Original Article

Development and validation of a food frequency questionnaire for consumption of polyphenol-rich foods in pregnant women

Izabele Vian*, Paulo Zielinsky*, Ana Maria Zilio*, Anne Mello*, Bruna Lazzeri*, Andressa Oliveira*, Kenya Venusa Lampert*, Antônio Piccoli*, Luis Henrique Nicoloso*, Guilherme Borges Bubols[†] and Solange Cristina Garcia[†]

*Fetal Cardiology Unit, Institute of Cardiology of Estado do Rio Grande do Sul, Porto Alegre, Brazil, and [†]Department of Toxicology, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Abstract

Previous studies have shown that maternal consumption of polyphenol-rich foods after the third trimester of pregnancy may interfere with the anatomical and functional activity of the fetal heart as, to our knowledge, there are no validated instruments to quantify total polyphenols in pregnant women. The aim of this study was to evaluate the reproducibility and validity of a food frequency questionnaire (FFQ), with 52 items, to assess the intake of polyphenol-rich foods in pregnant women in Brazil. This cross-sectional study included 120 pregnant women who participated in nutritional interviews in two moments. The intake of polyphenols estimated by the developed FFQ was compared with the average of two 24-h recalls (24HR), with the average intake measured by a 3-day food diary (D3days) and with the urinary excretion of total polyphenols. The triangular method was applied to calculate Pearson's correlation coefficients, intraclass correlation and Bland–Altman plots for the FFQ, using an independent biochemical marker, in addition to classification by quarters of consumption. The questionnaires were log transformed, adjusted for body mass index and gestational age. The adjustment for energy was applied only of 24HR and D3days. Analysis of the reproducibility between the FFQ showed a very high correlation ($r = 0.72$; $P < 0.05$). A low but significant association was observed between the FFQ and urinary excretion (0.23 ; $P = 0.01$). The association between the dietary survey methods was moderate to very high ($r = 0.36$ to $r = 0.72$; $P < 0.001$). In conclusion, this questionnaire showed reproducibility and validity for the quantification of consumption of total polyphenols in pregnant women.

Keywords: food frequency questionnaire, dietary intake assessment, validation, antioxidants, polyphenols and pregnancy.

Correspondence: Izabele Vian, Fetal Cardiology Unit, Institute of Cardiology of Estado do Rio Grande do Sul, Av. Princesa Isabel, 395, Santana, Porto Alegre, RS 90620-000, Brazil. E-mail: ped.nutri@yahoo.com.br

Introduction

There are evidences indicating that consumption of polyphenol-rich foods after the third trimester of pregnancy may interfere with the anatomical and functional activity of fetal heart (Zielinsky *et al.* 2011). Similar to non-steroidal anti-inflammatory drugs, these foods may have an inhibitory effect on

the synthesis of prostaglandins and are associated with cases of fetal ductus arteriosus constriction (Gordon & Samuels 1995; Norton 1997). These considerations stress the importance of assessing maternal exposure to that substance.

Few studies have been developed and validated in Brazil about food frequency questionnaires (FFQ) to assess usual consumption in pregnant women

(Rondó *et al.* 1999; Giacomello *et al.* 2008; Oliveira *et al.* 2010). Also, there are, to our knowledge, no studies on the frequency of consumption of all classes of total polyphenols in pregnant women. Therefore, there is a need for the development and validation of a dietary assessment tool that quantifies the presence of total polyphenols in the diet of pregnant women, including a large number of foods rich in this substance.

FFQ is an often-used method to evaluate usual dietary intake due to the easy administration. Food consumption can be evaluated during a long period of time, with low costs. In diet programmes, food intake is notoriously difficult to assess due to measurement errors and to the difficulty of estimating portion size. The FFQ must be validated to provide information on the accuracy of measurement in the target population (Willett 1998).

Validation studies may include biochemical markers of food intake, in addition to an often-used method in the diet (Nelson 1991), although the correlations between estimated consumption and biomarkers are usually weaker than the correlations between two dietary methods. The low correlation between dietary intake and biomarkers is explained by the influence of other factors in addition to consumption, such as individual differences in absorption and metabolism, genetics and changes resulting from biochemical adaptation of the organism to situations such as pregnancy (Willett & Lenart 1998; Arab & Akbar 2002; Arab 2003).

The aim of this study was to test the reproducibility and validity of a FFQ. This questionnaire measured the intake of foods rich in polyphenols by pregnant women.

Methods

Outline of the study

Cross-sectional study for the validation of a questionnaire of frequency of consumption of foods rich in polyphenols by pregnant women.

Study population and sample

Pregnant women from the public health system who volunteered for a fetal echocardiogram test, performed at the Institute of Cardiology in Porto Alegre, Brazil, participated in this study. The data were collected in May 2011. The calculation of sample size by the intraclass correlation (ICC) test, with 90% power, significance level ≤ 0.05 and a minimum correlation coefficient of 0.33, as reported in a study of Norwegian women (Brantsaeter *et al.* 2007), indicated a minimum number of 93 pregnant women. Inclusion criteria were gestational age ≤ 36 weeks, signing of a follow-up commitment form and delivery of the 3-day food diary (D3days). Pregnant women with abnormal fetal echocardiography, who could not read or write or refused to participate, were excluded from the study.

A total of 120 pregnant women who matched the inclusion criteria were initially selected. Data from these 120 pregnant women were used to assess the correlation of first moment questionnaires [FFQ and 24 h recall (24HR)] with excretion of total polyphenols in urine. After 15 days, 95 pregnant women returned for the second-period interview (FFQ and 24HR), but two of them did not deliver the D3days. Thus, the final sample included 93 pregnant women with complete data, i.e. urine sample,

Key messages

- The FFQ provides new valid estimates of consumption of polyphenol-rich foods by pregnant women in south Brazil.
- Correlations among the methods of dietary assessment were stronger than biomarkers and the results of the questionnaires.
- The results for intake of total polyphenols estimated by the FFQ were significantly higher than by 24HR and D3days.

responses to two FFQ and two 24HR and completed D3days.

