

# Reliability and relative validity of an FFQ for nutrients in the Tehran Lipid and Glucose Study

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## Abstract

**Objective:** To describe the relative validity and reliability of the FFQ used for assessing nutrient intakes of participants in the Tehran Lipid and Glucose Study (TLGS).

**Design:** A total of 132 subjects (sixty-one males and seventy-one females) were included in the study. Dietary data were collected monthly by means of twelve 24 h dietary recalls (24hDR). Subjects completed two, 168-item semi-quantitative FFQ. Blood and urine samples were taken every season for measurement of plasma biomarkers and urinary N and K.

**Results:** Mean age and BMI of the participants were 35.5 (SD 16.8) years and 25.5 (SD 5.2) kg/m<sup>2</sup>, respectively. The mean energy-adjusted and deattenuated correlation coefficients for overall nutrient intake between the 24hDR and FFQ2 were 0.44 and 0.37 in ≤35-year-olds and >35-year-olds, respectively, and for individual nutrients ranged from 0.24 to 0.71 in men (mean  $r = 0.53$ ) and from 0.11 to 0.60 in women (mean  $r = 0.39$ ). The mean energy-adjusted reliability coefficients varied from 0.48 in ≤35-year-olds to 0.65 in >35-year-olds, and ranged from 0.41 to 0.79 in men (mean  $r = 0.59$ ) and from 0.39 to 0.74 in women (mean  $r = 0.60$ ). The FFQ2 and 24hDR produced exact agreement rates ranging between 39.6% and 68.3% in men and between 39.6% and 54.1% in women. The ranges of questionnaire validity coefficients, with the sample correlation between the questionnaires and biochemical marker as the lower limit and the estimate obtained by the method of triads as the upper limit, were 0.21–0.56 (protein) and 0.37–0.61 (K).

**Conclusions:** The FFQ developed for the TLGS has reasonable relative validity and reliability for nutrient intakes in Tehranian adults.

**Keywords**  
Reliability and validity  
Nutrition assessment  
Questionnaire

The contribution of dietary factors to the development and prevention of non-communicable diseases is being increasingly recognized<sup>(1)</sup>. Epidemiological interest currently focuses on examining the association between disease and individual foods, food groups, food patterns, dietary nutrients or healthy eating indices<sup>(2–6)</sup>.

The measurement of dietary intake remains one of the most challenging tasks in nutritional epidemiology<sup>(7)</sup>. The FFQ is one of the most commonly used methods in epidemiological studies to assess individual long-term dietary intakes of foods and nutrients. Because of its ability to capture usual dietary patterns<sup>(8)</sup>, it is crucial to estimate the validity and reliability of an FFQ because, like any other type of dietary assessment, it is affected by error<sup>(9)</sup>. Information regarding validity and reliability is important

and indispensable in interpreting study results to enhance the interpretation of estimated diet–disease associations and to improve the translation of such associations into dietary recommendations<sup>(10,11)</sup>.

The performance of an FFQ is sensitive to the culture and ethnic background of the study population. Thus the validity and reliability for an FFQ needs to be evaluated for studies conducted in different study populations<sup>(12)</sup>.

In recent years, epidemiological studies in Tehran, the capital city of Iran, have shown a high prevalence of metabolic syndrome and CVD in the urban population<sup>(13,14)</sup>. The Tehran Lipid and Glucose Study (TLGS) was conducted to further investigate dietary relationships, among the other causes of high rates of CVD<sup>(15)</sup>. As part of TLGS, we administered a new FFQ (168 items)

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designed specifically to capture the dietary practices of the study participants. In the present study, we aimed to describe the validity and reliability of this FFQ for assessing nutrient intakes.

## Subjects and methods

### Subjects

The present study was conducted within the framework of the TLGS, a prospective study conducted in a sample of residents of district-13 of Tehran, to determine the prevalence of risk factors for non-communicable disease and to identify lifestyles to reduce these risk factors<sup>(15)</sup>. A random sample of 200 cohort members, aged 20 years and over, were requested to participate in a dietary assessment validation study and 162 subjects agreed. Sample size was determined by considering a confidence interval of 95%, study power of 80%, minimum expected correlation coefficient of 0.25 and attrition rate estimation of 50%. To minimize the effect of under- and over-reporting, we excluded subjects who had left more than seventy items blank on the FFQ and those who reported a total daily energy intake outside the range of 3360–17 640 kJ (800–4200 kcal) on either of the two questionnaires<sup>(16)</sup>; we also excluded those who did not provide a blood or urine sample. A total of 132 subjects (sixty-one males and seventy-one females) remained for the current analysis. The study was approved by the research ethical committee of the Research Institute for Endocrine Sciences of Shahid Beheshti University of Medical Sciences and informed written consent was obtained from each subject.

