Validity and reproducibility of a food frequency questionnaire to assess lipid and phytochemical intake

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VALIDITY AND REPRODUCIBILITY OF A FOOD FREQUENCY QUESTIONNAIRE TO ASSESS LIPID AND PHYTOCHEMICAL INTAKE

VALIDACIÓN Y REPRODUCIBILIDAD DE UN CUESTIONARIO DE FRECUENCIA DE CONSUMO ALIMENTARIO PARA VALORAR LA INGESTA DE LÍPIDOS Y FITOQUÍMICOS

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Abstract

Background: epidemiological studies have been related food intake with the incidence of non-transmissible chronic diseases.

Objective: the purpose of the present study was to analyze the validity and reproducibility of a food frequency questionnaire (FFQ) aimed at assessing lipid and phytochemical intake.

Material and Methods: FFQ was administered to 45 people of both sexes, aged between 20 and 72 years old and resident in Cordoba, Argentina. The FFQ included 257 questions referring to foods, their consumption frequency and portion size. Regarding consumption of fruit and vegetables, the season was also taken into account. The questionnaire was applied at two different periods (FFQ1 and FFQ2) with a break of six months in between. As a reference, the 24-hour dietary recall was used (24HDR) three times. The mid intake of FFQ1-FFQ2, the 24HDR-FFQ2 median intake, median difference, Mean Absolute Deviation from the median differences (MAD), Wilcoxon signed rank sum test and Spearman rank order correlation coefficients were calculated to analyze the accuracy of the FFQ data.

Results: the correlation coefficients for FFQ1-FFQ2 varied from 0.52 for 20:5 n3 eicosapentanoic (EPA) fatty acid to 0.89 for 4:0 butyric fatty acid (p<0.05). For 24HDR-FFQ2, the values ranged from 0.19 for lycopene to 0.93 for EPA fatty acid (p<0.05).

Conclusion: the analysis carried out showed an acceptable validity and reproducibility of the FFQ, thus enabling it to be used in research relating the intake of lipids and phytochemicals and the risk of non-transmissible diseases.

Key words: dietary recalls; validity; reproducibility; food frequency questionnaire; lipids; phytochemicals

Resumen

Estudios epidemiológicos sugieren que el consumo de ciertos alimentos puede reducir el riesgo de enfermedades no transmisibles (ENT).

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Objetivo: analizar la validez y reproducibilidad de un cuestionario de frecuencia de consumo alimentario (FFQ) destinado a evaluar la ingesta de lípidos y fitoquímicos.

Material y métodos: el FFQ fue administrado a 45 personas de ambos sexos, con edades entre 20 y 72 años de edad, residentes en Córdoba, Argentina. Incluyó 257 preguntas referidas a los alimentos, su frecuencia de consumo y tamaño de la porción. Se consideró la estacionalidad para el consumo de frutas y verduras. El cuestionario se aplicó en dos períodos diferentes (FFQ1 y FFQ2) con un intervalo de seis meses y se acompañó por un recordatorio de 24 horas (24HDR) utilizado en tres periodos de tiempo. Se realizó la estimación de: ingesta promedio FFQ1-FFQ2 y 24HDR-FFQ2, diferencia de medias, desviación absoluta de diferencias de medias, prueba de los rangos con signo de Wilcoxon y test de correlación de Spearman.

Resultados: los coeficientes de correlación para FFQ1-FFQ2 variaron de 0,52 para el ácido graso eicosapentanoico 20:5 n3 (EPA) a 0,89 para ácido graso butírico 4:0 (p<0,05). Para 24HDR-FFQ2, los valores de correlación oscilaron entre 0,19 para licopeno y 0,93 para el EPA (p <0,05).

Conclusión: el análisis realizado mostró una validez aceptable y reproducibilidad del FFQ, permitiendo así que sea utilizado en investigaciones sobre ingesta de lípidos y fitoquímicos y el riesgo de ENT.

