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Plasma retinol and total carotenes and fracture risk after long-term supplementation with high doses of retinol

Gina L. Ambrosini M.P.H., Ph.D. [a], Helman Alfonso Ph.D. [b], Alison Reid Ph.D. [c], Dorothy Mackerras M.P.H., Ph.D. [d,e], Alexandra P. Bremner Ph.D. [a], John Beilby Ph.D. [f,g], Nola J. Olsen B.Sc. [a,h], Arthur W. Musk M.D., M.Sc. [a,h], Nicholas H. de Klerk Ph.D. [i,*]

[a] School of Population Health, The University of Western Australia, Perth, Western Australia
[b] Western Australian Centre for Health and Ageing, The University of Western Australia, Perth, Western Australia
[c] Centre for Medical Research, The University of Western Australia, Perth, Western Australia
[d] Menzies School of Health Research, Institute of Advanced Studies, Charles Darwin University, Darwin, Northern Territory, Australia
[e] Food Standards Australia New Zealand, Canberra, Australian Capital Territory, Australia
[f] School of Pathology and Laboratory Medicine, The University of Western Australia, Perth, Western Australia
[g] Department of Molecular Genetics, PathWest Laboratories, Perth, Western Australia
[h] Department of Respiratory Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia
[i] Telethon Institute for Child Health Research, Centre for Child Health Research, The University of Western Australia, Perth, Western Australia

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ABSTRACT

Objective: Observational studies suggest that moderate intakes of retinol and increased circulating retinol levels may increase fracture risk. Easy access to supplements, combined with an aging population, makes this a potentially important association. The aim of this study was to investigate plasma retinol and total carotene concentrations in relation to fracture risk after long-term supplementation with retinol and/or beta-carotene in 998 adults between 1990 and 2007.
Methods: Participants were 663 men and 335 women in a cancer prevention program who were initially randomized to a retinol (7.5 mg RE/d) or beta-carotene (30 mg/d) supplement between 1990 and 1996. After 1996, all participants received the retinol supplement only. Plasma retinol and total carotene, medication use and various lifestyle factors were measured at annual clinic visits. Fractures were identified by self-report in 2007. The risk for any fracture or osteoporotic fracture was modeled using Cox proportional hazard models.
Results: Over a median follow-up of 7.8 y, 123 participants with plasma samples reported an incident fracture. Although plasma retinol concentrations were markedly higher than those reported in observational studies, no association was observed between plasma retinol and risk for any fracture (hazard ratio [HR], 0.86 μmol/L; 95% confidence interval [CI], 0.65–1.14) or osteoporotic fracture (HR, 0.97 μmol/L; 95% CI, 0.66–1.43). A lower risk for any fracture was suggested with increasing plasma total carotenes (HR, 0.85 μmol/L; 95% CI, 0.71–1.01).
Conclusions: This study does not support earlier reports of an increased fracture risk associated with increased plasma retinol concentration. The potential for carotenes to prevent fractures deserves further investigation.

Introduction

Retinol is an essential contributor to vitamin A status; however, it is well established that chronic, high doses of retinol have a deleterious effect on bone structure in animals and humans [1]. This may result from excess retinoic acid influencing the genetic expression of osteoblasts and osteoclasts, thus increasing bone turnover and fragility [2]. Interest in the effect of

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* Corresponding author. Tel.: +61 89 489 7735; fax: +61 89 489 7700.
E-mail address: Nickdk@ichr.uwa.edu.au (N. H. de Klerk).

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"subclinical hypervitaminosis A," due to moderate intakes of retinol either through food or supplements, was initiated in 1998 when a Swedish study reported a doubling in hip fracture risk with a dietary retinol intake >1.5 mg/d [3]. Subsequent reports have been inconsistent: The Nurse's Health Study reported increased fracture risk with dietary intakes of retinol >1.0 mg/d [4], whereas several other studies have reported null associations with similar ranges of intake [5–7]. However, dietary retinol intake is one of the most difficult dietary micronutrients to measure because it is highly concentrated in only some foods and so day-to-day intake can be extremely variable [8]. Furthermore, most previous studies have used food frequency questionnaires to estimate retinol intake, and these rely on multiple assumptions when estimating nutrient intakes. For these reasons, studies of objectively measured retinol biomarkers and fracture risk may be more reliable.

Relatively few studies have examined fracture risk and biomarkers of retinol status, and their results have been inconsistent. Some suggest a positive or U-shaped relationship [9,10] between serum or plasma retinol and fracture risk, whereas others report no association [11]. The possibility of a relationship between retinol status and fracture risk raises concerns in Western populations where food may be fortified and where retinol-containing supplements are commonly consumed and often encouraged for the elderly [12]. In the same populations, fractures due to osteoporosis impose significant health care costs and reductions in quality of life and longevity. Therefore, it is important to establish whether moderate retinol intakes confer any excess risk for bone fractures.

We conducted a secondary analysis of a cancer prevention study of retinol and beta-carotene supplements between 1990 and 2007 in Perth, Western Australia, to examine whether bone fracture risk was positively associated with plasma retinol concentration. Because some observational studies have suggested a protective relationship between beta-carotene intake and fracture risk [13,14], we hypothesized that fracture risk would be inversely associated with plasma total carotene concentration, and that plasma levels of alkaline phosphatase (ALP), as a marker of bone turnover, would be positively associated with fracture risk.

