Carotenoid dietary intakes and plasma concentrations are associated with heel bone ultrasound attenuation and osteoporotic fracture risk in the EPIC-Norfolk cohort.

Richard PG Hayhoe[1], Marleen AH Lentjes[2], Angela A Mulligan[2], Robert N Luben[2], Kay-Tee Khaw[2], and Ailsa A Welch[1].

[1]Department of Population Health and Primary Care, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, NR4 7TJ.
[2]Department of Public Health and Primary Care, Institute of Public Health, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN.

Names for PubMed indexing: Hayhoe, Lentjes, Mulligan, Luben, Khaw, Welch.

Corresponding Author: Dr Richard Hayhoe, Department of Population Health and Primary Care, Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, NR4 7TJ. Email: r.hayhoe@uea.ac.uk Tel: 01603 593852

Short title: Carotenoid intake, bone status and fractures.

Keywords: Carotenoids, nutrition, bone ultrasound, fractures, osteoporosis.

ABSTRACT

Carotenoids are found in abundance in fruits and vegetables and may be involved in the positive association of these foods with bone health. This study aimed to explore associations of dietary carotenoid intakes and plasma concentrations with bone density status and osteoporotic fracture risk in a European population. Cross-sectional analyses (n=14,803) of bone density status, using calcaneal broadband ultrasound attenuation (BUA), and longitudinal analyses (n=25,439) of fractures cases were conducted on data from the prospective EPIC-Norfolk cohort of middle-aged and older men and women. Health and lifestyle questionnaires were completed, and dietary nutrient intakes were derived from 7-day food diaries. Multiple regression demonstrated significant positive trends in BUA for women across quintiles of dietary alpha-carotene intake (p=0.029), beta-carotene intake (p=0.003), beta-cryptoxanthin (p=0.031), combined lutein and zeaxanthin (p=0.010), and lycopene (p=0.005). No significant trends across plasma carotenoid concentration quintiles were apparent (n=4,570). Prentice-weighted Cox regression showed no trends in fracture risk across dietary carotenoid intake quintiles (mean follow-up 12.5 years), except for lower risk of wrist fracture for women with higher lutein and zeaxanthin intake (p=0.022); nevertheless, inter-quintile differences in fracture risk were found for both sexes. Analysis of plasma carotenoid data (mean follow-up 11.9 years) showed lower hip fracture risk in men across higher plasma alpha-carotene (p=0.026) and beta-carotene (p=0.027) quintiles. This study provides novel evidence that dietary carotenoid intake is relevant to bone health in men and women, demonstrating that associations with bone density status and fracture risk exist for dietary intake of specific carotenoids and their plasma concentrations.

INTRODUCTION

Nutrition is an important modifiable factor influencing bone health[1], and thus an optimised diet could help reduce age-related osteoporotic bone deterioration and risk of fracture, an increasingly critical issue in our ageing population. The significance of dietary calcium and vitamin D to bone, especially during development, has been well established in the literature[2], although the true benefits of supplementation in later life has been subject to recent debate[3]. Research has now begun to appreciate that other nutrients may be similarly important. In particular, growing evidence supports the importance of micronutrients and antioxidants abundant in fruit and vegetables, including magnesium and potassium[4], and vitamin C[5].

Carotenoids are a class of phytochemicals found in particular abundance in yellow-orange and dark-green leafy vegetables[6]. Their chemical structure contains a conjugated double-bond chain forming a chromophore which confers a specific colour, e.g. yellow (lutein), orange (β-carotene), or red (lycopene), and provides antioxidant properties and potential for energy transfer reactions[6]. They were originally hypothesised to exert their effects on bone via provitamin A activity since Vitamin A, in its active form as retinoic acid, is known to regulate the balance between osteoblastic bone formation and osteoclastic bone resorption, upregulate vitamin D receptors, and have an anabolic effect on bone, except at high doses where it may accelerate bone resorption[7]. However, some carotenoids (lutein, zeaxanthin, and lycopene) do not possess provitamin A activity and thus the positive effect on bone health of non-provitamin A carotenoids supports the concept of a mechanism separate to vitamin A. Reactive oxygen species (ROS) have been shown by *in vitro* experiments, including those using human cell lines, and *in vivo* animal studies to be involved in multiple processes with the potential to adversely affect bone remodelling. These include suppressing osteoblastic differentiation[8], increasing osteoclastogenesis[9,10] and osteoclastic differentiation[10,11], and activating the transcription factor nuclear factor-κB involved in bone resorption signalling[11]. Thus the potent independent antioxidant activity of carotenoids has the potential to reduce bone resorption and lower fracture risk[12]. *In vitro* studies suggest that carotenoids may also have direct stimulatory effects on osteoblast proliferation and differentiation[13,14,15].

A number of epidemiological studies have investigated links between carotenoids and bone health. There is some evidence of associations between higher specific carotenoid intakes and greater bone density[16,17,18,19] or lower incidence of hip fractures[20,21], and that higher plasma

carotenoid concentrations are associated with greater bone density[22] and lower risk of developing osteoporosis[23,24]. However, these studies have had limited generalisability due to their focus on discrete population groups with small cohort size, and predominantly non-European participants. The current study thus aimed to explore potential associations of dietary carotenoid intakes and plasma concentrations (α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene) with bone density status and risk of osteoporotic fractures in a general UK population of middle-aged and older men and women. This was achieved using data from a large prospective cohort and performing cross-sectional analysis of broadband ultrasound attenuation of the heel bone in addition to longitudinal analysis of the occurrence of incident fractures of the hip, spine, and wrist.

MATERIALS AND METHODS

Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC) was established as a collaboration involving ten Western Europe countries. EPIC-Norfolk is one of the UK subcohorts, described in detail previously[25]. A baseline health-check was attended by 25,639 free-living men and women aged 39-79 years between 1993 and 1997. A second health-check was attended by 17,304 of the participants aged 42-82 years between 1998 and 2000. The Norfolk District Health Authority Ethics Committee approved all procedures and written informed consent was provided by participants according to the Declaration of Helsinki.

Exposure variables

Dietary carotenoids: Daily dietary intakes of α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, lycopene, and pre-formed retinol, were estimated from 7-day food diaries using the methodology described below for dietary covariates.

Plasma carotenoids: Blood was sampled by peripheral venepuncture at baseline, and plasma fractions with sodium citrate were stored in liquid nitrogen at -196ºC until analysed by reversed-phase high-performance liquid chromatography, to determine plasma α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, lycopene, and retinol, concentrations[26]. Correlation between matched dietary and plasma continuous scale variables was assessed by Pearson correlation coefficient.