Palabras claves: recordatorio de veinticuatro horas; validación; reproducibilidad; cuestionario de frecuencia alimentaria; lípidos; fitoquímicos

Introduction

Epidemiological studies have indicated strong associations between diet and health, mainly referred to chronic diseases such as cardiovascular disease, obesity and cancer, among others [1,2]. Nutritional research was previously based on a single nutrient intake while the dietary patterns sometimes emphasizing one or various nutrients are now preferred [3].

It is necessary to know a person dietary intake to understand the relationship between the diet as a modifying factor and the chronic diseases. The food frequency questionnaire (FFQ) is usually employed to get dietary information in a short time (1-2 hours). Like other instruments, the FFQ requires validation and reproducibility studies that guarantee its applicability in specific populations avoiding the measurement errors that would minimize the epidemiological outcome related to the development and progression of chronic diseases [4,5].

Dietary lipids are considered to have a significant impact on human health, not only in relation to cancer but also to cardiovascular diseases. Depending on their type and amount, they may promote or protect against tumors in different tissues [6]. It has been suggested that a high intake of total fat –especially saturated fat and cholesterol- promotes cancer in various organs, whereas the opposite effect has been found for polyunsaturated fatty acids [7,8].

Phytochemicals are bioactive non-nutrient plant compounds found in fruit, vegetables, grains, and other plant foods generally related to the prevention of chronic diseases [3]. Certain epidemiologic studies have consistently supported the association between high intake (5 servings of FV meaning at least 400 grams, excluding potatoes) of fruits and vegetables (FV) –rich in phytocemicals- and lower risk of cancer, heart disease, and stroke.

A clinical-epidemiological research protocol designed in Córdoba, Argentina, to study the association between hormone-dependent tumor development and dietary conditions -mainly lipids and phytochemicals- required a questionnaire adapted to the regional dietary habits. The purpose of the present study was to analyze the validity and reproducibility of a FFQ aimed at assessing the lipid and phytochemical intake.

Subjects, Materials and Methods

Subjects

Forty five voluntary adult subjects of both sexes, 27 women and 18 men, participated in the study. All of them lived in Córdoba -a Mediterranean city of Argentina-, belonged to the middle social and economic class, and had mixed alimentary habits. The subjects' characteristics are described in Table 1.

Participants signed the informed consent approved by Institutional Committee of Ethics in Health Research (CIEIS) of Province of Córdoba, Argentina. Besides, this study took into account national and international ethic laws, like the Argentine National Act 25,326 on protection of personal data (Hábeas Data).

Study design

Two trained nutritionists applied the following food information collecting methods:

* Two FFQ: they were administered with an interval of six months between the first (FFQ1) and the second (FFQ2) FFQ in order to evaluate seasonal intake in the Southern Hemisphere.

* Three 24-hour dietary recalls (24HDR): they were applied at the beginning of the week, at the end of the week and at midweek day, with an interval of four months between each of them.

The devices used for FFQ1, FFQ2 and for 24HDR –used as a reference method- are described below.

Instruments

Food frequency questionnaire and 24HDR

The FFQ included questions related to 257 types of food and drink consumed five years before the time of the interview, taking into account the latent period between the consumption of a particular food and its potential consequences on health [9]. The frequency references used were: never, the number of times per month, per week and per day. The portion size was described as small, medium or large. A photographic guide was used in order to better understand these portion size definitions [10]. For those that were not included in this guide, it was established that the medium size portion was the standard, with 50% bigger defined as a large portion, and 50% smaller as a small portion, respectively [11]. Moreover, home measures were also used (plates, spoons, cups).

Seasons were taken into account when referring to the consumption of vegetables and fruit. For instance: a fruit available six months a year and eaten three times a week during the season produces a frequency of approximately 78 times a year [12].

The 24HDR consisted in a personal interview carried out by well trained nutritionists in which the participants were asked about the food and drink intake during the previous twenty four hours. The photographic guide, portion size and home measures (plates, spoons, cups) were the same as for the FFQ. All items of FFQ and 24HDR –related to the consumed food- were codified and changed into daily intake of nutrients and phytochemicals by means of the software Interfood1 [13]. The 35 nutrients and phytochemicals taken into account in the research were grouped into: energy, proteins, carbohydrates, lipids, saturated fatty acids, polyunsaturated fatty acids, cholesterol, total fibers, alcohol, vitamins, minerals, lycopene and isoflavones.

