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Assessment of Omega-3 Fatty Acid Food Intakes and Their Association with Socioeconomic Status and Acculturation in Midwestern Latinas

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ASSESSMENT OF OMEGA-3 FATTY ACID FOOD INTAKES AND THEIR ASSOCIATION WITH SOCIOECONOMIC STATUS AND ACCULTURATION IN MIDWESTERN LATINAS

By

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Assessment of Omega-3 Fatty Acid Food Intakes and Their Association with Socioeconomic Status and Acculturation in Midwestern Latinas

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The purpose of this study was to assess the validity and reliability of a culturally appropriate food frequency questionnaire (FFQ) to measure total omega-3 (n-3) fatty acid, ALA, EPA and DHA intakes of Midwestern Latinas. In addition, the study examined the association of age, socioeconomic status (SES) and acculturation with intake of n-3 fatty acids. The n-3 FFQ was developed from preliminary interviews, analyzed for content validity and pilot tested. The final instrument containing 209 items (15 culturally-specific dishes) was tested with 162 first-generation Latinas. One-on-one interviews in Spanish were conducted to validate the FFQ. In addition, women provided sociodemographic information, and completed an acculturation questionnaire. The validity and reliability of the FFQ was assessed by Pearson correlation coefficient and Bland-Altman plots were constructed to assess agreement between the two methods and to increase the robustness of the validation. Correlation and regression analysis were conducted to test the association of age, SES and acculturation with n-3 fatty acid intakes. Mean daily intakes (±SD) of Total n-3, ALA, EPA and DHA (g) estimated by the mean of the two FFQs were 1.2±0.7, 1.1±0.6, 0.1±0.8, 0.1±0.1 respectively. Reliability of the n-3FFQ was 0.71 for Total n-3, 0.65 for ALA, 0.74 for EPA, 0.54 for DHA ($P < 0.01$). Validity correlation coefficients were 0.42 for Total n-3, 0.44 for ALA, 0.27 for EPA, and 0.24 for DHA ($P < 0.05$). Participant's age was negatively correlated with intakes of

Total n-3 and ALA. Education, income and acculturation were positively correlated with intakes of EPA+DHA ($P<0.05$). Participant's age was related to intake of Total n-3 and ALA after accounting for variation in education, income and acculturation. Income was related to intake of EPA+DHA when the other variables were taken into account ($P<0.05$). Findings from the present study suggest that the n-3 FFQ had acceptable reliability and adequate validity for the type of nutrients studied and may perform better to assess Total n-3 and ALA than to assess LC n-3 PUFA intakes in the study population.

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Introduction

Several studies have recognized the beneficial effects of omega-3 (n-3) fatty acids on cardiovascular health (1-5). Consumption of foods or dietary supplements rich in n-3 fatty acids lower the risk of coronary heart disease and protect against sudden cardiac deaths (6-8). An Adequate Intake of α-linolenic acid (ALA) has been set at 1.6 g/d for men and 1.1 g/d for women 19 y of age and older (9). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a minimum intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) combined of 500 mg/d for cardiovascular health (10). Food sources of ALA are flaxseeds, walnuts, and canola oil, while EPA and DHA can be found in seafood such as salmon, tuna, sardines, and poultry (11-13). Although greater benefit on cardiovascular health has been attributed to seafood sources of n-3 fatty acids compared to vegetable foods sources, explained in part by the rate of conversion of ALA to EPA and DHA in the body, controversy still exists (11-13).

Cardiovascular diseases (CVD) are the leading cause of death in the United States (US) (14). In Latinos living in the US, heart diseases account for almost 24% of the deaths in this population (15). Minority and low-income populations in the US suffer a disproportionate burden of chronic diseases such as heart diseases. Studies have reported that income and education, two indicators of socioeconomic status, account for an important part of the complex scenario of higher heart diseases in minority populations (16,17). Minority women are more likely to be in poorer health, suffer from premature death, disease, disabilities and face socioeconomic and cultural barriers that increase their chances for health disparities compared to Caucasian women (18).

Current intake of n-3 fatty acids for the US population based on data from the National Health and Nutrition Examination Survey (NHANES) 1999-2000 are 1.7 g/d and 1.3 g/d for men and women 20-59 y, respectively (19). Intakes of EPA and DHA range from 0.04-0.09 g/d and 0.03-0.07 g/d for mean and women 20-59 y, respectively (19). In contrast, lower mean intakes in the range of 1.1 to 1.2 g/d for ALA and 0.02-0.03 g/d for EPA and 0.05-0.06 g/d for DHA are reported for Mexican-American women 20 to 49 y (20).

Studies in minorities that assess the consumption of certain foods in which essential nutrients in high concentrations are found, such as the n-3 fatty acids, are limited. In addition, research has established that in minority/ethnic groups CVD risk factors are influenced not only by societal factors, but behavioral and cultural factors (21-24). Indeed, poor health practices in immigrants have been linked to the risk for chronic health conditions through a social mechanism called acculturation and migration to the new host country (25-26). Acculturation is defined as "the process by which immigrants adopt the attitudes, values, customs, beliefs, and behaviors of a new culture" (27).

Given the complex scenario of health disparities among minority and undeserved populations in part attributable to low diet quality, there is a need to investigate new methodologies for developing culturally appropriate assessment methods that will support effective dietary interventions in high risk populations towards behavior change, especially in minority women. Thus, the present study developed and tested a culturally appropriate dietary assessment method, a food frequency questionnaire, to assess n-3 fatty acid intakes in Latinas and examined intakes with societal and acculturation indicators.

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Objectives

The study had two objectives:

1. To assess the validity and reproducibility of a culturally appropriate food frequency questionnaire to estimate Total omega-3 fatty acid, α-alpha linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) intakes in urban Midwestern Latinas.
2. To examine the association of age, socioeconomic status and acculturation with intake of omega-3 fatty acids in urban Midwestern Latinas.

Review of Literature

1. Dietary Methods for Measuring Food Consumption of Individuals

There are two groups of methods to measure food consumption: quantitative daily consumption methods and retrospective consumption methods. Recalls or records designed to measure the quantity of the individual foods consumed over a 1-d or longer period are quantitative daily methods. "Usual" intakes of individuals can be obtained by increasing the number of measurement days of quantitative daily methods. Diet history and food frequency questionnaires are examples of retrospective consumption methods. These last two methods obtain retrospective information on the patterns of food consumption during longer periods of time than daily methods and can be used to assess the usual intake of foods (1).

1.1 Dietary or Food Records

For the dietary record approach, the respondent records the foods and beverages and the amounts of each food consumed over one or more days. Usually dietary or food records of not more than of 3 or 4 days are obtained when multiple and consecutive days are included in this methodology of dietary assessment (2), because of respondent fatigue (3). Typically, information is recorded on paper by the respondent and checked by the interviewer or nutritionist, but other methods such as dictaphones, computer recording and self recording scales have been used (4-7). Recently Di Noia et al. (6) developed a computer-administered instrument that potentially could be used for low-literacy groups in which the respondent selects the food consumed and the respective portion size via food photographs on the computer screen. The dietary, or food record, has the potential for providing quantitatively accurate information of food consumed during the recording period (1) because it lessens the problem of omission and the foods are fully described (2). Under and overreporting of macronutrient intakes using food or dietary records have been reported. In the 'Quality of Life after 65' study in Belgium, underreporting and overreporting occurred in 13.6% and 7.9% of food records, respectively, women were more likely to underreport energy intake, and men were more likely to overreport (8). Similarly, obese and overweight women increased their prevalence of underreporting energy intake after participating in a behavioral weight-loss self program. Intake was measured by 7-day food records and underreporting went from 39.7% at baseline to 60.3% after intervention (9). Food or dietary records can be classified as estimated food records and weighed food records.

1.2 Estimated Food Records

In this method, the respondent is asked to record, at the time of consumption, all foods and beverages eaten in household measures for a specified time period. Method of preparation, cooking and detailed descriptions of food and beverages are requested (1). Food portion sizes are estimated in standard household measuring utensils supplemented with rulers for foods such as cakes and meats. Unfortunately this method of measuring portion sizes may introduce errors as the respondent might quantify incorrectly and fail to convert volumes to weight correctly (1). Estimated records have been recently used to assess the effects of usual childhood dairy intake on adolescent bone health with data from the Framingham Children's Study (10), to analyze the dietary patterns of children with autism spectrum disorder (11), and to investigate iron intake and absorption in the diets of Swedish adolescents (12).

1.3 Weighed Food Records

The weighed food record is the most precise method available for estimating usual food and nutrient intakes of individuals. In this method, the respondent, or parent, or caretaker is instructed to weigh all foods and beverages consumed by the subject during a specific time period. Methods of preparation, description of foods, brand names of foods, and amounts of foods eaten at or away from home are recorded. Weighed records have been used in the National Diet and Nutrition Survey of British adults to examine the associations between the portion sizes of food groups (13), and to explore intake of diets with high a glycemic index (GI) or a low fiber content placing adults at risk for becoming overweight (14). The number, spacing and selection of days necessary to characterize the usual intake of food and nutrient intakes with weighed food records depends on the nutrient of interest, study population and seasonal variation of intake. It is advised that both weekend and weekend days should be included in the method. This type of food record requires the respondent to be motivated, numerate and literate. In addition, respondent burden for food records is higher than for 24-h recalls. Compared to estimated food records, weighed records have a greater reproducibility because portions sizes are weighed (1).

1.4 Dietary History

In 1947, Burke (15) introduced the dietary history as a method to evaluate intake in individuals. The dietary history method estimated the usual food intake and meal pattern of individuals over a long period of time. The time periods covered by the dietary history method vary, ranging from 1 month or less to up to a year (1,2), although the maximum time period has not been definitively established. Measurements of food intake over 1-y periods are probably unrealistic (1).

1.5 Twenty-Four-Hour Recalls

In this method, subjects or parents or caretakers, if the subject is a child or unable to provide information by himself, are asked by a trained interviewer to recall the subject's exact food intake during the previous 24-h period. It is generally accepted that a single 24-h recall is not sufficient to describe an individual's usual intake of food and nutrients, thus requiring multiple recalls over different days to achieve a usual intake (1). However, a single 24-h recall can be used to describe the average dietary intake of the group because the means are robust and unaffected by within-person variation (2). A fairly new method to estimate food and nutrient intake used in dietary methods to enhance recall and reduce respondent burden is the USDA-Automated Multiple Pass Method, a 5-step multiple-pass 24-h dietary recall method (16,17,18). This method has been used for the dietary collection entitled "What We Eat in America" in the National Health and Nutrition Examination Survey since 2002 (17). The 5-step multiple-pass approach consists of 1) Quick list (Collect a list of foods and beverages consumed the previous day), 2) Forgotten foods (Probe for foods forgotten during the Quick List), 3) Time and occasion (Collect time and eating occasion for each food), 4) Detail cycle (For each food, collect detailed description, amount, and additions. Review 24-hour day), and 5) Final probe (Final probe for anything else consumed) (19). In dietary assessment of population studies, any 24-h recall protocol needs to be standardized prior to use (1), particularly if the method is to be used in large national surveys and in comparisons across countries (20). Recall interviews are advised to be conducted in the respondent's home as the familiar environment improves the recall of foods consumed, and facilitates calibration of household utensil used (1), although telephone interviews are also used (21). The success of the 24-h recall depends on the subject's memory, ability of the subject to convey accurate estimates of portion sizes, motivation of the respondent and persistence of the interviewer (22). In contrast to record methods, dietary recalls occur after the food has been consumed, minimizing the potential for the assessment method to interfere with dietary behavior (2). Lately, two studies (23,24) have explored the validity of the 24-h recalls by comparing respondent's reports against the Goldberg equation (25,26) in which individuals are categorized as a low-or-high reporters based on their reported energy intake, basal metabolic rate, and physical activity. These studies found that only 60% of older, rural, low socioeconomic status women accurately reported their energy intake assesses by the 24-h recall method (24), and that 54.8% African-American preadolescent girls 54.8% were classified as underreporters. Nevertheless, the 24-h recall is a widely used method to report food and nutrient intake, accommodates to the responder food intake as it is open ended and allows for food, food source, and food processing specificity (27). In addition, 24-h recalls are useful for estimating intakes in cultural specific populations and does not require high literacy from participants (27).

1.6 Food Frequency Questionnaires

The food frequency questionnaire (FFQ) asks respondents to report their usual frequency of consumption of each food from a list of foods for a specific period of time (28,29). Information is collected on frequency and portion size of food eaten and overall nutrient intake estimates are derived by summing, over all foods, the products of the reported frequency of each food by the amount of nutrient in a specified serving to produce an estimated daily intake of nutrients (2). The underlying principle of the FFQ is that average long-term diet (intake over weeks, months or years) is the conceptually important exposure rather than intake on a few specific days (28), thus making the FFQ often used to measure the association of diet and disease (1). Commonly FFQs used in dietary research are the Health Habits Questionnaires or Block Questionnaires (30-34), the Harvard University Food Frequency Questionnaires or Willet Questionnaires (33-36), and The Fred Hutchinson Cancer Research Center Food Frequency Questionnaire (37,38). Other FFQs developed for specific populations will be covered later in this review of literature.

Food items included in a FFQ need to have three characteristics: first, the food must be used reasonably often by an appreciable number of individuals, second, the food must have a substantial content of the nutrient(s) of interest, and third, to be discriminating, the use of the food must vary from person to person (28). Several approaches can be used to compile a food list. It is good practice to start with examining published food composition tables and identify foods that contain a substantial amount of the nutrient of interest. Another approach is to start with a long list of foods that are potentially important nutrient sources and reduce the list systematically. A third approach to constructing a questionnaire is to use open ended dietary information as those provided by dietary recalls or diet records, to identify food that potentially can contribute to the absolute nutrient intake of the group as whole. The open-ended approach, although time consuming, has been supported by Willet (28) as an important contributor to nutrient intake because foods are unlikely to be missed, but not recommended by Gibson (1) as a preformatted lists of food categories in a FFQ act as a memory prompt. Lately, Block

(39) found that open-ended questions asked at the end of a FFQ administered in a large multiethnic cohort of women added little to nutrient estimates or rankings in an ethnic group.

An alternative to designing a questionnaire from the beginning is to use or modify an existing questionnaire. Willet (28) recommends that if the study population is somewhat different, such as an ethnic subgroup of a general population for which an existing questionnaire was designed, the addition of some foods to the existing questionnaire may be desirable; although the threshold for making changes to an existing questionnaire when the population is somewhat different is not clear. Among instruments that have been adapted from other questionnaires are a FFQ to assess the diets of Puerto Rican and non-Hispanic adults adapted from the National Cancer Institute/Block FFQ (40), a FFQ to assess the diets of Australians adults adapted from the Willet FFQ (41), and the Insulin Resistance Atherosclerosis Study (IRAS) (42) FFQ modified from the National Cancer Institute Health Habits and History Questionnaire to include ethnic and regional foods to assess the diets of a multicultural population cohort (43, 44). In addition, when developing a FFQ, the organization and structure of a food list is important in developing FFQs. Related items should be clustered together to maximize the comprehensiveness of a questionnaire while maintaining brevity by combining or collapsing several foods in single questions (28).

Because FFQs are intended to measure long-term dietary intake and for epidemiological purposes dietary intake over a number of years is the exposure of conceptual interest, FFQs tend to be the preferred dietary assessment method to describe the diets of populations over years (28) and its association with disease (45-47). The relevant reference period is a function of the physiology and pathophysiology of the outcome being studied and the metabolism of the dietary factor under investigation (28). Response formats in FFQs that address the reference period vary depending on the nutrients studied. An example of frequency distributions of responses is: never, once a month or less, 2-3 times a month, once a week, 2-4 times a week, 5-7 times a week, once a day, 2-3 times a day, 4 and more times a day (28). Another strength of FFQs is that they are relatively inexpensive to administer, are designed to be self-administered, although depending on the population studied other methods of administration can be used, may require 30-60 minutes to complete depending on the instrument and the respondent, and may cause less respondent burden compared to multiple diet records or recalls (30,37,48). Semi-quantitative FFQ, different from frequency questionnaires where portion sizes of the food items are not included, specify portion sizes of the food list in the FFQ (1,28). The general concept is to include portion sizes such as small, medium and large and ask the respondent their usual portion using food models (1), or providing pictures of different portion sizes (49,1). In addition, semi-quantitative FFQ are used in nutritional epidemiology because of its ability to rank subjects on the basis of their intakes, so subjects with low intakes, can be separated from those with high intakes (1,50-52). Thus, FFQ are not advised to be used for estimating quantitative parameters, such as the mean and the variance of a population's usual dietary intake (28,53-55) because of the error inherent in the food frequency approach (2).

Finally, the total intake of a nutrient can be calculated as the sum of the products of the frequency weight and the nutrient content for each food ($\sum$=frequency weight×nutrient content) (28). Frequency weights can be obtained from calculating the frequency of the food item eaten in the time frame that the FFQ assess (ie., once a month= 1, 2-3 times a month= 2.5).

2. Sources of Error in Food Frequency Questionnaires

Table 1 gives a summary of the potential sources of error and bias in estimating dietary intake using a FFQ.

Table 1. Sources of error or bias in dietary intakes estimates from Food Frequency Questionnaires

Source of Error	Type of Error
Participant	Memory Frequency judgments Question comprehension Response errors Portion size errors Social desirability bias
Questionnaire (investigator)	Food list Food groups Portion sizes Categorization of frequencies Poor design Database Data collection and programming errors Seasonal variation Unusual dietary patterns
Other	Intervention-associated bias

Adapted from Thompson FE and Subar AF (2).

Error in FFQ can be of two types: Random errors and systematic errors. Random errors refer to mistakes such as inadvertently marking the wrong frequency column, skipping questions, and lapses in judgment. Systematic errors refer to under or overreporting intake across the population and to person specific bias (2).

3. Validation and Reliability of Food Frequency Questionnaires

3.1 Reliability

Reliability refers to whether an instrument will measure an exposure (e.g., nutrient intake) in the same way twice at a sufficient time interval on the same respondents (1,2,28), and is assessed by comparing mean intake estimates from two administrations of a dietary method, in this case a FFQ in the same group of respondents. If the scores are reliable, the mean intake estimates should not vary substantially between the two administrations (2). The "test-retest" –an estimation of the correlation between two or more administrations of the same item (usually Pearson or Spearman coefficient) design has been used as the conventional approach to measure reliability in which the same assessment tool is repeated in the same subjects over the same period after a set interval (1). Time interval set to administered the second assessment varies depending on the nutrient investigated, but it is not recommended to administer the questionnaire at very short intervals (few day or weeks) as the subjects may remember their previous responses (1,28). Block and Hartman (56) recommend an interval time of 4 to 8 weeks for the second administration of the questionnaire.

The use of the "test-retest" approach to measure reliability in FFQ, although widely used, has some limitations. Reliability is a function of the true variation in daily nutrient intakes within individuals and random errors in measurement, thus efforts need to be made to reduce sources of random error to improve reliability of any dietary assessment method. Specifically, because FFQ are designed to assess the usual food intake of an individual over a long period of time, they are not sensitive to day-to-day intake variations (1). In general, the reproducibility of a FFQ may depend on the time frame of the method (e.g., seasonal variations), population group under study, nutrient of interest, technique used to measure foods and quantities and the between-and-within subject variation (1).

Several studies have developed, tested and examined the reliability of FFQs in diverse populations. Risica et al. (57) evaluated the reliability of the SisterTalk Food Habits Questionnaire (STFHQ) to measure fat intake using the test-retest reliability approach. After African-American participants completed the STFHQ 4 weeks apart the researchers found reliability correlations of 0.87. Villegas et al. (58) evaluated the reproducibility of a FFQ with 61,582 male residents of eight communities of urban Shanghai between the ages of 40 and 70 years after a time lapse of 1 year to measure their diets. Reliability correlations (Spearman) were moderate ranging from 0.39 to 0.64 for eggs and fruits. A short (10 items) FFQ to assess calcium intake in a multicultural sample of 11 to 14 year old children (n=248) in Minneapolis found that test-retest reliability assessed with intraclass correlations between calcium intake estimates derived from the first and second administration of the calcium FFQ was 0.74 (59). Willet et al. (36) evaluated the reliability of a self-administered, semi quantitative FFQ on 173 female nurses after a time lapse of 1 year. Willet found that mean daily nutrient intakes were similar. Intraclass correlation coefficient for nutrient intake scores, adjusted for energy intake, ranged from 0.49 for total vitamin A to 0.71 for sucrose.

In general, reported FFQs correlations for adults in the literature range from 0.5-0.8, depending of the study group and size, nutrient of interest and instruction associated with the instrument (1,2,28). Estimates of reliability give an upper bound to the accuracy of an instrument. Whereas a high reliability coefficient does not imply a high validity coefficient because high correlation can be the result of correlated error (systematic and within-person error), a low reliability coefficient means poor validity of the questionnaire and inability to measure long-term intake (2,28).

3.1.1 Statistical Assessment of Reliability of FFQs

- **Paired test of the mean or median intake**

  Paired t-test for normally distributed data or Wilcoxon matched-pairs test for non-normally distributed data are used to assess agreement between nutrient intakes of a group average. Non significant differences between the group mean or median intakes for the two dietary methods indicates satisfactory agreement and thus reproducibility (1). Harnack et al. (59) used paired t-test analysis to assess the reliability of a FFQ to estimate calcium intake in middle-school-aged children The researchers found that the calcium FFQ had a mean of 856 mg/d ($P<0.001$). Klohe et al. (60) reported using the Wilcoxon signed-ranks tests for comparisons of food group servings on time 1 and time 2 to assess the reliability of a FFQ for a triethnic population of children from low-income families. The confounding effect of the within-subject variation is not taken into account when a paired t-test or Wilcoxon matched-pairs test is used. In addition, when the within-subject variation is larger than the between-subject variation, the power of the t-test will be reduced and non-significant difference in the means may not indicate good reliability, but the confounding effect of large within-subject variation (61).

- **Correlation analysis**

  Correlation analysis for the test-retest method to assess reliability of a FFQ uses Pearson's product moment correlation for normally distributed data or Spearman's nonparametric rank correlation coefficients and/or intraclass correlation coefficients to assess agreement at the individual level (1). Ocké et al. (62) assessed the reliability of the Dutch European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ administered three times at 6-month intervals (1 y lapse) for different nutrient by using Pearson correlation coefficients between nutrient intakes energy, (macronutrients, dietary fiber, retinol, β-carotene, vitamin C, and vitamin E) assessed by repeated questionnaires. The correlation coefficients ranged from 0.70 to 0.94 among men (n=63) and from 0.59 to 0.94 among women (n=58). However, the same group of researchers (63) reported that when assessing the reliability of food groups (bread, fruit, fish, eggs, nuts and seeds, cereals, vegetable, potatoes, meat, cheese, milk and milk product, added fats sugar and sweet products, biscuits and pastry, raw vegetables, citrus fruit, and alcoholic and non-alcoholic beverages Spearman non-parametric rank order correlation coefficients between estimates of food group intake assessed by repeated questionnaires ranged from 0.45 to 0.92. Although the use of correlation analysis to estimate reliability of dietary methods is widely used, Gibson (1) advises that the correlation coefficient should be interpreted with caution and the use of other statistical measures to assess reproducibility

Finally, statistical methods for assessing reliability on a group average using a test-retest design include paired test of the mean (paired t-test) or the median (Wilcoxon matched-pairs, signed rank test) intakes. At the individual level, correlation analysis can be performed (1).

