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dieta de estudiantes de la
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10.20960/nh.02109

OR 2109

Valoración nutricional y exposición a fitoestrógenos en la dieta de estudiantes de la Universidad de Costa Rica

Nutritional assessment and phytoestrogen exposure in a diet of students of the University of Costa Rica

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Received: 08/06/2018

Accepted: 08/02/2019

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Resumen: este estudio presenta resultados respecto a dieta y un análisis más en profundidad de la exposición a estrógenos naturales (fitoestrógenos) en la ingesta, y como este tipo de compuestos influye y se relaciona con otros aspectos importantes que pueden modular funciones biológicas relacionadas con la salud en estudiantes universitarios.

Objetivos: valorar hábitos nutricionales y estimar la ingesta de fitoestrógenos de la población en estudio.

Material y métodos: población costarricense universitaria femenina (n: 211, edades $18,83 \pm 2,06$ años) y masculina (n: 199, edades $19,64 \pm 3,05$ años) de la Universidad de Costa Rica y se han aplicado pruebas antropométricas mediante el DEXA y cuestionarios de Frecuencia de Consumo de Alimentos (FFQ) y Recuerdos de 24 Horas (R-24).

Resultados: los errores nutricionales más graves fueron: la elevada ingesta de sodio, lípidos y proteínas de origen animal en hombres y mujeres, y el déficit

importante de fibra y ácido fólico en las mujeres. La ingesta total de fitoestrógenos referidos a la daizdeína fue de 0.23 ± 0.40 mg/día y 7.01 ± 11.94 mg/mes en mujeres y de 0.17 ± 0.13 mg/día y de 5.14 ± 3.96 mg/mes en varones; principalmente consumida en forma de lignanos 0.24 ± 0.12 mg/día (mujeres) y 0.23 ± 0.14 mg/día (hombres), mientras la ingesta de isoflavonas fue de 0.09 ± 0.38 mg/día (mujeres) y de 0.04 ± 0.08 mg/día (hombres).

Conclusiones: la población de estudio presentó porcentajes de grasa elevados nutricionalmente hablando, aunque el consumo de vegetales, cereales, granos enteros y frutas tiende un poco a la dieta mediterránea, su patrón alimentario se acerca mucho más a la Dieta occidental.

Palabras clave: Fitoestrógenos. Dieta. Salud. Cuestionario de Frecuencia de Consumo de alimentos. Recordatorio de 24 horas.

ABSTRACT

Introduction: this study presents the results regarding diet and an analysis of natural estrogens (phytoestrogens) intake and how they affect other important aspects, which can modulate biological health functions among university students.

Objectives: assessing nutritional habits and estimating the intake of phytoestrogens in the population under study.

Materials and methods: Costa Rican female (n: 211, 18.83 ± 2.06 years) and male (n: 199, 19.64 ± 3.05 years) university population of the University of Costa Rica applied anthropometric tests using DEXA, the Food Frequency Questionnaire (FFQ) and the 24-hour Reminder (R-24).

Results: the most serious nutritional bad habits were high ingestion of sodium, lipids and animal origin proteins in men and women and a deficit of fiber and folic acid in women. The total intake of phytoestrogens referred to: daidzein 0.23 ± 0.40 mg/day and 7.01 ± 11.94 mg/month in women and 0.17 ± 0.13 mg/day and 5.14 ± 3.96 mg/month in men; mainly consumed in the form of lignans 0.24 ± 0.12 mg/day (women) and 0.23 ± 0.14 mg/day (men). The intake of isoflavones was 0.09 ± 0.38 mg/day (women) and 0.04 ± 0.08 mg/day (men).

Conclusions: The study population presented high fat percentage although the consumption of vegetables, cereals, whole grains and fruits tends slightly to be a Mediterranean diet; their food pattern was much closer to the Western diet.

Key words: Phytoestrogens. Diet. Health. Food Frequency Questionnaire. 24-hour recall.

INTRODUCTION

For decades, and even presently, the prevention of chronic diseases has revealed a global importance. Hundreds of studies have tried to discover the ways in which various nutrients can affect health, to know in more detail how nutrients move inside the cell in the molecular level, how they interact between various nutrients, and how they influence (1).

Although today there is a large number of scientific studies related to human nutrition, food composition and intake of various nutrients, phytoestrogens have deserved special attention among researchers for several years. Both the scientific community and the food industry have been concerned, in recent years, by the possible dual role of these compounds, and some epidemiological studies have published important data (2-6).

Phytoestrogens are natural compounds presented in a great variety of vegetable foods. These compounds are found abundantly in soybeans, cereals, legumes, vegetables and fruits. The phytoestrogens are defined as any plant, substance or metabolite that induces biological responses in vertebrates and can mimic or modulate the estrogen endogenous actions, usually by joining the estrogen receptors (7).

In vegetables, the phytoestrogens are evident in their original state (inactive glycosylated) as precursors. By means of the enzymatic action of the intestinal bacteria, once these compounds are ingested, they lose the glucose molecule (transforming themselves into their active forms) and absorbed by the enterohepatic circulation of biliary acids being able to be excreted again by the bile. Its absorption by the intestinal mucosa is completely conditioned by the intestinal flora bacteria and; therefore, the use of antibiotics or gastrointestinal

diseases will affect the metabolism of these compounds. A high fiber intake can also hinder their absorption (8).

