applied sciences

MDPI

Article

Intake of Phytoestrogens and Estrogenic Effect of the Diet of Female University Students in Mexico

Diana Espino-Rosales [1,2], Leticia Heras-Gonzalez [1,3,4], Maria J. Jimenez-Casquet [1], Nicolás Olea [3,4,5], Fátima Olea-Serrano [1] and Miguel Mariscal-Arcas [1,4,*]

[1] Health Science and Nutrition Research (HSNR-CTS1118), Department of Nutrition and Food Science, School of Pharmacy, University of Granada, 18071 Granada, Spain; dradianaespino@gmail.com (D.E.-R.); leticia.heras.sspa@juntadeandalucia.es (L.H.-G.); mariajosejcf@correo.ugr.es (M.J.J.-C.); folea@ugr.es (F.O.-S.)
[2] Faculty of Physical Education and Sport Sciences, Autonomous University of Chihuahua, Chihuahua 31009, Mexico
[3] University Hospital of Virgen de las Nieves, 18014 Granada, Spain; nolea@ugr.es
[4] Instituto de Investigación Biosanitaria de Granada (ibs.GRANADA), 18012 Granada, Spain
[5] Radiology and Physical Medicine Department, University of Granada, 18016 Granada, Spain
* Correspondence: mariscal@ugr.es

Abstract: Phytoestrogens are components naturally occurring in plants and include many foods that are part of the regular diet of animals and humans. Phytoestrogens are xenoestrogens of plant origin that are not produced in the endocrine system. Phytoestrogens can act as either agonists or antagonists, depending on their tissue concentrations and the levels of endogenous estrogens at various life stages. The aim was to evaluate the intake of phytoestrogens and the estrogenic effect of the diet of women at university in Chihuahua (Mexico). In total, 400 female university students individually filled out a food frequency questionnaire (FFQ) that included 120 foods. Estimates of the intake of phytoestrogen (genistein, daidzein, biochanin A, formononetin, matairesinol, coumestrol, enterolactone, secoisoresinol, enterodiol) in the subjects' daily diet were based on published reports. Quantification of phytoestrogens was expressed in μg day^{-1}. The estrogenic effect of those compound identified according to the foods consumed was estimated using the in vitro E-SCREN test. SPSS v.22.0 (IBM, Chicago, IL, USA) was applied for statistical analysis following descriptive analysis and stepwise regression. $p < 0.050$ was taken as significant. The results of intake show that the majority of isoflavones are formononetin (median 110.60 (μg day^{-1}) and their estrogenic activity is 4.11 Eq. E2 (pmol day^{-1}); the majority of lignans are enterolactone (median 147.24 (μg day^{-1}), and their estrogenic activity is 4.94 Eq. E2 (pmol day^{-1}). The total phytoestrogen estrogenic effect is measured in pM of E2, with a mean of 28.28 (SD = 23.97) and median of 21.50. The mean consumption of phytoestrogens in Mexican university students is similar to the consumption found in similar studies in the United States, England, Germany, and Spain (<1 mg day^{-1}). Phytoestrogens can be beneficial in adult women during perimenopause and menopause due to their estrogenic effects, but they are less recommended for women in the fertile stage, as, for example, in the study presented here, because they could function as endocrine disruptors. They are not recommended as dietary supplements for young women or pregnant women.

Keywords: phytoestrogen; diet; Mexican women; endocrine disrupter

Academic Editor: Wojciech Kolanowski

Received: 19 November 2024
Revised: 16 January 2025
Accepted: 20 January 2025
Published: 22 January 2025

Citation: Espino-Rosales, D.; Heras-Gonzalez, L.; Jimenez-Casquet, M.J.; Olea, N.; Olea-Serrano, F.; Mariscal-Arcas, M. Intake of Phytoestrogens and Estrogenic Effect of the Diet of Female University Students in Mexico. *Appl. Sci.* **2025**, *15*, 1092. https://doi.org/10.3390/app15031092

1. Introduction

Phytoestrogen is a plant-derived xenoestrogen that is not created in the endocrine system but is instead consumed by consuming plants or manufactured foods. It is a diverse

group of naturally occurring, non-steroidal plant compounds that have the ability to cause estrogenic or anti-estrogenic effects. Phytoestrogens are not essential nutrients [1–6]. There are several phytoestrogen families, including coumestan, lignans, resorcylic acid lactones, and isoflavone, presents in many foods, such as fruits and vegetables, tea, and wine, including botanical dietary food supplements [7].

Phytoestrogens can attach to the same estrogen receptors. It is believed that phytoestrogens compete with estradiol for binding to intercellular estrogen receptors. The estrogenic action of phytoestrogens can be either agonistic or antagonistic according not only to their tissue concentrations but also to the endogenous estrogen levels occurring at different human life stages [8]. As a weak estrogen, isoflavone can compete with the more potent natural endogenous estrogens to act as an anti-estrogen, which has important implications for reducing breast cancer risk. The hormone action of these agents has been known for some time [9–11], and estrogenic and/or anti-estrogenic effects have been observed in farm and wild animals, in vivo and in vitro tests, and in humans [3,12–15]. In vitro assays have shown the estrogenic agonist activity of certain phytoestrogens at lower concentrations, which stimulate mammary cell proliferation and gene expression of estrogen-dependent genes, whereas at higher concentrations they may antagonize natural hormones [1,16–18].

Although evolutionary adaptation to phytoestrogens has occurred, exposure to high concentrations of some phytoestrogens may be associated with a risk [19–21] of alterations in cellular production, hormone metabolism or action, protein synthesis, and malignant cell multiplication or angiogenesis, as well as other functions [22–24]. It has been suggested that the high prevalence of hormone-dependent cancers and other disorders in Western populations could be associated with the decline in fruit and vegetable intake over the last 50 years [25,26]. Emerging evidence suggests that phytoestrogens may have a preventive role in chronic disorders, warranting further investigation into their dietary implications [27], and investigators continue to study the nutritional contribution of these compounds to metabolic functions as diverse as cholesterol regulation and the maintenance of postmenopausal bone density [23,28]. Certain phytoestrogens have antioxidant properties. This also means that, in addition to the potential health benefits of nutrients, they combat cell damage in the body which is connected to a wide spectrum of chronic disorders, also known as the anti-aging effect [29]. Recent studies have highlighted the importance of various dietary supplements, including boron, in maintaining bone health [30].

The most well-studied group is isoflavones, like genistein and daidzein, which appear to be the strongest and are found in a wide range of foods, especially cereals, legumes, vegetables, and fruits, with soybeans being the most plentiful human source [28,31,32]. Lignans are phytoestrogens found in nuts, grains, plants, seeds, tea, and wine. Lignans are claimed to have an antioxidant function when they are converted into estrogen-like substances by naturally occurring bacteria in the body [33]. Daidzein, genistein, and equol are powerful free radical scavengers and have high antioxidant activity in vitro [34]. Equol's characteristics decrease skin aging in conjunction with its anti-aging skin effects through the reduction in oxidative stress cascade events by a combination of a variety of biochemical/molecular mechanisms and actions to improve human skin health [35]. Lignans exert antioxidant and anti-inflammatory effects, as well as activity in estrogen receptor-dependent pathways [36]. The neuroprotective properties of phytoestrogens appear to be associated with both their antioxidant effects and their estrogen receptor interaction. The potential impact of phytoestrogens on the thyroid appears to have no significant side effects [37–39]. To understand the controversies surrounding phytoestrogens as endocrine disruptors, it is critical to have perspective, as they have both positive and negative connotations. The world's population is exposed to these substances that we ingest on a daily basis, regardless of gender, age, or geographic location [40].