Covariates

At each health-check height and weight were recorded according to standard protocols[25], and participants completed a health and lifestyle questionnaire (HLQ). Smoking status was categorised as *current*, *former*, or *never*; family history of osteoporosis was categorised as *yes* or *no*; menopausal status (women only) was categorised as *pre-menopausal*, *peri-menopausal (<1 year)*, *peri-menopausal (1-5 years)*, or *post-menopausal*; and HRT status (women only) was categorised as *current*, *former*, or *never* users. Physical activity over the preceding 12 months was assessed using a questionnaire which placed participants into *inactive*, *moderately inactive*, *moderately active*, and *active* categories by a method validated against heart-rate monitoring data [27]. A 7-day food diary was used to estimate dietary intake of each participant[28]; participants recorded the quantity and type of all food, drink, and supplements consumed within a 7-day period. Validation has shown this to be more accurate in estimating dietary nutrient intake than food-frequency questionnaires (FFQ) [25,29]. DINER (Data Into Nutrients for Epidemiological Research) software was used to record the 7-day food diary information[30], before further translation of the data for nutrient analysis using DINERMO[31]. All data entries were checked by nutritionists trained in use of the system[31]. The contribution of supplements was quantified using the Vitamin and Mineral Supplement (ViMiS) database[32].

Outcome variables

Quantitative ultrasound measurements of the calcaneus (heel bone) were taken at the second health-check using a CUBA (contact ultrasound bone analyser) device (McCue Ultrasonics, Winchester, UK) following standard protocols. Broadband ultrasound attenuation (BUA; dB/MHz) measurements were taken at least in duplicate for each foot of the participant, and the mean of the left and right measures was used for analysis. Each of the five CUBA devices used in the study was calibrated daily with its physical phantom. In addition, calibration between devices was checked monthly using a roving phantom. The coefficient of variation was 3.5%. The CUBA method of bone density assessment has been shown capable of predicting fracture risk[33], and is cheaper and simpler to conduct in general practice settings compared to the gold-standard of Dual X-ray absorptiometry (DXA).

Fracture incidence data were collected by questionnaire at each health-check, and the East Norfolk Health Authority database (ENCORE) of hospital attendances by Norfolk residents was also available for data linkage to corroborate self-reported data[34]. Incidence of all

125 osteoporotic fractures in the cohort, up to the end of March 2009, was thus determined by
126 retrieving data using each participant's NHS number and searching for events logged using
127 International Classification of Diseases 9 and 10 diagnostic codes for osteoporotic hip, spine,
128 or wrist fractures (the three most common sites of osteoporotic fracture[35]).
129
130 *Statistical analysis*
131 The High Performance Computing Cluster supported by the Research and Specialist
132 Computing Support service at the University of East Anglia was used for statistical data
133 analysis with STATA software (v.13; Stata Corp., Texas). Prior study of this population has
134 shown sex differences in age-related changes in bone, with greater deterioration evident in
135 women[33], and thus sex stratification was used in all our analyses. Differences between values
136 of variables for men and women were tested using t-test for continuous or chi-square for
137 categorical variables. Any p-values <0.05 were considered to be statistically significant in
138 individual analyses.
139
140 *Cross-sectional analyses*
141 Cross-sectional analyses were conducted using data taken at the second health-check,
142 combined with dietary or plasma data from the first health-check; 14,803 participants had
143 complete data for diet and ultrasound analyses, and 4,570 had complete data for plasma and
144 ultrasound analyses (see **Fig. 1**). Multivariable adjusted regression with ANCOVA was used
145 to investigate differences in calcaneal BUA across sex-specific dietary intake quintiles of
146 carotenoid or pre-formed retinol. Trend testing was achieved by treating the median values
147 for quintiles as a continuous variable[36]. Each model was adjusted for important biological,
148 lifestyle, and dietary factors: age, BMI, family history of osteoporosis, menopausal and HRT
149 status in women, corticosteroid use, smoking status, physical activity, calcium intake, total
150 energy intake, and calcium and vitamin D containing supplement use, known to influence
151 BUA in this population[33,37,38,39,40]. To help correct for dietary misreporting, days of food
152 diary completed, and the ratio of energy intake to estimated energy requirement[41], were
153 included in all diet models. A number of different models were also tested for comparison
154 purposes: models using residual adjustment for energy intake[42] where we adjusted for energy
155 prior to defining the nutrient quintiles, in place of using unadjusted nutrient quintiles and
156 adding energy as a covariate in the regression model; models including dietary fat or fibre as
157 covariates since evidence suggests these may affect dietary carotenoid absorption[43]; models
158 including a variable quantifying total fruit and vegetable intake; and models combining food

and supplement intakes, since excluding supplements may underestimate total nutrient intake[44]. Least square means for each quintile were calculated for all models. To minimise missing data exclusions, some missing values were recoded: missing menopausal status data (2.8%) as pre-menopausal if <50 y and never-user of HRT, or postmenopausal if >55 y or a current or former HRT user; missing smoking status data (0.7%) as former smokers. Participants missing data for other variables in the multivariable model were excluded. In separate analyses, calcaneal BUA was investigated across sex-specific plasma concentration quintiles of specific carotenoids in a model with the covariates described above, but excluding dietary and supplement use data.

Longitudinal analyses

Longitudinal analyses used data from the first health-check together with data of hospital recorded fractures for cohort participants (all cohort hip, spine, and wrist fracture cases up to 31st March 2009; follow-up time was calculated as the time between an individual's first health-check and this cut-off date, or death if earlier); data for diet and fracture analyses were available for 25,439 participants, and for plasma and fracture analyses for 7,474 participants (see **Fig. 1**). Prentice-weighted Cox regression was used to investigate associations between incidence of fractures and sex-specific quintiles of specific carotenoid or retinol dietary intakes, or plasma concentrations, using the same adjustments as BUA models. Missing values were treated in the same way as in BUA models. Total risk of hip, spine, or wrist fracture was calculated as the risk of the first occurrence of one of these fractures; this does not consider multiple fractures and therefore the sum of the specific-site fracture incidences does not sum to the total.

RESULTS

Selected characteristics are summarised in **Table 1.** The significant differences evident according to sex supports our use of sex-specific model analyses. Mean dietary and supplement derived intakes of specific carotenoids and pre-formed retinol are shown for the study population (α-carotene, β-cryptoxanthin, lutein and zeaxanthin, and lycopene supplement contributions were negligible; individual means ≤ 150 ng/day). However, no UK Reference Nutrient Intake (RNI) values[45] for carotenoids are currently available for comparison. Retinol plasma concentrations below 10 μg/dL are considered to indicate severe deficiency; 10 to 20 μg/dL indicates mild deficiency[46]. Three individuals (0.07%) with

plasma carotenoid data in the ultrasound cohort (n=4570) were mildly deficient according to these criteria and one (0.02%) was severely deficient; 11 individuals (0.15%) of the fracture cohort with plasma data (n=7474) were mildly deficient and three (0.04%) were severely deficient.

