

Development and Validation of a Food Frequency Questionnaire for Assessment of Diet in Lower and Middle Income Countries: A study among People Living with HIV/AIDS in Nepal

Dilip Upreti^{1,*}, Janet Kyle², Padam Simkhada³, Janine Thoulass⁴ and Geraldine McNeill⁵

^{1, 2, 5}Public Health Nutrition Group
Institute of Applied Health Sciences
University of Aberdeen
Scotland, UK

³Centre for Public Health
Liverpool John Moores University
Liverpool, UK

⁴Chronic Disease Research Group
Institute of Applied Health Sciences
University of Aberdeen
Scotland, UK

* Corresponding author's email: [dupreti \[AT\] abdn.ac.uk](mailto:dupreti [AT] abdn.ac.uk)

ABSTRACT---- *Assessing the validity of a Food Frequency Questionnaire (FFQ) by comparison with other established dietary assessment methods is essential prior to use of a new FFQ in different population groups. In this study a new FFQ for Nepal was developed with 73 food items and administered by an interviewer who then carried out a 24-h recall. 73 adults living with HIV/AIDS (43M and 30F) were purposively recruited. A nutrient database to estimate the intake of energy and macronutrients (fat, protein, and carbohydrate) and selected micronutrients (iron, β -carotene and vitamin C) was compiled from Nepalese, Indian and UK based on food composition tables. Comparison of mean intakes by the two methods showed good agreement between the FFQ and 24-h recall for the mean intake of energy, protein, carbohydrate, iron, vitamin C and Vitamin A (all $p > 0.20$) but fat intake was significantly lower by the FFQ than the 24-h recall ($p < 0.001$). Pearson correlation coefficients between the methods ranged from 0.50 to 0.60 for energy and macronutrients but were below 0.3 for micronutrients. Bland-Altman analyses showed reasonable agreement between the group means but wide individual differences between methods. The newly designed semi-quantitative FFQ can estimate the group mean intake for selected nutrients.*

Keywords--- HIV/AIDS; Food; Nutrition; Diet; FFQ; Validation; Nepal

1. BACKGROUND

Diet provides one of the foundations for health, with sufficient nutrient intake being important to maintain the immune system and promote health, particularly among those with living with HIV/AIDS (Friis, 2006; WHO, 2005). The HIV/AIDS epidemic has had a devastating impact on health, nutrition, food security and overall socioeconomic development in countries that have been most affected by the infection (UNAIDS, 2008). In Nepal approximately 40,720 people live with HIV/AIDS (PLHA), an estimated prevalence of 0.23% among the adult population (15-49 years) (NCASC, 2014).

South East Asian countries including Nepal are at the early stage of nutritional transition (Balarajan & Villamor, 2009). It has been reported that one in three people live below the poverty line in Nepal following a mainly plant based non-diverse diet and an estimated 40% of the population are undernourished (WFP, 2009; Brown, Worth & Shah, 1968). However there have been few surveys of dietary intake in Nepal, which may reflect the lack of tools for accurate dietary assessment in surveys in this population.

Methods for measuring dietary intake can be classified in to two major groups: prospective and retrospective techniques. Prospective methods include daily non-weighed and weighed food records while diet history, 24-h recall and Food Frequency Questionnaire (FFQ) techniques are retrospective techniques (Willett & Lenart, 1998; Xu *et al.*, 2006; Gibson, 1990). Dietary record and recall methods are generally labour-intensive for participants and researchers and can only

cover a very short period of time. FFQs are widely used as a more convenient tool to measure the habitual dietary intake in large-scale epidemiological studies (Wrieden *et al.*, 2003; Willett & Lenart, 1998; Gibson, 1990). The aim of an FFQ is to assess the frequency, e.g. daily, weekly or monthly, with which certain food items or food groups are consumed during the specified time period. Because FFQs lower the respondent burden, they are suitable for large-scale surveys in literate populations as they may be self-completed and can be sent and returned by mail. In populations with low literacy rates FFQs may be interviewer administered, which has the added benefit of reducing errors in completion of the FFQ. FFQs can be developed and modified according to the research requirements and the population in which they will be used (Willett & Lenart, 1998).

Development and validation of each FFQ in the target population is essential so that the FFQ can list all the foods, which are common in that community and can be used with confidence (Willett & Lenart, 1998). The main aim of this study was to assess the validity of an FFQ designed for assessing the diet of HIV positive people in Nepal with a 24-h recall as a reference method. Similarly, a repeated 24-h recall was carried out among the 20 sub-sample of the study population on two consecutive days to assess the variation of dietary behavior.