Leguminous seeds (peas, beans), especially soya products, are the most important sources of isoflavone in edible plants. Flaxseeds are found to contain the highest total phytoestrogen levels, closely followed by soya beans and tofu, while lignans are mostly present in flaxseeds. The levels vary within the same food group, e.g., soy beverages, depending on the process and type of soybeans used and soy-based products containing isoflavone. Plant-based products containing soya include miso, soymilk, edamame, and meat alternatives [7,41,42]. Dried fruits, such as dates, prunes, and dried apricots, are another good resource of phytoestrogens [28,43,44].

We have published many studies on the estrogenic effects of synthetic and natural molecules that are part of food, either as natural components, especially of vegetables, or due to contamination. Most of these molecules have been categorized as endocrine disruptors following analysis of their in vitro or in vivo behavior in biological media [3,15,17,45].

This study aimed to estimate the estrogenic effect of diet as a basis for its phytoestrogen content, applying a food frequency questionnaire (FFQ) to obtain data on the dietary phytoestrogen intake of foods and using the E-screen test to determine the estrogenicity of ED consumption. The aims of this research were to estimate the dietary exposure to phytoestrogens of women from the University of Chihuahua, Mexico, to estimate the potential estrogenic effects of the diet.

2. Materials and Methods

The study included 400 university students from the School of Social Work at the University of Chihuahua, Mexico (Table 1). All participants signed informed consent documents to participate in the study, which was approved by the Scientific Ethics Committee of the University of Chihuahua, Mexico (date: 5 October 2019) [46].

Table 1. Descriptive data of the population were collected.

		Age (Years)	Weight (kg)	Height (m)	BMI (kg/m^2)
Mean		21.34	63.85	1.61	24.70
Median		20.00	60.00	1.61	23.61
SD		3.57	15.67	0.07	5.53
Minimum		17.00	29.00	1.10	14.30
Maximum		47.00	131.00	1.86	56.20
Percentile	25	19.00	54.00	1.56	20.69
	50	20.00	60.00	1.61	23.61
	75	22.00	72.00	1.65	27.73

Face-to-face individual interviews were conducted at the University of Chihuahua. The questionnaire was administered during an academic year (February to May) prior to the COVID-19 pandemic. Each participant was administered 4 questionnaires by a specially trained interviewer (D.E.-R.) [46–48]. The first one collected sociodemographic information. The 2nd was a widely used semiquantitative questionnaire that covered the previous year and recorded food consumption as the number of times per day, week, or month and the amount consumed each time in g, mL, or household measures (e.g., full plates, full glasses, teaspoons, tablespoons, etc.). Daily food and nutrient consumption was calculated (in g or mL) by multiplying the standard portion size of the items by the frequency of consumption, categorized as follows: never = 0; 1–3 times/month = 0.07; 1–2 times/week = 0.21; 3–4 times/week = 0.50; 5–6 times/week = 0.80; 1 time/day = 1; and 2–3 times/day = 2.5. The FFQ involved 120 foods according to the dietary habits of the Mexican population ranked by food groups (i.e., ten dairy products, seven cereals, three eggs, six legumes, fourteen meats, five fish, seven fats/oils, fourteen vegetables, sixteen fruits, twelve desserts, six sweets/snacks, ten beverages/infusions, four nuts, six miscellaneous) [46,49,50]. The 3rd questionnaire was a 24 h recall of three

non-consecutive days, including one non-working day. To estimate nutrient and energy intake (EI), the Mexican Nutrikal dietary nutrient database was used, based on the dietary intake collected in the semiquantitative FFQ and estimating the amount of each nutrient per 100 g of food [46,51]. We estimated the intake of phytoestrogens (genistein, daidzein, enterodiol, biochanin A, formononetin, matairesinol, coumestrol, enterolactone, secoisoresinol) in the daily diet of the participants based on reports in the literature on their levels in foods [7,43,52].

Daily phytoestrogen consumption (microg day^{-1}) was estimated by multiplying the quantity of food (g day^{-1}) collected in the FFQ by the respective phytoestrogen values.

In determining the estrogenic activity of phytoestrogens, the chemicals used as standards for the analysis were genistein (Sigma-Aldrich, St. Louis, MO, USA), formononetin, daidzein, coumestrol, matairesinol, biochanin A, enterolactone, secoisoresinol, and enterodiol (Fluka, St. Louis, MO, USA). Stock solutions of chemicals were prepared in ethanol and stored in a cold room. Working solutions were prepared daily by diluting the stock solution with ethanol Chromasolv® for HPLC (≥99% ethanol).

Oestrogenicity assays of the tested compounds were performed using the scientific–technical service platform of the Scientific–Technical Department of the Instituto de Investigación Biosanitaria de Granada, Spain (ibs.GRANADA), supervised by Dr. N. Olea (N.O.). Briefly, MCF-7 cloned cancer cells were cultured for routine maintenance in Dulbecco's modified Eagle's medium (DME) supplemented with 10% fetal bovine serum (FBS) (BioWittaker, Walkersville, MD, USA) in a 5% CO_2/95% air atmosphere under saturated humidity at 37 °C. We subcultured cells at weekly intervals using a mix of 0.05% trypsin and 0.01% EDTA. Sex steroids were eliminated using charcoal and dextran extraction from the serum. For this purpose, a 5% charcoal suspension (Norit A, Sigma Chemical Co., St. Louis, MO, USA) was prepared with 0.5% dextran T-70 (Pharmacia-LKB, Uppsala, Sweden). Similar volume aliquots of the charcoal and dextran suspension were centrifuged at 1000× *g* for 10 min. The supernatant was removed, and the serum aliquots were mixed with the charcoal granules. This charcoal/serum mixture was kept in suspension by rolling for six cycles min^{-1} at 37 °C for 1 h. The suspension was then centrifuged at 1000× *g* for 20 min, and the supernatant was filtered through a 0.22 mm filter (Millipore, Billerica, MA, USA). Bovine and human sera treated with carbon dextran (CDFBS and CDHuS, respectively) were stored at −20 °C until needed; MCF7 cells were used for the estrogenicity test following a slight modification of the original technique [53]. Briefly, cells were trypsinized and seeded in 24-well plates (Limbro, McLean, VA, USA) at initial concentrations of 0.22 mm and 0.22 mm (Millerpore, Billerica, Billerica, MA, USA). At initial concentrations of 20,000 cells per well in 5% FBS in DME, the cells were allowed to settle for 24 h, and then the seeding medium was replaced by DME without phenol red supplemented with 10% CDFBS or CDHuS.

Various concentrations of phytoestrogen products were added, and the test was terminated after 144 h by withdrawing the medium from the wells, fixing the cells, and staining with sulphorhodamine-B (SRB), as previously described elsewhere [17,45]. The Linearity of the SRB test according to cell number was checked before the cell growth experiments. The 100% proliferative effect (PE) was estimated as the relationship between the greatest cell yield achieved with 50 pM estradiol and the proliferation of control cells without hormones. Each phytoestrogen was tested in triplicate with a negative (vehicle) and positive (50 pM estradiol) control on each plaque. The PE of the standard phytoestrogen was referenced to the maximum PE achieved with estradiol, converted to estradiol equivalent units (Eeq) and expressed as nM concentration reading from a dose–response curve generated with estradiol (concentration range = 0.1 pM to 10 nM). The average cell number did not differ significantly from that of the steroid-free controls at concentrations <1 pM estradiol, equivalent to 1 fmol in 1 mL of culture medium. Thus, 1 fmol of estradiol per well was the minimum detectable estrogen level in this test.

