

**Original Article****Development and Validation of a Food Frequency Questionnaire for Assessing Dietary Calcium in Children**Elham Zeynnejad<sup>1</sup>, Nasrin Omidvar<sup>\*1</sup>, Tirang Neyestami<sup>2</sup>, Anahita Houshiarrad<sup>2</sup>, Mohammad Reza Eshraghian<sup>3</sup>, Ame Stormer<sup>4</sup>

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**Received:** December 2014**Accepted:** February 2015**ABSTRACT**

**Background and Objectives:** This study aimed to develop and validate a food frequency questionnaire (FFQ) for measuring calcium intake in 9-13 years old children in Tehran, Iran.

**Materials and Methods:** A 56-item FFQ containing main contributors of calcium in Iranian diet was designed. Criterion validity of the calcium specific FFQ was evaluated through comparing its result with five 24-h recalls as the reference method. Reproducibility was measured by twice administration of FFQ, one month apart. The study was performed in the selected primary and middle schools of Tehran city. Subjects included 184 children aged 9-13 years (90 girls and 94 boys), who were recruited through two-stage systematic cluster sampling from 20 primary and middle schools.

**Results:** Mean calcium intake was  $922.8 \pm 322.3$  and  $876.0 \pm 491.8$  mg/d for the 24-h recalls and FFQ, respectively, indicating a mean difference of  $46.7 \pm 458.7$  mg/d ( $P < 0.001$ ). Corrected Pearson's correlation was 0.57. Cross-classification analysis of the FFQ and 24-h recalls classified 80% of the subjects in the same or adjacent category, and 6% in the extreme quartiles. The FFQ correctly identified 85% of the children consuming less calcium than the age-specific Recommended Dietary Allowance (RDA) (1300 mg/d). Pearson's correlation for repeated administrations was 0.65. Cross-classification analysis of the repeated administration of FFQ classified 81% of the subjects in the same or adjacent quartiles, and 3.3% in the extreme categories.

**Conclusions:** The FFQ underestimates mean calcium intake of a group, and has limited use to estimate calcium intake for individuals. However, it has acceptable validity and reproducibility for epidemiologic studies to assess a group's mean calcium intake.

**Keywords:** Validation, Food frequency questionnaire, Calcium intake, Children

**Introduction**

Calcium is an essential nutrient in achieving optimal bone health and overall growth. Adequate calcium intake promotes mineralization of skeleton and peak bone mass early in life, and stabilizes it during the young adulthood (1). During the adolescence, in a period of three to four years, an increased bone mass acquisition occurs, which results in 40% accumulation of total lifetime bone mass (2). Data from calcium balance studies suggest that for

most of 9-18 years old healthy children, the maximum net calcium balance is achieved with an intake of approximately 1300 mg per day (3). Data on Iranian households' food intake show that calcium is one of the most limiting nutrients in the Iranian diet, mainly due to inadequate intake of milk and dairy products (4). Yet very little information is available on calcium intake in Iranian youth.

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Iran's National Nutrition and Food Technology Research Institute's (NNFTRI) Calcium and Vitamin D Project was initiated to measure the prevalence of vitamin D deficiency in school-age children, and to assess the effectiveness of school milk fortification program in a trial comparing the effects of calcium + vitamin D-fortified milk, calcium + vitamin D-fortified fruit juice, and fruit juice plus calcium and vitamin D supplements (5). Assessing dietary calcium intake through a quick, practical and efficient method was, therefore, essential for identifying children with inadequate calcium intake, and evaluating the efficacy of the interventions aiming to improve calcium intake. No single method for assessment of an individual's usual intake is optimal under all conditions. The choice depends on the objectives of the study, the nutrient of the primary interest, individual versus group data, the need for relative or absolute estimates, and characteristics of the population (6). Twenty-four-hour dietary recalls (24-h recall) can be used with acceptable internal and external validity with children as informants, if they are 10 years of age or older (7). However, estimating individual usual intake needs completing multiple 24h recalls, which is very time-consuming (7). Among the other dietary assessment tools, food frequency questionnaire (FFQ) is shown to be a quick, practical and efficient approach to assess habitual diet over periods of time with lower investigator and respondent burden (6). Also the data from reproducibility studies suggest that FFQs provide enough accuracy in the studies of adolescents to permit individual diets to be related to subsequent health outcomes (8).