Materials and methods

Participants

The Vitamin A Program was a cancer prevention program designed to examine the efficacy of high-dose retinol and beta-carotene supplements in reducing the risk for malignant mesothelioma, lung cancer, and other cancers in individuals with previous asbestos exposure [15–17]. The study methods have been detailed elsewhere [18] and are reported here in brief. Study participants were former workers and former residents of the mining township of Wittenoom, a remote town in the northwest of Western Australia, where crocidolite (blue asbestos) was mined and milled by the Australian Blue Asbestos Company from 1943 to 1966 [19]. Follow-up of both cohorts has been ongoing since 1975 to document the occurrence of asbestos-related diseases including asbestosis, malignant mesothelioma, and lung cancer [20,21]. In 1990, all surviving cohort members who could be traced were invited to take part in the Vitamin A Program. Phase I of the study randomized former workers to a daily dose of either 7.5 mg retinol equivalents (RE) as retinyl palmitate or 30 mg synthetic *all-trans* beta-carotene. Former residents were randomized to a daily dose of either 30 or 0.75 mg beta-carotene, as many were women of childbearing age for whom a high dose of retinol was contraindicated [18]. Supplements were provided by Roche Pharmaceuticals and the study was single-blinded (participants).

By the end of Phase I (September 1996), the average supplementation period was 232 wk [18]. Participants randomized to 7.5 mg RE/d had a lower incidence of mesothelioma than those randomized to 30 mg/d beta-carotene (relative risk [RR], 0.24; 95% confidence interval [CI], 0.07–0.86) [16]. Simultaneously, international trials reported increased rates of lung cancer among smokers taking a similar dose of beta-carotene [22,23]. Therefore, supplementation with beta-carotene ceased in 1996 and all remaining participants in the Vitamin A Program (Phase I) subsequently received 7.5 mg RE/d as retinyl palmitate only (Phase II) [18]. Participants were continuously recruited until December 2007 when the program ended.

During the follow-up period, 2346 former workers and residents of Wittenoom participated in the program. Of 1791 surviving participants (1188 men and 603 women) who were sent the fracture questionnaire, 1125 (738 men and 387 women) returned a completed fracture history (response rate 62.8%). Of the remaining 666, 73 were lost to follow-up, 111 returned incomplete questionnaires, and 482 did not respond. One hundred and twenty-seven questionnaire respondents who did not have at least two plasma measurements were excluded, leaving 998 participants for this analysis.

All participants gave their informed consent at entry to the Vitamin A Program, and both the program and the present study were approved by The University of Western Australia's Human Research Ethics Committee and the Clinical Drug Trials Committee of the Sir Charles Gairdner Hospital, Western Australia.

Participants provided occupational, smoking, and medical histories at entry to the study. Thereafter, they were required to attend annual clinic visits and complete brief questionnaires to update their smoking history, information about prescribed medications, consumption of other vitamin supplements, physical activity ("Do you currently participate in any regular activity or program designed to improve or maintain physical fitness?" [24]), the development of any serious illnesses, compliance with the supplement, and possible side effects (liver function test and side-effects questionnaire).

Biochemistry

A 20 mL non-fasting blood sample was collected from each participant at program entry and at each annual clinic visit thereafter. After centrifuging, 3 × 2 mL samples of plasma were frozen at −80°C before reverse-phase high-performance liquid chromatography analyses of retinol and total carotene concentrations, which usually took place within 7 to 10 d of venipuncture [25]. After May 1994, the assay for total carotene was replaced by separate assays for alpha- and beta-carotene. From this point, plasma total carotene was estimated as the sum of plasma alpha- and beta-carotene concentrations. ALP enzyme activity was determined from the rate of formation of *p*-nitrophenol in an AMP buffer and its absorbance was measured at a wavelength of 404 nm on an Abbott Architect ci16200 automated analyzer [26]. For all concentrations below the limit of detection, a value midway between zero and the laboratory's limit of detection was assigned [27].

Identification of outcome

Fractures were reported in a self-administered questionnaire. The questionnaire was posted to all surviving program participants (N = 1791) for completion in June 2007. The questionnaire asked participants to record details of all fractures experienced over their lifetime, how each fracture occurred, whether or not they sought medical treatment, and if they were admitted to hospital. Details were also collected on lifetime use of vitamin supplements and prescribed medicines for bone health (i.e., those used for osteoporosis or renal dialysis), calcium, diuretics, and hormone replacement therapy (HRT).

Incident fractures, the outcome of interest, were those that occurred during follow-up, i.e., after study enrolment (and time of first blood sample), and were classified as osteoporotic or non-osteoporotic based on fracture site. Following a previous study [28], an osteoporotic fracture was defined as any fracture of the hip, neck, pelvis, ribs, shoulder, spine, sternum, or wrist. As information on trauma associated with the fracture was not available, classification was based on site only. Previous fractures were defined as those that occurred before enrollment in the study and the date of the first blood sample.

Statistical analyses

Participants included in the analyses were former workers or residents of Wittenoom who provided a fracture history and had plasma measurements available at baseline and one later time point before the end of follow-up or their first fracture. All incident fractures and incident osteoporotic fractures were analyzed as two separate outcomes. Both analyses were carried out in a similar fashion. Follow-up commenced at the time of the first blood sample and continued until date of first fracture, date of death, or date the fracture history questionnaire was received, whichever occurred first. The outcome was first incident fracture to occur during follow-up. Fractures reported only with the year of occurrence were assumed to have occurred midyear. Risks for any fracture or osteoporotic fracture according to plasma concentrations of retinol, total carotene, and ALP were estimated with Cox proportional hazards regression models for repeated measures using Stata [29]. This time-to-event analysis modeled all available measurements of retinol, total carotene, and ALP taken from baseline to end of follow up (i.e., outcome or censoring) simultaneously in a multivariate model. Potential confounders were included in multivariate models and those not statistically significant were excluded using a backward modeling strategy. Plasma retinol, total carotene and ALP, had approximately normal distributions