The study was approved by the Ethics Committee of the Institute of Cardiology of Estado do Rio Grande do Sul, Brazil, under number 447110. All pregnant women provided written informed consent, after having been fully informed of the purpose of the project. The study followed the guidelines of Resolution 196/96 from the Brazilian Health Council, which establishes principles for research with humans, with assurance of anonymity and privacy of participants.

Development of the food consumption frequency questionnaire

The FFQ was developed with the following question: 'During pregnancy, what is the frequency with which you have consumed or consume the following foods?' The FFQ included 52 polyphenol-rich foods, classified as those with content of polyphenol substances above the 75th percentile, i.e. with at least 30 mg of polyphenol per 100 g of food, as established by the American database (United States Department of Agriculture 2007). The median portion size of each food was established from this model of FFQ and a 24HR used previously in a pilot study, with 119 pregnant women. The results were described in absolute frequency, median and interquartile range interval for the determination of the median portion size of each food of FFQ.

The FFQ developed has eight categories of answers on the frequency of use of each food on the list, ranging from 'never' up to seven times, considering one unit of time (day, week, month, year and rarely). Forty-four of the 52 foods included in the FFQ were selected according to the American database (United States Department of Agriculture 2007), considering a concentration of polyphenols equal to or greater than 30 mg per 100 g of food (above the 75th percentile). Eight other foods of higher consumption and polyphenol content, according to a study about food in Brazil (Faller & Fialho 2009) were included.

The median portion size of each food of the FFQ was determined with the use of domestic measurement tools, identified by the pregnant women by pictures, according to a book of domestic measurement

of weight and volume (Vitolo 2008). Each pregnant woman described the portion size she consumed. The average portion of each food was described only as a guide for the person to determine if the portion consumed was equal, greater or smaller than the average, but the participant reported the exact size of each portion of each food consumed. For example, for the intake of 0.5 cup of tea of average size (average portion = 150 mL), a 75-mL intake was recorded. Portion sizes were different for each food item and the foods were not grouped together.

Quantification of polyphenol-rich foods recorded in 24HR and FFQ was initially accomplished through domestic measures, which were transformed into millilitres (mL) for volume and grams (g) for mass, also using as a reference the book on domestic measurement of weight and volume (Vitolo 2008). The results were put into a database using Microsoft Office Excel 2007, in which each polyphenol-rich food was a variable, with records of its consumption.

Logistics of the study

Data were collected through interviews in the outpatient clinic of Institute of Cardiology of Rio Grande do Sul, Brazil, on two occasions, with an interval of 15 days. In a first moment, identification and demographic data were collected with a socio-economic questionnaire with information about the family income, with four minimum wage classifications and education level assessed by the number of years of formal education. The FFQ developed in this study, with polyphenol-rich foods, and a 24HR prior to interview, were used for dietary analysis. On the same day, pregnant women received a D3days with clear and objective instructions, to be completed at home, a precision scale to weigh all food consumed and a measuring cup to measure the liquids ingested in the days of the registry.

Pregnant women were initially instructed to respond to the FFQ based on total period of gestation. In the case of foods that were not consumed during all the gestational period, an estimate of daily consumption was made by multiplying the reported portion by frequency of use, and dividing by the

number of days in the time unit (day, week, month or year; the year was counted as total days of gestation). All analyses were adjusted for gestational age.

In a second moment, 15 days after the first interview, the pregnant women returned to deliver the D3days and responded the same FFQ used in the first moment. The amount of food consumed during this period was also measured through domestic measures and estimated by photos (Vitollo 2008).

Calculation of total polyphenols and energy from food questionnaires

The measurement of total polyphenols from information collected with the food questionnaires was based on the American database (United States Department of Agriculture 2007), which presents the subclasses and contents of flavonoids in 385 foods, and on the French database (Phenol-Explorer 2009), containing more than 300 registered food, with the values of total polyphenols and their various subclasses for each food. The results of total polyphenols found in dietary questionnaires were described in milligrams (mg; Table 2).

Total polyphenols present in mate tea (infusion of yerba mate *Ilex paraguariensis*) were quantified by physical-chemical testing according to the Official Methods of AOAC International 18th edition, with a concentration of 47.4% and a temperature of 80°C. These parameters were used in order to reproduce the conditions of consumption of this drink among the population of southern Brazil (Kummer *et al.* 2005).

The total energy value of the methods of dietary assessment (24HR and D3days) was calculated through the Microsoft Excel 2007 software, using as a reference a Brazilian table of food composition (TACO 2006). The energy results found in dietary questionnaires were described in kilocalories (kcal; Table 3).

Anthropometric measurements and evaluation of nutritional status

Participants were weighed with an anthropometric digital scale, without shoes and without excess cloth-

ing. Height was measured using a vertical stadiometer attached to the scale, graduated every 0.5 cm and an extensive range between 95 and 195 cm, brand *Welmy* and model *W110h*. The participant was barefoot, with feet together and knees straight. The head and neck were aligned and hold in place by the researcher. The pre-pregnancy weight was obtained through information provided by the pregnant woman.

The nutritional status in gestation was diagnosed by calculating the current and pre-pregnancy body mass Index (BMI), with reference to gestational age, according to the classification of the World Health Organization 2006 (Atalah *et al.* 1997).

Urine collection and analysis

A volume of 50 mL of urine samples were randomly collected, in sterile containers, only in the first moment of the study and stored at -80°C protected from light until analysis.

