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Ambrosini GL, Bremner AP, Reid A, Mackerras D, Alfonso H, Olsen NJ, Musk AW, de Klerk NH (2013) No dose-dependent increase in fracture risk after long-term exposure to high doses of retinol or beta-carotene. *Osteoporosis International* 24(4):1285–1293

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This is pre-copy-editing, author-produced version of an article accepted for publication in *Osteoporosis International* following peer review.

The final publication is available at link.springer.com.

DOI: <http://dx.doi.org/10.1007/s00198-012-2131-6>

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No dose-dependent increase in fracture risk after long term exposure to high doses of retinol or beta carotene.

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The authors declare no competing interests.

Summary: Uncertainty remains over whether or not high intakes of retinol or vitamin A consumed through food or supplements, may increase fracture risk. This intervention study found no increase in fracture risk among 2,322 adults who took a controlled, high dose retinol supplement (25,000 IU retinyl palmitate/d) for as long as 16 years. There was some evidence that beta-carotene supplementation decreased fracture risk in men.

Abstract

Purpose: There is conflicting epidemiological evidence regarding high intakes of dietary or supplemental retinol and an increased risk for bone fracture. We examined fracture risk in a study administering high doses of retinol and beta carotene (BC) between 1990 and 2007.

Methods: The *Vitamin A Program* was designed to test the efficacy of retinol and BC supplements in preventing malignancies in persons previously exposed to blue asbestos. Participants were initially randomised to 7.5 mg retinol equivalents (RE)/day as retinyl palmitate, 30 mg/day BC or 0.75 mg/day BC from 1990 to 1996, after which all participants received 7.5mg RE/d. Fractures were identified by questionnaire and hospital admission data up until 2006. Risk of any fracture or osteoporotic fracture according to cumulative dose of retinol and BC supplementation was examined using conditional logistic regression models adjusting for age, sex, smoking, BMI, medication use and previous fracture.

Results: Supplementation periods ranged from 1 to 16 years. Of the 2,322 (664 female and 1,658 male) participants, 187 experienced 237 fractures. No associations were observed between cumulative dose of retinol and risk for any fracture (OR per 10g RE=0.83; 95% CI, 0.63-1.08) or osteoporotic fracture (OR per 10g RE=0.95; 95% CI 0.64-1.40). Among men, cumulative dose of BC was associated with a slightly reduced risk of any fracture (OR per 10g=0.89; 95% CI 0.81-0.98) and osteoporotic fracture (OR per 10g=0.84; 95% CI 0.72-0.97).

Conclusions: This study observed no increases in fracture risk after long term supplementation with high doses of retinol and/or beta carotene.

Keywords: vitamin A, retinol, carotenes, supplementation, fracture, osteoporosis, experimental study

Introduction

Dietary retinol is essential for growth, night vision, and normal cell differentiation [1].

However, it is well documented that chronic high doses of retinol induce osteopenia and fractures in laboratory animals [1, 2]. Excess retinol is thought to increase bone turnover and bone fragility via retinoic acid, the active metabolite that influences the genetic expression of osteoblasts and osteoclasts [3]. Excess retinoic acid is also thought to interact negatively with 1,25-dihydroxyvitamin D (vitamin D) and serum calcium levels [2, 4]. While most animal studies have administered very high chronic doses to young growing animals, ‘subchronic’ doses of retinol have also been shown to cause bone thinning and a tendency to fracture in mature animals [5].

In humans, impaired bone remodelling and bone abnormalities have in the main, been associated with cases of accidental overdosing or chronic retinol toxicity [6, 7]. However, the potential for subclinical retinol toxicity to increase fracture risk in humans has been recognised [8]. This is particularly relevant in developed countries with ageing populations, where osteoporosis is a public health problem, and where it is not difficult to achieve high retinol intakes owing to the common consumption of supplements that contain retinol.