With regard to the intake of phytoestrogens that provides the diet of different populations, Spain, Germany, England and the United States are countries where the intake of phytoestrogens is lower (< 1 mg/d), while Canada and Scotland present higher intakes < 1.34 mg/d). Oriental countries as Japan and Korea have an even higher intake (> 20 mg/d) (9). Only one single study was found (Mexico) that evaluates the intake of this class of compounds in Latin American populations; however, the sample used was very small (n: 50) for validating the fact of phytoestrogen intake (10). In Costa Rica, there are so far no related studies that have been published on this topic.

The exposure of these compounds in greater concentrations than acceptable levels (for example, changes in diet) could be associated with a certain level of risk (11). In addition, some alterations in the duration of the menstrual cycle are linked to high soy consumption (12). Additionally, the demographic level has a high incidence of hormone-dependent cancers (breast and prostate) as has been demonstrated. Thus, typical diseases in other Western societies could be related to the decline, over the past 30 to 40 years, of fruit and vegetable consumption.

Phytoestrogen consumption could modify the production of hormones and their metabolism, or their action at the cellular level, as well as influencing other functions such as protein synthesis, proliferation of malignant cells and/or angiogenesis. Therefore, it is important to understand the effects of these substances, particularly if their exposure is excessive, because this can promote hyperplasia or neoplasia in breast tissue. A recent study shows the importance of this effect (13).

In contrast, phytoestrogens, diphenols, lignans and isoflavones may have a role as protectors from cancer. Indeed, some epidemiological studies do tend to support this hypothesis in breast cancer, which correlates higher amounts of lignans and isoflavonoids excreted in urine with lower incidence of breast cancer (13).

Other authors have also described antiangiogenic activity and an ability to inhibit cellular differentiation, in addition to describing other properties, such as

the antioxidant action (14). In contrast, different *in vitro* tests have shown that some phytoestrogens have an agonist estrogenic activity in low concentrations, stimulating the proliferation of mammary cells and the expression of genes that are under the control of estrogen response elements. However, at higher doses, they have the ability to antagonize the effect of natural hormones (15).

Based on the previous arguments and considering the fact that in Costa Rica no studies have been conducted related to nutritional assessment and phytoestrogen intake, this research seeks to rate nutritional habits and evaluate exposure to phytoestrogens by diet, in students (men and women) of the University of Costa Rica.

METHODS AND MATERIALS

Subjects

A sample of university students (women n: 211; men n: 199) was recruited at the Human Movement Science Research Center (CIMOBU) of the University of Costa Rica on two occasions: a first session to perform the anthropometric measurements and a second one to complete the questionnaires. Before starting the research, the subjects were informed about the research design.

There were no selection criteria on health or demographic characteristics. The participants were requested to give information on medical history. The Ethical Committee of the University of Costa Rica, meeting the principles of the Declaration of Helsinki (52nd General Assembly; Edinburgh, Scotland, October 2000), approved the study, and a written informed consent, personal identification and personal data was obtained from all subjects. At the end of the study, the participants received the nutritional results via email.

Volunteers were accepted if they had a good health history, were 18 years or older, and signed an informed consent form in accordance with the ethical standards defined by the University of Costa Rica.

Measurement procedures

After the recruitment, the student participants were summoned to a scheduled session at CIMOBU. They were asked to wear sports apparel or normal indoor clothing (shorts, elastic fabrics, and/or T-shirts). They were also told not to wear

any jewelry or metal objects to complete the anthropometric tests (weight, height, waist and hip circumference, and fat percentage). Nutritional and physical activity surveys were obtained in a second meeting. The body composition studies were carried out in one morning, after overnight fasting and without previous exercise, alcohol or stimulant beverages. Nutritional and physical activity surveys (FFQ and 24-hour recall) were asked in a second meeting.

Instruments and questionnaires measuring

Anthropometric measures such as weight (kg), size (m), waist and hip circumference (cm), and fat percentage were taken at the CIMOHU, using the equipment listed below:

- Height: stadiometer (Novel Products Inc., Rockton, IL, USA) accuracy ± 0.5 cm.
- Body weight: (Tanita®, model BF-350, Arlington Heights, IL, USA) accuracy ± 0.1 kg.
- Fat percentage: this was obtained using a DEXA scan of full body (Lunar Prodigy Advance, General Electric, Madison, WI, USA). A certified equipment technician in accordance with the CIMOHU's standard protocol applied and analyzed every test. Manufacturer protocols and calibrations were accomplished out daily to ensure the quality of the equipment and measurements.

Waist and hip circumference measurement: a Gulick self-locking tape measure was used, based on the ACSM recommendations (16,17).

Questionnaires

The FFQ has been widely used and validated and was adapted to include items consumed by this population group (9,18,19,47,64) by a semi-quantitative estimation of intake of macronutrients and the energy level of the population, applying the same questionnaire at the beginning and at the end of the study. Spearman's correlation rank was less than $p < 0.05$ for energy from protein ($\rho = 0.67$), lipids ($\rho = 0.76$) and carbohydrates ($\rho = 0.67$) in all the correlations.

For the FFQ, similar questionnaires were used as have previously been used (9,18,19); adapted to a Costa Rican diet and including 144 foods. Some foods were included because of changes in feeding patterns (products low in fat, for example) and others were selected based on published data (20,21) and the database (PHYTOHEALTH Thematic Network) due to their richness in phytoestrogens. Specific food consumption or not, frequency of consumption (day/week/month) and amounts in homemade measurements were collected. To calculate food ration averages, raw weight data was used.

