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Original article

The validity and reproducibility of a semi-quantitative food frequency questionnaire used for measuring dietary vitamin A intake in under-five children in Addis Ababa, Ethiopia.

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Summary: A valid assessment of diet is crucial both for understanding disease causation and for launching control programs. Although there have been a number of studies that have evaluated and demonstrated the reproducibility and validity of food frequency questionnaires (FFQs) in the developed world, to our knowledge there have not been published reports of such studies from Africa. The purpose of the present study was to assess the validity and reproducibility of a 98-item FFQ used in a cohort study to evaluate dietary vitamin A intake as a risk factor for acute lower respiratory infections in children under five years of age in Addis Ababa, Ethiopia. Serum retinol determinations and seven days of 24-hour diet recall were compared with data from the FFQ completed three times at six month intervals in a randomly chosen sub-sample of 100 children from the larger cohort. Reproducibility was high for all nutrients between the second and third FFQ. The correlations ranged from 0.36 for folic acid to 0.63 for preformed vitamin A. The correlations between FFQ1 and FFQ2 ranged from 0.23 for total vitamin A to 0.39 for preformed vitamin A. Correlations between the mean of the three questionnaires and serum retinol were 0.22 for intake of total vitamin A, 0.25 for preformed vitamin A and 0.15 for provitamin A. All nutrient estimations from the multiple recalls were not appreciably correlated with serum retinol levels. Correlations between the multiple 24-hour recalls were 0.21 for total vitamin A, 0.11 for preformed vitamin A, 0.16 for provitamin A, 0.12 for folic acid and 0.27 for total caloric intake. Our results suggest that 98-item FFQ performs reasonably well for categorizing children by levels of vitamin A intake in Addis Ababa. The results also indicate that the seven-day multiple 24-hour recalls were inferior to the questionnaires in estimating long-term vitamin A intake in children and are probably best avoided as gold standard measure in this setting.[*Ethiop. J. Health Dev.* 1997;11:339-46]

Introduction

Acute and chronic under nutrition and communicable diseases are major problems of developing countries (1). The interactions between nutrition and health have been recognized for some time (2). A reasonably valid assessment of diet is crucial both for understanding disease causation and for launching control programs (3). In the past decade, a large number of epidemiologic studies in the industrial world have used the semi-quantitative food frequency questionnaire (FFQ) for dietary assessment in their search for causes of diseases (4). The FFQ assesses the frequency of consumption during a specified time period with a list of foods using defined portions sizes. The strengths and limitations of the FFQs have been reviewed (5,6). Short-term dietary assessment methods using the 24-hour recall or diet records, now less frequently used in the developed countries,

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are still widely used in developing countries including Ethiopia (7). Probably the two major advantages of use of the FFQs is that they measure the average long term diet (for example 6-12 months), which is more relevant for most disease causation than short term diet, and because they are relatively inexpensive and thus can be used for large scale epidemiologic studies (6). Although there have been a number of studies which have evaluated and demonstrated the reproducibility and validity of FFQs in the developed world (8-11), to our knowledge there have not been published reports of such studies from Africa.

Figure Time sequence of food frequency questionnaire (FFQ1-FFQ3) validation study using seven-day 24-hr recalls and serum retinol determination, conducted among 100 children under five years of age in Addis Ababa, Ethiopia 1993

1991	1992										
December	J	F	M	A	M	June	J	A	S	O	November
FFQ1						FFQ2			**		FFQ3

(September)
Seven-day multiple 24-Recalls
followed by
Serum collection

The purpose of the present study was to assess the validity and reproducibility of a 98-item FFQ used in a cohort study to evaluate dietary vitamin A intake as a risk factor for acute lower respiratory infections in children under five years of age in Addis Ababa, Ethiopia.

Methods

Population: Children in this study were a sample of a cohort of 1,992 children under five years of age participants of a cohort study, followed for one year, between November 1991 and December 1992, to study the risk factors for acute respiratory infections. Dietary information was collected using food frequency questionnaires developed locally, three times during the year, at six-month intervals . The FFQ were administered by trained and supervised interviewers. Mothers or other caretakers were asked about their children's usual intake in the previous six months for each specific item and portion size, and were asked to respond in terms of frequency of intake per day, week, month, past 6 months or never. For the validation study we selected a random sample of 100 children from the cohort study and collected multiple 24-hour diet recalls for seven days followed by venous blood for serum retinol determinations. The blood collection and recall data were obtained close to the mid-interval (September) of time between the administration the second and third FFQs (Figure). After the conclusion of the recall interviews mothers were asked to bring their children to the clinical chemistry laboratory of a nearby referral and teaching hospital (Tikur Anbessa Hosptial) where an experienced laboratory technician drew their blood. Because of technical difficulties serum retinol determinations could be completed in only 80 of study children. Thus, comparisons between the dietary data and serum retinol levels were limited to the 80 study children who had both measurements.