Correlations between dietary carotenoid intakes and plasma concentrations

A number of weak, but significant, correlations were identified between dietary carotenoid intakes and plasma concentrations. Dietary α-carotene intake was significantly correlated with plasma α-carotene concentration in both men ($r=0.497$, $p<0.001$, $n=2355$, ultrasound cohort; $r=0.496$, $p<0.001$, $n=2380$, fracture cohort) and women ($r=0.373$, $p<0.001$, $n=2201$, ultrasound cohort; $r=0.368$, $p<0.001$, $n=2219$, fracture case cohort). Dietary β-carotene intake was significantly correlated with plasma β-carotene concentration in both men ($r=0.311$, $p<0.001$, $n=2355$, ultrasound cohort; $r=0.311$, $p<0.001$, $n=2380$, fracture cohort) and women ($r=0.280$, $p<0.001$, $n=2201$, ultrasound cohort; $r=0.275$, $p<0.001$, $n=2219$, fracture case cohort). Dietary β-cryptoxanthin intake was significantly correlated with plasma β-cryptoxanthin concentration in both men ($r=0.395$, $p<0.001$, $n=2355$, ultrasound cohort; $r=0.397$, $p<0.001$, $n=2380$, fracture cohort) and women ($r=0.390$, $p<0.001$, $n=2201$, ultrasound cohort; $r=0.388$, $p<0.001$, $n=2219$, fracture case cohort). Dietary lutein and zeaxanthin intake was significantly correlated with plasma lutein and zeaxanthin concentration in both men ($r=0.211$, $p<0.001$, $n=2355$, ultrasound cohort; $r=0.212$, $p<0.001$, $n=2380$, fracture cohort) and women ($r=0.214$, $p<0.001$, $n=2201$, ultrasound cohort; $r=0.212$, $p<0.001$, $n=2219$, fracture cohort). Dietary lycopene intake was significantly correlated with plasma lycopene concentration in both men ($r=0.275$, $p<0.001$, $n=2355$, ultrasound cohort; $r=0.279$, $p<0.001$, $n=2380$, fracture cohort) and women ($r=0.294$, $p<0.001$, $n=2201$, ultrasound cohort; $r=0.293$, $p<0.001$, $n=2219$, fracture cohort). Pre-formed dietary retinol intake was not significantly correlated with plasma retinol concentration in either men ($r=0.039$, $p=0.056$, $n=2355$, ultrasound cohort; $r=0.038$, $p=0.062$, $n=2380$, fracture cohort) or women ($r=0.013$, $p=0.539$, $n=2201$, ultrasound cohort; $r=0.014$, $p=0.516$, $n=2219$, fracture cohort).

Associations between dietary carotenoid intakes and bone density

Mean calcaneal BUA values stratified by sex and quintiles of specific dietary carotenoid or pre-formed retinol intakes are shown in **Fig. 2** for the fully adjusted model (unadjusted data

are shown in **Supplementary Table 1**). In women, significant positive linear trends were apparent across quintiles of α-carotene intake (p=0.029), β-carotene intake (p=0.003), β-cryptoxanthin intake (p=0.031), combined lutein and zeaxanthin intakes (p=0.010), and lycopene intake (p=0.005), for fully adjusted BUA; a significant negative trend was apparent across retinol intake quintiles (p=0.037). Individual significant differences in fully adjusted BUA in quintiles *vs.* quintile 1 were also identified for women for quintiles 3 (1.5% higher; n=1662, p=0.023) and 5 (2.3% higher; n=1662, p=0.001) for β-carotene intake; and quintiles 4 (1.8% higher; n=1663, p=0.007) and 5 (1.7% higher; n=1662, p=0.011) for combined lutein and zeaxanthin intake (see Fig. 2). The associations described between BUA and carotenoid intake were no different when food and supplement contributions were combined in the model, except that with the combined intake data no trend in BUA across retinol quintiles was evident.

Associations between plasma carotenoid concentrations and bone density

Analysis of bone density measures according to plasma carotenoid concentration quintiles, adjusting for all covariates previously described, with the exception of dietary factors, showed no significant linear trends in BUA for either men or women (see **Fig. 3)**. Nevertheless, a significant difference in fully adjusted BUA was identified for men between quintile 2 and quintile 1 for plasma lutein and zeaxanthin (3.2% higher; n=473, p=0.015). Unadjusted data are shown in **Supplementary Table 2**.

Associations between dietary carotenoid intakes and fracture risk

Fully adjusted total risk of hip, spine, or wrist fractures showed a significant negative linear association in men with quintiles of dietary α-carotene (n=11510, p=0.040) and β-carotene (n=11510, p=0.044) intake. A significant negative trend was also present in women for the association between wrist fracture risk and lutein and zeaxanthin intake quintiles (n=13929, p=0.022). **Table 2** shows all trend p values and quintile 1 *vs.* 5 comparisons. In men, total hip, spine, and wrist fracture risk was lower in α-carotene intake quintile 5 vs. quintile 1 (0.71 (95% CI: 0.53, 0.95); p=0.020); and hip fracture risk was lower in α-carotene intake quintile 3 vs. quintile 1 (0.64 (95% CI: 0.42, 0.99); p=0.046), and β-cryptoxanthin intake quintile 5 vs. quintile 1 (0.65 (95% CI: 0.42, 0.99); p=0.046). In women, hip fracture risk was lower in lutein and zeaxanthin quintile 4 vs. quintile 1, (0.75 (95% CI: 0.58, 0.98); p=0.032). A negative linear association was evident across pre-formed retinol intake quintiles for wrist

fracture risk (n=11510, p=0.005) in men. Also in men, compared to dietary retinol quintile 1 total fracture risk was lower in quintile 5 (0.71 (95% CI: 0.52, 0.97); p=0.033); wrist fracture risk was lower in quintile 4 (0.44 (95% CI: 0.24, 0.81); p=0.008) and quintile 5 (0.33 (95% CI: 0.17, 0.65); p=0.001); and spine fracture risk was lower in quintile 3 (0.56 (95% CI: 0.33, 0.96); p=0.033).

The associations between carotenoid intakes and fracture risk were no different when food and supplement contributions were combined in the model. However, pre-formed retinol analyses showed a number of differences when supplements were included. There was no significant difference in total fracture risk in men between retinol quintile 1 and 5 with the combined intake data, although the differences in risk between quintile 2 and quintile 1 (0.67 (95% CI: 0.50, 0.90); p=0.008) and quintile 3 and quintile 1 (0.72 (95% CI: 0.53, 0.96); p=0.028) were significant. Other significant retinol inter-quintile differences, in addition to those found in diet only analyses, were: wrist fracture risk for men in quintile 3 vs. quintile 1 (0.37 (95% CI: 0.20, 0.69); p=0.002); spine fracture risk for men in quintile 2 (0.31 (95% CI: 0.17, 0.56); p=0.048), quintile 4 (0.59 (95% CI: 0.36, 0.96); p=0.036), and quintile 5 (0.54 (95% CI: 0.30, 0.97); p=0.040) vs. quintile 1; and wrist fracture risk for women in quintile 5 vs. quintile 1 (0.64 (95% CI: 0.43, 0.96); p=0.031).

Associations between plasma carotenoid intakes and fracture risk

In men, but not women, there was a significant linear trend for lower hip fracture risk across plasma α-carotene quintiles (p=0.026) and plasma β-carotene quintiles (p=0.027) (see **Table 3**). In women, fracture risk was significantly lower in α-carotene quintile 3 than quintile 1 in the fully adjusted model for both total fracture (0.70 (95% CI: 0.50, 0.96); p=0.028) and hip fracture (0.63 (95% CI: 0.41, 0.97); p=0.035); hip fracture risk in women was also lower in plasma retinol quintile 4 vs. quintile 1 (0.64 (95% CI: 0.41, 0.99); p=0.044).

