ± 0.01
Daidzein	0.413 ± 0.03	0.015 ± 0.01
Glycitein	0.089 ± 0.01	0.003 ± 0.01
Glycones		
Genistin	1.494 ± 0.09	0.056 ± 0.01
Daidzin	0.540 ± 0.03	0.020 ± 0.01

Mean daily supplement intake in children was 38.59 ± 2.5 g/day. The mean daily total intake of isoflavones was 3.49 ± 0.2 mg/day; this consumption corrected for BW was 0.130 ± 0.04 mg/kg BW/day (Table 8).

Discussion

Although BMI in boys and girls tends to be similar, variations in muscularity, adiposity and nutritional status can be attributed to several factors, such as race, eating habits, physical activity, socioeconomic status and gender. In addition, the patterns of fat storage are related to the sexual dimorphism attributed especially to the action of steroid hormones such as oestrogen, which increase fat storage, resulting in being greater in women than men.[18] On the other hand, it has been reported that, for boys and girls from mid-childhood onwards (after adiposity rebound), the increase in BMI/A has been attributed to the increase in fat-free mass relative to height[19] What could possibly have happened to the girls who consumed the nutritional supplement in this study, although macronutrient intake assessed from the acceptable macronutrient distribution range (AMDR) was within the recommended range. Changes in the intake of nutrients were greater for this group (data not shown), particularly in terms of protein intake.

Arm anthropometry has received considerable attention during the last decades but has not been widely adopted for the evaluation of body composition and nutritional status. A positive correlation between the triceps skinfold thickness (T) and upper arm fat area (F) with BMI for age was demonstrated in Argentinian boys and girls from 6 to 13 years of age[20] comparable with studies that emphasised the relationship of BMI to fat mass or fat percentage.[21] However, some studies have shown that, for growing children, BMI was influenced by a large increase in fat-free mass, with small changes in fat mass index in girls, contrary to boys, doubling the fat mass index and demonstrating small increases in the fat-free mass index.[22]

Recently, it has been shown, in boys and girls aged 5–12 years, that the upper arm muscle area (U) and upper arm circumference (C) of boys was higher than that of girls except at 5, 7 and 11 years of age.[23] This same tendency, but for M, was reported in children and adolescents 6–20 years old in India.[24] It has also been reported that the upper arm fat area is significantly higher in girls than in boys[23,24]; however, it has been shown that it decreases in children 5–7 years old and subsequently increases from 7 to 10 years old.[23] This latter trend was found in the control group of this study as a significant increase in the percentage of children in the 75–90 percentiles for upper arm fat area (Figure S2, Supporting Information).

Our results are comparable with others studies with infant formulas based on soy protein isolate.[25] According to the literature, genistein is between 52 and 67%, followed by daidzein, 24 to 42.4%, and finally glycitein within 5.2–12%. Some studies with Korean children aged 7–12 years showed that the total intake of isoflavones ranged from 3.67 to 12.6 mg/day[26,27]; other Asian regions reported daily intake of 5.4–20 mg.[28] In Australia, a study reported consumption of 6.62–14.05 mg/m^2 of body surface area/day for infants and 22.7 mg/m^2 of body surface area/day for

adults.[29] In western countries, a study reported that 9-year-old girls in the USA have an intake of 1.7–2.2 mg/day[6]; in German girls aged 6–7, intake was 4–19.1 μg/day[5] and 0.5 mg/day for a German population aged 3–18 years.[30] In our study, consumption of isoflavones from the supplement (0.130 ± 0.04 mg/kg BW/day or 3.49 mg/day) is below that reported for children aged 7–12 years in Korea (8.3 mg/day)[26] and Italian boys 7–9 years (1.1 mg/kg BW/day).[31]

A high intake of isoflavones seems to be more likely in the Asian region due to the consumption of traditional soy-based foods such as tofu, soy drinks, soy sauce and red pepper paste.[11,27] However, food consumption based on soy protein isolates and soy protein concentrates, used in many western countries for the preparation of infant formulas and nutritional supplements, could increase the consumption of isoflavones when these products are incorporated into the diet.