The reported information in the R-24 recall and the FFQ were analyzed with a DNA sequencing software (food diet and nutrition) Academic Version 3.3.0., created by Ph.D. Luis García Diz and in collaboration with PhD Mataix Verdú J, Sierra Cinos JL, Murcia Tomás MA and Martinez M, in 2012. Based on this analysis, the consumption of macronutrients SFA and cholesterol, minerals, and vitamins were obtained. All values were compared to the international intake recommendations i.e., dietary reference intakes (22).

The total energy intake consumed calculated by means of FFQ and R-24 was compared to the total energy expenditure calculated for the sample according to their age, weight and height using these formulas:

$$\begin{aligned} \text{GET (women)} &= 354 - (6.91 * \text{age [years]}) \pm 1.2 * (9.36 * \text{weight [kg]}) \pm (726 * \\ &\quad \text{size [m]}) \\ \text{GET (men)} &= 662 - (9.53 * \text{age [years]}) \pm 1.2 * (15.91 * \text{weight [kg]}) \pm \end{aligned}$$

To extract values to calculate the amount of phytoestrogens contained in many foods (and taking into account the current information available in the literature) food was classified in nine categories according to their phytoestrogens content, based on concentrations of: a) isoflavones: daizein, formononetin, genistein, biochanin A; b) coumestans: coumestrol, matairesinol, secoisolariciresinol; and c) lignans: enterolacton and enterodiol. When in academic literature different values were found for the same food, the highest value was chosen. Phytoestrogen estimate intake per day was calculated by multiplying the amount of food, corresponding to the desired compound values, expressed as mg/day (20,21). To weight the total daily consumption of phytoestrogens by subject, calculations were done to standardize the values referring to daidzein as a model substance.

Data analysis

The statistical analysis was performed with the SPSS 21.0 (SPSS Inc., Chicago, IL, USA) program. For the quantitative variables, descriptive techniques were used such as averages, standard deviations and a normality distribution test; for categorical variables, the tests used were frequencies expressed in percentages and the Chi-square test. For the inferential study, parametric and non-parametric contrasts were used: Student's t test, ANOVA test, contingency tables, and Spearman's correlation coefficients. The statistical significance level for every test was $p \leq 0.05$.

RESULTS

Anthropometric characteristics

Table I presents young adults with anthropometric characteristics that differ significantly ($p \leq 0.001$) according to sex. It is highlighted that, in general, they have suitable anthropometric characteristics except for the high fat percentage, a contrasting value with the average of the BMI and the relation between the waist-hip values of subjects.

Nutritional assessment of the population under study

Table II shows, through the Student's t analyses, the most important weekly food consumptions in the diet of the participants (women and men) with a reference value based on the required consumption; in this case, of weekly recommendations for intake in accordance with the Food Guides for the Spanish population (23) and the Food Guides for the Costa Rican population (24). A comparison of the weekly frequency consumption among men and women is also presented.

Focusing on a comparison with the weekly requirement, women presented no significant differences in the intake of floury vegetables, pasta, eggs, legumes, fish, and fruit with respect to the weekly-required intake. However, they consume significantly ($p \leq 0.01$) fewer dairy products and cereals, and they drink little coffee. In contrast, they consumed significantly ($p \leq 0.01$) higher rations of white bread, red and white meat, vegetables and refined sugar. Men

show significant differences in all the results ($p \leq 0.05$), including a lower consumption of dairy products, cereals, fish and fruit, while drinking a little coffee. In contrast, men had a higher consumption of white bread, vegetables, pasta, eggs, and red and white meat.

Based on a comparative analysis by sex, men had significantly higher intake ($p \leq 0.05$) of almost all foods compared to women while based on the 24-hour recall, the analysis of Student's t was used to compare the average to DRIs (22) reference value. Classified nutrient results are detailed in tables II-VI.

Based on these results (Table III), women had a significant lipid and protein intake ($p \leq 0.001$) in contrast to a low fiber intake ($p = 0.031$) while men only had an adequate fiber intake. The protein, carbohydrate and lipid intake was significantly ($p \leq 0.05$) lower than the recommended intake value. Moreover, men obtained a low total energy consumption as they consumed approximately 80% of the required energy to meet their needs.

Based on the outcomes associated with the intake of fatty acids and cholesterol in the participant population's diet (Table IV), no significant difference ($p > 0.05$) in the intake of saturated fats between men and women was revealed, but we did find more saturated fat intake in women than the recommended value ($p \leq 0.001$). Mono-unsaturated fat intake and cholesterol, for both men and women, was less than the recommended value ($p \leq 0.001$). In addition, higher cholesterol intake in women compared to men ($p = 0.006$) was found. In relation to the intake of poly-unsaturated fats, in both men and women were similarly higher ($p \leq 0.05$) than the recommended values.

According to these results (Table V), women had a significantly higher consumption ($p = 0.001$) of iron, phosphorus, sodium, and selenium, and a significantly lower consumption ($p < 0.01$) of calcium, iodine, magnesium, and potassium based on daily intake recommendations. Only zinc intake coincided with the recommendations. On the other hand, men have higher intakes of iron, phosphorus, sodium, and selenium, and very low intakes of calcium, iodine, magnesium, zinc and potassium based on daily intake recommendations ($p < 0.001$).