DISCUSSION

This study has shown significant associations between dietary carotenoid intake and a quantitative measure of bone density exist in a UK population cohort, after adjustment for important biological, lifestyle and other dietary covariates. In women, dietary intake quintiles of dietary α-carotene, β-carotene, β-cryptoxanthin, combined lutein and zeaxanthin, and lycopene were all positively linearly associated with calcaneal BUA, such that individuals

with higher intake of each of these carotenoids had higher BUA measurements; pre-formed retinol was negatively associated. Significant associations of BUA with quintiles of plasma carotenoid concentration were much more limited, with no significant trends apparent, and only a single inter-quintile association evident for lutein and zeaxanthin in men. Nevertheless, the magnitude of the effects seen with the dietary analyses is highly relevant to bone health[33], for example the difference between the median β-carotene intakes in quintiles 5 and 1 for women (3462 and 792 μg/day) could be accounted for by the additional intake of just one small carrot and yet is associated with 2.3% greater BUA. Moreover, this study included longitudinal analysis of the risk of osteoporotic fracture, demonstrating significant linear trends for lower risk of wrist fracture across dietary retinol quintiles in men and dietary lutein and zeaxanthin quintiles in women, and lower hip fracture risk across plasma α- and β-carotene concentration quintiles in men. A number of significant differences in fracture risk were also shown between individual quintiles of dietary carotenoid intake or plasma concentration. These include lower total hip, spine, and wrist fracture risk in the highest versus lowest intake quintiles of dietary α-carotene in men, as well as lower hip fracture risk in the highest β-cryptoxanthin intake quintile in men and with higher lutein and zeaxanthin intake in women. This study is to our knowledge the first comprehensive epidemiological analysis of the relevance of specific dietary and plasma carotenoids with bone density status and risk of osteoporotic fractures in a large European mixed-sex cohort. The findings thus provide an important advance to the current research evidence.

Inclusion of a variable quantifying total fruit and vegetable intake in our regression models caused an attenuation of the associations of carotenoids with BUA (data not shown), suggesting potential effects of other components in fruits and vegetables in addition to carotenoids. However, despite this attenuation, the associations of carotenoids with BUA remained significant, indicating that the effects of carotenoids independent of total fruit and vegetable consumption are important. The mechanisms by which carotenoids may influence bone metabolism are not fully understood, although a number of theories have been proposed. Some, but not all carotenoids have pro-vitamin A activity and therefore may have effects on bone health via this mediator[7], all have antioxidant activity likely to be protective of bone[12], and members of the carotenoid family have also been shown experimentally to have direct stimulatory effects on osteoblast proliferation and differentiation at physiologically relevant concentrations[20].

Our results suggest that the effects on bone health may differ for specific carotenoids, a situation also evident in previous carotenoid research[16,17,21]. In the Framingham Osteoporosis Study, participants had lower risk of hip fracture or non-vertebral fracture if they were in the highest tertile of total carotenoid or lycopene intake, respectively, but no associations were evident for α- or β- carotene, β-cryptoxanthin, or lutein and zeaxanthin[21]. It is possible that this occurrence may be due to differing ranges and magnitude of intakes for different carotenoids. Indeed, specific carotenoids are found in differing concentrations in different fruits and vegetables: unpublished composition analysis conducted for the EPIC-Norfolk cohort showed α-carotene predominantly sourced from root vegetables, especially carrots (65% of total); β-carotene also sourced significantly from carrots (35%) and other root, dark green leafy, and fruiting vegetables; β-cryptoxanthin from citrus fruits, mainly oranges; lutein mainly from peas (16%), with broccoli, cabbages, and other leafy vegetables providing approximately 10% each; zeaxanthin mostly from citrus fruits (19% from oranges), apples (>10%), and green leafy and fruiting vegetables; and lycopene from fruiting vegetables, mainly tomatoes (35%) and tinned beans in tomato sauce (15%). However, it is also possible that underlying mechanisms of action may be different and more potent for some carotenoids compared to others. We know that all carotenoids are capable of antioxidant activity with potential to counter the negative influence of oxidative stress on bone health[12], but others, for example β-cryptoxanthin[7], have been shown to have direct effects on bone metabolism. The fact that differing magnitudes of effects appear to exist leads us to speculate that the universal antioxidant activity may not be the dominant mechanism for all carotenoids. Another factor is the potential for differential absorption which may affect interpretation, but makes the plasma data presented in this study particularly useful. Indeed, although low serum concentrations of α- and β-carotene, lycopene, β-cryptoxanthin, and zeaxanthin have been demonstrated in a study of Italian women with osteoporosis, and likewise for lycopene and β-cryptoxanthin in US women[12], only one small Japanese study has been published detailing a longitudinal analysis of serum carotenoids and bone health, observing lower risk of osteoporosis development with higher serum β-carotene and β-cryptoxanthin[23].

Our findings showed correlation between dietary intakes of carotenoids and their plasma concentrations, corroborating previous studies[47,48]. The relatively weak nature of these correlations has also been noted previously and attributed to various influences including

seasonality, obesity, and day to day variation in an individual's dietary intake and plasma concentrations[49]. No correlation was identified for dietary retinol intake and plasma retinol concentration. Between extremes of severe deficiency and excess, plasma retinol is tightly homeostatically controlled[50] which could explain the lack of correlation with dietary intake in our data[44]. Our results for bone density status in women confirm the detrimental effects of higher dietary vitamin A retinol-equivalent intakes reported elsewhere[51], and although not directly replicated in associations of diet and fracture risk, plasma retinol data corroborates this with a lower comparative risk of fracture in quintile 4 vs. 1 than quintile 5 vs. 1.

Strengths and Limitations

This study provides important observational evidence of associations between specific carotenoid dietary intakes or plasma concentrations and bone health, in the largest European study on this subject to date. Nevertheless, we were limited in the data available for analysis. In particular, plasma carotenoid data was only available for a smaller subset of the full cohort which may have reduced the power of our analyses. In terms of anthropometric indices, blood pressure and blood lipids, the EPIC-Norfolk cohort is representative of the UK population[25]. We acknowledge that hospital admission data may underestimate fracture incidence, particularly of spine fractures, and this could differ by sex. Furthermore, record linkage used to determine fracture cases precluded the ability to discriminate between low and high trauma fractures. The influence of this on our findings is expected to be small, as the proportion of high trauma fracture cases in this demographic group is likely to be low[52]. It is an advantage of our study that data for both sexes were analysed since different effects were evident in men and women, a situation often apparent in bone health. For example, data from a Chinese cohort study showed that total carotenoid and α- or β-carotene and lutein/zeaxanthin were all inversely associated with hip fracture risk in men, but no significant associations were identified for women[20]. Our data similarly shows the strongest associations for fracture risk in men, although the ultrasound data is conversely more significant in women. Sex differences in fruit and vegetable consumption or reporting may be responsible for differences in the associations with bone identified here and in previous studies[53], although since carotenoids are fat-soluble the different adiposity of men and women could also influence their bioavailability and effects.