Quantification of total polyphenols in urine was performed as described and validated by Medina-Remón *et al.* (2009). Briefly, the urine samples stored at -80°C, were thawed for 3 h in an ice bath and centrifuged 4°C for 10 min. Samples were then diluted and acidified, and processed for solid phase extraction with Waters Oasis MAX 30-mg cartridges (Milford, MA, USA). Fifteen μ L of the eluates were added to 170 μ L of Milli-Q water (Millipore, Bedford, MA, USA) in 96-well microplates for reaction with 12 μ L of the Folin-Ciocalteu reagent 2 M and 30 μ L 20% sodium carbonate for 1 h. This reaction detects total phenolic groups present in the samples, thus allowing quantification of the broad array of dietary polyphenols excreted in urine. After incubation, 50 μ L of Milli-Q water were added and optical density was read in a plate reader Spectramax M2 (Molecular Devices, Sunnyvale, CA, USA), at 765 nm. Urinary creatinine was determined according to the modified method of Jaffé (1986) by spectrophotometry using commercial kits (Doles Reagents, Goiânia, GO, Brazil). Total polyphenols excreted in urine were expressed in milligrams (mg) of gallic acid equivalents per gram (g) of creatinine.

Statistical analysis

General characteristics are presented in absolute frequency and categorical variables as percentage. Continuous variables with symmetrical distribution are expressed as mean and standard deviation (SD), and those with asymmetrical distribution, as median and interquartile range. Statistical differences between the median consumption of total polyphenols determined by the FFQ and the average consumption determined by the other methods were evaluated with the paired *t*-test. Statistical data were analysed with the Statistical Package for the Social Sciences software, version 19.0 (SPSS Inc., Chicago, IL, USA). The paired *t*-tests assessed the difference between total polyphenols of the FFQ and the average of the other dietary survey methods.

Comparison of the dietary methods and between the dietary methods and the biomarker for the relative validity and reproducibility was performed with the Pearson's correlation coefficients (used to assess the linear proximity relation between both methods) and ICC, to evaluate the concordance between methods, with 95% confidence interval (95% CI). Due to the attenuation caused by the daily intrapersonal variation (IV) in dietary intake, the Pearson's and ICC coefficients were corrected by the ratios of variance of the two 24HR (Willett 1994; Zanolli *et al.* 2009).

The FFQ was also validated by means of concordance analysis between the methods: number of pregnant women classified by consumption quartiles, by Kappa analysis and Bland–Altman plots (Bland & Altman 1995; Cade *et al.* 2002; Hirakata & Camey 2009). The results with $P < 0.05$ were considered significant. All data have been log transformed prior to analysis to improve the uniformity.

Pearson's correlations were adjusted for BMI, gestational age and total energy value. The total energy value was adjusted only for the 24HR and D3days questionnaires. The correction was made computing the residues of regression models, in which the energy intake, BMI and gestational age were considered independent variables, and the total polyphenols intake was considered the dependent variable (Willett & Stampfer 1986). For assessment of the

validity of the instrument, concordances and correlations were evaluated with the results of the first evaluation questionnaire (FFQ1).

The correlation coefficients are described as follows: 0–0.1, insubstantial; 0.1–0.3, low; 0.3–0.5, moderate; 0.5–0.7, high; 0.7–0.9, very high and 0.9–1.0, close to the ideal (Cohen 1988; Hopkins *et al.* 2009).

Results

Mean maternal age was 27 years ($SD \pm 6.67$) and mean gestational age was 27.2 ($SD \pm 5$) weeks of pregnancy. A proportion of 56.67% had studied for periods between 8 and 11 years and 68.33% had a household income of less than three minimum official Brazilian wages. Considering the nutritional status, 53% had an adequate pre-gestational weight and 39% of them had a nutritional diagnosis of obesity, considering the gestational age at the first interview (Table 1).

Table 2 presents total polyphenols of food consumed according to the FFQ, to the 24HR, applied in the year 2010, and food quantified in Brazil. These data were used for the development of the FFQ validated in the present study, aiming at determining the size of the median portion of each polyphenol-rich food mentioned in the FFQ.

Table 3 shows the results on total polyphenol consumption obtained by the FFQ, by the average of the two 24HR and the average of the D3days. However, the total polyphenol consumption of FFQ was significantly higher, when compared with the average of the 24HR and the average of the three records. The paired *t*-test for differences between the FFQ and the average of the other methods of dietary survey showed statistically significant differences for the intake of total polyphenols ($P < 0.001$).

Reproducibility, analysed by Pearson's correlation coefficient after adjusted for energy, showed very a high correlation between the FFQ (0.728; $P < 0.001$). Pearson's correlation coefficient showed a low but significant association between the amounts of total polyphenols obtained by FFQ and 24HR with the urinary excretion, (0.22 and 0.23, respectively,

Table 1. Socio-demographic characteristics and nutritional status of 120 pregnant women in the State Rio Grande do Sul, Brazil

Characteristic	Mean (standard deviation)
Age (years)	26.99 (6.67)
GA* (weeks)	27.2 (5)
	<i>n</i> (%)
Education (%)	
Up to 8 years	36 (30)
8 to 11 years	68 (56.67)
11 to 15 years	13 (10.83)
>15 years	3 (2.5)
Family income† (%)	
Up to 3	82 (68.33)
3 to 5	30 (25)
5 to 10	6 (5)
>10	2 (1.67)
PG‡ nutritional status (%)	
Low weight	4 (3.33)
Eutrophy	53 (44.17)
Overweight	43 (35.84)
Obesity	20 (16.67)
Current nutritional status (%)	
Low weight	5 (6)
Eutrophy	30.83 (37)
Overweight	31.67 (38)
Obesity	32.5 (39)

*Gestational age. †Family income in minimum wage. ‡Pre-gestational.

$P < 0.05$). The association between the questionnaires was moderate to very high ($r = 0.47$ to 0.728 ; $P < 0.001$; Table 4).

The results obtained with the ICC test were similar to the Pearson's correlation. The analysis of reproducibility between the two FFQ showed a very high correlation ($r = 0.726$; $P < 0.001$). The dietary parameters also showed moderate to very high concordance ($r = 0.35$ to $r = 0.75$; $P < 0.001$; Table 5).

The mean exact concordance (percentage of subjects classified in the same quartile) between the FFQ and the average of the other dietary surveys and between the questionnaires and recalls compared with the two moments was 40.42%. On average, 84.14% of the pregnant women were classified in the same or in adjacent quartiles and 15.86% were classified in opposite quartiles for the dietary methods. The average value of the quadratic kappa ranged from 0.068 ($P = 0.25$) between the FFQ and D3days,

up to 0.425 ($P < 0.001$) between the first- and second-moment FFQ (Table 6).

