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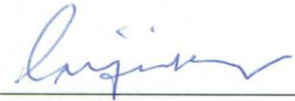
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This thesis, THE EFFECTIVENESS OF A SHORT FOOD FREQUENCY QUESTIONNAIRE IN DETERMINING THE ADEQUACY OF VITAMIN D INTAKE IN CHILDREN, by Caitlin S. Russell was prepared under the direction of the Master's Thesis Advisory Committee. It is accepted by the committee members in partial fulfillment of the requirements for the degree Master of Science in the College of Health and Human Sciences, Georgia State University. The Master's Thesis Advisory Committee, as representatives of the faculty, certify that this thesis has met all standards of excellence and scholarship as determined by the faculty.


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ABSTRACT

THE EFFECTIVENESS OF A SHORT FOOD FREQUENCY QUESTIONNAIRE IN DETERMINING THE ADEQUACY OF VITAMIN D INTAKE IN CHILDREN

by
Caitlin S. Russell

Background: Studies have consistently found a high prevalence of vitamin D deficiency in adolescents. Few validated dietary intake assessment tools for vitamin D exist for adolescents.

Objective: The aim of this study was to determine if a short food frequency questionnaire (SFFQ) can be used to effectively assess vitamin D intake in adolescents compared to a previously validated long food frequency questionnaire (LFFQ).

Participants/setting: 140 healthy 6-12 year old (male $n=81$) Caucasian and African American ($n=94$) children from Pittsburgh, Pennsylvania completed a SFFQ and LFFQ at two time points 6 months apart.

Main outcome measures: Reliability and validity of a SFFQ by comparison with a previously validated LFFQ for children and adolescents.

Statistical analysis: Reliability, validity, sensitivity, specificity, positive, and negative predictive values were assessed using Pearson correlation coefficients.

Results: Mean vitamin D intake from the SFFQ (range, 434 to 485 IU) was higher than the LFFQ (range, 320 to 378 IU). Overall association between the SFFQ and the LFFQ for vitamin D intake was modest ($r=0.36$, $P<0.001$). When stratified by race, the overall degree of association was weak for African Americans ($r=0.26$, $P=0.001$) and moderate for Caucasians ($r=0.57$, $P<0.001$). Overall reliability testing results were modest and

significant for the LFFQ ($r=0.28$, $P=0.002$) and SFFQ ($r=0.33$, $P<0.001$). Association between mean vitamin D intake from LFFQs and SFFQs was used to determine validity. The association for validity was found to be modest ($r=0.51$, $P<0.001$). Sensitivity, specificity, positive predictive value, and negative predictive value for the SFFQ were 90%, 64%, 0.78, and 0.58, respectively.

Conclusion: The SFFQ was found to be modestly valid and reliable in an early adolescent population. Associations between African Americans were not as strong as Caucasians which may be due to errors in reporting dietary consumption related to higher body weight.

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ABBREVIATIONS

1,25(OH) ₂ D	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
AAP	American Academy of Pediatrics
AI	Adequate Intake
BMI	Body Mass Index
cm	centimeter
CSFII	Continuing Survey of Food Intakes by Individuals
DRI	Dietary Reference Intake
EAR	Estimated Average Requirement
FFQ	Food Frequency Questionnaire
GA	Georgia
IOM	Institute of Medicine
IU	International Units
kg	kilogram
L	liter
LFFQ	Long Food Frequency Questionnaire
µg	microgram
mL	milliliter
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
nmoL	nanomole

NPV	Negative Predictive Value
oz	ounce
PA	Pennsylvania
PPV	Positive Predictive Value
RDA	Recommended Dietary Allowance
SBNM	School-Based Nutrition Monitoring
SFFQ	Short Food Frequency Questionnaire
UPMC	University of Pittsburgh Medical Center
U.S.	United States of America
USDA	United States Department of Agriculture
UVB	Ultra-violet B

CHAPTER I

THE EFFECTIVENESS OF A SHORT FOOD FREQUENCY QUESTIONNAIRE IN DETERMINING THE ADEQUACY OF VITAMIN D INTAKE IN CHILDREN

Introduction

Several studies have reported the excessive prevalence of vitamin D insufficiency among children (1-9). The primary role of vitamin D in humans is to maintain serum calcium and phosphorus concentrations at levels that support proper cell function and bone mineralization (10,11). In children, deficiency of vitamin D is associated with rickets, when growing bones fail to mineralize. When a subject is deficient in vitamin D, calcium absorption is only 10 – 15% of what is normally absorbed, resulting in decreased bone mineralization and secondary hyperparathyroidism (12). Maintaining adequate vitamin D and calcium status during childhood may reduce the risk of developing chronic diseases such as osteoporosis, multiple sclerosis, hypertension, osteomalacia, and certain cancers, as well as reduce the risk of bone fracture, falls, depression, certain autoimmune diseases, muscle weakness, and cardiovascular disease later in life (11,13-15). Immune function and inflammation reduction are also regulated by vitamin D (16). Several factors determine vitamin D status and these include dietary intake of vitamin D, skin pigmentation, season, and latitude of residence (14,16). Because vitamin D is found in very few foods, fortified food and beverages provide the U.S. population with most of its dietary vitamin D. Fish liver oil, fatty fish, and egg yolks are natural sources of vitamin D (15,16). Researchers have measured the prevalence of vitamin D deficiency in healthy adolescents using serum 25-hydroxyvitamin D (25(OH)D) as a marker and dietary

assessment methods (2-4). These studies have consistently found a high prevalence of vitamin D deficiency or insufficiency in adolescents. Subjects with the lowest serum 25(OH)D tended to be African American, obese, and those who did not consume vitamin D-rich foods (2).

There is a range of dietary assessment methods that help determine individual and population nutritional intakes and habits. These methods play an essential role in establishing when a segment of the population is at risk for nutritional problems. There are limited data available on validated dietary assessment tools for adolescents (17-20). Previously validated tools include a food frequency questionnaire (FFQ) that provided reasonable estimates on vitamin D intake in children when compared to 3-day diaries (21), a calcium FFQ that was moderately associated with estimates from 24-hour dietary recalls in middle-school-aged children (22), a FFQ that showed good test-retest reliability for assessing food intake in schoolchildren when compared with observed food intake (23), and a FFQ that suitably ranked food and nutrient intake of adolescents when compared with food records (24). Fumagalli and colleagues (25) measured dietary intake in 5-10 year olds comparing 3-day dietary records with a FFQ that had previously been validated for use in adults. They found that the FFQ appears to overestimate usual energy and nutrient intakes in children and that portion sizes need to be adjusted before adoption of this instrument in children. When designing a valid assessment tool for this age group, consideration should be given to the populations' age, reading level, ability to understand questions, ability to understand conceptual thinking, and whether an adult caretaker will assist in completing the assessment (26).

At the Primary Care Center of the Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center (UPMC), Rajakumar and colleagues conducted a Vitamin D and Sunlight study between June 2006 and March 2008. This study was designed to determine the seasonal variation of vitamin D insufficiency in healthy 6-12 year old pre- and early adolescent African American and Caucasian children residing in Pittsburgh, PA. Included in this initial study was an evaluation of nutrition intake using both a long food frequency questionnaire (LFFQ) and a short food frequency questionnaire (SFFQ). For the current study, a secondary analysis was performed on the data generated from Rajakumar's initial investigation. By way of validity and reproducibility tests, the secondary analysis aimed to determine if the SFFQ could effectively assess vitamin D intake in adolescents compared to the LFFQ. It is important to use validity and reproducibility components because any interpretation of a FFQ should include the degree to which a questionnaire can truly measure dietary intake (17). We hypothesized that there will be no difference between adolescents' vitamin D intake calculated from a SFFQ when compared to a validated LFFQ.

CHAPTER II

Literature Review

Vitamin D Requirements in Humans

The primary role of vitamin D in humans is to maintain cellular calcium concentrations within the homeostatic 8-10 mg/dL range. Vitamin D, or its biologically active form 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}$), regulates calcium and phosphorus metabolism in the intestine and bone. Without adequate vitamin D, the small intestines only absorb about 10 – 15% of dietary calcium (15). Low serum calcium stimulates parathyroid hormone (PTH) which, along with $1,25(\text{OH})_2\text{D}$, increases decalcification of bone leading to osteoporosis in adults and rickets in children (27). Having a sufficient concentration of serum vitamin D has been shown to reduce diastolic and systolic blood pressures in hypertensive patients and possibly decrease the risk of developing type 1 diabetes, cardiovascular disease, osteoporosis, and certain cancers such as prostate, breast, colon, and esophagus (10,15).

