Abstract

Vitamin A deficiency is recognized as a public health problem in the Philippines as confirmed by the 1993 National Nutrition Survey which showed an increasing prevalence of vitamin A deficiency among schoolchildren. This proposed study aims to determine whether the intake of fruits and vegetables that are rich in provitamin A carotenoids will result in an improvement in vitamin A liver stores of 7-10 year old children who are malnourished in vitamin A. To meet this objective, vitamin A malnourished children (serum retinol, <25 μg/dL) in two villages (experimental and control) will be identified and enrolled. Deworming will be done one month before the start of the study. Vitamin A status measurements - the deuterated retinol dilution method, serum retinol, dietary vitamin A intake, and conjunctival impression cytology (CIC) procedure - will be done at baseline. Then the experimental group (n = 25) will be fed with fruits/vegetables to provide 2.4 mg of β-carotene, i.e., 400 μg retinol equivalents (RE) daily, for five days a week for twelve weeks. The control group (n = 25) will be fed an equivalent amount of calories and no vitamin A or carotenoids. At the end of the feeding period, all measurements for determining vitamin A status will be repeated. Other determinations before and after intervention, will include total blood haemoglobin, and serum ferritin, albumin, transthyretin, and C-reactive protein concentrations. The proposed study will provide information regarding the adequacy of vitamin A measures, and the efficacy of food-based intervention programs in improving the vitamin A status of malnourished populations.

1. SCIENTIFIC BACKGROUND AND SCOPE OF THE PROJECT

1.1. Background

Vitamin A deficiency continues to be a public health problem in many developing countries including the Philippines. It is estimated that 17 children in the Philippines go blind everyday as a result of vitamin A deficiency [1]. As shown in Table I, vitamin A deficiency signs and symptoms were found in many regions of the country [2-7]. Among schoolchildren 7-14 years of age, 1.3% experienced night blindness, and 0.2% had Bitot’s spots [7]. In certain regions, low serum retinol concentrations (<20 μg/dL) were highly prevalent [2, 6, 8-11]. For example, a study done by the Nutrition Center of the Philippines in 1993 showed that about 30% of rural schoolchildren 6-16 years old, in Santo Tomas, Batangas, had serum retinol levels that were <20 μg/dL [11]. In rural areas, dietary vitamin A intake was only 73% of the Filipino recommended daily intake, and was obtained mostly from pro-vitamin A carotenoid sources.
1.2. Study Objectives

This study aims to determine whether the intake of fruits and vegetables that are rich in pro-vitamin A carotenoids, i.e., 2.4 mg β-carotene (400 µg RE), for 5 days a week for 12 weeks, will result in an improvement in the vitamin A status of 7-10 year old children who are malnourished in vitamin A. The results of the deuterated retinol dilution method will be compared with other measures of assessing vitamin A status, i.e., serum retinol concentrations, total dietary vitamin A intake from pre-formed vitamin A and pro-vitamin A carotenoids, and the CIC procedure.

1.3. Deuterated Retinol Dilution Method (DRD)

A most promising indirect approach for estimating total body stores of vitamin A appears to be the stable isotope dilution method using deuterated vitamin A [12-15]. The method is based on the principle that when a tracer dose of deuterated vitamin A is administered, it reaches a pseudo equilibrium with the existing body pool of vitamin A, and the ratio of deuterated to non-deuterated retinol in serum can be used to calculate total body stores, 90-95% of which is in the liver [16,17]. Since deuterated vitamin A is non-radioactive, there are no risks involved by ingesting it. Earlier studies with animals employed tracer doses of radioactive (3H) vitamin A to validate the use of the isotope dilution method as a measure of total body stores of vitamin A [18-21]. Few studies have been done in humans. Sauberlich et al. used (C14) vitamin A to study the vitamin A body pool and utilization rate in young adult males [16]. Furr et al. validated the use of the isotope dilution assay using tetradeuterated vitamin A by comparing the calculated vitamin A liver concentrations with values obtained by HPLC measurements of surgical liver biopsies [12]. They found that the correlation coefficient between these values was 0.88, and the Spearman’s rank correlation coefficient was 0.95 (p<0.002). They concluded that the isotope dilution assay can provide a valid estimate of total body stores of vitamin A in humans. As one might expect, Furr et al. found no correlation in the satisfactory range of vitamin A status, between the results of the stable isotope dilution method and serum retinol levels, since serum retinol is homoeostatically controlled over a wide normal range of liver vitamin A levels [12, 22].

