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Original article

Blood docosahexaenoic acid and eicosapentaenoic acid in vegans: Associations with age and gender and effects of an algal-derived omega-3 fatty acid supplement

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SUMMARY

Background & aims: Several studies have demonstrated that vegetarians and vegans have much lower plasma concentrations of omega-3 fatty acids (i.e., docosahexaenoic and eicosapentaenoic acids) when compared to those who eat fish. The purposes of this study were 1) to define the age and/or sex-specific docosahexaenoic plus eicosapentaenoic acids levels in red blood cell membranes (expressed as a percent of total fatty acids; hereafter the omega-3 index) in long-term vegans, and 2) to determine the effects of a vegetarian omega-3 supplement (254 mg docosahexaenoic plus eicosapentaenoic acids/day for 4 months) on the omega-3 index.
Methods: A sample ($n = 165$) of vegans was recruited, and their omega-3 index was determined using a dried blood spot methodology. A subset of 46 subjects with a baseline omega-3 index of <4% was given a vegetarian omega-3 supplement for 4 months and then retested.
Results: The mean ± SD omega-3 index was 3.7 ± 1.0% which was similar to that of a cohort of omnivores (deployed US soldiers) from a recently-reported study. Among the vegan cohort, the index was significantly higher in females than males (3.9 ± 1.0% vs. 3.5 ± 1.0%; $p = 0.026$) and was directly related to age (p for trend = 0.009). The omega-3 index increased from 3.1 ± 0.6% to 4.8 ± 0.8% ($p = 0.009$) in the supplementation study.
Conclusions: We conclude that vegans have low baseline omega-3 levels, but not lower than omnivores who also consume very little docosahexaenoic and eicosapentaenoic acids. The vegans responded robustly to a relatively low dose of a vegetarian omega-3 supplement.

1. Introduction

The main omega-3 fatty acid in the vegan diet is alpha-linolenic acid (ALA), which is derived from foods such as soybeans, flaxseed and walnuts. ALA is very sparingly and inefficiently converted into the long-chain omega-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA).[1,2] Several studies have demonstrated that vegetarians and vegans have much lower plasma concentrations of DHA and EPA when compared to those who eat fish.[3–6] Supplementation with ALA increases plasma EPA to a small extent, but it has little effect on DHA.[2,3]

A substantial body of research (in essentially omnivorous populations) over the past several decades has documented the health benefits of increased omega-3 fatty acid intakes, suggesting that baseline levels could be considered deficient.[7] Because fish and fish oils were, up until recently, the only concentrated sources of preformed DHA and EPA, individuals who do not eat fish or fish oils could be at risk of low omega-3 fatty acid status. Whether this translates into increased risk for disease (e.g., cardiovascular) in vegans is unknown as their risk is already lower than that of omnivores. A recent meta-analysis found a 29% reduction in risk for death from ischemic heart disease and 18% reduction in risk for cancer in vegetarians compared to non-vegetarians,[8] and the EPIC-Oxford study reported similar benefits in British vegetarians.[9] Until randomized trials with omega-3 fatty acids are conducted in

Non-standard abbreviations: ANOVA, analysis of variance; ALA, alpha-linolenic acid; ASA24, Automated Self-Administered 24-h Recall; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; RBC EPA + DHA, omega-3 index; RBC, red blood cells.

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vegetarian/vegan cohorts, the question of whether higher blood levels of these nutrients will reduce risk for disease will remain unanswered. Nevertheless, at our present state of knowledge, it is possible that low omega-3 status does place vegetarians/vegans at increased risk for disease, which then presents a dilemma for these individuals.

There were 2 purposes of this study. The first (Phase 1) was to determine omega-3 status in long-term vegans and to investigate age and/or sex-related differences. We also sought to compare a vegan with an omnivore cohort with respect to omega-3 status. As we were unable to recruit two cohorts simultaneously in this investigation, we have used published data (also from our laboratory) from omnivores who met most of the same inclusion/exclusion criteria as did the vegans.[10] The second purpose was to determine the extent to which omega-3 status can be improved in vegans by the administration of a vegan DHA + EPA supplement (Phase 2). Throughout we have used DHA + EPA content of red blood cells (RBC) expressed as a percent of total RBC fatty acids (hereafter called the omega-3 index[11]), as the biomarker of omega-3 status.