Data Analysis

Analysis of reproducibility of the FFQ

Non-parametric methods were used since the nutrient intake did not have a normal dis-

Variable	Mean	SD [1]	VC [2]	Min	Max
Age (years old)	37.53	13.91	37.05	20.00	72.00
Weight (kg)	68.89	15.17	22.02	48.00	104.00
Height (m)	1.70	0.09	5.01	1.55	1.92
BMI (weight/height^2)	23.57	3.40	14.41	17.64	30.79

Table 1: Characteristics of subjects according to age, weight, height and body mass index
SD: standard deviation
VC: variance coefficient

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tribution. FFQ1 and FFQ2 reproducibility was analysed through the median intake, median difference, Mean Absolute Deviation from the median differences (MAD) [12]. Wilcoxon signed rank sum test and Spearman rank order correlation coefficients [14].

The median difference is an estimate of the average errors of FFQ1 in relation to FFQ2, while MAD gives information about the average value of individual differences and might be understood in relation to the median intake of the two measures [12].

The Wilcoxon signed rank sum test was used to verify if the median difference of the nutrient and phytochemical intake is equal to zero (null hypothesis).

The Spearman rank order correlation coefficients between FFQ1-FFQ2 were estimated in order to determine the reproducibility of both questionnaires.

Analysis of the validity of the FFQ
The comparison between FFQ2 with the reference method 24HDR was used to test the validity. To carry this out, the median intake, median difference, Mean Absolute Deviation from the median differences (MAD) [12], Wilcoxon signed rank sum test y Spearman rank order correlation coefficients [14] were estimated.

Furthermore, the median for each tercile of consumption was estimated in order to describe the distribution of nutrient intake. In order to analyze the error level in the classification of terciles, the number of subjects in each of the terciles according to FFQ2 with respect to the same tercile obtained through 24 HDR was estimated. The classification was: in the same category, in an adjacent category or in an extreme category.

The software packages that were used for the statistical analysis of reproducibility and validation were InfoStat 2005p.1[18] and StatGraphics Plus v5.1 [19].

Results
Reproducibility of the FFQ
The median intake, median difference and MAD estimated for FFQ1 y FFQ2 are shown in Table 1.

When comparing the median intake of the 35 items (nutrients and phytochemicals) between FFQ1 and FFQ2, it was observed that 67% of them had differences of less than 15%. Out of the remaining 33%, five had a difference of 16-30% between FFQ1 and FFQ2 and the other five varied by more than 60%.

With respect to the average intake of nutrients and phytochemicals estimated between FFQ1 and FFQ2, the MAD value differed greatly for the majority of them. For instance, the median intake of energy was 37.65g, while the MAD was 156.91g. These results also showed a great intra-individual variability.

The median difference between FFQ1-FFQ2 was positive, being only significantly different to zero for butiric, palmitic, palmitoleic, oleic, linolenic and arachidonic fatty acids. For these nutrients, the FFQ2 tended to estimate lower than the FFQ1. For example, the difference in intake for oleic fatty acids was 1.06 g lower in FFQ2 than in FFQ1. For the remaining nutrients and phytochemicals, no significant differences between medians were observed.

The Spearman rank order correlation coefficients between FFQ1 and FFQ2 (Table 3) showed that there was no correlation less than 0.5 for any nutrient or phytochemical. In nineteen of these items, a moderate correlation of 0.5-0.7 was found, while in the rest this was greater then 0.8.

Validity of the FFQ

The median intake, median difference and MAD estimated between 24HDR and FFQ2 are shown in Table 2.

As regards the consumption of nutrients, lower values were observed with 24HDR than with FFQ2, except for butiric, lauric, nervonic and clupanodonic fatty acids, carbohydrates and isoflavones (daidzein). When comparing the median intake between 24HDR y FFQ2, seventeen nutrients had a difference of less than 15%, eleven of them between 16 and 30%, with the remaining seven greater than 30%.