2. METHODS AND MATERIALS

DEVELOPMENT OF THE FFQ

Literature review: Literature relating to development and validation of FFQs were reviewed (Roumelioti & Leotsinidis, 2009; Shahare *et al.*, 2003; Cade *et al.*, 2002; Willett & Lenart, 1998). A MEDLINE, EMBASE, PUBMED, SCOPUS, COCHRANE Library, CINAHL and Google Scholar search was completed using search terms (FFQ, 24-h recall, diet, food, study, Nepal, India, Pakistan and Asia). A food list with 54 different foods was developed from existing questionnaires used in studies of diet in Nepal (Ohnoet *et al.*, 2006; Ohnoet *et al.*, 2005; Sudoet *et al.*, 2005; Ohnoet *et al.*, 1997; Hirai *et al.*, 1993), India (Bharathiet *et al.*, 2008; Pandey *et al.*, 2005) and Pakistan (Saleheen *et al.*, 2009) was developed.

Identification of commonly consumed food items: An open ended self-administered questionnaire was used to identify the common foods in the diet of Nepal among 30 individuals in the Nepalese community in Aberdeen, Scotland; preference was given to those who were newly arrived from Nepal. Interviewer administered 24-h recalls were conducted among 34 subjects from the same community to identify additional foods in their diet. All subjects were asked about their habitual portion size using standard household measurement units, e.g.; ladle, bowl, plate, thal (traditional dinner plate in Nepal), glass, teaspoon, table spoon, cup and mug.

Finalization of foods list for FFQ: The common foods from the Nepalese community in Aberdeen and foods identified from existing FFQs found in the literature review were combined to produce a final list of 73 food items. These foods were divided into eight different food groups which were listed in order of frequency of consumption: tea and hot beverages; cereals and starchy foods; daal (lentil soup), vegetable and mixed curries; green salads and pickle; fish and meat; milk and milk products; fruit and high sugar and fat foods (sweets, cookies, savoury fried foods and nuts). The FFQ also included a section that allowed participants to report any foods or beverages not included in the questionnaire so that the nutrient intake from these food items could be included in the analysis. Table 1 outlines the number of food items in each of the eight groups.

Table 1: Food categories and total food items in the initial FFQ

Categories of food	Number of food items
Tea and hot beverages	2
Cereals and starchy food	13
Daal (lentils), vegetable and curry	7
Green salads and pickle	6
Fish and meat	10
Milk and milk products	4
Fruits	12
Sweets, cookies, fried foods and nuts	19
Total number of food items	73

Because the diet in Nepal varies with season, for each food the seasonality of consumption was divided into four different categories: 'almost never' (less than once a month throughout the year), 'at festival times only' ("dasain & tihar"; 15 days per year), 'seasonal only' (3 months per year) and 'all year round'. For foods eaten at festival times, in season or

all year the frequency of consumption during the appropriate season for each food item was recorded using eight frequency categories: 1/month, 2-3/month, 1/week, 2-4/week, 5-6/week, 1/day, 2/day and 3+/day.

Food portion size: Food measure units and weight per serving were taken from Nepalese food tables where possible (DFTQC, 2007; DFTQC, 2004). If the food was not listed in Nepalese food tables then Indian food tables were used (Chiplonkar & Agte, 2007; Gopalan *et al.*, 2007; Pasricha & Rebello, 1982; CFTRI, 1981). For some food items (e.g. sugar, boiled egg) the UK food portion size was used (FSA, 2002; Davies & Dickerson, 1991). The food weight per serving unit was not available for a few foods e.g. tomato chutney, mixed chutney, beaten rice (rice flakes), mutton curry or dalmouth (savory fried lentils and split gram). The weights of typical portions of these food items were measured on three separate occasions in different Nepalese homes in Aberdeen, using digital kitchen scales with the average value being used for the calculation of nutrient intake from the FFQ. Weights of typical portions were later measured in Nepalese households, which showed good agreement with the values from the UK Nepalese households. Colour food photographs of the unit of measurement of common foods (e.g. vegetable curry, rice and daal) and standard Nepalese household items (e.g. ladle, cup, katori (small bowl) and plate) were also produced to assist the assessment of uniform portion size during administration of the FFQ.

The draft FFQ was piloted among 25 Nepalese residents of Aberdeen to test the food list and identify commonly used measures for estimating portion size and to help to identify any possible problems that could arise during the process of data collection in Nepal. No potential problems were identified at this stage. The HIV status of these participants was not ascertained.