Vitamin D can be obtained through the diet and from supplements. Vitamin D is synthesized in the epidermis of the skin after its exposure to ultraviolet-B (UVB) radiation from sunlight, turning 7-dehydrocholesterol (provitamin D_3) into previtamin D_3 , which later becomes thermodynamically stable vitamin D_3 . This stable form of vitamin D is called cholecalciferol and later becomes $25(\text{OH})\text{D}$ (calcidiol) (15). However, when skin is protected from UVB radiation by melanin pigment, sunscreen, and clothing, cutaneous vitamin D production is greatly diminished (14). Living at a high latitude reduces the amount of vitamin D that can be synthesized in the skin since fewer solar

UVB photons reach the earth than for those living at a lower latitude. Natural sources of vitamin D include cod liver oil and oily fish such as salmon and sardines. In the U.S., milk, infant formulas, some orange juices, cereals, and breads are fortified with vitamin D. Obtaining accurate estimates of average dietary intakes of vitamin D can be difficult because of the variability of vitamin D content of fortified foods (11). Vitamin D supplements are available without a prescription and many supplements generally provide 400 IU (10 µg) of vitamin D.

Whether vitamin D is made in the skin or ingested in the diet, it enters circulation and is transported to the liver by vitamin D-binding protein. In the liver, vitamin D undergoes hydroxylation and becomes 25(OH)D, the major circulating form of vitamin D. To become biologically active, it is then hydroxylated to 1,25(OH)₂D in the kidneys and other tissues.

According to the Institute of Medicine, the Dietary Reference Intake (DRI) for vitamin D is 600 IU/day (15 µg) for 1-70 year olds (28). The Estimated Average Requirement (EAR) for vitamin D is 400 IU/day (10 µg) for 1-71+ year olds. The American Academy of Pediatrics (AAP) recommends 400 IU/day of vitamin D intake for all infants, children, and adolescents (13). Vitamin D status in humans is measured using 25(OH)D (11,15).

Vitamin D Status in Children and Adolescents

Vitamin D deficiency is defined by most experts as 25(OH)D \leq 20 ng/mL (5,6,14,29-30). Numerous studies report vitamin D deficiency and insufficiency among children and adolescents (1-9). Using data from the National Health and Nutrition Examination Survey (NHANES) 2001-2004, Kumar and colleagues (2) analyzed data on

children and adolescents and found that 25(OH)D deficiency was common among 1-21 year old persons. In this nationally representative segment of the U.S. population, 61% or 50.8 million of the children had 25(OH)D concentrations between 15 and 29 ng/mL. Children with lower 25(OH)D concentration were girls, non-Hispanic blacks, obese and those who spent more time in front of a television, playing video games, or using a computer. Vitamin D deficiency was also associated with hypertension, low HDL concentrations, and elevated PTH concentrations. Suboptimal vitamin D status was also common among healthy, low-income, minority children in Atlanta, GA (9). Cole and colleagues reported that 22% of the children in their study ($n=290$) had vitamin D deficiency and 74% had insufficiency. The study authors predicted that vitamin D intake would not influence vitamin D status in this study which was true. At a Boston primary care center, Gordon and colleagues (5) studied 380 pediatric patients and found that 12% were vitamin D deficient and 40% were below an accepted optimal threshold. Among the participants who had vitamin D deficiency, 32.5% showed evidence of demineralization after wrist and knee radiographic assessments. In light of growing data that support vitamin D's immunomodulatory effects, study authors stated that their findings support recommendations that vitamin D supplementation should be made available for all young children. The Atlanta and Boston studies contradict the assumption that children living at lower latitudes exhibit better vitamin D status than children living at higher latitudes.

Using ≤ 15 ng/mL as deficient, Gordon and colleagues (3) found that 24.1% ($n=307$) of 11-18 year old children were vitamin D deficient, 42% were vitamin D insufficient (≤ 20 ng/mL), and 4.6% were severely vitamin D deficient (≤ 8 ng/mL). They found that African American adolescents were more likely to be vitamin D deficient than

other ethnic groups, which corresponds with lower vitamin D production among individuals with darker skin pigmentation. They also suggested additional research on health outcomes among children and adolescents after vitamin D supplementation. In order to estimate the proportion of vitamin D insufficiency among the pediatric African American population, Rajakumar and colleagues (4) studied the response among 42 preadolescent African American children who were given 400 IU of vitamin D daily for one month. They found that 49% of the subjects were vitamin D insufficient (10-20 ng/mL) at baseline. After one month of supplementation, the mean 25(OH)D concentration significantly increased in the vitamin D insufficient group, however, 18% persisted to be vitamin D insufficient. Study authors explained that the short duration of the study and minimal dosage of vitamin D supplementation might explain the persistent insufficiency at the end of the trial. They concluded that preadolescent African Americans residing in the Northeast were at risk of low vitamin D concentrations. In a southeastern U.S. prospective study, Willis and colleagues (8) found that vitamin D status declined with age for African American and Caucasian girls aged 4-8 years old. In this population, 18% of the participants had 25(OH)D concentration below 20 ng/mL. They found an inverse relationship between circulating 25(OH)D and muscle mass, indicating that an increase in fat-free soft tissue may have an increased utilization of vitamin D. This raises the question of whether more vitamin D is required during periods of growth among adolescents.

Dietary Assessment Methods in Children

As children grow from birth through adolescence, they undergo enormous social, emotional, physical, and cognitive development. There are many factors that influence

this growth, including environmental factors, genetics, and nutritional status (26). To understand the dietary intake habits of adolescents, it is important to monitor their nutritional behavior using methods that quantify what is consumed. An important prerequisite for monitoring the nutritional status in children is the accurate assessment of nutrient intake using validated tools that have been shown to accurately portray consumption patterns. There are various methods used to assess children's dietary intake, ranging from food records, 24-hour dietary recalls, FFQs, and combinations of all three (18-25,31-44). Due to the challenging nature of dietary assessment tools, some of which require concerted effort to complete on behalf of the respondent, these methods are prone to reporting error, and dietary intakes may be erroneously interpreted (45). Subjects must be able to retrieve dietary information from memory and accurately estimate portion sizes. When a child is too young to complete an assessment on his own, parents and caregivers must be able to reliably estimate what the child has consumed. Parents and caregivers may be able to report intake from the home setting but may not accurately assess what is consumed at school and outside the home. It is essential to develop valid assessment tools for adolescents, which reduce response burden and achieve precise estimation of actual nutrient intake (18,20,37).

Dietary assessment methods can assess macro- and micronutrient components of the diet. Variability is lowest among nutrients that are consumed on a regular basis such as protein, fat, and carbohydrate. Micronutrient intake, such as vitamin D, is the most variable and requires many days of records to accurately capture true intake, though it is not known exactly how many days are needed (45).

24-Hour Dietary Recall

A 24-hour dietary recall is a snapshot of what a person has recently eaten. It is usually a consultation, either in person or on the telephone, between a participant and trained personnel who interview the subject about everything he has eaten and drank over the past 24-hour period (18). The participant does not have to be motivated to complete any sort of dietary record when participating in a 24-hour dietary recall. A nutrient analysis program is needed in order to assess intake after a skilled nutritionist records the information. Limitations of this method include subjects not recalling all consumed foods, reporting information they feel the clinician wants to hear, and needing to collect multiple recalls in order to determine usual intake (17). As diet is known to vary from day to day, a 24-hour recall does not give an accurate portrayal of long-term eating habits for an individual, but it does provide estimates of population means for nutrients (18). Validity of 24-hour recalls depends on how accurately an individual can recall his intake in terms of both food eaten and portion sizes. Validity is also dependent on how accurately the trained personnel codes the intake recorded during the interview and how comprehensive the nutrient database is that is used to analyze the recorded food (17). Faggiano and colleagues (46) completed a validation trial in which subjects' ($n=103$) actual food consumption was compared with their recollection of consumption on the following day. All food chosen for consumption was recorded and weighed before and after the meal. For the recall, researchers used photographs of each type of dish served, including seven different portion sizes for each dish. The following day, subjects were interviewed on what they consumed during the prior meal. Subjects overestimated portion sizes by $>20\%$ for six foods and underestimated portion sizes by $<20\%$ for four foods. Those who ate more than average tended to underreport and those who ate less

than average tended to overreport intake. This phenomenon is generally referred to as *flat slope syndrome*. In a 2009 validation study, Baxter and colleagues (47) investigated the effects of the elapsed time between a meal and the interview (retention interval) on the accuracy of 4th graders reporting dietary intake during 24-hour recalls. The children were observed eating two school meals and later interviewed to obtain 24-hour recall. Two target periods were used: prior 24 hours (immediately preceding the interview) and previous day (midnight to midnight of the day before the interview). Interviews were conducted in the morning, afternoon, and evening. Researchers found that accuracy was better for prior 24 hours than previous day recalls and the best accuracy was obtained in the afternoon and evening. After analyzing data in 2010 from the same 2009 retention-interval validation study, Baxter and colleagues (48) concluded that shortening the retention interval of dietary intake and report increases accuracy for reporting macronutrients among this age-group.