The semi-quantitative food frequency questionnaire: An experienced dietitian from the Ethiopian Nutrition Institute was invited to participate in the process of identifying the relevant food items to be included in the FFQ, with the aim of measuring intakes of total calories and vitamin A in Addis Ababa. Food composition tables for use in Ethiopia, East Africa and Africa (12-16) were consulted to work out an initial extensive food list of over 400 items. Of these 99 items deemed relevant and able to categorize children by past nutritional intake were selected. For each of the 99 food items listed, mothers were asked to estimate their children's use of that food in terms of a standard portion size. We have made this portion sizes to correspond to children's 'natural' units (for example, a quarter of *Injera*, the staple, but fairly large, flat bread). The list included mixed dishes as the number of such foods were not extensive in this population and food composition data on these foods were available. This FFQ was then pilot tested on 50 children under-five in a sub-district outside of the study area before it was used in the cohort study.

A computer program was developed to calculate nutrient values from the FFQ using

Ethiopian and African food composition table data. Daily, weekly or monthly frequencies for each item in the FFQ were first converted into daily frequencies of consumption. These were multiplied by the specific food composition data base reflecting the nutrient content of one portion of each item in the questionnaire. The values were then summed up for all items in the FFQ giving total daily intake of the various nutrients for each child in the study.

Multiple 24-hour recalls: Using a diet recall form developed for the purpose, trained and supervised field workers made visits to the selected households and interviewed caretakers on the child's diet intake during the previous 24-hours. This was repeated for seven consecutive days. For the 24-hour recalls, the dietitian from the Ethiopian Nutrition Institute calculated the nutrient values manually but following the same steps as described above for the FFQs.

Serum retinol determination: Serum retinol determinations were conducted using high pressure liquid chromatography (HPLC) at the laboratory of the Ethiopian Nutrition Institute using the method described by Bieri et al (17). Blood was collected by venous puncture without anti coagulant and serum was separated and stored in the dark at -20^{0}C until analysis.

Quantitation Retinol and retinyl acetate standards were prepared in absolute ethanol. Concentrations of the standard solutions were determined using a Beckman Du-60 (Irvine, CA, USA) spectrophotometer. The retinol concentration was computed from a standard curve of peak area ratios. To prepare a standard curve, we combined a constant amount of the internal standard with five different concentrations of retinol. These mixtures of retinol and retinyl acetate were chromatographed, and the peak area ratios (peak area retinol and retinyl acetate) and concentration ratios (concentration of retinol: concentration of retinyl acetate) were determined. The inter-assay coefficient of variation for retinol determination was 5.2%.

Statistical analysis: Means and standard deviations were calculated on total nutrient intakes from the three FFQs and the recalls. Spearman's correlation coefficients were used in the statistical analysis. Correlations coefficients were computed before and after adjustment for total caloric intake: total caloric intake ($\log_e$ transformed) was used as the independent variable in a regression model with the nutrient score ($\log_e$ transformed) as the dependent variable. The rationale for energy-adjustment is given elsewhere (6), an additional reason was because we have used energy-adjusted nutrient estimates in evaluating dietary risk factors in the ALRI cohort study. Multivariate regression models were also fitted to evaluate the ability of the FFQs to predict serum retinol levels.

Table 1: **Age, sex and nutritional status of children under five years of age participating in the Addis Ababa validation sub-study and ALRI Study cohort, 1993**

Variable	Validation Study (n = 100) %	Total Cohort (n = 1,992) %
Age		
Under 6 months	5.0	5.5
6-12 months	8.0	9.0
13-24 months	9.0	19.0
> 24 months	78.0	66.5
Sex		
Male	51.0	53.1
Female	49.0	46.9
Weight-for-height (wasting)		
More than -2.0 Sd*	90.0	88.8
Between -2.0 and -3.0 Sd	1.0	1.7
Less than -3.0 SD	9.0	9.9
Height-for-age (stunting)		
More than -2.0 SD	47.0	48.3
Between -2.0 and -3.0 Sd	22.0	22.2
Less than -3.0 SD	31.0	29.4

* Z scores were computed to categorize levels of weight-for-height, and height-for-age based on the NCHS standards.

The ability of FFQs in discriminating children with serum retinol values of 10 mcg/dl or less was examined by dichotomizing serum retinol values into high and low ($\leq$ 10 mcg/dl) and fitting a logistic regression model to the data, with serum retinol as the dependent variable. Odds ratios and 95% confidence intervals were then estimated. Test for trend was calculated by including the nutrient as continuous variables in the model.