Development of the food composition database

As this semi quantitative FFQ was designed to assess the diet of PLHA, the main aim was to estimate the intake of energy and macronutrients (protein, fat and carbohydrate) and some selected micronutrients (iron, carotene and vitamin C), which may influence immune function. Nepalese food composition tables were used as a main source of nutrient composition data to develop the nutrient database for cooked and raw food as appropriate (DFTQC, 2007; DFTQC, 2004). If foods were not available in the Nepalese food tables, the Indian (Chiplonkar & Agte, 2007; Gopalan *et al.*, 2007), then the UK (McCance & Widdowson, 2006) food tables were used. Table 2 shows the details of the source of nutrient composition of food items included in the FFQ database. As long as the food composition was likely to be similar (e.g. tea, doughnuts, biscuits) then the UK and Indian food composition tables were used. The nutrient composition of a few foods had to be estimated from similar foods or from ingredient values. For example the energy value for gundruk (a traditional Nepalese curry made from fermented green leaves for example mustard leaves) was estimated using mustard leaves. Local recipes were calculated for six food dishes (chicken curry, mutton curry, sarbat (home-made juice), tomato chutney, radish chutney and gundruk chutney).

Table 2: Sources of food composition data in the nutrient database

Nutrients	Nepal ^a	India ^b	UK ^c	Recipe calculation	Estimated from similar food ^c	Total
FFQ	Number of food items					
Energy (kcal)	36	6	26	4	1	73
Fat (g)	37	6	26	4	0	73
Protein (g)	37	6	26	4	0	73
Carbohydrate (g)	37	6	26	4	0	73
Iron (mg)	33	10	26	4	0	73
Carotene (µg)	21	17	31	4	0	73
Vitamin C (g)	21	18	30	4	0	73
24 h recall						
Energy (kcal)	35	6	26	6	-	73
Fat (g)	35	6	26	6	-	73
Protein (g)	35	6	26	6	-	73
Carbohydrate (g)	35	6	26	6	-	73
Iron (mg)	31	10	26	6	-	73
Carotene (µg)	25	15	27	6	-	73
Vitamin C (g)	29	11	27	6	-	73

^aDFTQC (2006/7), ^a DFTQC (2006/7a) and ^a DFTQC (2004)

^bChiplonkar (2007)

^cMcCance and Widdowson's (2006)

24-h RECALL

All the subjects were asked to recall their exact food intake during the preceding day (from waking in the morning to going to bed in the evening). All foods and beverages consumed by respondents including quantity (number of portions), frequency, brand name (where known) and cooking method were recorded by the researcher. Probing was used to help the respondent remember all foods consumed throughout the day including any additional food or nutrient supplements (Thompson & Byers, 1994; Gibson, 1990), as one study found that respondents with interviewer probing reported 25% higher dietary intake in comparisons without probing (Campbell & Dodds, 1967).

VALIDATION STUDY

This study involved cross-sectional data collection. As literacy in the population was not guaranteed, the FFQ and 24-h recall were designed for interviewer administration. FFQ and 24-h recall were conducted with a total of 73 PLHA from the Kathmandu valley and the Terai high-way regions of Nepal. A repeated 24-h recall was carried out two days after the first recall. Every subject was requested for a repeated interview however only 20 (8M and 12F) subjects were willing and available. Participants were recruited from local PLHA networks by convenience sampling.

Data collection: Data was collected January – March 2011, all subjects were invited to visit fieldworkers in local training and care centers to complete the study. The FFQ was embedded in a larger questionnaire covering general health and demographic information. The interview was completed in three stages. After consent was taken, the first stage was to collect personal information (demographic and socio-economic) and knowledge on nutrition. Stage two was to collect dietary information using the newly designed FFQ followed by a 24-h recall. In the third stage the participants' height and weight were measured following standardized measurement procedure (Opsomer, Jensen & Pan, 2003) to allow calculation of Body Mass Index (BMI).

Data and Statistical analyses: All questionnaire and 24hr recall data were entered into SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) for analysis. Demographic information was summarized and the average two readings of height and weight were calculated to measure the BMI of the subjects as $\text{weight (kg)} / \text{height (m)}^2$. Frequency and portion size of each food was linked with the newly compiled food composition data base to calculate daily nutrient intake for both the FFQ and 24-h recall.

To assess the quality of data entry and nutrient analysis from FFQ and 24-h recall 10% (N=8) participants were randomly selected. Nutrient values for each participant were manually calculated and all these manually calculated values were cross-checked with electronic data sheet of nutrients. There was not any difference in the result.

The intake of energy, fat, protein and carbohydrate were normally distributed, while those for iron, carotene and vitamin C were not normally distributed. Parametric and non-parametric statistical methods were therefore used as appropriate (Mean \pm standard deviation (SD), median and inter quartile range (IQR)). The difference between the estimated nutrient intakes for each dietary assessment method was tested using either a paired sample t test or Wilcoxon's signed rank test for paired data. The agreement between methods was assessed by Bland and Altman plots (Bland & Altman, 1986). Finally, agreement in ranking between the two methods was assessed by Pearson and Spearman rank correlation coefficients.

