



Published in final edited form as:

*J Am Diet Assoc.* 2009 March ; 109(3): 479–485.e3. doi:10.1016/j.jada.2008.11.025.

## Validation of a Food Frequency Questionnaire for Calcium and Vitamin D Intake in Adolescent Girls with Anorexia Nervosa

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### Abstract

**Background**—Assessing calcium and vitamin D intake becomes important in conditions associated with low bone density such as anorexia nervosa (AN). Food records (FR) that assess intake over a representative time period are used in research and sometimes clinical settings. However, compliance in adolescents can be suboptimal.

**Objectives**—This study was undertaken to determine the validity of a food frequency questionnaire (FFQ) for assessing calcium and vitamin D intake in adolescent AN and healthy girls compared to a validated FR assessing intake over a four-day period, the hypothesis being that intake would be adequately predicted by the FFQ.

**Design**—Thirty-six girls with AN and 39 healthy girls 12–18 years old completed both the FR and the FFQ. An additional 31 subjects (20 AN, 11 controls) completed the FFQ, but not the FR, and one AN girl completed the FR, but not the FFQ.

**Results**—Subjects demonstrated greater compliance with the FFQ (99%) than the FR (71%). Daily calcium and vitamin D intake calculated using the FR and FFQ did not differ, although the FFQ tended to under-report vitamin D intake corrected for energy intake. Using quartile analysis, no gross misclassification was noted of calcium or vitamin D intake calculated using the FR or FFQ in AN. Strong correlations were observed of daily vitamin D intake derived from the FFQ versus the FR, particularly in AN ( $r=0.78$ ,  $p<0.0001$ ). Less robust correlations were observed for calcium intake ( $r=0.65$ ,  $p<0.0001$ ).

**Conclusion**—The FFQ used in this study can be effectively used to assess daily calcium and vitamin D intake in adolescent girls suffering from AN

### Keywords

adolescents; anorexia nervosa; calcium; vitamin D; questionnaires; food record; bone density

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The authors have no conflict of interest to report

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## INTRODUCTION

Anorexia nervosa (AN) is the third most common chronic illness amongst teenage girls (1). Low bone mineral density (BMD) is a significant co-morbidity, and decreased bone accrual during adolescence may cause suboptimal peak bone mass and increased fracture risk (2–5). In addition to maintaining normal weight and optimal estradiol, growth hormone and insulin like growth factor -1 levels, adequate calcium and vitamin D intake is key to optimizing BMD (6,7). It is therefore important to assess calcium and vitamin D intake in adolescents with AN and to identify individuals with deficient intake such that this can be optimized.

Standard methods to assess intake include food records (FR) and food frequency questionnaires (FFQ). FRs assess intake over a representative time period, for example three weekdays and one weekend day (8). Records are completed by subjects at home and subsequently analyzed. Conversely, FFQs are administered by dietitians real-time, and used to assess recalled intake over a predetermined period. Whereas FRs are less prone to recall bias, they assess dietary intake over a short duration. In contrast, FFQs assess the subject's recalled intake over a longer period, and may reveal nutritional habits not evident from a FR. In adolescents, there may be decreased compliance with the FR compared to a FFQ because the former requires taking responsibility to remember to complete these records over several days at home. In fact, in the concurrent study, fewer subjects completed the FR than the FFQ. Given the relatively poor compliance with FR completion, validating the use of a FFQ for calcium and vitamin D intake in adolescents with AN would provide a simpler method of tracking intake of these micronutrients.

The validity of FFQs has been tested within groups based on socioeconomic class, geographical location, and other factors (9–16), and also in adolescents (17). However, studies have not specifically validated the use of FFQs in determining dietary calcium and vitamin D intake in adolescent AN. This study was undertaken to determine the validity of a FFQ for assessing calcium and vitamin D intake in adolescent AN girls and healthy girls compared to a validated FR, with the hypothesis that calcium and vitamin D intake would be adequately predicted by the FFQ.