### Assessment of dietary intake

Usual dietary intake was assessed twice using a 168-item semi-quantitative FFQ, one year apart (FFQ1 and FFQ2), all administered by the same trained dietitians for each participant, who had at least 3–5 years of experience in the Nationwide Food Consumption Survey project<sup>(17)</sup> and the TLGS<sup>(18)</sup>, for assessing intra-rater reliability<sup>(19)</sup>. The FFQ consisted of a list of foods with a standard serving size commonly consumed by Iranians. Participants were asked to report their frequency of consumption of a given serving of each food item during the previous year, on a daily (e.g. bread), weekly (e.g. rice, meat) or monthly (e.g. fish) basis. The reported frequency for each food item was converted to a daily intake. Portion sizes of consumed foods were converted to grams using household measures<sup>(20)</sup>. Dietary data were also collected by means of 24 h dietary recalls (24hDR), repeated twelve times; 24hDR interviews were performed every month for 12 months by the same trained dietitians according to a standardized protocol and lasted 20 min on average. For all subjects, the recall days included one day of the weekend in Iran (Thursday and Friday); the other days of

the week were recalled twice. The first recall was completed one month after FFQ1 administration and the last recall was completed one month before administration of FFQ2. All recall interviews were performed at the subjects' homes to measure the volume of commonly used household measures. The same interviewer interviewed each subject throughout the study. All 24hDR were reviewed by the investigators and any questions raised were resolved with participants. Because the Iranian food composition table (FCT) is incomplete (limited to only raw materials and a few nutrients), each food and beverage was analysed for energy and nutrient intake using the US Department of Agriculture's (USDA) FCT. For mixed dishes, nutrients were calculated according to their ingredients. The energy and macronutrients of breads and fruits are almost similar to alternative food items in the USDA FCT, with correlation >0.9. We used the Iranian FCT only for food items like '*kasbk*' which was not listed in the USDA FCT<sup>(21)</sup>.

### Biochemical measurements

Every season, a blood sample was drawn into Vacutainer tubes between 07.00 and 09.00 hours from all study participants in a non-fasting state. Thus we collected four samples (one for each season) for each person. Blood samples were taken in a sitting position according to a standard protocol and centrifuged within 30 to 45 min of collection. Separated plasma was stored at  $-70^{\circ}\text{C}$  for up to 17 months until analysed. Serum total cholesterol and TAG concentrations were measured by commercially available enzymatic reagents (Pars Azmoon Inc., Iran) adapted to the Selectra autoanalyser. Plasma concentrations of retinol,  $\beta$ -carotene and  $\alpha$ -tocopherol were measured by the HPLC technique, adapted from Craft *et al.*<sup>(22)</sup>. All samples were analysed when internal quality control met the acceptable criteria. The intra- and inter-assay CV was respectively 2.9% and 3.2% for  $\alpha$ -tocopherol, 6.8% and 7.1% for  $\beta$ -carotene and 5.9% and 6.8% for retinol. Also, every season, all participants were asked to provide a 24 h urine collection. Urine was collected in 1-litre plastic bottles containing 5 mg boric acid. On delivery, participants were questioned about the completeness of urine collection; they were then asked to repeat the collection if there was >50 ml loss. Total urinary N was determined by the Kjeldahl technique and urinary K was measured by flame photometry; the reference for urinary K measurement was 25–120 mEq/24 h.

### Statistical methods

The SPSS statistical software package version 13 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Normality of the distributions of dietary intake variables was assessed by the Kolmogorov–Smirnov test. When the variables were not normally distributed, we used log-transformed data. All log-transformed variables were normal. All analyses were conducted on the mean of

energy and nutrient intake from the twelve 24hDR. Means and standard deviations were calculated for energy and all nutrient intakes from both FFQ and from the twelve 24hDR. The paired *t* test was conducted to show differences between the two FFQ and between FFQ2 and 24hDR. Pearson correlation coefficients were estimated between energy and nutrient intake variables from FFQ2 and 24hDR. Energy- and age-adjusted nutrient intakes were calculated to remove variation due to energy and age, using the residual method<sup>(23)</sup>. Deattenuated correlation coefficients were reported by using Rosner and Willett's formula to correct within-person variation in the twelve 24hDR<sup>(8,23,24)</sup>. Crude and energy-adjusted intraclass correlation coefficients were calculated to assess the 1-year reliability<sup>(19)</sup> of the FFQ. We divided the daily intakes from dietary recalls into thirds and compared them with thirds calculated from FFQ2, expressing the results as agreement, adjacent agreement and complete disagreement percentages.