A number of observational studies have examined dietary and supplementary intakes of retinol and fracture risk. In the Swedish Mammography Cohort, dietary retinol intakes greater than 1.5 mg/d were associated with a doubling in hip fracture risk (RR=2.05, 95% CI 1.05-3.98) [3]. In the US Nurses Health Study, dietary retinol intakes >1 mg/d were also associated with an increased hip fracture risk (RR=1.69; 95% CI, 1.05-2.74) [9]. Similarly, two cohort studies have reported an increased fracture risk among men [10] and women [11] with high concentrations of serum retinol. Whereas, other cohort studies, including the

Women's Health Initiative Observational Study [12], the Danish Osteoporosis Prevention Study [13], the Iowa Women's Health Study [14] and a UK prospective study of hip fracture in elderly women [15] found no relationships between dietary retinol and fracture risk. The Iowa Women's Health Study reported an increased hip fracture risk among vitamin A supplement users only (RR=1.18, 95 %CI, 0.99-1.41) [12]. The only published human experimental study (to date) reported no difference in biomarkers of bone turnover between healthy men, aged 18 to 58 years, who were randomised to a placebo or 7.5 mg/d of retinol (as retinyl palmitate) for six weeks [16]. Although retinol and beta-carotene both contribute to vitamin A intake, not all beta-carotene is converted to retinol, and so they do not necessarily have the same effect on bone. A protective relationship has been suggested between beta carotene (a precursor of vitamin A) and fracture risk in two observational studies [17, 18], possibly through its antioxidant capability [17].

Given the lack of large, extended experimental studies examining retinol and bone health, we conducted an exploratory analysis of fracture risk as a secondary endpoint, using data from a cancer-prevention study that administered high doses of retinol and/or beta carotene supplements to asbestos-exposed members of the general population between 1990 and 2007. We hypothesised that (i) longer supplementation with a high dose of retinol would be associated with an increased fracture risk and (ii) longer supplementation with a high dose of BC would be associated with a decreased fracture risk.

Methods

The *Vitamin A Program* was an intervention study designed to test the efficacy of high dose retinol and beta carotene supplements for reducing the risk of mesothelioma and lung cancer (primary outcomes) in persons previously exposed to crocidolite (blue asbestos). The study was conducted in Perth, Western Australia (WA) between 1990 and 2007 [19, 20].

Participants were eligible for the study if they had experienced occupational and/or environmental crocidolite exposure as former workers or residents of the remote town of Wittenoom (WA), where crocidolite was mined and milled by the Australian Blue Asbestos Company from 1943 until 1966 [21]. Since 1979, former Wittenoom worker and resident cohorts have been followed up for incident asbestos-related diseases; asbestosis, malignant mesothelioma and lung cancer [22, 23]. In July 1990, all surviving cohort members were invited to take part in the *Vitamin A Program* [20].

Consenting former Wittenoom workers were randomly assigned to take either 30 mg/d of synthetic *all-trans* beta carotene (BC) or 7.5 mg retinol equivalents (RE)/d as retinyl palmitate (25,000 IU). Many members of the former residents cohort were women of reproductive age for whom a high-dose retinol supplement was considered contraindicated. For this reason, all former residents were initially randomised to either 30 mg/d or 0.75 mg/d synthetic *all-trans* BC. These two parallel regimens, conducted between 1990 and 1996, represent Phase 1 of the *Program* (Figure 1). All supplements were provided by Roche Pharmaceuticals and the study was single-blinded (participants).

At the end of 1995, preliminary analyses showed a reduced risk of mesothelioma among participants in the study who were randomised to 7.5 mg RE/d retinol (RR=0.24, 95% CI 0.07-0.86) [20]. At around the same time, adverse effects were being reported from similar

doses of BC in trials examining lung cancer risk [24, 25]. Therefore, in September 1996 administration of BC supplements ceased and all study participants were requested to stop taking their BC supplements. The *Vitamin A Program* ceased briefly but recommenced in July 1997, from which point all study participants were administered 7.5 mg RE/d of retinol as retinyl palmitate (Phase 2, Figure 1). Women of child bearing age who had not undergone surgical sterilisation and participants with abnormal liver function were supplemented with 1.5 mg RE/d of retinol as retinyl palmitate (5,000 IU). A very small number of participants had to change dose after commencing the *Vitamin A Program* due to changes in individual circumstances and participants were continually recruited into the study.