Concerning cell proliferation in the E-screen bioassay for the listed compounds, MCF-7 cells were prepared with these compounds at the indicated concentrations. Results are reported as the highest cell multiplication rate caused by the test sample (proliferative effect). This was measured as the ratio between the maximal proliferation rate obtained for the test sample and the multiplication rate achieved by the negative control.

SPSS v.22.0 (IBM, Chicago, IL, USA) was applied to the statistical analysis. A descriptive analysis was performed to obtain means, standard deviations (SDs), medians, maximum and minimum values, and stepwise regression. $p < 0.050$ was assumed to be significant.

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was used throughout the study to guide the writing of this article.

3. Results

The estimated phytoestrogens in the diet of the population correspond to the following groups: (1) isoflavones: daidzein, genistein, formononetin and biochanin A; (2) coumestans: coumestrol; (3) lignans: enterolactone, enterodiol, matairesinol, and secoisolariciresinol.

The average food servings were estimated from the semiquantitative FFQ, which was previously validated. The phytoestrogen intake per day was estimated by multiplying the amount of food reported in the semiquantitative FFQ (Table 2).

Table 2. Foods (g day^{-1}) used to estimate phytoestrogen.

Food (g day^{-1})	Mean	Median	SD	Maximum
Cereals (total)	120.09	93.72	117.04	580.00
White bread	20.31	12.60	37.43	270.00
Other bread	23.34	12.60	35.56	270.00
Breakfast cereal	9.24	5.88	15.17	126.00
Rice	13.84	10.00	16.11	90.00
Flour Tortillas	6.16	0.00	16.15	75.00
Potatoes	15.97	5.60	27.39	175.00
Pasta	26.92	14.70	35.63	175.00
Legumes (total)	46.54	43.00	38.44	223.40
Lentils	2.45	0.90	6.16	75.00
Chickpeas	0.79	0.00	1.90	14.70
Pea	1.89	0.00	5.50	50.00
Kidney beans	2.92	0.00	10.86	70.00
Beans	39.04	40.00	31.08	80.00
Fruit (total)	241.76	164.38	271.92	1744.82
Apples	45.40	22.26	74.46	477.00
Pears	16.85	4.02	33.70	335.00
Oranges	27.34	15.96	37.19	342.00
Bananas	34.01	22.89	43.24	490.50
Tangerines	13.98	5.12	19.74	64.00
Strawberries	20.39	5.40	36.09	180.00
Grapes	19.14	3.42	57.79	513.00
Peaches	13.79	2.31	38.63	346.50
Melon	20.38	4.32	34.88	144.00
Watermelon	19.04	4.29	33.82	143.00
Mangoes	23.44	9.92	32.25	124.00
Sweets (total)	29.43	16.64	39.24	180.00
Jam	2.65	0.90	5.60	30.00
Pastries/Pastry	10.87	2.70	20.47	90.00
Sweet cookie	7.29	4.83	7.86	23.00
Alcoholic Drinks (total)	35.73	9.90	119.66	1009.80
Beer	33.68	9.90	90.94	1485.00

Table 2. *Cont.*

Food (g day^{-1})	Mean	Median	SD	Maximum
Red wine	1.31	0.00	6.22	80.00
White wine	0.74	0.00	3.15	40.00
Natural juice	72.60	19.20	145.55	1080.00
Vegetables (total)	207.23	138.36	267.75	1037.22
Tomato	8.73	3.61	12.55	77.40
Onion/garlic	11.05	4.79	15.34	102.60
Green pepper	1.27	0.00	3.95	40.00
Cabbage/Cabbage	23.24	6.39	43.62	213.00
Cauliflower	18.89	6.39	42.47	532.50
Lettuce	11.41	7.56	15.17	90.00
Cucumber	29.55	13.28	35.44	129.48
Pumpkin	22.00	4.68	37.53	390.00
Carrot	24.20	10.16	31.66	127.00
Mushroom	3.22	0.00	14.63	202.50
Spinach	4.98	0.00	14.49	225.00
Broccoli	11.66	3.39	21.92	113.00
Brussels sprouts	10.61	3.39	19.57	113.00
Nuts (total)	7.63	2.84	14.03	94.50
Walnuts	1.72	0.81	3.83	27.00
Peanuts	6.57	2.03	18.14	303.75
Other (total)	130.97	120.87	134.26	945.00
Pizza	24.69	10.80	45.75	360.00
Burritos	107.72	49.14	114.79	585.00

The value corresponding to the desired phytoestrogen is expressed in μg day^{-1}. Table 3 details the mean amounts and standard deviations of the daily intake of phytoestrogens, following the classification by food groups. Table 2 does not include foods of animal origin (meat, eggs, fish, and dairy products) and sweets and fats since their phytoestrogen values have not been published so far; it is likely that, in raw foods, they are not present in these types of compounds.

Table 3. Total estimated intake (μg day^{-1}) of each phytoestrogen.

	Mean	Median	SD	Minimum	Maximum
ISOFLAVONES					
Diadzein (μg day^{-1})	126.65	10.55	340.14	0.00	3654.46
Genistein (μg day^{-1})	106.93	109.71	83.45	1.34	289.67
Biochanin A (μg day^{-1})	3.01	2.90	2.85	0.00	22.43
Formononetin (μg day^{-1})	106.80	110.60	85.04	1.37	247.66
LIGNANS					
Enterolactone (μg day^{-1})	182.57	147.24	171.69	0.19	813.13
Eneterodiol (μg day^{-1})	134.57	121.78	118.29	0.16	648.71
Eecoisolariciresinol (μg day^{-1})	173.16	121.21	199.91	0.22	1389.96
Matairesinol (μg day^{-1})	22.96	18.26	24.12	0.00	199.17
COUMESTANS					
Coumestrol (μg day^{-1})	0.29	0.08	0.77	0.00	8.30

Average food intake according to the classification by group is expressed in g day^{-1}. Table 2 shows that the fruit groups had the highest average intake (241.75 g day^{-1}), followed by vegetables (207.23 g day^{-1}), cereals (120.09 g day^{-1}), and legumes (46.54 g day^{-1}). The participants do not consider the consumption of soybean and its derivatives of interest, not reaching 0.5% of the population, with only sporadic mention (less than 1 v/month), while the intake of nuts (7.63 g day^{-1}), sweets, and candies accounts for 29.43 g day^{-1}, and these groups correspond to several others, for example, pizza. Burritos, included as a potential source of phytoestrogens, present mean intake values of 130.97 g day^{-1}. The

group of alcoholic beverages is not important based on their consumption, but if it is of interest, natural fruit juices have an average value of 72.6 g day^{-1}.

The quantification of phytoestrogens (µg day^{-1}) was carried out by multiplying the amount of food by the values corresponding to each compound (Table 3).

The contribution of each food item to the exposure to phytoestrogens (Figure 1) was estimated using the stepwise regression test values collected in Tables 4 and 5.