3.2 Validity

Validity refers to the accuracy of an instrument (2) and describes the degree to which a dietary method measures what it is supposed to measure (56). Validity can be absolute and relative. Absolute validity is determined in studies that involve a limited number of subjects or only cover a short period of time and observations of food intake during and either before or after of the study time frame are compared making the protocol difficult (1). Then, relative validity assessment of a dietary method is a practical approach to estimate the accuracy of the instrument and is defined as the comparison of the “test” method, in this case a FFQ, with another method, or called “reference” method (usually dietary recalls or records) which has a greater degree of demonstrated validity (1). Relative validity is also known as convergent validity (2) and in this type of study, bias is generally assessed by comparing the mean estimates from a FFQ to those of the reference method in the same respondents (2). This comparison allows determining whether nutrient intake estimates from a FFQ appear to be generally under- or overerreported in comparison to the criterion measure (64). In a convergent validity study, precision of the test method is the correlation coefficient between nutrient intake estimates from the FFQ and the reference method (2). The rationale behind convergent validity is that the major sources of errors associated with FFQs are independent of those associated with dietary recalls or records, which avoids high estimates of validity resulting from correlated errors (2).

3.2.1 Factors Involved in the Design of the Validation of FFQs

- **Selection of a population for a validation study**

  The subjects in a validation study should be a representative sample of the study population in which the questionnaire is being used (1,28). Examples of FFQs developed and validated for specific populations are the University of Arizona FFQ and the Southwest FFQ, to capture the diverse diet of Latinos and Native Americans (48,65,66).

- **The comparison method for the validation study**

  Diet records have been recognized as an optimal comparison method because the sources of error are independent of error of a dietary questionnaire. However, when cooperation or literacy of study subjects is limited, multiple 24-h recalls are the best alternative (28). Validation of FFQ studies that have used 24-h recalls as the reference method in ethnically diverse populations are a FFQ for assessing calcium intake in postmenopausal Vietnamese women (n=140) (67), a 47-item semiquantitative soy FFQ designed to measure the usual intake of soy isoflavones among Chinese women in Hong Kong (n=145) (68), and the EPIC semiquantitative FFQ developed and tested in a cohort of Dutch subjects (n=121) (62)

- **Appropriate time frame**

  It is important that the reference method chosen reflect the time frame of the test method. For instance, if variation in intake exists due to seasonality, then it is appropriate to have multiple measurements during the time frame chosen for the study. In addition, collection of data from multiple days per subject is essential because intake of food varies from day to day and to measure the true between-subject variation (28).

- **Sequence of data collection**

  Gibson (1) advises to administer the test method prior to the reference method in a validation study and that the test and reference method must be carefully spaced so that completion of the test method does not influence the responses to the reference method. This approach has been followed in many validation designs (58,62,67,68) but not other validation designs in which the reference method was collected after the test method (36,59).

- **Independent errors**

  Errors in the reference dietary method should be independent of those in the test method and also of the true intake (69).Therefore, the selected reference method must be different form the test method. To illustrate, errors associated with FFQs are a fixed food list imposed by a FFQ, perception of portion sizes and the challenge of accurately assessing food consumption over a broad time frame (2,28). These errors are minimally shared by reference methods such as 24-h recalls and diet records, which are open ended and do not depend on memory.

- **Socioeconomic status, ethnicity, and health status**

  Kristal et al. (70) reported that socioeconomic status (SES) and ethnicity affected the outcome of the validation of a FFQ. In this study, validity of the FFQ when compared to food records, as reference method, was lower among Blacks than among Whites, did not differ between Latinos and Whites, and was lower among women with fewer years of education. Similarly, Gary et al (71) reported that fat intake assessed by the Block FFQ (72) among African-American adults showed that people with more education and higher incomes had a higher average daily fruit intake and that older people, women, participants with higher SES and those who were physically active consumed healthier foods.

3.2.2 Statistical Assessment of the Validity of FFQs

Literature shows that there is not an uniform statistical method to assess the validity of a dietary instrument (73). Gibson (1) advises to use different validation methods and to cautiously compare results.

- **Pearson correlation coefficients**

  Correlation analysis is the most commonly used method to measure the strength of the relationship at the individual level, between the intakes of the test and the reference method (1). The data should be transformed, if its not normally distributed, to increase normality before the computation of the correlation coefficients. Because random errors and within-person variability weaken the correlations between a FFQ and the reference method (diet records or 24-h recalls) it is advised to calculate the deattenuated (e.g., corrected) Pearson coefficients to increase the correlations (28,74).

  To accomplish that, the variance ratio (ratio of within-subject (s^2_w) to the between-subject (s^2_b) variation) can be calculated. Different factors affect the variance ratio. Hartman et al. (75) and Palaniappan et al. (76) have reported that variance ratios depend on the nutrient, sample size, dietary methodology, age, gender, education and sociocultural group. Gender (76) affects the variance ratio for fat intake in Canadian women compared to men and was found to be higher (range: 2.56 to 2.95). In addition, nutrients found in high concentrations in few foods that are consumed occasionally, such as polyunsaturated fatty acids (PUFA), have larger variance ratios than other nutrients due to its high within subject variation making it more difficult to obtain precise estimates of the usual intakes of these nutrients for individuals (1). Beaton (74) has also reported high variance ratios for PUFAs (1.7 for males and 2.0 for females) compared to other nutrients (energy, protein, total carbohydrate, total fat and monounsaturated fats: 1 to 1.2). Several studies have reported the use of the variance ratio to improve correlation coefficients in the validation of FFQs (77,78).

- **Other correlations**

  Spearman rank correlations coefficients are used for nonnormally distributed data. Intraclass correlations are also used and thought to be a better measure of association than the Pearson coefficient. Values from intraclass correlations are normally less than for Pearson correlation coefficients and values above 0.4 indicate good agreement (1).

- **Test of means or medians**

  A paired t-test is used to examine if the means of the two dietary methods are statistically different, provided the data are normally distributed. The data should be normalized if nutrient(s) intakes are skewed before testing the means (1). If the data fails to be normalized by log transformation, the median and selected percentile should be used to describe intakes and their variability. The Wilcoxon's signed rank test for paired data can be used to test the comparability of the medians and thus the relative validity of the test method (1). Several studies have reported the use of the paired t-test to compare mean nutrient(s) intake between a FFQ and the reference method in Vietnamese women (67), rural population (79), United States (US) school children (57) and Koreans (80).

- **Mean and standard deviation of the difference**

  The use of the mean and the standard deviation of the difference between the test and the reference method for each nutrient has been supported by Bland and Altman (81) and widely used in FFQ validation designs (68,79,82,83). In the Bland-Altman approach, two plots should be drawn for each nutrient to validate the dietary method. First the results of the test method are plotted against those of the reference method and a line of equity is drawn. The plot will highlight outliners in the data and indicate bias in the test method. The second plot depicts the means of the test and reference intake for each subject plotted against the difference between each pair of observations. If there is not bias in the test method the differences will cluster along the horizontal line and the mean difference will be close to zero (1).

Finally, the approach taken in most studies is to examine the concordance of food frequency responses with multiple 24-h dietary recalls or diet records to estimate correlations between nutrient intakes measured by FFQs and the reference method. For most foods and nutrients, such correlations are in the range of 0.40–0.70 (34). According to Willet (84) correlation coefficients greater than 0.7 are rare and may suggest a “ceiling of validity” because as even with a major expansion in length of the questionnaire and careful attention to recent changes in the food supply, the results would not be appreciably better than those obtained using a considerably shorter and out-of-date questionnaire. Moreover this ceiling is probably related to the inherent complexity of diet that cannot be fully captured by a structured questionnaire (84)

4. Dietary Assessment in Latino Populations

Culturally diverse populations with variable dietary and disease patterns provide a unique opportunity to conduct epidemiology studies to identify the role of diet in the etiology of chronic diseases (85). Ethnic groups have different dietary patterns based on their geographical locations and cultural influences. (86). Although epidemiologic studies in culturally diverse populations are generally similar in design to those in a homogenous population, knowledge of eating patterns of each ethnic group in the study is needed to develop an appropriate dietary questionnaire (85). For instance, if the dietary method requires an interview, interviewers of the same ethnic or cultural background are preferable so that dietary information can be more effectively communicated (2). If dietary information is to be quantified into nutrient estimates, it is necessary to examine the recipes and assumptions underlying the nutrient composition of certain ethnic foods (87), making it necessary to obtain detailed recipe information for all ethnic mixtures reported (2) when constructing a dietary assessment method such as a FFQ.

Use of FFQs developed for the majority population may be suboptimal for many individuals with ethnic eating patterns (2,85,86). Many members of ethnic groups consume both foods common in the mainstream culture and foods that are specific to their own ethnic group. A food list can be developed either by modifying an existing food list based on judgment of the diet of the study population or by examining the frequency of reported foods in the population from a set of dietary recalls or records (2).

Specifically, research on dietary intakes of Latinos in the US has involved assessment of intake by the use of recalls and food records as well as the development of tailor-made FFQs aiming to characterize and provide individual and group intakes of this ethnic group. Examples of FFQs that have been validated for Latino audiences include the Block FFQ for Hispanics (87-89), The Southwestern FFQ developed by the University of Arizona (48), a FFQ validated among low-income Mexican Americans in Texas (90), and a FFQ for elderly Puerto Rican population (91). Other validation studies have included multiethnic populations such as Asian, Latino and non-Latino White youth (92), African-American and Latinos youth (93), and adult Latino, African-American and White women (70).

As described in this literature review, validity correlations coefficient of FFQs depend on many factors such as SES and education level. Fitzgerald et al. (94) reported that SES was positively related to the intake of fruits, vegetables, and meats assessed by a FFQ adapted for Latinos (95). Similarly, Kristal et al. (70) indicated that the validity of a FFQ to assess the feasibility of a low-fat intervention was lower among Latinas with fewer years of education. In addition, two studies (96,97) have reported lower validity correlation coefficients for their respective FFQs in Latinos compared to Blacks and Whites. Coates et al. (96) reported validity correlation coefficients of –0.02 between a fat intake FFQ and diet recalls in a sample of mostly Latinas. Likewise, Block et al. (97) reported very low correlations between a FFQ for use among participants in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) and three 24-h recalls in the Latino group. In this context, the development and validation of culture-specific FFQs needs to take into account a culture-specific food list, culturally defined portion sizes and be administered in a cultural appropriate context to successfully grasp the diets of the population study.

5. Omega-3 Fatty Acids

The omega-3 (n-3) fatty acids (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) make up a family of essential polyunsaturated fatty acids (PUFA) for humans indispensable for different physiological processes (98-101). In humans, ALA (18:3n–3) is the precursor and thus can be converted to the long-chain (LC) n-3 PUFA EPA (20:5n–3), DPA (22:5n–3) and DHA (22:6n–3) by alternating desaturation, elongation, and partial β-oxidation processes. (98,102,103).

The efficiency of the ALA conversion into the LC n-3 PUFAs has been researched widely (98,104-111). Some studies support the concept that enzymatic conversion efficiencies vary among species and are relatively inefficient in humans (98). Research has shown that more than 15-35% of dietary ALA is rapidly catabolized to CO_2 (104,105,106) for energy and that less than 1% is converted to DHA (107,108). In addition, it has been reported that the fractional conversion of ALA to EPA varies between 0.3 to 0.8% in males and the conversion of ALA to DHA in less than 4% or is undetectable in males (106,109-111). In females, the conversion to EPA (up to 21%) and DHA (up to 9%) seems to be more efficient (105). Contrary, the work of Barceló-Coblijn et al. **(**112) have found an increase in erythrocyte total phospholipid ALA (2 fold), EPA

(1.3-1.4 fold) and DPA (1.3 fold) in males after consumption of capsules containing either 2.4 or 3.6 g flax oil/d supplemented for twelve weeks. Similarly, the work of Harper et al. **(**113**),** showed that African-Americans fed 3 g ALA/d from flaxseed oil capsules for twelve weeks in a randomized, double-blind trial increased plasma EPA by 60% (from 24.09 ± 16.71 to 38.56 ± 28.92 μmol/L) and DPA by 25% (from 19.94 ± 9.22 to 27.03 ± 17.17 μmol/L) at 12 week. However, Plasma DHA levels did not increase.

The n-3 fatty acids are major structural components of membrane phospholipids of tissues and influence membrane fluidity and ion transports. These PUFA are rich in the brain, myocardium and retina, are essential for proper functioning and growth, and modulate many physiological processes (100). Another important feature of n-3 fatty acids is their role in the prevention of cardiovascular disease (CVD) (98,100,101,114-116). The strongest evidence of a relation between n-3 fatty acids and disease is the inverse relation between the amount of n-3 fatty acids in the diet and in blood and tissues and the occurrence of coronary heart disease (117). Mechanisms proposed to explain how EPA plus DHA might benefit cardiovascular health include prevention of arrhythmias, lowering plasma triacylglycerols, decrease of blood pressure, decrease in platelet aggregation, improving vascular reactivity and decreasing inflammation (118). In that regard, the beneficial effects of n-3 fatty acids on coronary heart disease have been shown in several epidemiologic and interventional trials (119-123). The existing controversy on the efficiencies of conversion of ALA to EPA and DHA in humans could be in part explained by the concept that consumption of food sources of EPA and DHA are associated with decreased cardiovascular death, whereas consumption of plant sources of ALA is not as effective (124,118), leading to recommendations of EPA and

DHA for CVD reduction (125). However, more research is needed in this area to draw conclusions.

The predominant food sources of n-3 fatty acids in the diet are vegetable oils and fish. Fish is the major source of EPA and DHA, and vegetable oils (canola, soybean, flaxseed/linseed, olive) are the major source of ALA. Other sources include nuts and seeds, vegetables and some fruit, egg yolk, poultry, and meat (126,127). Diets enriched with fish oil (128,129), extracts of algae oils (130,131), and mixtures of fish oil and linseed, canola and sunflower oil (132) have been fed to hens to produce enriched eggs as a source of n-3 fatty acids for humans. The National Academy of Sciences/Food and Nutrition Board has set Adequate Intakes (AI) for ALA for females ≥ 19 years at 1.1 g/d, and an Acceptable Macronutrient Distribution Range (AMDR) of 0.6-1.2 % energy for ALA for adults with up to 10% of this amount provided by EPA and DHA (99). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a healthy intake of ALA of 0.7% energy, and a minimum intake of EPA and DHA combined of 500 mg/d for cardiovascular health (125). The Food and Drug Administration classifies intake of up to 3 g per day of n-3 fatty acids from fish as GRAS (Generally Regarded as Safe) (133). A statement of the American Heart Association declared that people who have elevated triglycerides may need 2 to 4 g of EPA and DHA per day provided as a supplement, but cautions patients taking more than 3 g of supplemental n-3 fatty acids because it could cause excessive bleeding (120). The Dietary Guidelines for Americans 2005 report suggest consumption of two servings of fatty fish per week may reduce the risk of mortality from coronary heart disease (127), a dietary strategy that can lead to achieve the 500 mg/d recommendation given by ISSFAL (125).

6. Intakes of Omega-3 Fatty Acid Foods in Diverse Populations in the US

Dietary intakes of n-3 fatty acids in the US have been assessed either by a tailor-made n-3 FFQ (135) or by other dietary methods such as 24-h recalls, food records, or a previously validated FFQs to measure diet intake (136-140).

Literature reports that only one n-3 FFQ has been validated to measure intakes in cardiovascular patients in the US Midwest (135). The study reports a mean daily intake of 2.47±1.93 g assessed by the n-3 FFQ. Table 2 shows selected mean daily n-3 intakes in diverse populations in the US assessed by different dietary methods. Only one study (141) has reported the ALA, EPA and DHA intakes of Mexican-Americans living in the US.

Table 2. Mean daily omega-3 intakes in diverse populations in the US[a]

	Total n-3	ALA	EPA	DHA	Dietary method
			g		
Male (142)					24-h recall
20-39 y (n=635)		1.7±0.06	0.04±0.005	0.07±0.006	
40-49 y (n=577)		1.7±0.07	0.06±0.009	0.02±0.003	
Females (142)					24-h recall
20-39 y (n=635)		1.3±0.05	0.03±0.004	0.06±0.006	
40-49 y (n=577)		1.3±0.05	0.04±0.008	0.07±0.012	
Females (n=29) (143)		1.5±0.60	0.15±0.0333	0.047±0.047	Diet record
Mexican American females (141)					24-h recall
20-29 y (n=643)		1.2	0.02	0.05	
30-39 y (n=517)		1.1	0.03	0.06	
40-49 y (n=361)		1.1	0.03	0.06	
Midwestern pregnant females (n=30) (144)	1.060 ± 0.030				24-h recall
Physically active adults (n=12) (145)	0.887 ± 0.121				Food record
Eskimos (n=686) (146)	4.9±4.3	1.8±1.1			FFQ
Young children (n=404) (147)	1.18±0.58				Willet FFQ (28)
Male substance abusers (n=24) (148)	1.40±0.78	1.24±0.71			NIHDHQ FQ (149)
Males and females (n=87) (150)		3.3±2.1	$6x10^{-5}$	$1.5x10^{-4}$	FFQ
Females (n=32470) (151)	1.4				Willet FFQ (28)

[a]Mean ± standard deviation.

7. Omega-3 Fatty Acids Effects on Cardiovascular Disease and Risk in Women

CVD is the largest single cause of death among women worldwide, and in the US more women than men die every year due to this condition (152). In the US, 38.2 million women (34%) live with CVD, and the population at risk is even larger (153). The America Heart Association in its 2007 update of Evidence-Based Guidelines for Cardiovascular Disease Prevention in Women (152) suggested that “As an adjunct to diet, n-3 fatty acids in capsule form (approximately 850 to 1000 mg of EPA and DHA) may be considered in women with coronary heart disease, and higher doses (2 to 4 g) may be used for treatment of women with high triglyceride levels."

Albert et al. (154) examined the association between dietary intake of ALA assessed via a FFQ (155) and the risk of sudden cardiac death (SCD) and nonfatal myocardial infarction (MI) among more than 76,000 women participating in the Nurses' Health Study. ALA was the predominant n-3 fatty acid consumed, with intake ranging from 0.37% of total energy intake in the lowest quintile to 0.74% in the highest; corresponding percentages for combined EPA and DHA were 0.03% to 0.23%, respectively. The median absolute intake of ALA was 0.66 g/d in the lowest and 1.39 g/d in the highest quintile. During 18 years of follow-up, the intake of ALA was inversely associated with the risk of SCD but not with the risk of other fatal coronary heart disease or nonfatal MI, and risk of sudden cardiac death was 38-40% lower in the highest two quintiles of dietary intake.

Lopez-Garcia et al. (156) investigated the effect of n-3 fatty acid consumption in a sample of apparently healthy 727 female nurses (43-69 y) enrolled in the Nurse's Health Study with relation to endothelial activation, which is an early event in the development of atherosclerosis, and biomarkers of inflammation (C-reactive protein, interleukin-6 (IL-6), soluble tumor necrosis factor receptor 2(sTNFR-2), E-selectin, and soluble cell adhesion molecules (sICAM-1 and sVCAM-1). N-3 fatty acid intake was assessed by a validated FFQ (148). C-reactive protein levels were 29% lower among women in the highest quintile of total n-3 fatty acids intake (ng/d). Compared with the lowest quintile of intake, all the biomarkers but soluble tumor necrosis factor receptor 2(sTNFR-2), were lower (7% to 23% lower). The intake of ALA was inversely related to plasma concentrations of C-reactive protein and E-selectin after controlling for age, BMI, physical activity, smoking status, alcohol consumption, and intake of linoleic acid and saturated fat. EPA and DHA concentrations were inversely related to sICAM-1 and sVCAM-1. The researchers concluded that the association of n-3 fatty acids with lower levels of inflammation and endothelial activation might explain in part the effect of n-3 fatty acids in preventing CVD in this group.

A third study that included a larger subsample from the Nurse's Health Study (n=84,688, 34-59 y) was assessed longitudinally for sixteen years to investigate the association between fish and LC n-3 PUFA intake and risk of CHD (122). Fish intake was assessed by a validated FFQ (36). After follow-up, intake of LC n-3 PUFA was primarily from fish (87%), and secondary from chicken (7%) and liver (2%). Compared with women who ate fish <1 per month, those with a higher intake of fish (≥5 times a week) had a lower risk of CHD. After adjustment for age, smoking, and other cardiovascular risk factors, and trans-fat, fiber, and the ratio of PUFA to saturated fat, the multivariable relative risks of CHD were 0.79 for fish consumption one to three times per month, 0.72 for once per week, 0.72 for two to four times per week, and 0.69 for 5 or more times per week. The lower relative risk observed with higher fish consumption led to the conclusion that n-3 fatty acid intakes is associated with lower risk of CHD.

A study of predominately White postmenopausal women (n=229) participating in an Estrogen Replacement and Atherosclerosis trial examined the association between fish intake and the progression of coronary artery atherosclerosis in women with coronary artery disease with a mean age of 64 y (157). Usual fish intake was estimated with a FFQ

(36). The study showed that consumption of ≥ 2 servings of fish or ≥ 1 serving of tuna or dark fish per week was associated with smaller increases in the percentage of stenosis.

Research has also shown that not only frequency of fish consumption but type of fish meals may be related to cardiac benefits. Mozaffarian et al. (158) in a cohort study, assessed usual fish consumption at baseline among 3910 males and females aged ≥ 65 years and free of known CVD. Usual intake of "fried fish or fish sandwich (fish burger)," "tuna fish/tuna salad/tuna casserole," and "other fish (broiled or baked)" was assessed by a picture-sort version of the National Cancer Institute FFQ (159). Consumption of tuna and other broiled or baked fish (mean intake: 2.2 servings per week) correlated with plasma phospholipid LC n-3 fatty acids, whereas consumption of fried fish or fish sandwiches (mean intake: 0.7 serving per week) did not. After adjustment for potential confounders, consumption of tuna or other broiled or baked fish, but not fried fish/fish sandwich, was associated with lower risk of total ischemic heart disease death among all participants and with being female.

Patade et al. (160) investigated the effect of either 30 g of flaxseed/d, flaxseed and oat fiber consumption for a period of 3 months in the diet of mild to moderately hypercholesterolemic Native American postmenopausal women (n=55). Dietary flaxseed supplementation lowered total cholesterol (7%) and low-density lipoprotein cholesterol (LDL-C) (10%) blood concentrations. However, the concentrations of high-density lipoprotein cholesterol (HDL-C) and triglyceride remained unaltered.

The effect of flax seed supplementation on CVD markers in menopausal women has also been examined in the work of Dodin et al. (161). Women (n=179) were randomly assigned to consume 40 g daily of flaxseed or wheat germ placebo for twelve months. Several CVD markers (fatty acids, apolipoproteins A-1 and B, lipoprotein(a), low-density lipoprotein particle size, fibrinogen, C-reactive protein, insulin, and glucose) were measured at baseline and at twelve months. Flaxseed increased plasma ALA, DPA and total n-3 and raised apolipoproteins A-1 (4.4 % vs 11.6%) and B (3% vs 7%) moderately compared to wheat germ.