The concordances between the dietary survey methods and between the surveys and the biomarker were assessed using Bland–Altman plots. The results showed a bias (distance of the differences from the value of zero) of 0.65 (95% CI: 0.59 to 0.71) and an error (dispersion of the points of differences around the average) of 0.39 for the FFQ compared with the urine; a bias of 0.29 (95% CI: 0.22 to 0.36) and an error of 0.31 for the FFQ compared with the mean 24HR; and a bias of 0.30 (95% CI: 0.22 to 0.36) and an error of 0.32 for the FFQ compared with the mean D3days, in addition to outliers and trends. This concordance observed on the Bland–Altman plots by linear regression of the difference, indicated a linear trend comparing the FFQ with two methods in the diet. The graphs show a dependence of the difference between the methods and the average, showing that the extreme estimates are expected to be a higher magnitude of error (Figs 1–3).

Discussion

This is the first study to develop and validate a dietary assessment tool to quantify total dietary ingestion of polyphenols during pregnancy. In addition, a large number of foods, i.e. 52 polyphenol-rich food items, were evaluated to determine the validity between methods. The results validated the FFQ, showing association and concordance with other dietary survey often-used methods.

The FFQ used to estimate total polyphenols consumption by pregnant women developed and validated in this study presented low association with urinary excretion of polyphenols, as previously reported in a systematic review of studies aiming to validate dietary questionnaires in pregnant women (Ortiz-Andrellucchi *et al.* 2009). The low correlation between dietary instruments and biomarkers is due to the influence of other factors in addition to consumption, such as individual differences in absorption and metabolism, genetics and changes in biochemical adaptation of the organism, such as pregnancy (Willett 1998; Arab & Akbar 2002; Arab 2003). The assessment of the dietary intake of pregnant women is

Table 2. Total amount of polyphenols of food consumed in food frequency questionnaire (FFQ) and 24HR applied in the year 2010 and food quantified in Brazil

Foods	<i>n</i> [¶]	Median of consumption (g or mL)	Total polyphenols/100 g** (mg)	Total polyphenols/portion (mg)
Bitter black chocolate	5	4	1859.88	74.4
Fruit tea*	25	86	1025.00	881.5
Black chocolate or milk chocolate powder	77	11	854.34	94.0
Black plum with skin	21	21	409.79	86.1
Raw strawberry	29	10	289.20	28.9
Orange	88	69	278.60	192.2
Red apple with peel	96	74	201.50	149.1
Tangerine	84	70	192.00	134.4
Raw red grape	34	41	184.97	75.8
Raw cabbage	31	4	176.67	7.1
Raw sweet cherry	5	4	173.10	6.9
Blackberry*	4	3	135.40	4.1
Mate [‡]	65	250	126.00	315.0
Black tea	7	60	104.48	62.7
Green spice	73	5	89.27	4.5
Radish leaves*	7	9	78.09	7.0
Raw red onion	16	11	75.70	8.3
Natural grape juice	12	52	68.00	35.4
Green tea	11	21	61.86	13.0
Raw lime	4	14	59.80	8.4
Olive oil	42	3	55.14	1.7
Natural orange juice	76	115	48.88	56.2
Tomato with skin	101	86	45.06	38.8
Soy beans*	4	3	37.41	1.1
Industrialised orange juice [‡]	42	74	—	—
Industrialised grape juice [‡]	51	57	—	—
Natural passion fruit juice [§]	—	—	20	2.86
Industrialised passion fruit juice [§]	—	—	20	2.86
Natural pineapple juice [§]	—	—	35.85	5.12
Industrialised pineapple juice [§]	—	—	21.70	3.1
Natural lemon juice [§]	—	—	21.13	3.01
Industrialised lemon juice [§]	—	—	18	2.57
Natural apple juice [§]	—	—	33.9	4.84
Industrialised apple juice [§]	—	—	30	4.28
Natural strawberry juice [§]	—	—	132.10	18.87
Industrialised strawberry juice [§]	—	—	132.10	18.87
Red plum [§]	—	—	409.79	58.54
Banana [§]	—	—	154.70	22.10
Papaya [§]	—	—	57.6	8.22
Pineapple [§]	—	—	147.91	21.13
Kaki [§]	—	—	0.80	0.11
Raw white onion [§]	—	—	45.5	6.5
Tomato boiled [§]	—	—	45.06	6.43
Broccoli [§]	—	—	198.55	28.36
Raw cabbage [§]	—	—	348.02	49.71
Carrot [§]	—	—	57.82	8.26
Beet [§]	—	—	164.10	23.44
Lettuce [§]	—	—	65.92	9.41
Tea Boldo*	—	—	24.05	3.43
Tea chamomile*	—	—	22.80	3.25
Black coffee*	—	—	104.48	14.92

*Database for the Flavonoid Content of Selected Foods Release (2007). [†]Bracesco *et al.* 2011. [‡]There are no references for total polyphenol contents of these foods. Only flavonoids are quantified. [§]Food quantified in Brazil (Arabbi *et al.* 2004; Faller & Fialho 2009). [¶]Number of citations of each food in the FFQ used in 2010. ^{**}Database on polyphenol content in foods, Phenol-Explorer (2009).

Table 3. Daily intake of total polyphenols evaluated by the food frequency questionnaire (FFQ), 24-h recall (24HR) and 3-day food diary (D3days). The statistical differences between the FFQ and the average of the other dietary survey methods were evaluated through paired *t*-test

	FFQ	24HR	FFQ – 24HR (<i>n</i> = 95)
Polyphenols (mg) median (IQR)	1048.30 (356.46–361.87)	490.44 (313.75 – 761.63)	557.86 (42.71–600.24)*
	FFQ	D3days	FFQ – D3days (<i>n</i> = 93)
Polyphenols (mg) median (IQR)	1048.30 (356.46–361.87)	587.25 (88.57–90.47)	461.05 (267.89–571.4)*

IQR, interquartile range. **P* ≤ 0.001 in paired *t*-test for the difference between total polyphenols between FFQ and the average of the other dietary survey methods.