As for the intake of vitamins in the study population (Table VI), women had a significantly elevated consumption ($p = 0.001$) of A, C and B-complex vitamins

and a significantly low consumption ($p < 0.01$) of vitamin D and folic acid based on the daily intake recommendations. Only the intake of vitamin E and thiamin agreed with the recommendations. In the case of men, statistically significant differences were found ($p < 0.01$) for all outcomes. Thus, elevated intakes of vitamins A, C, E, and B-complex were found, as well as very low vitamin D, thiamine and folic acid levels, based on daily intake recommendations.

As already mentioned, the quantification of phytoestrogens per day was calculated by multiplying the amount of food by the corresponding values to the desired compound. In addition, to weigh the daily consumption, calculations were conducted by multiplying the molecular weight to standardize the values and comparing those results to daidzein as a pattern substance. Table VII shows the average values of phytoestrogen-intake by day.

A significant difference ($p \leq 0.05$) in the total phytoestrogen, lignan and isoflavone intake was found. In such cases, women showed greater intakes. With respect to the aforementioned daily and monthly daidzein intake, no differences were found ($p = 0.054$) in men or women. In addition, it was observed that in both groups the lignans, enterodiol and enterolactom were the most representative phytoestrogens in the sample diet of the study, while both coumestrol and biochanin A presented the lowest intake values.

As part of the inferential analysis, the Spearman's correlation coefficient (ρ) was applied for the variables of daidzein, genistein, biochanin A, formononetin; coumestrol, matairesinol, secoisolariciresinol; enterolactom and enterodiol for each food group (Table VIII). The correlation matrix reveals the relationship between each phytoestrogen (mg/d) studied and the food intake, classified by group (g/d). It is highlighted that all correlations were significant and positive ($p < 0.001$, $p < 0.05$). Cereals, legumes, fruits, vegetables, and sweets, for example, correlated significantly ($p < 0.001$, $p < 0.05$) with nine types of phytoestrogens. As for soybeans, the unique phytoestrogen, which did not have a significant correlation, was biochanin A. Drinks, although presenting weak relations to six of the phytoestrogen analyzed, did not relate significantly with genistein, biochanin A and coumestrol.

DISCUSSION

Based on the results (Table I), the sample showed similar anthropometric features to sample used in Spanish (25), and other foreign studies (6,26-28). This allows the deduction that the phytoestrogen nutrient intake can, therefore, be comparable.

The valued food intake using the FFQ (Table II indicates that women follow the recommendations in consuming legumes, fish, fruit, pasta, and eggs. Nonetheless, they consume an excess ($p \leq 0.05$) of white and red meats, sugar and vegetables. They also show a deficit ($p \leq 0.05$) intake of dairy products, bread, rice, and cereals for breakfast while men have a nutritional profile statistically different ($p \leq 0.05$, $p \leq 0.001$) in all food groups. For instance, they eat foods such as carbohydrates, eggs, legumes, red and white meats, vegetables and sugar, while having lower daily intakes of fish and fruits. These results could also explain the argument mentioned above that even when many of the participants under study do not follow a strict diet, they probably limit the intake of bread, potatoes and carbohydrates. Due to this idea of being fit, they replace them with less caloric foods such as vegetables, fruits and fish (29-32) that means, the Mediterranean diet (33,34).

On the other hand, men reveal a different statistical nutritional profile in all food groups ($p \leq 0.05$, $p \leq 0.001$) consuming carbohydrates, eggs, legumes, red and white meat, vegetables and sugar in higher proportions and less dairy, fish and fruits. However, in both women and men a lack of dairy and the abuse of consuming meat and sugar can be dangerous for health (35-38).

As a complement, the macronutrient intake and energy from the R-24 was analyzed showing that the media energy intake in men was 2,219.45 kcal/day. That reveals an approximate deficit of 20% of the DRIs, based on the age and the weight range, while women usually had the recommended energy intakes (2,056.69 kcal/day), taking into account that other authors have concluded that populations with low-calorie diets are associated with healthy lifestyles (32). In any case, the possible energy intake undervaluation (or overvaluation) may suggest two possible situations. First, there is a likely concern about body image; stereotypes enforced by relatives, and television, among others, lead into eating disorders, putting at risk the individual's health (39). Secondly, the method depends on the data provided by the individual, which are most

susceptible to mistakes since they tend to approximate their intake levels to those they believe are normal, especially if they consider their level to be excessive (40).

In women, *protein* intake significantly exceeded 80.96 g/d, the value representing 105.8% of daily recommendations, while in men the intake only showed an 88.8% of the recommendations, with an intake of 92.40 g/d. According to some authors, the intake of some nutrients such as calcium, iron, zinc, vitamin A and riboflavin is related to the diet's protein quality (41). Therefore, even when the nutritional objectives (42) are intended to involve a decreased in terms of proteins, many studies in different populations revealed that the current trend in diet includes generous amounts of proteins.

The trend by gender considering the carbohydrate daily total was similar. Women had intakes of 313.93 g/d (111.98% of the recommended) while men ate 336.62 g/d under the obtained values in other studies (43,44), representing 88.25% of the ideal consumption of this macronutrient according to the nutritional objectives already mentioned. These data corroborate the low intake trend of these foods from the 40s until today (45,46).

For this reason, one may think that people's psychological perception may interfere with different food beliefs, even when those beliefs may negatively alter the intake of important macronutrients (47,48). However, valid arguments highlight the benefits in a rich carbohydrate diet over anthropometric variables related to weight loss and health (49,50).