Values significantly different to zero median intake were obtained for total fat, palmitic fatty acid, total monounsaturated fatty acids, oleic and eicosaenoic fatty acids, polyunsaturated fatty acids, cervonic fatty acid, zinc and daidzein (Table 2).

Table 2: Median intake of nutrients and phytochemicals according to FFQ1, FFQ2 y 24HDR. Median difference and MAD for comparison between FFQ1-FFQ2 and 24HDR*-FFQ2

Variables	Median intake			Median difference[d]		MAD[e]	
	FFQ1[a]	FFQ2[b]	24HDR[c]	FFQ1 versus FFQ2	24HDR versus FFQ2	FFQ1 versus FFQ2	24HDR versus FFQ2
Energy (kcal)	2213.03	1953.24	1850.99	-37.65	131.99	156,91	255.65
Carbohydrates(g)	248.27	232.43	224.08	-7.35	-0.21	23,76	31.94
Proteins(g)	83.73	79.68	72.31	0.85	6.72	7,01	8.16
Total fats(g)	84.29	80.72	65.54	0.87	12.61[f]	7,55	8.33
Saturated fatty acids(g)	34.84	33.32	30.20	1.29	2.72	4,30	4.45
Butyric C4:0	0.37	0.47	0.56	-0.02[f]	-0.04	0,06	0.19
Lauric C12:0	0.25	0.29	0.30	0.00	-0.01	0,07	0.12
Myristic C14:0	3.43	3.02	3.03	0.14	0.03	1,05	1.63
Palmitic C16:0	18.03	15.72	12.95	0.70[f]	1.98[f]	2,09	2.38
Stearic C18:0	13.45	13.03	12.22	-0.02	0.40	2,09	2.73
Araquidic C20:0	0.10	0.10	0.08	0.00	0.00	0,06	0.09
Monounsaturated fatty acids (g)	34.89	30.13	21.58	0.83	4.84[f]	3,72	5.13
Palmitoleic C16:1 n7	2.46	2.34	1.90	0.13[f]	0.55	0,50	0.56
Oleic C18:1 n9	27.35	25.31	18.98	1.06[f]	4.16[f]	2,82	3.63
Eicosaenoic C20:1 n9	0.67	1.33	1.56	0.00	0.22[f]	1,43	1.23
Nervonic C24:1 n9	0.33	0.33	0.40	0.00	-0.04	0,11	0.10
Polyunsaturated fatty acids (g)	15.76	17.58	11.68	-0.63[f]	3.90[f]	1,94	3.17
Linoleic C18:2 n6	11.76	14.62	10.10	-0.42	2.36[f]	1,51	2.80
Linolenic C18:3 n3	0.73	0.64	0.46	0.10[f]	0.15	0,11	0.14
Arachidonic C20:4 n6	0.33	0.23	0.13	0.01[f]	0.07	0,07	0.11
Eicosapentanoic C20:5 n3	0.00	0.00	0.00	0.00	0.00	0,003	0.002
Clupanodonic C22:5 n3	0.02	0.02	0.02	0.00	-0.003[f]	0,01	0.01
Docosahexaenoic C22:6 n3	1.60	161	0.61	0.00	1.03[f]	0,71	1.12
Cholesterol (mg)	245.04	255.87	245.55	-2.50	12.47	26,77	47.35

a First food frequency questionnaire
b Second food frequency questionnaire
c Dietary recalls
d Median difference between both questionnaires
e Mean absolute deviation from the median differences (MAD)
f Median difference significantly different from zero (p< 0.05), Wilcoxon signed rank sum test
* Median of three 24HDRs

The Spearman rank order correlation coefficients between 24HDR and FFQ2 are shown in Table 3. Ten items showed a correlation

>0.5, 23 had a moderate correlation and the rest were >0.8. Inter-personal variation in nutrient intake for the three 24 HDRs weakened the correlation coefficients.