Accurate estimation of dietary nutrient intake is critical to the validity of the findings of this type of study. The quantitative 7-day food diary method used here has been validated

previously and is expected to provide more precise dietary intake figures compared to FFQs or 24-hour recall methods[31]. Dietary and lifestyle data used in longitudinal analyses were collected at baseline and thus variation in food consumption and lifestyle behaviours could have influenced our findings. We have focused our attention on models using nutrient composition data from food intake only, thus potentially underestimate total nutrient intakes including supplements. Carotenoids from supplements have been suggested to have greater bioavailability than those derived from foods and thus may make an important contribution to plasma carotenoid concentrations[6]. In this cohort, no fundamental differences were apparent between models combining food and supplement contributions and those using food contributions only, although some additional inter-quintile differences in fracture risk were apparent for pre-formed retinol analyses when supplements were included, a likely result of extension of the upper intake range. Previous studies have shown absorption of carotenoids is positively associated with dietary lipid intake, in particular monounsaturated fatty acids, and may also be affected by dietary fibre[43]. However, in our dietary BUA model, the effect of inclusion of dietary fat or fibre was minimal (data not shown). Food preparation may also affect carotenoid stability, which combined with food carotenoid content variability due to cultivation practices, season, and ripening status[6] may have reduced the accuracy of carotenoid intake estimations from the food diaries used in this study. In addition to the direct influence of dietary carotenoid intake, plasma carotenoid concentrations are influenced by the rate of uptake into, and efflux from, other tissues[54]. Inter-individual variability in these processes may thus make plasma concentrations less reliable as a biomarker of dietary intake and may partly explain the discrepancies between diet and plasma results presented here. Indeed it has been suggested that adipose tissue concentrations are likely to give a better indication of long-term carotenoid status[55,56]. Metabolism and absorption of carotenoids and thus their measurable plasma concentrations may also be influenced by other physiological or lifestyle factors, including inflammatory profile[57], adiposity[58], and smoking[59]. Inflammatory profile may be particularly relevant to the cohort analysed here, since chronic low-grade inflammation is common in older populations, and thus should be investigated by future studies with reference to bone health.

Conclusions

This study has shown positive associations of dietary intake and plasma concentration of specific carotenoids with a quantitative ultrasound measure of bone density status and lower fracture risk in a general population group. The results are insufficiently consistent to make

425 definitive conclusions, but are nevertheless supportive of the hypothesis that dietary intakes of
426 fruit and vegetables rich in carotenoids and other antioxidants are beneficial to adult bone,
427 which once confirmed by clinical trial may provide a valuable approach for public health
428 strategies to improve bone health in our ageing population.
429
430 **Acknowledgements**
431 The authors thank the EPIC staff and the participants enrolled in the study.
432
433 **Financial Support**
434 The EPIC-Norfolk study received grants from the Medical Research Council (G9502233) and
435 Cancer Research UK (SP2024-0201 and SP2024-0204).
436
437 **Conflictof Interest**
438 None.
439
440 Authorship
441 AAW developed the research question with RPGH who analysed the data and drafted the
442 manuscript. AAW also arranged data collection in conjunction with RNL, who implemented
443 record linkage. MAHL and AAM prepared dietary and supplement data. K-TK is principal
444 investigator of the EPIC-Norfolk Study. All authors contributed to data interpretation, review
445 of the manuscript and its approval.

REFERENCES

1. Mitchell PJ, Cooper C, Dawson-Hughes B *et al.* (2015) Life-course approach to nutrition. *Osteoporos Int*.

2. Gennari C (2001) Calcium and vitamin D nutrition and bone disease of the elderly. *Public Health Nutr* **4**, 547-559.

3. Michaelsson K (2015) Calcium supplements do not prevent fractures. *BMJ* **351**, h4825.

4. Hayhoe RP, Lentjes MA, Luben RN *et al.* (2015) Dietary magnesium and potassium intakes and circulating magnesium are associated with heel bone ultrasound attenuation and osteoporotic fracture risk in the EPIC-Norfolk cohort study. *Am J Clin Nutr* **102**, 376-384.

5. Finck H, Hart AR, Jennings A *et al.* (2014) Is there a role for vitamin C in preventing osteoporosis and fractures? A review of the potential underlying mechanisms and current epidemiological evidence. *Nutr Res Rev* **27**, 268-283.

6. Arscott SA (2013) Food Sources of Carotenoids. In *Carotenoids and Human Health* [SA Tanumihardjo, editor]. New York: Springer.

7. Yamaguchi M (2012) Role of carotenoid beta-cryptoxanthin in bone homeostasis. *J Biomed Sci* **19**, 36.

8. Mody N, Parhami F, Sarafian TA *et al.* (2001) Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radic Biol Med* **31**, 509-519.

9. Garrett IR, Boyce BF, Oreffo RO *et al.* (1990) Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. *The Journal of clinical investigation* **85**, 632-639.

10. Lee NK, Choi YG, Baik JY *et al.* (2005) A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. *Blood* **106**, 852-859.

11. Bai XC, Lu D, Liu AL *et al.* (2005) Reactive oxygen species stimulates receptor activator of NF-kappaB ligand expression in osteoblast. *The Journal of biological chemistry* **280**, 17497-17506.

12. Tanumihardjo SA, Binkley N (2013) Carotenoids and Bone Health. In *Carotenoids and Human Health* [SA Tanumihardjo, editor]. New York: Springer.

13. Kim L, Rao AV, Rao LG (2003) Lycopene II--effect on osteoblasts: the carotenoid lycopene stimulates cell proliferation and alkaline phosphatase activity of SaOS-2 cells. *J Med Food* **6**, 79-86.

14. Park CK, Ishimi Y, Ohmura M *et al.* (1997) Vitamin A and carotenoids stimulate differentiation of mouse osteoblastic cells. *J Nutr Sci Vitaminol (Tokyo)* **43**, 281-296.

15. Uchiyama S, Yamaguchi M (2005) beta-cryptoxanthin stimulates cell differentiation and mineralization in osteoblastic MC3T3-E1 cells. *Journal of cellular biochemistry* **95**, 1224-1234.

16. Wattanapenpaiboon N, Lukito W, Wahlqvist ML *et al.* (2003) Dietary carotenoid intake as a predictor of bone mineral density. *Asia Pac J Clin Nutr* **12**, 467-473.

17. Sahni S, Hannan MT, Blumberg J *et al.* (2009) Inverse association of carotenoid intakes with 4-y change in bone mineral density in elderly men and women: the Framingham Osteoporosis Study. *Am J Clin Nutr* **89**, 416-424.

18. Sugiura M, Nakamura M, Ogawa K *et al.* (2011) Dietary patterns of antioxidant vitamin and carotenoid intake associated with bone mineral density: findings from post-menopausal Japanese female subjects. *Osteoporos Int* **22**, 143-152.