The beneficial effect of EPA and DHA supplementation on inflammation, and CVD risk in a group of overweight women was reported by Browing et al. (162). The study was a randomized crossover design, with two twelve-week intervention phases, separated by a four-week washout period. Participants in the treatment group ingested five 1 g oil capsules (1.3 g EPA and 2.9 g DHA) per day in the treatment phase or 2.8 g linoleic and 1.4 g oleic acid per day during the placebo phase. Women in the LC n-3 PUFA supplementation phase showed significantly higher plasma EPA and DHA and lower triacylglycerols at four and twelve weeks. Inflammatory markers: C-reactive protein and interleukin-6 were significantly lower after 12 weeks LC n-3 PUFA supplementation compared to baseline, thus lowering CVD risk.

8. Omega-3 Fatty Acid Intakes and Cardiovascular Disease in Latinos

Studies involving n-3 fatty acids food consumption of ethnic groups living in the US are limited. Currently, studies focusing on the n-3 fatty acid food behavior intakes of Latinos, and in particular Latinas, are lacking. Latino-Americans have been reported to have higher rates of obesity, elevated blood pressure, elevated triglyceride levels, dyslipidemia, and diabetes which in turn increases their risk factors and rates for CVD, different from other ethnic/racial groups (163). It has been widely reported that Latinos compared to Whites have more of the major contributing factors for CVD (164). For instance, greater odd ratios for heart disease (165) and lower awareness of heart disease as their leading cause of death compared to White women (152) have been reported in Latinas. Data from the third National Health and Nutrition Examination Survey (1988–1994) found that Black and Mexican-American women (65-84 y) had significantly higher prevalences of type II diabetes than White women making these groups at the greatest risk for CVD (166).

Mexican-Americans have been reported to have higher triglycerides and lower high-density lipoprotein cholesterol concentrations than Anglo Americans (167) and higher total fat, saturated fat and cholesterol intake than other Latino groups (168). In the same fashion, studies have indicated that Mexican-Americans have significantly lower knowledge than Anglo Americans about the role of eating a low fat diet in preventing a heart attack and are reported to engage in fewer behaviors specifically aimed toward coronary heart disease prevention compared to Anglo Americans (169).

For instance, dietary fat intake from fish, principal source of EPA and DHA, has been reported to be low in postmenopausal Latinas compared to postmenopausal White women (55). Low intake of EPA and DHA as reported in the frequency of fish consumption of 0.3±0.2 times/d has been found in Latinas living in Connecticut (170). Contrary, Latina college students had higher fish intake (median 2.8 servings/week) compared to their White and Black counterparts (56). The Dietary Intake of Macronutrient, Micronutrient and other Dietary Constituents in the United States, 1988-1994 reported that intakes of ALA, EPA and DHA of Mexican-American women 20 to 49 y were in the range of 1.1 to 1.2 g/d, 0.02 to 0.03 g/d, and 0.05 to 0.06 g/d, respectively (33). These intakes seems to achieve the AI for ALA recommended for women ≥ 19 y by the National Dietary Reference Intakes (99), but fails to meat the 500 mg/day of EPA and DHA combined for CVD reduction (125).

Research has established that in minority/ethnic groups CVD risk factors are influenced by behavioral, cultural and societal factors (171-174). In addition, a recent study has reported that Latino ethnic groups living in the US have different risk factors and prevalence for subclinical CVD (163). For instance, in Dominican Americans, hypertension was highly prevalent compared to Mexican, Puerto Rican, and other Latino Americans, and Mexican-Americans risk factor profile were found to have the highest levels of nearly all of the measures of subclinical CVD (163). It is clear that the risk factors and morbidity prevalence associated with the development of CVD in Latinos is a heterogeneous and complex scenario that needs to be addressed properly into a cultural framework. Moreover, there is a need for more studies that address correlation factors such as education, knowledge, SES, acculturation and beliefs regarding the n-3 fatty acid food intakes of Latinos and in particular Latinas.

9. Validity and Reliability of FFQs to Measure Omega-3 Fatty Acid and Fish Intakes

Several studies have assessed n-3 fatty acids and fish intakes in different populations (136, 139,146,175-177) by using validated FFQs, but not many studies have developed and validated a food frequency method that specifically assesses fish or n-3 fatty acid intakes.

In that regard, two studies have validated FFQs to measure fish consumption in the Australian population (178,179) and three have looked into the validation of a FFQ that assesses LC n-3 fattty acids in Australians (83,180) and total n-3 fatty acid intakes in US Midwestern cardiac patients (135).

Woods et al. (178) validated a semiquantitative FFQ for measuring fish intake originally developed to assess dietary intake in the Australian population and modified it to indicate the frequency of steamed, grilled, baked, fried and tinned fish consumption. The study used plasma fatty acids as biomarkers of validation in a cohort of 174 adults (26-49 y). The median total daily intake of fish was 40 g/d, the intraclass correlation test for reliability was 0.62, 0.35, 0.63, 0.60 and 0.68 for steamed, grilled, baked, fried, tinned, total and total non-fried fish, respectively. Plasma n-3 and DHA fatty acids were significantly correlated with all types of fish except fried fish (n-3 range: 0.20 to 0.33, DHA range: 0.27 to 0.34), and EPA concentrations were significantly correlated with intakes of steamed, grilled or baked fish and total non-fried fish (EPA range: 0.16 to 0.20).

Another FFQ designed to measure habitual fish and seafood consumption has been validated in a sample of 91 Australians (21-75 y) by Mina et al. (179). The questionnaire included different species of fresh fish, processed fish and seafood, fresh seafood, n-3 fortified foods and supplements. Mean servings per week of fresh/frozen fish were 2.16, processed fish and seafood 1.35, seafood 0.60, and fortified foods (n-3 fortified eggs, bread, milk and margarine) 0.40. Validity of the FFQ was assessed with linear regression techniques. Variation in n-3 fatty acids was best accounted for by a model containing variables representing different categories of fish and seafood consumption (R^2 0.484 for EPA and R^2 0.203 for DHA).

Sullivan et al. (83,180) developed a FFQ to estimate LC n-3 fatty acids for the Australian population. The researchers validated the FFQ against food records (83) and red blood cells and plasma LC n-3 PUFA (EPA, DPA and DHA) as biomarkers (180) separately with 53 participants. The 28-items FFQ contained the following food groups: fish and seafood, meats, fat spreads, dairy, eggs, including LC n-3 PUFA-fortified products such as breads, milks, margarine and eggs. Fish oil capsules were also included in the questionnaire. In the biomarker validation, average daily intake (mg/d) of Total LC n-3 PUFA, EPA, DPA and DHA were 214±203, 61±59, 45±41, and 109±118, respectively. The validation test showed significant Spearman's correlation coefficients between the FFQ intakes and red blood cells, and FFQ intakes and plasma fatty acids for Total LC n-3 PUFA, EPA, and DHA were 0.50 and 0.54, 0.39 and 0.54, and 0.40 and 0.48, respectively. DPA was not significantly correlated.

In a dietary validation study, Sullivan et al. (83) tested the FFQ against 3 day weighed food records and determined its reproducibility assessed 4 to 6 weeks apart. Median intake (mg/d) of the FFQ and food records for Total LC n-3 PUFA, EPA, DPA and DHA were 212 and 234, 58 and 65, 48 and 40 and 108 and 125, respectively. Spearman correlation coefficients between intakes estimated using the FFQ and food records for LC n-3 PUFA, EPA, DPA and DHA were 0.75, 0.64, 0.62 and 0,72, respectively. Reproducibility of the FFQ assessed by Spearman correlation coefficients for intakes of LC n-3 PUFA, EPA, DPA and DHA were 0.88, 0.88, 0.90 and 0.87, respectively.

In the US, a quantitative FFQ to measure Total n-3 fatty acid intakes in cardiac patients was validated against three 24-h recalls and tested for reliability (135). The 152-item FFQ was developed from foods that provided equal or more than 10 mg/d of n-3 fatty acids per medium serving. Food groups in the FFQ were seafood and fish, meat, eggs, dairy products, vegetables, fruits and fruit juices, nuts and seeds, breads and cereals, fats and oils, legumes, and herbs and spices. Mean daily Total n-3 fatty acids intake assessed by the recalls was 1.76±1.95 g with a range of 0.21 to 8.15 g/d. Mean daily Total n-3 fatty acids intake assessed by the first FFQ was 2.47±1.93 g with a range of 0.34 to 7.64 g/d. Pearson correlation coefficient for the FFQ and the recalls was 0.42 ($P<0.05$) and paired t-test indicated no significant differences between the two methods. In the reliability part of the study, the α coefficient for Total daily n-3 intake was 0.83.

In summary, n-3 fatty acid intakes assessed by a FFQ specifically developed for that purpose and tested against a reference dietary method have shown validity correlations for Total n-3 of 0.42, and 0.75 for Total LC n-3 PUFA. Correlations for EPA, DPA and DHA were 0.64, 0.62 and 0.72, respectively. Only one study (83) has validated a FFQ for LC n-3 PUFAs. Reliability correlation coefficients have been reported to be 0.83 and 0.88 for Total n-3 and LC n-3 PUFA, respectively, and 0.88, 0.90 and 0.87 for EPA, DPA and DHA, respectively.

10. The role of Acculturation and Diet in Latinos

The ethnic category Hispanic or Latino is defined as “a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term “Spanish-origin” can also be used in addition to “Hispanic or

Latino" (181). Latinos will represent 25% of the US population by the year 2050 (182). Dietary intake is an important determinant of chronic health conditions among Latinos in the US and a healthful diet is important in chronic disease management (183). It has been reported that the link between poor health practices and risk for chronic health conditions is the social mechanism called acculturation process and migration (183,184).

Acculturation is defined as "the process by which immigrants adopt the attitudes, values, customs, beliefs, and behaviors of a new culture" (185) It is a long term and complex process (186) that can be defined both at the societal and the individual level (187). The process of "individual acculturation involves the accommodation to a host culture on the part of a member of a migrant culture" (187). Proxy indicators of acculturation used in research of diet intakes and Latinos are birthplace, language use, and number of years in the US (188). In addition, several acculturation scales have been used in research with Latino populations (189-194).

Reviews on the process of acculturation and diet among Latinos affirms that greater acculturation to the US nutritional habits is associated with less healthful dietary intake and dietary behaviors (188,195), while other studies conclude that being less acculturated is associated with poorer dietary habits (196,197). Different studies have examined the influence of acculturation on food habits and/or nutrient intakes among Latinos (198-202) however, this review of literature will only cover the association of acculturation and dietary fat intake in both male and female Latinos as studies with only Latinas is limited.

Neushouser et al. (199) examined the effect of acculturation on fat intake among Mexican-Americans (n=735) by administration of a four-item acculturation scale that included questions about language most often spoken, language most often used for thought, ethnic identification of self and birthplace of self (194) and the Fat-Related Diet Habits questionnaire (203,204). The researchers found that highly acculturated Latinos had slighter higher, but not statistically significant, scores of fat intake compared to low acculturated Latinos and that the fat changes made in acculturation were adding fat at the table to bread and potatoes.

Increased acculturation among Mexican-American women was found to be associated with a higher percentage of energy from fat intakes in a sample of 1,245 women of Mexican descent (Mexican born and US born) (205). Acculturation was measured by the proxy country of birth and the Short Acculturation Scale developed by Marin et al. (189) Mexican-born women had lower energy from fat intakes compared to US-born women. Hence, Mexican-born women predominately Spanish-speaking and predominately English-speaking had percentage of energy from fat of 31.9±4.1 and 32.1±3.0, respectively, compared to US-born women predominately Spanish-speaking and predominately English-speaking whose percentage of energy from fat was 33.5±4.7 and 33.6±3.9, respectively.

Gregory-Mercado et al. (206) in a cross-sectional study reported the association of acculturation, measured by five acculturation items from the Acculturation Rating Scale for Mexican-Americans (ARSMA) (191) and total fat intake measured by 24-h recalls in a sample of Mexican American women (n = 260). The researchers reported no significant association between acculturation status and total fat intake.

A positive association of acculturation and intake of dietary fat was reported by Winkleby et al. (207) in a sample of White and Latino youth and adults. The acculturation proxy for Latinos used in the study was primary language spoken at home. In this study, although White adults were significantly more likely than Latino adults to have eaten high-fat foods in the last 24 hours, more acculturated Latino (English-speaking) were intermediate between less acculturated Latino (Spanish-speaking) and Whites in their dietary intake.

Mazur et al. (208) also reported a positive association between acculturation measured by primary language spoken by the parents and dietary fat intake in a national sample of youth from the third National Health and Nutrition Examination Survey (1988–1994). There was no significant difference in the percentage of energy from fat between male and female youths. Parents' exclusive use of Spanish at home was associated with lower intakes than was their use of both Spanish and English or English only for percentage of energy from fat.

Polednak (209) reported differences in gender, acculturation and dietary fat intake in a sample of Latinos in Connecticut. Acculturation was measured by using four items from the ARSMA (184) regarding language (English or Spanish) spoken by the respondent, language preferred to speak, read better and wrote better. Fat intake was assessed by the 13-item brief screen for dietary fat by Block et al. (210). The study reported mean daily fat intake (g) higher for males (32.9±1.0) compared to females (29.5±1.1) and the acculturation score had positive association with fat intakes in males but not in females. Food items that contributed most to fat intakes were whole milk for both genders and hamburgers, ham and cheese for males and hot dogs, eggs and margarine for females.

A review of the relationship between acculturation and diet among Latinos by Ayala et al. (183) concluded that across several studies, dietary fat and percentage of energy from fat was not associated with acculturation, despite evidence that fat related behaviors seems to differ between less and more acculturated Latinos. It is summarized that less acculturated Latinos consume more milk, use fat in food preparation, consume more fruit, rice and beans and less sugar and sugar-sweetened beverages, whereas more acculturated consume more fast foods, snacks and added fats (183).

Summarizing, it is evident that acculturation influences health behaviors and outcomes; however, acculturation is not the only social mechanism that accounts for dietary behavior intakes in Latinos. Other factors such as SES that account for more education, employment status, income, neighborhood environment (183), and psychological status (e.g., depression) (188) need to be accounted for when drawing relationships on the acculturation process and dietary choices of Latinos in the US.

11. Association of socioeconomic status with diet quality and the risk for heart disease in Latinos

Socioeconomic position, or SES, is typically operationalized as education, income and education (211,212). These three social indicators affect diet by different pathways. Education may influence food choice by affecting an individual’s ability to understand the information transmitted, occupation may affect diet through social networks and work-based cultures, and income influences dietary quality by making healthy foods affordable (213). Several studies have examined the association of operators or indicators of SES with food intake and diet quality in different populations (71,214-222). Higher single indicators of SES (education, income, employment), or a combination of them, have been linked to higher consumption of fruits, vegetables, grains, (214-219) fiber and micronutrients (220), fish (221), and PUFA (222) in different populations. Thus, individuals with lower SES compared to those with higher SES are more likely to have poorer diet quality (219).

Shimakawa et al. (220) examined dietary intake of several nutrients assessed by a FFQ in relation to race, gender and educational attainment in more than 15,000 middle age Black and White participants. Researchers found that higher educational attainment was associated with lower energy intakes of meats, eggs, chicken with skin, and whole milk and higher intake of foods such as fruits, vegetables and fish. The study found that fish was consumed infrequently especially among men, but participants with higher educational attainment had greater consumption of fish.

The contribution of certain foods to the development of a potential atherogenic diet and its relation to age, gender and education was studied in a multiethnic population of White, Black and Latinos living in New York State (214). Participants' diets were evaluated by a FFQ designed to assess intake of saturated fat and cholesterol. Results of the study revealed that consumption of beef and whole milk tended to be greater among Latinos than among Whites and Blacks, and fruit consumption was higher among females, better educated and older participants. Compared to Whites and Blacks, Latinos had the lower level of education (38% not completing high school).

Xie et al. (222) examined the effect of gender, ethnicity, income and parent's education on dietary patterns of 11-20 year-old adolescents in California, US. Dietary intake was assessed by a validated FFQ in a multiethnic sample of more than 3,000 youth. Boys had significantly higher intake of energy, total fat, saturated fat, monounsaturated fat and calcium. Latinos and Asians had significantly higher cholesterol intakes than Blacks and Whites. Intakes of PUFA, protein, calcium and iron were increased with family income, while intake of total fat, saturated fat, monounsaturated fat and cholesterol decreased significantly with education levels of the parents. An interesting finding was that fish comprised 7% of the meat and main dishes consumed among all youth. The researcher concluded that adolescents from families that had higher educational attainment were more likely to meet the recommendations of dairy, fruits and vegetables intake.

A negative association between income and diet quality among first (n=475) and second (n=898) generation Mexican-American women, and White Women (n=2326) has been reported by Guendelman and Abrams (223). Dietary intake was assessed by a single 24-h recall to evaluate intake of several nutrients. First-generation Mexican American women where the least educated and the poorest, the least likely to work for earnings, and least likely to perceive their health as excellent or very good. The researcher found that less acculturated women consumed more protein, carbohydrates, vitamins and calcium, and while income did not have an effect on diet quality on second generation Mexican Americans, it had an opposite effect on first-generation women compared to White women. Food choices of first generation Mexican-American women deteriorated as income increased explained by the women having fewer adherences to the traditional Mexican diet.

A study to describe the association of diet and SES (education and occupation) was conducted in Switzerland with 35-74 year old males and females (224). Dietary intake was assessed with a FFQ developed for the target population. In females, lower education (9-12 y of school or less vs. ≥13 y of school) and lower occupation (non-manual and manual occupation vs. professional profession) were significantly associated with lower intake of fish and vegetables and higher consumption of fried foods.

Specifically in Latinos, different indicators of SES have been associated with intake of foods and nutrients (70,225). Dubowitz et al. (225) in a study that evaluated the SES of the neighborhood and its association with fruit and vegetable intake in a multiethnic sample of low-educated, low-income Whites, Blacks and Mexican American found that neighborhood SES was positively associated with combined fruit and vegetable intake, fruit but not vegetable intake in Mexican Americans. Fruit and vegetable intake did not differ significantly between Mexican Americans and Whites, but did between Blacks and Whites. The researchers concluded that socioeconomic characteristics of the neighborhood where individuals live may be one of the mechanisms through which neighborhood SES affects health.

Fitzgeral et al. (94) reported that in Latinas with and without type 2 diabetes, higher SES, assessed by educational attainment, was a determinant of greater nutrition knowledge (knowing about MyPyramid), but not food label use. The researchers concluded that Latinas with greater nutrition knowledge were more likely to use food labels suggesting that the association between education attainment and food label use could be explained by nutrition knowledge.

Mazur et al. (208) examined the influence of acculturation and socioeconomic variables on the diet of households in which US Hispanic/Latino youths lived. Household food insufficiency decreased with less acculturation and increased with low income. The households in which the Hispanic/Latino youths lived were more likely to be disadvantaged in socioeconomic terms and to have food-related problems than were the households of non-Hispanic Whites. Households in which Spanish was the only language spoken, had higher poverty indexes compared to households where only English or Spanish and English were the language spoken. Several indicators of low SES (low education, low income, higher poverty index ratio, less likely to be employed in semi-skilled, skilled, or professional occupations compared to non-Hispanic Whites) were associated with an increase in the risk of food insufficiency. Poorer Spanish-speaking-only households had higher intakes of energy, protein, and sodium.

There is an agreement that an individual's SES is a strong predictor of the person's mortality and morbidity (226,227). Research has established that in minority/ethnic groups CVD risk factors are influenced by behavioral, cultural and societal factors (171-174). Income and education, two indicators of SES, are recognized underlying factors that affect the rate of heart diseases in minorities and low-income populations increasing their mortality rate for CVD (228). Minority women are more likely to be in poorer health, suffer from premature death, disease, disabilities and face socioeconomic and cultural barriers that increase their chances for health disparities (229). For instance, immigrant Latinas have been reported to be more likely to receive less information about heart disease prevention due to language barriers, lack of access to health care and cultural role expectations (230,231). Greater odd ratios (165) and lower awareness of heart disease as their leading cause of death compared to White women (152) have been reported in Latinas. Although White and Black females have higher prevalence for hypertension and CVD, Mexican-American women have a prevalence of age-adjusted hypertension of 21% (232) and CVD of 34% (233).

Similar results on the effect of SES with multiple risk factors for heart disease in Latinas have been reported by Hayes et al. (165). The study found that prevalence of risk factors increased with age, decreased with educational attainment, income and employment among Black, Native American, Latino and White women. Latinas had lower multiple risk factors for heart diseases compared to White, Black and Native American women after adjusting for age, income, education and health coverage.

A sample of Black, Mexican-American and White women from the third National Health and Nutrition Examination Survey 1988-1994 were examined for the association of SES (educational attainment) and CVD risk factors (234). CVD risk factors (body mass index (BMI), systolic pressure, leisure-time physical activity, smoking, diabetes) were higher among Black and Mexican-American women. After adjusting for years of education, significant differences in blood pressure, BMI, physical inactivity and diabetes remained for Mexican-American women. Mexican-American women with higher education (> 12 y of school) had lower CVD risk factors compared to the women with lower education (< 9 y of school).

Several epidemiologic and interventional studies have demonstrated the beneficial effects of n-3 fatty acids on CVD (119-123). An Adequate Intake for ALA for females ≥ 19 y (99), and a minimum intake of EPA and DHA combined of 500 mg/d for cardiovascular health (125) have been recommended for the general population. Recommended intakes for ALA can be achieved by consuming flaxseed, flaxseed oil, walnuts, and canola oil, while consumption of two fatty fish meals a week is advised as a strategy to achieve recommended intakes of EPA and DHA (235). Ethnic minorities in the US because of the several factors affecting their diet quality may be at higher risk for not meeting dietary recommendations that consequently could increase their odds for several diseases such as CVD. For instance, lower consumption of fish with low SES of the individual has been reported in Latinos. Fitzgeral et al. (236) reported an association of low SES with less frequency of fish consumption in Puerto Rican women. Similarly, low intake of EPA and DHA as reported in the frequency of fish consumption of 0.3±0.2 times/d has been found in Puerto Rican females living in poverty in Connecticut (170).

Studies involving n-3 fatty acids food consumption of ethnic groups living in the US are limited. Currently, studies focusing on the n-3 fatty acid food behavior intakes of Latinos and in particular Latinas are lacking. Latino Americans have been reported to have higher rates of obesity, elevated blood pressure, elevated triglyceride levels, dyslipidemia, and diabetes which in turn increase their risk factors and rates for CVD, different from other ethnic/racial groups (163). Given the multiple environmental factors that affect food intake and thus diet quality in an individual, there is need to improve the understanding of how societal and cultural factors affect the risk for CVD in minority women.