The fiber estimation trend, on the contrary, seemed to be in reverse. Results showed that women under study were at levels of 2,338 g, representing 93.52% of DRIs, while men reached an intake percentage of 108.24% (27.06 g/d). This data, low in the case of women, followed the same population behavior of other samples evaluated in Spain and in other countries (51-55).

It is important that *fiber* intake was increased. Insufficient fiber intake is associated with an increased risk of suffering short-term illnesses such as constipation and diverticulosis (56), and in the long term, it seems to be linked to incidence and worsening of diabetes, cardiovascular disease and cancer (57-59). In fact, it has been shown how beneficial and protective fiber can be against colon and rectum cancer (60). Unfortunately, the intake of food rich in

fiber, such as beans and nuts, sometimes is reduced in favor of fast foods, especially in young people, which affects the diet quality. In spite of this, many studies, specifically made for women, have verified that motivated individuals can have great changes in eating habits to prevent future diseases (61,62). In this sense, it has been verified that when public health offices execute policies and projects trying to improve nutritional habits, the quality of the diet improves significantly (54).

The intake of *fatty acids and cholesterol* valued according to R-24 indicates that, in the case of men, the MUFA and the PUFA are the only ones that fit statistically ($p = 0.102$ and $p = 0.146$, respectively) within the dietary goals, while the intake of MUFA for both men and women is higher than recommended and of cholesterol, lower than recommended. In the case of women, the PUFA ingestion represents 119.2%, whereas the MUFA reaches 139.8%, in agreement with other studies (44) and simultaneously resisting other Spanish samples (63).

Concerning *mineral* intake, statistically significant differences were found between the real intake and DRI recommendations. Under 100% were calcium, magnesium, potassium, zinc and iodine in women, whereas phosphorus, selenium, iron and sodium intakes surpassed 100% of the recommended. On the other hand, zinc intake comes near expected intake daily recommendation. High phosphorus intake is due to the large consumption of different foods such as carbonated drinks. Coke and sugary drinks today are replacing other types of beverages such as water or milk (64). This data is similar with the one found in children, which differs with the one presented in adolescents' intakes (64). In early ages, the family seems to be in charge of meals. This fact suggests that a deficiency of nutrients in adult diets would be related to childhood diet. However, families tend to lose the control of the diet as they grow older.

For *vitamins* consumption, the average values of vitamin C, thiamine, riboflavin, niacin, pyridoxine, and vitamin A were over 100% of the DRIs in both groups, which means that in all cases they did not agree with the values found in a study made in Cataluña (65) with intakes below recommendations in niacin, vitamin A, vitamin D, and folic acid. For riboflavin, and vitamin A the consumption is superior to the ones found for other scientists. In regard to acid

folic intake, both men's and women's intakes were 86.37% and 84.61%, respectively.

This fact could be worrisome because for most the possibility of being pregnant is latent and the adequate ingestion of this nutrient is essential to prevent fetus congenital malformations. Folates are mainly found in fruits, legumes and green vegetables whereas vitamin E fundamentally is consumed from oil and vegetable. If vitamin E is not consumed in suitable rations, a lack of vitamins will occur. The results show low values of *phytoestrogen ingestion* 0.23 ± 0.40 mg/d and 7.01 ± 11.94 mg/m for women and 0.17 ± 0.13 mg/d and 5.14 ± 3.96 mg/m in men, mostly consumed in the form of lignans; 0.24 ± 0.12 mg/d (women) and 0.23 ± 0.14 mg/d (men), while the ingestion of isoflavones 0.09 ± 0.38 mg/d and of 0.04 ± 0.08 mg/d for women and men, respectively. The total consumption of isoflavones was 0.23 mg/d, of lignan 1.07 mg/d and, according to our analysis, coumestrol intake was 0.001 mg/d. These conclusions are consistent with the earlier published data on phytoestrogens intake in Western populations (2,3,5). This data differs significantly when we compare daily intakes with Oriental diets (11). In addition, when performing a comparative analysis by sex of the estimation of phytoestrogen intake, any of the analyses supported significant statistical differences ($p > 0.05$) between men and women, which are consistent with the current research.

Based on these results, some important aspects of our data should be considered. We used a semi-quantitative standardized FFQ as a measurement instrument, designed to quantify the monthly dietary intake of food groups in terms of frequency and food portions. This has an advantage because its validity has been widely proven, and has even given reliable results when these results have been contrasted with other types of complex measurements such as the analysis of biomarkers (66). According to Boker et al. (20), the analysis of blood, urine and plasma usually represents only a short period intake (usually up to 48 hours) and its results depend on the bio-availability and the influence of phytoestrogens, while the digestion of them is affected by innumerable reasons (intestinal microflora, use of antibiotics, gender, etc.).

However, the results may be underestimated because it has not taken into account the overlapping sources of soy, which for a long time has been used in food production systems, e.g., beverages and fermented food products, cereal mixtures or pastry shop, processed meats, or stock cube soup. In addition, according to Boker et al. (20), the lack of data confirms that the presence and the content of lignans in food products might lead to inaccuracies, especially when evaluating the ingestion of phytoestrogens in Western populations because they consume more frequently lignans than Oriental populations.