19. de Jonge EA, Kiefte-de Jong JC, Campos-Obando N *et al.* (2015) Dietary vitamin A intake and bone health in the elderly: the Rotterdam Study. *Eur J Clin Nutr* **69**, 1360-1368.

20. Dai Z, Wang R, Ang LW *et al.* (2014) Protective effects of dietary carotenoids on risk of hip fracture in men: the Singapore Chinese Health Study. *J Bone Miner Res* **29**, 408-417.

21. Sahni S, Hannan MT, Blumberg J *et al.* (2009) Protective effect of total carotenoid and lycopene intake on the risk of hip fracture: a 17-year follow-up from the Framingham Osteoporosis Study. *J Bone Miner Res* **24**, 1086-1094.

22. Sugiura M, Nakamura M, Ogawa K *et al.* (2008) Bone mineral density in post-menopausal female subjects is associated with serum antioxidant carotenoids. *Osteoporos Int* **19**, 211-219.

23. Sugiura M, Nakamura M, Ogawa K *et al.* (2012) High serum carotenoids associated with lower risk for bone loss and osteoporosis in post-menopausal Japanese female subjects: prospective cohort study. *PLoS One* **7**, e52643.

24. Yang Z, Zhang Z, Penniston KL *et al.* (2008) Serum carotenoid concentrations in postmenopausal women from the United States with and without osteoporosis. *Int J Vitam Nutr Res* **78**, 105-111.

25. Day N, Oakes S, Luben R *et al.* (1999) EPIC-Norfolk: study design and characteristics of the cohort. European Prospective Investigation of Cancer. *Br J Cancer* **80 Suppl 1**, 95-103.

26. Al-Delaimy WK, van Kappel AL, Ferrari P *et al.* (2004) Plasma levels of six carotenoids in nine European countries: report from the European Prospective Investigation into Cancer and Nutrition (EPIC). *Public Health Nutr* **7**, 713-722.

27. Wareham NJ, Jakes RW, Rennie KL *et al.* (2003) Validity and repeatability of a simple index derived from the short physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Public Health Nutr* **6**, 407-413.

28. Bingham SA, Gill C, Welch A *et al.* (1997) Validation of dietary assessment methods in the UK arm of EPIC using weighed records, and 24-hour urinary nitrogen and potassium and serum vitamin C and carotenoids as biomarkers. *Int J Epidemiol* **26 Suppl 1**, S137-151.

29. Riboli E, Hunt KJ, Slimani N *et al.* (2002) European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* **5**, 1113-1124.

30. Welch AA, McTaggart A, Mulligan AA *et al.* (2001) DINER (Data Into Nutrients for Epidemiological Research) - a new data-entry program for nutritional analysis in the EPIC-Norfolk cohort and the 7-day diary method. *Public Health Nutr* **4**, 1253-1265.

31. Lentjes MA, McTaggart A, Mulligan AA *et al.* (2013) Dietary intake measurement using 7 d diet diaries in British men and women in the European Prospective Investigation into Cancer-Norfolk study: a focus on methodological issues. *Br J Nutr*, 1-11.

32. Lentjes MA, Bhaniani A, Mulligan AA *et al.* (2011) Developing a database of vitamin and mineral supplements (ViMiS) for the Norfolk arm of the European Prospective Investigation into Cancer (EPIC-Norfolk). *Public Health Nutr* **14**, 459-471.

33. Welch A, Camus J, Dalzell N *et al.* (2004) Broadband ultrasound attenuation (BUA) of the heel bone and its correlates in men and women in the EPIC-Norfolk cohort: a cross-sectional population-based study. *Osteoporos Int* **15**, 217-225.

34. Moayyeri A, Kaptoge S, Dalzell N *et al.* (2009) Is QUS or DXA better for predicting the 10-year absolute risk of fracture? *J Bone Miner Res* **24**, 1319-1325.

35. Kanis JA, Oden A, Johnell O *et al.* (2001) The burden of osteoporotic fractures: a method for setting intervention thresholds. *Osteoporos Int* **12**, 417-427.

36. Chiuve SE, Sampson L, Willett WC (2011) The association between a nutritional quality index and risk of chronic disease. *Am J Prev Med* **40**, 505-513.

37. Jakes RW, Khaw K, Day NE *et al.* (2001) Patterns of physical activity and ultrasound attenuation by heel bone among Norfolk cohort of European Prospective Investigation of Cancer (EPIC Norfolk): population based study. *BMJ* **322**, 140.

38. Willett WC, Howe GR, Kushi LH (1997) Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* **65**, 1220S-1228S; discussion 1229S-1231S.

39. Flynn A (2003) The role of dietary calcium in bone health. *Proc Nutr Soc* **62**, 851-858.

40. Heaney RP (2000) Calcium, dairy products and osteoporosis. *J Am Coll Nutr* **19**, 83S-99S.

41. Institute of Medicine of the National Academies (2002) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: National Academies Press.

42. Willett W (1998) *Nutritional epidemiology*. 2nd ed. ed. New York ; Oxford: Oxford University Press.

43. Goltz SR, Ferruzzi MG (2013) Carotenoid Bioavailabiity: Influence of Dietary Lipid and Fiber. In *Carotenoids and Human Health* [SA Tanumihardjo, editor]. New York: Springer.

44. Lentjes MA, Mulligan AA, Welch AA *et al.* (2015) Contribution of cod liver oil-related nutrients (vitamins A, D, E and eicosapentaenoic acid and docosahexaenoic acid) to daily nutrient intake and their associations with plasma concentrations in the EPIC-Norfolk cohort. *J Hum Nutr Diet* **28**, 568-582.

45. Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. *Report on Health and Social Subjects No 41*. London: HSMO.

46. WHO (2011) Serum retinol concentrations for determining the prevalence of vitamin A deficiency in populations. Vitamin and Mineral Nutrition Information System. http://www.who.int/vmnis/indicators/retinol.pdf (accessed 16/12/2015

47. Brady WE, Mares-Perlman JA, Bowen P *et al.* (1996) Human serum carotenoid concentrations are related to physiologic and lifestyle factors. *J Nutr* **126**, 129-137.

48. Talegawkar SA, Johnson EJ, Carithers TC *et al.* (2008) Carotenoid intakes, assessed by food-frequency questionnaires (FFQs), are associated with serum carotenoid concentrations in the Jackson Heart Study: validation of the Jackson Heart Study Delta NIRI Adult FFQs. *Public Health Nutr* **11**, 989-997.

49. Scott KJ, Thurnham DI, Hart DJ *et al.* (1996) The correlation between the intake of lutein, lycopene and beta-carotene from vegetables and fruits, and blood plasma concentrations in a group of women aged 50-65 years in the UK. *Br J Nutr* **75**, 409-418.

50. Tanumihardjo SA (2011) Vitamin A: biomarkers of nutrition for development. *Am J Clin Nutr* **94**, 658S-665S.

51. Binkley N, Krueger D (2000) Hypervitaminosis A and bone. *Nutrition reviews* **58**, 138-144.

52. Finck H, Hart AR, Lentjes MA *et al.* (2015) Cross-sectional and prospective associations between dietary and plasma vitamin C, heel bone ultrasound, and fracture risk in men and women in the European Prospective Investigation into Cancer in Norfolk cohort. *Am J Clin Nutr* **102**, 1416-1424.