Sample correlations and estimated validity coefficients between the mean of four measurements of urinary and plasma biomarkers and dietary intakes of comparable nutrients from FFQ2 and the mean of twelve 24hDR were calculated using the method of triads<sup>(25)</sup>. The correlations between the mean of twelve 24hDR and the mean of four urinary and plasma measurements were corrected for within-person variation<sup>(23,24)</sup>. Plasma levels of retinol,  $\beta$ -carotene and  $\alpha$ -tocopherol were adjusted for plasma concentration of cholesterol and TAG.

## Results

The mean age of the participants was 35.6 (SD 16.8) years, 39.8 (SD 18.8) years for men and 33.4 (15.4) years for women; and their mean BMI was 25.5 (SD 5.2) kg/m<sup>2</sup>, 24.7 (SD 3.8) kg/m<sup>2</sup> for men and 26.0 (SD 5.8) kg/m<sup>2</sup> for women. Mean daily intakes from the twelve 24hDR and the two semi-quantitative FFQ are shown in Table 1. The FFQ tended to overestimate intake compared with the 24hDR, especially in women, with the largest discrepancy being seen for  $\beta$ -carotene and vitamin C in men and for vitamin D and  $\beta$ -carotene in women.

Crude, energy-adjusted and deattenuated correlation coefficients between mean nutrient intakes of the 24hDR and FFQ2 are shown in Table 2. Overall, adjusted and deattenuated correlation coefficients between the 24hDR and FFQ2 ranged from 0.24 for vitamin A to 0.71 for P in men and from 0.11 for  $\beta$ -carotene to 0.60 for fibre in women. Correlation coefficients were generally higher in men than in women, except for carbohydrate. Mean adjusted and deattenuated correlation coefficients were 0.37 and 0.44 in two age categories,  $\leq 35$  and  $> 35$  years, respectively.

Intraclass correlations between the two FFQ, after adjusting for age and energy intake, are presented in

Table 3. Age- and energy-adjusted intraclass correlation coefficients between the two FFQ, administered at a 1 year interval, ranged from 0.41 (monounsaturated fat) to 0.79 (protein) in men and from 0.39 (monounsaturated fat) to 0.74 (saturated fat) in women. Mean adjusted intraclass correlation coefficients between the two FFQ were 0.48 and 0.65 in two age categories,  $\leq 35$  and  $> 35$  years, respectively.

Table 4 shows the agreement, adjacent agreement and complete disagreement in nutrient intakes between the 24hDR and FFQ2. The agreement percentages ranged from 39.6% (vitamin C) to 68.3% (P) in men and from 39.6% (K) to 54.1% (fibre) in women. The complete disagreement ranged from 0 (protein) to 16.3% ( $\beta$ -carotene) in men and from 1.2% (thiamin) to 16.2% (Ca) in women.

The estimated validity coefficients of protein ranged from 0.38 to 1.16. The ranges of questionnaire validity coefficients, with the sample correlation between the questionnaires and biochemical marker as the lower limit and the estimate obtained by the method of triads as the upper limit, were 0.21–0.56 (protein), 0.37–0.61 (K), 0.38–0.50 ( $\beta$ -carotene), 0.31–0.95 (cholesterol), 0.21–0.55 (retinol) and 0.28–0.38 ( $\alpha$ -tocopherol; Fig. 1).

## Discussion

In the present study we examined the reliability and relative validity of the FFQ developed for the TLGS. We used twelve 24DR to compare nutrient intakes from the FFQ and 24hDR, and to compare serum and urine biomarkers as well. Reliability of the FFQ, as assessed by intraclass correlation coefficients between the results of the two FFQ, was also obtained from the same population. The results showed reasonable relative validity based on true estimated validity coefficients and good reliability of the FFQ over a 1-year period. Cross-classification between these two methods was reasonably acceptable.