Table 2: **Mean (± standard deviation) absolute daily vitamin A intake estimated by three food frequency questionnaires (FFQ1-FFQ3), and seven days 24-hr recalls among 100 children under five of age in Addis Ababa, Ethiopia, 1993**

Variable	*FFQ1*	*FFQ2*	*FFQ3*	*FFQ1-FFQ3**	*Recall*	*ALRI Cohort (n = 1,992)*
Total vitamin A (RE/d)	409 ± 285	556 ± 259	556 ± 259	469 ± 204	389 ± 203	444 ± 238
Preformed vitamin A (RE/d)	27 ± 47	28 ± 40	28 ± 49	28 ± 36	16 ± 24	33 ± 43
Provitamin A (RE/d)	387 ± 115	444 ± 237	537 ± 257	448 ± 203	372 ± 205	420 ± 232
Folic acid (mcg/d)	191 ± 111	188 ± 92	212 ± 140	191 ± 82	176 ± 167	173 ± 90
Total caloric intake (kcal/d)	1495 ± 667	1657 ± 720	1720 ± 844	1582 ± 590	1096 ± 274	1479 ± 602

*Average intake estimated from all three FFQs

Results for only the first FFQ and the mean of the first and second FFQs are presented because these were used in the ALRI cohort study.

As the purpose of this report is to quantify measurement error rather than test hypothesis, p values for correlation coefficients are not presented. Data were entered onto a computer using the EPI-INFO program, version 5, CDC, Atlanta GA, and analyzed using the Statistical Analysis System program, version 6.04, SAS Institute, Cary NC.

Scientific and ethical clearance for the study was given by the Research and Publication Committees of the Department of Community Health, the Faculty of Medicine, and the Research and Publication Office of Addis Ababa University.

Results

Age, sex, and the degrees of wasting and stunting among children participating in the validation study were comparable with these distributions in the overall cohort (Table 1). The proportion of children over 24 months of age was somewhat higher in the validation group than the larger cohort.

We compared the means for average daily nutrient intakes from the seven days of multiple 24-hour recalls and from questionnaires 1 to 3 individually and in combination for the 100 children included in the validation study (Table 2). On the average, nutrient estimates from the three questionnaires were higher than those from recalls. This over estimation ranged between 4.8% for total vitamin A and 3.8% provitamin A for FFQ1 to 36% for total caloric intake with FFQ3. The mean intakes of all nutrients, except preformed vitamin A were slightly lower for the whole cohort (n = 1,992) compared with responses from questionnaire from the sample. This reduction varied between 2.3% for performed vitamin A and 8% for total caloric intake.

The reproducibility of the FFQ was examined by calculating Spearman's correlations between the three FFQs completed at six-months intervals in one year (Table 3). Reproducibility was high for all nutrients when the second and third FFQ were compared. These ranged from 0.36 for folic acid to 0.63 for preformed vitamin A. Correlation coefficients, though, were lower with FFQ1.

Table 3: **Correlations (Spearman's coefficients) of food frequency questionnaires (FFQ1-FFQ3), completed three times at 6 months interval, among 1992 children under five years of age in Addis Ababa, Ethiopia, 1993**

Variable	*FFQ1 versus FFQ2*		*FFQ1 versus FFQ3*		*FFQ2 versus FFQ3*	
	Crude	Adjusted*	Crude	Adjusted	Crude	Adjusted
Total vitamin A	0.23	0.11	0.23	0.01	0.43	0.42
Preformed vitamin A	0.39	0.39	0.39	0.38	0.63	0.61
Provitamin A	0.29	0.14	0.22	0.04	0.41	0.46
Folic acid	0.30	0.17	0.20	0.16	0.36	0.25
Total caloric intake	0.29	-	0.18	-	0.60	-

* Intakes adjusted for total caloric intake using regression analysis

The mean (± SD) of serum retinol in the 80 children was 15.5 (± 12.2) mcg/dl, and levels ranged between 6 and 64.1 with a median of 14.6 mcg/dl. Comparison of energy-adjusted nutrient intake measurements from the questionnaire with serum retinol values show the highest correlation for preformed vitamin A (r = 0.25) from the mean of all three questionnaires (Table 4). For preformed vitamin A correlations ranged between 0.09

for FFQ1 and 0.25 for the mean of all three questionnaires. For provitamin A these ranged between 0.06 for FFQ1 and 0.18 for the mean of FFQ2 and FFQ3. For total vitamin A the range was between 0.04 for FFQ1 and 0.22 for the mean of all three questionnaires. All nutrient estimations from the multiple recalls were not appreciably correlated with serum retinol levels.