Ethical Approval: This study was reviewed and approved by the University of Aberdeen College of Life Sciences and Medicine Ethics Review Board (ref no: CERB/2010/5/459) and Nepal Health Research Council, Government of Nepal (ref: 1382). Written consent from participants was taken after the participant information sheet was read out by the interviewer and witnessed by another observer.

3. RESULTS

Demographic characteristics: Seventy three (M43; F30) participants were recruited to complete the validation study. Most of the subjects (85%) were of the economically active age group 20-39 years and 64% were taking Anti-Retroviral Therapy (ART). The participants' self-reported health status was good (14%) fair (61%) or poor (25%) and 88% were literate. The median BMI values for men and women were 20.4 kg/m² (IQR: 19.5; 23.8) and 20.9 kg/m² (IQR: 19.1; 24.5); respectively. 14% of subjects (M: 9% and F: 5%) were below the BMI cutoff point for underweight of 18.5 kg/m².

Daily nutrient intake: The nutrient intake of the population as estimated from the FFQ and 24-h recalls is shown in Table 3. The daily energy intake from the FFQ and 24-h recall ranged from 938 kcal to 3602 kcal and 1042 kcal to 2909 kcal, respectively. Average daily intakes of energy did not differ significantly between the two methods; 2029 \pm 505 kcal from FFQ and 2046 \pm 469 kcal from 24-h recall ($p = 0.745$). Similarly, there was no significant difference in the average intake of vitamin C ($p = 0.829$), carbohydrate ($p = 0.827$) or protein ($p = 0.705$) between the two methods. Carbohydrates accounted for more than 74% of total energy intake by both methods. However, the FFQ fat intake estimates were significantly lower than the 24-h recall values ($p < 0.001$). Fat contributed only 13% of the total energy intake by the FFQ

and 16% from 24-h recall. Iron and carotene intakes were higher by the FFQ though the differences were not statistically significant ($p = 0.204$ and 0.341 ; respectively).

Table 3: Nutrient intake from FFQ and 24-h recall among all participants, men and women.

	FFQ		24-h recall		Significant difference (p)	
	Mean±SD	Median (IQR)	Mean ± SD	Median(IQR)	between means	between medians
All (n=73)						
Energy (kcal/day)	2030 ± 505	1973(1737;2381)	2046±469	2034 (1763;2447)	0.745	0.769
Fat (g/day)	20.0 ± 13.0	25.8 (20.4;39.4)	36.2±18.3	35.2 (24.8;45.1)	0.001** *	0.001***
(% of energy/day)	12.69 ± 4.2	12.3(9.9;15.1)	15.6 ±7.1	14.8 (11.7;18.2)	0.001** *	0.001***
Protein (g/day)	60.0 ± 15.8	60.2 (49.2; 67.8)	60.7±16.1	60.1 (51.2;72.4)	0.705	0.504
(% of energy/day)	11.9 ±1.5	11.8(10.8;12.6)	11.8 ±2.1	11.6 (10.4;12.9)	0.924	0.915
Carbohydrate (g/day)	385.5± 96.5	376.9 (324.8;445.9)	383.2±86.5	380.4 (323.0;447.7)	0.827	0.603
(% of energy/day)	76.1 ±5.4	77.3(73.1;79.8)	75.5 ±8.0	76.24 (71.7;80.8)	0.523	0.433
Iron (mg/day)	18.0 ± 5.3	17.8 (14.2; 21.0)	16.6±8.9	14.8 (9.2;22.8)	0.204	0.118
Carotene (µg/day)	2219± 661	2165 (1746; 2626)	2082±1160	1838 (1225;2459)	0.341	0.046 *
Vitamin C (g/day)	48.8 ± 15.1	47.5 (37.9; 57.9)	49.4±20.1	49.9 (35.2; 61.5)	0.829	0.993
Men (n=43)						
Energy (kcal/day)	2186± 517	2133 (1877; 2499)	2266±439	2364 (2059; 2603)	0.320	0.232
Fat (g/day)	32.0 ± 13.5	29.4 (22.0;41.5)	37.5±17.9	36.5 (26.2;43.0)	0.061	0.061
(% of energy/day)	13.1 ±4.2	12.6 (10.4;15.2)	14.4 ±5.5	13.6 (11.6;16.9)	0.177	0.176
Protein (g/day)	65.3 ±15.7	64.4 (57.2;75.3)	65.8± 7.3	67.0 (56.4;76.9)	0.867	0.491
(% of energy/day)	12.0 ±1.6	12.1 (10.9;13.1)	11.5 ±1.9	11.4 (10.2;12.4)	0.092	0.114
Carbohydrate (g/day)	411.4±101.0	415.7 (332.2;493.0)	424.0 ± 82.3	414.8 (382.3;502.1)	0.436	0.546
(% of energy/day)	75.3±5.6	75.9 (71.8;79.7)	75.3 ±7.3	76.1 (72.0;80.2)	0.996	0.847
Iron (mg/day)	18.3± 4.8	17.9 (14.9;20.5)	18.2±8.4	17.6 (10.8;25.1)	0.946	0.744
Carotene (µg/day)	2170 ± 568	2140 (1821;2569)	2252± 1221	1968 (1380;3008)	0.677	0.629
Vitamin C (g/day)	50.3± 15.2	48.5 (37.6; 61.6)	51.5± 18.2	50.2 (40.2; 62.1)	0.736	0.772
Women (n=30)						
Energy (kcal/day)	1804 ± 394	1829(1640;2003)	1731± 306	1815 (1537;1894)	0.159	0.192
Fat (g/day)	24.6± 11.1	21.4(17.8;32.2)	34.3± 18.8	30.5 (20.2;53.3)	0.001** *	0.001**
(% of energy/day)	12.1 ±4.3	11.1(8.9;15.1)	17.4 ±8.7	16.9 (11.8;24.6)	0.001** *	0.000***
Protein (g/day)	52.3±12.7	52.8(43.6;61.6)	53.3± 13.7	53.2 (47.6;63.2)	0.669	0.861
(% of energy/day)	11.6±1.5	11.6(10.8;12.2)	12.3 ±2.4	12.5 (10.5;13.6)	0.146	0.106
Carbohydrate (g/day)	348.4± 77.7	353.2(320.2;385.8)	325.4± 54.0	328.3 (294.7;346.7)	0.042*	0.072
(% of energy/day)	77.3 ± 5.0	77.9(74.5;81.1)	75.8 ±9.0	76.3 (70.6;81.4)	0.326	0.339
Iron (mg/day)	17.7 ± 6.1	16.9(12.6;21.2)	14.2± 9.2	10.3 (8.2;18.6)	0.076	0.045*
Carotene (µg/day)	2287 ± 781	2229(1654;2847)	1837± 1038	1577 (1072;2377)	0.027*	0.014**
Vitamin C (g/day)	46.6 ± 14.9	46.3 (37.1; 55.6)	46.2 ± 22.6	47.7 (25.2;61.3)	0.911	0.658