The values of correlation coefficients were almost the same between men and women for several nutrients but for some nutrients there were differences between sexes. This may be due to the same portion sizes being used for men and women; in studies in which portion sizes are self-defined, there tend to be differences in portion size between men and women, and furthermore correlations in validity studies tend to be highest when subjects are able to describe their own portion sizes<sup>(26)</sup>. The overestimation of the FFQ compared with the mean of the 24hDR may be due to the seasonal availability of food items (like fruits and vegetables when the FFQ was completed), infrequent items with large variation frequency, over-reporting healthy food choices and defining food groups like breads and cereals in great detail; considering that breads and rice are staple foods leads to

**Table 1** Daily intake of energy and nutrients estimated by twelve 24 h dietary recalls (24hDR) and two FFQ: Tehran Lipid and Glucose Study

Nutrient	Men (n 61)								Women (n 71)							
	24hDR		FFQ1		FFQ2		% difference*	% difference†	24hDR		FFQ1		FFQ2		% difference*	% difference†
	Mean	SD	Mean	SD	Mean	SD			Mean	SD	Mean	SD	Mean	SD		
Energy (kJ)	10422	2569	11849	4878	11686 <sup>a</sup>	3406	12	-1	7134	1703	8899	3046	8996 <sup>a</sup>	3234	26	1
Protein (g)	81.3	19	88.0	37	84.4	24	5	-4	55.0	12	67.0	28	68.0 <sup>a</sup>	24	21	0.9
Carbohydrate (g)	360	88	443	161	389 <sup>a,c</sup>	162	8	-3	254	60	311	112	307 <sup>a</sup>	109	21	-1
Fat (g)	80	18	92	25	85 <sup>b</sup>	23	11	-3	58	17	72.0	22	76.0 <sup>a</sup>	22	30	5
Cholesterol (mg)	225	75	227	76	210	70	-8	-14	163	48	174	66	178	70	12	13
Saturated fat (g)	23.7	5	28.0	12	25.0 <sup>b</sup>	8	11	-10	16.0	5	21.6	7	22.9 <sup>a</sup>	9	32	6
Monounsaturated fat (g)	30.3	7	24.5	28	31.2	9	6	31	21.2	6	26.4	10	27.2 <sup>a</sup>	10	28	3
Polyunsaturated fat (g)	19.3	5	22.0	7	20.0	6	7	-2	14.1	14	16.8	6	17.6 <sup>a</sup>	6	26	3
Fibre (g)	41.6	18	51.4	28	45.2	20	9	-2	33.0	10	33.4	12	37.0 <sup>a</sup>	15	-17	5
Vitamin C (mg)	103	41	176	94	159 <sup>a</sup>	86	54	-4	98	31	138	69	138 <sup>a</sup>	64	39	-7
Folate (µg)	532	123	745	320	644 <sup>a,d</sup>	176	19	-6	388	99	512	156	518 <sup>a</sup>	134	29	-6
Zn (mg)	11.5	2	12.8	4	12.3	2	9	-7	8.1	2	9.7	3	10.0 <sup>a</sup>	3	21	-1
Mg (mg)	353	75	436	154	422 <sup>a</sup>	119	18	-5	273	67	323	99	343 <sup>a</sup>	94	25	1
Ca (g)	0.99	0.30	1.29	0.56	1.19 <sup>a</sup>	0.40	17	-5	0.79	0.25	0.91	0.35	1.06 <sup>a</sup>	0.38	37	13
K (g)	3.26	0.78	3.94	1.3	3.84 <sup>a</sup>	1.22	21	-5	2.69	0.69	3.26	1.09	3.43 <sup>a</sup>	1.05	26	2
Vitamin A (RAE)	370	178	505	255	464 <sup>b</sup>	204	12	-15	342	176	462	207	461 <sup>a</sup>	233	23	-9
P (mg)	1290	242	1594	488	1522 <sup>a</sup>	322	16	-8	942	229	1203	410	1258 <sup>a</sup>	383	30	1
Thiamin (mg)	2.1	0.6	2.6	0.9	2.3	0.6	14	-11	1.4	0.3	1.7	0.5	1.7	0.5	21	0
Vitamin D (µg)	1.2	0.7	1.8	1.1	1.6	0.9	33	-11	1.2	0.7	1.6	2.0	1.9	2.7	58	17
β-Carotene (µg)	2004	1058	3172	1918	3264 <sup>a</sup>	1988	72	-12	1987	993	3740	3249	3241 <sup>a</sup>	1818	58	-16
Riboflavin (mg)	1.8	0.6	2.2	0.8	2.0 <sup>d</sup>	0.6	5	-13	1.4	0.4	1.7	0.6	1.7 <sup>a</sup>	0.6	23	0

RAE, retinol activity equivalent.

Mean value was significantly different (paired *t* test) between: <sup>a</sup>24hDR and FFQ2 ( $P < 0.01$ ), <sup>b</sup>24hDR and FFQ2 ( $P < 0.05$ ), <sup>c</sup>FFQ1 and FFQ2 ( $P < 0.01$ ), <sup>d</sup>FFQ1 and FFQ2 ( $P < 0.05$ ).

\*Percentage difference between intakes calculated with FFQ2 and the average of twelve 24hDR.