Annual Follow Ups

At entry to the *Vitamin A Program*, participants were interviewed and provided occupational, smoking and medical histories. Height and weight were measured and a chest x-ray was taken. Thereafter, participants were invited to attend an annual clinic visit to collect information on compliance with the administered supplement (self-report and capsule count), consumption of other vitamin supplements, prescribed medications, physical activity, serious illnesses and smoking habits. Possible side effects were monitored annually using a symptom questionnaire and blood tests for liver function. Participants unable to attend their annual interview returned questionnaires by mail.

Fractures

Fracture risk was not a primary outcome in the *Vitamin A Program*. An interest in fracture risk was brought about by earlier reports of high vitamin A intake and increased fracture risk in observational studies [3, 9]. Fracture incidence was estimated using hospital admissions for fracture by linking the resident and worker cohorts with the Western Australian hospital

morbidity database (Data Linkage Western Australia [26]) which at that time, was current up until 31st December 2005. Admissions for fracture were identified using International Classification of Diseases (ICD) codes: ICD 10 M80.0 - M81.9 (Osteoporosis with and without pathological fracture), M84.3, M84.4, S02 - S02.9, S12, S22, S32, S42, S52, S62, S72, S82, S92, T02, T08, T10, T12, T14.2 (from July 1999) and ICD 9 733.0 733.1 800-829 (from 1990 – July 1999). Hospital admissions ascertain the most serious fractures. Because not all fractures require hospitalisation, a self-administered questionnaire on fracture history (modelled on one used in a previous Australian study of fractures [27]) was posted to all surviving *Program* participants for completion in June 2007 ($n=1791$). Respondents were asked to record details of all fractures during their lifetime, how the fracture occurred, if they sought medical treatment, and if they were hospitalised. This questionnaire also collected information on serious illnesses, smoking history, lifetime vitamin supplement use and prescribed medication use, specifically: thyroid drugs, diuretics, corticosteroids (inhaled and oral), anabolic steroids, hormone replacement therapy, sedatives, tranquilisers, anti-coagulants, chemotherapy and medications for bone health (i.e. those used for calcium deficiency, osteoporosis or renal dialysis), epilepsy, depression and anxiety. Self reported fractures occurring at the spine, hip, femur, arm, ribs or wrist were classified as osteoporotic fractures [28]. Where self-reported fracture dates differed from hospital admission records, the latter was used.

All participants gave their informed consent to participate in the Vitamin A Program. The study, including record linkage and study questionnaires, was approved by The University of Western Australia's Human Research Ethics Committee and the Clinical Drug Trials Committee of the Sir Charles Gairdner Hospital, Nedlands, Western Australia.

Statistical Analyses

Participants were followed up from the date they commenced until withdrawal from the study, date of death, or 31st December 2005, whichever occurred first. Only incident fractures were included as outcomes, i.e. those that occurred after commencement of the study and before 31st December 2005, corresponding to the date that record linkage follow up ended. Fractures occurring before commencing the study were treated as ‘previous’ fractures.

Two primary outcomes were analysed: the risk of fracture occurring at an osteoporotic site, and the risk of any fracture. Study participant characteristics were compared by fracture outcome using chi-squared tests and t-tests in PASW Statistics (v18.0.0). Conditional logistic regression models with matching for calendar year were used to examine the risk of any fracture and osteoporotic fracture according to cumulative doses of administered retinol and BC supplements (Stata, v10.1). It is computationally quicker and easier to fit models with time varying covariates using conditional logistic regression than Cox regression and the partial likelihood in the Cox’s method and the conditional likelihood from the conditional method are algebraically equivalent[29]. Repeated measurements of the following were treated as time-varying covariates: annual cumulative dose of retinol and BC supplements, age, body mass index (BMI), smoking pack-years, and previous fracture (which increased in the case of having more than one fracture during follow up). Cumulative doses of retinol and BC supplements were estimated by summing the number of days the allocated supplement was taken between each annual follow up (taking account of compliance and any changes in supplement or dose), multiplying by the dose administered, and adding to the previous year’s total. Cumulative doses of retinol were analysed in units of 10g RE (retinol was converted from IU assuming 10,000 IU retinyl palmitate is equivalent to 3 mg RE [30]). Cumulative doses of BC were analysed in units of 10g. Gender, ever smoked, and the use of medications

for bone health were examined as fixed variables. Other medication types were not considered owing to their being very few users. Age, gender, BMI and smoking were included in all models as these are known risk factors for osteoporosis [31]. All tests for statistical significance used an alpha level of 0.05.