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Manuscript I

Validity and Reliability of a Food Frequency Questionnaire to Measure Omega-3 Fatty Acid Intakes in Midwestern Latinas

Abstract

The purpose of this study was to assess the validity and reliability of a culturally appropriate food frequency questionnaire (FFQ) to measure total omega-3 fatty acid (Total n-3), ALA, EPA and DHA intakes of Midwestern Latinas (n=162). The n-3 FFQ was developed from preliminary interviews, analyzed for content validity and pilot tested. The final instrument contained 209 items (15 culturally-specific dishes). Participants were healthy first-generation Latinas, 20-50 y, non-pregnant and non-lactating at the time of the study. One-on-one interviews in Spanish were conducted to validate the FFQ. Two FFQ, four weeks apart, and three, nonconsecutive 24-h recalls (within the four-weeks) were collected. The validity and reliability of the FFQ was assessed by Pearson correlation coefficient. Correlations with correction for the attenuating effect in the 24-h recalls were calculated. Bland-Altman plots were constructed to assess agreement between the two methods. Daily intakes (±SD) of Total n-3, ALA, EPA and DHA (g) estimated by the mean of the two FFQs were 1.2±0.7, 1.1±0.6, 0.1±0.8, 0.1±0.1, respectively. Reliability of the n-3FFQ was 0.71 for Total n-3, 0.65 for ALA, 0.74 for EPA, 0.54 for DHA ($P < 0.01$). Corrected and adjusted correlations between the mean of the two FFQs and the mean of the recalls were 0.42 for Total n-3, 0.44 for ALA, 0.27 for EPA, and 0.24 for DHA ($P < 0.05$). Bland-Altman plots showed good agreement between the two methods. The n-3 FFQ had acceptable reliability and adequate validity for the type of nutrients studied. Although validity for EPA and DHA were low, the coefficients were similar to those previously reported for PUFAs.

INTRODUCTION

The omega-3 (n-3) fatty acids (α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential for different physiological processes in humans (1-4). Several epidemiologic and interventional studies have demonstrated the beneficial effects of n-3 fatty acids on cardiovascular disease (CVD) (5-9) and it is documented that consumption of foods or dietary supplements rich in n-3 fatty acids lower the risk of coronary heart disease and protects against sudden cardiac deaths (8,10,11). Examples of food sources to achieve recommended ALA intakes are flaxseeds, walnuts, and canola oil, while consumption of two fatty-fish meals per week is recommended to meet EPA and DHA recommendations (12). Greater benefit on cardiovascular health has been attributed to seafood sources of n-3 fatty acids compared to vegetable foods sources, explained in part by the rate of conversion of ALA to EPA and DHA in the body, although controversy still exists (1,12,13).

Heart diseases were identified as the leading causes of death of Latinos in the United States (US) in 2002 accounting for almost 24% of the deaths in this population (14,15). In the US, compared to White and Asian females, Latinas have greater odds ratios for heart disease and stroke risk factors (16), and lower awareness of heart disease as their leading cause of death when compared to White women (17). The Dietary Intake of Macronutrient, Micronutrient and other Dietary Constituents in the US, 1988-1994 reported that intakes of ALA, EPA and DHA of Mexican-American women 20 to 49 y are in the range of 1.1 to 1.2 g/d, 0.02 to 0.03 g/d, and 0.05 to 0.06 g/d, respectively (18). Recently, intakes of 33 to 58 mg/d of DHA in women in New Mexico has been reported (19). Low intake of EPA and DHA found mainly in marine sources may explain low intakes in this cultural group as reported in the frequency of fish consumption of 0.3±0.2 times/d of Latinas in Connecticut (20).

The food frequency questionnaire (FFQ) is a method of measuring food consumption and is often used to measure the association of diet and disease (21). FFQs have become widely used because of their feasibility to assess intake over a long period of time, reduce error introduced from day-to-day variability in 24-hr recalls, and reduce response burden in the study population compared to other dietary assessment methods (22).

Two FFQs to measure n-3 fatty acid intakes have been validated against a dietary reference method. An Australian FFQ that estimates long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) was validated (n=53) using as reference method 3 d weighed food records and found high reproducibility correlation coefficients (≥0.87) and strong validity correlations of 0.75, 0.64, 0.62, and 0.72 for Total LC n-3 fatty acids (EPA, DPA and DHA), EPA, DPA (docosapentaenoic acid) and DHA fatty acids, respectively (23). The validation of a quantitative FFQ to estimate n-3 fatty acid intakes in cardiovascular patients (n=28) in the US Midwest (24) reported validity correlation coefficient of 0.42 when tested against three 24-hr recalls, and high reliability (0.83).

Dietary intakes of n-3 fatty acids in the US Midwest have been reported to be low (25-27). Low-income Midwestern pregnant women, physically active adults, hypercholesterolemic subjects consuming a low fat diet with no eggs, and cardiac patients living in the Midwest reported intakes of 1.060 ± 0.030 g/d (93% ALA, 2% EPA, 5% DHA), 0.887 ± 0.121 g/d, 0.620 ± 0.710 g/d, and 0.18 to 10.15 g/d, respectively (24,28-30). The National Academy of Sciences/Food Nutrition Board has set Adequate

Intakes (AI) for ALA for females ≥ 19 years at 1.1 g/d, and an Acceptable Macronutrient Distribution Range (AMDR) of 0.6-1.2 % energy for ALA for adults with up to 10% of this amount provided by EPA and DHA (2). In addition, The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a healthy intake of ALA of 0.7% energy, and a minimum intake of EPA and DHA combined of 500 mg/d for cardiovascular health (31). Little is known of the n-3 fatty acid food intakes of Latinos living in the Midwest, their intake of fish oil supplements or consumption of foods that are good sources of EPA and DHA. In addition, it has been reported that in this part of the Midwest marine-food consumption as dietary source of EPA and DHA is low (24,25).

Latinos will comprise 15.8% of the population by 2015 and are the fastest growing ethnic group in the US (32) still suffering the burden of health disparities. Poor lifestyle behaviors, including lack of healthy food choices, among other factors, contribute to racial/ethnic health disparities (33-37) that in Latinos increases morbidity rate for illnesses such as heart diseases. There is a need for culturally appropriate dietary instruments that capture the diets of specific ethnic/racial groups in a nation that is becoming more racially and ethnically diverse. Although adequate n-3 fatty acid intakes are recognized to prevent and treat CVD (5-9), currently there is not a culturally appropriate FFQ to assess Total n-3, ALA and LC n-3 PUFA intakes in Latinos that could be used for nutrition interventions in this population.

Therefore, the objective of this study was to assess the validity and reproducibility of a culturally appropriate FFQ to estimate Total n-3 fatty acid, ALA and LC n-3 PUFA (EPA, DPA and DHA) intakes in a sample of urban Midwestern Latinas.

METHODS

Sample and Study Design

This study was conducted in a sample of 162 Latinas living in the urban cities of Lincoln and Omaha in Nebraska. Participants were recruited from Latino community centers, churches, and physical activity community service project sites. Convenience and snowball sampling were employed to facilitate recruitment. These types of non-probabilistic sampling have been previously used with success in a study about quality of life of Latinos in Nebraska (38). Participants were first-generation Latinas, 20 to 50 years, not pregnant or lactating at the time of the study. Participants were apparently healthy and did not report having current illness(s) or smoking. Only one participant was recruited from each household or family.

To validate the FFQ one-on-one interviews were conducted with each participant between October 2007 and August 2008 by a trained bicultural interviewer at the participant's home, church, community center or community project site. Community health workers and social workers served as liaisons. Participants were interviewed to provide dietary intake and sociodemographic information. Each participant met with the interviewer twice, four weeks apart. Interviews took approximately 1 hour to complete and were conducted in Spanish. Participants who completed the study received a $30 gift card as compensation for their participation. The study was approved by the Institutional Review Board for the Protection of Human Subjects at the University of Nebraska-Lincoln.

Dietary intake methods

Two methods of dietary intake assessment were used; two FFQs, four weeks apart, and three, non-consecutive 24-hr recalls within the four-week period were collected. Participants met with the interviewer to complete the FFQ and 24-hr recalls twice (at the beginning and end of the four weeks) and provided one 24-hr recall by phone in the mean time. Interviews lasted from 45 min to 1 h to complete. Dietary intakes were analyzed using the Nutrition Data System for Research software version NDSR 2007 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN (39).

FFQ development and assessment

A convenience sample of five Latinas was interviewed to obtain preliminary dietary information to modify a previously validated FFQ to measure n-3 fatty acid intakes in cardiac patients (24) and to develop a culturally competent dietary instrument that could include foods relevant to the study population. Participants responded to questions: what foods and meals are part of your daily diet?, what foods do you prepare or cook for yourself?, where and what groceries do you buy for you and your family?, when you and your family, relatives or friends dine out, what food(s) do you order?, and, if you are enrolled in food assistance programs, what foods do you receive that are part of your usual diet? In addition, participants responded to questions regarding the types of oils or fats they used to prepare meals, and fish, seafood and other meats they would cook for them and their families. Participants in the preliminary interviews phase received a $15 gift card as compensation for their participation.

Food items from the original FFQ (24) plus dietary information gathered from the preliminary interviews in the form of food items and prepared dishes (culturally-specific dishes including) were analyzed for Total n-3 fatty acid, ALA, EPA, DPA and DHA content in grams per medium serving. Serving sizes (small: 0.5, medium:1, and large:1.5) were defined for each food item using the US Department of Agriculture MyPyramid daily recommendation guidelines (40). Culturally-specific prepared dishes (ie., enchiladas, tacos dorados) and their respective portion sizes included in the FFQ were developed based on recipes and amounts that the recipe would serve to a family. Frequency of consumption was classified as none, once a month, two to three times a month, one to two times a week, three to four times a week, five to six times a week, and daily (once or twice a day). Foods included in the FFQ provided ≥ 10 mg Total n-3 fatty acid/medium serving.

Then, the FFQ containing 167 food items and culturally-specific dishes was analyzed first for content validity with three nutrition experts and then pilot tested in a convenience sample of 10 Latinas to evaluate its effectiveness. Food models were used to facilitate recall and to help estimate portion size. Pilot testing participants provided feedback on the instructions, understanding of portion and frequency of food consumption and readability of the FFQ. An open-ended question; “Are there any other foods that you eat at least once a month?” was asked to include missing foods. In addition recipes and amounts for the culturally-specific dishes were reviewed for accuracy. Changes were made and as a result, the FFQ contained 183 single food items and 26 prepared dishes (15 culturally-specific dishes). Food items were grouped as meats, fish and seafood, eggs, diary products, vegetables, fruits, breads and cereals, snacks, oils and fats, nuts and seeds, beans and prepared dishes.

Therefore, the newly developed 209-item FFQ contained foods that provided Total n-3, ALA and LC n-3 PUFA (EPA, DPA and DHA). The instrument was translated and back-translated to and from Spanish by the principal researcher and tested in a sample of 162 Latinas twice for the reproducibility study. Food models were used to illustrate portion sizes for foods on the FFQ. A template developed in Excel 2003 (Microsoft Corp, Seattle, WA) was used to estimate n-3 fatty acid food intakes for each participant. Estimated intakes of Total n-3, ALA and individual long chain n-3 PUFA on the FFQ were calculated for each food by multiplying the frequency of consumption times by the amount eaten by that food for the respective n-3 fatty acid content of that food. When the participant ate a portion larger than the defined large portion size, the amount entered was calculated based on the portion size for that food in the FFQ.

24-hr recalls

The 24-h recall was chosen as the reference method in this validation study (21,41). During the first interview, participants were instructed about serving sizes and scheduled for a phone call to provide a 24-hr recall. Three nonconsecutive 24-hr recalls (including a weekend day) were collected in a month.

Sociodemographic questionnaire

A sociodemographic questionnaire adapted from the Behavioral Risk Factor Surveillance System 2006 Questionnaire, Spanish version (42), was used to obtain information regarding age, country of birth, ethnicity, marital status, number of children living at home, number of adults living at home, years of schooling, annual household income, language spoken at home, employment status, age when first came to the US, and years of permanency in the US of each participant. Sociodemographic information was obtained during the first interview.

Statistical analysis

Statistical analysis was conducted using SAS (version 9.1 TS level 1M3 2006, SAS Institute, Inc., Cary, NC). Descriptive statistics of sociodemographic information and of means, standard deviations and medians were calculated for Total n-3, ALA, EPA, DPA, DHA and food groups for the FFQs and the 24-h recalls. Data were examined for normality. The nutrients and food groups were not normally distributed (skewed to the right), therefore, all dietary intakes were log-transformed to improve the normality of the distribution with the formula log (x + 1) since intakes values could be zero (43). Reliability was assessed with a test-retest approach. Pearson correlation coefficients were calculated with the normalized data to compare correlation coefficients between the intakes derived from the first and second administration of the FFQ by food groups and by Total n-3, ALA, EPA, DPA and DHA in the test-retest approach. Convergent validity was assesses in the present study. Convergent validity estimates if constructs, the n-3 FFQ and 24-h recalls, are theoretically related to each other. To assess the validity of the n-3 FFQ, corrected Pearson correlation coefficients were computed between the mean of the two FFQs and compared to the mean of the three 24-h recalls. To account for random errors and within-person variability that may weaken the correlations between the FFQ and the recalls, the variance ratio (ratio of within-subject (S^2_w) to the between-subject (S^2_b) variation) was calculated to deattenuate (adjust) the correlations of the 24-h recalls (21,41,44,45) using the following formula (41):

$$r_t = r_o \{1 + [(s^2_w / s^2_b)/n]\}^{0.5}$$

Where:

r_t = true correlation

r_o = observed correlation

s^2_w / s^2_b = the within-subject variance divided by the between-subject variance

n= 3 (number of repeated measures of the 24-h recalls)

Correlation coefficients were statistically significant for reliability ($P<0.01$), and validity ($P<0.05$) analyses. Bland-Altman plots were constructed to assess agreement between the FFQ and the recalls. The difference of the FFQ and recalls was plotted against the average of the two methods. Corrected (log-transformed) dietary intake data was used for the plots. Mean difference (md) and standard deviation of the difference (s) were calculated with the formula md±2s to set the "limits of agreement" (LOA) as recommended by Bland and Altman (46).

RESULTS

The present study had as the population Midwestern Latinas (n=162) recruited from two urban cities in Nebraska. Sociodemographic characteristics of Latinas participating in the study are shown in Table 1. Nearly 40% of participants were in the 20-30 y range, 68% of the women were from Mexico, 61% had high school or less and 43% had annual incomes in the range of $10,000 to $20,000. Proxy indicators of acculturation showed that most women spoke Spanish at home (82%), had a mean age of arrival to the US of 25.1±9.0 y and had lived in the US at the time of the study for 9.3±6.4 y.

Mean intakes

Mean intakes of Total n-3, ALA, EPA, DPA and DHA estimated by the mean of the FFQs and the mean of the three 24-h recalls are presented in Table 2. Mean intakes of Total n-3, ALA, EPA, DPA and DHA (g) estimated by the mean of the two FFQs were 1.2±0.7, 1.1±0.6, 0.1±0.8, 0.02±0.04 0.1±0.1, respectively. Daily mean intake of EPA+DHA assessed by the mean of the two n-3 FFQ was 0.12±0.10 g/d. The n-3 FFQ estimated 99% of the intake of Total n-3 from the recalls, 96% from ALA, and overestimated EPA, DPA, and DHA intakes by 330, 200 and 160 percent, respectively. ALA intake accounted for 90% and 92% of the Total n-3 intakes estimated by the mean n-3 FFQ and mean of recalls, respectively. Mean intakes of Total n-3, ALA, EPA, DPA and DHA showed consistency in the food groups that contributed the most to Total n-3 and each LC n-3 PUFA intake to the participants in FFQ1 and FFQ2 (Table 3). The oils and fats, meats, and prepared dishes groups provided the most Total n-3 intakes; while oils and fats, prepared dishes, meats and breads provided the most ALA intakes. The seafood, and bread groups provided the most EPA intakes, while meats, and seafood provided the most of DPA intakes. For DHA, eggs, seafood, and meats were the food groups that provided the most intakes to the participants. Table 4 shows the rank listing of individual foods that provided 100% of Total n-3 in the study population.

Reliability

Median comparison of Total n-3, ALA, EPA, DPA and DHA in the two FFQs, mean of the recalls and 25-75th percentile of intake for Total n-3 and n-3 LC PUFA and Pearson correlation coefficients for reliability are shown in Table 5. Median values are presented as the distribution of the intakes in the FFQs and recalls were skewed. Pearson correlations among Total n-3, ALA, EPA, DPA and DHA in FFQ1 vs. FFQ2 for the corrected (log transformed) values were 0.71 for Total n-3, 0.65 for ALA, 0.74 for EPA, 0.695 for DPA, and 0.54 for DHA ($P < 0.01$). Lowest to highest correlation for the food groups between FFQ1 and FFQ2 ranged from 0.47 for nuts and seeds to 0.71 for eggs for Total n-3, 0.47 for nuts and seeds to 0.63 for dairy for ALA, 0.55 for seafood to 0.94 for meat for EPA, 0.46 for meat to 0.74 for prepared dishes for DPA, and 0.08 for prepared dishes to 0.65 for eggs for DHA ($P < 0.01$) (Table 6).

Validity

Validity correlation of uncorrected, corrected and adjusted values for Total n-3, ALA, EPA, DPA and DHA between the mean of the two FFQs and the mean of the three 24-h recalls are presented in Table 7. Total n-3 and ALA correlation coefficients were corrected and adjusted. EPA and DHA correlation coefficients were not adjusted since they had high within-subject variation (s^2_w) and near to zero between-subject variation (s^2_b) which made the adjusted values unrealistic. Therefore, validity correlation coefficients were 0.42 for Total n-3, 0.44 for ALA, 0.27 for EPA, and 0.24 for DHA ($P < 0.05$). DPA correlation coefficient of 0.12 was not significant ($P > 0.05$), thus it could not be validated in this study. Bland-Altman plots between the FFQ and the recalls for Total n-3, ALA, EPA and DHA (Figures 1, 2, 3, 4) showed no systematic variation between the two dietary methods. The plots highlighted outliers, but not bias as data from the FFQs did not fall either above or below the line of equity (mean difference), was scattered along the line and within the LOA (46,21).

DISCUSSION

The purpose of the present study was to develop and evaluate the performance of a culturally appropriate FFQ to measure Total n-3, ALA, EPA, DPA and DHA food intakes in Midwestern Latinas by comparing the nutrients intakes from the FFQ with those obtained from the recalls. Correlation coefficients scores of the n-3 FFQ had acceptable reliability and adequate validity for the nutrients studied. To our knowledge, this is the first study purposely developed to measure n-3 intakes in Latinas. ALA mean intake of 1.1±0.6 g/d in the study population assessed by the mean of two n-3 FFQs was similar to a range of intake of 1.1 to 1.2 g/d reported for 20-49 year-old Mexican American women (18) and ALA intake accounted for 90% of Total n-3 intake. These findings suggest that the n-3 FFQ was useful in identifying foods choices sources of n-3 fatty acids in Latinas. Mean ALA intakes of 1.3 g/d for US women (20-59 y) (47) have been reported previously, and the National Academy of Sciences/Food Nutrition Board Adequate Intakes (AI) for ALA for females ≥ 19 years was set at 1.1 g/d. (2). Mean ALA intakes of Latinas in the present study met the AI for ALA for their age group. In the current study, mean intakes of EPA, DPA and DHA assessed by the n-3 FFQ overestimated intakes by 330, 200 and 160 percent, respectively, compared to the mean of 24-h recalls. Similar findings have been reported by studies of validation of FFQs that used as the reference method 24-h recalls where Latinos were part of the study population. Hernandez et al. (45), reported lower mean intakes for different macronutrients such as carbohydrates, protein, fiber, calcium and others when compared to sixteen 24-h recalls in the diets of women in Mexico City. The validity of the Insulin Resistance Atherosclerosis Study (IRAS) FFQ tested in a multiethnic population of women showed lower mean intakes for energy, total and saturated fat, vitamin A, vitamin C and vitamin E in Latinas when compared to eight 24-h recalls (48). Similarly, Tucker et al. (49) reported lower mean consumption of carbohydrate, vitamin B-12 and vitamin D in a sample of 52-91 year-old Latinos assessed by 24-h recalls. In the present study, we collected three 24-h recalls within a month and compared to studies that used a larger number of recalls, the mean of EPA, DPA and DHA assessed by the recalls were lower and in agreement with previous studies. PUFA and n-3 fatty acid intakes estimated by other FFQs compared to other reference methods in validation studies have been mixed. Mean PUFA intakes measured by two FFQs were overestimated by 173% and 143% compared to 24-h recalls and food intake records, respectively, in Latinas and in a multiethnic population of women (48,50). In contrast, 24-h recalls overestimated mean PUFA intakes by 213% compared to a FFQ in women living in Mexico City (45). Two studies have reported higher n-3 fatty acid intakes when compared to food records and recalls in the validation of a FFQ to measure n-3 fatty acid intakes in Australians and cardiac patients in the US (23,24). Sullivan et al. (23) reported higher mean EPA and DPA intakes assessed by a FFQ compared to food records of 73±63 mg/d vs. 71±65 mg/d (103%) and 52±41 mg/d vs. 38±40 mg/d (137%), respectively. Ritter-Gooder et al. (24) indicated higher mean n-3 fatty acid intakes assessed by a FFQ compared to 24-h recalls of 2.47±1.9 g/d vs. 1.8±1.9 g/d (140%), and a paired-t test showed not statistically significant difference between the two methods.

The women in the present study failed to meet the recommendation of 0.5g/d of EPA and DHA combined for cardiovascular health (31). Mean intakes for EPA, DPA and DHA assessed by the mean n-3 FFQ were 0.10±0.75 g/d, 0.02±0.04 g/d and 0.08±0.06

g/d, respectively, and mean intake of EPA and DHA was 0.12±0.10 g/d. Similar findings were reported for Mexican-American women 20 to 49 y who had a range of intake of 0.02 to 0.03 g/d, and 0.05 to 0.06 g/d for EPA and DHA, respectively (18). Recently, intakes of 0.03 to 0.06 g/d of DHA in women in New Mexico have been reported (19). Several observations could help clarify this phenomenon in our study population. The low intake of EPA and DHA in the participants could be explained by the low socioeconomic status (SES) and acculturation of the participants. Acculturation is defined as “the process by which immigrants adopt the attitudes, values, customs, beliefs, and behaviors of a new culture” (51). Latinas in the present study had mean years of education of 10.7±4.1 y, 43% had annual incomes in the range of $10,000 to $20,000, and 48% were unemployed/homemaker. In addition, indicators of acculturation of the participants’ revealed that 82% of participants preferred to speak Spanish at home and had a mean of 9.3±6.4 y. of permanency in the US implying that Latinas in this study were not fully acculturated to the US. These social indicators could have favored low intakes of food sources of EPA and DHA generally more costly, and thus could have been less affordable for the majority of the participants. For instance, foods sources of LC n-3 PUFA in the FFQ such as chicken, tilapia and shrimp did not provide considerably to Total n-3 intake of the participants (<5%). In addition, low educational attainment could have led to reduced nutrition knowledge and awareness of the health benefits of food sources of LC n-3 PUFA, situation enhanced by limited English language skills as most of participants spoke only Spanish. The low dietary acculturation of the majority of participants could have also counteracted with their adoption of eating patterns of the host country as a food such as salmon is more linked to a Western type of diet. Other studies have reported similar effects of SES and acculturation on intake of different foods and nutrients (53-58).