†Percentage difference between intakes calculated with FFQ1 and FFQ2.

**Table 2** Pearson correlation coefficients of nutrient intake estimated by the average of twelve 24 h dietary recalls (24hDR) and the second FFQ: Tehran Lipid and Glucose Study

Nutrient	Correlation coefficient between 24hDR and FFQ2*							
	Men (n 61)		Women (n 71)		Age ≤35 years (n 72)		Age >35 years (n 60)	
	Crude	Adjusted†	Crude	Adjusted†	Crude	Adjusted‡	Crude	Adjusted‡
Energy	0.55	0.55	0.46	0.46	0.64	0.49	0.67	0.45
Protein	0.64	0.65	0.48	0.50	0.68	0.44	0.67	0.47
Carbohydrate	0.38	0.39	0.47	0.47	0.63	0.42	0.60	0.43
Fat	0.62	0.59	0.40	0.38	0.49	0.35	0.60	0.36
Cholesterol	0.47	0.44	0.35	0.41	0.53	0.34	0.60	0.51
Saturated fat	0.61	0.58	0.37	0.34	0.50	0.33	0.64	0.54
Monounsaturated fat	0.55	0.49	0.39	0.34	0.44	0.23	0.58	0.38
Polyunsaturated fat	0.37	0.33	0.35	0.32	0.39	0.25	0.47	0.25
Fibre	0.68	0.67	0.61	0.60	0.56	0.43	0.65	0.71
Vitamin C	0.42	0.43	0.25	0.28	0.38	0.31	0.35	0.38
Folate	0.68	0.69	0.45	0.45	0.68	0.40	0.68	0.43
Zn	0.59	0.59	0.46	0.47	0.63	0.37	0.62	0.41
Mg	0.63	0.65	0.38	0.39	0.53	0.35	0.58	0.42
Ca	0.66	0.67	0.32	0.33	0.55	0.43	0.56	0.47
K	0.33	0.33	0.31	0.32	0.41	0.27	0.44	0.37
Vitamin A	0.22	0.24	0.38	0.20	0.25	0.30	0.38	0.37
P	0.70	0.71	0.42	0.42	0.58	0.44	0.65	0.44
Thiamin	0.69	0.70	0.53	0.55	0.75	0.45	0.71	0.35
Vitamin D	0.61	0.63	0.65	0.43	0.53	0.56	0.68	0.66
β-Carotene	0.33	0.31	0.22	0.11	0.12	0.12	0.40	0.33
Riboflavin	0.64	0.65	0.42	0.43	0.46	0.40	0.59	0.51
Mean§	0.54	0.53	0.41	0.39	0.51	0.37	0.58	0.44

\*Dietary data were collected by means of twelve 24hDR repeated monthly. The first recall was completed one month after FFQ1 administration and the last recall completed one month before administration of FFQ2.

†Age- and energy-adjusted and deattenuated.

‡Sex- and energy-adjusted and deattenuated.

§Mean of correlation coefficients.

overestimation of carbohydrates and energy. In addition, relative under-reporting of energy intake was shown by 24hDR compared with doubly labelled water methods<sup>(9)</sup>. However the problem of our questionnaire estimates of absolute intake should be of less concern when they are applied, because energy-adjusted values are used rather than absolute values.

This is the second validation study in Iran; the first was done in Golestan, a province in the north of Iran, as part of the Golestan cohort study of oesophageal cancer<sup>(27)</sup>. In the Golestan study, the correlation coefficients between the dietary recalls and the FFQ ranged from 0.49 to 0.82 and the intraclass correlation between four FFQ ranged from 0.66 to 0.89, but the energy-adjusted correlation coefficients were not calculated. The results of our study have similar ranges of correlation coefficients for the validation of an FFQ as for cohort studies in Japan<sup>(10)</sup>, northern Sweden<sup>(11)</sup>, Canada<sup>(28)</sup> and the Dutch<sup>(29)</sup> and German<sup>(30)</sup> parts of the European Prospective Investigation into Cancer and Nutrition (EPIC).