Table 3: Spearman rank order correlation coefficients of intake of nutrients and phytochemicals between FFQ1-FFQ2 and 24HDR-FFQ2

bles	FFQ1		FFQ2		24HDR		FFQ1 versus FFQ2	24HDR versus FFQ2
	Mean	SD	Mean	SD	Mean	SD	r^a	r^b
gy (kcal)	2140.21	718.26	2080.93	634.91	1941.7	573.11	0.88	0.44
ohydrates(g)	252.58	103.87	246.71	90.80	232.53	72.97	0.86	0.54
ins(g)	84.56	27.39	80.40	21.84	75.33	21.13	0.85	0.41
fats(g)	89.00	29.28	85.75	28.28	74.33	27.67	0.87	0.75
rated fatty acids(g)	38.57	13.89	36.26	13.16	34.20	12.30	0.79	0.74
ic C4:0	0.43	0.31	0.48	0.34	0.61	0.35	0.89	0.28[(1)]
c C12:0	0.35	0.32	0.32	0.25	0.32	0.16	0.89	0.27[(1)]
tic C14:0	3.95	2.79	3.72	3.50	3.95	3.02	0.80	0.32
itic C16:0	18.71	7.26	17.08	6.33	15.19	7.48	0.83	0.71
ic C18:0	15.02	6.31	14.59	5.42	14.03	6.34	0.74	0.60
idic C20:0	0.12	0.15	0.11	0.12	0.15	0.36	0.67	0.67
unsaturated fatty (g)	34.96	12.42	32.76	11.18	27.41	12.18	0.83	0.67
itoleic C16:1 n7	2.96	1.45	2.59	1.16	2.42	1.96	0.75	0.68
C18:1 n9	29.14	10.28	26.93	8.89	23.3	11.19	0.86	0.66
aenoic C20:1 n9	2.50	3.18	2.91	3.70	2.06	2.30	0.53	0.80
nic C24:1 n9	0.29	0.19	0.33	0.24	0.39	0.26	0.76	0.70
nsaturated fatty (g)	15.45	5.58		6.65	12.76	6.31	0.80	0.55
eic C18:2 n6	12.47	5.09	16.72	5.39	10.82	5.54	0.84	0.43
enic C18:3 n3	0.81	0.35	13.23 0.69	0.31	0.61	0.45	0.83	0.64
idonic C20:4 n6	0.34	0.20	0.28	0.15	0.28	0.29	0.79	0.62
apentanoic C20:5	3.3E-03	0.01	0.01	0.01	0.01	0.01	0.52	0.92

a Spearman correlation coefficients (p<0.001)
b Spearman correlation coefficients (p<0.05)
(1)   p<0.010
(2)   p>0.010

When analysing the distribution of the nutrient and phytochemical intake between 24 HDR and FFQ2, an overestimation for the lowest terciles and a tendency to underestimate the highest terciles were observed (Table 4). For some groups of nutrients and phytochemicals, an increase in the median intake from the 1st to the 3rd tercile was not found when classified through FFQ2. For instance, the median intake of lycopene from the 1st to the 3rd tercile was 41.79 g, 32.18 g, 32.66 g, respectively, for FFQ2 and 36.00 g, 48.01 g, 75.25 g for 24HDR (wrong classification of FFQ2).

The margin of error in the classification, related to the intake from the FFQ2, was calculated as the number of subjects similarly classified in the same tercile, adjacent or opposing, according to the 24 HDR (Table 5). The number of subjects similarly classified by both devices oscillated between 33% (proteins, among others) and 84% (eicosapentanoic fatty acid), with the majority of values being between 40-50%. The number of subjects similarly classified in the adjacent category oscillated between 26.67% (alcohol) and 51.1% (total fat). The classification of extreme values was rare, with 11.11% being the highest value (cervonic fatty acid).