## SUBJECTS AND METHODS

### Subjects and Subject Selection

Calcium and vitamin D intake was assessed using a four-day FR and a FFQ in 107 girls 12–18 years. Of these, one AN girl did not complete the FFQ, but did complete the FR, whereas 31 girls (20 AN, 11 controls) did not complete the FR, but did complete the FFQ. Thirty-six AN and 39 controls completed both the FR and the FFQ, and data on these subjects are reported. Subjects not completing the FFQ or FR did not differ from the group that completed both for baseline characteristics such as age ( $15.9 \pm 0.3$  vs.  $16.0 \pm 0.2$  years) and BMI ( $19.3 \pm 0.6$  vs.  $19.5 \pm 0.3$  kg/m<sup>2</sup>). All AN subjects met the DSM-IV criteria for the disorder, and were enrolled in outpatient treatment programs. Healthy controls were >90% of ideal body weight based on the 50<sup>th</sup> percentile of BMI for age, and had no previous or present history of eating disorders. Mean chronological age was  $16.6 \pm 0.3$  in AN and  $15.4 \pm 0.2$  years in controls. All subjects spoke English as their first language. AN girls included 31 White, one Black, two Asian and two mixed race girls, and controls included 31 White, one Black, no Asian and seven mixed race girls (p not significant). The study was approved by the Partners HealthCare Institutional Review Board, and subjects and parents signed informed consent and assent.

## Experimental Protocol

Subjects were weighed wearing hospital gowns on an electronic scale. Heights were measured with a wall-mounted stadiometer and average of three measurements taken. BMI was calculated as the ratio of weight (kg) to height (meters)<sup>2</sup>, and standard deviation scores (SDS) calculated from CDC data (18).

The FFQ used was a modified version of previously reported questionnaires (19) (20), adapted for our subjects by our General Clinical Research Center (GCRC) Bionutrition department (Appendix). The modernized version is designed to take into account changes in dietary habits and includes items such as calcium-fortified orange juice. The FFQ was administered to subjects by a research dietitian and average daily intake of calcium and vitamin D calculated (Ca-FFQ and Vit D-FFQ). The frequency (per month, week or day) with which subjects consumed each item on a list 40 food items was determined, and subjects asked about supplement intake.

The FR used in this study was a four-day record that included three weekdays and one weekend day, that has been validated for use in young women (21). Records were given to subjects at the screening visit with detailed verbal and written instructions provided by registered GCRC dietitians. Subjects were instructed to return completed records at the subsequent visit, when the FR was reviewed with subjects for completeness. Subjects were asked to describe portion sizes and preparation methods for food items included in the FR. Nutrient intake was calculated using the Minnesota Nutrition Data System software (version 4.03; nutrient database 31) by GCRC dietitians.

In this analysis, only calcium and vitamin D intake from food, but not supplements, is reported. Several girls reported use of supplements on the FFQ but not the FR. It would warrant further study to determine whether subjects did not take supplements over the four-day period when they completed the FR, or forgot to include supplement use. For the purpose of this study, it is sufficient to determine whether the FFQ accurately predicts calcium and vitamin D intake from food.

## Statistical Analysis

Data are described as means  $\pm$  SEMs. Student t-tests were used to compare baseline characteristics. However, because data regarding calcium and vitamin D intake were not normally distributed, comparisons between groups were performed using the Wilcoxon rank sum test, a non-parametric test. Analyses were performed for calcium and vitamin D intake reported from the FR (Ca-FR and Vit D-FR) and FFQ (Ca-FFQ and Vit D-FFQ), and additionally after adjusting nutrient intake per 1,000 kcal. Calcium and vitamin D intake determined using the FR and FFQ was then divided into quartiles, and quartiles cross-classified to assess degree of misclassification. The Fisher's exact test was used to compare proportions. Spearman's Rank Correlation was used to compare intake from the two assessment methods as data were not normally distributed. A stepwise regression model was developed to describe the predictive ability of the FR by the FFQ. Other predictors included in this model were age and BMI. Analyses were performed using JMP software (version 5; SAS).

## RESULTS

### Clinical Characteristics

As expected, AN had lower BMI ( $17.4 \pm 0.2$  vs.  $21.4 \pm 0.5$  kg/m<sup>2</sup>;  $p < 0.0001$ ), and BMI Z-scores ( $-1.1 \pm 0.1$  vs.  $0.04 \pm 0.1$ ,  $p < 0.0001$ ) than controls. AN and controls did not differ for caloric intake based on their FR ( $2056 \pm 143$  vs.  $1879 \pm 89$ ,  $p$  not significant). Both the FFQ and the FR showed that average calcium and vitamin D intake was higher among AN than controls,

although this difference did not persist for Ca-FFQ after adjusting for caloric intake (adjusted Ca-FFQ) (Table 1).