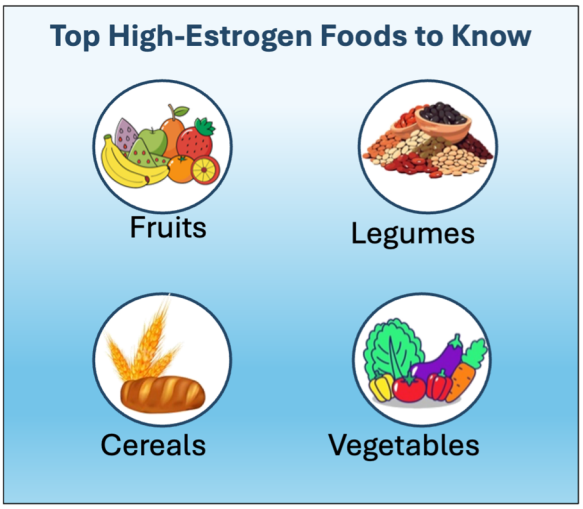

Figure 1. Contribution of each food group to phytoestrogen exposure.

Table 4. Stepwise regression was used to estimate the predictor variables (food) involved in exposure to phytoestrogens according to the study population.

Daidzein	r2	Genistein	r2	Secoisolariciresinol	r2	Formononetin	r2
Fruit	0.271	Legumes	0.903	Cereals	0.719	Other *	0.327
Vegetables	0.322	Nuts	0.990	Fruit	0.937	Fruit	0.415
Various	0.330	Fruit	0.995	Nuts	0.967	Cereals	0.450
Legumes	1.000					Legumes	1.000
Matairesinol	r2	Biochanin A	r2	Enterolactone	r2	Enterodiol	r2
Cereals	0.892	Legumes	0.985	Vegetables	0.876	Fruit	0.675
Fruit	0.929	Nuts	0.990	Fruit	0.990	Vegetables	0.949
Sweets	0.945			Natural juice	0.994	Other *	0.986
Various	0.956						

* Other: pizza, burritos.

Table 5. Micrograms of phytoestrogens per gram day^{-1} of food estimated from semiquantitative FFQ.

	(g day^{-1})	Daidzein (µg day^{-1})	Genistein (µg day^{-1})	Formononetin (µg day^{-1})	Biochanin A (µg day^{-1})	Matairesinol (µg day^{-1})	Seicoresinol (µg day^{-1})	Enterolactone (µg day^{-1})	Enterodiol (µg day^{-1})	Coumestrol (µg day^{-1})
					Cereals					
Mean	120.09	2.51	126.07	127.08	0.08	19.08	138.11	7.82	11.48	
Median	93.72	1.32	78.88	80.73	0.05	10.93	79.78	5.20	7.72	
SD	117.04	3.62	203.48	208.33	0.13	26.35	181.88	11.85	17.81	
Maximum	580.00	27.36	1685.81	1730.04	1.07	200.79	1362.32	107.08	161.71	
					Legumes					
Mean	46.54	235.88	97.75	107.63	5.37	--	0.20	26.86	186.79	0.19
Median	43.00	4.32	99.46	108.48	3.85	--	0.06	0.00	168.08	0.05
SD	38.44	1247.45	77.62	85.48	12.52	--	0.71	221.87	194.59	0.69
Maximum	223.40	16,743.65	225.16	350.46	239.18	0.01	8.50	4391.42	2818.73	8.27
Fruit										
Mean	241.76	0.57	3.92	0.01	--	2.22	55.54	63.23	67.42	--
Median	164.38	0.24	2.25	0.00	--	0.55	22.27	37.23	36.23	--
SD	271.92	0.81	5.28	0.01	--	5.10	111.41	93.12	97.44	--
Maximum	1744.82	4.24	62.20	0.05	--	78.7	1632.29	843.68	682.94	--
					Vegetables					
Mean	207.23	0.62	1.62	--	--	0.77	16.00	150.67	68.68	--
Median	138.36	0.41	0.72	--	--	0.35	9.32	92.01	42.56	--
SD	267.75	0.86	2.98	--	--	1.59	23.47	209.67	87.43	--
Maximum	1037.22	7.77	30.15	--	--	18.35	255.16	1985.53	712.73	--

Table 5. *Cont.*

	(g day^{-1})	Daidzein (μg day^{-1})	Genistein (μg day^{-1})	Formononetin (μg day^{-1})	Biochanin A (μg day^{-1})	Matairesinol (μg day^{-1})	Seicoresinol (μg day^{-1})	Enterolactone (μg day^{-1})	Enterodiol (μg day^{-1})	Coumestrol (μg day^{-1})
					Sweets					
Mean	29.43	0.08	0.02	--	--	1.09	2.82	0.94	0.94	--
Median	16.64	0.03	0.01	--	--	0.59	1.13	0.26	0.26	--
SD	39.24	0.14	0.07	--	--	1.44	4.75	2.80	2.80	--
Maximum	180.00	0.89	0.94	--	--	8.43	50.44	38.96	38.96	--
					Natural juice					
Mean	72.60	0.22	0.05	--	--	--	1.42	7.72	6.28	--
Median	19.20	0.05	0.01	--	--	--	0.34	1.87	1.52	--
SD	145.53	0.47	0.11	--	--	--	2.98	16.26	13.23	--
Maximum	1080	3.02	0.73	--	--	--	19.32	105.24	85.62	--
					Alcoholic Beverages					
Mean	35.73	0.03	0.07	0.16	0.06	0.22	2.84	--	--	--
Median	9.90	0.01	0.02	0.04	0.01	0.00	0.00	--	--	--
SD	119.66	0.08	0.21	0.47	0.16	1.79	23.31	--	--	--
Maximum	1009.80	0.96	2.70	5.98	2.04	35.33	461.22	--	--	--
					Nuts					
Mean	7.63	1.09	7.87	0.33	0.33	0.11	14.36	3.44	--	--
Median	2.84	0.34	2.43	0.10	0.10	0.04	4.66	1.06	--	--
SD	14.03	2.92	21.74	0.91	0.91	0.32	37.24	9.51	--	--
Maximum	94.50	48.87	364.51	15.19	15.19	3.32	613.41	159.47	--	--
					Other *					
Mean	130.97	142.05	0.12	0.06	--	0.68	5.01	2.87	9.86	--
Median	120.87	117.14	0.05	0.05	--	0.26	4.02	1.83	7.62	--
SD	134.26	194.32	0.34	0.08	--	2.12	6.66	5.60	12.93	--
Maximum	945.00	1056.62	6.26	0.43	--	38.8	37.88	93.86	76.29	--

* Other: pizza and burritos.

We performed an estimation of the estrogenic capacity of a diet from the intake of phytoestrogens. The estrogenic effects of phytoestrogens identified according to the foods consumed by the study population were assessed.

Table 6 presents data on the concentration in which each phytoestrogen tested showed its maximum proliferative effect on the E-screen (concentration). We determined the maximum proliferation rate for each compound at the optimal concentration (proliferative effect) and estimated the relative proliferative potency (PPR) and relative proliferative efficacy (RPE). According to these results, it was observed that daidzein, genistein, biochanin A, and formononetin exhibit a medium agonist estrogenic effect, since they behaved in the E-screen test in the same way as the E2 test at a maximum response concentration of 10^{-10} M.

Table 6. Estrogenicity of phytoestrogen intake by the population.