1.4. Study Significance

Data from this study will provide information regarding the efficacy of food-based intervention programs in improving the vitamin A status of malnourished populations. It will indicate how the deuterated retinol dilution assay compares with other measures of assessing vitamin A status, i.e., total dietary vitamin A intake, serum retinol concentrations, and the CIC procedure. Correlations with total dietary intakes will show at which level of intake low body vitamin A stores (<20 µg/g liver) are associated with.

2. METHODS

2.1. Preliminary procedures

These include identification of the study sites which are two elementary schools located in two villages of similar socio-economic characteristics; conferring with local government, school and health officials; obtaining study approval from the Ethics Review Board of the Philippine Council for Health and Research Development; conferring with various agencies regarding food supplies; and setting up of a “work station” at the study sites.
2.2. Recruitment of subjects

Elementary school children of both sexes will be recruited to participate. Informed consent will be obtained from their parents or guardians.

2.3. Screening

2.3.1. Procedures and exclusions

Volunteer subjects (total n = 400 or 200 per school) will undergo screening procedures as follows: information will be collected on medical history, use of medications, nutritional supplements, age, parents' smoking habits, and demographic characteristics (e.g., family size, parents' education, etc.). Eye examinations will be conducted by an ophthalmologist, and children found to have clinical signs of xerophthalmia (Bitot's spots and conjunctival xerosis) will be treated and excluded from participation [22]. A complete physical examination will be done; heights and weights will be obtained. General nutritional state will be determined by use of Filipino standard tables of weight-for-age, and weight-for-height, and height-for-age measurements. Those who are <60% of weight-for-age gender-specific standards will be considered severely protein-energy malnourished, and will not be eligible to participate. The children who will be admitted into the study will be 7-10 years of age who do not have any major diseases, prolonged diarrhea, acute or chronic infections, and prolonged febrile conditions. They should agree to provide blood samples during the study, and should be permanent residents in the area.

2.3.2. Conjunctival impression cytology (CIC)

As part of the screening procedure, in addition to an eye examination for signs of xerophthalmia, a cytologic assessment will be done. The CIC procedure is a non-invasive and sensitive histological technique of obtaining surface cells on the bulbar conjunctiva with the use of a special vacuum pump applicator that gently applies a filter paper on the temporal surface of each eye and removes it by suction in one quick step [23]. The specimen is fixed, stained and examined under light microscopy for the presence of abundant mucin-secreting goblet cells and sheets of small epithelial cells. Impressions from xerophthalmic children will show a marked reduction or absence of goblet cells and the appearance of enlarged keratinized epithelial cells [24].

2.3.3. Serum retinol measurements

To identify vitamin A-deficient children, blood will be obtained from ~300 children (~150 per school). Serum will be separated, frozen, and transported to the HNRCA in Boston for serum retinol analyses using reverse-phase HPLC procedures (25,26). Those with serum retinol values of <25 µg/dL will be considered vitamin A-deficient and thus eligible to participate in the intervention study.

2.4. Study design

Children in the experimental group (n=25) whose serum retinol values are found to be <25 µg/dL, will be matched (for age, gender, weight for age, family size, serum retinol value) to children in the control group (n=25). The following procedures will be done: i) deworming; ii) baseline measurements of vitamin A status and other biochemical measurements in blood; iii) feeding fruits and vegetables during the intervention period to the experimental group, and an equivalent amount of calories from foods containing no vitamin A or carotenes to the control group; iv) and at post-intervention, repeat of vitamin A status measurements and other blood measurements.
2.4.1. Deworming

About one month before the start of the intervention phase, the participants will be treated for parasites, since parasites may adversely affect carotenoid absorption and vitamin A status.