Reliability, which is also referred to as reproducibility or precision, expresses the degree to which an investigator is able to obtain the same results when a method is repeated under similar conditions (17). If a certain method is used to assess dietary intake and it does not give consistent results after repeated use under the same conditions, the method cannot be considered reliable. Using a USDA-funded School-Based Nutrition Monitoring (SBNM) secondary-level student questionnaire, Hoelscher and colleagues (49) analyzed reproducibility results for 8th grade middle school students' food choice behavior. Using test-retest study design for reproducibility, researchers administered the survey to students at two timepoints. They found that most questions on the SBNM questionnaire had acceptable reproducibility. For questions on food choices from the

previous day, agreement was 70% or greater ($r=0.66$). As this questionnaire only represented one day and was not typical intake at the individual level, authors emphasized that it is appropriate to use their study findings to characterize groups, as in cross-sectional studies, but it is not appropriate for tracking individuals' food intake over time. Also using a test-retest study design for reproducibility of questionnaire items, Penkilo and colleagues (50) measured reproducibility coefficients of an elementary school-level SBNM questionnaire among 4th graders. Food and meal choice questions had relatively good reproducibility, however mean percent agreement was lower for the elementary school-level SBNM questionnaire versus the secondary school-level SBNM questionnaire mentioned previously (78.4% vs. 86.0% respectively). The authors concluded that the questionnaire could be a valuable tool for epidemiological studies when it is necessary to assess dietary intake in a large number of children in a relatively short time period.

Food Record

Similar to the 24-hour dietary recall, the food record is based on actual foods and amounts consumed by an individual on one or more specific days (17). As with 24-hour recalls, the food record is used primarily as a means to assess the validity of a FFQ when the questionnaire is used as the primary collection instrument (17,21,24-26,32-34,40,42-44,51). There is considerable flexibility afforded for dietary data analysis when using a food record since this method can accommodate any level of diverse food description detail, which is also true with the 24-hour recall method. Also known as a food diary, the food record consists of a detailed listing of all food and beverages consumed by an individual for one or more days. To reduce relying on memory, intake should be recorded

by the subject at the time of consumption and the subject should be trained on the proper way to keep an accurate log (21,37). The food record is then reviewed by trained personnel in order to ensure sufficient detail of food descriptions, preparations, and amounts (17,32). An advantage of using a food record is that, if subjects are compliant with instructions, the foods and amounts eaten are recorded immediately and relying on memory is not necessary, unlike with 24-hour recalls. Keeping a food record requires a high level of motivation for the subject, which can lead to poor response rates if subjects are not inspired to keep accurate records. Taylor and colleagues (32) designed a study to determine the validity of a food frequency questionnaire compared to a 4-day food record for assessing calcium and vitamin D intake in adolescent girls with and without anorexia nervosa. Daily calcium and vitamin D intake did not differ between the food record and questionnaire. They found that there was better compliance with the questionnaire than with the food record, which reflects the general sentiment that food records are burdensome to complete. Multiple food record days are needed in order to correctly assess usual individual intake. The number of days necessary for an accurate estimation of intake depends on the daily variability of the nutrient of interest. Basiotis and colleagues (52) used food records from 29 participants who completed a 365 consecutive-day food record to determine the number of days of intake needed to estimate average nutrient intakes for individuals and groups of individuals. Food energy and 18 nutrients, except vitamin D, were analyzed. The number of days of food intake records needed to predict the usual nutrient intake of an individual varied substantially among individuals for the same nutrient and within individuals for different nutrients. Food energy required 31 days to predict usual intake (the least) and vitamin A required 433 days (the most).

For estimating mean nutrient intake for the group, 3 days were needed for food energy and 41 days were needed for vitamin A. Authors concluded that for even larger groups, fewer days of food records would be necessary. The Basiotis study, as well as a paper by Nelson and colleagues (53) that presents a comprehensive picture of within- and between-subject variation in nutrient intake, suggest that the minimum number of days of intake required for characterizing usual intake for energy and macronutrients ranges from 3-10 days. For food components, such as vitamins and minerals, with large day-to-day variability, the number of days required may range from 20 to >50 days (17). Participant motivation and logistics make this situation impractical for most researchers. In order to assess intake of energy and macronutrients, 4-5 days of food record intake are customarily selected as a reasonable compromise. For nutrients with high within-person variability that are found in few food sources such as vitamins and minerals, FFQs specifically designed to assess these micronutrients are usually selected as the method of choice.

Food Frequency Questionnaire

The 24-hour dietary recall and the food record differ in their methods of data collection from the FFQ. Whereas the 24-hour recall and the food record report specific foods and amounts consumed on a particular day, the FFQ is based on an individual's perception of typical intake over a period of time, usually from 1 month to a year (17,18). When designing a FFQ, decisions about whether to measure intake of specific nutrients or whether there is a need for a more comprehensive dietary assessment are critical. There are many factors to consider when designing a questionnaire, which include whether the primary objective is to rank individuals or to get a measure of absolute intake

of a nutrient. Also, the questionnaire must include food items that are consumed reasonably often by a majority of individuals. Developing the food list for a questionnaire takes careful consideration and can be approached by various methods: a review of published food composition tables using foods that contain large amounts of the nutrient of interest; or start with a long list of foods and reduce the list by pilot testing the questionnaire. Stepwise regression is a more sophisticated approach and has been used, among other approaches, to develop questionnaires that measured a fairly comprehensive list of nutrients that needed to fit in a relatively small amount of space (54,55). FFQs can be “short” or “long” depending on the number of questions included. In large epidemiological studies, it may not be feasible to collect multiple days of dietary intake via the 24-hour dietary recall or the food record. Therefore, the FFQ is generally the most appropriate method for measuring intake in this situation. A number of studies have used FFQs to investigate relationships between dietary intake and disease outcomes or to just assess energy, macronutrient, and micronutrient intakes in a pediatric population (19-25,32,33,36-44). In order to determine validity of their FFQ, most of these studies compared their questionnaire with either food records or dietary recalls. Only three studies (21,32,33) were found that focused on vitamin D intake among adolescent populations, and the studies are summarized in Table 1. Using 3-day diaries for reference, Marshall and colleagues (21) investigated the relative validities of the Iowa Fluoride Study questionnaire and the Block Kids’ Food Questionnaire in assessing vitamin D intakes. Children completed the Iowa Fluoride Study nutrient questionnaire ($n=223$), the Block Kids’ Food Questionnaire ($n=129$) and 3-day diaries during similar time periods. Nutrient correlations between intakes estimated from 3-day diaries and questionnaires

were similar for vitamin D intake. The authors concluded that a dietary assessment method that targets specific foods or nutrients could be as effective at estimating intakes as a more comprehensive assessment tool. They also stated that it is important to consider major dietary sources of the relevant nutrient and dietary habits of the population of interest when designing a targeted nutrient tool. Using a 3-day food record as a reference, Marshall and colleagues (33) designed a study to evaluate the validity of a beverage frequency questionnaire in assessing calcium and vitamin D intakes. They found that their questionnaire could provide a relative estimate of vitamin D intake. As consumers are decreasing their milk intake and increasing their soft drink and energy drink intake, they are consuming fewer beverages that contribute vital nutrients to the total diet such as calcium and vitamin D.