Based on the FFQ, 31% controls and 61% AN met the DRI for calcium intake, and 5% controls and 17% AN met the DRI for vitamin D. Using the FR, 15% controls and 61% AN met the DRI for calcium intake, and 8% controls and 39% AN met the DRI for vitamin D.

### **Comparison of Calcium and Vitamin D Intake as Assessed by the FR and FFQ (Table 1)**

Ca-FR and Vit D-FR did not differ from Ca-FFQ and Vit D-FFQ for the whole group or subgroups considered separately. However, when nutrients were adjusted for energy intake (per 1,000 kcal), adjusted Ca-FFQ was greater than adjusted Ca-FR ( $p=0.04$ ) for controls, and adjusted Vit D-FFQ lower than adjusted Vit D-FR for AN ( $p=0.04$ ).

### **Quartile Classification (Supplemental Table 1 (online) and Table 2)**

To assess the extent of misclassification, quartiles of calcium and vitamin D intake using the FFQ and FR were compared. Table 2 indicates the number and proportion of calculated values for Ca-FR vs. Ca-FFQ and for Vit D-FR vs. Vit D-FFQ that fell in the same quartile, within one quartile, or were grossly misclassified ( $>$  two quartiles apart). For calcium intake, we observed 0% gross misclassification. Only one subject was grossly misclassified for vitamin D intake. After adjusting for caloric intake, more misclassification was noted, with 7% and 4% of adjusted calcium and vitamin D intake, respectively, being grossly misclassified. Bland Altman analysis comparing the FR and FFQ is shown in Supplemental Figure 1 (online).

AN did not differ from controls for quartile classification for calcium intake and adjusted calcium and vitamin D intake. However, a larger proportion of AN than controls ( $p=0.01$ ) fell within the same quartile for calcium intake assessed using the FR and FFQ, and a larger proportion tended to fall within one quartile ( $p=0.07$ ).

### **Correlation Analyses**

For the group as a whole, Ca-FR correlated positively with Ca-FFQ ( $r=0.60$ ,  $p<0.0001$ ); as did Vit D-FR with Vit D-FFQ ( $r=0.75$ ,  $p<0.001$ ). Among AN, correlations were stronger for calcium ( $r=0.65$ ,  $p<0.0001$ ) and vitamin D ( $r=0.78$ ,  $p<0.0001$ ) (Supplemental Figure 2: online). Among controls, correlations were weaker for both calcium ( $r=0.45$ ,  $p=0.004$ ) and vitamin D ( $r=0.47$ ,  $p=0.003$ ). Overall, correlations were weaker for adjusted Ca-FR and adjusted Ca-FFQ ( $r=0.32$ ,  $p=0.006$ ), but stronger for adjusted Vit D-FR and adjusted Vit D-FFQ ( $r=0.62$ ,  $p<0.0001$ ).

### **Regression Modeling**

To determine whether older girls and those with lower BMI were more likely to accurately report intake, regression modeling was performed (Supplemental Table 2 (online)). Ca-FR or Vit D-FR was designated the dependent variable, and Ca-FFQ or Vit D-FFQ, age and BMI designated independent variables. For the entire group, BMI independently predicted Ca-FR and Vit D-FR such that girls with lower BMI had higher calcium and vitamin D intake, and the FFQ and BMI together contributed to 44% and 56% of the variability of Ca-FR and Vit D-FR respectively.

In controls, age and BMI independently predicted Ca-FR, such that older girls with lower BMI were more likely to report higher Ca-FR. Ca-FFQ, age and BMI together contributed to 29% of the variability in Ca-FR. In AN, age independently predicted Ca-FR, and younger girls were more likely to have higher calcium intake. Ca-FFQ and age contributed to 46% of Ca-FR variability. Age and BMI were not independent predictors of Vit D-FR within controls or AN.

## DISCUSSION

These data indicate better compliance with the FFQ than the FR in adolescent AN. Daily calcium intake calculated using the FR and FFQ does not differ, although the FFQ tends to under-report energy intake adjusted vitamin D intake in AN, and over-report adjusted calcium intake in controls. Importantly, quartile analysis indicates no gross misclassification of calcium or vitamin D intake calculated using the FFQ in adolescent AN. Vit D- FFQ correlates strongly with Vit D-FR, particularly in AN. Less robust correlations were observed for calcium intake.