Table 4: **Spearman correlation coefficients of vitamin A intake calculated from three food frequency questionnaires (FFQ1-FFQ3), and seven-day 24-hour recalls results with serum retinol determinations among 80 children under five years of age in Addis Ababa, Ethiopia, 1993**

	Total vitamin A		Preformed vitamin A		Provitamin A	
	Crude	Adjusted*	Crude	Adjusted	Crude	Adjusted
FFQ1	0.11	0.04	0.10	0.09	0.13	0.06
FFQ2	0.07	0.12	0.16	0.20	0.09	0.12
FFQ3	0.07	0.12	0.17	0.10	0.05	0.11
Mean of FFQ1 & FFQ2	0.12	0.14	0.18	0.21	0.27	0.12
Mean of FFQ2 & FFQ3	0.09	0.19	0.25	0.22	0.09	0.18
Mean of FFQ1, FFQ2 & FFQ3	0.09	0.22	0.24	0.25	0.10	0.15
Seven 24-hr Recalls	0.05	0.04	0.08	0.03	0.02	0.02

* Intakes adjusted for total caloric intake using regression analysis

The questionnaires were also compared with the multiple 24-recalls (Table 5). Correlation estimates were generally lower for total vitamin A. For preformed vitamin A the correlations ranged between 0.07 for FFQ2 to 0.27 for FFQ3, and for preformed vitamin A they ranged between 0.11 for FFQ2 and 0.22 for FFQ3. Adjustment for total energy intake decreased correlation coefficients. These were 0.21 for total, 0.11 for preformed, 0.16 for pro vitamin A, 0.12 for folic acid. The correlation was 0.27 for total caloric intake.

The ability of the FFQs in discriminating children with low level of serum retinol ($\leq$ 10 mcg/dl) was also examined (Table 6). There was a statistically significant association between dietary intake of preformed vitamin A levels and serum retinol levels. Children in the highest quintile of dietary preformed vitamin A intake had a 30% lower chance of having low levels of serum retinol compared to the children in the lowest quintile, FFQ1: (Adjusted RR: 0.70, 95% CI: 0.17, 2.84, P for trend:0.04); mean of FFQ1 and FFQ2: (Adjusted RR: 0.44, 95% CI: 0.11, 1.74, P for trend:0.003).

Discussion

We compared individual nutrient intakes estimated by a 98-item food frequency questionnaire completed three times in a year with serum retinol determinations and seven 24-hour diet recalls completed in the second half of the year. Correlations between serum retinol and the mean of the three questionnaire was 0.22 for total vitamin A, 0.25 for preformed vitamin A and 0.15 for provitamin A.

Energy adjustment of nutrients improved correlations in the studies by Willett et al (8); the effect of adjustment by total caloric intake was minimal in the study by Pietinnen et al (18). In our study such adjustment improved the correlation of nutrient estimates from the questionnaire with serum retinol value but attenuated those with multiple 24-hour recalls. The higher correlations with serum retinol after energy adjustment supports the biological rationale for this procedure (6), which is the primary reason for taking energy intake into account.

Table 5: **Spearman correlation coefficients for three food frequency questionnaires (FFQ1-FFQ3) and seven day 24-hr recalls among 100 children under-five years of age in Addis Ababa, Ethiopia, 1993.**

	Total vitamin A		Preformed Vitamin		Provitamin A		Folic acid		Calories
	Crude	Adj.a	Crude	Adj	Crude	Adj.	Crude	Adj	Crude
24-Recalls Versus									
FFQ1	0.02	0.11	0.07	0.08	0.12	0.15	0.15	0.02	0.05
FFQ2	0.04	0.19	0.28	0.03	0.11	0.13	0.16	0.06	0.24
FFQ3	0.11	0.01	0.27	0.08	0.22	0.04	0.15	0.13	0.16
FFQ1&FFQ2	0.07	0.18	0.17	0.08	0.17	0.18	0.20	0.09	0.18
FFQ2 & FFQ3	0.11	0.18	0.26	0.08	0.17	0.08	0.25	0.13	0.27
FFQ1,FFQ2 & FFQ3	0.14	0.21	0.19	0.11	0.21	0.16	0.19	0.12	0.27

* Intakes adjusted for total caloric intake using regression analysis

Table 6: Odds ratios (95% confidence interval) of low serum retinol level according to the distribution of nutrient intakes from food frequency questionnaires among 80 children under five years of age in Addis Ababa, Ethiopia.