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ^a 19- 50 years

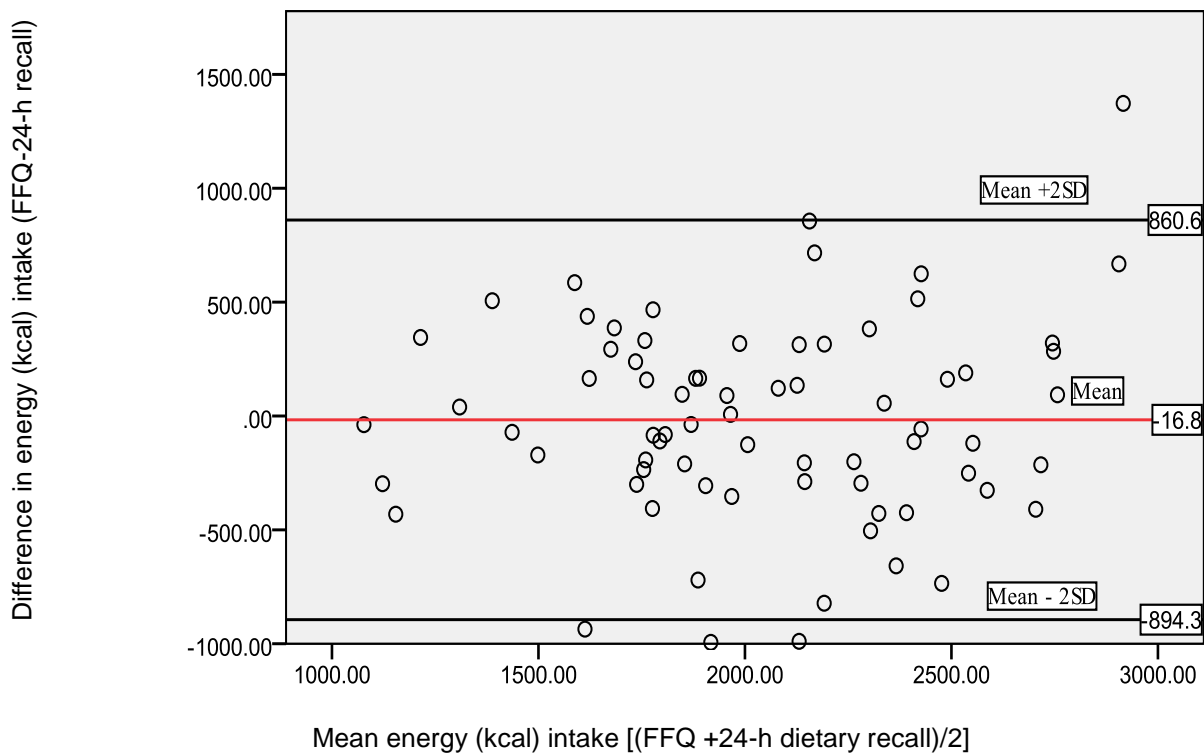
Individual agreement from Bland and Altman test: Reasonable agreement between the mean nutrient intakes from the FFQ and 24-h recall is demonstrated by the Bland and Altman method at group level. However, the 95% confidence intervals (CI) were fairly wide at individual level (Table 4). Figure 1 illustrates the individual agreement for energy between the two methods along with the 95% CI for the agreement.