Table 4. Pearson's correlation coefficients for total polyphenols, with the data log transformed between the dietary parameters from the first moment with the polyphenols excreted in the urine and between averages of the dietary parameters

	Raw correlation	Adjustment BMI	Adjustment GA	Adjustment TEV	Adjustment IV [†]
FFQ × Urine	0.231*	0.256*	0.255*	–	–
24HR × Urine	0.221*	0.244*	0.245*	0.219*	–
FFQ 1 × FFQ 2	0.727**	0.728**	0.724**	–	–
FFQ × 24HR	0.522**	0.511**	0.511**	0.511**	0.595**
FFQ × D3days	0.515**	0.584**	0.515**	0.458**	–

BMI, body mass index; D3days, 3-day food diary; FFQ, food frequency questionnaire; GA, gestational age; TEV, total energy value. **P* ≤ 0.05. ***P* ≤ 0.001. [†]Correlations corrected for intrapersonal variation (IV) in the two 24-h recall (24HR).

Table 5. Intraclass correlation coefficients (ICC), with the data log transformed between the dietary parameters from the first moment with polyphenols excreted in the urine and between averages of the dietary parameters

	ICC	95% CI	<i>P</i> -value	Adjustment IV*	95% CI
FFQ × urine	0.230	0.000–0.393	0.00	–	–
24HR × urine	0.199	0.000–0.365	0.01	–	–
FFQ 1 × FFQ 2	0.726	0.616–0.809	<0.001	–	–
FFQ × 24HR	0.349	0.000–0.591	<0.001	0.397	0.000–0.673
FFQ × D3days	0.489	0.318–0.629	<0.001	–	–

CI, confidence interval; D3days, 3-day food diary; FFQ, food frequency questionnaire. *Correlations corrected for intrapersonal variation (IV) in the two 24-h recall (24HR).

complicated because of various factors depending on the period of pregnancy. Poor correlation between instruments may be partly explained by appetite fluctuations and nausea, which may also influence the long-term diet reports (Erkkola *et al.* 2001).

The FFQ, however, showed strong association and concordance with the other questionnaires. A Norwegian study with pregnant women validated a food questionnaire which considered only one subclass of polyphenols, namely flavonoids, and with only three

food groups: fruits, vegetables and teas. That study was validated with correlation coefficients of 0.33 with flavonoids in urine (Brantsaeter *et al.* 2007), a result similar to the present study. However, the absorption of flavonoids and total polyphenols may not be a comparable measure. The most common phenolic compounds in human diet are not always the most biologically active, for different reasons such as low intrinsic activity, reduced intestinal absorption or fast metabolism and excretion. The metabolites

Table 6. Classification of participants (%) by quarters of consumption of total polyphenols between the averages of dietary survey methods

	<i>n</i>	Exact classification in the same quarter (%)	Classification in the same or adjacent quarter (%)	Classification in opposite quarters (%)	Kappa	<i>P</i> -value
FFQ × 24HR	95	37.9	83.2	16.8	0.171	0.04
FFQ × D3days	93	30.2	84	16	0.068	0.25
FFQ1 × FFQ2	95	56.8	91.5	8.5	0.425	<0.001

24HR, 24-h recall; D3days, 3-day food diary; FFQ, food frequency questionnaire.

Fig. 1. Bland–Altman plot: comparison of the concordance of total polyphenol consumption evaluated by food frequency questionnaire (FFQ) with the total amount of polyphenols excreted in the urine, after natural log transformation, in 120 pregnant women from the south of Brazil.

found in the blood, in target organs or as a result of gastrointestinal and hepatic activity, may have biological activity different from the native forms (Manach *et al.* 2004). A recent study has compared the total polyphenol excretion after the collection of 24-h urine or spot urine samples corrected by creatinine levels, indicating a correlation of 0.211 of 24-h urine and 0.113 of urinary total polyphenol excretion expressed by creatinine (Zamora-Ros *et al.* 2011). These correlations are similar to our study, and also cover the general class of total polyphenols.

The values for the intake of total polyphenols estimated by the FFQ were significantly higher than those estimated by the 24HR and D3days (Table 3). This is due to the fact that the FFQ includes a fixed list of foods. In the present study, the FFQ developed was composed only by polyphenol-rich foods, which excludes higher-calorie foods, such as complex carbo-

hydrates and fats, resulting in lack of relevance of the analysis and adjustment of energy in the FFQ. Adjusting for energy increases the correlation coefficient when the variability of nutrient consumption is related to energy intake (Willett 1998). Therefore, there was no need to analyse energy intake in the FFQ because only polyphenol contents of 52 foods were considered and this nutrient does not influence energy consumption.

Correlations were stronger among the methods of dietary survey than between biomarkers and the results of the questionnaires (Table 4). The correlations observed between the different methods in the dietary assessment were within the range observed in other validation studies in pregnant women (Ortiz-Andrellucchi *et al.* 2009), and lower than those reported in non-pregnant women (Jackson *et al.* 2011).

Fig. 2. Bland–Altman plot: comparison of the concordance of total polyphenol consumption evaluated by the food frequency questionnaire (FFQ) with total polyphenol consumption obtained by the average of two 24-h recall (24HR), after natural log transformation, in 95 pregnant women in south Brazil.

Fig. 3. Bland–Altman plot: comparison of the concordance of total polyphenol consumption evaluated by the food frequency questionnaire (FFQ) with total consumption of polyphenols obtained through the average of three food diaries (D3days), after natural log transformation, in 93 pregnant women from south Brazil.

The graphical visualisation of the concordance between total polyphenol consumption assessed by the FFQ and total polyphenols excreted in urine, or estimated in the average of the 24HR and of the three food diaries, was verified for 120 pregnant women in Brazil. This concordance was observed on the Bland–Altman plots (Figs 1–3). The estimated polyphenol consumption by FFQ was higher than the average of the 24HR and the average of the D3days, but similar

to other FFQ validation studies during pregnancy (Erkkola *et al.* 2001; Pinto *et al.* 2010; Barbieri *et al.* 2012).