Table 1. Characteristics of studies that focused on vitamin D intake among adolescent populations

Author/year publication	Participants/age group	Dietary method(s)/ reference method	Findings	Limitations
Taylor <i>et al.</i> (32) (2009)	57 girls with anorexia nervosa 50 healthy girls 12-18 years	FFQ 4d food record	Greater compliance with FFQ (99%) than food record (71%). Daily vitamin D intake from food record and FFQ did not differ. Strong correlations observed for daily vitamin D intake derived from FFQ vs food record ($r=0.78$, $P<0.0001$).	Vitamin D concentrations were not measured as biomarker of intake. Socioeconomic status and parental education, which can affect dietary reporting, were not collected.
Marshall <i>et al.</i> (21) (2008)	223 children completed Iowa Fluoride Study questionnaire and 3 d food record at 9 years; 129 children completed the Block Kids' Food Questionnaire and 3 d food record at 8.3 years	Iowa Fluoride Study targeted nutrient questionnaire Block Kids' Food Questionnaire 3 d food record	Correlations with food records for vitamin D intakes reported on Iowa Fluoride Study questionnaires were similar to correlations with vitamin D reported on Block Kids' Food Questionnaire. Percentage of exact agreement was lower for vitamin D for intakes reported on Iowa Fluoride Study questionnaires compared to Block Kids' Food Questionnaires relative to food records.	Targeted nutrient questionnaires were not administered before standard 3 d food record, leading to potential bias. Parents reported data and may not know what/how much children are eating. Subjects reporting data may not remember specifics of intake. Participants primarily Caucasian from narrow geographic region – not a representative sample of the general population. Results not generalizable to wider population.
Marshall <i>et al.</i> (33) (2003)	240 children 6 months-5 years	Quantitative beverage questionnaire 3 d food record	Correlations between mean daily nutrient intakes estimated from questionnaires and food record were 0.60-0.80 for vitamin D.	Targeted beverage questionnaire was not administered before standard 3 d food record, leading to potential bias. Parents reported data and may not know what/how much children consumed. Participants primarily Caucasian from narrow geographic region – not a representative sample of the general population. Results should not be generalized to wider population.

Vitamin D Intake in Children and Adolescents

The EAR for vitamin D is 400 IU/day (10 µg) for children age 1-13 years and the RDA is 600 IU/day (15 µg) (28). According to food consumption data collected in NHANES 1988-1994 (NHANES III), the 1994-1996 Continuing Survey of Food Intakes by Individuals (CSFII 1994-1996), and the 1998 Supplemental Children's Survey, the majority of Americans are not consuming the Adequate Intake (AI) of vitamin D (56). Although dairy was the largest food source of calcium and vitamin D, some avoided dairy products due to taste or lactose intolerance. For these persons, fortified juices can provide an alternative source of vitamin D. Using NHANES 2005-2006 data, Bailey and colleagues (57) estimated vitamin D intakes from food and dietary supplements. Participants completed two 24-hour recalls and a questionnaire. Among 9-13 year olds, 24% of males and 32% of females used a daily supplement containing vitamin D. Mean vitamin D intake among this age group was 8.4 µg or 336 IU/day for males and 8.0 µg or 320 IU/day for females. The prevalence of meeting the AI for vitamin D through diet was 53% and 47% among 9-13 year old males and females, respectively. When dietary supplement use was included, 66% of 9-13 year old males met the AI for vitamin D and 53% of 9-13 year old females met the AI for vitamin D. Decreased dairy consumption relates to inadequate intake of key nutrients, including vitamin D (58). Using data from CSFII 1994-1996, 1998 and NHANES 1999-2000 reports, Fulgoni and associates (59) showed that African American males across all age groups had significantly lower dairy intake than did their non-African American counterparts. Likewise, African American females age 2-18 years consumed significantly less dairy when compared with non-African Americans. In a segment of the population that is already at risk of low serum

vitamin D concentrations due to darker skin pigmentation, these results indicate a need for increased education about the role vitamin D plays in our health and the risks of not consuming enough vitamin D-fortified foods and beverages or a vitamin D supplement.

FFQs are typically validated by comparing results with food records as the reference method (18,21,24-26,32-34,36-40,43,44). Statistical tests are used to determine whether the two instruments reported similar values for nutrients. These tests assess reliability and validity, and statistical analysis includes paired sample *t* tests, Pearson's correlation coefficients, Spearman correlations, Fisher's exact test, Wilcoxon rank sum test, weighted κ statistics, and percentages of exact agreement.

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CHAPTER III

Manuscript in style of Journal

1 **AUTHORS' PAGE**

2 The Effectiveness of a Short Food Frequency Questionnaire in Determining the

3 Adequacy of Vitamin D Intake in Children

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23 **ABSTRACT**

24 **Background:** Studies have consistently found a high prevalence of vitamin D deficiency
25 in adolescents. Few validated dietary intake assessment tools for vitamin D exist for
26 adolescents.

27 **Objective:** The aim of this study was to determine if a short food frequency
28 questionnaire (SFFQ) can be used to effectively assess vitamin D intake in adolescents
29 compared to a previously validated long food frequency questionnaire (LFFQ).

30 **Participants/setting:** 140 healthy 6-12 year old (male $n=81$) Caucasian and African
31 American ($n=94$) children from Pittsburgh, Pennsylvania completed a SFFQ and LFFQ at
32 two time points 6 months apart.

33 **Main outcome measures:** Reliability and validity of a SFFQ by comparison with a
34 previously validated LFFQ for children and adolescents.

35 **Statistical analysis:** Reliability, validity, sensitivity, specificity, positive, and negative
36 predictive values were assessed using Pearson correlation coefficients.

37 **Results:** Mean vitamin D intake from the SFFQ (range, 434 to 485 IU) was higher than
38 the LFFQ (range, 320 to 378 IU). Overall association between the SFFQ and the LFFQ
39 for vitamin D intake was modest ($r=0.36$, $P<0.001$). When stratified by race, the overall
40 degree of association was weak for African Americans ($r=0.26$, $P=0.001$) and moderate
41 for Caucasians ($r=0.57$, $P<0.001$). Overall reliability testing results were modest and
42 significant for the LFFQ ($r=0.28$, $P=0.002$) and SFFQ ($r=0.33$, $P<0.001$). Association
43 between mean vitamin D intake from LFFQs and SFFQs was used to determine validity.
44 The association for validity was found to be modest ($r=0.51$, $P<0.001$). Sensitivity,

45 specificity, positive predictive value, and negative predictive value for the SFFQ were
46 90%, 64%, 0.78, and 0.58, respectively.

47 **Conclusion:** The SFFQ was found to be modestly valid and reliable in an early
48 adolescent population. Associations between African Americans were not as strong as
49 Caucasians which may be due to errors in reporting dietary consumption related to higher
50 body weight.

51 The Effectiveness of a Short Food Frequency Questionnaire in Determining the
52 Adequacy of Vitamin D Intake in Children

53 **INTRODUCTION**

54 Several studies have reported the excessive prevalence of vitamin D insufficiency
55 among children (1-9). The primary role of vitamin D in humans is to maintain serum
56 calcium and phosphorus concentrations at levels that support proper cell function and
57 bone mineralization (10,11). In children, deficiency of vitamin D is associated with
58 rickets, when growing bones fail to mineralize. When a subject is deficient in vitamin D,
59 calcium absorption is only 10 – 15% of what is normally absorbed, resulting in decreased
60 bone mineralization and secondary hyperparathyroidism (12). Maintaining adequate
61 vitamin D and calcium status during childhood may reduce the risk of developing chronic
62 diseases such as osteoporosis, multiple sclerosis, hypertension, osteomalacia, and certain
63 cancers, as well as reduce the risk of bone fracture, falls, depression, certain autoimmune
64 diseases, muscle weakness, and cardiovascular disease later in life (11,13-15). Immune
65 function and inflammation reduction are also regulated by vitamin D (16). Several factors
66 determine vitamin D status and these include dietary intake of vitamin D, skin
67 pigmentation, season, and latitude of residence (14,16). Because vitamin D is found in
68 very few foods, fortified food and beverages provide the U.S. population with most of its
69 dietary vitamin D. Fish liver oil, fatty fish, and egg yolks are natural sources of vitamin D
70 (15,16). Researchers have measured the prevalence of vitamin D deficiency in healthy
71 adolescents using serum 25-hydroxyvitamin D (25(OH)D) as a marker and dietary
72 assessment methods (2-4). These studies have consistently found a high prevalence of
73 vitamin D deficiency or insufficiency in adolescents. Subjects with the lowest serum

74 25(OH)D tended to be African American, obese, and those who did not consume vitamin
75 D-rich foods (2).