53. Myint PK, Welch AA, Bingham SA *et al.* (2007) Fruit and vegetable consumption and self-reported functional health in men and women in the European Prospective Investigation into Cancer-Norfolk (EPIC-Norfolk): a population-based cross-sectional study. *Public Health Nutr* **10**, 34-41.

54. Burri BJ, Neidlinger TR, Clifford AJ (2001) Serum carotenoid depletion follows first-order kinetics in healthy adult women fed naturally low carotenoid diets. *J Nutr* **131**, 2096-2100.

55. Wallstrom P, Wirfalt E, Lahmann PH *et al.* (2001) Serum concentrations of beta-carotene and alpha-tocopherol are associated with diet, smoking, and general and central adiposity. *Am J Clin Nutr* **73**, 777-785.

56. Kardinaal AF, van 't Veer P, Brants HA *et al.* (1995) Relations between antioxidant vitamins in adipose tissue, plasma, and diet. *American journal of epidemiology* **141**, 440-450.

57. Wood AD, Strachan AA, Thies F *et al.* (2014) Patterns of dietary intake and serum carotenoid and tocopherol status are associated with biomarkers of chronic low-grade systemic inflammation and cardiovascular risk. *Br J Nutr* **112**, 1341-1352.

58. Hosseini B, Saedisomeolia A, Skilton MR (2017) Association between Micronutrients Intake/Status and Carotid Intima Media Thickness: A Systematic Review. *J Acad Nutr Diet* **117**, 69-82.

59. Handelman GJ, Packer L, Cross CE (1996) Destruction of tocopherols, carotenoids, and retinol in human plasma by cigarette smoke. *Am J Clin Nutr* **63**, 559-565.

Fig. 1 – Study population flowchart.

Fig. 2 – Fully adjusted calcaneal BUA of 6490 men and 8313 women from the EPIC-Norfolk cohort, stratified by sex and dietary intake quintiles of specific carotenoids or retinol.

Full Model: age, BMI, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, smoking status, physical activity, calcium intake, total energy intake, calcium and vitamin D containing supplement use, days of food diary completed, and the ratio of energy intake to estimated energy requirement.

Retinol as pre-formed intake only.

Data plotted as mean ± SD. * = P value <0.05 *vs.* Quintile 1; ** = P value <0.01, according to ANCOVA.

α-carotene intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 406 ± 363; Q1, 40 ± 36; Q2, 188 ± 41; Q3, 339 ± 46; Q4, 515 ± 60; Q5, 948 ± 399. *Women:* mean, 403 ± 356; Q1, 50 ± 40; Q2, 196 ± 40; Q3, 337 ± 44; Q4, 509 ± 60; Q5, 922 ± 416.

β-carotene intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 2069 ± 1207; Q1, 757 ± 254; Q2, 1366 ± 146; Q3, 1871 ± 150; Q4, 2472 ± 212; Q5, 3877 ± 1199. *Women:* mean, 2036 ± 1206; Q1, 758 ± 247; Q2, 1352 ± 139; Q3, 1832 ± 142; Q4, 2428 ± 206; Q5, 3813 ± 1294.

β-cryptoxanthin intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 406 ± 569; Q1, 15 ± 9; Q2, 56 ± 17; Q3, 168 ± 52; Q4, 447 ± 123; Q5, 1343 ± 622. *Women:* mean, 455 ± 570; Q1, 25 ± 13; Q2, 89 ± 29; Q3, 243 ± 61; Q4, 540 ± 124; Q5, 1380 ± 613.

Lutein and zeaxanthin intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 1095 ± 870; Q1, 334 ± 127; Q2, 642 ± 72; Q3, 899 ± 80; Q4, 1244 ± 130; Q5, 2355 ± 1144. *Women:* mean, 1136 ± 930; Q1, 363 ± 123; Q2, 659 ± 71; Q3, 915 ± 80; Q4, 1263 ± 132; Q5, 2482 ± 1256.

Lycopene intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 1428 ± 1671; Q1, 126 ± 117; Q2, 556 ± 121; Q3, 1028 ± 160; Q4, 1693 ± 242; Q5, 3735 ± 2416. *Women:* mean, 1289 ± 1365; Q1, 147 ± 116; Q2, 524 ± 104; Q3, 932 ± 134; Q4, 1546 ± 233; Q5, 3297 ± 1764.

Retinol intake (mean ± SD; μg/day) per quintile (Q). *Men:* mean, 773 ± 1297; Q1, 177 ± 52; Q2, 295 ± 29; Q3, 403 ± 35; Q4, 561 ± 68; Q5, 2431 ± 2212. *Women:* mean, 622 ± 1159; Q1, 138 ± 41; Q2, 233 ± 22; Q3, 309 ± 25; Q4, 425 ± 45; Q5, 2004 ± 2069.

Fig. 3 – Fully adjusted calcaneal BUA of 2362 men and 2208 women from the EPIC-Norfolk cohort, stratified by sex and plasma concentration quintiles of specific carotenoids or retinol.

Full Model: age, BMI, smoking status, physical activity, family history of osteoporosis, menopausal and HRT status in women, and corticosteroid use.
Data plotted as mean ± SD. * = P value <0.05 *vs.* Quintile 1, according to ANCOVA.
α-carotene (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 7.7 ± 5.7; Q1, 2.5 ± 0.8; Q2, 4.6 ± 0.5; Q3, 6.5 ± 0.6; Q4, 8.9 ± 0.9; Q5, 16.0 ± 7.3. *Women:* mean, 10.2 ± 6.9; Q1, 3.5 ± 1.1; Q2, 6.1 ± 0.6; Q3, 8.5 ± 0.8; Q4, 12.0 ± 1.2; Q5, 20.8 ± 7.2.
β-carotene (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 20.0 ± 12.4; Q1, 7.9 ± 2.1; Q2, 12.9 ± 1.2; Q3, 17.4 ± 1.5; Q4, 23.5 ± 2.1; Q5, 38.3 ± 14.1. *Women:* mean, 26.7 ± 16.2; Q1, 10.7 ± 2.8; Q2, 17.4 ± 1.6; Q3, 23.4 ± 1.7; Q4, 31.0 ± 2.6; Q5, 50.9 ± 18.3.
β-cryptoxanthin (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 7.6 ± 6.1; Q1, 2.2 ± 0.7; Q2, 4.0 ± 0.5; Q3, 6.0 ± 0.6; Q4, 8.8 ± 1.0; Q5, 17.0 ± 7.0. *Women:* mean, 10.8 ± 8.6; Q1, 3.2 ± 0.9; Q2, 5.7 ± 0.7; Q3, 8.5 ± 0.9; Q4, 12.5 ± 1.6; Q5, 23.9 ± 10.3.
Lutein & zeaxanthin (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 19.8 ± 8.5; Q1, 10.5 ± 2.0; Q2, 14.9 ± 1.0; Q3, 18.2 ± 1.0; Q4, 22.8 ± 1.6; Q5, 32.8 ± 7.8. *Women:* mean, 21.1 ± 9.4; Q1, 11.0 ± 2.0; Q2, 15.5 ± 1.1; Q3, 19.5 ± 1.1; Q4, 24.0 ± 1.6; Q5, 35.5 ± 8.9.
Lycopene (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 30.0 ± 17.7; Q1, 10.3 ± 3.5; Q2, 19.0 ± 2.1; Q3, 26.7 ± 2.4; Q4, 36.6 ± 3.5; Q5, 57.5 ± 14.4. *Women:* mean, 32.0 ± 18.3; Q1, 10.9 ± 3.5; Q2, 20.4 ± 2.4; Q3, 28.9 ± 2.6; Q4, 39.4 ± 3.6; Q5, 60.3 ± 14.0.
Retinol (mean ± SD; μg/dL) per quintile (Q). *Men:* mean, 52.8 ± 12.2; Q1, 37.9 ± 4.8; Q2, 46.1 ± 1.7; Q3, 51.4 ± 1.7; Q4, 57.7 ± 2.1; Q5, 70.8 ± 9.6. *Women:* mean, 49.7 ± 12.0; Q1, 35.0 ± 3.9; Q2, 43.1 ± 1.6; Q3, 48.5 ± 1.6; Q4, 54.6 ± 1.9; Q5, 67.4 ± 9.6.