Table 4: Individual agreement while comparing FFQ and 24-h recall from Bland and Altman test (n=73)

Daily intake	Mean Difference (\pm SD)	95% CI limits of agreement ^a
Energy (kcal)	-16.8 \pm 438.7	860.6; -894.2
Fat (g)	-7.2 \pm 16.7	26.2; -40.7
Fat % of energy	-2.9 \pm 6.7	10.5; -16.3
Protein (g)	-0.7 \pm 15.6	30.6; -32.0
Protein % of energy	0.03 \pm 2.2	4.5; -4.4
CHO (g)	2.3 \pm 87.9	178.1; -173.6
CHO % of energy	0.6 \pm 7.9	16.4; -15.2
Iron (mg)	1.5 \pm 9.8	21.0; -18.1
Carotene (μ g)	136.5 \pm 1217	2571; -2298
Vitamin C (mg)	-0.5 \pm 21.4	42.3; -43.4

^aUpper and lower value at 95% of CI (\pm 2SD) from Bland-Altman test

Figure 1: Bland-Altman plot showing agreement between the FFQ and 24-h dietary recall for energy (kcal) (n=73).

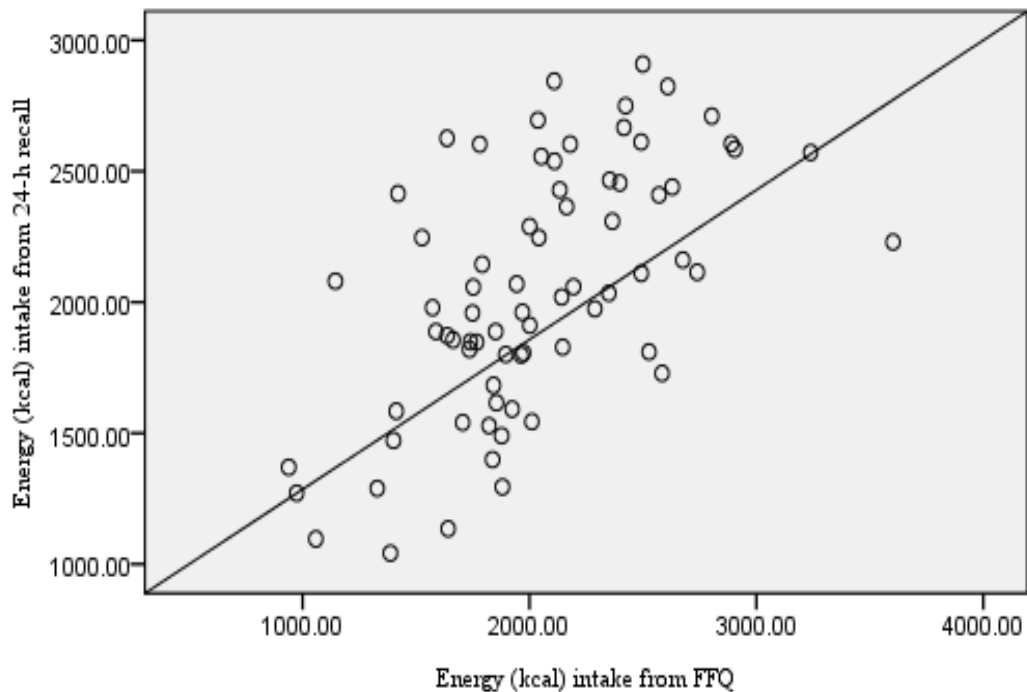


Correlation: Table 5 shows the correlation coefficient for intakes of nutrients by the two methods, which show the agreement in ranking of nutrient intake. Pearson correlation coefficients ranged from 0.47 to 0.60 for energy and macronutrients, which is considered reasonable agreement for using the FFQ for ranking. Figure 2 shows the correlation between the two methods for energy intake ($r = 0.596$, $p < 0.001$). Spearman's correlation coefficients were used to see the agreement in ranking for iron, carotene and vitamin C. For the micronutrients there was not sufficiently good correlation to use the FFQ intake data in a study using ranking.