Product	Concentration of Maximum Proliferative Effect	Proliferative Effect	PPR (%)	EPR (%)
Positive control E2 (1×10^{-10} M)	1×10^{-10} M	6.61 ± 0.30	100.00	100.00
Negative control (Culture medium)	---	1.00 ± 0.14	---	---
Daidzein	1×10^{-5} M	6.24 * ± 0.02	0.001	94.40
Genistein	1×10^{-5} M	6.37 ± 0.12	0.001	96.36
Biochanin A	1×10^{-5} M	7.11 ± 1.32	0.001	107.56
Formononetin	1×10^{-5} M	6.53 ± 1.25	0.001	98.78
Coumestrol	1×10^{-5} M	3.09 * ± 0.18	0.001	46.74
Enterolactone	1×10^{-5} M	1.16 ± 0.05	0.001	17.54
Matairesinol	1×10^{-5} M	1.59 * ± 0.14	0.001	24.05
Enterodiol	1×10^{-5} M	1.62 ± 0.04	0.001	24.50

* Significant difference versus negative control, estimating growth = 1; $p < 0.05$.

With these results and knowing the average exposure to these molecules in the daily diet, the potentially estrogenic effect attributable to phytoestrogens was estimated using estradiol (17-β E 2) as a reference. The results of this estrogenic effect are presented in Table 7.

Table 7. Estrogenic effects were estimated using the mean intake of phytoestrogens.

	Mean	Median	SD	Minimum	Maximum
ISOFLAVONES					
Diadzein (μg day^{-1})	126.65	10.55	340.14	0.00	3654.46
* Eq. E2 (pmol day^{-1})	4.98	0.41	13.87	---	14.37
Genistein (μg day^{-1})	106.93	109.71	83.45	1.34	289.67
Eq. E2 (pmol day^{-1})	3.85	4.06	3.08	0.50	10.12
Biochanin A (μg day^{-1})	3.01	2.90	2.85	0.00	22.43
Eq. E2 (pmol day^{-1})	0.11	0.10	0.80	----	0.77
Formononetin (μg day^{-1})	106.80	110.60	85.04	1.37	247.66
Eq. E2 (pmol day^{-1})	3.97	4.11	3.16	0.05	9.21
LIGNANS					
Enterolactone (μg day^{-1})	182.57	147.24	171.69	0.19	813.13
Eq. E2 (pmol day^{-1})	6.12	4.94	5.95	0.006	21.25
Eneterodiol (μg day^{-1})	134.57	121.78	118.29	0.16	648.71
Eq. E2 (pmol day^{-1})	4.45	4.03	3.91	0.005	21.45
Secoisolariciresinol (μg day^{-1})	173.16	121.21	199.91	0.22	1389.96
Eq. E2 (pmol day^{-1})	4.18	3.34	5.52	0.006	38.36
Matairesinol(μg day^{-1})	22.96	18.26	24.12	0.00	199.17
Eq. E2 (pmol day^{-1})	0.64	0.51	0.67	---	5.56
COUMESTANS					
Coumestrol (μg day^{-1})	0.29	0.08	0.77	0.00	8.30
Eq. E2 (pmol day^{-1})	0.001	0.003	0.03	---	0.31

* Total refers to pM of E2, with a mean of 28.28 (SD = 23.97) and median of 21.50.

4. Discussion

The effects of phytoestrogens are very varied [23], with the following properties described: anticancer effects [25], cardiotonic effects attributed to the flavonoid quercetin and, to a lesser extent, to genistein and lutein [54], improving the resistance of capillaries and helping prevent them from breaking (hesperidin, rutin, and quercetin) [55], the ability to prevent the formation of thrombi in blood vessels, and the ability to lower the concentration of cholesterol and triglycerides [25,56]; hesperidin has anti-inflammatory and analgesic properties [57], but only its antioxidant effect has been demonstrated with persistent results [36] in addition to estrogenic/anti-estrogenic effects [34].

The dietary intake of phytoestrogens has been reported to be <1 mg day^{-1} in Europe and the United States [44,58] and considerably higher (>20 mg day^{-1}) in Japan and Korea, attributable to the elevated consumption of soy derivatives such as natto, miso, and tofu [59–62].

Our research group has published numerous studies on the estrogenic effects of natural and synthetic molecules in foods, especially vegetables. Many of these molecules have been classified as endocrine disrupters after analyses of their behavior in biological media in vitro or in vivo [3,15,17,45].

There has been considerable research on the hormonal effects of phytoestrogens and interest in their intake in the diet or as supplements. We describe here a technique to estimate the estrogenic effect of diet as a function of its phytoestrogen content using a food frequency questionnaire (FFQ) to collect data on the dietary intake of phytoestrogens and apply the E-screen test to establish the estrogenicity of the phytoestrogens consumed.

The diet of this population of Mexican women was estimated to have a mean total estrogenic capacity of 0.129×10^{-10} eq.E2 (12.9 pmol day^{-1}). The mean was elevated and equivalent to the hormonal capacity of individuals who produce between 0.3 and 14 pmol day^{-1} according to age and other factors [63]. Regarding the estrogenic effect, the effects of this additional burden are highly controversial, and no definitive conclusion has been reached. Exposure to the estrogenic activity of these chemicals can be positive or negative, and exposure is considered a risk at any age [64–66]. However, many phytoestrogens are

considered endocrine disruptors, suggesting that they may also have an adverse effect on health. The response is probably complex and may depend on age, health status, and even the presence or absence of specific gut microflora [67–69].

Phytoestrogens are present in numerous dietary supplements and are widely marketed as a natural alternative to estrogen replacement therapy. However, the true effect of these substances has not yet been established. The consumption of isoflavone and fiber by menopausal women has not been definitively demonstrated to have a protective effect against vasomotor symptoms, and almost all recent research suggests that their favorable or unfavorable effects can only be verified in studies of very large populations [38]. Some authors have shown that the intake of traditional plant extracts rich in phytoestrogens, such as red clover, soybean, and hops, can reduce menopausal symptoms [11,20].

The only adverse effect of soy intake has been the occurrence of endometrial hyperplasia in some investigations, although analysis of the clinical and pharmacological data indicates that this disease does not occur when soy isoflavones are administered at the usual therapeutic doses [70]. In summary, the novel contribution of this study was to estimate the average dietary intake of phytoestrogens in this population from FFQs and literature data on the isoflavone content of foods, applying the E-screen method to determine the estrogenicity of the assessed isoflavone content, using cell proliferation as an endpoint. The data on the intake of specific populations may allow progress in elucidating the implications of phytoestrogen consumption, while in other foods, phytoestrogens are present in quantifiable amounts, highlighting significant amounts of genistein and seicoresinol in cereals and formononetin in legumes. Although soybeans are an important source of phytoestrogen, our subjects do not consider the consumption of soybean and its derivatives of interest, not reaching 0.5% of the population with only sporadic mention (less than 1 v/month).

5. Conclusions

Phytoestrogen can be beneficial in adult women during perimenopause and menopause due to their estrogenic effects, but they are less recommended in women in the fertile stage, as, for example, in the study presented here, because they could function as endocrine disruptors. They are not recommended as dietary supplements for young women or pregnant women.

Phytoestrogens, as components of plant foods, are included in any healthy diet rich in vegetables, such as the Mediterranean diet, so it would be a good recommendation to abandon the Western diet and introduce, as far as possible, habits of the Mediterranean diet rich in vegetables.

In relation to the limitations of this study, caution should be taken in extrapolating the present 400, highly specific studied subjects, young women with university status, to the general population, given the specific nature of this study sample, which was drawn from healthy women not receiving estrogen supplements. The exclusion of soy products due to low consumption (<0.5%) in the sample misses an important source of phytoestrogens that could offer comparative insights. Reliance on self-reported dietary data through the FFQ can introduce recall bias, potentially skewing the results. The current study did not explore the long-term health implications of phytoestrogen intake, which limits its applicability to chronic health outcomes.