76 There is a range of dietary assessment methods that help determine individual and
77 population nutritional intakes and habits. These methods play an essential role in
78 establishing when a segment of the population is at risk for nutritional problems. There
79 are limited data available on validated dietary assessment tools for adolescents (17-20).
80 Previously validated tools include a food frequency questionnaire (FFQ) that provided
81 reasonable estimates on vitamin D intake in children when compared to 3-day diaries
82 (21), a calcium FFQ that was moderately associated with estimates from 24-hour dietary
83 recalls in middle-school-aged children (22), a FFQ that showed good test-retest reliability
84 for assessing food intake in schoolchildren when compared with observed food intake
85 (23), and a FFQ that suitably ranked food and nutrient intake of adolescents when
86 compared with food records (24). Fumagalli and colleagues (25) measured dietary intake
87 in 5-10 year olds comparing 3-day dietary records with a FFQ that had previously been
88 validated for use in adults. They found that the FFQ appears to overestimate usual energy
89 and nutrient intakes in children and that portion sizes need to be adjusted before adoption
90 of this instrument in children. When designing a valid assessment tool for this age group,
91 consideration should be given to the populations' age, reading level, ability to understand
92 questions, ability to understand conceptual thinking, and whether an adult caretaker will
93 assist in completing the assessment (26).

94 At the Primary Care Center of the Children's Hospital of Pittsburgh of the
95 University of Pittsburgh Medical Center (UPMC), Rajakumar and colleagues conducted a
96 Vitamin D and Sunlight study between June 2006 and March 2008. This study was

97 designed to determine the seasonal variation of vitamin D insufficiency in healthy 6-12
98 year old pre- and early adolescent African American and Caucasian children residing in
99 Pittsburgh, PA. Included in this initial study was an evaluation of nutrition intake using
100 both a long food frequency questionnaire (LFFQ) and a short food frequency
101 questionnaire (SFFQ). For the current study, a secondary analysis was performed on the
102 data generated from Rajakumar's initial investigation. By way of validity and
103 reproducibility tests, the secondary analysis aimed to determine if the SFFQ could
104 effectively assess vitamin D intake in adolescents compared to the LFFQ. It is important
105 to use validity and reproducibility components because any interpretation of a FFQ
106 should include the degree to which a questionnaire can truly measure dietary intake (17).
107 We hypothesized that there will be no difference between adolescents' vitamin D intake
108 calculated from a SFFQ when compared to a validated LFFQ.

109 **METHODS**

110 **Study Design**

111 The Vitamin D and Sunlight study was an observational study designed to
112 determine the seasonal variation of vitamin D insufficiency, and to assess its risk factors
113 and metabolic impact in healthy 6-12 year old pre-and early adolescent African American
114 and Caucasian children residing in Pittsburgh, PA. A total of 140 participants were
115 enrolled in the initial study between the summer months of June-September 2006 and the
116 winter months of December 2007-March 2008. There was no intervention and
117 participants were seen at 2 time points, with the initial visit being either in the summer or
118 winter and the follow-up visit occurring 6 months later. Blood was drawn at both visits to
119 determine serum concentrations of calcium, phosphorus, albumin, serum PTH, 25(OH)D
120 and 1,25(OH)₂D. Subjects completed a LFFQ and a SFFQ at both timepoints in order to
121 assess sunlight exposure and dietary intake of vitamin D and calcium. The initial study
122 was approved by the Institutional Review Board of the University of Pittsburgh. Written
123 informed consent was obtained from parents at recruitment of the initial study and
124 participants' assent was obtained prior to enrollment. The Institutional Review Board at
125 Georgia State University approved the secondary analysis study.

126

127 **Subjects**

128 The initial study used a convenience sample of patients with a mean age of 9.1
129 years at the time of recruitment. Participants were recruited from the Primary Care Center
130 of the Children's Hospital of Pittsburgh of the University of Pittsburgh Medical Center
131 (UPMC) and from the surrounding area. Inclusion criteria included healthy 6-12 year old

132 pre- and early adolescent African American and Caucasian children residing in
133 Pittsburgh, PA. Children with hepatic or renal disease, metabolic rickets, malabsorptive
134 disorders, cancer, or those on treatment with anticonvulsants or systemic glucocorticoids
135 were excluded.

136

137 **Data Collection**

138 Study participants received a clinical exam at their initial and six month visit to
139 gather anthropometric information including weight (kg), height (cm) and body mass
140 index (BMI) (kg/m^2). BMI was calculated from weight and height at baseline and follow-
141 up. Dietary intake was recorded by participants at both time points using a SFFQ and a
142 LFFQ. BMI was evaluated using the 2000 Centers for Disease Control and Prevention
143 growth charts, where overweight is determined by a BMI value between the 85th and 95th
144 percentile and obesity >95th percentile (27,28).

145

146 *The SFFQ.*

147 Rajakumar and colleagues adapted a questionnaire, developed by Dr. Michael
148 Holick (Boston University Medical Center) that assessed vitamin D intake in adults, to
149 address vitamin D intake and sunlight exposure in pre- and early adolescents. The
150 questionnaire included 21 questions for the patient/parent to complete. Subjects were
151 asked if they take a multivitamin, vitamin D supplement, or cod liver oil, how many
152 servings of milk, cheese, yogurt, and vitamin D-fortified orange juice they consume per
153 day, and how often they consume fish and dried mushrooms per month. The remaining
154 questions documented sun exposure and sunscreen use.

155

156 *The LFFQ.*

157 Subjects also completed a LFFQ at baseline and at the follow-up visit. The LFFQ
158 is an eating survey designed by the Harvard Medical School, copyright ©1995 Brigham
159 and Women’s Hospital. The LFFQ was used as the gold standard against the SFFQ. The
160 LFFQ is a semiquantitative FFQ that consists of 7 food groups with 152 questions and
161 requests information on dietary intake over the past year.

162

163 *Data Analyses.*

164 In order to quantify the dietary intake of vitamin D, completed SFFQs were
165 analyzed using the Food Processor SQL (version 10.4.0, ESHA Research; Salem, OR) by
166 the Clinical Nutrition Department at the Children’s Hospital of Pittsburgh of UPMC.
167 LFFQ data forms were analyzed at Brigham and Women’s Hospital. Nutrient intake data
168 comprised 17 nutrients including total energy, dietary calcium, and dietary vitamin D.

169 For the current study, variables and data distribution were analyzed for normality
170 using the Kolmogorov-Smirnov test ($P \leq 0.05$). Normality testing revealed that most
171 anthropometric data were not normally distributed (Table 1). Mean values were used for
172 age and vitamin D intake while median values were used for weight, height and BMI.
173 The Wilcoxon Signed-Rank test was used to examine differences in BMI between the
174 initial and 6 month visit. The Mann-Whitney U test was used to determine if there was a
175 significant difference between BMI by race at each time point. A summary of SFFQ
176 responses for multivitamin, calcium supplement, and vitamin D supplement use, as well
177 as dairy, and vitamin D-fortified orange juice consumption was determined using

178 frequency statistics. Vitamin D intake (IU) was found to be normally distributed for both
179 the LFFQ and SFFQ for most groups. Pearson's correlation was used to determine the
180 correlation coefficient between the LFFQ and the SFFQ for vitamin D intake. The
181 correlation coefficient "*r*" indicates the strength of the relationship and the value can
182 range from -1.0 to +1.0. A correlation coefficient of near +1.0 implies perfect
183 correlation. Correlations were interpreted using the following guidelines: 0.0–0.3 as
184 weak, 0.3–0.6 as modest and 0.6–1.0 as good. Reliability testing was done using
185 Pearson's correlation between the LFFQ and SFFQ completed at the initial visit and
186 corresponding LFFQ and SFFQ completed 6 months later. Validity of the SFFQ and
187 LFFQ was evaluated by comparing mean vitamin D intake from the two LFFQs with the
188 two SFFQs. External validity of the SFFQ was evaluated via sensitivity, specificity,
189 positive predictive value (PPV), and negative predictive value (NPV) equations.
190 Sensitivity refers to the test instrument (SFFQ) and describes the proportion of
191 participants who were correctly identified as having met the American Academy of
192 Pediatrics' (AAP) recommendation for vitamin D (400 IU/day). Specificity also refers to
193 the test instrument, but describes the proportion of participants who were correctly
194 identified as not having met the AAP's recommendation for vitamin D. PPV measures
195 the probability that a person who tests positive for meeting the AAP's recommendation
196 truly consumed 400 IU/day. NPV measures the probability that a person who tests
197 negative for meeting the AAP's recommendation actually consumed less vitamin D.
198 Statistical analyses were done using IBM® SPSS® Statistics 18 (version 18, 2010, IBM
199 Corp, Armonk, NY).