In response to the need for a calcium questionnaire tailored for food consumption patterns and cultural characteristics of Iranian children and adolescents, as part of the NNFTRI's Calcium and Vitamin D Project (5), this study aimed to develop and validate a FFQ for estimating calcium intake in 9-13 years old students in the city of Tehran, Iran. It was hypothesized that calcium intake assessed by a calcium specific FFQ would be positively associated with calcium intake assessed by the 24-h recall method.

## Materials and Methods

**Development of Food Frequency Questionnaire (FFQ):** The FFQ developed included 56 food items

(Table 1), as the main contributors of calcium intake in Iranian diet based on the latest national food consumption survey results (4).

**Table 1.** Food items included in the Ca FFQ as the main sources of Calcium in children, Tehran

Food item
1. Lavash bread
2. Barbari bread
3. Sangak bread
4. Taftoon bread
5. French bread
6. Other breads
7. Rice, cooked
8. Lentil rice
9. Lentil soup (Adasi)
10. Beans, any type, cooked
11. Split peas
12. Milk
13. Chocolate milk/coffee milk/flavoured milk
14. Yogurt (plain or drained)
15. Cheese (all kinds)
16. Keshk (liquid/dried)
17. Doogh (yogurt drink)
18. Eggs
19. Red meat (stew/broth/muscle/kebab, minced meat)
20. Chicken
21. Fish, any type of fresh, frozen, or canned
22. Hamburger/kebab
23. Tomatoes (cooked or raw)
24. Cucumber
25. Lettuce (salad/leaves)
26. Cabbage (white/red)
27. Cauliflower/broccoli/Brussel sprout
28. Green leafy vegetables, raw as side dish
29. Vegetable kish (kookou)
30. Green leafy vegetables, cooked in dish
31. Spinach (raw/cooked)
32. Oranges
33. Tangerine
34. Apple
35. Dates
36. Dried fruits
37. Akhteh
38. Fruit rolls(lavashak)
39. Tammarines
40. Gharaghoroot
41. Icecream, dairy based
42. Cookies/biscuits
43. Cakes
44. Pastries
45. Danish roll
46. Chocolate/bars
47. Walnuts
48. Almonds
49. Peanuts
50. Pistachios & hazelnuts
51. Raisons and dried peas
52. Soy bean, dried
53. Seeds (sunflower, watermelon, pumpkin)
54. Wheat, dried hemp,
55. Sesame
56. Tahini, Halva Shekari (sesame sweets)

Major food groups that were identified included dairy products, cereals, green leafy and non-leafy vegetables, citrus and other fruits, eggs, and beans, which account for 97% of calcium intake in Iranian diet. Also data on the commonly consumed food items among children in Tehran were analyzed (9). Additionally, other food items, which were not in the above food groups, but nonetheless were rich in calcium (based on food composition data), including sesame seeds, nuts, and fish were included. The food list was then converted into a quantitative FFQ by asking two questions on each food item: the frequency of intake (never or rarely, times per month, per week, or per day, as appropriate), and amount of consumption (commonly consumed portion size) during the previous month. See appendix for the list of foods included on the FFQ.

**Validity and Reproducibility:** The developed questionnaire was first reviewed by a panel consisted of three experts on dietary intake assessment methods in NNFTRI. Then a pilot study on 50 students aged 9-13 years who were not the study subjects was performed to identify the optimal format, and sequencing of the questions on FFQ (*face and content validity*).

*Criterion validity* of the calcium specific FFQ was evaluated through comparing its results with five 24-h recalls as the reference method. Results from numerous validation studies provide support for the use of 24-h recalls, as reference method for assessing the dietary intake of children (6, 7). Since the second administration of FFQ (FFQ2), and not the first one, was based on the calcium intake of the same month through which five 24-h recalls were obtained, for validity evaluation, the results from FFQ2 were compared to the reference method.

*Reproducibility* of the questionnaire was assessed through comparing the results of two administrations of FFQ, one month apart.