Author Contributions: The study was designed by F.O.-S., N.O. and M.M.-A.; data were collected and analyzed by D.E.-R., L.H.-G., M.J.J.-C. and M.M.-A.; data interpretation and manuscript preparation were undertaken by D.E.-R., L.H.-G., F.O.-S., N.O. and M.M.-A. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by FEDER-ISCIII PI14/01040 and FEDER-ISCIII PI17/01758, by the Counselling of Economic Transformation, Industry, Knowledge and Universities-Junta de Andalucía (P18-RT-4247) and by the High Council for Sports (CSD), Spanish Ministry of Culture and Sport, through the NESA NETWORK "Spanish Network of Sports Care at Altitude (RADA)" Ref. 19/UPB/23.

Institutional Review Board Statement: All of these volunteers signed an informed consent form for participation in the study, which was approved by the Scientific Ethics Committee of the University of Chihuahua, Mexico (cod.14-01040, Date: 5 October 2019). The study was conducted in accordance with the Declaration of Helsinki.

Informed Consent Statement: All of these volunteers signed informed consent forms to participate in this study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. There are restrictions on the availability of data for this trial, due to the signed consent agreements around data sharing, which only allow access to external researchers for studies following the project's purposes. Requestors wishing to access the trial data used in this study can make a request to mariscal@ugr.es.

Acknowledgments: This paper will be part of Diana Espino Rosales's doctoral thesis, being completed as part of the "Nutrition and Food Sciences Program" at the University of Granada, Spain.

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Olea, N.; Pazos, P.; Fernández, M.F.; Rivas, A.; Olea-Serrano, F.; Pedraza, V. Phyto and mycoestrogens (Xenoestrogens) as a preventable cause of breast cancer. *Med. Biol. Environ. Int. J.* **1999**, *27*, 55–60.
2. Yildiz, F. *Phytoestrogens in Functional Foods*; CRC Press: Boca Raton, FL, USA, 2005; pp. 210–211. ISBN 978-1-57444-508.
3. Hernandez-Elizondo, J.; Monteagudo, C.; Murcia, M.A.; Olea, N.; Olea-Serrano, F.; Mariscal-Arcas, M. Assessment of the estrogenicity of the diet of a healthy female Spanish population based on its isoflavone content. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.* **2013**, *30*, 627–633. [CrossRef] [PubMed]
4. Křížová, L.; Dadáková, K.; Kašparovská, J.; Kašparovský, T. Isoflavones. *Molecules* **2019**, *24*, 1076. [CrossRef] [PubMed]
5. Domínguez-López, I.; Yago-Aragón, M.; Salas-Huetos, A.; Tresserra-Rimbau, A.; Hurtado-Barroso, S. Effects of Dietary Phytoestrogens on Hormones throughout a Human Lifespan: A Review. *Nutrients* **2020**, *12*, 2456. [CrossRef] [PubMed]
6. Heras-González, L.; Espino, D.; Jimenez-Casquet, M.J.; Lopez-Moro, A.; Olea-Serrano, F.; Mariscal-Arcas, M. Influence of BPA exposure, measured in saliva, on childhood weight. *Front. Endocrinol.* **2022**, *13*, 1040583. [CrossRef]
7. Forslund, L.C.; Andersson, H.C. *Phytoestrogens in Foods on the Nordic Market: A Literature Review on Occurrence and Levels*; Nordic Council of Ministers, Nordic Council of Ministers Secretariat: Copenhagen, Denmark, 2017; ISBN 978-92-893-5047-1. [CrossRef]
8. Bhakta, D.; Higgins, C.D.; Sevak, L.; Mangtani, P.; Adlercreutz, H.; McMichael, A.J.; Dos Santos Silva, I. Phyto-oestrogen intake and plasma concentrations in South Asian and native British women resident in England. *Br. J. Nutr.* **2006**, *95*, 1150–1158. [CrossRef]
9. Kaldas, R.S.; Hughes, C.L., Jr. Reproductive and general metabolic effects of phytoestrogens in mammals. *Reprod. Toxicol.* **1989**, *3*, 81–89. [CrossRef]
10. Wagner, J.; Jiang, L.; Lehmann, L. Phytoestrogens modulate the expression of 17alpha-estradiol metabolizing enzymes in cultured MCF-7 cells. *Adv. Exp. Med. Biol.* **2008**, *617*, 625–632. [CrossRef]
11. Rietjens, I.M.C.M.; Louisse, J.; Beekmann, K. The potential health effects of dietary phytoestrogens. *Br. J. Pharmacol.* **2017**, *174*, 1263–1280. [CrossRef]
12. Mäkelä, S.; Santti, R.; Salo, L.; McLachlan, J.A. Phytoestrogens are partial estrogen agonists in the adult male mouse. *Environ. Health Perspect.* **1995**, *103* (Suppl. S7), 123–127. [CrossRef]
13. Saarinen, N.M.; Huovinen, R.; Wärri, A.; I Mäkelä, S.; Valentín-Blasini, L.; Sjöholm, R.; Ammälä, J.; Lehtilä, R.; Eckerman, C.; Collan, Y.U.; et al. Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. *Mol. Cancer Ther.* **2002**, *1*, 869–876. [PubMed]
14. Jiang, Q.; Payton-Stewart, F.; Elliott, S.; Driver, J.; Rhodes, L.V.; Zhang, Q.; Zheng, S.; Bhatnagar, D.; Boue, S.M.; Collins-Burow, B.M.; et al. Effects of 7-O substitutions on estrogenic and anti-estrogenic activities of daidzein analogues in MCF-7 breast cancer cells. *J. Med. Chem.* **2010**, *53*, 6153–6163. [CrossRef] [PubMed]