A correlation was observed among the results of the quantification of total polyphenols obtained with the FFQ, 24HR, D3days and urinary excretion. As already mentioned, in general, validation studies of food surveys show a low correlation with biomarkers (Brantsaeter *et al.* 2007; Jackson *et al.* 2011). Thus, the

correlations found in the present study are consistent with data from the literature.

Currently, some studies suggest that biomarkers are not adequate for dietary evaluation of pregnant women. This can indicate that biomarkers are not sensitive to the changes in food consumption during the quarters of pregnancy (Pinto *et al.* 2010). According to a systematic review of food questionnaires in pregnancy, biomarkers are not considered useful for dietary evaluation in pregnant women, except for folic acid. The FFQ seems to be more sensitive than biomarkers to evaluate the intake of certain nutrients in pregnancy, both in short and long term (Ortiz-Andrellucchi *et al.* 2009).

The FFQ is considered the most practical, informative and the most used instrument to investigate previous diet as it can classify individuals according to their usual eating patterns. It is also low cost and easy to use, which enables its application in population studies (Willett 1998). In 1973, the FFQ was recommended by the American Public Health Association as one of the dietary assessment methods (Zulkifli & Yu 1992).

Ideally, the 24HR should be compared with the 24-h urine collection, which represents all the urine eliminated in a period of 24 h. However, this examination was not feasible as it was not possible to identify previously the pregnant women who would participate of the study, and also due to the inconvenience of collecting urine during 24 h in late pregnancy and the need for appropriate temperature and light storing conditions of the sample during the 24 h of collection. A recent study has compared the total polyphenol excretion after the collection of 24-h urine or spot urine samples corrected by creatinine levels, indicating that despite the obvious advantages of analysing the entire 24-h urine volume, analysis of creatinine-corrected spot urinary samples was also suitable, especially relevant in epidemiological studies, in which samples from a large population are analysed (Zamora-Ros *et al.* 2011). Therefore, a random spot urine collection was analysed in the present study after proper correction by creatinine levels, similar to approaches used in clinical and epidemiological studies (Medina-Remón *et al.* 2009).

Green tea, which is rich in catechins, as well as other polyphenol-rich foods included in this FFQ, which may present an enormous variety of polyphenolic compounds, have in common the low bioavailability demonstrated for these compounds, which is variable according to the contents and variety of polyphenols in foods, as demonstrated by studies on animals (Chen *et al.* 1997; Mata-Bilbao *et al.* 2008) and humans (Chow *et al.* 2001, 2003; Urpi-Sarda *et al.* 2010). Therefore, the concentration of the metabolites in the blood circulation or excreted in the urine are much lower than the amount of polyphenols ingested. Also, the analysis of urine excretion of total polyphenols does not take in consideration the metabolites that are distributed in the tissues or the biliary elimination, for example (Borges *et al.* 2010; Urpi-Sarda *et al.* 2010).

The main limitation of this study is related to the lack of information about the content of total polyphenols in some foods produced in Brazil. The investigation of associations between dietary surveys and biomarkers is hampered by the lack of studies on the content of polyphenols in industrialised juices, soy juice and drinks commonly consumed in Brazil, such as the mate and Boldo tea. In the present study, tables of quantification of flavonoids and total polyphenols in food produced on American and French soils, respectively, were employed. Polyphenol content of only eight of the 52 foods included in the questionnaire are quantified in Brazilian soil. Similarly, we could not find in the literature any report on the quantification of total polyphenols for all foods included in the questionnaire. For most of them, only information on the amount of flavonoids is available (United States Department of Agriculture 2007). The possibility of investigating the correlation of food questionnaires with results on polyphenols excreted in the urine is thus considerably reduced.

Another limitation of the present study is the investigation of an association of FFQ with the average of only two 24HR results. More repeated measures of the 24HR could allow a better understanding of intrapersonal variability and improve the investigation of correlations between methods (Ortiz-Andrellucchi *et al.* 2009). Other studies in pregnant

women using Pearson's correlation showed correlation coefficients lower than expected, ranging from 0.42 for vitamin B12 to 0.46 for iron (Forsythe & Gage 1994), and from 0.01 for saturated fat to 0.47 for calcium (Giacomello *et al.* 2008). These low correlations can be explained by high intrapersonal variability in estimating energy and nutrients during pregnancy, thereby reducing concordance between the methods when a small number of 24HR are employed as a standard of comparison (Baer *et al.* 2005). In the present study, however, correlations were adjusted for IV in two 24HR.

Another limitation of the present study was related to the period referred to in each food questionnaire. The FFQs considered the total period of gestation, while the 24HR and records were based on pregnancy quarter. Ideally, the application of 24HR and D3days in each trimester of pregnancy, but that would imply following pregnant women since the first quarter, which probably would lead to losses over the course of the study. Therefore, in the present, we decided to ensure at least the sample calculated, abbreviating the sampling period (2 weeks). A new study is being developed to analyse the amount of total polyphenols in food produced in Brazilian soil. The use of more 24HR measurements to investigate the association with the new FFQ and 24-h urine collection also are being considered for improving the analysis of associations.

Several foods and drinks mentioned by the pregnant women have high concentrations of polyphenols and are consumed freely throughout pregnancy. The fact that there is no proper control for the use of these substances is of concern because in the third trimester of pregnancy, they may be associated with functional and anatomical changes of the fetal heart (Zielinsky *et al.* 2011). Currently, there is no recommendation on the daily amount of polyphenols that should be consumed during pregnancy.

Validation of the dietary intake in pregnant women becomes more complex in terms of weight gain and important metabolic changes. However, statistically significant correlations are observed among dietary intake assessed with the new FFQ and the other food survey methods considered as reference. This study indicates that the FFQ offers new valid estimates of

intake of polyphenol-rich foods in pregnant women in Brazil, and may be used to classify individuals in the target population. The FFQ developed in the present study proved reproducible and valid for the quantification of total polyphenols consumed by pregnant women.