Correlations comparing nutrients from the dietary recalls with nutrients from FFQ2 might be slightly higher than correlations for nutrients from FFQ1. This difference may reflect some learning bias and change of dietary intake over the years may account for the lower correlations observed with FFQ1<sup>(12,31)</sup>. However, similar to Rimm *et al.*'s study<sup>(31)</sup>, the FFQ2 used in our study

represents the time period during which the 24hDR were collected. Also in our study there was no significant difference between the dietary intake from FFQ1 and FFQ2, except for carbohydrate, folate and riboflavin. Energy-adjusted and deattenuated correlation coefficients were not very different from the crude ones in our study, similar to the findings in a Greek study<sup>(32)</sup>. Ocke *et al.*<sup>(29)</sup> reported higher correlation particularly among men, findings in line with our study. Marks *et al.* showed that among personal characteristics, sex was most commonly associated with intake estimate errors for food groups<sup>(26)</sup>. We calculated agreement and disagreement percentages to ascertain the usefulness of the FFQ for categorizing individuals based on their levels of consumption. Studies on diet-disease relationships frequently divide nutrient intakes into categories and in epidemiology the primary need is often to place individuals in correct ranking order, rather than to make accurate estimate of absolute intake<sup>(33)</sup>. High exact agreement and low complete disagreement percentages were seen in accordance with high and low correlation coefficients, respectively, in our study; hence our FFQ might have the capability to estimate the usual intake at population level. Other validation studies with 24hDR and FFQ showed average exact agreement proportions of 28%<sup>(34)</sup> or ranging between 25% and 58%<sup>(30)</sup>.

Using 24hDR as the reference method may be one of the limitations of our study, but we did so because they

**Table 3** Intraclass correlation for energy and nutrients among the two FFQ\*: Tehran Lipid and Glucose Study

Nutrient	Men (n 61)		Women (n 71)		Age ≤35 years (n 72)		Age >35 years (n 60)	
	Crude	Age- and energy-adjusted	Crude	Age- and energy-adjusted	Crude	Sex- and energy-adjusted	Crude	Sex- and energy-adjusted
Energy	0.73	–	0.78	–	0.79	–	0.84	–
Protein	0.80	0.79	0.75	0.69	0.80	0.39	0.84	0.67
Carbohydrate	0.71	0.45	0.75	0.47	0.78	0.41	0.82	0.63
Fat	0.61	0.43	0.74	0.42	0.69	0.57	0.72	0.48
Cholesterol	0.81	0.64	0.75	0.67	0.79	0.69	0.78	0.60
Saturated fat	0.80	0.52	0.78	0.74	0.72	0.48	0.85	0.79
Monounsaturated fat	0.50	0.41	0.74	0.39	0.67	0.58	0.65	0.45
Polyunsaturated fat	0.56	0.56	0.65	0.62	0.59	0.42	0.42	0.40
Fibre	0.70	0.53	0.78	0.70	0.66	0.44	0.87	0.86
Vitamin C	0.86	0.76	0.72	0.63	0.77	0.62	0.80	0.76
Folate	0.75	0.63	0.75	0.57	0.75	0.46	0.81	0.61
Zn	0.84	0.53	0.76	0.70	0.80	0.50	0.85	0.76
Mg	0.85	0.61	0.70	0.59	0.72	0.37	0.85	0.75
Ca	0.84	0.74	0.64	0.56	0.66	0.41	0.80	0.65
K	0.81	0.66	0.77	0.64	0.77	0.55	0.84	0.75
Vitamin A	0.77	0.59	0.59	0.41	0.49	0.33	0.75	0.51
P	0.83	0.48	0.68	0.68	0.74	0.48	0.81	0.59
Thiamin	0.68	0.65	0.74	0.71	0.74	0.44	0.83	0.71
Vitamin D	0.62	0.57	0.71	0.71	0.63	0.63	0.68	0.64
β-Carotene	0.79	0.67	0.59	0.51	0.55	0.49	0.80	0.65
Riboflavin	0.85	0.58	0.71	0.59	0.69	0.39	0.85	0.71
Meant	0.75	0.59	0.72	0.60	0.70	0.48	0.78	0.65

\*Dietary data were collected by means of twelve 24 h dietary recalls repeated monthly. The first recall was completed one month after FFQ1 administration and the last recall completed one month before administration of FFQ2.  
 †Mean of correlation coefficients.