Table 5: Correlation coefficients between nutrient intake by FFQ and 24-h recall (n=73)

Nutrients	Pearson's correlation (r)	Sig (p)	Spearman's correlation (r)	Sig (p)
Energy	0.596	<0.001	0.604	<0.001
Fat	0.470	<0.001	0.539	<0.001
Fat % energy	0.31	0.001	0.392	0.001
Protein	0.547	<0.001	0.543	<0.001
Protein % energy	0.282	0.016	0.346	0.003
CHO	0.543	<0.001	0.534	<0.001
CHO % energy	0.358	0.002	0.316	0.006
Iron	0.120	0.310	0.162	0.172
Carotene	0.196	0.096	0.267	0.022
Vitamin C	0.287	0.014	0.245	0.036

Figure 2: Correlation of energy intake from FFQ and 24-h recall (n=73)



Repeated 24-h recall

Twenty participants (8M and 12F) completed repeated (second) 24-h recalls and table 6 shows the differences between mean intakes of selected nutrients and the mean±SD and 95% CI for the difference between the intakes of two 24-h recalls by the Bland and Altman method for 24-h recall. The average energy intake from the two days were not significant different (p = 0.384). Similarly, there was no significant difference in the daily intake of fat (p= 0.285), protein (p= 0.514), carotene (p= 0.63), iron (p=0.093) and vitamin C (p= 0.405) between the two recalls.

Table 6: Difference in nutrients intake (mean ± SD) and individual agreement from Bland-Altman test for repeated 24-h recall (n=20)

Nutrients	1 st recall (Mean ± SD)	2 nd recall (Mean± SD)	Sig diff (p) between means	Mean difference (±SD) between the two 24-h recall	95% limits of agreement ^a
Energy (kcal)	1947 ± 336	2030 ± 282	0.384	-83.1±416.5	750.0; -961.1
Fat (g)	35.7 ± 18.9	30.8 ± 14.7	0.285	4.9± 2.0	44.9; -35.0
Fat % energy	16.5 ± 9.2	13.3 ± 5.2	0.128	3.3± 9.2	21.6; -15.1
Protein (g)	62.8 ± 16.1	59.7 ± 14.0	0.514	3.1± 20.7	44.6; -38.4
Protein % energy	12.9 ± 2.5	11.8 ± 2.3	0.125	1.1± 3.1	7.3; -5.1
Carbohydrate (g)	361.8 ± 69.0	388.8 ± 50.3	0.151	-27.1± 80.8	134.6; -188.7
Carbohydrate % energy	74.4 ± 8.8	76.9 ± 6.1	0.230	-2.5±9.0	15.4; -20.4
Iron (mg)	14.9 ± 7.9	19.0 ± 6.7	0.093	-4.2±10.5	16.84; -25.1
Carotene (µg)	1965 ± 1001	2118 ± 997	0.631	-153.3±1403	2653; -2959
Vitamin C (mg)	43.9 ± 18.1	48.9 ± 18.3	0.405	-5.0± 26.4	47.8; 57.9

^aUpper and lower value at 95% of CI(± 2SD) from Bland-Altman test

4. DISCUSSION

This study was undertaken due to the lack of availability of a valid method to measure the dietary intake in a large-scale survey of HIV positive people in Nepal. Generally, the FFQ is considered as one of the most appropriate methods for measuring habitual diet and its less time consuming and inexpensive method in comparison with 24-h recall and food records (Willett & Lenart, 1998).

The results of this study provide evidence that this FFQ can be used in the target population for selected applications. There was no difference in the mean or median intake between the two methods for the estimation of energy, protein, carbohydrate, iron, carotene and vitamin C intake, suggesting that the FFQ can be used to estimate group mean intakes of these nutrients in this population. Furthermore, the correlation coefficient between the methods for nutrient intakes ranged from 0.47 to 0.60 for macro nutrients which reasonable agreement (Willett & Lenart, 1998) and similar with other validation studies conducted in adult population (Bautista, Herran & Pryer, 2005; Chen *et al.*, 2004; Decarliet *et al.*, 1996) including studies among pregnant women (Erkkola *et al.*, 2001; Wei *et al.*, 1999) but were less than 0.30 for iron, carotene and vitamin C, suggesting that the FFQ can be used to assess ranking of intake for macronutrients but not for micronutrients. Though there was good agreement in the average intake estimates for energy, protein, carbohydrate, iron, carotene and vitamin C, mean fat intake was significantly higher by the FFQ than by the reference method, the 24-h recall.