Results

Of the 2346 former workers and residents of Wittenoom who participated in the *Program*, 2322 (99%) had a known fracture outcome for one or more years of follow-up (664 females and 1658 males). The study population was predominantly male (71%) and the mean age when joining the study was 55 years (**Table 1**). Follow up times ranged from 1 to 16 years (median, 7 years). The maximum cumulative dose of retinol was 42g RE, equivalent to taking 7.5 mg RE/d for 15.3 y. For BC, the maximum cumulative dose was 80.6g, equivalent to taking 30 mg BC/d for 7.3 y. The mean cumulative dose of retinol and mean number of annual follow ups was higher among those who experienced a fracture during follow up, compared with those without fracture. Those who had fractures were also more likely to have had a fracture before commencing the *Program* (overall 22.6% reported at least one prior fracture) and to have taken medication for bone health (indicated for calcium deficiency, osteoporosis or renal dialysis).

Side effects related to skin dryness, nausea or headache were reported by 12% of participants and of these, 1.2% withdrew from the study. Increased liver function (raised gamma glutamyl transferase or aspartate aminotransferase concentration) was observed in 12% of the participants after commencing the study and of these, 1.3% withdrew from the study as a result.

Of the 2322 participants with known fracture status, 187 experienced at least one fracture during follow up; 30 participants had two fractures and one had six (**Table 2**). Most fractures (63%) occurred in men. Forty percent of fractures (n=95) were at osteoporotic sites. Thirty nine percent (39.3%) of participants (of which, 90% were male) were administered a retinol and a BC supplement at some stage during follow up, 33.4% (of which, 76% were male) were administered retinol only, and 27.3% (of which, 67% were male) were administered BC only; the fracture rates within these groups were 13.6%, 6.6% and 1.9%, respectively.

Univariate conditional logistic regression analyses (n=2,322 subjects) showed no significant relationships between supplementation with retinol or BC and fracture risk (any or osteoporotic fracture) (Table 3). However, an increased odds of fracture was associated with female gender (Any fracture OR=1.67, 95% CI 1.28-2.19, $p<0.001$; Osteoporotic fracture OR=1.72, 95% CI, 1.15–2.63, $p=0.009$), each prior fracture (Any fracture OR=1.46, 95% CI 1.34-1.58, $p<0.001$; Osteoporotic fracture OR=1.42, 95% CI, 1.25–1.61, $p<0.001$) and taking medications for bone health (Any fracture OR=1.97, 95% CI 1.37-2.84, $p<0.001$; Osteoporotic fracture OR=2.43, 95% CI, 1.44 – 4.08, $p<0.001$).

The multivariate conditional logistic regression model (n=1743 subjects) included age, sex, smoking pack-years, BMI, medications for bone health, previous fractures, and cumulative dose of retinol and BC supplement at each follow up. Functional forms of these covariates and interactions were investigated and only an interaction between sex and BC was statistically significant ($p=0.002$). No relationship was observed between any fracture or osteoporotic fracture risk and cumulative dose of retinol (per 10g RE: Any fracture OR =0.83, 95% CI 0.63-1.08, $p=0.17$; Osteoporotic fracture OR =0.95, 95% CI 0.64-1.40, $p=0.79$) in males and females. No relationship between fracture risk and cumulative does of BC was