Acknowledgements

The authors would like to thank the students from Fetal Cardiology Unit of Institute of Cardiology of Estado do Rio Grande do Sul, Brazil, the nutrition academics that helped in the application of food questionnaire, the Department of Toxicology team from the Federal University of Rio Grande do Sul, Brazil and the nutritionists and nutrition techniques from the Service nutrition of Institute of Cardiology of Estado do Rio Grande do Sul, Brazil.

Source of funding

This study was supported in part by grants of CNPq (National Council of Technological and Scientific Development), FAPERGS (State of Rio Grande do Sul Agency for Research Support) and FAPICC (Institute of Cardiology Fund for Research and Culture Support), Brazil.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Contributions

PZ and AMZ were involved in all stages of the project. AM and BL participated in the collection of data and the preparation of the study. AO and KVL participated in the collection of data and collaborated with analyses, calculations of questionnaires and revision of the manuscript. AP and LHN participated in the discussion and review of the manuscript. GBB and SCG coordinated the collection and analysis of urine, in addition to scientific writing that refers to this biomarker. IV designed the project, trained and supervised the team to collect data, analysed the data

and wrote this manuscript, which was reviewed and approved by all the authors, who also agreed with the submission of the manuscript to this journal.

References

- Arab L. (2003) Biomarkers of fat and fatty acid intake. *The Journal of Nutrition* **133**, 925S–932S.
- Arab L. & Akbar J. (2002) Biomarkers and the measurement of fatty acids. *Public Health Nutrition* **5**, S865–S871.
- Arabbi P.R., Genovese M.I. & Lajolo F.M. (2004) Flavonoids in vegetable foods commonly consumed in Brazil and estimated ingestion by the Brazilian population. *Journal of Agricultural and Food Chemistry* **52**, 1124–1131.
- Atalah S.E., Castillo C. & Castro R. (1997) Propuesta de un nuevo estándar de evaluación nutricional en embarazadas. *Revista Médica de Chile* **12**, 1429–1436.
- Baer H.J., Blum R.E., Rockett H.R.H., Leppert J., Gardner J.D., Saito C.W. *et al.* (2005) Use of a food frequency questionnaire in American Indian and Caucasian pregnant women: a validation study. *BMC Public Health* **5**, 135.
- Barbieri P., Nishimura R.Y., Crivellenti L.C. & Sartorelli D.S. (2012) Relative validation of a quantitative FFQ for use in Brazilian pregnant women. *Public Health Nutrition* **16**, 1–8.
- Bland J.M. & Altman D.G. (1995) Comparing methods of measurement: why plotting difference against standard method is misleading. *Lancet* **345**, 1085–1087.
- Borges G., Mullen W., Mullan A., Lean M.E.J., Roberts S.A. & Crozier A. (2010) Bioavailability of multiple components following acute ingestion of a polyphenol-rich juice drink. *Molecular Nutrition & Food Research* **54**, S268–S277.
- Bracesco N., Sanchez A.G., Contreras V., Menini T. & Gugliucci A. (2011) Recent advances on Ilex Paraguariensis research: minireview. *Journal of Ethnopharmacology* **136**, 378–384.
- Brantsaeter A.L., Haugen M., Rasmussen S.E., Alexander J., Samuelsen S.O. & Meltzer H.M. (2007) Urine flavonoids and plasma carotenoids in the validation of fruit, vegetable and tea intake during pregnancy in the Norwegian Mother and Child Cohort Study (MoBa). *Public Health Nutrition* **10**, 838–847.
- Cade J., Thompson R., Burley V. & Warm D. (2002) Development, validation and utilization of food-frequency questionnaires – a review. *Public Health Nutrition* **5**, 567–587.
- Chen L., Lee M.J., Li H. & Yang C.S. (1997) Absorption, distribution, elimination of tea polyphenols in rats. *Drug Metabolism and Disposition* **25**, 1045–1050.
- Chow H.H.S., Cai Y., Alberts D.S., Hakim I., Dorr R., Shahi F.F. *et al.* (2001) Phase I pharmacokinetic study of tea polyphenols following single-dose administration of epigallocatechin gallate and polyphenon E. *Cancer Epidemiology, Biomarkers & Prevention* **10**, 53–58.
- Chow H.H.S., Cai Y., Hakim I.A., Crowell J.A., Shahi F., Brooks C.A. *et al.* (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clinical Cancer Research* **9**, 3312–3319.
- Cohen J. (1988) *Statistical Power Analysis for the Behavioral Sciences*. Lawrence Erlbaum: Hillsdale, NJ.
- Erkkola M., Karppinen M., Javanainen J., Rasanen L., Knip M. & Virtanen S.M. (2001) Validity and reproducibility of a food frequency questionnaire in early pregnancy. *American Journal of Epidemiology* **154**, 466–476.
- Faller A.L.K. & Fialho E. (2009) Polyphenol availability in fruits and vegetables consumed in Brazil. *Revista de Saúde Pública* **43**, 211–218.
- Forsythe H.E. & Gage B. (1994) Use of a multicultural food-frequency questionnaire with pregnant and lactating women. *American Journal of Clinical Nutrition* **59**, S203–S206.
- Giacomello A., Schmidt M.I., Nunes M.A.A., Duncan B.B., Soares R.M., Manzolli P. *et al.* (2008) Validation of a food frequency questionnaire in pregnant users of services of the Sistema Único de Saúde in two municipalities in Rio Grande do Sul, Brazil. *Revista Brasileira de Saúde Materno Infantil* **8**, 445–454.
- Gordon M. & Samuels P. (1995) Indomethacin. *Clinical Obstetrics and Gynecology* **38**, 697–705.
- Hirakata V.N. & Camey S.A. (2009) Concordance analysis between Bland–Altman methods. *Revista HCPA* **29**, 261–268.
- Hopkins W.G., Marshall S.W., Batterham A.M. & Hanin J. (2009) Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise* **41**, 3–13.
- Jackson M.D., Walker S.P., Younger N.M. & Bennett F.I. (2011) Use of a food frequency questionnaire to assess diets of Jamaican adults: validation and correlation with biomarkers. *Nutrition Journal* **10**, 1–11.
- Jaffé M. (1986) Über den Niederschlag, welchen Pikrinsäure in normalen Harn erzeugt und über eine neue Reaktion des Kreatinins. *Zeitschrift für Physikalische Chemie* **10**, 391–400.
- Kummer C., Moura M. & Almeida R. (2005) *Yerba mate*. Regional University of the Northwestern of the Rio Grande do Sul State. Department of Physics, Statistics and Mathematics. Available at: http://www.projetos.unijui.edu.br/matematica/modelagem/erva_mate/index.html (Accessed 7 October 2009).