15. Heras-González, L.; Latorre, J.A.; Martinez-Bebia, M.; Espino, D.; Olea-Serrano, F.; Mariscal-Arcas, M. The relationship of obesity with lifestyle and dietary exposure to endocrine-disrupting chemicals. *Food Chem. Toxicol.* **2020**, *136*, 110983. [CrossRef] [PubMed]
16. Villalobos, M.; Olea, N.; Brotons, J.A.; Olea-Serrano, M.F.; Ruiz de Almodovar, J.M.; Pedraza, V. The E-screen assay: A comparison of different MCF7 cell stocks. *Environ. Health Perspect.* **1995**, *103*, 844–850. [CrossRef]
17. Olea, N.; Olea-Serrano, M.F. Oestrogens and the environment. *Eur. J. Cancer Prev.* **1996**, *5*, 491–496.
18. Xin, M.; Wang, Y.; Ren, Q.; Guo, Y. Formononetin and metformin act synergistically to inhibit growth of MCF-7 breast cancer cells in vitro. *Biomed. Pharmacother.* **2019**, *109*, 2084–2089. [CrossRef]
19. Albertazzi, P.; Purdie, D.W. Reprint of The nature and utility of the phytoestrogens: A review of the evidence. *Maturitas* **2008**, *61*, 214–226. [CrossRef]
20. Kwack, S.J.; Kim, K.B.; Kim, H.S.; Yoon, K.S.; Lee, B.M. Risk assessment of soybean-based phytoestrogens. *J. Toxicol. Environ. Health A* **2009**, *72*, 1254–1261. [CrossRef]
21. Costa, E.M.; Spritzer, P.M.; Hohl, A.; Bachega, T.A. Effects of endocrine disruptors in the development of the female reproductive tract. *Arq. Bras. Endocrinol. Metabol.* **2014**, *58*, 153–161. [CrossRef]
22. Adlercreutz, H.; Mazur, W. Phyto-oestrogens and Western diseases. *Ann. Med.* **1997**, *29*, 95–120. [CrossRef]
23. Desmawati, D.; Sulastri, D. Phytoestrogens and Their Health Effect. *Open Access Maced. J. Med. Sci.* **2019**, *7*, 495–499. [CrossRef] [PubMed]
24. Hall, J.M.; Powell, H.A.; Rajic, L.; Korach, K.S. The Role of Dietary Phytoestrogens and the Nuclear Receptor PPARγ in Adipogenesis: An In Vitro Study. *Environ. Health Perspect.* **2019**, *127*, 37007, Erratum in *Environ. Health Perspect.* **2019**, *127*, 109002. [CrossRef] [PubMed]
25. Adlercreutz, H. Phytoestrogens: Epidemiology and a possible role in cancer protection. *Environ. Health Perspect.* **1995**, *103* (Suppl. S7), 103–112. [CrossRef] [PubMed]
26. Bhakta, D.; Silva, I.d.S.; Higgins, C.; Sevak, L.; Kassam-Khamis, T.; Mangtani, P.; Adlercreutz, H.; McMichael, A. A semiquantitative food frequency questionnaire is a valid indicator of the usual intake of phytoestrogens by south Asian women in the UK relative to multiple 24-h dietary recalls and multiple plasma samples. *J. Nutr.* **2005**, *135*, 116–123. [CrossRef]
27. Canivenc-Lavier, M.C.; Bennetau-Pelissero, C. Phytoestrogens and Health Effects. *Nutrients* **2023**, *15*, 317. [CrossRef]
28. Rodríguez-García, C.; Sánchez-Quesada, C.; Toledo, E.; Delgado-Rodríguez, M.; Gaforio, J.J. Naturally Lignan-Rich Foods: A Dietary Tool for Health Promotion? *Molecules* **2019**, *24*, 917. [CrossRef]
29. Liu, T.; Li, N.; Yan, Y.; Xiong, K.; Liu, Y.; Xia, Q.; Zhang, H.; Liu, Z. Recent advances in the anti-aging effects of phytoestrogens on collagen, water content, and oxidative stress. *Phytother. Res.* **2020**, *34*, 435–447. [CrossRef]
30. Rondanelli, M.; Faliva, M.A.; Peroni, G.; Infantino, V.; Gasparri, C.; Iannello, G.; Perna, S.; Riva, A.; Petrangolini, G.; Tartara, A. Pivotal role of boron supplementation on bone health: A narrative review. *J. Trace Elem. Med. Biol.* **2020**, *62*, 126577. [CrossRef]
31. Knight, D.C.; Eden, J.A. A review of the clinical effects of phytoestrogens. *Obstet. Gynecol.* **1996**, *87 Pt 2*, 897–904.
32. Agradi, E.; Vegeto, E.; Sozzi, A.; Fico, G.; Regondi, S.; Tomè, F. Traditional healthy Mediterranean diet: Estrogenic activity of plants used as food and flavoring agents. *Phytother. Res.* **2006**, *20*, 670–675. [CrossRef]
33. Senizza, A.; Rocchetti, G.; Mosele, J.I.; Patrone, V.; Callegari, M.L.; Morelli, L.; Lucini, L. Lignans and Gut Microbiota: An Interplay Revealing Potential Health Implications. *Molecules* **2020**, *25*, 5709. [CrossRef] [PubMed]
34. Kładna, A.; Berczyński, P.; Kruk, I.; Piechowska, T.; Aboul-Enein, H.Y. Studies on the antioxidant properties of some phytoestrogens. *Luminescence* **2016**, *31*, 1201–1206. [CrossRef] [PubMed]
35. Lephart, E.D. Skin aging and oxidative stress: Equol's anti-aging effects via biochemical and molecular mechanisms. *Ageing Res. Rev.* **2016**, *31*, 36–54. [CrossRef] [PubMed]
36. Jang, W.Y.; Kim, M.Y.; Cho, J.Y. Antioxidant, Anti-Inflammatory, Anti-Menopausal, and Anti-Cancer Effects of Lignans and Their Metabolites. *Int. J. Mol. Sci.* **2022**, *23*, 15482. [CrossRef] [PubMed]
37. Divi, R.L.; Chang, H.C.; Doerge, D.R. Anti-thyroid isoflavones from soybean: Isolation, characterization, and mechanisms of action. *Biochem. Pharmacol.* **1997**, *54*, 1087–1096. [CrossRef]
38. Doerge, D.R.; Sheehan, D.M. Goitrogenic and estrogenic activity of soy isoflavones. *Environ. Health Perspect.* **2002**, *110* (Suppl. S3), 349–353. [CrossRef]
39. Gorzkiewicz, J.; Bartosz, G.; Sadowska-Bartosz, I. The Potential Effects of Phytoestrogens: The Role in Neuroprotection. *Molecules* **2021**, *26*, 2954. [CrossRef]
40. Lephart, E.D. Phytoestrogens (Resveratrol and Equol) for Estrogen-Deficient Skin-Controversies/Misinformation versus Anti-Aging In Vitro and Clinical Evidence via Nutraceutical-Cosmetics. *Int. J. Mol. Sci.* **2021**, *22*, 11218. [CrossRef]
41. Mulligan, A.A.; Welch, A.A.; McTaggart, A.A.; Bhaniani, A.; Bingham, S.A. Intakes and sources of soya foods and isoflavones in a UK population cohort study (EPIC-Norfolk). *Eur. J. Clin. Nutr.* **2007**, *61*, 248–254. [CrossRef]
42. Thompson, L.U.; Boucher, B.A.; Liu, Z.; Cotterchio, M.; Kreiger, N. Phytoestrogen content of foods consumed in Canada, including isoflavones, lignans, and coumestan. *Nutr. Cancer* **2006**, *54*, 184–201. [CrossRef]