It has been well documented that the oestrogenic activity of these phytoestrogens depends not only on the amount consumed but also on other factors such as the timing of exposure, oestrogen receptor status, hormonal profiles (endogenous oestrogen levels) and source of isoflavones (glycones or aglycones) consumed.[7] Isoflavones are found in soy and its derivatives, mainly as glycones, the biologically inactive form, which are hydrolysed by intestinal beta glucosidases to release the biologically active glycone. These are absorbed by the small intestine, and some are metabolised in the large intestine, to obtain metabolites such as equol, orthodimethylangolysin, dihydrogenistein and other less abundant species, which along with the unmetabolised aglycones circulate through the bloodstream and are excreted through the urine.[32]

Some studies have reported that factors associated with diet and intestinal microbiota status, specifically related to some microorganisms involved in phytoestrogen metabolism, could vary the bioavailability of aglycones and their metabolites,[33,34] which appears to be a relevant factor on oestrogenic effects. A case–control study reported a relationship between precocious puberty and high serum levels of isoflavones (daidzein and genistein) in 6–10-year-old Korean girls.[7] This agonist effect has also been found in girls fed at an early age with soy-based beverages, who presented menarche at an earlier age, with respect to girls who were fed primarily with breast milk.[1] Dietary patterns or serum concentrations of isoflavones were not evaluated in this study, which could account for the ability of this population to hydrolyse glycones to aglycones and determine their bioavailability. Taking into account that the oestrogenic effect of the isoflavones could be conditioned by the state of the intestinal microbiota for hydrolysis, depending on specific dietary patterns in each region, it is conceivable that this could affect the bioavailability of aglycones in the blood and would therefore be a possible explanation for the results in this study.

In children, no studies so far have been reported on the association between isoflavone consumption and the time of puberty. Although most studies involve girls, the results have shown great variability in oestrogenic effects. Particularly in this study, no evidence of these effects was found in the intervention group, which is probably associated with the bioavailability of aglycones in blood and factors associated with diet that were not evaluated and would be relevant for future studies.

This study has also some limitations. First, this was a limited sample because the population was a subsample of a randomised controlled trial with 109 participants aged 2–9 years attending public dining rooms and receiving the nutritional supplement for 12 months. Second, in terms of follow-up, losses were about 20% in the original study, which may lead to a bias in our results. Further studies are necessary with longer periods of supplementation and more participants both in the intervention and control groups.

Conclusion

In this study, the isoflavone intake measured for the intervention group was lower than that reported by boys and girls in Asian countries and the maximum recommended intake for Italian children but higher was than that for girls in western countries. This consumption of SPS for 12 months did not affect sexual maturation and the onset of puberty. Regarding nutritional status, BMI/A, W/A and H/A were higher in girls from the intervention group and significantly different compared to the control group but remained normal according to WHO parameters. No statistically significant differences were found in the body composition parameters evaluated.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Percentage distribution in percentiles of the study groups (intervention (1) and control (0) for triceps fold by gender (boys (1) and girls (2)) through time (0, 6, 12 months).
Fig. S2. Percentage distribution in percentiles of the study groups (intervention (1) and control (0) for fat area by gender (boys (1) and girls (2)) through time (0, 6, 12 months).
Fig. S3. Percentage distribution in percentiles of the study groups (intervention (1) and control (0) for muscle area by gender (boys (1) and girls (2)) through time (0, 6, 12 months).
Fig. S4. Percentage distribution in percentiles of the study groups (intervention (1) and control (0) for muscle circumference by gender (boys (1) and girls (2)) through time (0, 6, 12 months).
Fig. S5. Percentage distribution in percentiles of the study groups (intervention (1) and control (0) for arm circumference, by sex (boys (1) and girls (2)) through time (0, 6, 12 months).
Table S1. Nutritional supplement composition.