2.4.2. Baseline measurements

2.4.2.1. Deuterated retinol dilution test

Subjects will start an isotope dilution test by oral ingestion of 4 mg of tetradeuterated vitamin A (all-trans-retinyl-10, 19, 19, 19-[2H4]acetate) in corn oil. After a pseudo equilibrium period of 21 days, 5 mL of blood will be drawn; serum will be separated, frozen, and transported to the HNRCA in Boston for HPLC quantitation of retinol [25,26], isolation of retinol from serum, conversion of isolated retinol to tert-butyldimethylsilyl derivatives [14], and determination of deuterated and non-deuterated retinol in derivatized preparations by GC/MS [12-15]. Total body stores of vitamin A will be calculated using the formula of Bausch and Rietz as modified by Furr et al. [12, 18].

2.4.2.2. Dietary Vitamin A intake assessment

A detailed assessment of the child’s dietary intakes of vitamin A, pro-vitamin A carotenoids, protein, fat, and energy, will be obtained by a dietitian. Retrospective methods for recalling past actual intake (e.g., 24-hour food recall) and for determining past usual intake through a food frequency questionnaire based on Philippine food composition tables will be used.

2.4.2.3. Other biochemical measurements in blood

In addition, the following measurements will be done. Serum carotenoids will be quantitated using HPLC procedures that measure both pro-vitamin A carotenoids (α- and β-carotene, cryptoxanthin) and non-pro-vitamin A carotenoids (lycopene, lutein, zeaxanthin) [25,26]. Total haemoglobin in whole blood, and serum ferritin, albumin, transthyretin, and C-reactive protein will be measured. Serum ferritin will be determined using the Ciba Corning MAGIC ferritin (125I) radioimmunoassay kit (Cat. No. 472329, Ciba Corning Diagnostics Corp., Medfield, MA). Serum albumin will be measured using the Roche Reagent for Albumin kit (Order No. 42332, Roche Diagnostic Systems, Branchburg, NJ) which uses a modification of the bromocresol green binding assay of Doumas et al. [27]. C-reactive protein will be measured with the Cobas FARA II in conjunction with a reagents kit from Atlantic Antibodies (Document AM-0039, Atlantic Antibodies, Inc., Stillwater, MN). Except for haemoglobin determinations, all of these assays will be conducted at the HNRCA in Boston.

2.4.3. Food-intervention phase: Effect of fruit and vegetable intake on measures of vitamin A status

Five days a week for twelve weeks, the experimental group will be fed with fruits/vegetables with rice, to provide 2.4 mg of β-carotene (i.e., 40 μg RE) per day; the control group will be fed an equivalent amount of calories in the form of cereal/rice products and jam devoid of vitamin A and carotenoids. After the study period of 12 weeks, all measurements done at baseline, the CIC procedure, and serum retinol measurements will be repeated. For the isotope dilution test, octadeuterated retinyl acetate will be administered (instead of tetradeuterated retinyl acetate) in order to avoid interference from residual tetradeuterated retinol from the previous dose.
Throughout the intervention phase, morbidity data will be obtained, and weekly eye examinations will be done in both experimental and control groups. Subjects who develop clinical signs of xerophthalmia will be treated and removed from the study. At the end of the 12-week period, all children will be given a 200 000 IU dose of vitamin A before they are discharged from the study. (All schoolchildren in the two villages who are identified to have low serum retinol levels will be provided with a 200 000 IU vitamin A capsule upon completion of the study, and every 6 months thereafter, for a period of 2 years.)

Whether pro-vitamin A carotenoids from plants can improve vitamin A status in humans remains controversial [28]. A recent study by de Pee et al. showed no improvement in vitamin A status of breast feeding Indonesian women supplemented daily with 100-150 g of vegetables (cassava leaves, water spinach, spinach, or carrots) for 12 weeks, whereas a similar amount of β-carotene from a simpler matrix (enriched wafer) produced a strong improvement [29]. In the group supplemented with vegetables, serum β- and α-carotene increased, but no changes were observed in retinol concentrations in serum or breast-milk or in MRDR test results. In our study, we will use several measures of vitamin A status, including serum retinol concentration, the CIC procedure, total dietary vitamin A status, and the deuterated retinol dilution method. It is our hope that the latter procedure will prove to be a reliable index of vitamin A body stores and more sensitive index of vitamin A states in humans.