2. Methods

2.1. Subjects

This study was approved by the University of San Diego Institutional Review Board. Subjects were recruited from vegan websites (e.g., vegsource.com, vegnews.com, healthscience.org), with an announcement directing interested individuals to a brief screening questionnaire on the study website. To qualify for the study subjects were required to be 20 years of age or older, vegan (consuming no meat, fish, eggs or dairy) for at least one year and currently not taking any essential fatty acid supplements. Subjects who met these criteria were contacted by study staff and invited to complete a consent form online (at nutritionalresearch.org). After doing so subjects were enrolled in the study and received an email with their personal identification number and password to use to complete the online study demographic questionnaire. This questionnaire included information on age, gender, height, weight, race/ethnic group, education, and length of time on a vegan diet, and nutritional supplements used currently and in the past year. In addition to collecting a blood sample, participants were instructed to complete 3 days of dietary recall online (described below).

Participants who completed the online consent form and study questionnaire were sent an omega-3 index test kit by regular mail. The kit contained a letter welcoming participants to the study and giving general instructions for completing the study. The kit also included blood sample collection instructions, all necessary supplies, a sample collection card with study ID, and a test request form and prepaid return envelope for the blood sample. Participants were instructed to collect a drop of blood from a fingerstick and mail it immediately to the laboratory (OmegaQuant Analytics, Sioux Falls, SD). The samples were analyzed on the day of arrival as described below. (This is the same procedure that was used in the omnivore study noted above.)

A total of 296 people filled out the screening questionnaire and were qualified to participate in Phase 1 of the study. There were an additional 619 who were qualified to participate but, because we had reached the quota of 27 subjects per gender/age group (see Section 2.4), these individuals were put on a waiting list. Of the 296 who were originally qualified for the study, 59 did not complete the online dietary survey within a period of two months. These individuals were notified that they would be removed from the study, and those on the waiting list in the corresponding sex*age groups were invited to participate. Recruitment occurred between February and August of 2012. Enrolled participants received a $20 gift certificate from a national department store chain.

In Phase 2 of the study we invited a sample of Phase 1 participants who had been tested early in Phase 1 and who had an omega-3 index of 4.0% or below to take a vegan omega-3 supplement for 4 months. Of 61 invited individuals, 48 chose to participate. These individuals were sent a 4-month supply of a vegan omega-3 supplement (Life's DHA plus EPA, DSM Nutritional Products, Inc., Parsippany, NJ) providing 172 mg DHA and 82 mg EPA per 0.75 mL. They were instructed to take this amount daily via a graduated bottle dropper. The blood collection protocol used in Phase 1 was repeated after 4 months.

2.2. Fatty acids

The omega-3 index home test kit was used to measure subjects' essential fatty acid levels in a dried blood spot as previously described.[10] Briefly, fatty acids ($n = 24$) are identified using capillary column gas chromatography with an internal-standard-based, three-point calibration curve approach. Upon receipt in the laboratory, paper punches of dried blood are transferred to a reaction vial. Fatty acid methyl esters are generated with boron trifluoride for 10 min at 100 °C, extracted into hexane after the addition of water and analyzed by gas chromatography by using a GC2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with a SP2560, 100-m column (Supelco, Bellefonte, PA). Fatty acids are identified by comparison with a standard mixture of fatty acids (GLC 727, Nucheck Prep, Elysian, MN). The omega-3 index (an erythrocyte-specific metric) is calculated from the dried blood spot EPA + DHA value using an equation determined from a study comparing these two values from 49 subjects. The correlation coefficient between RBC and dried blood spot EPA + DHA was 0.96 ($p < 0.0001$). The laboratory coefficient of variation for the omega-3 index is 5–6%.

2.3. Dietary assessments

In Phase 1 dietary intake data were collected and analyzed using the Automated Self-Administered 24-h Recall (ASA24) system, version 1, developed by the National Cancer Institute, Bethesda, MD. ASA24 is a freely available, web-based software tool.[12] It consists of a respondent web site used by participants to enter recall data and a researcher web site to manage study logistics and obtain analyses. The format and design of the Respondent Web site are modeled on the interviewer-administered Automated Multiple Pass Method 24-h recall developed by the US Department of Agriculture. Nutrient intakes were calculated by the ASA24 program based on the USDA's Food and Nutrient Database for Dietary Studies. Participants were instructed to complete at least two, and if possible three, days of dietary recall with a separation of 2–3 days between each recall. Regular reminders were sent to participants who did not complete the recalls within a month of enrollment in the study.

The ASA24 database did not include chia and hemp seeds, two food sources of ALA that might be consumed on a regular basis by vegans. These were therefore included in the online study survey, and the ALA provided was calculated as the grams of hemp/chia seed per teaspoon × frequency per week × grams ALA per teaspoon.

2.4. Statistical analysis

In phase 1, participants were initially stratified into five age categories by gender (20–39, 40–59, 60–69, 70–79, and 80+). Recruitment by internet proved to be difficult for those aged 70 and above, therefore the age categories of 70–79 and 80+ were