The percentage of carbohydrate contributing to the total energy intake of the Nepalese diet was previously reported to be 76% (Brown, Worth & Shah, 1968), which is similar to the values of 75% for both male and females in this study. Rice is usually eaten at least twice a day and is the main source of energy in the Nepalese diet and 57% and 60% of carbohydrate was derived from rice in 24-h recall and FFQ respectively; high fat foods are eaten very rarely (DFTQC, 2007; Dahan *et al.*, 2005; Brown, Worth & Shah, 1968). The data suggests that protein intake is satisfactory among the participants at 77% and 23% from plant and animal sources respectively, which is similar with other studies in Nepal (Brown, Worth & Shah, 1968) but the percentage of fat in the total energy intake was very low at 15% and 17% for men and women respectively (FAO, 2007), where fat contributes around 37% of energy for UK population (FAO, 2007).

In this study, we have described the process used for the development of an FFQ for HIV positive people in Nepal. The food list used to develop the FFQ reflects the dietary habits of the target population so could potentially be used in other population groups, within Nepal. However, there could be slightly differences due to local food habits and cultural practices so further testing would be needed before use in other population groups (Teufel, 1997). However, internal migration is very common in Nepal, with migration towards urban areas for employment, good education and access to medical facilities (CBS, 2003). When people migrate they bring their regional food and dietary habits. Hence there are similarities in dietary pattern across the country though they have different culture and beliefs (Dahan *et al.*, 2005; CBS, 2003).

The strengths of this study lie in the setting and design. This was a population-based cross-sectional study which is, to our knowledge, the first validation study of a FFQ developed for Nepal. Representation of both sexes was satisfactory and there was a wide age range in the participants. Wherever possible Nepalese food tables were used for the nutrient composition database. Bearing in mind the importance of regional food composition databases (FAO, 2007; FAO, 2004), alternative food composition data was taken from Indian tables in the first instance, with UK data used only for foods considered likely to be similar in UK and south Asia. Similarly, the unit and portion size was finalised based on Nepal, India and UK references and double checked with measurements made in the field. Other nutrients could not be estimated due to the lack of local food composition data, but could be added if data were to become available in the future.

A limitation of this study was that only one 24-h recall was used as a reference method for comparison with the FFQ. The repeated 24-h recall in 20 participants suggested that there was little day-to-day variation in energy and macronutrient intake in this population at group level. However, there was substantial variation in the mean intake of diet at individual level, as shown by the wide 95% limits of agreement (Table 6). Consequently, the single recall should have been a reasonable estimate of habitual intake at group level but more days of 24-h recall records in the reference method would have been given a better estimate of habitual intake of each individual and increased the correlation between energy and nutrient intakes by the FFQ and the reference method.

Fat intake was slightly higher in 24-h recall than in the FFQ in this population. A possible reason for this maybe that interviews were scheduled in towns where the clinic or ART centers were based and participants' from rural may have travelled a long distance to attend the clinic and consequently they may have eaten more high-fat foods, which are luxury items, then in their usual diet. Similarly, some of volunteers were completing residential training in the center for few days during the current study and they might have had meat and special foods during that time.

After the completion of validation study based on a combination of participant feedback and the author's practical experience in the field, minor amendments were made to the questionnaire. The food list was presented without any food group headings in an order, which reflected the timing of food consumption over the day and the frequency of food intake. Other suggestions (for example; local name of foods, portion size and unit) from study participants about the FFQ were incorporated to make the questionnaire easier to use in future studies of nutrition in PLHA in Nepal.

In conclusion this new FFQ can estimate the group mean intake for energy, protein, carbohydrate, iron, carotene and vitamin C in this population. The questionnaire could be adapted for use in other groups, e.g. general population, children or adults in Nepal, though further validation in each group would be required. The FFQ could also be extended to include new foods and other nutrients if reliable local food composition data becomes available. Furthermore, the process of development and validation of FFQ could be used in other lower and middle income countries.

5. LIST OF ABBREVIATIONS USED

FFQ: Food Frequency Questionnaire; BMI: Body Mass Index; HIV: Human Immune Deficiency Virus; AIDS: Acquired Immune Deficiency Syndrome; ART: Anti-Retroviral Treatment; PLHA: People Living with HIV/AIDS; WHO: World Health Organization; M: Male; F: Female; SD: Standard Deviation; CI: Confidence Interval.

6. COMPETING INTERESTS

All authors declare that they have no conflict of interest.

7. AUTHORS CONTRIBUTION

DU was main designer of this project and was responsible for the field work. JK supervised the development of the nutrient database, nutrient analysis and advised on the statistical analysis. PS and JT advised on the protocol for recruitment and field work. GMcN conceived the study and advised on all stages. All the authors were involved in the preparation and revision of the manuscript and read and approved the final version.

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