Table 4: Distribution of the intake of nutrients and phytochemicals into terciles according to 24HDR* and FFQ2 in relation to 24HDR

Nutrients/Phytochemicals	Median 24HDR, 24HDR-cutpoints			Median 24HDR, FFQ2-cutpoints		
	Terciles			Terciles		
	1	2	3	1	2	3
Energy (kcal)	1578.88	2055.35	3598.72	1603.79	1627.24	3577.47
Carbohydrates(g)	188.10	239.40	497.42	102.35	159.79	450.15
Proteins(g)	65.36	78.58	123.81	86.41	88.61	133.43
Total fats(g)	53.17	72.34	156.59	84.12	123.00	170.84
Saturated fatty acids(g)	23.65	39.88	68.81	24.43	26.13	72.54
Butyric C4:0	0.30	0.60	1.68	0.02	0.51	0.70
Lauric C12:0	0.19	0.31	1.04	0.06	0.48	0.29
Myristic C14:0	1.41	3.01	12.39	5.23	1.79	7.05
Palmitic C16:0	8.97	14.09	42.13	22.71	23.64	33.76
Stearic C18:0	8.16	14.80	41.69	14.69	22.06	10.21
Araquidic C20:0	0.05	0.10	0.54	0.06	0.17	0.10
Monounsaturated fatty acids(g)	17.30	23.89	76.42	36.76	19.76	61.23
Palmitoleic C16:1 n7	0.53	1.70	10.95	3.10	4.38	4.44
Oleic C18:1 n9	13.94	18.93	63.87	18.13	17.12	50.91
Eicosaenoic C20:1 n9	0.00	1.33	9.33	2.00	4.67	14.00
Nervonic C24:1 n9	0.00	0.22	1.58	0.34	0.22	1.58
Polyunsaturated fatty acids(g)	9.65	12.91	35.47	15.58	10.70	37.07
Linoleic C18:2 n6	7.64	11.62	27.37	9.77	6.93	25.39
Linolenic C18:3 n3	0.23	0.43	2.23	0.69	1.00	1.16
Arachidonic C20:4 n6	0.02	0.11	1.68	0.25	0.05	0.51
Eicosapentanoic C20:5 n3	0.00	0.00	0.03	0.00	0.01	0.03
Clupanodonic C22:5 n3	0.01	0.03	0.08	0.01	0.02	0.08
Docosahexaenoic C22:6 n3	0.06	0.17	6.34			
Cholesterol (mg)	182.15	333.79	707.47	1.61	1.61	9.53
Total fiber (g)	4.01	5.53	10.11	206.88	365.89	244.80
Lycopene (ug)	36.00	48.01	75.25	4.77	5.12	5.70
Isoflavones (g)	0.00	0.00	47.00	41.79	32.18	32.66
Genistein (g)	0.00	0.00	22.71	0.03	13.45	20.17
Daidzein (g)	0.00	0.00	22.44	0.00	2.45	9.73
Alcohol (g)	0.22	2.37	23.57	2.16	0.01	9.62
				2.41	1.68	9.84

Table 5: Error analysis in the classification of subjects according to terciles of consumption of intake of nutrients and phytochemicals (FFQ2/24HDR)

Variable	FFQ2 / 24HDR		
	Same category (1)	Adjacent category (1)	Extreme category (1)
Energy	42.22	42.22	2.22
Carbohydrates	48.89	35.56	2.22
Proteins	33.33	40.00	4.44
Total fats	40.00	51.11	2.22
Saturated fatty acids	48.89	48.89	2.22
Butyric C4:0	46.67	37.78	0.00
Lauric C12:0	33.33	35.56	2.22
Myristic C14:0	44.44	35.56	2.22
Palmitic C16:0	37.78	48.89	2.22
Stearic C18:0	53.33	42.22	0.00
Araquidic C20:0	42.22	48.89	0.00
Monounsaturated fatty acids	44.44	35.56	0.00
Palmitoleic C16:1 n7	35.56	46.67	0.00
Oleic C18:1	40.00	35.56	0.00
Eicosaenoic C20:1 n9	68.89	22.22	4.44
Nervonic C24:1 n9	48.89	37.78	0.00
Polyunsaturated fatty acids	46.67	35.56	2.22
Linoleic C18:2 n6	48.89	40.00	0.00
Linolenic C18:3 n3	37.78	40.00	0.00
Arachidonic C20:4 n6	33.33	42.22	0.00
Eicosapentanoic C20:5 n3	84.44	11.11	0.00
Clupanodonic C22:5 n3	46.67	33.33	0.00
Docosahexaenoic C22:6 n3	33.33	31.11	11.11
Cholesterol	46.67	48.89	0.00
Total fiber	40.00	35.56	8.89
Lycopene	42.22	46.67	2.22
Isoflavones	51.11	33.33	0.00
Genistein	43.33	36.67	0.00
Daidzein	46.67	31.11	0.00
Alcohol	66.67	26.67	0.00

