Multinational study of major breast milk carotenoids of healthy mothers

Supporting information

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Supported by a grant from Wyeth Nutrition,
Philadelphia, PA, USA.

Summary Background
Carotenoids in serum vary between countries and within populations with evidence suggesting a qualitative relationship to diet. Breast milk carotenoids furnish a source of vitamin A and potentially provide immunoprotection and other health benefits for infants. There have been numerous studies of milk carotenoid concentrations in undernourished populations; however, carotenoid concentrations have not previously been compared in populations of well-nourished mothers. Aim of Study To compare concentrations of five major carotenoid groups: α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, and lycopene in breast milk of healthy women from Australia, Canada, Chile, China, Japan, Mexico, the Philippines, the United Kingdom, and the United States, and to qualitatively compare patterns of dietary intake with milk carotenoid concentrations.

Methods Breast milk collected from healthy lactating women was analyzed for concentrations of five carotenoids and retinol and quantitated relative to total milk lipid. All determinations were performed in a single research laboratory using standardized methodology. Mothers consumed their usual diets and provided a single 24-h dietary recall. Results Breast milk carotenoid concentrations varied greatly among countries, with the greatest differences in β-cryptoxanthin (~ 9-fold) and the least in α-carotene and lycopene (~3-fold). Breast milk retinol concentrations varied ~ 2-fold across countries. The provitamin A carotenoids α-carotene, β-carotene, and β-cryptoxanthin as a group accounted for > 50% of the carotenoids measured. Total breast milk carotenoids were highest in Japanese and lowest in Philippine mothers. Breast milk β-carotene concentrations were highest in Chile and lowest in the Philippines. Conclusions Patterns of breast milk carotenoids were unique to each country and qualitative patterns reflected the dietary carotenoid supply.

Key words breast milk – carotenoids – healthy mothers
Introduction

As constituents of breast milk, the provitamin A carotenoids (β-carotene, α-carotene and β-cryptoxanthin) provide a significant source of vitamin A for the nursing infant. Furthermore, 30 additional carotenoids identified in breast milk have no known provitamin A activity but many have been associated with health benefits. For example, lycopene, a powerful antioxidant, is associated with decreased risk for ovarian [1] and breast cancer [2], while the macular pigments lutein and zeaxanthin are associated with decreased risk for age-related macular degeneration and cataracts [3]. Carotenoids as a group may enhance the immune system [4] and provide protection against chronic diseases [5]. Carotenoids delivered in breast milk may enhance immune defenses of the infant and protect against chronic disease later in life.

Breast milk is the preferred source of nutrition for infants. In addition to providing nutrients for growth, it contains biological components such as antibodies, enzymes and hormones, all of which contribute to infant development and protection of health. However, breastfeeding is not always possible. When artificial feedings are required, the nutrient composition of the infant formula should reflect the nutrient composition of breast milk as closely as possible. Unfortunately, many infant formulas currently on the market contain no measurable levels of carotenoids, and those that do, contain predominately β-carotene and β-cryptoxanthin [6].

Previously, most studies of carotenoids in breast milk have been of healthy U.S. mothers or mothers consuming diets low in vitamin A [7–12]. Milk carotenoid concentrations of healthy mothers in other countries have not been compared with those of U.S. mothers and in early studies, carotenoid concentrations in breast milk from U.S. mothers were assumed to be the standard “normal” concentrations [13]. If breast milk carotenoid composition did not vary between populations it would then be appropriate to use U.S. data as a reference against which to compare breast milk carotenoids in vitamin A low-to-deficient populations [14–18], and for the design of infant formula. However, if patterns of breast milk carotenoids within a population reflect the carotenoids in the local food supply, data from a single country would not provide a worldwide reference for healthy mothers. Therefore, to assess the range of carotenoid intakes for healthy breast-fed infants worldwide, comparative baseline data are needed. For this study, we chose nine countries that varied in climate and cultures and in which we were able to identify a pediatrician who had research experience and was involved with a successful lactation program. Five of the countries selected were in Asia or the Pacific Rim, three were in the Americas and one was in Europe (UK).

Subjects and methods

Study design

The study was a single, cross-sectional survey of carotenoids in milk from apparently healthy, well-nourished lactating women in nine countries: Australia, Canada, Chile, China, Japan, Mexico, the Philippines, the United Kingdom, and the United States. Study sites/investigators were selected using the following criteria: 1) participation by a practicing pediatrician, 2) clinic, health center or hospital-based, 3) access to a large group of healthy, lactating women, 4) available staff experienced in collection of breast milk, and 5) adequate facilities for the collection and storage of breast milk.

All mothers signed written, informed consent in their native language prior to enrolment in the study.

Subject Selection

All participants were 18 to 40 years of age, mothers of a healthy full-term singleton infant, and between 1–12 months postpartum at the time of milk collection. The sample collection period at each site was limited to three months. Our goal was to obtain samples from 50 women in each country. Inclusion criteria required: 1) a minimum 8.5 kg prenatal weight gain to assure that women had not followed a restrictive diet during pregnancy, 2) frequency of breast-feeding at least 5 times per day to assure that infants were primarily breast-fed, 3) consumption of at least 3 servings of fruits and vegetables (combined) per day, and 4) ability to comply with an afternoon appointment time. Exclusion criteria disallowed mothers who smoked, were receiving steroid medications, taking vitamins or other supplements containing carotenoids or vitamin A > 8000 IU/day, or who had given birth to more than 5 infants. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Human Subjects Committee of the University of Arizona and the Human Ethics Committees associated with each participating institution.

Prior to the study, a clinical scientist (KP) and/or the research investigator (LC) travelled to each site to train nursing personnel on the proper use of the electric breast pump, using a videotape created for that purpose. The study procedure for recruitment/enrolment, milk collection, sample handling and dietary interviewing were also discussed. Mothers were recruited during an appointment with their pediatrician, signed Informed Consent forms, and made an appointment to return mid-afternoon on the following day to express their milk. On the day of sample collection, nurses interviewed mothers for a health history for their infants and themselves and completed a 24-h dietary recall with...