Food groups in the n-3 FFQ that provided the most Total n-3 intakes were oils and fats, meats, and prepared dishes. Oils and fats, prepared dishes, meats and breads provided the most ALA intakes. The seafood, and bread groups provided the most EPA intakes, while meats and seafood provided the most DPA intake. For DHA, eggs, seafood, and meats were the food groups that provided the most intakes to the participants. The Continuing Survey of Food intakes by Individuals 1994-1996 (59) reported that apart from fats and oils; grains, meat, poultry and fish accounted for most of the intake of ALA, while meat, poultry and seafood contributed to most EPA and DHA in US women. Food items that provided most Total n-3 intakes in the present study were vegetable and canola oil, regular mayonnaise, walnuts and beef. Interestingly, pinto beans, tortilla, and shrimp considered foods frequently eaten by Latinos subgroups were part of the items that provided the most Total n-3 to the study population. These findings are useful in determining the food groups that provide most of the intake of Total n-3, ALA and LC n-3 PUFA to this type of population. Oils and fats, prepared dishes and meats, but not seafood, were the main sources of Total n-3 and ALA intakes. ALA intakes accounted for 90% of Total n-3 intakes in Latinas in this study as assessed by the mean FFQ and this finding may help explain the similarity in the food groups sources of Total n-3 and ALA in the study population. These results are significant when developing culturally sensitive nutrition education interventions that could benefit from using a dietary instrument validated in the same population such as the n-3 FFQ as a strategy to increase diet quality.

The correlation coefficients between the two administrations of the FFQ showed acceptable reliability. Corrected correlation in the present study ranged from 0.74 for EPA 0.71 to 0.54 for DHA. Reported FFQs reliability correlations for adult populations in the literature ranged from 0.5-0.8, depending of the study group and size, nutrient of interest and instructions associated with the instrument (21,41,60). Reliability correlations for n-3 fatty acids reported in three studies ranged from 0.62 for Total n-3 fatty acids to 0.90 for DPA (23,24,61). Cronbach's alpha coefficient of 0.83 for Total n-3 was found in 28 cardiac patients in the US (24). Spearman correlation coefficients of 0.88, 0.88, 0.90 and 0.87 were found for Total LC n-3 PUFA, EPA, DPA and DHA, respectively, in 53 high-educated middle aged Australians (23). Intraclass correlation coefficient for Total n-3 fatty acids was 0.62 in 113 pregnant Finnish women (61).

Food groups that had higher reliability correlation coefficients were different for Total n-3 ALA, EPA, DPA and DHA. Beans, eggs and meat had the highest correlations for Total n-3. Beans, eggs and dairy showed the highest correlation for ALA. Meats and prepared dishes, prepared dishes and seafood, and eggs and meats were the food groups that had highest reliability correlation coefficients for EPA, DPA and DHA, respectively. Correlation coefficients for food groups ranged from 0.54 to 0.94. This finding indicates that the food groups that had highest reliability included foods that were part of the women's diet. Of particular importance are the sources of EPA and DHA, which are known to be found in seafood and other types of meats. Greater benefit on cardiovascular health has been attributed to seafood sources of n-3 fatty acids compared to vegetable foods sources. In the present study, the seafood group had one of the highest reliability correlations for DPA only, while meats, eggs and prepared dishes had the highest reliability of intake for EPA and DHA. Given the recommendation of two fatty-fish meals per week to meet EPA and DHA recommendations as these foods are the greatest sources of LC n-3 PUFA (12), Latinas intakes in the present study did not follow that guideline as seafood was not identified as asource of EPA and DHA in this study. This finding is in agreement with the low mean intakes of 0.12±0.10 g/d for EPA+DHA in the current study.

Administration of the FFQs was carried out by one-on-one interviews and conducted by the same bicultural trainer interviewer over a one month period to minimize variation in the women' diets, minimize systematic errors and increase recall memory that could improve reliability as it has been reported that test-retest correlation coefficients diminish with time between the administration of the test method (21,41,62). Thus, the acceptable reliability correlation coefficients in the present study translate as the n-3 FFQ was able to estimate intakes of n-3 fatty acids in Latinas in a constant manner given a one month period and took into account methodology errors that could have affected the test-retest.

Given the high variability in intake of certain nutrients such as PUFA, and differences in food choices in males and females reported previously (63,64), dietary assessment tools designed to measure these types of nutrients need to be designed and administered keeping in mind that intakes of nutrients such as n-3 fatty acids change over time and are affected by gender. The sample population in the present study was women with low educational attainment, low income, unemployed and low acculturation. In addition, marine sources of LC n-3 PUFAs are scarce in this geographical area of the US Midwest which in turn affects intakes of n-3 fatty acids. Taking into account the characteristic of the participants and the high variability in intake of LC n-3 PUFAs (EPA and DHA) we suggest that higher reliability correlation coefficients would have been difficult to obtain and that in dietary instruments tested longer than a month it might decrease over time. Moreover, higher correlation coefficients between the two FFQs do not imply higher intakes as the consumption of food sources of EPA, DPA and DHA was low, but still had acceptable high coefficients. This observation is supported by the low mean intakes of EPA, DPA and DHA assessed by the mean of the two FFQs.

The higher validity correlation coefficients in the n-3 FFQ were for Total n-3 (0.42) and ALA (0.44), while lower coefficients were observed for EPA (0.28) and DHA (0.24). Correlation coefficients obtained from the present study are comparable to those obtained for reported n-3 fatty acids. Intakes of Total n-3 from a 152-item FFQ were compared to three 24-h recalls (24). Pearson uncorrected correlation between the two methods was 0.42. Sullivan et al. (23) obtained Spearman correlation coefficients of 0.88, 0.88, 0.90, and 0.87 for LC n-3 PUFA (EPA+DPA+DHA), EPA, DPA and DHA, respectively, when they compared a 28-item FFQ that estimated LC n-3 PUFAs with 3 d weighed food records in Australians. Corrected Person correlation coefficients of 0.59 and 0.55 for EPA and DHA have been reported when comparing a 129-food item FFQ with 7 d weighed records designed to measure dietary fatty acid in the UK (65). Intakes of Total n-3 from a 181-item FFQ developed to estimate food consumption in pregnant Finnish women was compared to 5 d food records (61). Pearson corrected correlation between the two methods was 0.30 for Total n-3. Correlation coefficients for PUFAs have been reported to be 0.12 in women living in Mexico City (45), 0.21 for rural Hispanic women in the US (48), and 0.55 for low-income Mexican-Americans (66). The higher correlation coefficients obtained for EPA and DHA in the Australian and UK studies compared to the present study could be attributed to several reasons. Food records, considered the best reference method to validate a FFQ, were used as reference method (21). The subjects of the Australian and UK studies were highly educated university staff and students and contrary, to the studies that had Latinos as study population, the lower correlations for PUFAs was explained by the lower educational attainment (48). In addition, the Australian and UK studies used a considerably lower-item FFQ compared to the present study that could have influenced respondent burden (21).

The 24-h recall is a dietary method used to assess an individual's intakes, it is relatively easy to administer, useful for estimating intakes in cultural specific populations, does not require high literacy from participants and it is less costly compared to other assessment methods (21,67,68). Several FFQ validation studies involving Latino populations have used the 24-h recall as the reference method (48,45,69,70). However, the 24-h recall does not always provide a reliable estimate of an individual's intake due to day-to-day variation or within-subject variance (71). The magnitude of the within-subject variance depends on how frequently the measurements are made and it is not easy to minimize as it is a property of the individual being studied (71). In the present study, three non-consecutive 24-h recalls were collected in a one month period in an attempt to control within-subject variance that could affect correlation coefficients. In addition, the between-subject variance was very low or near to zero making the variance ratio high. The high variance ratio implies that n-3 fatty acid intakes in Latinas were irregular, occasional and similar among them. Several factors could have contributed to the high variance ratio in the present study. Palaniappan et al. (64) found higher within-subject and lower between-subject variability in women compared to men for several nutrients, but was especially high for fat explained by inconsistent use of low fat products or less consumption of fat containing products. In addition, age, gender, education and family size were found to affect most foods and nutrients intakes. Higher within- than between-subject variance has been reported in pregnant Malawian females for energy and several nutrients (72) explained by infrequent consumption of food sources of certain nutrients. The sources of variance in 24-h recalls assessed for different nutrients in males and females have been discussed by Benton et al. (63). Gender was found to be a major contributor to total variance of absolute intake of 19% for fat and 8.9% for PUFA, females had higher mean intake of PUFA on weekend days compared to weekdays, and the variance ratio was highest for PUFA compared to other nutrients in females, and it was higher in females compared to males. Gibson et al. (73) have reported that within- and between-subject variance is a function of the nutrient of interest. Few foods that are consumed occasionally in which high concentrations of nutrients are found, such as ALA, within-subject variation is high, complicating accurate estimates of usual intake. Participants in the present study shared similar characteristics that contributed to the high variability in their intakes of n-3 fatty acids and this phenomenon could be explained in two ways: From the participants perspective, food sources of n-3 fatty acids could have been avoided as they may have been seen as fattening and thus leading to infrequent consumption, social indicators of low SES and low acculturation of the majority of participants, systematic errors that lead to under or overreporting of intakes, and social desirability to fit a “healthy diet profile” could have affected their intake and contributed to high variance ratio. Considering the nutrients, the high variance ratio reported for PUFA in other studies with high within-subject variance suggest a wide variation in day-to-day selection of Latina's diets intensified by the characteristic that few foods are sources of n-3 fatty acids, therefore making intake highly variable.

The Bland-Altman plots were constructed to increase the robustness of the validation study. The method does not make assumptions about what method is better and uses the mean and standard deviation of the difference between the test and the reference method. The Bland-Altman plots constructed for Total n-3, ALA, EPA and DHA showed no systematic variation between the two dietary methods.

Several conclusions arise from the present study:

1. The FFQ had acceptable reliability and adequate validity for the nutrients studied. Validity correlation coefficients for EPA and DHA were low; however, the coefficients were similar to those previously reported for PUFA.
2. Underreporting or overreporting of intake, personal bias, social desirability to report consumption of foods considered "healthy" could have created high within-person variation and therefore affecting reliability and validity correlation coefficients in the present study.
3. The validity correlation coefficients in the n-3 FFQ for Total n-3 and ALA were higher than the coefficients for EPA and DHA. ALA intake assessed by the n-3 FFQ accounted for 90% of Total n-3 intakes implying that ALA was the most consumed form of n-3 fatty acids in Latinas. Higher and regular intakes of ALA could have accounted for less within-person variability in ALA and thus in Total

n-3 and may have contributed to their higher validity correlation coefficients compared to EPA and DHA.

4. The n-3 FFQ did not perform well in validating EPA, DPA and DHA due to the high variability in these nutrients observed in the population studied. EPA and DHA intakes in the reference method could not be adjusted for within-person variation. The within-person variation relative to between-person was very high when conducting the adjustments. This scenario could be explained as intakes of EPA and DHA varied considerable in the same participant (within-subject) and were similar –and low– across all participants (between-subjects).
5. The comprehensive characteristic of the n-3 FFQ with inclusion of ethnic-specific dishes made it possible to capture a wide range of intakes of Total n-3 and ALA of participants in the present study. Mean intake of ALA, EPA and DHA in Latinas were in agreement with intakes for similar populations.
6. The n-3 FFQ may be better used to assess Total n-3 and ALA and not LC n-3 PUFA. The low validity correlation coefficients observed for EPA and DHA were the result of high within-subject variability and low intake of food sources of LC n-3 PUFA in the study population. The social characteristics of the study population (low educational attainment, low income, unemployed and low acculturation) along with systematic errors may have affected reported intake of n-3 fatty acid foods.
7. The n-3 FFQ validated in the present study could be used as a research tool to estimate n-3 fatty acid intakes in Midwestern Latinas for nutrition interventions. Given the observation that the n-3 FFQ provided good insight of Total n-3 and

ALA intakes, referenced intakes of LC n-3 PUFAs could be approximated by difference.

Significance

This is the first study that attempts to measure n-3 fatty acid intakes in Latinas using a dietary instrument purposely developed for this population. The n-3 FFQ performed acceptably in identifying food sources of n-3 fatty acids and had adequate reliability and acceptable validity for the type of nutrients studied. Given the recognized beneficial cardiovascular effects of n-3 fatty acids, the n-3 FFQ is a culturally appropriate instrument that could be used for nutrition interventions in this population.

Recommendations

The present study evaluated the reliability and validity of a culturally appropriate n-3 FFQ in Latinas. Taking into account the findings of the study the following recommendations for professional practice are suggested:

1. It is fundamental to establish connections with Latino community representatives to facilitate recruitment of participants.
2. Convenience and snowball sampling, although non-probabilistic sampling methods, performed well in obtaining participation in the population studied.
3. Administration of food questionnaires that are extensive as the n-3 FFQ, perform well in one-on-one interviews with trained bicultural interviewers. It is recommended that the interviewer complete the questionnaire to avoid miscalculation of portion sizes, question miscomprehension, and responses errors.
4. The n-3 FFQ required approximately 45 m to 1 h to complete because of it comprehensiveness. It is important that the interviewer assist the participants with

food models that are relevant to the study population. Conventional foods model are not always ethnic/culturally appropriate. In that case, interviewers need to supply themselves with real foods (i.e., sardines canned in tomato sauce, condensed milk) that participants could indentify as their food choices.

5. It is advised to include ethnic/culturally representative ethic dishes in a food questionnaires that asses intakes of nutrient(s) in a cultural group. In present study the inclusion of ethnic prepared dishes made possible to capture a better representation of Latinas of n-3 fatty acid food intakes.
6. An item analysis of the foods contained in the n-3 FFQ may help reduce the length of the questionnaire and thus interview time. It is recommended that if food items are removed from the original n-3 FFQ, a reliability analysis needs to be conducted to assess the association or correlation between the questionnaires.
7. It is important to consider that test-retest assessment of nutrients with high within subject variability have to be conducted in a relatively short time period as reliability could diminish with time between the administrations of the test method.
8. When validating food questionnaires that assess intake of nutrients such as n-3 fatty acids that are found in high concentrations in few foods and have high within-subject variability, it is advised to increase the number of repeated measures of the reference method. This methodology will increase the likelihood to cover more days of intake and possible reduce variations in intake.
9. Whenever it is possible, sociodemographic characteristics of the population study need to be collected. SES, anthropometric measurements, and acculturation

indicators, if working with immigrant/cultural/ethnic groups, are advised to be estimated to indicate association of intake with social characteristics.

10. Directions for further investigation on the intake of n-3 fatty acids in minority populations in the US would be related to food behavior. An n-3 fatty acid food related behavior questionnaire would provide insight of behavior patters of intake. A food behavior questionnaire and the presently developed n-3 FFQ may be useful in nutrient education interventions to aim diet quality towards prevention of heart diseases in Latinos.
11. If possible, the n-3 FFQ should be tested in a larger sample with similar sociodemographic and cultural characteristics than the population of the present study to examine if n-3 fatty acid intakes are similar. Taking into consideration that the results of the present study were affected by the high variability of the nutrients studied and the social characteristics of the participants, perhaps higher validity correlation coefficients would be difficult to obtain for this type of population. Future research on this area would need to verify the recommendation given.

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Table 1. Sociodemographic characteristics of Latinas (n=162)

Age[a]	34.3±8.2
Age range (%)	
20-30 y	39.5
31-40 y	35.2
41-50 y	25.3
Country of birth (%)	
Mexico	68
Other countries[b]	32
Marital status (%)	
Married/live-in partner	74
Other[c]	26
Number of children living at home (%)	
None	18.5
1 to 2	54.4
> 3	27.1
Number of adults living at home[a]	2.1±1.1
Schooling (y)[a]	10.7±4.1
Never went to school (%)	1.2
High school (%)	61.1
1 to 3 y college (%)	17.3
College graduate (%)	20.4
Annual household income (%)	
< $10,000	11.8
$ 10,000 to $20,000	42.6
$ 21,000 to $ 35,000	31.5
> $35,000	13.6
Language spoken at home (%)	
English	8
Spanish	80.2
Spanish and English	11.7
Employment status (%)	
Full-time employed	32.7
Part-time employed	19.8
Unemployed/homemaker	47.5
Years of permanency in the US (y)[a]	9.3±6.4

[a]Mean ± SD.
[b]Argentina, Chile, Cuba, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Panama, Peru, Puerto Rico, Santo Domingo.
[c]Divorced, widow, separated, single

Table 2. Mean intakes (±SD) total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) estimated by the two FFQs and the recalls.

	FFQ	**24-h recalls**	**FFQ (plus recalls)**
		g/d	
Total n-3	1.22±0.70	1.23±0.78	90
ALA	1.10±0.65	1.14±0.74	96
EPA	0.10±0.75	0.03±0.05	333
DPA	0.02±0.04	0.01±0.02	200
DHA	0.08±0.06	0.05±0.09	160

Table 3. Mean intakes (±SD) of total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) estimated by food groups in the mean of the two FFQs.

	Total n-3	**ALA**	**EPA**	**DPA**	**DHA**
			g/d		
Meat	0.15±0.10	0.12±0.09	0.01±0.04	0.01±0.09	0.02±0.01
Seafood	0.11±0.12	0.01±0.03	0.04±0.04	0.01±0.01	0.05±0.06
Eggs	0.04±0.05	0.04±0.05	0.003±0.047	–	0.08±0.08
Dairy	0.09±0.09	0.09±0.09	0.01±0.14	–	–
Vegetables	0.09±0.06	0.09±0.06	0.01±0.09	–	–
Fruits	0.05±0.03	0.05±0.04	0.01±0.07	–	–
Bread	0.12±0.08	0.12±0.08	0.02±0.20	–	–
Snacks	0.03±0.05	0.03±0.05	–	–	–
Oils and fats	0.29±0.38	0.29±0.38	0.01±0.07	–	–
Nuts and seeds	0.05±0.12	0.05±0.12	–	–	–
Beans	0.09±0.10	0.09±0.10	0.004±0.048	–	–
Prepared dishes	0.13±0.14	0.13±0.14	0.003±0.031	0.001±0.006	0.002±0.007

Table 4. Rank order listing of foods that provided 100% of total omega-3 (Total n-3) intake[a] in the study population (n=162).

Rank order	Food	% Each food provided to Total n-3 intake
1	Vegetable oil	16
2	Canola oil	13
3	Mayonnaise regular	8
4	English Walnuts	7
5	Beef steak	7
6	Pinto beans	6
7	Salad dressing "Ranch type"	6
8	Fresh or frozen salmon	5
9	Whole milk	5
10	Chicken burger breaded	5
11	Beef cuts	5
12	Corn tortilla	4
13	Chicken leg no skin	4
14	Tilapia	4
15	Shrimp	4

[a]Intake assessed by the mean of the two FFQs

Table 5. Median comparison of total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA) in the two FFQs, mean of 24-h recalls and correlations[a] between the two FFQs.

	FFQ1		**FFQ2**		**24-h recalls**		**Correlation**
	Median	**25-75 percentile**	**Median**	**25-75 percentile**	**Median**	**25-75 percentile**	**FFQ1 vs FFQ2**
Total n-3	1.41	0.79-1.78	0.94	0.66-1.32	1.01	0.69-1.68	0.709
ALA	1.11	0.71-1.63	0.95	0.55-1.21	0.92	0.61-1.48	0.654
EPA	0.03	0.01-0.05	0.10	0.01-0.04	0.01	0.00-0.02	0.740
DPA	0.02	0.01-0.03	0.02	0.01-0.02	0.01	0.00-0.01	0.695
DHA	0.06	0.03-0.12	0.04	0.03-0.09	0.03	0.01-0.05	0.537

[a]Correlations were calculated based on log-transformed values.
All correlations were significant ($P<0.01$).

Table 6. Pearson correlations[a] of food groups by total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) between the two FFQs.

	FFQ1 vs. FFQ2				
Food group	**Total n-3**	**ALA**	**EPA**	**DPA**	**DHA**
Meat	0.645	0.588	0.936	0.455	0.542
Seafood	0.525	0.517	0.550	0.504	0.495
Eggs	0.709	0.701	1	1	0.651
Dairy	0.633	0.632	1	1	NC
Vegetables	0.543	0.545	1	1	NC
Fruits	0.607	0.589	1	1	NC
Bread	0.589	0.579	1	1	NC
Snacks	0.540	0.532	NC	NC	NC
Oils and fats	0.584	0.568	1	1	NC
Nuts and seeds	0.470	0.470	NC	NC	NC
Beans	0.794	0.782	1	1	NC
Prepared dishes	0.594	0.595	0.928	0.744	0.083

[a]Correlations were calculated based on log-transformed values.

NC= non computed

All correlations were significant (*P*<0.01).

Table 7. Correlations on total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) between the FFQs and the recalls.

	Uncorrected	**Corrected[a]**	**Adjusted[a,b]**
Total n-3	0.271*	0.324*	0.423*
ALA	0.284*	0.337*	0.436*
EPA	0.264*	0.275*	0.275*
DPA	0.103	0.115	0.115
DHA	0.192*	0.236*	0.236*

[a]Calculated on log-transformed values.
[b]Adjusted for variation in the 24-h recalls (deatteanuated)
Significantly (P<0.05).