**Participants:** A sample size of 86 girls and 86 boys was estimated based on the correlation coefficient ( $r=.4$ ) between two methods or two administrations of a single method and power of 90% (10). Data were collected in the selected primary and middle schools of Tehran city from November 2007 to March 2008. A total of 206 students (103 girls and 103 boys) aged 9-13 years were recruited based on the two-stage

systematic cluster sampling. In the first stage, 10 primary and 10 middle schools were selected through systematic random sampling from all nineteen districts of the Department of Education in Tehran city. In the second phase, from each school, 10 to 11 students from grades four and five in primary schools and grades one and two in middle schools were recruited. All subjects were healthy, did not use any medication or supplements (including calcium), and were not on any special diet.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Research Council and the Ethical Committee of NNFTRI. Written informed consent was obtained from the parents of all children.

**Study Design:** Prior to enrollment, all the participating children and their parents were fully informed about the objectives and methods of the study, and signed a written consent.

During the next visit in the school, the first food frequency questionnaire (FFQ1) was completed through face-to-face interview with children. Then five non-consecutive 24-h recalls were collected within the following 30 days in five random typical days (four weekdays and one weekend) with at least four-day intervals between the recalls. The FFQ was administered for the second time (FFQ2), 30 days after the first administration. The interviewers included two nutritionists who had been trained in a 7-day pilot study designed specifically to train the nutritionists. The same nutritionist interviewed the same subject in different stages of the study. All procedures were approved by the Ethical Committee of NNFTRI.

#### **Data Collection**

**Food Frequency Questionnaire:** FFQs were completed through face-to-face interviews with each child as respondent, thus literacy was not an issue since the children were not reading and completing the FFQ on their own. Although self administered questionnaire would be a faster and easier mode of data collection, due to low accuracy of intake quantity reported by this age group, direct interview was used as the method of choice. The FFQ measured relative intake of calcium during the month prior to the interview. Visual aids, including a food album (11),

and common household measures were used to approximate the serving sizes of various foods. Calcium content of food items was determined based on the revised edition of the Iranian Food Composition Table (12). Estimates of calcium intake per day were obtained using Microsoft Office Excel 2007, by multiplying frequency per day by the calcium content per gram and gram weight of eaten food, and then summing over all intakes.

**24-Hour Recalls:** The five 24-h recalls were administered in person through face-to-face interviews over a month period (four week days and one week-end). The multi-pass interview technique was applied in collecting 24-h recalls to reduce under-reporting in dietary intake (13). The multi-pass approach involves asking the respondents about whatever they ate or drank the previous day in several passes rather than all at once. The child served as the primary respondent, and mothers were called upon only when a child was unable to provide needed food detail, such as food-preparation information. Briefly, in the first pass, a quick list was obtained to provide an outline of what was eaten in a chronological order. Then the quick list was reviewed to identify the forgotten foods. In the third pass, some details were asked, including the name of meal, as well as the location and amount of each item consumed. To improve the accuracy of food descriptions, common household measures and a food album (11) were used during the interviews. At the end of each interview, recorded information was reviewed to confirm the entries, and add possible forgotten items. For analyzing food intake data, portion size of the eaten foods was translated to gram weight of ingredients of the food. Then every food item was coded based on the revised edition of Iranian Food Composition Table. To determine nutritional value, the obtained data were analyzed by Microsoft Office Access 2007, in which the database of the revised edition of Iranian Food Composition Table in NNFTRI had been added. As mentioned before, the same database was used to derive the calcium content of the food items included in the FFQ.

**Statistical Analysis:** All analyses were restricted to the participants who had completed both administrations of the FFQ and at least three 24-h recalls. The participants, whose reported average

energy intake, based on the mean of 24-h recalls, was above or below “mean energy intake  $\pm$  3SD”, were excluded from the analysis.

Tests for normality were performed using the Kolmogorov-Smirnov’s test. In the validity study, the difference between mean calcium intakes was tested using the paired t test, and the association between the two methods for calcium intake was described using Pearson’s correlation coefficient and corrected correlation coefficient for within-individual variation (6). Agreement between the 24-h recalls and FFQ at individual level was assessed using mean difference and standard deviation of the difference, which was visually shown in a Bland and Altman plot (14). Individual results for calcium intake estimates by the 24-h recalls and FFQ were classified into quartiles to assess the questionnaire’s ability to assign individuals to the same quartiles as the 24-h recalls. To assess the discriminatory power of the FFQ, the subjects’ distributions within the quartiles of dietary recalls with those from FFQ were compared.

*Sensitivity* was defined as the proportion of those with a daily calcium intake (on the basis of 24-h recalls) less than the Dietary Reference Intake (DRI) of 1300mg/d (3), who were identified correctly by the FFQ.