Dietary variable	Quintile of intake					P(trend)*
	1	2	3	4	5	
FFQ1						
Total vitamin A	1.00	0.73 (0.19, 2.77)	0.95 (0.25, 3.55)	1.14 (0.30, 4.33)	1.52 (0.40, 5.80)	0.6
Preformed vitamin A	1.00	0.46 (0.11, 1.81)	0.28 (0.07, 1.15)	0.38 (0.09, 1.49)	0.70 (0.17, 2.84)	0.04
Provitamin A	1.00	0.45 (0.11, 1.80)	0.93 (0.25, 3.51)	1.91 (0.47, 7.63)	1.56 (0.41, 5.96)	0.7
Mean of FFQ1 & FFQ2						
Total vitamin A	1.00	0.67 (0.22, 1.97)	0.35 (0.10, 1.17)	1.20 (0.36, 4.05)	1.02 (0.32, 3.22)	0.1
Preformed vitamin A	1.00	0.73 (0.19, 2.81)	0.92 (0.23, 3.58)	0.64 (0.15, 2.65)	0.44 (0.11, 1.74)	0.003
Provitamin A	1.00	0.65 (0.18, 2.36)	0.71 (0.19, 2.57)	1.99 (0.52, 7.66)	1.26 (0.34, 4.59)	0.8

In well nourished populations the liver stores at least 90% of the total body reserve of vitamin A and buffers serum levels over a wide range of dietary vitamin A intake (9). On the other hand, serum retinol may be more sensitive indicator of vitamin A status in under nourished populations. Ethiopia is categorized globally among "category I" countries having physiologically significant vitamin A deficiency (19). Several surveys have also demonstrated this fact (7,20-22). The deficiency of the Ethiopian diet in protein, calorie and vitamin A was also shown by early studies (23-25). A survey of 31,876 children under six years of age in 1988 has shown a Bitot's spot rate of 0.6% in Addis Ababa (26). The present study was conducted in a population with low socio-economic status and poor nutritional status (Paper II). Thus, it is assumed that serum retinol values may be more sensitive indicator of prolonged low intake of vitamin A. Several studies from developing countries (27) and Ethiopia (28) have shown the association between intake of vitamin A and serum retinol levels.

Our finding that nutrients estimated by questionnaire correlated with serum retinol levels, but not with multiple 24-hour recall, suggest that the questionnaire better reflects vitamin A status of children than multiple 24-hour recalls. The availability and intake of food sources of vitamin A is affected by seasons in many countries particularly in Ethiopia due to agricultural practices (28) and the religious practice of fasting. Most of the 80% orthodox Christians fast between 150 and 200 days each year, and most of the Moslem population also fast for 30 days each year (29,30). Furthermore, unlike adults, as children grow, their dietary intake changes. This variation of intake of vitamin A will have impact on serum retinol levels. Also, the seasonal variation of dietary vitamin A intake is probably better measured by the three food frequency questionnaire which reflect longer period of intake (one to one-half year), than the multiple 24-hour recalls which covered a period of only seven days. This is also indicated by the very low correlation between serum retinol levels and the multiple 24-hour recall in our study.

The reproducibility of the FFQ was variable for total, preformed and provitamin A. This is probably due to the fact that the FFQs were reflecting in part a true variation in dietary intake among the study. For the orthodox christian population, fasting involves avoidance of meat and dairy products including eggs, and for the more conservative, fish. In this study fewer than 0.5 % of children were reported as having eaten fish during the study period. On the other hand, variations in the intake of vegetables, particularly food containing pepper (*Capsicum anuum*), which is widely and regularly eaten (28), may not be as marked as meat and dairy products.

Our food frequency questionnaire was designed to be used in a cohort study of children under five years of age in Addis Ababa to evaluate the relationship of acute lower respiratory tract infections (ALRI) and dietary vitamin A intake. Comparisons of children in extreme quintiles of distribution of dietary vitamin A intake were used to evaluate relative risks of ALRI (31).

Our data also provided the opportunity to compare the performances of both the food frequency questionnaire and diet recalls with serum retinol determinations. The results indicate that the seven day multiple 24-hour recalls were inferior to the questionnaires in estimating long-term vitamin A intake in children and probably best avoided as gold standard measures in this setting. The value of a greater number of 24-hour recalls or recalls more dispersed in time is unknown in the Ethiopian environment.

In conclusion, we have found the 98-item food frequency questionnaire used in children under five years of age to be predictive of serum retinol levels among children in Addis Ababa. These result indicate that the food frequency questionnaire can be used to evaluate associations between dietary intake of vitamin A and risk of ALRI. Further research on the FFQs is required if they are to be used for estimation of other nutrients in children in Ethiopia.

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