200 RESULTS

201 The study sample ($n=140$) consisted of 67% African American ($n=94$). At the 6
202 month follow-up ($n=122$), the sample consisted of 68% African American ($n=83$). For
203 the 6 month visit, 18 participants were lost due to follow-up. At both time points, 58% of
204 the participants were boys. The majority of data is missing for one participant in the 6
205 month follow-up group and has been excluded from the data analysis. In African
206 Americans, BMI ranged from 13.8 to 35.3 kg/m² for the initial and 6 month visits, with
207 46% of observations above the 85th percentile at the initial visit and 50% at the 6 month
208 visit. For Caucasians, BMI ranged from 14.3 to 32.0 kg/m², with 25% and 28% of
209 observations above the 85th percentile at the initial visit and 6 month visit, respectively.
210 At the initial visit, 24% of African Americans were obese compared with 12% of
211 Caucasians. Six months later, 29% of African Americans were obese compared with 12%
212 of Caucasians. A significant difference in BMI was found between the initial and 6
213 month visits for Caucasians (16.3 vs. 17.1 kg/m², $P<0.01$) and African Americans (18.3
214 vs. 19.1 kg/m², $P<0.001$) (Table 1). There was a significant difference between BMI by
215 race at the initial and at the 6 month visit ($P<0.05$).

216 At the initial visit, 24% ($n=34$) of participants reported taking a multivitamin,
217 while 33% ($n=39$) reported multivitamin use at the follow-up visit. Only 1 participant
218 reported taking a vitamin D supplement at the initial and 6 month visit. Reported intake
219 of milk, dairy products, and vitamin D-fortified orange juice from the SFFQ is shown in
220 Table 2. Median milk intake per day for participants at the initial and six month visit was
221 two 8-oz servings of milk. Median daily cheese consumption was one 1-oz serving for
222 the initial visit and one and one-half 1-oz servings at the follow up visit. Median daily

223 intake of other dairy products (i.e. Lactaid[®], chocolate milk, yogurt) was minimal. Mean
224 vitamin D intake stratified by visit and race is shown in Table 3. Mean values for vitamin
225 D intake for the LFFQ were significantly lower than for the SFFQ at both timepoints
226 ($P<0.001$). There are intra-group differences between LFFQs and SFFQs at the initial and
227 6 month follow-up visits.

228 Associations between the LFFQ and the SFFQ for vitamin D intake by race and
229 visit timepoint are shown in Table 4. With the exception of African Americans at the 6
230 month visit, the associations between the 2 FFQs for vitamin D intake were significant
231 ($P<0.01$). For all pairs, the degree of association between the LFFQ and the SFFQ was
232 modest ($r=0.36$). After stratifying the population by race, the degree of correlation
233 between the LFFQ and the SFFQ was weak ($r=0.26$) for African Americans and modest
234 for Caucasians ($r=0.57$).

235 Reliability test results for vitamin D intake between the two LFFQs administered
236 6 months apart and the two SFFQs administered 6 months apart are shown in Table 5.
237 With the exception of African Americans for the LFFQs, the reliability of the 2
238 questionnaires is modest and is statistically significant ($P<0.01$) for all groups.

239 Association or validity between the average vitamin D intake from the two LFFQs
240 and the two SFFQs administered 6 months apart ($n=119$) was modest ($r=0.51$) and
241 significant ($P<0.001$). Probability statistics for predicting whether the SFFQ is able to
242 identify participants who have or have not met the AAP's recommendation for vitamin D
243 are shown in Table 6. The SFFQ was tested against the LFFQ for sensitivity, specificity,
244 PPV, and NPV. In this evaluation, 90% of participants were correctly identified as having
245 met the AAP's recommendation for vitamin D (400 IU/day). Of these participants, the

246 probability (PPV) that they actually consumed ≥ 400 IU/day was 0.78. This indicates that
247 the SFFQ is very sensitive and that the probability of the result being true is high.
248 According to the SFFQ, 64% of participants were correctly identified as not having met
249 the AAP's recommendation; the probability that these participants actually consumed
250 < 400 IU/day was 0.58. This indicates that the SFFQ is less specific than it is sensitive;
251 however, out of those who did not consume 400 IU/day, the likelihood of this result
252 being false is low.

253

254 **DISCUSSION**

255 This study was designed to evaluate the reliability and validity of a SFFQ in
256 determining vitamin D intake among adolescents using a LFFQ as a reference. There are
257 limited data available on adolescent behavior in regards to dietary consumption habits
258 (18,19). The adolescents' level of independence and their caretakers' unawareness of the
259 foods they consume further complicate assessing adolescent dietary consumption (29).
260 Developing a valid tool that accurately measures vitamin D consumption in children is
261 important in order to assess factors that optimize adequate vitamin D status. Shortening a
262 long FFQ to a targeted short FFQ would provide a quick and accurate estimate of the
263 nutrient of interest. Constructing and evaluating a less burdensome tool (the SFFQ) that
264 specifically targets a certain nutrient (vitamin D) is important in order to assess the
265 vitamin D status in a vulnerable segment of the population.

266 Rajakumar's initial study is significant because there are a limited number of
267 dietary assessment instruments that are specifically designed for assessing vitamin D
268 intake in adolescents (18,19,30). Accurately assessing the dietary intake of the adolescent

269 population is essential in order to monitor their nutritional status. Valid assessment is also
270 crucial for determining links between diet and health (31). It is important to evaluate the
271 effectiveness of Rajakumar's SFFQ because this instrument could be used in future large
272 epidemiological studies to estimate the vitamin D intake of an adolescent population.
273 Estimating vitamin D consumption in adolescents is important because of the significant
274 role this nutrient plays in establishing peak bone mass, as well as potentially preventing
275 or delaying adult-onset diseases (10,13). Since studies have shown that vitamin D
276 deficient status in US children is prevalent, it is important to have an instrument that will
277 quickly assess an adolescent's vitamin D intake. Subsequent intervention can take place
278 if subjects are found to be deficient.

279 For the total population in our study, the mean vitamin D intake, assessed by the
280 SFFQ, was 142 IU and 90 IU higher than the LFFQ at the initial visit and 6 month
281 follow-up visit, respectively. This is not surprising given that our SFFQ's primary focus
282 was on vitamin-D-rich foods, whereas the LFFQ assessed all foods eaten over a one year
283 period, not concentrating specifically on one nutrient. Despite the higher reported vitamin
284 D intake by the SFFQ, associations between the short and long FFQs ranged from modest
285 for the entire population ($r=0.36$) and weak for African Americans ($r=0.26$) to modest for
286 Caucasians ($r=0.57$). Using a 3-day diary for validation purposes, Marshall and
287 colleagues (21) found that vitamin D intake estimates were lower on targeted nutrient
288 questionnaires compared to food diaries. Our findings differ perhaps due to the open-
289 ended nature of food diaries, which allow a more comprehensive assessment of nutrient
290 intake and thus accumulate more nutrient-specific data. FFQs assess specific foods or
291 nutrients and may leave out a food that is routinely eaten by a participant.

292 At the 6 month visit, we found a weak relation ($r=0.19$) between the LFFQ and
293 SFFQ for African Americans. Kaaks and colleagues (32) report that, when compared to
294 normal weight subjects, obese subjects usually underreported food intake regardless of
295 dietary assessment technique used. Similarly, Bandini and colleagues (33) found that
296 obese adolescents underreported food intake significantly more than nonobese
297 adolescents. This could explain the weak relation seen in the African American segment
298 of our study.

299 Reliability was assessed by comparing vitamin D intake data from the same
300 questionnaire administered at two separate time points. Associations were weak for the
301 entire population for the LFFQs administered six months apart ($r=0.28$) and modest for
302 the entire population for the SFFQs administered 6 months apart ($r=0.33$). Validity was
303 assessed by comparing vitamin D intake data collected using different methods (LFFQ
304 and SFFQ) at two time points. Using unadjusted values of vitamin D intake, validity
305 testing revealed modest association ($r=0.51$). Based on sensitivity and specificity, it is
306 deduced that the SFFQ is more accurate in measuring the proportion of people who met
307 the AAP's recommendation for vitamin D than measuring the proportion of people who
308 did not meet the AAP's recommendation.