We have to be careful regarding the consumption of diets rich in phytoestrogens in higher concentrations since the consequences of an exaggerated consumption remains unknown. Among the population groups that can be affected, children who drink soy milk (as a substitute for maternal or cow milk) are more susceptible, since the concentration of isoflavones in the blood is 1,000 times higher than that found in children's blood when nursed by mothers who consume diets rich in soybean (67,69). Studies made with wildlife and other animals show that the fetal or perinatal exposure to endocrine disruptors, such as phytoestrogens, originate an altered sexual differentiation and urogenital malformations, which lead to reproductive disorders in adult life (70); besides, a recent meta-analysis evidences that phytoestrogen intake via food might, at least in part, be responsible for sperm concentration trends (71) . Therefore, it is crucial to mention that exposure to phytoestrogens in one's diet should not be high, especially in development and reproduction stages, as in the sample studied.

CONCLUSIONS

In summary, the studied population has a normal BMI, but with high fat percentages for their age. The nutritional profile differs significantly from the weekly recommendations (practically in all the food groups) according to the nutritional guides in Costa Rica and Spain.

In general terms, men consumed smaller percentages of CHO and lipids while women exceeded caloric intake, according to expenditure, exceeding the intake percentage recommended in all the macronutrients. In relation to the consumption of fatty acids and cholesterol, women exceed by more than 100%

the recommended intake of SFA and PUFA while they have intakes under 70% of the recommendation regarding MUFA and cholesterol. On the other hand, men only consume PUFA above the 100% recommended.

In the particular case of the sample included in this study, it does not seem that the intake of phytoestrogens can be defined as harmful. These results are consistent with similar intakes in other Occidental countries.

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Table I. University students' anthropometric characteristics

<i>Variables</i>	<i>Average ± SD</i>		<i>p</i>
	♀	♂	
Age (years)	18.83 ± 2.06	19.64 ± 3.05	0.002 [†]
Height (cm)	160.98 ± 6.18	173.65 ± 6.84	0.0011 [‡]
Weight (kg)	58.32 ± 10.84	71.52 ± 12.74	0.0011 [‡]
BMI (kg/m ²)	22.46 ± 3.62	23.53 ± 3.90	0.018*
Percentage of fat (%)	36.53 ± 7.35	23.00 ± 9.00	0.0011 [‡]
Waist (cm)	71.75 ± 8.26	81.81 ± 10.36	0.0011 [‡]
Hip (cm)	98.08 ± 7.63	98.92 ± 7.66	0.350
WHR	0.74 ± 0.04	0.82 ± 0.05	0.0011 [‡]
BMD (g/cm ²)	1.09 ± 0.08	1.16 ± 0.08	0.0011 [‡]

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belong to University of Costa Rica. WHR: waist to hip ratio, BMD: bone mineral density, ♀: university women; ♂: university men; ±SD: standard deviation; BMI: body mass index.

Comparative analysis using Student's t test. Significance according to *p ≤ 0.05, **p ≤ 0.01, †p ≤ 0.001, ‡p ≤ 0.0011.

Table II. Comparison of the participants weekly frequency intake based on the required intake recommended by sex

Food	Required intake	Average \pm SD		<i>p</i>		Gender
		♀	♂	Requirement ♀	Requirement ♂	
Dairy products	14	12.04 \pm 7.54	12.32 \pm 7.15	0.001 [†]	0.002 [†]	0.724
Bread	7	9.99 \pm 7.69	11.55 \pm 6.87	0.001 [†]	0.001 [†]	0.053 [†]
Cereals	7	3.62 \pm 4.49	3.73 \pm 4.08	0.001 [†]	0.001 [†]	0.814
Potatoes, banana, yucca	8	7.42 \pm 5.36	10.02 \pm 8.15	0.179	0.001 [†]	0.001 [†]
Rice	18	11.54 \pm 8.07	14.31 \pm 8.95	0.001 [†]	0.001 [†]	0.003 [†]
Pasta	2	1.99 \pm 2.06	2.60 \pm 2.81	0.969	0.005 [†]	0.027*
Eggs	4	3.95 \pm 3.11	5.43 \pm 4.34	0.869	0.001 [†]	0.001 [†]
Legumes	7	7.65 \pm 6.34	10.02 \pm 8.39	0.193	0.001 [†]	0.004 [†]
Meat (red)	3	3.65 \pm 2.48	4.35 \pm 3.18	0.001 [†]	0.001 [†]	0.028*
Chicken, turkey, Pork	4	4.62 \pm 3.36	5.28 \pm 3.77	0.021*	0.001 [†]	0.096
Fish	4	3.57 \pm 3.04	3.42 \pm 3.16	0.080	0.018*	0.666
Fruits	21	20.01 \pm 12.07	19.15 \pm 12.19	0.307	0.047*	0.522
Vegetables	14	29.63 \pm 16.05	25.16 \pm 16.38	0.001 [†]	0.001 [†]	0.013*
Sugar	7	10.07 \pm 8.20	10.63 \pm 8.92	0.001 [†]	0.001 [†]	0.554
Coffee	14	3.06 \pm 4.72	4.44 \pm 6.55	0.001 [†]	0.001 [†]	0.031*

Source: produced by Hernández-Elizondo J. Comparative analysis using Student's t test. Results of the analyzed sample belongs to University of Costa Rica. ♀: university women; ♂: university men; ±SD: standard deviation.

Significance according to * $p \leq 0.05$, ** $p \leq 0.01$, † $p \leq 0.001$.