observed in females (Table 3). However, the interaction revealed a reduction in fracture risk with increasing cumulative dose of BC in males (per 10g BC: Any fracture OR = 0.89, 95% CI 0.81-0.98, $p=0.014$; Osteoporotic fracture OR = 0.84, 95% CI 0.72-0.97, $p=0.022$) (Table 3). Each previous fracture (Any fracture OR=1.27, 95% CI 1.14-1.42, $p<0.001$; Osteoporotic fracture OR=1.31, 95% CI 1.12 – 1.55) and the use of medications for bone health (Any fracture OR=1.81, 95% CI 1.16-2.82, $p=0.009$; Osteoporotic fracture OR=2.03, 95% CI 1.06 – 3.90) were associated with greater fracture risk, but age ($p=0.48$; $p=0.61$ for any and osteoporotic fracture, respectively), sex ($p=0.39$; $p=0.57$), smoking pack-years ($p=0.35$; $p=0.51$) and BMI ($p=0.69$; $p=0.92$) were not associated with fracture risk in the multivariate models.

Discussion

In this intervention study we did not observe positive associations between fracture risk and duration of supplementation with high doses of retinyl palmitate, for up to 16 years.

However, a decreased risk of fracture (any and osteoporotic site) was associated with a greater duration of beta carotene supplementation in men.

There have been very few experimental studies of supplemental retinol and bone health in humans. Kawahara et al randomised 80 screened, healthy men aged 18 to 58 years of age to a placebo or 25,000 IU (7.5 mg RE) of retinol palmitate per day, the same daily dose of retinol administered in the present study, for six weeks [16]. There were no differences in bone turnover (serum osteocalcin, bone-specific alkaline phosphatase, and N-telopeptide of type-1 collagen) between the supplemented and placebo groups at weeks 2, 4 and 6. However, this study was short in relation to the 4 to 6 month turnover of the bone remodelling unit [32] and the young, healthy male subjects were at a low risk for osteoporosis.

A case control study examined the use of prescription vitamin A analogues (isotretinoin and acitretin as well as topical vitamin A analogues) using a population-based registry of drugs sold at pharmacies and hospitalisation for fractures for the whole of Denmark in 2005 [33]. They found no increase in fracture risk with dose of retinoid drug prescribed, duration of therapy, or cumulative dose. However, unlike the retinol supplement administered in the present study, synthetic analogues of retinol are used as therapeutic agents and do not occur naturally in foods nor are they added to foods or vitamin supplements.

Observational studies examining vitamin A supplement use in relation to fracture risk have been mixed. A slightly increased risk for self-reported hip fracture, but not all fractures, was suggested among vitamin A supplement users (RR=1.18, 95 %CI, 0.99-1.41) in the Iowa Women's Health Study, which followed up over 34,000 postmenopausal women for an average of 9.5 years [14]. However, no dose-response relationship was demonstrated, and hip fracture was not associated with dietary or total retinol intake [14]. In the placebo arm of a UK trial of bisphosphonate clodronate to prevent hip fracture in elderly women, 312 fractures were identified (of which 92 were hip fractures) during an average of 3.7 years of follow up. Vitamin A supplement users were reported to have a decreased risk for any fracture (HR=0.76, 95% CI, 0.60-0.96) but not hip fracture (HR=0.85, 95% CI 0.56-1.33) [15]. In a small case-control study of 229 post-menopausal women living in a geographically defined area of Iowa ($n=98$ cases), vitamin A supplement users ($n=89$) did not have an increased risk for self-reported fracture [34].

Two large cohort studies have reported positive associations between dietary retinol intake and fracture risk. The Swedish Mammography Cohort Study included 60,651 women aged 40 to 76 years resident in two Swedish counties [3]. Retinol intakes were estimated using an evaluated FFQ that collected information on the consumption of 60 foods over the previous 6 months and assumed standard portion sizes. A nested case-control analysis included 247 women hospitalised for a hip fracture within 64 months of study enrolment and 873 age-matched controls. Dietary retinol intake above 1.5 mg/d was associated with an increased risk for hip fracture (RR=2.05, 95% CI 1.05-3.98), after adjustments for BMI, leisure time physical activity, smoking, HRT use, cortisone use, other supplement use, diabetes, menopause factors, previous fractures and total energy intake. This study also reported an inverse cross-sectional association between retinol intake and bone mineral density. The US