As anticipated, compliance was better for the FFQ than the FR, and FFQs were completed by all but one AN subject. The FR is more time consuming and requires girls to remember to complete this over several days. Of 107 subjects, 31 did not complete the FR, and two thirds of non-completers were AN girls. One may speculate that this reflects reluctance amongst AN girls to discuss intake; or that filling out a FR over a four-day period is an additional burden for these girls who are in active treatment programs that require multiple visits to multiple care providers, causing poor compliance. These data demonstrate the value of a FFQ in maximizing useful information regarding calcium and vitamin D intake, thereby allowing for timely recommendations.

Interestingly, Vit D-FFQ correlated better with Vit D-FR than did Ca-FFQ with Ca-FR, even though Vit D-FFQ was lower than Vit D-FR, whereas calcium intake derived from the two methods did not differ. This may reflect differences in short-term (FR) versus longer-term (FFQ) assessment of micronutrient intake. It is reassuring that quartile analysis demonstrated no gross misclassification of calcium or vitamin D intake in AN girls, although some misclassification was noted in controls. Overall, greater correlations of micronutrient intake derived from the FR and FFQ in AN compared with controls likely reflect greater attention to intake in AN, and that these girls are in active treatment programs focusing extensively on improving intake. In addition, AN girls have a trained knowledge and recall of food portions, quantity and detail compared to controls, who have less need to focus on food. The variability of food intake in controls compared with AN may also cause difficulties in recall.

Higher reported calcium intake in younger AN girls and overall in girls with lower BMI may indicate greater self or parental awareness of and attention to diet in younger and lower weight girls. Interestingly, the age association was reversed in controls, with older girls demonstrating higher intake, the implication of which is unclear.

A study limitation is that vitamin D levels were not measured as a biomarker of intake. Importantly, given the great seasonal variability of vitamin D levels, this may not be a perfect biomarker of intake unless subjects are assessed in the same season. Calcium levels were measured and did not differ between groups (data not reported). Another limitation is that data that could potentially impact dietary reporting, such as socioeconomic status and parental education, were not collected.

### Conclusions

These data demonstrate that the FFQ used in this study is associated with better compliance than the FR, and is a useful tool to assess calcium and vitamin D intake in adolescent girls with AN. Data derived from this questionnaire agree well with those from the FR, and it is proposed that this FFQ can be effectively used to assess daily calcium and vitamin D intake in an outpatient or research setting in adolescent AN girls.

### Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

We thank the Bionutrition Core of the GCRC of Massachusetts General Hospital for their contribution to this study. Catherine Taylor, Kimberly Kruczek and Brooke Lammarello worked on data collection and entry, data analysis and the writing of the manuscript. Ellen Andersen and Jane Hubbard worked on the food frequency questionnaire and food records, analyzed the output, and helped with manuscript preparation. Madhusmita Misra worked with study design, recruitment, data analysis and manuscript preparation.

This work was supported in part by NIH grants R01 DK 062249, K23 RR018851 and M01-RR-01066

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**Table 1**  
Comparison of calcium and vitamin D intake assessed using the FR or FFQ in all subjects (n=75), anorexia nervosa (AN) subjects (n=36) and healthy controls (n=39)

	Food Frequency Questionnaire	Food Record	P (Wilcoxon rank sum test)
<b>Daily intake</b>			
<b>Calcium (mg/d)</b>			
All subjects	1255±68	1185±72	0.35
AN	1429±101	1485±114	0.58
Controls	1095±84	908±63	0.07
p (AN vs. controls)	0.01	0.0001	
<b>Vitamin D (IU/d)</b>			
All subjects	197±17	252±21	0.07
AN	246±26	327±33	0.08
Controls	151±19	182±19	0.19
p (AN vs. controls)	0.007	0.001	
<b>Intake per 1000 calories</b>			
<b>Calcium (mg/d)</b>			
All subjects	718±64	601±26	0.14
AN	819±119	725±38	0.84
Controls	623±52	501±24	0.04
p (AN vs. controls)	0.09	<0.0001	
<b>Vitamin D (IU/d)</b>			
All subjects	101±8	124±9	0.047
AN	117±13	153±14	0.04
Controls	86±10	100±10	0.21
p (AN vs. controls)	0.06	0.001	



**Table 2**  
Cross-Classification of Quartiles for All Subjects (n=75), Subjects with Anorexia Nervosa (AN) (n=36) and healthy controls (n=39)