43. Boker, L.K.; Van der Schouw, Y.T.; De Kleijn, M.J.; Jacques, P.F.; Grobbee, D.E.; Peeters, P.H. Intake of dietary phytoestrogens by Dutch women. *J. Nutr.* **2002**, *132*, 1319–1328. [CrossRef] [PubMed]
44. Zamora-Ros, R.; Knaze, V.; Luján-Barroso, L.; Kuhnle, G.G.; Mulligan, A.A.; Touillaud, M.; Slimani, N.; Romieu, I.; Powell, N.; Tumino, R.; et al. Dietary intakes and food sources of phytoestrogens in the European Prospective Investigation into Cancer and Nutrition (EPIC) 24-hour dietary recall cohort. *Eur. J. Clin. Nutr.* **2012**, *66*, 932–941. [CrossRef] [PubMed]
45. Perez, P.; Pulgar, R.; Olea-Serrano, F.; Villalobos, M.; Rivas, A.; Metzler, M.; Pedraza, V.; Olea, N. The estrogenicity of bisphenol A-related diphenylalkanes with various substituents at the central carbon and the hydroxy groups. *Environ. Health Perspect.* **1998**, *106*, 167–174. [CrossRef] [PubMed]
46. Espino-Rosales, D.; Lopez-Moro, A.; Heras-González, L.; Jimenez-Casquet, M.J.; Olea-Serrano, F.; Mariscal-Arcas, M. Estimation of the Quality of the Diet of Mexican University Students Using DQI-I. *Healthcare* **2023**, *11*, 138. [CrossRef] [PubMed]
47. Tur, J.A.; Romaguera, D.; Pons, A. The Diet Quality Index-International (DQI-I): Is it a useful tool to evaluate the quality of the Mediterranean diet? *Br. J. Nutr.* **2005**, *93*, 369–376. [CrossRef]
48. Liu, L.; Wang, P.P.; Roebothan, B.; Ryan, A.; Tucker, C.S.; Colbourne, J.; Baker, N.; Cotterchio, M.; Yi, Y.; Sun, G. Assessing the validity of a self-administered food-frequency questionnaire (FFQ) in the adult population of Newfoundland and Labrador, Canada. *Nutr. J.* **2013**, *12*, 49. [CrossRef]
49. Bountziouka, V.; Panagiotakos, D.B. Statistical methods used for the evaluation of reliability and validity of nutrition assessment tools used in medical research. *Curr. Pharm. Des.* **2010**, *16*, 3770–3775. [CrossRef]
50. Dubois, L.; Diasparra, M.; Bédard, B.; Colapinto, C.K.; Fontaine-Bisson, B.; Morisset, A.S.; Tremblay, R.E.; Fraser, W.D. Adequacy of nutritional intake from food and supplements in a cohort of pregnant women in Québec, Canada: The 3D Cohort Study (Design, Develop, Discover). *Am. J. Clin. Nutr.* **2017**, *106*, 541–548. [CrossRef]
51. Marván, L.; Pérez, A.B. NutrirKcal VO (v.1.1). 2005. Available online: www.nutrikcal.com (accessed on 6 October 2019).
52. Pillow, P.C.; Duphorne, C.M.; Chang, S.; Contois, J.H.; Strom, S.S.; Spitz, M.R.; Hursting, S.D. Development of a database for assessing dietary phytoestrogen intake. *Nutr. Cancer* **1999**, *33*, 3–19. [CrossRef]
53. Soto, A.M.; Chung, K.L.; Sonnenschein, C. The pesticides endosulfan, toxaphene, and dieldrin have estrogenic effects on human estrogen-sensitive cells. *Environ. Health Perspect.* **1994**, *102*, 380–383. [CrossRef]
54. Alshehri, M.M.; Sharifi-Rad, J.; Herrera-Bravo, J.; Jara, E.L.; Salazar, L.A.; Kregiel, D.; Uprety, Y.; Akram, M.; Iqbal, M.; Martorell, M.; et al. Therapeutic Potential of Isoflavones with an Emphasis on Daidzein. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 6331630. [CrossRef]
55. De Kleijn, M.J.; van der Schouw, Y.T.; Wilson, P.W.; Grobbee, D.E.; Jacques, P.F. Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in postmenopausal U.S. women: The Framingham study. *J. Nutr.* **2002**, *132*, 276–282. [CrossRef]
56. Tang, S.; Du, Y.; Oh, C.; No, J. Effects of Soy Foods in Postmenopausal Women: A Focus on Osteosarcopenia and Obesity. *J. Obes. Metab. Syndr.* **2020**, *29*, 180–187. [CrossRef]
57. Chakraborty, D.; Gupta, K.; Biswas, S. A mechanistic insight of phytoestrogens used for Rheumatoid arthritis: An evidence-based review. *Biomed. Pharmacother.* **2021**, *133*, 111039. [CrossRef]
58. Rizzolo-Brime, L.; Caro-Garcia, E.M.; Alegre-Miranda, C.A.; Felez-Nobrega, M.; Zamora-Ros, R. Lignan exposure: A worldwide perspective. *Eur. J. Nutr.* **2022**, *61*, 1143–1165. [CrossRef]
59. Surh, J.; Kim, M.J.; Koh, E.; Kim, Y.K.; Kwon, H. Estimated intakes of isoflavones and coumestrol in Korean population. *Int. J. Food Sci. Nutr.* **2006**, *57*, 325–344. [CrossRef]
60. Xu, Y.; Le Sayec, M.; Roberts, C.; Hein, S.; Rodriguez-Mateos, A.; Gibson, R. Dietary Assessment Methods to Estimate (Poly)phenol Intake in Epidemiological Studies: A Systematic Review. *Adv. Nutr.* **2021**, *12*, 1781–1801. [CrossRef]
61. Shirabe, R.; Saito, E.; Sawada, N.; Ishihara, J.; Takachi, R.; Abe, S.K.; Shimazu, T.; Yamaji, T.; Goto, A.; Iwasaki, M.; et al. Fermented and nonfermented soy foods and the risk of breast cancer in a Japanese population-based cohort study. *Cancer Med.* **2021**, *10*, 757–771. [CrossRef]
62. Kim, Y.; Kim, D.W.; Kim, K.; Choe, J.S.; Lee, H.J. Usual intake of dietary isoflavone and its major food sources in Koreans: Korea National Health and Nutrition Examination Survey 2016–2018 data. *Nutr. Res. Pract.* **2022**, *16* (Suppl. S1), S134–S146. [CrossRef]
63. Stricker, R.; Eberhart, R.; Chevailler, M.C.; Quinn, F.A.; Bischof, P.; Stricker, R. Establishment of detailed reference values for luteinizing hormone, follicle stimulating hormone, estradiol, and progesterone during different phases of the menstrual cycle on the Abbott ARCHITECT analyzer. *Clin. Chem. Lab. Med.* **2006**, *44*, 883–887. [CrossRef]
64. Velentzis, L.S.; Woodside, J.V.; Cantwell, M.M.; Leathem, A.J.; Keshtgar, M.R. Do phytoestrogens reduce the risk of breast cancer and breast cancer recurrence? What clinicians need to know. *Eur. J. Cancer* **2008**, *44*, 1799–1806. [CrossRef] [PubMed]
65. Batool, M.; Ranjha, M.M.A.N.; Roobab, U.; Manzoor, M.F.; Farooq, U.; Nadeem, H.R.; Nadeem, M.; Kanwal, R.; AbdElgawad, H.; Al Jaouni, S.K.; et al. Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (*Cucurbita* sp.). *Plants* **2022**, *11*, 1394. [CrossRef] [PubMed]

66. Birsa, M.L.; Sarbu, L.G. Hydroxy Chalcones and Analogs with Chemopreventive Properties. *Int. J. Mol. Sci.* **2023**, *24*, 10667. [CrossRef] [PubMed]
67. Sklenickova, O.; Flesar, J.; Kokoska, L.; Vlkova, E.; Halamova, K.; Malik, J. Selective growth inhibitory effect of biochanin A against intestinal tract colonizing bacteria. *Molecules* **2010**, *15*, 1270–1279. [CrossRef]
68. Ağagündüz, D.; Cocozza, E.; Cemali, Ö.; Bayazıt, A.D.; Nanì, M.F.; Cerqua, I.; Morgillo, F.; Saygılı, S.K.; Canani, R.B.; Amero, P.; et al. Understanding the role of the gut microbiome in gastrointestinal cancer: A review. *Front. Pharmacol.* **2023**, *14*, 1130562. [CrossRef]
69. Feng, Z.J.; Lai, W.F. Chemical and Biological Properties of Biochanin A and Its Pharmaceutical Applications. *Pharmaceutics* **2023**, *15*, 1105. [CrossRef]
70. Hüser, S.; Guth, S.; Joost, H.G.; Soukup, S.T.; Köhrle, J.; Kreienbrock, L.; Diel, P.; Lachenmeier, D.W.; Eisenbrand, G.; Vollmer, G.; et al. Effects of isoflavones on breast tissue and the thyroid hormone system in humans: A comprehensive safety evaluation. *Arch. Toxicol.* **2018**, *92*, 2703–2748. [CrossRef]