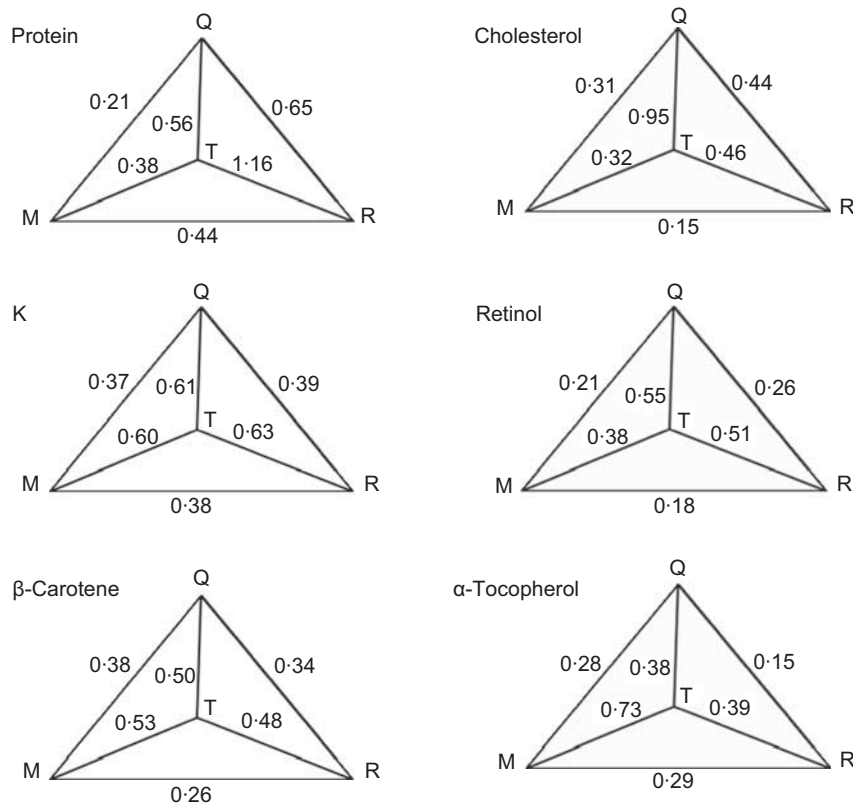
**Table 4** Percentages of agreement, adjacent agreement and complete disagreement according to tertile classification of daily nutrient intakes based on the average twelve 24 h dietary recalls and the second FFQ\*: Tehran Lipid and Glucose Study

Nutrient	Men (n 61)			Women (n 71)		
	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)	Agreement (%)	Adjacent agreement (%)	Complete disagreement (%)
Energy	59.1	36.4	4.5	50.6	42.3	7.1
Protein	62.8	37.2	0	45.4	48.8	5.9
Carbohydrate	67.5	23.3	9.3	52.3	44.3	3.5
Fat	65.8	29.5	4.5	43.5	47.1	9.5
Cholesterol	48.8	46.6	4.7	46.6	38.4	15.2
Saturated fat	56.8	40.9	2.3	43.5	45.8	10.6
Monounsaturated fat	56.8	31.8	11.4	49.4	41.4	9.4
Polyunsaturated fat	46.6	46.6	7.0	53.6	35.7	10.7
Fibre	52.3	40.9	6.8	54.1	43.6	2.4
Vitamin C	39.6	46.6	14.0	40.8	45.4	14.0
Folate	63.6	27.1	9.1	53.0	37.7	9.5
Zn	61.4	36.4	2.3	50.6	43.6	5.9
Mg	61.3	29.5	9.1	44.2	47.2	8.1
Ca	58.1	30.3	11.6	51.2	32.6	16.2
K	50.0	34.1	15.9	39.6	49.6	10.5
Vitamin A	46.6	42.2	11.1	47.7	37.2	15.1
P	68.3	25.1	6.8	48.2	45.9	5.9
Thiamin	65.9	31.7	2.4	52.9	46.4	1.2
Vitamin D	48.9	44.2	7.0	52.9	36.5	10.6
β-Carotene	46.5	37.2	16.3	41.3	43.5	14.9
Riboflavin	62.0	23.9	14.3	53.6	40.3	6.1
Mean	56.6	35.3	8.1	48.3	42.5	9.2

\*Dietary data were collected by means of 24 h dietary recalls repeated monthly. The first recall was completed one month after FFQ1 administration and the last recall completed one month before administration of FFQ2.

are less expensive, have high response rate, are easier to use in our population and do not interfere much with the normal dietary habits of subjects. However, their dependence on memory and the inability to incorporate direct measurements are the major limitations of 24hDR. Some

previous studies used dietary recalls<sup>(35–39)</sup> and other studies used diet records<sup>(31,40)</sup>. It seems that characteristics of the study population are important for choosing the reference method, as the diet record is probably not applicable in populations with low or moderate education,