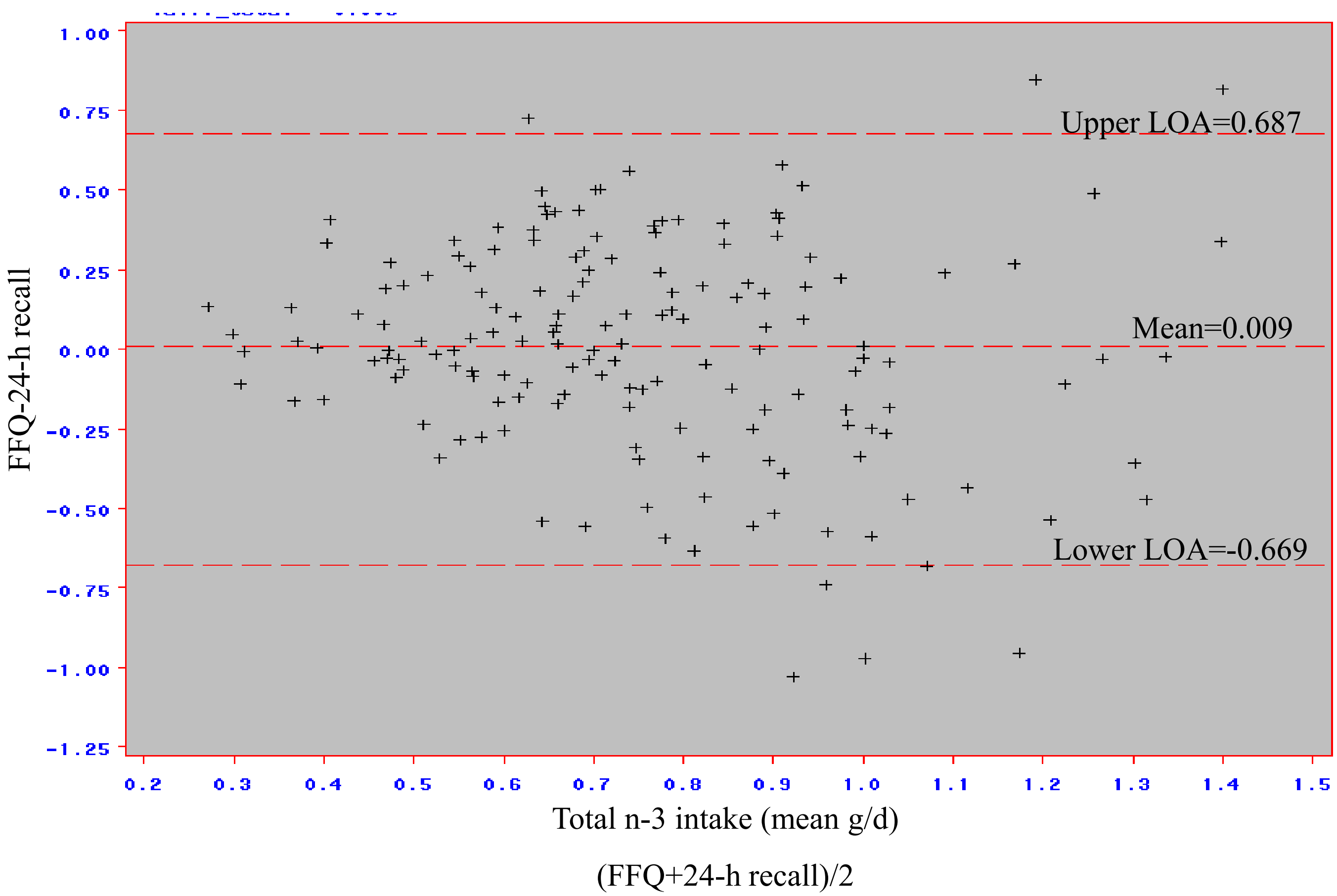

Figure 1. Bland-Altman Plot for Total n-3

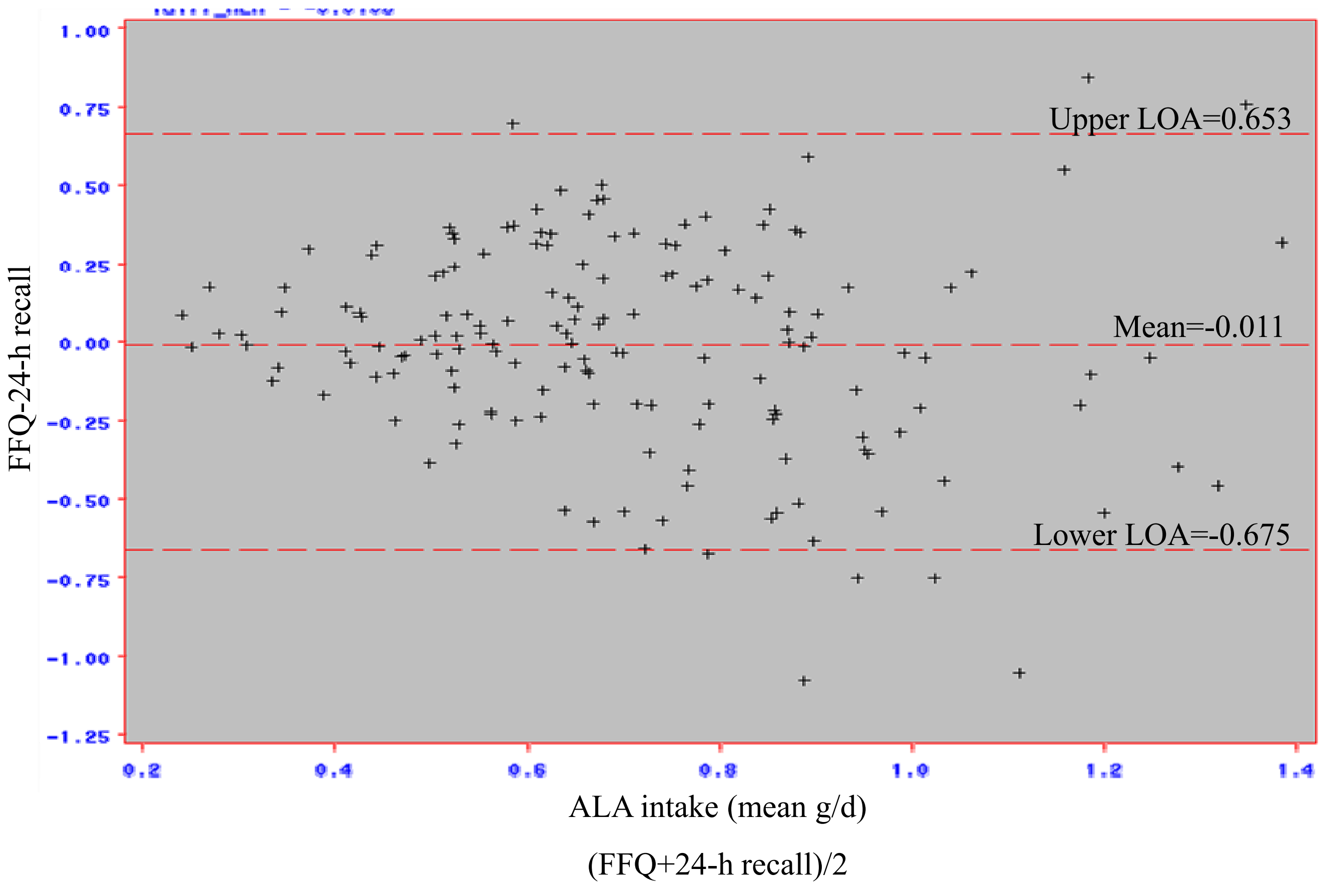

Figure 2. Bland-Altman Plot for ALA

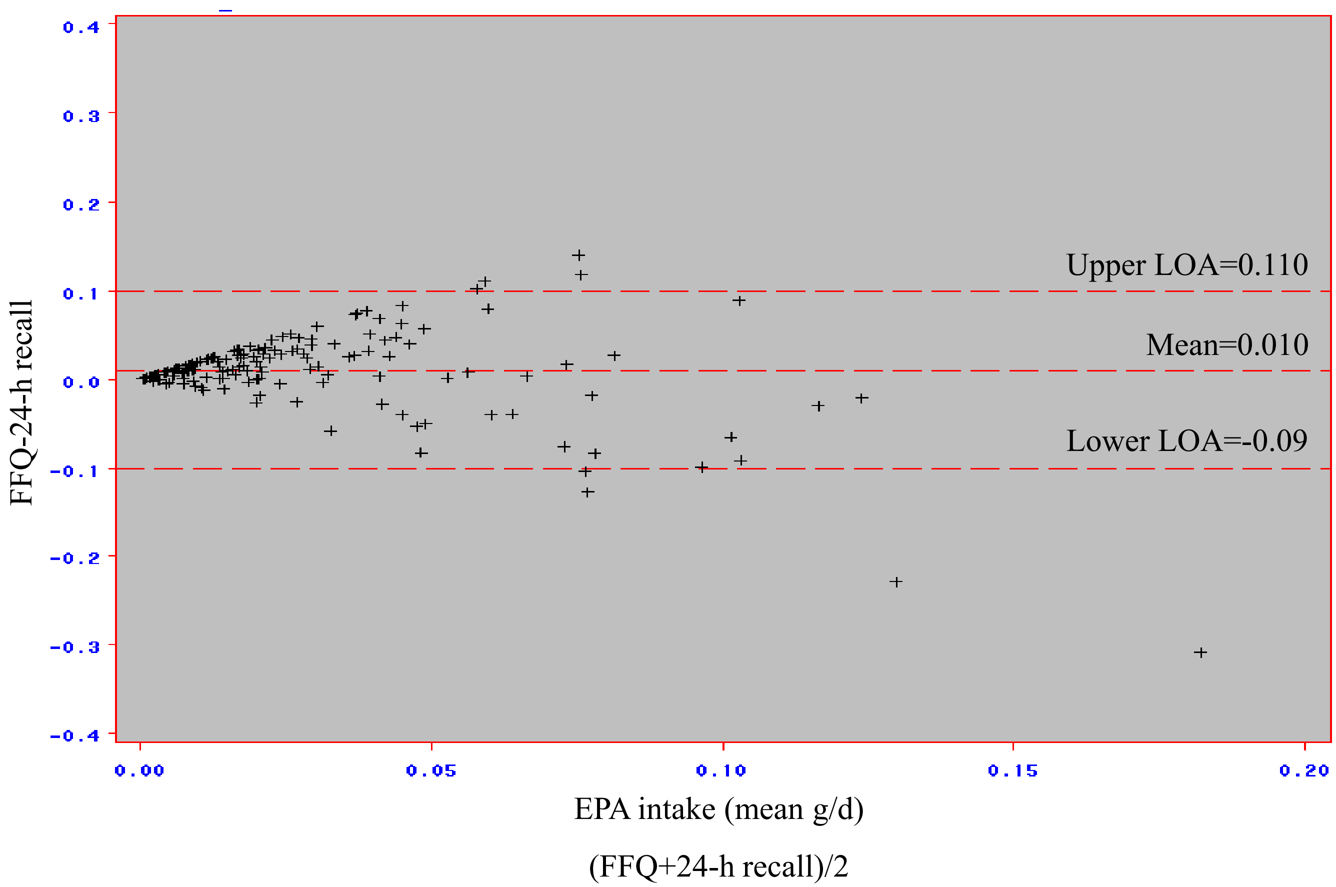

Figure 3. Bland-Altman Plot for EPA

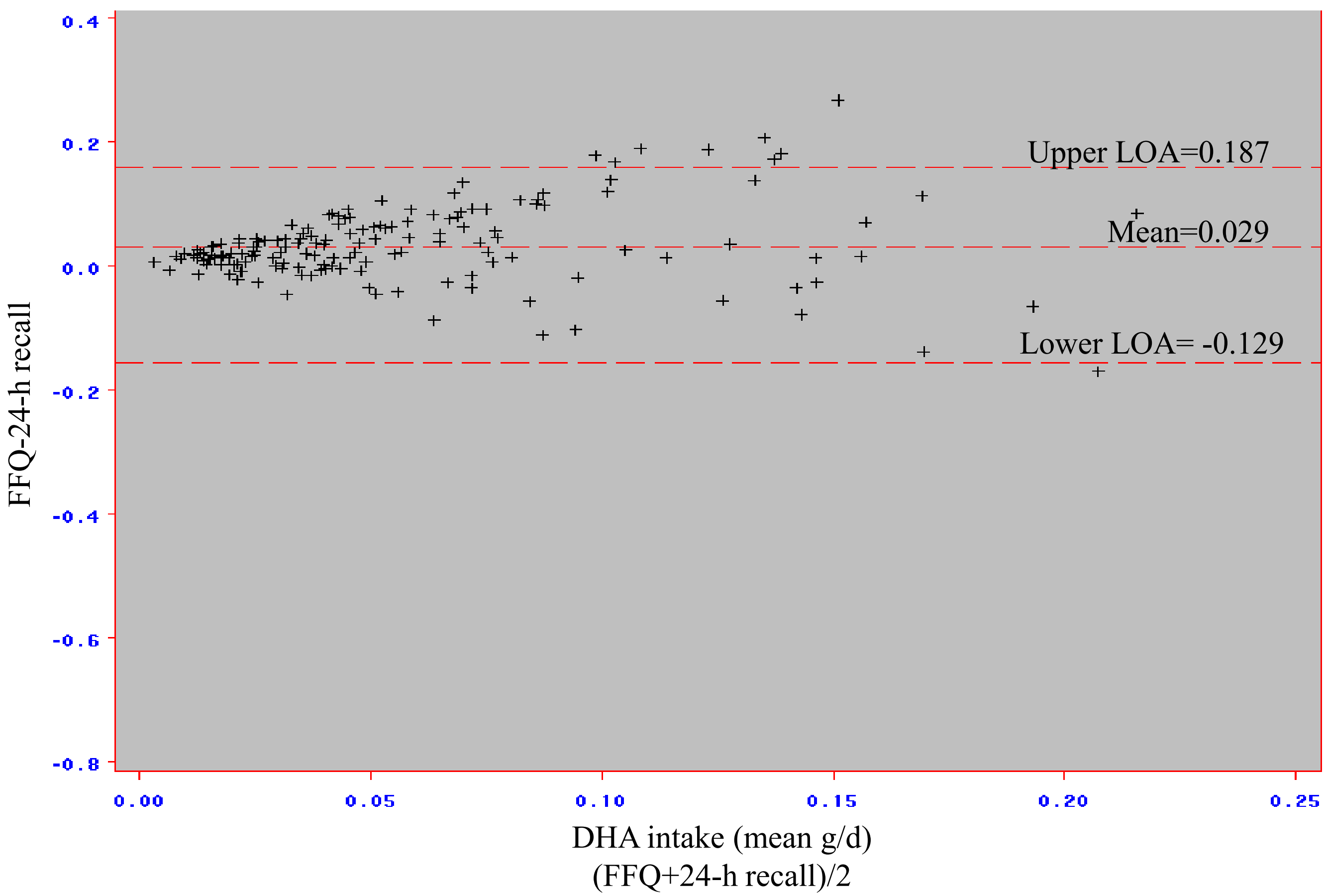

Figure 4. Bland-Altman Plot for DHA

Manuscript II

Association of Age, Socioeconomic Status and Acculturation with Intake of Omega-3 Fatty Acids in Midwestern Latinas

Abstract

The purpose of this study was to examine the association of age, socioeconomic status (education, income, employment) and acculturation on total omega-3 fatty acid (Total n-3), α-linolenic acid (ALA) and long chain (LC) n-3 polyunsaturated fatty acid (PUFA) (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) food intakes of Midwestern Latinas. Participants (n=162) were apparently healthy first generation Latinas, 20 to 50 y, who were not pregnant or lactating at the time of the study. Women provided sociodemographic information, dietary intake by use of a validated n-3 food frequency questionnaire and completed an acculturation scale. Correlation and regression analysis were conducted. Intakes of Total n-3, ALA and LC n-3 PUFA (g) were 1.2±0.7, 1.1±0.6, and 0.1±0.1, respectively. Acculturation scores classified 83% of the participants as low acculturation. Age was negatively correlated with Total n-3 and ALA, intakes. Education, income and acculturation were positively correlated with LC n-3 PUFA intakes ($P<0.05$). Latinas in the younger age category, 20-30 y, compared to the older age category, 41-50 y, consumed 1.2 and 1.3 more times of Total n-3 and ALA respectively. Latinas who completed ≥ 4 y of college compared to those with ≤ 12 y of education, had incomes ≥$50,000 compared to those with incomes of <$10,000, and were highly acculturated consumed 1.5, 1.7 and 1.5 more times LC n-3 PUFA, respectively. Age was related to intake of Total n-3 and ALA after accounting for variation in education, income and acculturation; however, only income was related to intake of LC n-3 PUFA when the other variables were taken into account ($P<0.05$). Participants met the Adequate Intake for ALA for women ≥ 19 y, but had intakes less than recommended 500 mg/d for EPA and DHA.

INTRODUCTION

An individual's socioeconomic status (SES) is a strong predictor of their mortality and morbidity (1,2). Income and education, two indicators of SES, are recognized underlying factors that affect the rate of heart diseases in minorities and low-income populations increasing their mortality rate for cardiovascular diseases (CVD) (3). Minority women are more likely to be in poorer health, suffer from premature death, disease, disabilities and face socioeconomic and cultural barriers that increase their chances for health disparities than Caucasian women (4). For instance, Latinas have a greater chance of experiencing heart disease (5) and lower awareness of heart disease as their leading cause of death compared to White women (6). Although White and Black females have higher prevalence for hypertension and CVD, Mexican-American women have a prevalence of age-adjusted hypertension of 21% (7) and CVD of 34% (8). Several studies have reported the association of low SES with higher occurrence of cardiovascular disease in Latinos and other disadvantaged populations (2, 9-11). Associations of lower intake of fruits, vegetables and fat with low SES (educational attainment) have been reported in Latinos and other racial and ethnic populations (12-14).

The omega-3 (n-3) fatty acids (α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential for different physiological processes in humans (15-18). Several epidemiologic and interventional studies have demonstrated the beneficial effects of n-3 fatty acids on CVD (19-23). The Dietary Intake of Macronutrient, Micronutrient and other Dietary Constituents in the United States, 1988-1994 showed that Mexican-American women 20 to 49 y met the current Adequate Intake for ALA for females ≥ 19 years (16), but were in the range of 0.02 to 0.03 g/d, and

0.05 to 0.06 g/d for EPA and DHA, respectively (24). Recently, intakes of 33 to 58 mg/d of DHA in women in New Mexico has been reported (25). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommends a minimum intake of EPA and DHA combined of 500 mg/d for cardiovascular health (26). Studies involving n-3 fatty acid food consumption of ethnic groups living in the United States (US) are limited. Currently, studies focusing on the n-3 fatty acid food behavior intakes of Latinos and in particular Latinas are lacking. Latino Americans have been reported to have higher rates of obesity, elevated blood pressure, elevated triglyceride levels, dyslipidemia, and diabetes which in turn increase their risk factors and rates for CVD compared to other ethnic/racial groups (27).

Research has established that in minority/ethnic groups CVD risk factors are influenced by behavioral, cultural and societal factors (11,28-30). Latinos will represent 25% of the US population by the year 2050 (31). Dietary intake is an important determinant of chronic health conditions among Latinos in the US and a healthful diet is important in chronic disease management (32). It has been reported that the link between poor health practices and risk for chronic health conditions is the social mechanism called acculturation and migration (32,33). Acculturation is defined as "the process by which immigrants adopt the attitudes, values, customs, beliefs, and behaviors of a new culture" (34). Reviews on the process of acculturation and diet among Latinos affirms that greater acculturation to the US nutritional habits is associated with less healthful dietary intake and dietary behaviors (35,36), while other studies conclude that being less acculturated is associated with poorer dietary habits (37,38). Results on intake of dietary fat and acculturation in Latinos have shown positive (39,40) and negative associations (41-43).

Culturally diverse populations with variable dietary and disease patterns provide a unique opportunity to conduct epidemiology studies to identify the role of diet in the etiology of chronic diseases (44). It is evident that acculturation influences health behaviors and outcomes; however, acculturation is not the only social mechanism that accounts for dietary behavior intakes in Latinos. Other factors, such as SES that account for education level, employment status, and income level (32), need to be considered when studying relationships on the acculturation process and SES on dietary choices of Latinos in the US. The objective of this study was to examine the association of age, SES (education, income, employment) and acculturation on intake of n-3 fatty acids in Latinas.

METHODS

Participants

The study population consisted of 162 apparently healthy first-generation Midwestern Latinas from the cities of Lincoln and Omaha in Nebraska. Participants were recruited from Latino community centers, churches, and physical activity community service project sites. Convenience and snowball sampling were employed to facilitate recruitment. Participants met the inclusion criteria of being between the ages of 20 to 50 years, and not being pregnant or lactating at the time of the study. Only one participant was recruited from each household or family.

Latinas were interviewed one-on-one by a trained bicultural interviewer to obtain sociodemographic information, n-3 fatty acid intakes, and completed an acculturation questionnaire between October 2007 and August 2008. Interviews were conducted at the participants' homes, churches, community centers and community project sites. Community health workers and social workers served as liaisons with project staff. Each participant met with the interviewer twice, four weeks apart. Interviews took approximately 1 hour to complete and were conducted in Spanish. Participants who completed the study received a $30 gift card as compensation for their participation. The study was approved by the Institutional Review Board for the Protection of Human Subjects at the University of Nebraska, Lincoln.

Sociodemographic Questionnaire

A sociodemographic questionnaire adapted from the Behavioral Risk Factor Surveillance System 2006 Questionnaire, Spanish version (45), was employed to obtain information regarding age, country of birth, ethnicity, marital status, number of children living at home, number of adults living at home, years of schooling, annual household income, language spoken at home, employment status, age when first came to the US, and years of permanency in the US from each participant. Sociodemographic information was obtained during the first interview.

Assessment of Omega-3 Fatty Acid Intakes

N-3 fatty acid intakes of participants was assessed by a culturally appropriate food frequency questionnaire (FFQ) validated in this population. The n-3 FFQ contained 209 items (15 culturally-specific dishes) and was developed to estimate Total n-3 fatty acid, ALA, EPA, and DHA intakes. Foods included in the FFQ provided ≥ 10 mg Total n-3 fatty acid/medium serving. Participants met with the interviewer to complete the n-3 FFQ twice, one month apart, as part of the validation study. Interviews lasted from 45 min to 1 h to complete. Dietary intakes were analyzed using the Nutrition Data System for

Research software version NDSR 2007 developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN (46).

Acculturation Measurement

The Short Acculturation Scale for Hispanics (SASH) developed by Marin et al. (47) was used as instrument to assign participants as low or high levels of acculturation. The SASH is originally a 12-item scale. In this study, the shorter 5-item version of the SASH was used as it has been reported to be a valid and reliable scale that can be used for Latinos in large studies (47). Additionally, we added an extra question to the 5-item acculturation scale from a validated acculturation questionnaire based on the SASH developed by Martinez (48) in order to increase the understanding of the acculturation instrument tested in this population. Each question in the acculturation questionnaire (6 items) was scored on a 5-point Likert scale ranging from “Only Spanish” (1) to “Only English” (5) to assess the Latinas acculturation based on language spoken. The acculturation questionnaire allocated respondents as low or high acculturation based on a dichotomous variable. The acculturation score was calculated by assigning the mean acculturation score (range 1 to 5) of each individual. A < 2.5 score indicated low acculturation and ≥ 2.5 score indicated high acculturation. The Holligshead scale (49) based on occupation and income was used to assign participants to low and high SES.

Statistical Analysis

Descriptive, correlation and regression analyses were conducted. Descriptive statistics characterized the study population. Age, education, household income, employment and acculturation information were each grouped in ranges and used as independent categorical variables. For instance, there were three age ranges: 20-30 y, 31-

40 y and 41-50 y. Education had four range groupings, household income had eight range groupings, employment had four range groupings and acculturation had two range groupings. Intakes of Total n-3 fatty acid, ALA, EPA and DHA were log transformed with the formula log (x+1) to improve normality and the corrected dietary data were used as dependent variables. EPA and DHA intakes were grouped together and named LC n-3 PUFA. Pearson correlation analyses were used to estimate the correlation among the independent and dependent variables. Then, variables that were significantly correlated ($P<0.05$) were checked for association between independent and dependent variables that were correlated to test the association of age, SES and acculturation with intakes of n-3 fatty acids. All statistical analyses were performed with SAS (version 9.1 TS level 1M3 2006, SAS Institute, Inc., Cary, NC).