*Specificity* was calculated as the proportion of those with a daily calcium intake (based on the 24-h recalls) more than 1300mg/d, who were identified correctly by the FFQ.

*Predictive value* was the proportion of those with a calcium intake less than 1300mg/d based on the FFQ whose actual intake (on the basis of 24-h recalls) was less than 1300mg/d, too (15).

*Reproducibility* of the FFQ in estimating daily calcium intake was evaluated by paired t test and Pearson’s correlation coefficient, mean difference and standard deviation, and cross-classification analysis for calcium intakes between the first and second administrations of FFQ.

All analyses were carried out using the SPSS software (version 14, 2005, SPSS Ins, Chicago, IL).

## Results

Out of the 206 subjects participating in this study, 184 completed both FFQs and at least three 24-h recalls (89%, 10% and 1% of the subjects provided five, four and three 24-h recalls, respectively). A total

of 897 24-h recalls and 368 FFQs (two administrations of FFQ for each subject) were analyzed. The mean age for total sample was 11±1.14 years. There were no significant difference in calcium intake between the subjects from primary schools (grades four and five) with mean age of 10 years and middle-schools (grades one and two) with a mean age of 12 years in the present study. Since the recommendation for calcium intake in the age group of 9-13 years is identical (3), the results for both groups were pooled. Demographic characteristics of the participants are shown in Table 2.

**Validity Study:** Calcium intake derived from both methods was normally distributed. Mean calcium intake calculated from the FFQ2 and 24-h recalls, and correlation coefficients between the results of the two methods for both sexes are shown in Table 3. There

were no significant differences between the mean calcium intake derived from the FFQ2 and 24-h recalls in both sex. Calcium intakes estimated from FFQ2 and 24-h recalls were positively and significantly correlated and the strength of this association was greater for males compared with females. The correlation coefficients increased when they were corrected for intra-variability in both sex.

The mean difference in calcium intake between the two methods (24-h recalls minus FFQ2) was 47±459 mg/d (95% CI: 13, 80), demonstrating that the FFQ under-estimated the calcium intakes compared with the 24-h recalls. The FFQ assessed calcium intakes from 871mg/d above to 964mg/d below (mean± 2 standard deviation) the 24-h recall method. This is graphically shown in a Bland & Altman plot in Figure 1.

**Table 2.** Demographic characteristics and weight status of the study sample in the validation study of calcium food frequency questionnaire in 9-13 years old children (n=186)

	Girls		Boys		Total	
	n	%	n	%	n	%
Grade						
Primary school (grades 4-5)	47	52.2	47	50	94	51.1
Middle school (grades 6-7)	43	47.8	47	50	90	48.9
Total	90	100	94	100	184	100
Weight status (based on BMI)						
Not overweight	70	77.8	58	61.7	128	69.9
At risk for overweight	16	17.8	14	14.9	30	16.3
Overweight	4	4.4	22	23.4	26	14.1
Total	90	100	94	100	184	100

BMI= Body Mass Index, calculated as kg/m<sup>2</sup>. Categories were defined as follows: Not overweight: <85th percentile; at risk for overweight (equivalent to obese in adults): 85th -94th percentile; and overweight (equivalent to overweight in adults): ≥ 95th percentile, according to age and sex based on the 2000 Centers of Disease Control and Prevention Growth Charts.

**Table 3:** Mean calcium intake (mg/d) based on the second administration of food frequency questionnaire (FFQ2) compared with the mean of five 24-hour dietary recalls and correlation coefficient between them in the validation study of calcium food frequency questionnaire in 9-13 years old children

Variables		FFQ2	Mean of recalls		Correlation coefficient		
		Mean±SD	Mean±SD	P Value	r	P Value	Corrected r
Total	(n=184)	876.0±491.8	922.8±322.3	NS <sup>a</sup>	0.42	0.0001	0.57
Girl	(n=90)	860.3±430.8	947.3±336.6	NS	0.35	0.001	0.47
Boy	(n=94)	891.1±545.8	899.4±308.0	NS	0.50	0.0001	0.67