309 The United States Department of Agriculture's (USDA) MyPyramid recommends
310 2-3 cups from the milk group per day for children (34). In general, 1 cup in the milk
311 group is defined as 1 cup of milk or yogurt, 1.5-oz of natural cheese, or 2-oz of processed
312 cheese. The reported median intake our sample consumed from the milk group per day
313 was 3.05 cups at the initial visit and 3.65 cups at the 6 month follow up visit, which
314 slightly exceeds the USDA guideline.

315 This is the first study to assess vitamin D-only intake data from a SFFQ designed
316 specifically to measure vitamin D consumption in adolescents. Other studies have
317 assessed total macronutrient and select micronutrient intakes or types of foods eaten in
318 children (23-25,35); however, only 4 studies included relations between mean daily
319 vitamin D intake estimated from questionnaires and food records in children and young
320 adults (21,36-38). Taylor and colleagues (37) performed a study to determine the validity
321 of a FFQ for assessing calcium and vitamin D intake in 12-18 year old anorexic and
322 healthy girls. Researchers validated the FFQ with 4-day food records. They found that
323 subjects demonstrated greater compliance with the FFQ than with the food record. They
324 also observed a strong association ($r=0.78$) between daily vitamin D intake derived from
325 the FFQ and the food record. The researchers concluded that the FFQ was useful in
326 maximizing the information regarding calcium and vitamin D intake in a segment of the
327 population that is vulnerable to nutrient deficiencies. Our SFFQ had similar or better
328 validity than reported in other studies of one or more micronutrient-focused FFQs
329 (22,38,39). Hacker-Thompson and colleagues (39) compared 2 calcium-focused
330 questionnaires to 3-day diet records. They found modest association between the
331 questionnaires and the diet records ($r=0.37$). Harnack and colleagues (22) found good
332 reliability ($r=0.74$) of a calcium FFQ administered twice, with 1 week between
333 administrations. Validity showed a modest association between the calcium FFQ and the
334 dietary recall ($r=0.43$). Wu and colleagues (38) evaluated the validity of a FFQ for
335 assessing vitamin D and used a 7-day food diary for reference. Validity of the FFQ was
336 good ($r=0.60$).

337 Using a 3-day diary as a reference, Marshall and colleagues (21) investigated the
338 relative validities of the Iowa Fluoride Study questionnaire and the Block Kids' Food
339 Questionnaire in assessing vitamin D intakes. Children completed the Iowa Fluoride
340 Study nutrient questionnaire ($n=223$), the Block Kids' Food Questionnaire ($n=129$), and
341 3-day diaries during similar time periods. Associations between intakes estimated from 3-
342 day diaries and the 2 questionnaires were modest for vitamin D intake and virtually
343 identical to our validation findings ($r=0.51$). Study authors concluded that a dietary
344 assessment method that targets specific foods or nutrients can be as effective at
345 estimating intakes as a more comprehensive assessment tool. They also stated that it was
346 important to consider major dietary sources of the relevant nutrient and dietary habits of
347 the population of interest when designing a targeted nutrient tool. Using a 3-day food
348 record as reference, Marshall and colleagues (36) designed a study to evaluate the
349 validity of a beverage frequency questionnaire in assessing calcium and vitamin D
350 intakes. They found that their questionnaire could provide a relative estimate of vitamin
351 D intake. The validity of our vitamin D-focused SFFQ compared favorably with the
352 results of Marshall's beverage questionnaire. Researchers stated that as beverage intake
353 patterns change, with decreased milk intake and increased soft drink and energy drink
354 intake, this leaves fewer beverages contributing vital nutrients such as calcium and
355 vitamin D to the total diet. A study by Araujo and colleagues (24) estimated the validity
356 of dietary intake data from a FFQ designed specifically for adolescents living in Brazil
357 and demonstrated that their FFQ was a suitable tool for ranking adolescent's energy and
358 nutrient intake. Study authors stated that aiding tools such as food models and
359 photographs should be used to reduce bias when reporting food intake portions.

360 Estimating vitamin D intake in children is important because of the role of
361 vitamin D in various important biological functions that include accruing peak bone
362 mass. Increased melanin causes decreased cutaneous production of previtamin D₃, putting
363 African Americans at higher risk of vitamin D deficiency (40). For those who live ≥ 40
364 degrees north all year or ≤ 40 degrees from November to early March, there is
365 insufficient ultraviolet B (UVB) radiation from the sun for vitamin D synthesis (11).
366 Experts disagree about what constitutes vitamin D deficiency (1,3,9,10,13,14,16,41-42).
367 Proposed cutoff values for deficient 25(OH)D range from 10 ng/mL to 30 ng/mL.

368 In November 2010, the Institute of Medicine (IOM) revised the Dietary Reference
369 Intakes (DRIs) for vitamin D. The new recommendations include an EAR of 400 IU/day
370 for all age groups. The Recommended Dietary Allowance (RDA) is 600 IUs for persons
371 age 1-70 years (43). In a 2008 publication, the American Academy of Pediatrics (AAP)
372 revised their recommendation that all infants, children, and adolescents have a minimum
373 daily intake of 400 IU of vitamin D, up from 200 IU/day (13). In our total study
374 population using the SFFQ, 54% reportedly met 400 IU/day and 30% met 600 IU/day at
375 the initial visit. At the follow-up visit, these percentages reduced to 46% and 23%,
376 respectively.

377

378 **Vitamin D Insufficiency in Children**

379 Among the scientific community, there is mounting consensus that vitamin D
380 insufficiency is more widespread than originally thought (1-9). Research has shown that a
381 large percentage of adolescents living in the northern hemisphere are frequently vitamin
382 D deficient, particularly African Americans. Otherwise healthy children may not show

383 clinical signs of vitamin D deficiency, but when serum 25(OH)D concentration is
384 measured, vitamin D deficiency is present in many US adolescents. Using data from the
385 National Health and Nutrition Examination Survey (NHANES) 2001-2004, Kumar and
386 colleagues (1) analyzed data on children and adolescents and found that vitamin D
387 deficiency was common among 1-21 year old persons and was associated with adverse
388 cardiovascular risks. In this nationally representative sample of the U.S. population, 61%
389 (50.8 million) of the children had vitamin D concentrations between 15 and 29 ng/mL,
390 considered vitamin D deficient in this study. Only 4% of the children had taken 400 IU of
391 vitamin D/day for 30 days. In our study, only 1 child out of 140 reported taking a vitamin
392 D supplement on a regular basis. In Kumar's study, children with lower vitamin D
393 concentrations were girls, non-Hispanic blacks, obese, and those who spent more time in
394 front of a television, playing video games, or using a computer. Deficiency was also
395 associated with hypertension, low HDL concentration, and elevated PTH. Suboptimal
396 vitamin D status was also common among healthy, low-income, minority children in
397 Atlanta, GA (8). Cole and colleagues reported that 22% of the children in their study
398 ($n=290$) had vitamin D deficiency (≤ 20 ng/mL) and 74% had insufficiency (≤ 30 ng/mL).
399 Fortified milk provided most dietary vitamin D (62%), comparing favorably with our
400 study where milk was the most consumed source of dietary vitamin D. Cole and
401 colleagues concluded that vitamin D intake did not influence vitamin D status. At a
402 Boston primary care center, Gordon and colleagues (4) studied 380 pediatric patients and
403 found that 12% were vitamin D deficient (≤ 20 ng/mL) and 40% were below an accepted
404 optimal threshold (≤ 30 ng/mL). Multivitamin use was reported in 25% of subjects and
405 69% of subjects reported occasional consumption of fortified milk. Among the

406 participants who had vitamin D deficiency, 32.5% showed evidence of demineralization
407 after wrist and knee radiographic assessments. In light of growing data that support
408 vitamin D's immunomodulatory effects, study authors stated that their findings support
409 recommendations that vitamin D supplementation should be made available for all young
410 children. The Atlanta and Boston studies contradict the assumption that children living at
411 lower latitudes exhibit better vitamin D status than children living at higher latitudes.