RESULTS

Table 1 shows the demographic characteristic of Latinas participating in the study. The mean age of participants was 34.3±8.2 y with 39.5% of Latinas in the 20-30 y age category. Sixty eight percent of the women were born in Mexico. Participants had a mean age of first arriving in the US of 25.1±9.0 y and mean years of permanency in the US of 9.3±6.4 y. Spanish was largely the language spoken at home (80.2%), 74% of women were married or had a live-in partner and 54% had an average of two children living at home. SES indicators of education, income and employment revealed that the majority of women had completed 12 y of schooling, had incomes in the range of $ 10,000 to $20,000, and identified themselves as unemployed/homemaker. The Holligshead scale of socioeconomic status, where I=high and V=low assigned majority of participants (43.8%) to the IV scale. Eighty three percent of Latinas were classified as low acculturation. Mean daily intakes (±SD) of Total n-3, ALA, and LC n-3 PUFA assessed in FFQ1, FFQ2 and mean of two FFQs are presented in Table 2. Participants had mean intakes of Total n-3, ALA and LC n-3 PUFA of 1.22±0.70 g/d, 1.10±0.65 g/d and 0.12±0.10 g/d, respectively assessed by the mean of the two FFQs. Table 3 presents correlations of age, SES, and acculturation, and significant associations in the multiple regression models with intakes of Total n-3, ALA, and LC n-3 FUFA. Pearson correlation coefficients showed that age range was negatively correlated with intake of Total n-3 and ALA, while education range, income range and acculturation were positively correlated with intakes of LC n-3 PUFA ($P<0.05$). Independent variables that were correlated with intakes of omega-3 fatty acids were tested in multiple regression models. In the first model, age range, education range, income range and acculturation range were included to test their relation to Total n-3 and ALA intakes. Only age range was significantly related to Total n-3 and ALA intakes after accounting for variation in education, income and acculturation ($P<0.05$). In the second model, education range, income range and acculturation range were included to test their relationship to LC n-3 PUFA intakes. Income was the only independent variable significantly related to LC n-3 PUFA intake when education range and acculturation range were taken into account ($P<0.05$). Table 4 was constructed in order to show intakes of omega-3 fatty acids of the independent variables that were significantly correlated in the population study. Latinas in the 20-30 y age category compared to the 41-50 y age category consumed 1.2 and 1.3 more times of Total n-3 and ALA, respectively. Latinas who completed ≥ 4 y of college compared to those with ≤ 12 y of education, had incomes ≥ $35,000 compared to those with incomes of < $10,000, and were high acculturation consumed 1.5, 1.7 and 1.5 more times LC n-3 PUFA respectively. Age, the indicators of SES, and acculturation were correlated among each other ($P<0.05$). Rank order listing of foods sources of LC n-3 PUFA that were eaten by most women are presented in Table 5.

DISCUSSION

This study examined the association of age, SES (education, income, employment) and acculturation with intake of n-3 fatty acids in Midwestern Latinas. Results showed that age was negatively correlated with intake of Total n-3 and ALA, and this association was significant after accounting for variation in education, income and acculturation. Previous studies have shown lower, although not significant, intake of a variety of food sources of protein and carbohydrate in middle age (20-64 y) compared to young (12-19 y) Latinos (39), and in a multiethnic population of White, Black and Latinos of 50-60 y compared to 20-34 y (50). Results of this study showed that Latinas in the 20-30 y age category compared to the 41-50 y age category consumed 1.2 and 1.3 more times of Total n-3 and ALA, respectively.

Latinas in this study had a mean daily intake of Total n-3 of 1.22±0.70 g, ALA of 1.10±0.65 g, EPA of 0.10±0.75 g and DHA of 0.08±0.06 g. Similar intakes of ALA in the range of 1.1 to 1.2 g/d, but lower intakes of EPA and DHA in the range 0.02 to 0.03 g/d, and 0.05 to 0.06 g/d, respectively, have been reported for Mexican-American women 20 to 49 y (24). Participants in the present study met the Adequate Intake of ALA for women ≥19 y (16), but failed to meet the recommended 0.5 g/day of EPA and DHA combined for CVD reduction (26). The validated n-3 FFQ used in this study to asses the diets of these women covered a wide range of foods and contained culturally ethnic dishes of this group. This characteristic of the n-3 FFQ allowed capturing a good representation of the participant's diets. A second finding of this study is that education, income and acculturation were significantly positively correlated with intakes of LC n-3 PUFA; however, only income was related to intake after taking into account the other social variables. Income accounted for a higher intake of LC n-3 PUFA (1.7 more times) in Latinas with higher incomes (≥ $50,000 compared to those with incomes of < $10,000) compared to the effect of education and acculturation.

Minority and low-income populations in the US have a disproportionate burden of heart diseases. In Latinos, CVD accounts for the leading cause of death in this group. (3). Studies have reported that income and education, two indicators of SES, account for an important part of the complex scenario of higher heart diseases in minority populations (51,52). Several studies have reported the association between SES and risk for chronic diseases in disadvantaged populations experiencing higher occurrence of cardiovascular disease (2,53). For instance, low educational attainment has been associated with higher prevalence and risk factors for chronic diseases in Latinos and Whites (39).

In addition, different indicators of SES have been associated with the intake of foods and nutrients in Latinos and African-Americans (12-14). Higher single indicators of SES or a combination of them have been linked to higher consumption of fruits, vegetables, grains, (50-55) fiber and micronutrients (56), fish (57), and PUFA (58) in different populations. Therefore, individuals with lower SES compared to those with higher SES are more likely to have poorer diet quality (55). Fitzgerald et al. (59) reported an association of low SES with less frequency of fish consumption in Puerto Rican women. Similarly, low intake of EPA and DHA as reported in the frequency of fish consumption of 0.3±0.2 times/d has been found in Puerto Rican females living in poverty in Connecticut (60).

In the present study, although income was associated with intakes of LC n-3 PUFA when other social variables where taken into account; education, income and acculturation were positively correlated with intake of this nutrient. This finding can be a reflection of having a higher educational attainment and may imply higher nutrition knowledge and awareness of the health benefits of food sources of LC n-3 PUFA. Additionally, in the current study the higher income of some participants may be due to greater affordability of more expensive foods and, a high acculturation could have affected the adoption of dietary practices of the host country (36). Findings from this study could also be explained as good foods sources of LC n-3 PUFA in the n-3 FFQ, such as salmon and sardines, were generally more costly or were not culturally relevant to Latinas making them less affordable and not a food choice for the majority of the participants. A previous study has reported the influence of income on food purchasing of relatively high-priced food such as fish, fresh fruit and vegetables (61). Research supports a positive association of education, age, employment status, and preferred language spoken at home with nutrition knowledge among Latinos (62). The findings of the present study are also supported by the low educational attainment of the majority of participants that may have influenced their intake of food sources of LC n-3 fatty acids such as fish.

Dietary acculturation “is a process by which a racial/ethnic group, usually a minority, adopts the cultural patterns of a dominant host” (36). Several studies have reported positive (63-65) and negative (43,66) associations of diet quality and acculturation of Latino immigrants to the US. In this study, more acculturated Latinas had higher intakes of LC n-3 PUFA and acculturation alone was significantly related to education and income. Higher intake of food sources of LC n-3 PUFA in high acculturation Latinas with higher incomes could imply that they had greater awareness of healthful foods, higher adoption of the food patterns of the host country, and higher educational attainment that may have produced higher incomes. Although findings from the present study indicate that acculturation is positively correlated with intake of LC n-3 PUFA, other studies have not reported significant association of acculturation on dietary fat intake in Latinos (41-43). The majority of participants in the present study lived in low-income Spanish neighborhoods close to ethnic shops. This observation could in part explain the low acculturation of the majority of participants as it has been reported in other study that immigrants who situate in ethnic enclaves may acculturate slower (67). In addition it has been reported that longer years of permanency in the US is less related to higher levels of acculturation of Latinos living in areas with higher concentration of similar ethnicity (68). In the present study, high acculturation women were more likely to engage in intake of food sources of LC n-3 PUFA such as fish consumption than low acculturated; however, income remained the main factor to affect n-3 fatty acid intakes in these women. An interesting finding from this study is that chicken without skin, shrimp, tuna canned in water, boiled eggs and tilapia were the principal foods sources of LC n-3 PUFA of Latinas as they were eaten by at least 50% or more of the participants. This finding is significant to understand the cultural and social aspects that affect food intake of Latinas in the present study. Seafood such as tilapia and shrimp are commonly eaten in

Mexico (69). The FFQ showed that foods such as shrimp and to a lesser extent tilapia were the main marine food sources of LC n-3 PUFA in this group. Shrimp was eaten by 79% while tilapia was eaten by 49% of the participants. Although these foods are good sources of LC n-3 PUFA, they have a lower amount of EPA and DHA compared to salmon, which is a food more linked to a Western type of diet. Therefore, the fish intake of these women were most likely to resemble their traditional food habits explained by they low acculturation and a mean of permanency in the US of less than 10 y. The majority of Latinas in this study had completed a 12 y educational attainment, identified themselves as unemployed or homemaker, had low incomes, were married or had live-in partners, and had a mean of two children living at home. Parents and particularly mothers with higher educational attainment are likely to be more health literate, adopt healthier dietary habits and thus affect the diet quality and food selection of their offspring. In the Latino community, the mother plays a central role in the family and influences the food intake selection of family members. For instance, Mexican-American women tend to be responsible for the food preparation in the home (69). In addition, it has been reported that the degree of acculturation of Mexican-American mothers affects child feeding strategies (70). The majority of Latinas in this study were mothers. It is necessary to ensure that ethnic minorities such as Latinos with low SES who are experimenting the process of acculturation are aware that healthy food sources of essential nutrients such as n-3 fatty acids are available at relatively low cost.

Nevertheless, taking into account that socioeconomically disadvantaged groups are at higher risk for heart diseases, there is a need to increase promotion of food sources of n-3 fatty acids in low SES Latinos. It is paramount to support nutrition knowledge as a route to raise awareness of the healthy benefits of LC n-3 PUFA and promote foods such as tuna, sardines and eggs which are available at relatively low cost as an strategy to decrease socioeconomic disparities in diet quality in minorities. It is important to encourage and educate immigrants, such as Latinas in the present study, on the adoption of healthy dietary behaviors of the host country while supporting maintenance of the healthy dietary practices of their country of origin.

In conclusion, research has established that in minority/ethnic groups, CVD risk factors are influenced by behavioral, cultural and societal factors (11,28-30). Low intakes of LC n-3 PUFA found in the present study may be in part explained by low accessibility to fish and seafood choices in this geographic area of the Midwest, lack of knowledge of health benefits of n-3 fatty acids, higher cost of food sources of LC n-3 PUFA, and low dietary acculturation of the majority of participants that counteracted with their adoption of eating patterns of the host country. Given the recognized association of low SES on risk factors for CVD, the effect of acculturation on diet quality in Latinos and the beneficial effects of n-3 fatty acid intakes on cardiovascular health, this study provides insight on social determinants of adequate intake of this nutrient. It is necessary to increase promotion of relatively low cost food sources of n-3 fatty acids in low SES Latinos as a possible strategy to decrease diet related risk for CVD and increase diet quality.

Significance

The present study examined the association of age, and societal and acculturation indicators on the intake of n-3 fatty acids in Midwestern Latinas. Given the recognized beneficial cardiovascular effects of n-3 fatty acids, it is important to understand the environmental mechanisms that affect food intake in undeserved minorities to complement culturally appropriate nutrition interventions towards behavior change and thus increase in diet quality.

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Table 1. Selected sociodemographic characteristics of Latinas (n=162)

Age[a]	34.3±8.2
Age range (%)	
20-30 y	39.5
31-40 y	35.2
41-50 y	25.3
Country of birth (%)	
Mexico	68
Other countries[b]	32
Schooling (y)[a]	10.7±4.1
Never went to school (%)	1.2
High school (%)	61.1
1 to 3 y college (%)	17.3
College graduate (%)	20.4
Annual household income (%)	
< $10,000	11.8
$ 10,000 to $20,000	42.6
$ 21,000 to $ 35,000	31.5
$ 36,000 to $ 50,000	10.5
> $50,000	3.1
Language spoken at home (%)	
Spanish	80.2
English or Spanish and English	19.7
Employment status (%)	
Full-time employed	32.7
Part-time employed	19.8
Unemployed/homemaker	47.5
Socioeconomic status (%)[c]	
I	1.2
II	5
III	11.7
IV	43.8
V	38.3
Acculturation distribution (%)	
Low acculturated	83
High acculturated	17
Years of permanency in the US (y)[a]	9.3±6.4

[a]Mean ± SD.
[b]Argentina, Chile, Cuba, Costa Rica, Cuba, Dominican Republic, Ecuador, El Salvador, Guatemala, Honduras, Panama, Peru, Puerto Rico, Santo Domingo.
[c]By Hollingshead Scale based on occupation and income, where I=high, V=low.

Table 2. Mean intakes (±SD) of total omega-3 (Total n-3), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and long chain polyunsaturated omega-3 fatty acids (LC n-3 PUFA): eicosapentaenoic acid (EPA) + docosapentaenoic acid (DHA) estimated by the FFQs.

	FFQ1	**FFQ2**	**Mean FFQ**
		g/d	
Total n-3	1.41±0.91	1.01±0.65	1.21±0.70
ALA	1.28±0.87	0.95±0.61	1.10±0.65
EPA	0.10±0.76	0.10±0.77	0.10±0.75
DHA	0.09±0.08	0.07±0.07	0.08±0.06
LC n-3 PUFA	0.13±0.12	0.11±0.11	0.12±0.10

Table 3. Correlations of age, socioeconomic status (SES), and acculturation, and significant associations in the regression models with intakes of total omega-3 (Total n-3), α-linolenic acid (ALA), and long chain polyunsaturated omega-3 fatty acids (LC n-3 PUFA): eicosapentaenoic acid (EPA) + docosapentaenoic acid (DHA).

	Mean FFQ		
	Total n-3	**ALA**	**LC n-3 PUFA**
Age range[a]	–0.2236*	–0.2473*	0.0604
Education range[b]	0.1396	0.1162	0.2129*
Income range[c]	0.1007	0.0752	0.1862*
Employment range[d]	-0.032	-0.0331	-0.0723
Acculturation[e]	0.0361	0.0044	0.1913*
Model 1[f]			
Age range[a]	0.0014*	0.0009*	–
Model 2[g]			
Income	–	–	0.0148*

[a]Age range= 20-30 y, 31-40 y, 41-50 y
[b]Education range= never went to school, high school graduate, 1 to 3 y of college, 4 y of college or more.
[c]Income range= less than $10,000 to more than $75,000.
[d]Employment range= full time employed, part-time employed, unemployed, homemaker
[e]Acculturation= low acculturation, high acculturation.
[f]Model adjusted for education, income and acculturation.
[g]Model adjusted for education and acculturation.
*P<0.05.

Table 4. Mean intakes (±SD) of total omega-3 (Total n-3), α-linolenic acid (ALA), and long chain polyunsaturated omega-3 fatty acids (LC n-3 PUFA): eicosapentaenoic acid (EPA) + docosapentaenoic acid (DHA) by age, socioeconomic status (SES) indicators and acculturation[a].

	Age range		**Education range**		**Income range**		**Acculturation**	
	20-30 y	**41-50 y**	**12 y of school**	**≥ 4 y of college**	**<$10,000**	**>$50,000**	**Low acculturation**	**High acculturation**
Total n-3	0.84±0.29	0.69±0.26	–	–	–	–	–	–
ALA	0.78±0.29	0.62±0.25	–	–	–	–	–	–
LC n-3 PUFA	–	–	0.09±0.08	0.14±0.09	0.10±0.07	0.18±0.10	0.10±0.08	0.16±0.09

[a]Acculturation= low acculturation, high acculturation as determined by Marin et. al. (48)
Mean intakes were significantly (P<0.05) correlated with age range (negative correlated), education range, income range and acculturation (positive correlated).

Table 5. Rank order listing of foods sources of long chain polyunsaturated omega-3 fatty acids (LC n-3 PUFA): eicosapentaenoic acid (EPA) + docosapentaenoic acid (DHA) in the study population (n=162).

Rank order	Food	% participants who ate the food
1	Chicken breast no skin	78
2	Shrimp	77
3	Chicken leg no skin	74
4	Tuna canned in water	61
5	Egg boiled	56
6	Tilapia	49
7	Mole with chicken[b]	35
8	Turkey	33
9	Chicken burger breaded	30
10	Fresh or frozen salmon	30
11	Turkey hot dogs	27
12	Chicken burger grilled	27
13	Chicken nuggets	27
14	Fish fillets breaded	24
15	Chicken leg with skin	23
16	Tuna canned in oil	19
17	Chicken breast with skin	12
18	Sardines canned in oil	10
19	Tuna sandwich "Subway"	6
20	Salmon pink canned	4
21	Sardines canned in water	3

[a]Intake assessed by the mean of the two FFQs
[b]Traditional Mexican dish

APPENDIX A

Institutional Review Board Letters

Nebraska UNIVERSITY OF
Lincoln

IRB#2007-07-410 EP
Date Approved: 08/08/07
Valid Until: 08/07/08

COLLEGE OF EDUCATION AND HUMAN SCIENCES
Department of Nutrition and Health Sciences

INFORMED CONSENT FORM

Dear participant:

You have been selected to participate in this study. Thanks you for being part of this important research study. You are being asked to participate because you are a Latino or Hispanic woman. Please read the information provided below before proceeding to answering the questions. Participation in this study is voluntary and does not pose any risk to you. All information will be kept strictly confidential.

Identification of Project:
Validity and reliability of a quantitative Food Frequency Questionnaire to measure omega-3 fatty acid foods intake of urban Midwestern Latino women - Phase I **/preliminary interviews.**

Omega-3 fatty acid foods are important for your health. They can protect against coronary heart disease. A Food Frequency Questionnaire is a dietary tool to measure amount and frequency of food consumption. We are conducting this study to know how much and how often you are eating foods that contain omega-3 fatty acids and that could provide you good cardiovascular health.

Purpose of the Research:
This is a research project that aims to develop a questionnaire to measure food intakes in a sample of urban Midwestern Latino women. To participate you must be between the ages of 20 to 50 years, born in a Latin American country, and not pregnant at the time of the study. You are being asked to participate because you are a Latino or Hispanic woman.

Procedures:
Participation in this study will require approximately 5-8 minutes of your time. You will be asked some questions about foods that are part of your habitual diet, foods that you prepare/cook to eat and are eaten by the household members, types of groceries you usually purchase to prepare meals, type of meals ordered/eaten at local restaurants, and foods provided by local agencies, supplemental food programs, or food boxes and are part of your habitual diet. These questions would need short answers. You will respond to these questions orally. You will not have to write your answers. Participants will be required to 1) read and sign the consent form, and 2) answer the questions. Your name will be kept in secret. This study will take place at either one of the following locations in Lincoln, NE: El Centro de las Americas, Lincoln Catholic Social Services-Emergency Clinic, or Cristo Rey Church.

Risks and/or Discomforts:
There are no known risks or discomforts associated with this research.

Benefits:
This study may not provide a direct benefit to you. However, the information gained from this study will help us to conduct a food related research that is helpful to the Latino community and make it more appropriate to the Latino audiences.

110 Ruth Leverton Hall / P.O. Box 830806 / Lincoln, NE 68583-0806 / (402) 472-3716 / FAX (402) 472-1587

IRB#2007-07-410 EP
Date Approved: 08/08/07
Valid Until: 08/07/08

Confidentiality:
Any information obtained during this study which could identify you will be kept strictly confidential. The data will be stored a file cabinet in a locked room and will only be seen by the principal and secondary investigators during the study and for three to five years after the study is complete and thereafter destroyed. The information obtained in this study may be published in scientific journals or presented at scientific meetings but your identity will be kept strictly confidential.

Compensation:
You will receive a $10 gift certificate from Wal-Mart for participating in this study.

Opportunity to Ask Questions:
You may ask any questions concerning this research and have those questions answered before agreeing to participate in or during the study. If you need more information or have questions about this research, please call the principal investigator, Karina Lora, office phone, (402) 472-7984 or by email at klora1@bigred.unl.edu. If you have questions concerning your rights as a research subject that have not been answered by the investigator or to report any concerns about the study, you many contact the University of Nebraska-Lincoln Institutional Review Board, telephone (402) 472-6965.

Freedom to Withdraw:
You are free to decide not to participate in this study or to withdraw at any time without adversely affecting your relationship with the investigators, the University of Nebraska. Your decision will not result in any loss or benefits to which you are otherwise entitled.

Consent, Right to Receive a Copy:
You are voluntarily making a decision whether or not to participate in this research study. Your signature certifies that you have decided to participate having read and understood the information presented. You will be given a copy of this consent form to keep.

Signature of Participant:

______________________________ ______________

Signature of Research Participant *Date*

Name and phone number of investigator(s)
Karina R. Lora, Principal Investigator *Office: (402) 472-7984*
Nancy Lewis Ph.D. Secondary Investigator *Office (402) 472-4633*

UNIVERSITY OF Nebraska Lincoln

COLLEGE OF EDUCATION AND HUMAN SCIENCES
Department of Nutrition and Health Sciences

INFORMED CONSENT FORM

Dear participant:

You have been selected to participate in this study. Thanks you for being part of this important research study. You are being asked to participate because you are a Latino or Hispanic woman. Please read the information provided below before proceeding to answering the questions. Participation in this study is voluntary and does not pose any risk to you. All information will be kept strictly confidential.

Identification of Project:
Validity and reliability of a quantitative Food Frequency Questionnaire to measure omega-3 fatty acid food intake of urban Midwestern Latino women - Phase I /**dietary assessments.**

Omega-3 fatty acid foods are important for your health. They can protect against coronary heart disease. A Food Frequency Questionnaire is a dietary tool to measure amount and frequency of food consumption. We are conducting this study to know how much and how often you are eating foods that contain omega-3 fatty acids and that could provide you good cardiovascular health.

Purpose of the Research:
This is a research project that aims to test a questionnaire to measure intakes of certain types of foods in a sample of urban Midwestern Latino women. You must be between the ages of 20 to 50 years, born in a Latin American country, and not pregnant at the time of the study. You are being asked to participate because you are a Latino or Hispanic woman.

Procedures:
For this study you would need to complete two methods of dietary assessment. In these two methods you would report type and amount of foods that you eat. The first dietary assessment method is a questionnaire. You would complete two questionnaires one month apart. Participation in the questionnaire will require approximately 15 to 20 minutes of your time to complete each. To complete the questionnaire you would need to mark an answer that best provides your food intake. The second dietary assessment method is a 24-hr food recall. You would need to complete three 24-hr recalls, one week apart each. Participation in the 24-hr food recall will require approximately 10 to 15 minutes of your time to complete each. To complete the 24-hr food recall you will provide short oral answers.

Participants will be required to 1) read and sign the consent form, and 2) answer the questions. Your name will be kept in secret. This study will take place at either one of the following locations in Lincoln, NE: El Centro de las Americas, Lincoln Catholic Social Services-Emergency Clinic, or Cristo Rey Church.

Risks and/or Discomforts:
There are no known risks or discomforts associated with this research.

110 Ruth Leverton Hall / P.O. Box 830806 / Lincoln, NE 68583-0806 / (402) 472-3716 / FAX (402) 472-1587

Benefits:
This study may not provide a direct benefit to you. However, the information gained from study will help us to conduct food related research that is helpful to the Latino community. By conducting this study we will gain insight of amount and frequency of food consumption of certain foods that urban Latino women eat and are beneficial for their health.

Confidentiality:
Any information obtained during this study which could identify you will be kept strictly confidential. The data will be stored in a file cabinet in a locked room and will only be seen by the principal and secondary investigators during the study and for three to five years after the study is complete and thereafter destroyed. The information obtained in this study may be published in scientific journals or presented at scientific meetings but your identity will be kept strictly confidential.