**Fig. 1** Tehran Lipid and Glucose Study: sample correlations and estimated validity coefficients between the mean of four measurements of urinary and plasma biomarkers (M) and dietary intakes of comparable nutrients from FFQ2 (Q) and the mean of twelve 24 h dietary recalls (R) using the method of triads; (T) true intake variable. Reference measurements were based on mean values of twelve 24 h dietary recalls (24hDR), completed monthly. Measurements were obtained by a semi-quantitative questionnaire (FFQ) administered twice. The first recall was completed one month after FFQ1 and the last recall was completed one month before FFQ2. Measurements of 24 h urinary N and K and plasma concentrations of  $\beta$ -carotene, cholesterol, retinol and  $\alpha$ -tocopherol were taken every season. The correlation between the mean of the 24hDR and the mean of the four urinary and plasma measurements and the other variables were corrected for within-person variation. In addition, plasma levels of retinol,  $\beta$ -carotene and  $\alpha$ -tocopherol were adjusted for plasma concentration of cholesterol and TAG. The estimated validity coefficients of protein ranged from 0.38 to 1.16. The ranges of questionnaire validity coefficients, with the sample correlation between the questionnaires and biochemical marker as a lower limit and the estimate obtained by the method of triads as an upper limit, were 0.21–0.56 (protein), 0.37–0.61 (K), 0.38–0.50 ( $\beta$ -carotene), 0.31–0.95 (cholesterol), 0.21–0.55 (retinol) and 0.28–0.38 ( $\alpha$ -tocopherol)

or in populations not very experienced in recording food intake, such as the population in the present study. The population in our study was somehow familiar with the dietary recall as it was a subgroup of the TLGS. Regardless of the kind of reference method, under- and over-estimation biases and random errors might affect any of the methods normally used as reference in validation studies. Therefore, we used twelve 24hDR, administered monthly for a year, to minimize random errors due to day-to-day variations in food intake and to cover seasonal variations throughout a 1-year period. In addition to repeated dietary recalls, biomarkers from urine and blood samples were used as part of our validation study, as they are not correlated with errors in dietary methods. Using the method of triads, which enables a triangular comparison between questionnaire, reference and biochemical marker measurements, an estimate of the validity coefficient of the FFQ was obtained. In the case of protein,

the estimated coefficient of the dietary recall was  $>1$ ; as a Heywood case that may be acceptable, because a positive correlation between random errors of the FFQ and 24hDR would produce validity coefficients that are overestimated for the FFQ and 24hDR and underestimated for the biochemical marker measurement<sup>(25)</sup>. The correlation of FFQ measurements of nutrients with biochemical markers as a lower limit and the true estimated coefficient of the FFQ as an upper limit showed that FFQ measurements of K,  $\beta$ -carotene and cholesterol might be reasonably accurate ( $>0.3$ ) and FFQ measurements of protein, retinol and  $\alpha$ -tocopherol appear to be less accurate; however the lower validity correlation of these nutrients may be underestimated<sup>(24,25)</sup>.

The FFQ administered in the present study was semi-quantitative, such as the FFQ in the Nurses' Health Study<sup>(40)</sup>. However, the portion size was different and we used portion sizes commonly used by Iranians.

To mention other limitations, the methods used for dietary assessment are subject to variations<sup>(29,41)</sup> and therefore comparison between the two methods may not be precise; however, we evaluated the relative validity<sup>(42)</sup> to partly overcome this problem. Another limitation is that social characteristics, smoking and dietary supplement intake and BMI were not considered in our study. Only a few validity studies<sup>(26,43)</sup> for FFQ have shown results for subgroups other than gender, like BMI groups, and highlight the need to assess FFQ validity in a sample size that is large enough to ascertain differences among subgroups. Using the USDA FCT is another limitation of our study. Not having any complete Iranian FCT with which to compare, we do not know how this affects our results concerning the correlations of dietary intakes of the 24hDR and FFQ with urine and plasma biomarker values.

One of the strengths of the present study is that data were analysed separately in men and women and both men and women were included in the study. Another strength is the reporting of both energy-adjusted and deattenuated correlation coefficients, which reduces the random error due to within-person variation. Clear reasons exist why nutritional epidemiology should focus on energy-adjusted nutrient intakes, which is the nutrient composition of diets in relation to disease occurrence<sup>(44)</sup>.

Over the years investigators have come to recognize that the reported values from FFQ are subject to substantial errors (intake-related bias, person-specific bias and within-person variation) that profoundly affect the interpretation of studies in nutritional epidemiology. It is suggested to structure new models of dietary measurement error for estimation of relative risk based on validation/calibration sub-studies in large epidemiological investigations that include urinary N as a biomarker for protein intake.

In conclusion, the present study shows that using combinations of twelve repeated 24hDR, two FFQ, biochemical markers in serum and urine samples, and true estimated validity coefficients for agreement between two methods, the FFQ used in the TLGS has reasonable relative validity and reliability for nutrient intakes in Tehranian adults and appears to be an acceptable tool for assessing nutrient intakes in this population.

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