Nurses Health Study examined self-reported hip fracture ($n=603$) during 18 years of follow up (860,355 person-years) [9]. Estimates of dietary retinol were made on five occasions between 1980 and 1994 using a validated semi-quantitative FFQ. Vitamin supplement use was common, and the mean retinol intake from food and supplements was 1.2 mg RE/d, ranging from 0.09-8.8 mg RE/d. After adjustment for BMI, smoking, leisure time activity, HRT and diuretics use, those in the highest quintile of dietary retinol intake (>1.0 mg RE/d) had a RR of 1.69 (95% CI, 1.05-2.74, p for trend=0.05) for incident hip fracture. Being in the highest quintile of retinol intake including food and supplements (>2 mg RE/d) was associated with a RR of 1.89 (95% CI, 1.33-2.68, p for trend <0.001).

More recent cohort studies have not observed associations between dietary or supplementary retinol intake and fracture risk. The Women's Health Initiative Observational Study included over 75,000 postmenopausal women, in which 10,400 self-reported fractures were identified during an average of 6.6 y of follow up [12]. Total retinol intake (food and supplements) was measured on two occasions with a semi-quantitative FFQ, and was not associated with risk for any fracture or hip fracture, although medication use was not considered in the analysis. There was however, a slightly elevated total fracture risk (RR=1.15, 95% CI, 1.03-1.29) among women in the highest quintile of estimated total retinol intake (≥ 1.43 mg/d) who had a low vitamin D intake (≤ 11 ug/d). The Danish Osteoporosis Prevention Study examined self-reported fractures and dietary retinol intake measured using a food record [13]. Their nested case-control analysis included 163 cases with 6 controls matched to each case according to HRT use. Fracture risk was not associated with dietary retinol intake or total retinol intake before or after adjustment for lifestyle factors and medication use, however retinol intakes in this study were lower than those reported in other studies.

It has been hypothesised that carotenoids may reduce fracture risk by counteracting oxidative stress, which can adversely affect bone mineral density [17]. Our finding of a decreased fracture risk with longer duration of BC supplementation, albeit in men only, (possibly because of the small number of women in our study) is in line with these observational studies. Based on 100 hip fractures occurring during 17 years of follow up, the Framingham Osteoporosis Study reported a lower fracture risk (HR=0.54, 95% CI 0.32-0.90) among subjects having the highest total dietary carotenoid intakes [17]. The Utah Study of Nutrition and Bone Health, a case-control study of 1,215 fractures, reported a significantly lower risk for osteoporotic fracture among smokers in the highest quintile of beta carotene intake (OR=0.39, 95% CI 0.23-0.68) [18].

To our knowledge, no other intervention studies have been published to date where controlled, high doses of retinol have been administered over many years. The doses administered in this study are well above typical population level dietary intakes and recommended intakes. In Australia, the recommended dietary intake (RDI) for vitamin A is 700 ug RE/d for non-pregnant females and 900 ug RE/d for males [35]. The daily dose of 7.5 mg RE administered in this study is equivalent to 8 to 10 times these recommended amounts and almost four times larger than the highest level of total retinol intake associated with an increased fracture risk in observational studies (2 mg RE/d [9]). Thus, the magnitude of retinol administered in the present study increased the likelihood of detecting an increased risk of fracture and avoids the measurement error associated with estimated dietary intakes.

To date, all of the observational studies that have investigated dietary or supplementary retinol and fracture risk have included women only, with the majority focussing on post-menopausal women. The present study includes both men and women of a wide range of

ages. Whereas the majority of studies have relied on self-report, the present analysis used both self-report and hospital admission data to ascertain fracture incidence. In addition, the long follow up in this study increased the likelihood of detecting incident fractures.

Although this study benefits from an experimental design, it was not possible to utilise a randomised controlled trial design in this analysis. The primary purpose of the original study (cancer prevention in a very high risk population) and nature of the exposure (beta carotene and retinol are nutrients found widely in food) did not support use of a placebo.