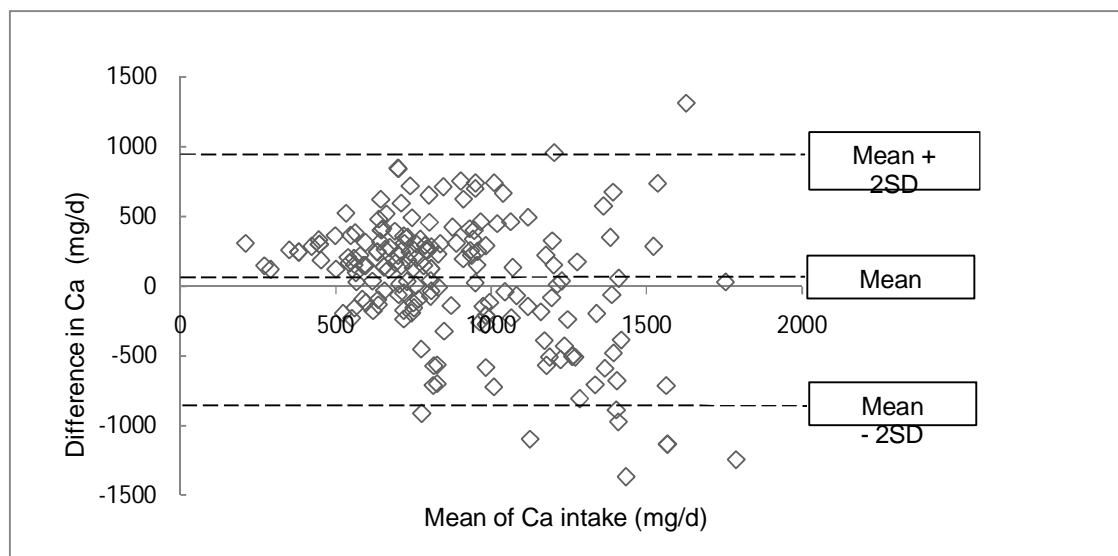
SD=Standard Deviation

NS=Not Significant

r=Pearson's correlation coefficient

Corrected r=Correlation coefficient corrected for intra-variability

<sup>a</sup> Paired t test



**Figure 1.** Bland-Altman Plot of agreement between the FFQ and the 24-hour recall for calcium intake estimates. “Difference in calcium” (y-axis) is the difference in calcium intake by the FFQ minus that by the 24-hour recall, whereas “mean Ca intake” (x-axis) is the mean of calcium intake by the two methods. The mean±2SD (broken lines) of the difference is shown.

Cross-classification analysis for calcium intake derived from the mean of 24-h recalls and FFQ indicated that 11 subjects (6%) were grossly misclassified (lowest quartile for one method and highest quartile for the other). While 66 subjects (36%) were classified correctly, and 81 subjects (44%) were classified in the adjacent quartiles. Thus 80% of the participants were classified in the same or adjacent category.

Assessing the discriminatory power of FFQ (Table 4) showed a progressive increase in calcium intake between the first and fourth quartiles; however, it did not differ significantly between all categories. Overall, the questionnaire could distinguish between the lower quartiles (first and second quartiles) and the

extreme higher quartile (fourth quartile), and also between the higher quartiles (third and fourth quartiles) and the extreme lower quartile (first quartile) significantly ( $P < 0.05$ ), but it could not differentiate between the adjacent categories (first and second; second and third; and third and fourth quartiles).

When the cut-off level for calcium intake was set at RDA of 1300 mg/d, the prevalence of calcium intake less than this level measured by the 24-h recall method was 90.2%. The specificity, sensitivity and predictive value of the FFQ were 85%, 33% and 92%, respectively. The specificity of the FFQ was estimated 6%, and its sensitivity error was 13%.

**Table 4:** Cross-classification analysis for calcium intake (mg/d) based on the second administration of food frequency questionnaire (FFQ2) compared with the mean of five 24-hour dietary recalls (mean of recalls) in the validation study of calcium food frequency questionnaire in 9-13 years old children

Quartiles of daily Ca intake based on FFQ2 <sup>a</sup>	Quartiles of daily Ca intake based on the mean of recalls <sup>a</sup>				Total	Agreement of quartiles (%)
	Q1 <689.6	Q2 689.6-884.7	Q3 >884.7-1085	Q4 >1085		
Q1 (<546)	21	13	6	6	46	45
Q2 (546-733)	12	15	11	8	46	32
Q3 (>733-1118)	8	14	11	13	46	23
Q4 (>1118)	5	4	18	19	46	41
Total	46	46	46	46	184	