412

413 **Food Frequency Questionnaires**

414 FFQs that assess nutrient intake in adolescents are uncommon. Although dietary
415 recalls and food records allow for a comprehensive evaluation of dietary habits, they
416 require a substantial commitment from the subjects which can lead to noncompliance and
417 increased dropout rates (21). Therefore, the FFQ has become the primary method for
418 assessing intake in large, population-based studies. The FFQ can also be specialized to
419 identify specific nutrient intake (44). The FFQ estimates food intake over a period of time
420 and is usually less expensive and takes less time to complete than a 24-hour recall and a
421 food record (18). Nutrition assessment instruments like the SFFQ in our study are easy to
422 administer, low-cost, simple to complete, reproducible, and accurate (19). The use of
423 FFQs can be a consistent and precise method for describing habitual dietary intake.
424 Before a FFQ is used in an epidemiological study, it should be validated to see if it
425 actually measures the aspect of the diet it was intended to assess (17). Although Klohe
426 and colleagues did not exclusively determine vitamin D status, they developed and
427 validated a FFQ for low-income children, and found that the FFQ yielded excellent
428 reliability and acceptable validity when compared to a 3-day diet record (45). This

429 demonstrates that FFQs can be reliable tools for assessing dietary consumption patterns
430 in youth.

431 Due to variations in adolescents' literacy levels and their limited knowledge of
432 foods, developing an assessment tool that accurately reports nutrient intake is challenging
433 (46). We were unable to adjust the analysis for energy intake because energy intake was
434 not mentioned in the SFFQ. However, no association was found between calories and
435 vitamin D for the LFFQ; we felt using the unadjusted values for the SFFQ was justified.
436 Another limitation was the relatively small sample size of our study, which may have
437 affected the statistical power for vitamin D intake assessment. In addition to the SFFQ
438 and the LFFQ, a 3-day food record was completed by fewer than half of the participants.
439 The food record was to be used to further validate the SFFQ. However, the data from
440 many of the food records were incomplete, and did not represent an accurate description
441 of macro- and micronutrient consumption. Therefore, it was determined not to use the
442 food record data in this secondary analysis.

443 Future studies should involve continued evaluation of this micronutrient
444 assessment instrument, especially among populations vulnerable to vitamin D deficiency.
445 Using biological markers such as 25(OH)D could improve the reliability and validity of
446 this instrument. As FFQs can provide valuable feedback, which is useful for evaluating
447 micronutrient policy initiatives, the SFFQ used in this study could be used in large-scale
448 surveys to monitor vitamin D intake in segments of the population at risk for deficiency.
449 Additional studies are needed to document the effects of screening otherwise healthy
450 children for and treatment of deficient vitamin D status.

451 **Conclusion**

452 The SFFQ is a reasonably valid and reliable tool that can be used to assess
453 vitamin D intake in adolescents. The SFFQ would serve well as a tool for identifying
454 children with low vitamin D intake who may benefit from a vitamin D nutrition
455 intervention designed for increasing vitamin D consumption.

456 **Tables**

457 Table 1. Mean age and median weight, height and body mass index measures by visit and
 458 race

Initial Visit	Total (n=140)	African American (n=94)	Caucasian (n=46)
Age (y) ^a	9.1 ± 1.7	8.9 ± 1.7	9.5 ± 1.7
Weight (kg) ^b	32.1 (26.8-39.4)	34.5 (27.5-39.1)	31.2 (24.9-41.6)
Height (cm) ^b	133.5 (126.8-140.8)	131.9 (127.0-139.8)	135.4 (126.5-145.6)
Body mass index (kg/m ²) ^b	17.8 (16.2-20.4)	18.3 (16.6-21.0)*‡	16.3 (15.4-19.2)**
6 Month Visit	(n=122)	(n=83)	(n=39)
Age (y) ^a	9.6 ± 1.7	9.5 ± 1.7	10.0 ± 1.8
Weight (kg) ^b	34.1 (28.8-42.8)	35.0 (29.0-42.7)	33.4 (27.8-45.7)
Height (cm) ^b	136.3 (129.5-143.2)	135.5 (129.5-142.2)	137.9 (128.2-145.6)
Body mass index (kg/m ²) ^b	18.2 (16.6-21.6)	19.1 (16.7-22.4)‡	17.1 (15.8-20.0)

459 ^aMean ± SD

460 ^bMedian (Interquartile range of 25%-75% of the study population)

461 *Comparison of median BMI between initial and 6 month visit ($P < 0.001$): Wilcoxon

462 Signed-Rank test

463 **Comparison of median BMI between initial and 6 month visit ($P < 0.01$): Wilcoxon

464 Signed-Rank test

465 ‡Comparison of BMI by race ($P < 0.05$): Mann Whitney U test

466 Table 2. Reported intake of milk, dairy products, and vitamin D-fortified orange juice
 467 per day from the short food frequency questionnaire

	n	Range	Median (25%-75%) ^a
Initial visit			
Milk (8 oz)	137	0.0 – 10.0	2.0 (1.0-3.0)
Soy, Lactaid [®] or chocolate milk (8 oz)	106	0.0 – 4.5	0.0 (0.0-1.0)
Cheese (1 oz or 1 slice)	133	0.0 – 8.0	1.0 (1.0-2.0)
Yogurt (1 cup)	129	0.0 – 5.0	0.05 (0.0-1.0)
Fortified orange juice (8 oz)	107	0.0 – 3.0	0.20 (0.0-1.0)
6 month visit			
Milk (8 oz)	119	0.0 – 10.0	2.0 (1.0-3.0)
Soy, Lactaid [®] or chocolate milk (8 oz)	92	0.0 – 4.0	0.0 (0.0-1.0)
Cheese (1 oz or 1 slice)	110	0.0 – 8.0	1.5 (1.0-2.0)
Yogurt (1 cup)	106	0.0 – 4.0	0.15 (0.0-1.0)
Fortified orange juice (8 oz)	92	0.0 – 4.0	0.35 (0.0-1.0)

468 ^aInterquartile range of 25%-75% of the study population

469 Table 3. Vitamin D intake for the long (LFFQ) and short food frequency questionnaire
 470 (SFFQ) by visit and race^a

Initial Visit	Total (n=140) IU	African American (n=94) IU	Caucasian (n=46) IU
LFFQ	332 \pm 180*	320 \pm 168*	357 \pm 201*
SFFQ	474 \pm 288	485 \pm 319	452 \pm 215
6 Month Visit	(n=122)	(n=83)	(n=39)
LFFQ	349 \pm 200*	334 \pm 185*	378 \pm 227
SFFQ	439 \pm 234	440 \pm 222	434 \pm 263

471 ^aValues are mean \pm SD

472 *Mean values for the LFFQ were lower than for the SFFQ ($P < 0.01$ to 0.001): Paired-
 473 Samples *t*-Test

474 Conversion equation: 40 IU = 1 μ g

475 Table 4. Association between the long (LFFQ) and short food frequency questionnaires
 476 (SFFQ) for vitamin D intake by visit and race

	n	LFFQ and SFFQ	
		r^a	P value ^b
Total	257	0.36	<0.001
African American	172	0.26	0.001
Caucasian	85	0.57	<0.001
Initial Visit			
Total	138	0.35	<0.001
African American	92	0.32	0.002
Caucasian	46	0.48	0.001
6 Month Visit			
Total	119	0.38	<0.001
African American	80	0.19	0.087
Caucasian	39	0.66	<0.001

477 ^aPearson correlation coefficient

478 ^bSignificance for r statistic

479 Table 5. Reliability test for vitamin D intake between two long food frequency
 480 questionnaires (LFFQs) administered 6 months apart and two short food frequency
 481 questionnaires (SFFQs) administered 6 months apart

	LFFQs ^a			SFFQs ^a		
	n	<i>r</i>	<i>P</i> value	n	<i>r</i>	<i>P</i> value
Total	119	0.28	0.002	120	0.33	<0.001
African American	79	0.09	0.428	80	0.31	0.006
Caucasian	40	0.52	0.001	40	0.41	0.008

482 ^aTest for reliability: Pearson correlation coefficient

483 Table 6. Probability statistics results for the short food frequency questionnaire (SFFQ)

	Total (258 pairs)
Sensitivity ^a	0.896
Specificity ^b	0.644
Positive Predictive Value ^c	0.776
Negative Predictive Value ^d	0.578

484 ^aProportion of participants who were correctly identified as having met AAP vitamin D
485 recommendation (400 IU/day)

486 ^bProportion of participants who were correctly identified as not having met AAP
487 recommendation

488 ^cProbability that a person who tested positive for meeting AAP recommendation truly
489 consumed ≥ 400 IU/day

490 ^dProbability that a person who tested negative for meeting the AAP recommendation
491 actually consumed < 400 IU/day

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