Randomisation was not possible beyond Phase 1 and therefore, confounding of the relationships between retinol and beta carotene and fracture risk cannot be ruled out. Our estimates of compliance with the administered supplement and hence, estimated cumulative doses, depended on self-report, may have been subject to error or reporter bias. The circumstances (traumatic or non-traumatic) of all fractures was not determined, however we conducted separate analyses of fractures typically classified as osteoporotic. We were unable to collect information on self-reported fractures from those study participants who withdrew or died before the fracture questionnaire was administered in 2007. This may have resulted in a some survivor bias among those who were surveyed and led to the underestimation of self-reported fracture rates in the study population. However, we were able to objectively ascertain hospitalised fractures, i.e. the most serious fractures, such as hip fracture, for all study participants up until the end of 2005. The relatively low number of fractures in this study may be due to the slightly younger age of participants and the high proportion of men (71%). However, the doses of retinol administered were well above intakes reported in observational studies and the long follow-up allowed for fractures to be detected, yet there was no suggestion of a positive relationship between retinol or BC supplementation and fracture risk.

In conclusion, this intervention study observed no increases in fracture risk after supplementation with beta carotene or retinol for as long as 16 years. As such, this study does not support previous reports of an increased fracture risk with higher intakes of dietary retinol.

Acknowledgements

The authors wish to express their sincere thanks to all participants in the *Vitamin A Program*. We are also very grateful to Lynne Watts, Naomi Hammond, Meralyn Pearce and Diane Jacoby who collected data for this study, Jan Sleith for data entry and data cleaning, Robin Mina who oversaw database management, and staff from the Perth Chest Clinic.

This study was funded by project grants from the National Health and Medical Research Council of Australia (Project ID: Ambrosini 458608, Musk 403930 and Musk 139047), the Western Australian Department of Health, and the Worker's Compensation and Rehabilitation Commission of Western Australia.

A.W.M, N.H.D, G.L.A, A.R and D.M designed research. G.L.A and N.J.O conducted research. A.P.B and A.R. analysed data. G.L.A, A.P.B, D.M., A.R, N.H.D. and H.A. wrote the paper. N.H.D and A.P.B. had primary responsibility for the final content. All authors have critically reviewed and approved the final manuscript and declare no conflicts of interest.

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Figure 1. The Vitamin A Program

RE; retinol equivalents (retinyl palmitate), BC; beta carotene

Table 1. Participant characteristics by fracture status

	No fracture		Any Fracture			Osteoporotic Fracture		
	<i>n</i>	<i>mean (SD)</i>	<i>n</i>	<i>mean (SD)</i>	<i>p-value</i> ^a	<i>n</i>	<i>mean (SD)</i>	<i>p-value</i> ^a
Age at first visit (y)	2135	52.6 (12.4)	187	57.7 (13.5)	<0.001	82	59.4 (12.8)	<0.001
Body mass index	1748	27.8 (4.4)	173	28.1 (4.4)	0.45	72	28.1 (4.1)	0.62
Cumulative smoking pack-years (y)	2050	26 (35)	178	22 (30)	0.19	79	27 (36)	0.78
Number of visits	2135	7.6 (5.1)	187	11 (4.2)	<0.001	82	11.3 (4.2)	<0.001
Cumulative Retinol Dose (g RE)	2135	10.6 (11.5)	187	16.8 (10.8)	<0.001	82	18.6 (10.9)	<0.001
Cumulative BC dose (g)	2135	20.0 (27.7)	187	25.1 (30.9)	0.03	82	25.1 (31.4)	0.15
	<i>n</i>	<i>%</i>	<i>n</i>	<i>%</i>	<i>p-value</i> ^b	<i>n</i>	<i>%</i>	<i>p-value</i> ^b
Former Wittenoom Worker	1198	56.1	94	50.3	0.12	43	52.4	0.51
Former Wittenoom Ex-resident	937	43.9	93	49.7		39	47.6	
Females	597	28.0	67	35.8	0.02	32	39.0	0.03
Males	1538	72.0	120	64.2		50	61.0	
Ever smoker	1530	71.7	125	66.8	0.16	53	64.6	0.16
Previous fracture	456	21.4	168	89.8	<0.001	76	92.7	<0.001
Bone health medication	136	13.3	30	22.4	0.004	18	29.0	0.001