(1) All values correspond to % of subjects

Discussion

The food frequency questionnaire analysed presented a strong correlation between estimations of both FFQs (reproducibility) and between the 24HDR and the FFQ2 (validation), for macro and micro-nutrients, especially for lipids and phytochemicals. Hong et al found similar results referred to macro and micronutrients in a study involving Corean people suffering from type 2 diabetes. However, the correlation between both methods was slightly lower when compared to a Western diet maybe due to the differences in intake amount and season as well as the food type [20].

In the questionnaire presented, the portion size was specified and this was then combined with the consumption frequency, since Block y Hartman noticed that the complexity of a questionnaire is an issue that could have a great impact on reproducibility [21].

With respect of validation, three 24 HDRs were used as an alternative to a multiple dietary recall. Results showed an underestimation of the median intake of 24HDR in relation to FFQ2. That is why it is advisable to use more 24 HDRs for the analysis of validation or its combination with biomarkers [22,23].

The Spearman rank order correlation coefficients showed a high reproducibility for the majority of nutrients and phytochemicals, especially between FFQ1 and FFQ2. Moreover, values were comparable to the ones observed in other research [23]. The main correlation coefficients between FFQ1-FFQ2 and 24HDR-FFQ2 for nutrients (macro and micronutrients) and chosen phytochemicals were 0.32-0.92 ($p<0.05$) and 0.66-0.84 ($p<0.001$), respectively, whereas other studies showed substantially lower correlation coefficients [30]. Values between 0.4-0.8 seem to be typical reproducibility coefficients for FFQs applied among middle-aged subjects [21,22,25].

Compared to the 24HDR, the FFQ showed a moderate overestimation for some of the investigated nutrients, especially for polyunsaturated fatty acids (23%), while a small underestimation was noticed for butyric and myristic fatty acids, cholesterol and some vitamins.

With respect to this, Navarro et al reported slightly higher levels of fatty acids [26]. In other study in Argentina by Elorriaga et al, a modest correlation for energy-adjusted for fatty acids and cholesterol was observed [27].

The average intake of genistein and daidzein was 0.05 and 0.16 mg/day, respectively, while Maskarinec in Hawaii reported an intake of 7 to 4 mg/day for the same phytochemicals [28]. Concerning the correlation coefficients, values lower than those found by Kirk were noted (0.70 and 0.69). He had previously observed a value of 0.89 for both genistein and daidzeína. From our results, the intake of these two phytochemicals was highly reproducible between 24HDR and FFQ2, which

could be a consequence of a low intake of food based on soy in the studied population. The main consumption source of phytoestrogen was breaded soy, while other studies referred to tofu and soy milk as the most consumed food [11].

The classification of percentiles strongly depends on the distribution of the real levels of intake in the population study, and also on the ability of the FFQ to correctly classify subjects. Therefore, it is open to interpretative difficulties. However, the observed alterations in classification might be related to the tendency of the FFQ to either overestimate or underestimate the intake of some nutrients. When analysing data, it is necessary to correctly define the intake categories, otherwise estimates of relative risks might be inaccurate [29].

The number of subjects included in the study was not high but coincident with that considered in other investigations [30,31]. While in our study were two DR, according to the recommendations of Hong it should also be taken into account the fluctuation within individuals by comparing the DR performed 4 times per year with the FFQ method [20].

Summing up, the analysed device showed an acceptable validity and reproducibility, which enables it to be used in research examining the relation between lipid and phytochemical intake and the risk of chronic diseases in Argentina and other regions of Latin America which present similar food consumption, irrespectively of their quantity and frequency.

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