<sup>a</sup>Number of subjects in each category

**Reproducibility Study:** Calcium intakes derived from the two administrations of FFQ was normally distributed. Table 5 represents mean calcium intake estimates from two administrations of FFQ and correlation coefficient between them. Mean calcium intake estimates resulting from FFQ1 were significantly higher ( $P < 0.001$ ) than those from FFQ2. Similar discrepancies were observed across both sexes. The correlation was similar for males and females. The mean difference in calcium intake between the two administrations of FFQ (FFQ1 minus FFQ2) was  $221 \pm 434$  mg/d (95% CI: 189, 253), and

the lower and higher limits (mean  $\pm$  2 standard deviation) for mean difference in calcium intake obtained from the two administrations of FFQ were -647 to 1090, respectively. This is graphically shown in a Bland & Altman plot in Figure 2.

Cross-classification analysis for calcium intake derived from the two administrations of FFQ indicated that 6 subjects (3%) were grossly misclassified, while 81 (44%) were classified correctly, and 149 (81%) were classified correctly or in the adjacent category.

**Table 5:** Mean calcium intakes (mg/d) based on the two administrations of food frequency questionnaire and correlation coefficient between them in the validation study of calcium food frequency questionnaire in 9-13 years old children

Variables		FFQ1 <sup>a</sup>	FFQ2 <sup>b</sup>	P Value <sup>c</sup>	Correlation coefficient	
		Mean $\pm$ SD	Mean $\pm$ SD		r <sup>d</sup>	P Value
Total	(n=184)	1101.8 $\pm$ 542.6	876.0 $\pm$ 491.8	<0.01	0.65	0.0001**
Girl	(n=90)	1086.1 $\pm$ 457.5	860.3 $\pm$ 430.8	<0.01	0.64	0.0001**
Boy	(n=94)	1116.8 $\pm$ 615.3	891.1 $\pm$ 545.8	<0.001	0.65	0.0001**

SD= Standard Deviation

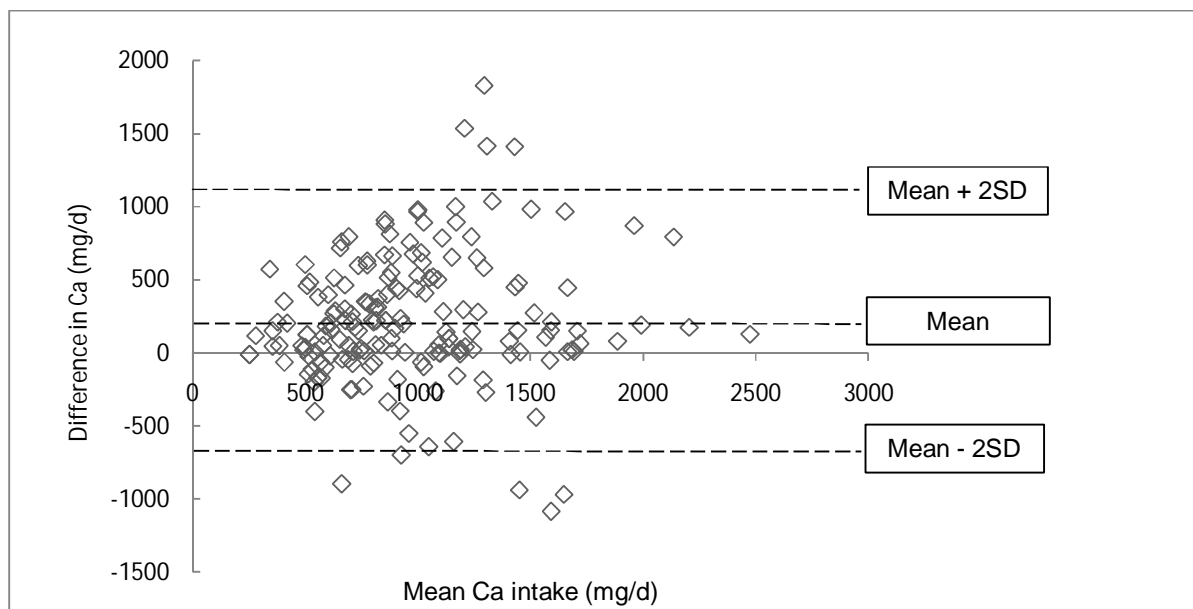
<sup>a</sup>FFQ1= First administration of FFQ