BC, beta carotene; ^a p-value, t-test, compared to no fracture group; ^b p-value, chi-squared test, compared to no fracture group;

Table 2. Incident fractures in the Vitamin A Program

	<i>N</i>	Female		Male	
	<i>fracture</i> (%)	<i>N</i> <i>fracture</i>	<i>Mean</i> <i>Age (SD)</i>	<i>N</i> <i>fracture</i>	<i>Mean</i> <i>Age (SD)</i>
<i>Fracture Site</i>					
Ankle	34 (14.3)	13	55.2 (15.3)	21	63.7 (12.8)
Foot and toe	22 (9.3)	10	55.3 (18.0)	12	50.6 (14.8)
Hand and finger	25 (10.5)	5	50.5 (13.7)	20	51.9 (11.1)
Head and neck	10 (4.2)	3	61.0 (6.8)	7	44.8 (12.8)
Knee/leg	17 (7.2)	7	61.3 (7.3)	10	53.0 (10.7)
Pelvis	7 (3.0)	5	58.2 (14.6)	2	69.9 (12.6)
Shoulder	15 (6.3)	6	70.6 (4.9)	9	54.7 (15.0)
Unspecified	12 (5.1)	5	71.7 (5.5)	7	69.6 (6.1)
Sub-total	142 (59.9)	54		88	
<i>Osteoporotic Fracture Site:</i>					
Arm	9 (3.8)	5	55.3 (9.5)	4	39.7 (17.3)
Femur	5 (2.1)	0	.	5	65.3 (14.2)
Hip	6 (2.5)	4	59.6 (15.6)	2	61.3 (8.3)
Rib	32 (13.5)	10	64.9 (13.8)	22	59.3 (11.4)
Spine	10 (4.2)	2	48.6 (8.3)	8	59.8 (18.3)
Wrist	20 (8.4)	11	59.4 (11.0)	9	55.5 (8.3)
Osteoporosis unspecified*	13 (5.5)	3	69.4 (6.0)	10	71.6 (7.9)
Sub-total	95 (40.1)	35		60	
Total	237 (100)	89		148	

*hospital admission record

Table 3. Fracture risk according to cumulative dose of retinol and BC supplement

All fractures (n=237 fractures)	Odds Ratio	95% CI	p-value
<i>Unadjusted model^a</i>			
Retinol supplement (per increase of 10 g RE)	0.83	0.68 – 1.02	0.08
BC supplement (per increase of 10 g)	0.95	0.90 – 1.00	0.05
<i>Adjusted model^b</i>			
Retinol supplement (per increase of 10 g RE)	0.83	0.63 - 1.08	0.17
BC supplement (per increase of 10 g): Males	0.89	0.81 – 0.98	0.01
BC supplement (per increase of 10 g): Females	1.09	0.99 – 1.19	0.08
Osteoporotic fracture site (n=95 fractures)	Odds Ratio	95% CI	p-value
<i>Unadjusted model^a</i>			
Retinol supplement (per increase of 10 g RE)	0.92	0.68 – 1.23	0.60
BC supplement (per increase of 10 g)	0.92	0.85 - 1.00	0.06
<i>Adjusted model^b</i>			
Retinol supplement (per increase of 10 g RE)	0.95	0.64 - 1.40	0.79
BC supplement (per increase of 10 g): Males	0.84	0.72 - 0.97	0.02
BC supplement (per increase of 10 g): Females	1.08	0.94 – 1.23	0.28

^a Conditional logistic regression matching on calendar year

^b Conditional logistic regression matching on age and adjusting for BMI, smoking pack-years, cumulative dose of retinol and BC supplements and previous fracture (as time-varying covariates), gender and medications for bone health. The effect of BC was examined separately for males and females owing to a significant interaction (p=0.002).