Compensation:
You will receive a $30 gift certificate from Wal-Mart for participating in this study.

Opportunity to Ask Questions:
You may ask any questions concerning this research and have those questions answered before agreeing to participate in or during the study. If you need more information or have questions about this research, please call the principal investigator, Karina Lora, office phone, (402) 472-7984 or by email at klora1@bigred.unl.edu . If you have questions concerning your rights as a research subject that have not been answered by the investigator or to report any concerns about the study, you may contact the University of Nebraska-Lincoln Institutional Review Board, telephone (402) 472-6965.

Freedom to Withdraw:
You are free to decide not to participate in this study or to withdraw at any time without adversely affecting your relationship with the investigators, the University of Nebraska or LPS. Your decision will not result in any loss or benefits to which you are otherwise entitled.

Consent, Right to Receive a Copy:
You are voluntarily making a decision whether or not to participate in this research study. Your signature certifies that you have decided to participate having read and understood the information presented. You will be given a copy of this consent form to keep.

Signature of Participant:

________________________________ ____________________

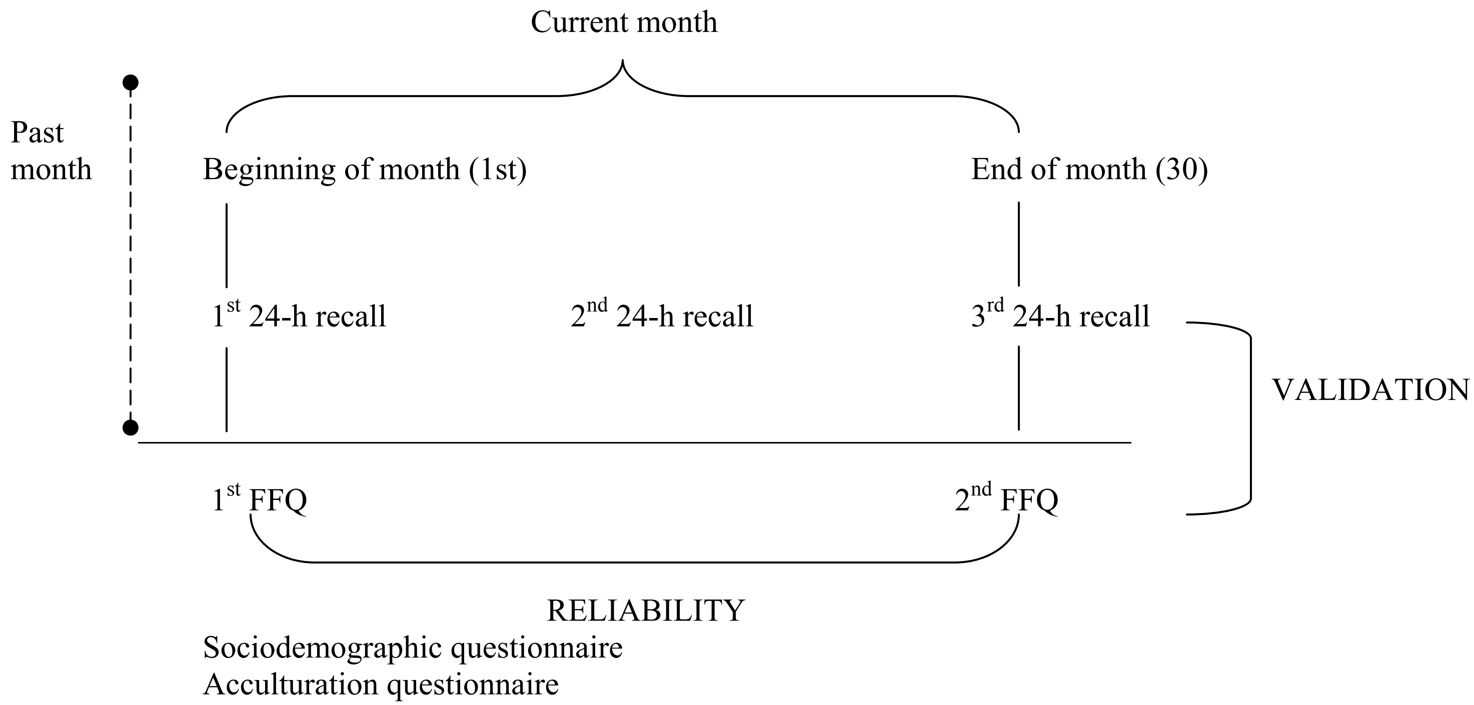
Signature of Research Participant *Date*

Name and phone number of investigator(s)
Karina R. Lora, Principal Investigator *Office: (402) 472-7984*
Nancy Lewis Ph.D. Secondary Investigator *Office (402) 472-4633*

APPENDIX B

Study Design

Study Design



Reliability: 1st FFQ vs. 2nd FFQ
Validity: Mean FFQs vs. Mean of three recalls

APPENDIX C

Letter to Institution Requesting Participation

Date

Dear Sir/Madam,

My name is Karina Lora. I am a PhD student in the Department of Nutrition and Health Sciences at the University of Nebraska-Lincoln, under the guidance of Dr. Nancy Lewis.

I would like to request your permission to survey Latinas/Hispanic women at **El Centro de las Americas** you direct for my study. My research aims to assess the intake of omega-3 fatty acids in Latinas/Hispanic women. The participants will be asked to complete two food questionnaires in one month. To accomplish that, I will meet with each participant twice, at the beginning and at the end of the month. I the first meeting, I will interview the participant about the foods she ate in the last month. In our second meeting I will conduct a similar interview I did in our first meeting. Participants will complete a sociodemographic and an acculturation questionnaire during the first interview. Participants for this study should be a) between the ages of 20 to 50 years, b) born in a Latin America country, and c) not being pregnant at the time of the study. Participation in this research study does not pose any risk to the participants or anyone else.

In order for me to survey the participants I will need to visit your institution and survey the participants there. I will require a table and chairs for the participants to sit and complete the questionnaire.

If you agree to grant me permission to carry out this study at your clinic, please let me know so by signing the attached letter of consent. The letter of consent needs to be printed on your letter head for use in our university's Institutional Review Board (IRB) process.
I would greatly appreciate your willingness to participate in this research study.

Thank you,
Sincerely,

Karina R. Lora, M.S.
(Principal investigator)
Graduate student
Department of Nutrition
& Health Sciences
University of Nebraska-Lincoln
Lincoln, Nebraska 68588 – 0806
Email: klora1@bigred.unl.edu

Dr. Nancy Lewis Ph.D. R.D.
(Secondary investigator)
Professor
Department of Nutrition &
& Health Sciences
University of Nebraska - Lincoln
Lincoln, Nebraska 68588 - 0806
Email: nlewis2@unl.edu

APPEDIX D:

Letter of Consent from Institution to Recruit Participants

LETTER OF CONSENT

Date:

Karina R. Lora M.S.
Graduate student
312 Ruth Leverton Hall
Department of Nutrition and Health Sciences
University of Nebraska Lincoln
Lincoln, Nebraska 68588 – 0806

Ms Lora,

□ I authorize and support the inclusion of subjects at El Centro de las Americas for your study.

□ I am sorry to inform you that I do not authorize and support the inclusion of subjects at El Centro de las Americas for your study.

Thank you for interest in our institution for your research study.

Good luck in your endeavors.

Sincerely,

______________________________________ _______________
Signature Date

____
Designation

____

____

____
Address

APPENDIX E

Design of Poster Used for Recruitment

Tell me about what you eat and ...

EARN A $10 WAL-MART GIFT CERTIFICATE!!!

A nutrition study to know how much of certain types of foods you eat that may be healthy for your heart

What do you need to do?

1. Fill two questionnaires
2. I will interview you three times to tell me what you eat the day before
3. It will take 15 to 20 minutes to complete each

If you are a <u>Hispanic/Latino woman</u> and are:

a) Between the ages of 20 to 50 years
b) Born in a Latin American country, and
c) You are <u>not</u> pregnant

You can participate!!!

Location:
Days:
Time:
Contact person: Karina Lora 770-4580

This is a study from the Department of Nutrition and Health Sciences, University of Nebraska-Lincoln

APPENDIX F:

Preliminary Interviews Participant's Recruitment Sheet

Preliminary Interviews
Omega-3 Latinas study

Code	Date	Participant's name	Would you like to be contacted again?	Best time to call	Phone number	Address

APPENDIX G

Preliminary Interviews

code________

Preliminary Interviews
Omega-3 Latinas Study

Date: _________________

1. What foods and meals are part of your daily diet?

____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________

2. What foods do you prepare or cook for?

Yourself	**Family**
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________
____________________	____________________

code________

3. Where and what groceries do you buy for you and your family?

4. When you and your family, relatives or friends dine out, what food(s) do you order?

1. ______________________________
2. ______________________________
3. ______________________________
4. ______________________________
5. ______________________________
6. ______________________________
7. ______________________________

5. If you are enrolled in food assistance programs, what foods do you receive that are part of your usual diet?

APPENDIX H

Pilot Test Participant's Feedback

Date__________   Code __________

Pilot test-Omega-3 FFQ

1. Were the instructions easy to understand?

   ____

2. Did you know how to mark your answer?

   ____

3. Do you think or feel there were too many options to mark your answer?

   ____

4. How hard was to answer the questions?

   ____

5. I saw that you hesitated to answers some questions, what did you think at that moment?

   ____

   ____

6. Did you find the questionnaire interesting?

   ____

7. Was there a time you wanted to stop answering the questions? Why?

   ____

8. Did any question offend you?

   ____

APPENDIX I

Sociodemographic Questionnaire

Date_______________ code ____________

Sociodemographic questionnaire
Omega-3 Latinas study

Please answer the following:

1. Name ____

2. Age_______________ (y) Country of birth ______________________

3. Are you Hispanic or Latino? Yes _____ No_____ Do not know _____

4. At this moment,
   Are you pregnant? Yes _____ No_____ Do not know _____

   Are you lactating? Yes _____ No_____ Do not know _____

5. You are?

_____ Married
_____ Divorced
_____ Widowed
_____ Separated
_____ Never married
_____ A member of an unmarried couple

6. How many children less than 18 years of age live in your household?

______ (number of children) ______ None

7. How many adults older than 18 years of age including yourself live in your household?

______ (number of adults)

8. What is the highest year of school you completed?

_____ Years of education

_____ Never attended school or only kindergarten

_____ Grade 12 or GED (high school graduate) code ___________

_____ College 1 year to 3 years (some college or technical school)

_____ College 4 years or more (college graduate)

_____ Refuse to answer

9. Is your annual household income from all sources?

   a. Less than $10,000
   b. Between $10,000 and $15,000
   c. Between $15,000 and $20,000
   d. Between $20,000 and $25,000
   e. Between $25,000 and $35,000
   f. Between $35,000 and $50,000
   g. Between $50,000 and $75,000
   h. $75,000 or more
   i. Do not know/he/she is not sure
   j. Refuse to answer

10. What is the primary language spoken at home?

_____ English _____ Spanish Other______

11. You are

_____ Full time employed
_____ Part-time employed
_____ Unemployed
_____ Homemaker

12. How old were you when you first arrived to the United States?

______ y

13. How long have you lived in the United States?

______ y.

APPENDIX J

Twenty-four Hour Recall

Date:	Number:	Code:
Date of record:	Weekday:	Weekend:

24-h recall Omega-3 Latinas study

Type of food	Amount	Cooking method
Example: Potato	1 medium	Boiled

APPENDIX K

Acculturation Questionnaire

Questionnnaire A

Code _______________

Please mark the box that better represent your answer

1 In general, what language(s) do you speak?

☐	☐	☐	☐	☐
Only Spanish	Spanish better than English	Both Equally	English better than Spanish	Only English

2 In general, in what language(s) do you read?

☐	☐	☐	☐	☐
Only Spanish	Spanish better than English	Both Equally	English better than Spanish	Only English

3 In general, what language(s) do you usually speak at home?

☐	☐	☐	☐	☐
Only Spanish	More Spanish than English	Both Equally	More English than Spanish	Only English

4 In which languages do you usually think?

☐	☐	☐	☐	☐
Only Spanish	More Spanish than English	Both Equally	More English than Spanish	Only English

5 What language(s) do you usually speak with your friends?

☐	☐	☐	☐	☐
Only Spanish	More Spanish than English	Both Equally	More English than Spanish	Only English

6 What was the language you used as a child?

☐	☐	☐	☐	☐
Only Spanish	More Spanish than English	Both Equally	More English than Spanish	Only English

APPENDIX L

Omega-3 Food Frequency Questionnaire

Food Frequency Questionnaire

Date________________ Code_____________

This form asks about your usual dietary intake over the past month. It will require approximately 15 to 20 minutes of your time to complete. Please use the following instruction:

1. Read each food item. If you have not eaten this food in the past month, mark "none" and move onto the next food item.
2. Indicate whether you think your usual serving is small (S), medium (M), or large (L) by marking the correct serving size box. The dietary interviewers will show you food models for visual reference of servings.

   Note: A small (S) serving is equal to half (1/2) the usual serving
   A medium (M) is equal to the medium servings listed on the form
   A large (L) is equal to one and a half (1 ½) times as much or mote of the medium serving

3. Think over the past month. How often do you usually eat each of the following items? Again, mark the box under the correct heading. Answer each question as best as you can; estimate if you are not sure.

Thanks!

						MONTH		WEEK			DAILY	
Meats		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Turkey	3 ounces											
Ground beef, 70% or 93% lean	3 ounces											
Ground beef, 75% or 95% lean	3 ounces											
Ground beef, 80%, 85% or 90% lean	3 ounces											
Ground beef, unknown % fat, stewed or boiled	3 ounces											
Beef cubes, boiled	3 ounces											
Breakfast beef patty	1 small											
Beef steak	3 ounces											
Beef liver	3 ounces											
Chicken leg, no skin	1 medium											
Chicken leg with skin	1 medium											
Chicken breast, no skin	3 ounces											
Chicken breast, with skin	3 ounces											
Ham	3 ounces											
Deli ham	0.5 ounce											
Deli turkey	0.3 ounce											
Ground pork	3 ounces											
Pork cubes, boiled	3 ounces											
Bacon	1 strip											
Sausage	3 ounces											
Pork hotdogs/franks	1 link											
Turkey hotdogs/franks	1 link											
Pork ribs	3 ounces											
Pork chop	3 ounces											

						MONTH		WEEK			DAILY	
Seafood		Never	S	M	L	Once a month	2-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	1 time	2 or more
Tuna canned in oil	3 ounces											
Tuna canned in water	3 ounces											
Sardines canned in tomato sauce	3 ounces											
Sardines canned in water	3 ounces											
Salmon canned	3 ounces											
Fresh salmon	3 ounces											
Tilapia	3 ounces											
Fish sticks	1 ounce											
Breaded fillet fish	3 ounces											
Shrimp	3 ounces											

						MONTH		WEEK			DAILY	
Eggs		Never	S	M	L	Once a month	2-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	1 time	2 or more
Egg, boiled	1 medium											
Egg yolk, boiled	1 medium											
Fried egg, vegetable oil	1 medium											
Fried egg, canola oil	1 medium											
Fried egg, corn oil	1 medium											
UNL n-3 enriched egg	1 medium											
Eggland Best n-3 egg	1 medium											

						MONTH		WEEK			DAILY	
Dairy		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Vitamin D whole milk	1 cup											
2% milk	1 cup											
1% milk	1 cup											
Evaporated milk	2 tbsp											
Condensed milk	2 tbsp											
Cheddar cheese shredded	1 ounce											
Cheddar cheese, slice	0.75 ounce											
Swiss Cheese, slice	0.75 ounce											
American cheese regular, slice	2/3 ounce											
2% American cheese, slice	0.75 ounce											
Monterrey cheese, shredded	1 ounce											
"Mexican blend" cheeses shredded (cheddar, monterrey, asadero, blanco)	1 ounce											
Mozarella cheese, whole milk, shredded	1 ounce											
Mozzarella cheese part skim milk, shredded	1 ounce											
Mozarella cheese 2% string	1 string											
Mexican queso fresco cheese	1 ounce											
Parmessan cheese	1 ounce											
Feta cheese	1 ounce											
Cream cheese, regular	1 tsp											
Cream cheese, light	1 tsp											
Yogurt, light	6 ounces											
Yogurt, original o regular	6 ounces											
Ice cream, regular	1/2 cup											
Ice cream, light	1/2 cup											
Butter	1 tsp											

						MONTH		WEEK			DAILY	
Vegetables		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Potato, boiled, no skin	1 medium											
French fries	1 small serving											
Hash browns	1/2 cup											
Broccoli, raw	1/2 cup											
Broccoli, fresh cooked	1/2 cup											
Broccoli, frozen cooked	1/2 cup											
Green peas, frozen boiled	1/2 cup											
Spinach, fresh	1/2 cup											
Spinach, fresh cooked	1/2 cup											
Spinach, frozen, cooked	1/2 cup											
Cauliflower, raw	1/2 cup											
Cauliflower,cooked	1/2 cup											
Carrots, frozen cooked	1/2 cup											
Corn, frozen cooked	1/2 cup											
Corn on cob	1 medium											
Corn, can	1/2 cup											

						MONTH		WEEK			DAILY	
Vegetables		Never	S	M	L	Once a month	2-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	1 time	2 or more
Green beans, fresh	1/2 cup											
Green beans, canned	1/2 cup											
Green peppers, chopped	1/2 cup											
Red pepper, chopped	1/2 cup											
Vegetable mix, frozen, boiled	1/2 cup											
Asparagus, frozen, boiled	1/2 cup											
Asparagus, fresh, boiled	1/2 cup											
Red radish	1/2 cup											
Romaine lettuce	1/2 cup											
Iceburg lettuce	1/2 cup											
Green olives	1 ounce											
Black olives	1 ounce											
Green soy beans	1/2 cup											
Tomato spaghetti sauce	1/2 cup											
Tomatillo, raw	1/2 cup											
Avocado	1/2 of medium											

Fruits		Never	S	M	L	MONTH Once a month	MONTH 2-3 times a month	WEEK 1-2 times a week	WEEK 3-4 times a week	WEEK 5-6 times a week	DAILY 1 time	DAILY 2 or more
Banana	1 medium											
Strawberries	1/2 cup											
Cranberry juice	3/4 cup											
Apple	1 medium											
Apple juice ready to drink	3/4 cup											
Orange	1 medium											
Orange Juice	3/4 cup											
Red/green grapes	1/2 cup											
Grape Juice	3/4 cup											
Grapefruit juice	3/4 cup											
Blueberries, frozen	1/2 cup											
Blueberries, fresh	1/2 cup											
Pineapple	1/2 cup											
Mango	1/2 cup											
Kiwi fruit	1 medium											
Papaya	1/2 cup											
Cantaloupe/muskmelon	1/2 cup											
Honeydew Melon	1/2 cup											
Fruit cocktail	1/2 cup											

						MONTH		WEEK			DAILY	
Breads, cereals		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Corn tortilla	1 tortilla											
Flour tortilla	1 tortilla											
Whole wheat tortilla	1 tortilla											
Whole wheat bread	1 slice											
White bread	1 slice											
Hotdog bun	1 bun											
Mexican pan dulce	1/2 half of each											
Mexicano bolillo	1 whole											
Oatmeal, instant	1 pack											
Oatmeal, regular	1 pack											
Special K cereal	1/2 cup											
Life cereal	1/2 cup											
Cherrios cereal	1/2 cup											
Honey bunch cereal	1/2 cup											
Raisin Bran cereal	1/2 cup											
Granola cereal	1/2 cup											
White rice	1/2 cup											
Noodles	1/2 cup											
Ramen noodles	1 pack											
Mac and cheese	1/2 cup											
Waffle, frozen	1 waffle											
Pancake from mix	1 panck											

						MONTH		WEEK			DAILY	
Bread,cereals		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Cinammon/sugar cookies	1 medium											
Oreo cookies	1 cookie											
Bagel	1/2 bagel											
Donut, plain	1/2 of medium											
Garlic bread	1 slice											

						MONTH		WEEK			DAILY	
Condiments, snacks		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Mayo, regular	1 tsp											
Mayo, light	1 tsp											
Popcorn natural/butter	1 cup											
Lay's clasic potatoes, different flavors	17 chips											
Doritos	17 chips											
Tortilla chips	17 chips											

						MONTH		WEEK			DAILY	
Oils		Never	S	M	L	Once a month	2-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	1 time	2 or more
Butter spread	1 tsp											
Smart Balance spread	1 tsp											
Soybean oil	1 tsp											
Canola oil	1 tsp											
Corn oil	1 tsp											
Vegetable oil	1 tsp											
Vegetable blend oil	1 tsp											
Olive oil	1 tsp											
Smart Balance omega 3 oil	1 tsp											
Lard	1 tsp											
Ranch dressing	1 tsp											
Italian salad dressing	1 tsp											

						MONTH		WEEK			DAILY	
Nuts and seeds		Never	S	M	L	Once a month	2-3 times a month	1-2 times a week	3-4 times a week	5-6 times a week	1 time	2 or more
English Walnuts	1 ounce											
Pumpkin seeds	1 ounce											
Pecans	1 ounce											
Cashews	1 ounce											
Hazelnuts	1 ounce											
Brazil nuts	1 ounce											
Pistachios	1 ounce											
Sunflower seeds	1 ounce											

						MONTH		WEEK			DAILY	
Beans		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Soybeans, boiled	1/2 cup											
Garbanzo beans, boiled	1/2 cup											
Lentils, boiled	1/2 cup											
Pinto beans, cooked	1/2 cup											
Black beans, canned or boiled	1/2 cup											
Navy beans, canned or boiled	1/2 cup											
Kidney beans, canned or boiled	1/2 cup											
Northen beans,canned or boiled	1/2 cup											
Blackeye beans, canned or boiled	1/2 cup											
Refried beans	1/2 cup											
Soy milk	1 cup											
Peanut Butter	1 Tbsp											

						MONTH		WEEK			DAILY	
Prepared dishes		**Never**	**S**	**M**	**L**	**Once a month**	**2-3 times a month**	**1-2 times a week**	**3-4 times a week**	**5-6 times a week**	**1 time**	**2 or more**
Tuna Subway	6 inch											
Chicken sandwich "Subway"	6 inch											
Double cheese burger	1 burger											
Single cheese burger	1 burger											
Bacon cheese burger	1 burger											
Chicken burger, breaded	1 burger											
Chicken burger, grilled	1 burger											
Chicken nuggets (small portion)	4 units											
Mole	1 serving											
Pozole	1 cup											
Picadillo	1 serving											
Quesadilla	1 serving											
Sope	1 serving											
Tacos dorados	1 taco											
Tamales with pork	1 tamale											
Beef taquito	1 taquito											
Beef with chile	1 serving											
Enchilada	1 enchilada											
Frijol charro	1/2 cup											
Beef and bean burrito	1 burrito											
Bean and cheese burrito	1 burrito											
Spanish rice	1/2 cup											
Atole (half water,half milk)	1 cup											
Cornmeal	1/2 cup											
Pizza, pepperoni "Pizza Hut"	1 slice											
Pizza, suprema "Pizza Hut"	1 slice											
Cheese pizza "Pizza Hut"	1 slice											