- Manach C., Scalbert A., Morand C., Rémésy C. & Jiménez L. (2004) Polyphenols: food sources and bio-availability. *The American Journal of Clinical Nutrition* **79**, 727–747.
- Mata-Bilbao M.L., Andrés-Lacueva C., Roura E., Jáuregui O., Escribano E., Torre C. *et al.* (2008) Absorption and pharmacokinetics of green tea catechins in beagles. *The British Journal of Nutrition* **100**, 496–502.
- Medina-Remón A., Barrionuevo-González A., Zamora-Ros R., Andres-Lacueva C., Estruch R., Martínez-González M.A. *et al.* (2009) Rapid Folin-Ciocalteu method using microtiter 96-well plate cartridges for solid phase extraction to assess urinary total phenolic compounds, as a biomarker of total polyphenols intake. *Analytica Chimica Acta* **634**, 54–60.
- Nelson M. (1991) The validation of dietary assessment. In: *Design Concepts in Nutritional Epidemiology* (eds B.M. Margetts & M. Nelson), pp 241–272. Oxford University Press: Oxford.
- Norton M.E. (1997) Teratogen update: fetal effects of indometacin administration during pregnancy. *Teratology* **56**, 282–292.
- Oliveira T., Marquitti F.D., Carvalhaes M.A.B.L. & Sartorelli D.S. (2010) Development of a quantitative food frequency questionnaire for pregnant women attending primary care in Ribeirão Preto, São Paulo State, Brazil. *Caderno de Saúde Pública* **26**, 2296–2306.
- Ortiz-Andrellucchi A., Doreste-Alonso J., Henríquez-Sánchez P., Cetin I. & Serra-Majem L. (2009) Dietary assessment methods for micronutrient intake in pregnant women: a systematic review. *The British Journal of Nutrition* **102**, S64–S86.
- Phenol-Explorer (2009) *Database on Polyphenol Content in Foods*. Available at: <http://www.phenol-explorer.eu> (Accessed 9 October 2009).
- Pinto E., Severo M., Correia S., Santos I.S., Lopes C. & Barros H. (2010) Validity and reproducibility of a semi quantitative food frequency questionnaire for use among Portuguese pregnant women. *Maternal & Child Nutrition* **2**, 105–119.
- Rondó P.H., Villar B.S. & Tomkins A.M. (1999) Vitamin A status of pregnant women assessed by a biochemical indicator and a simplified food frequency questionnaire. *Archivos Latinoamericanos de Nutrición* **49**, 322–325.
- TACO (2006) *Brazilian Table of Food Composition*. Center of study and research in foods. Version 2. 2nd. Ed. Campinas: State University of Campinas (UNICAMP).
- United States Department of Agriculture (2007) *USDA Database for the Flavonoid Content of Selected Foods Release* Available at: <http://www.nal.usda.gov/fnic/foodcomp/Data/Flav/Flav02-1.pdf> (Accessed 13 August 2008).
- Urpi-Sarda M., Llorach R., Khan N., Monagas M., Rotches-Ribalta M., Raventos-Lamuela R. *et al.* (2010) Effect of milk on the urinary excretion of microbial phenolic acids after cocoa powder consumption in humans. *Journal of Agricultural and Food Chemistry* **58**, 4706–4711.
- Vitolo M.R. (2008) *Nutrition of Pregnancy to Aging*. 2nd edn. Rio de Janeiro, Rubio.
- Willett W.C. (1994) Future directions in the development of food-frequency questionnaires. *The American Journal of Clinical Nutrition* **59**, 171–174.
- Willett W.C. (1998) Food frequency methods. In: *Nutrition Epidemiology* (ed. W. Willett), 2nd edn, pp 74–100. Oxford University Press: Oxford.
- Willett W.C. & Lenart E. (1998) Reproducibility and validity of food frequency questionnaires. In: *Nutritional Epidemiology* (ed. W. Willett), pp 101–147. Oxford University Press: Oxford.
- Willett W.C. & Stampfer M. (1986) Total energy intake: implications for epidemiologic analyses. *American Journal of Epidemiology* **124**, 17–27.
- Zamora-Ros R., Rabassa M., Cherubini A., Urpi-Sarda M., Llorach R., Bandinelli S. *et al.* (2011) Comparison of 24-h volume and creatinine-corrected total urinary polyphenol as a biomarker of total dietary polyphenols in the Invecchiare InCHIANTI study. *Analytica Chimica Acta* **704**, 110–115.
- Zanolla A.F., Olinto M.T.A., Henn R.L., Wahrlich V. & Anjos L.A. (2009) Assessment of reproducibility and validity of a food frequency questionnaire in a sample of adults living in Porto Alegre, Rio Grande do Sul State, Brazil. *Caderno de saúde Pública* **25**, 840–848.
- Zielinsky P., Piccoli A.L., Manica J.L., Nicoloso L.H., Menezes H., Busato A. *et al.* (2011) Maternal consumption of polyphenol-rich foods in late pregnancy and fetal ductus arteriosus flow dynamics. *Journal of Perinatology* **30**, 17–21.
- Zulkifli S.N. & Yu S.M. (1992) The food frequency method for dietary assessment. *Journal of the American Dietetic Association* **92**, 681–685.