Table III. Macronutrient energy intake from R-24 and intake percentages of university students under study according to DRIs

	Intake average \pm SD (%)	DRI	Required %	% Intake based on requiremen ts	t	p
Women participants (♀)						
Energy (kcal)	2,056.69 \pm 444.74	2,040.		101.0	0.44	0.655
		53 ^s				
Protein (g)	80.96 \pm 25.44 (15.87)		15	105.8	2.15	0.033 *
Carbohydrates (g)	313.93 \pm 229.29 (61.59)		55	111.98	1.79	0.075
Lipids (g)	74.63 \pm 25.76 (32.92)		30	109.73	3.16	0.002 †
Fiber (g)	23.38 \pm 9.13	25		93.52	-2.17	0.031 *
Men participants (♂)						
Energy (kcal)	2,219.45 \pm 467.96	2773. 79 ^s		80.01	-	0.001 15.2 †
					6	
Protein (g)	92.40 \pm 44.35 (13.32)		15	88.8	-3.37	0.001 †
Carbohydrates	336.62 \pm 246.37		55	88.25	-2.34	0.020

(g)	(48.54)				*
Lipids (g)	77.91 ± 31.97	30	84.26	-5.86	0.001
	(25.28)				†
Fiber (g)	27.06 ± 46.36	25	108.24	0.57	0.567

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belongs to University of Costa Rica. ♀: university women; ♂: university men, ±SD: standard deviation.

Significance according to *p ≤ 0.05, **p ≤ 0.01, †p ≤ 0.001. [§]Calculated for the range based on age and weight for the study population.

Table IV. Fatty acids and cholesterol intake based on R-24 and percentage intake according to the nutritional goals of the university population

	Consumption of fatty acids and cholesterol				DRIs (%)	% Intake based on DRIs		p		
	Intake average ± SD (%)		Kcal			♀	♂	Requirement		Gender
	♀	♂	♀	♂				♀	♂	
AGS (g)	21.64 ± 9.22 (9.54)	23.45 ± 9.36 (7.61)	194.77 ± 83.04	211.13 ± 84.30	8	119.	95.1	0.001 [†]	0.102	0.083
AGM (g)	2,010 ± 9.05 (8.87)	21.67 ± 15.96 (7.03)	180.93 ± 81.49	195.07 ± 143.66	15	59.1	46.8	0.001 [†]	0.001 [†]	0.483
AGP (g)	15.84 ± 6.89 (6.99)	18.93 ± 31.10 (6.14)	142.61 ± 62.04	170.40 ± 279.90	5	139.	122.	0.001 [†]	0.146	0.461
Cholesterol (mg)	215.85 ± 116.36	260.03 ± 161.47			< 300 (mg/d)	71.9	86.6	0.001 [†]	0.002 [†]	0.049*

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belong to University of Costa Rica. ♀: university women; ♂: university men; ±SD: standard deviation.

Comparative analysis by Student's t test. Significance according to * $p \leq 0.05$, ** $p \leq 0.01$, † $p \leq 0.001$.

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Table V. Minerals intake comparison in the university participants to recommended intake percentages according to DRIs

	Intake average \pm SD		DRI		% Intake based on the DRI		p	
	♀	♂	♀	♂	♀	♂	♀	♂
Iron (mg/d)	27.05 \pm 43.08	26.44 \pm 38.41	18	8	150.2	7	0.011*	0.001 [†]
Magnesium (mg/d)	288.73 \pm 103.78	299.28 \pm 96.17	310	400	93.13	74.82	0.013*	0.001 [†]
Zinc (mg/d)	8.02 \pm 3.55	8.63 \pm 3.51	8	11	100.2	5	0.944	0.001 [†]
Calcium (mg/d)	643.13 \pm 273.42	695.66 \pm 307.55	1,000	1000	64.31	69.56	0.001 [†]	0.001 [†]
Iodine (ug/d)	58.97 \pm 38.86	62.80 \pm 55.06	150	150	39.31	41.86	0.001 [†]	0.001 [†]
Phosphorus (mg/d)	1,150.76 \pm 383.62	1,246.03 \pm 374.19	700	700	164.3	178.0	0.001 [†]	0.001 [†]
Potassium (mg/d)	3,251.21 \pm 1,131.78	3,220.95 \pm 1,064.84	4,700	4700	9	0	0.001 [†]	0.001 [†]
Sodium (mg/d)	2,624.64 \pm 1,015.75	2,919.95 \pm 961.97	1,500	1500	69.17	68.53	0.001 [†]	0.001 [†]
Selenium (ug/d)	76.81 \pm 35.50	85.12 \pm 38.67	45	45	174.9	170.6	0.001 [†]	0.001 [†]
					189.1	8		
					5			

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belong to University of Costa Rica. ♀: university women; ♂: university men; \pm SD: standard deviation.

Comparative analysis using Student's t test as requested. Significance according to *p \leq 0.05, **p \leq 0.01, [†]p \leq 0.001.