Nutrient	n (%) Same Quartile	n (%) Same or within one quartile	n (%) Grossly misclassified
<b>Daily Calcium Intake (mg/d)</b>			
All subjects	37 (49)	65 (87)	0 (0)
AN	19 (53)	29 (81)	0 (0)
Controls	14 (36)	32 (82)	2 (5)
<b>Daily Vitamin D Intake (IU/d)</b>			
All subjects	45 (60)	70 (93)	1 (1)
AN	23 (64)*	35 (97) <sup>+</sup>	0 (0)
Controls	14 (36)	33 (85)	3 (8)
<b>Calcium Intake per 1,000 kcal (mg/d)</b>			
All subjects	20 (27)	56 (75)	5 (7)
AN	14 (39)	23 (64)	1 (3)
Controls	16 (41)	31 (79)	3 (8)
<b>Vitamin D Intake per 1,000 kcal (IU/d)</b>			
All subjects	36 (48)	67 (89)	3 (4)
AN	17 (47)	31 (86)	0 (0)
Controls	16 (41)	31 (79)	3 (8)

AN versus controls:

\* p=0.02;

<sup>+</sup> p=0.07

## Appendix

## Calcium and Vitamin D Frequency Questionnaire

Vit. D (IU)	Ca+ (mg)	Food	Serving	sv/day	sv/wk
		cold cereal	1 cup		
92.4	300	Milk (whl, LF, skim, choc, soy)	8 oz		
46.4	150	Milk over cereal	4 oz		
11.6	35	Milk, cream in coffee	1 oz		
2	275	Buttermilk, whole	8 oz		
0.8	325	Yogurt (fruited or flavored)	8 oz		
0.8	435	Yogurt (plain)	8 oz		
6.4	95	Ice cream or ice milk	1/2c		
6.4	320	Frozen yogurt	8 oz		
7.2	65	Ice cream bar, fudgsicle	1 each		
2.4	135	American or mozzarella	1 oz		
3.2	25	Cream cheese	1 oz		
3.6	200	Hard cheese (cheddar, swiss, prov)	1 oz		
25	175	Macaroni and cheese, lasagna	1 cup		
30.4	400	Ravioli, quiche	1 cup		
3.6	280	Cheese pizza	1/8 large		
0.4	135	Cottage cheese	1 cup		
0	150	cheese food or spread	1 oz		
44.4	125	Pudding or custard made with milk	1/2 cup		
40	180	Cream soup, chowders, cream sauces	1 cup		
0	45	Broccoli	1/2 cup		
0	85	Greens: mustard, turnip, collard, beet, spin.	1/2 cup		
0	290	Calcium Fortified Juice (orange, others)	8 oz		
0	20	Bread (white/wheat/pita)	1 slice		
11.6	75	Muffins	1 medium		
20.4	145	biscuit, cornbread	2" cube		
12.8	15	Pancakes/waffles frozen	4"		
0.4	155	Pancakes/waffles-HM	4"		
4	50	Red, pinto, lima, black-eye	1 cup		

Vit. D (IU)	Ca+ (mg)	Food	Serving	sv/day	sv/wk
0	260	Tofu, regular	1 cup		
0	55	Spaghetti w/tomato sauce	1 cup		
26	25	Eggs	1 each		
0	85	fast food hamburger	1 each		
0	150	Fast Food Cheeseburger	1 each		
0	60	Oysters, shrimp, crab, crawfish, herring	3 oz		
663	195	Canned Salmon w/Bones	3.75oz can		
1730.8	350	Sardines	3.75oz can		
0	35	Cake	3×3×2		
0	85	Almonds	1/4 cup		
0	85	Milk Chocolate	1.6oz bar		
		Other food items(Ensure, Boost, Power bars, etc)			
		Subtotal Calcium (food only)/day			
		Subtotal Vit D (food only)/day			
400	200	Multivitamin	1 Tab		
200	500	Calcium supplement (list: viactiv chew etc)	1 Tab		
		Vitamin D supplement			
		Subtotal Calcium Supplement			
		Subtotal Vit D supplement			
		<b>Total Calcium Food and Supplement</b>			
		<b>Total Vit D Food and Supplement</b>			

The FFQ is designed as an Excel worksheet to determine calcium and vitamin D intake in real time. When in use, the Registered Dietitian questions the subject and fills in the columns on the right. The left hand columns (not shown) in the working Excel worksheet are used to tally the number of food portions and the corresponding calcium and vitamin D value. Please contact Ellen Anderson (eanderson1@partners.org) or Jane Hubbard (jhubbard1@partners.org) for more information regarding the FFQ