2.4.3.1. Food source, preparation, and ingestion

In order to minimize non-compliance, mothers will be consulted regarding the fruit/vegetable preferences of their children, and the information will be used in the selection of fruits/vegetables for the intervention phase. The foods will be obtained in bulk from the International Institute of Rural Reconstruction (IIRR), a pioneer of bio-intensive gardening in the Philippines, which will be contracted by the Nutrition Center of the Philippines to grow the vegetables. A vegetable viand containing 1.4 mg of β-carotene will be consumed with rice during lunch at school. Oil will be used in the food preparation. Two 0.5-mg β-carotene portions of fruits/vegetables will be consumed as snacks during the mid-morning and mid-afternoon.

The control group will be given an equivalent amount of calories in the form of cereal/rice products, and jam containing no vitamin A or carotenoids.

Aliquots of homogenates of foods for the experimental and control groups will be analyzed for their carotenoid content by HPLC at the HNRCA, Boston.

3. RESULTS

3.1. In the Philippines

We have identified the study sites (experimental and control) which are two villages of similar socioeconomic characteristics: i) the village of Hukay, in the municipality of Silang, province of Cavite, about 50 km south of Manila, and ii) the village of Santa Elena, in the municipality of Santo Tomas, province of Batangas, about 70 km from Manila. The two villages are far apart, thus there will be no sharing of information among villagers regarding the study. They are similar in road conditions, economic status of families, and school population. Secondary nutrition data of the Silang Health Center in 1995 show that Hukay has a high prevalence of moderately and severely underweight pre-schoolchildren. Hukay Elementary School records (1995) reveal that 48 (i.e., 27%) of the 179 school children in Grades 1 to 6 are moderately and severely underweight. In Santo Tomas, Batangas, a study done by the Nutrition Center of the Philippines in 1993 showed that ~30% of schoolchildren have serum retinol levels <20 μg/dL [11].
We have submitted the study for approval by the Ethics Review Board of the Philippine Council for Health and Research Development, and approval has been granted.

We have conferred with local government, school, and health authorities in the two villages.

We are arranging for the procurement of fruits and vegetables from the IIRR. The Nutrition Center of the Philippines will contract the IIRR to grow the vegetables for the study, in order to have a single source of vegetables, thus minimizing variability in their nutrient composition.

The Nutrition Center of the Philippines has many years of experience in conducting nutrition surveys and vitamin A studies in children, including clinical and biochemical assessments for xerophthalmia, CIC measurements, and dietary intake assessments [3,6,8,11].

3.2. In Boston

We are making arrangements for the synthesis of tetradeuterated- and octodeuterated retinyl acetate.

The HNRCA laboratory in Boston routinely measures serum retinoids and carotenoids by HPLC, and has done initial studies of GC/MS retinol measurements [25,26].

4. PLANS FOR FUTURE WORK

We hope to complete all preliminary work and the field work in the Philippines during the first 18 months (November 1995 to April 1997), and all analyses (biochemical, dietary, cytological, statistical) during the following year.

The school calendar in the Philippines starts in mid-June and ends in mid-March. In order to avoid disruption of the study during the period of school vacation, we plan to start the study at the beginning of the school year. Screening procedures and blood drawing will be done in July/August 1996. Serum samples (n~300) will be transported to Boston and analyzed for retinol in September/October 1996. Children identified to have low serum retinol levels will be invited to participate in the study. They will be dewormed in November - one month prior to the start of the study. Baseline isotope dilution tests will be conducted in December 1996, and the intervention phase will proceed from January to March 1997. We hope to complete the post-intervention isotope dilution tests and other tests by April 1997. We expect to finish the writing-up of study results for publication in scientific journals by late 1997 or early 1998.

An evaluation of the efficacy of nutrition programmes aimed at improving the health of vulnerable populations is essential. Factors affecting the bioavailability of carotenoids and vitamin A are not well understood. We hope that this study is the first of many more future collaborative studies on carotenoid and vitamin A nutrition between the Nutrition Center of the Philippines and the HNRCA at Tufts University in Boston, Massachusetts, USA.
REFERENCES


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*Project Area

Source: National Nutrition Survey, Food and Nutrition Research Institute, 1993