Table VI. Comparison of vitamin intake in the university study population to recommended intake percentages according to DRIs

	Intake average \pm SD		DRI		% Intake based on DRI		p	
	♀	♂	♀	♂	♀	♂	♀	♂
Vitamin A (ug/d)	994.59 \pm	994.66 \pm	70	90	142.0	110.	0.001 [†]	0.048*
Ascorbic acid (mg/d)	224.44 \pm	220.57 \pm	75	90	299.2	245.	0.001 [†]	0.001 [†]
Vitamin D (ug/d)	126.58	137.90	15	15	5	07	0.001 [†]	0.001 [†]
Vitamin E (mg)	5.58 \pm 11.30	5.00 \pm 10.28	15	15	37.2	3	0.974	0.043 *
Thiamin (mg/d)	15.01 \pm 6.19	16.10 \pm 6.97	1	1	6	33	0.075	0.001 [†]
Riboflavin (mg/d)	1.77 \pm 4.64	1.50 \pm 0.66	1	2	160.9	125.	0.001 [†]	0.001 [†]
Niacin (mg/d)	1.55 \pm 0.71	1.72 \pm 0.89	1	1	0	00	0.001 [†]	0.001 [†]
Pyridoxine (mg/d)	28.99 \pm 9.85	32.21 \pm	14	16	207.0	201.	0.001 [†]	0.001 [†]
Cyanocobalamin (ug/d)	5.06 \pm 4.23	5.77 \pm 4.12	4	4	7	31	0.001 [†]	0.001 [†]
					176.1	187.	0.001 [†]	0.001 [†]
					3	69	0.001 [†]	0.001 [†]
					210.8	240.	0.001 [†]	0.001 [†]
					4	41		

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belongs to University of Costa Rica. ♀: university women; ♂: university men; \pm SD: standard deviation. Comparative analysis using Student's t test as requested. Significance according to *p \leq 0.05, **p \leq 0.01, [†]p \leq 0.001.

Table VII. Phytoestrogen daily intake of the university participants

	Media and (SD)		p
	♀	♂	
Daidzein	0.03 ±	0.01 ±	0.058
	0.14	0.03	
Genistein	0.05 ±	0.02 ±	0.057
	0.24	0.04	
Formononetin	0.002 ±	0.002 ±	0.440
	0.003	0.002	
Biochanin A	0.0012 ±	0.0012 ±	0.156
	0.0011	0.0012	
Coumestrol	0.0012 ±	0.0012 ±	0.828
	0.0011	0.0012	
Matairesinol	0.009 ±	0.008 ±	0.569
	0.008	0.007	
Secoisolariciresinol	0.05 ±	0.05 ±	0.281
	0.04	0.03	
Enterolactom	0.10 ±	0.10 ±	0.442
	0.05	0.06	
Enterodiol	0.06 ±	0.06 ±	0.773
	0.03	0.04	
*Total phytoestrogens	0.23 ±	0.17 ±	0.054
(mg/day)	0.40	0.13	
*Total phytoestrogens	7.01 ±	5.14 ±	0.054
(mg/month)	11.94	3.96	
*Total isoflavones	0.09 ±	0.04 ±	0.057
	0.38	0.08	
†Total lignans and	0.24 ±	0.23 ±	0.442
precursors	0.12	0.14	

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belong to University of Costa Rica. *Data referred to the daizdein as substance pattern. †Data referred to the enterolacton as substance pattern. ♀: university women; ♂: university men; ±SD: standard deviation. Comparative analysis by using Student's t test by sex.

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Table VIII. Spearman's correlation (r_{sp}) between phytoestrogen (mg/d) and the participants' food group intake (g/d) per day

		Daizdei	Genistei	Formono	Biochani	Coume	Mataire		Enterola	Enterodi
		n	n	n	n A	st	sinol	Secoiso	c	ol
Cereals	r_{sp}	0.247 [†]	0.231 [†]	(0.362) [†]	0.189 [†]	-0.179 [†]	0.268 [†]	0.224 [†]	0.199 [†]	0.284 [†]
	Sig.	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Legumes	r_{sp}	0.313 [†]	0.266 [†]	0.545 [†]	0.323 [†]	0.402 [†]	0.195 [†]	0.229 [†]	0.326 [†]	0.323 [†]
	Sig.	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Fruits	r_{sp}	0.462 [†]	0.439 [†]	0.452 [†]	0.324 [†]	0.440 [†]	0.434 [†]	0.632 [†]	0.584 [†]	0.577 [†]
	Sig.	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Vegetables	r_{sp}	0.550 [†]	0.544 [†]	0.346 [†]	0.338 [†]	0.546 [†]	0.466 [†]	0.549 [†]	0.813 [†]	0.842 [†]
	Sig.	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Soy	r_{sp}	0.287 [†]	0.324 [†]	0.138*	0.092	0.155 [†]	0.184 [†]	0.168 [†]	0.121*	0.154 [†]
	Sig.	0.001	0.001	0.012	0.097	0.005	0.001	0.002	0.029	0.005
Sweets	r_{sp}	0.218 [†]	0.202 [†]	0.281 [†]	0.237 [†]	0.126*	0.328 [†]	0.231 [†]	0.183 [†]	0.199 [†]
	Sig.	0.001	0.001	0.001	0.001	0.023	0.001	0.001	0.001	0.001
Beverages	r_{sp}	0.132*	0.071	0.177 [†]	0.082	0.082	0.142*	0.246 [†]	0.233 [†]	0.260 [†]
	Sig.	0.018	0.203	0.001	0.136	0.137	0.010	0.001	0.001	0.001

Source: produced by Hernández-Elizondo J. Results of the analyzed sample belong to the University of Costa Rica. \pm SD: standard deviation; r_{sp} : Spearman's correlation. Significance according to * $p \leq 0.05$, ** $p \leq 0.01$, [†] $p \leq 0.001$