<sup>b</sup>FFQ2= Second administration of FFQ

<sup>c</sup>Paired t test

<sup>d</sup>r=Pearson's correlation coefficient

\* P<0.01 \*\* P<0.001



**Figure 2.** Bland-Altman Plot of mean difference in calcium intake between FFQ1 and FFQ2 for calcium intake estimates. "Difference in calcium" (y-axis) is the difference in calcium intake in two administrations of FFQ1 minus that by FFQ2, whereas "mean Ca intake" (x-axis) is the mean of calcium intake by twice administration. The mean $\pm$ 2SD (broken lines) of the difference is shown.

## Discussion

Results of the present study suggest that the calcium FFQ has acceptable validity and adequate reliability for population surveillance of calcium intake among 9-13 years old children. Calcium intake estimates in this study (923 mg/d in 9-13 years old children) are comparable with those of other studies on the similar age groups in Iran (9) and in other nations (16, 17).

**Validity Study:** Mean calcium intake estimates from the FFQ2 did not differ significantly from those assessed by the 24-h recall method. Similarly, Jensen and colleagues reported no significant difference between the mean calcium intake from the second administration of FFQ and the mean of two 24-hour dietary recalls in a subgroup of 10-13-year-olds (17). However, it is worth noting that the FFQ in Jensen's study was self-administered, which can have effect on the results. In another study on children with a mean age of 8 years, Marcotte and colleagues found no significant difference between the mean calcium intakes estimated from the calcium checklist and the parentally reported one-day 24-h recall (18). This is also common in the majority of previous studies on other age groups comparing the mean calcium intake derived from the FFQ and the reference method (19-24). Although, in a number of recent studies, the calcium FFQ was found to underestimate (16, 25, 26) or overestimate calcium intake compared with the reference method (27-29).

However, as demonstrated in the Bland-Altman plot (Figure 1), the FFQ developed in the present study tended to underestimate group calcium intake compared to the 24-h recall method. Large standard deviations of the difference between the intakes assessed by the two methods indicated limitation of the FFQ to estimate calcium intake for individuals. Looking-over the graph shows a divergence, which means there is higher differences in higher mean calcium intakes, and greater difficulty in estimating usual dietary calcium intake in higher intakes of calcium. This is in line with the findings of other studies that the degree of agreement between the two methods may depend on the actual levels of calcium intake, with differences widening at higher intakes (25-26, 30-31).

Corrected correlation coefficient between the two methods in this study was 0.57. In validation studies of calcium FFQs with the reference 24-h recall method, the correlation coefficients of 0.31 to 0.59 have been reported (16, 17, 29). Better validity of FFQ in boys versus girls in the present study compares well with the results obtained by other researchers (16, 25, 30-32). However, it is in contrast with the findings of Harnack and colleagues (16).

The findings of cross-classification analysis were in the range, which were reported previously in similar studies, and were higher than expected (62.5%) for correctly classified within one quartile due to chance alone (25-27).

Evaluating the discriminatory power of FFQ showed that the FFQ could reliably distinguish the extremes of calcium intakes. There is a variety of findings in previous studies evaluating the discriminatory power of FFQ in different age and sex groups (25, 26). The present study showed that the short Calcium FFQ would correctly identify 85% of children with actual daily intakes less than the DRI of 1300 mg. Therefore, for intervention purposes, 13% of all children would lose the possibility of receiving a required intervention due to specificity error, while 6% of the children would be provided with an unneeded intervention and will be classified as having inadequate intake despite of adequacy (sensitivity error). Results from similar studies evaluating the specificity and sensitivity of calcium FFQ showed lower specificity and predictive value, and higher sensitivity value in comparison with the present study (25, 27).

**Reproducibility Study:** At the second administration of the FFQ, a lower mean calcium intake was found in comparison with the first one, which was analogous to the studies by Harnack and colleagues (16) on middle-school-aged (11 to 14 years of age) children, and Jensen and colleagues (17) on the similar age group as our study (10 to 13 years old subgroup).

Lower calcium intake estimates resulting from the second, compared with the first administration of the questionnaire, may be due to measurement fatigue or lack of motivation in completing the second administration and putting less effort by choosing



“never or rarely consumed” to reduce the time required for completing FFQ. On the other hand, there is a possibility of more careful and accurate answering to the FFQ2 because of previous acquaintance with the questionnaire and increase in sensitivity about what they ate after the first administration of FFQ1 (6) and five 24-h recalls that were collected between the two FFQs. Presumably, the accuracy of portion size estimates could have been improved at the final stage of the study.

The other reason could be the FFQ format in which the reference period of time was one month. Since two administrations of FFQ for measuring the reproducibility of FFQ occurred at an interval of one month, they did not measure calcium intake in a concurrent period of time, so a real difference in consumption of food items and consequently calcium intake might exist (6).

In reproducibility studies, the coefficients of correlation have generally ranged from 0.5 to 0.7 (6). In the present study, the coefficient of correlation between the first and second administrations was 0.65. The correlation coefficient obtained by Jensen et al. (17) in 10-13 years old children with two administrations of FFQ during Week 1 and Week 4 was 0.67, and the correlation coefficient in the study by Harnack et al. (16) in 11-14 years old children with one week interval between the two administrations was 0.74. Overall, correlation coefficient does not measure agreement between the two administrations; rather it only measures the degree to which the two administrations are related. The strength of correlation is also dependent on the range of values in population, which itself can be partly influenced by the sample size (34).

Bland-Altman method indicated that in the second administration, the FFQ underestimated group calcium intake in comparison with the first administration, and estimated calcium intakes from 647 mg/d above to 1090 mg/d (Mean $\pm$ 2 SD) below the first FFQ. The divergence in the plot shows a higher difference for higher mean individual intake values.

The present study has a number of limitations that should be considered when interpreting the findings. For this validation study, due to the lack of an absolute reference method, we rather had to compare

the results of the new FFQ with those of another method (the 24-h recall) that is judged to be superior. Ideally, the reference method should have the least correlated errors with the instrument under evaluation. Major sources of errors associated with FFQ are due to the restrictions imposed by a fixed list of foods, memory, perception of portion sizes, and interpretation of questions (6). Errors related to the 24-h recall tend to be more correlated with the errors in the dietary questionnaire; for example, memory issues with the 24-h recall. However, they are less demanding for the participants than diet recording, and less likely to influence the actual diet of the subjects; so in many situations such as when the subjects are illiterate or less than highly motivated, multiple 24-h recalls may be the only reasonable option (6, 34). In addition, results from numerous validation studies provide support for the use of 24-h recalls for assessing the dietary intake of children (35-38).

In comparison to other validation studies in children or adolescents (16-18, 32), the present study has the advantage of having completed sufficient number of 24-h recalls (with a mean of 4.8 recalls per subject) and representing an average intake in the time interval corresponding to the questionnaire, given the study design. In addition, implementing the multi-pass interview technique in collecting 24-h recalls reduced probable under-reporting in dietary intake (13).

This is the first validation study on a calcium specific FFQ for children in Iran. In this study, the FFQ underestimated group mean calcium intake as compared with the 24-h recalls (reference method). Given its fairly good ability to classify subjects into extremes of calcium intake and to indicate children having calcium intake lower than the RDA, the FFQ presented in this study seems reasonable for gross categorization of subjects into groups with intakes above or below a specified threshold but not to associate specific levels of intake with specific health outcomes. Therefore, it can serve as a good screening tool to identify those with inadequate intake. Other methods such as multiple 24-h recalls or weighted dietary records may be better in estimating the calcium intakes of individuals.

## Acknowledgement

The authors would like to thank all the subjects who participated in this study. We are grateful to the staff of the Nutrition Research Department in NNFTRI. None of the authors had any personal or financial conflicts of interest. This study was part of a large study of "Vitamin D Study in School Children of Tehran (VDST)", and it was supported by "National Nutrition and Food Technology Research Institute" of Shahid Beheshti University of Medical Sciences.

## Financial disclosure

None of the authors had any personal or financial conflicts of interest.

## Funding/Support

This work was supported by "National Nutrition and Food Technology Research Institute" of Shahid Beheshti University of Medical Sciences (Tehran/Iran).

**Ethics of human subject participation:** This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Research Council and the Ethical Committee of the National Nutrition and Food Technology Research Institute. Written informed consent was obtained from all subjects/patients.

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