Table 1 – Selected characteristics of the ultrasound analysis cohort (n=14803) and the fracture cohort (n=25,439) from EPIC-Norfolk, stratified by sex.

Selected Characteristics	**Ultrasound cohort**[a]			**Fracture cohort**[b]		
	Men	**Women**		**Men**	**Women**	
	n=6490	**n=8313**	***P***[c]	**n=11510**	**n=13929**	***P***[c]
Age (years)	62.9 ± 9.0	61.6 ± 9.0	*<0.001*	59.7 ± 9.3	58.9 ± 9.3	*<0.001*
BMI (kg/m^2)	26.9 ± 3.3	26.5 ± 4.4	*<0.001*	26.5 ± 3.3	26.2 ± 4.3	*<0.001*
BUA (dB/MHz)	90.1 ± 17.5	72.1 ± 16.5	*<0.001*	--	--	

Dietary derived intake						
Alpha-carotene (µg/day)	406 ± 363	403 ± 356	*0.601*	390 ± 366	389 ± 387	*0.862*
Beta-carotene (µg/day)	2069 ± 1207	2036 ± 1206	*0.108*	1988 ± 1220	1958 ± 1291	*0.061*
Beta-cryptoxanthin (µg/day)	406 ± 569	455 ± 570	*<0.001*	378 ± 574	426 ± 557	*<0.001*
Lutein & zeaxanthin (µg/day)	1095 ± 870	1136 ± 930	*0.006*	1048 ± 884	1087 ± 1013	*0.001*
Lycopene (µg/day)	1428 ± 1671	1289 ± 1365	*<0.001*	1385 ± 1750	1238 ± 1470	*0.001*
Retinol[d] (µg/day)	773 ± 1297	622 ± 1159	*<0.001*	780 ± 1571	610 ± 1239	*<0.001*
Calcium intake (mg/day)	942 ± 289	784 ± 243	*<0.001*	919 ± 298	766 ± 249	*<0.001*
Total energy intake (kcal/day)	2285 ± 502	1731 ± 379	*<0.001*	2240 ± 527	1694 ± 395	*<0.001*
Supplement derived intake						
Beta-carotene (µg/day)	39 ± 673	68 ± 833	*0.023*	41 ± 706	65 ± 804	*0.012*
Retinol (µg/day)	202 ± 402	256 ± 421	*<0.001*	180 ± 383	238 ± 417	*<0.001*
Ca containing supplement use	102 (1.6)	505 (6.1)	*<0.001*	165 (1.4)	746 (5.4)	*<0.001*
VitD containing supplement use	1621 (25.0)	2773 (33.4)	*<0.001*	2570 (22.3)	4273 (30.7)	*<0.001*
Plasma concentration						
Alpha-carotene (µg/dL)	7.7 ± 5.7[e]	10.2 ± 6.9[f]	*<0.001*	7.2 ± 5.6[g]	9.7 ± 7.4[h]	*<0.001*
Beta-carotene (µg/dL)	20.0 ± 12.4[e]	26.7 ± 16.2[f]	*<0.001*	19.2 ± 12.0[g]	25.7 ± 16.1[h]	*<0.001*
Beta-cryptoxanthin (µg/dL)	7.6 ± 6.1[e]	10.8 ± 8.6[f]	*<0.001*	7.2 ± 5.9[g]	10.5 ± 9.0[h]	*<0.001*
Lutein & zeaxathin (µg/dL)	19.8 ± 8.5[e]	21.1 ± 9.4[f]	*<0.001*	19.2 ± 8.5[g]	20.9 ± 9.6[h]	*<0.001*
Lycopene (µg/dL)	30.0 ± 17.7[e]	32.0 ± 18.3[f]	*<0.001*	29.0 ± 19.6[g]	30.7 ± 18.4[h]	*<0.001*
Retinol (µg/dL)	52.8 ± 12.2[e]	49.7 ± 12.0[f]	*<0.001*	52.5 ± 12.8[g]	50.1 ± 12.7[h]	*<0.001*
Smoking			*<0.001*			*<0.001*
Current	555 (8.6)	721 (8.7)		1471 (12.8)	1691 (12.1)	
Former	3609 (55.6)	2697 (32.4)		6233 (54.2)	4446 (31.9)	
Never	2326 (35.8)	4895 (58.9)		3806 (33.1)	7792 (55.9)	
Physical activity			*<0.001*			*<0.001*
Inactive	1792 (27.6)	2188 (26.3)		3549 (30.8)	4232 (30.4)	
Moderately inactive	1626 (25.1)	2714 (32.6)		2833 (24.6)	4469 (32.1)	
Moderately active	1615 (24.9)	1990 (23.9)		2650 (23.0)	3096 (22.2)	
Active	1457 (22.5)	1421 (17.1)		2478 (21.5)	2132 (15.3)	
Family history of osteoporosis			*0.001*			*0.001*
No	6313 (97.3)	7792 (93.7)		11203 (97.3)	13120 (96.6)	
Yes	177 (2.7)	521 (6.3)		307 (2.7)	809 (3.4)	
Corticosteroid use			*0.391*			*0.077*
Current or former (>3 months)	272 (4.2)	426 (5.1)		351 (3.0)	480 (3.4)	
Never (<3 months)	6218 (95.8)	7887 (94.9)		11159 (97.0)	13449 (96.6)	
Menopausal status						
Pre-menopausal	--	484 (5.8)		--	2342 (16.8)	
Peri-menopausal (<1 y)	--	272 (3.3)		--	754 (5.4)	
Peri-menopausal (1-5 y)	--	1461 (17.6)		--	2494 (17.9)	
Post-menopausal	--	6096 (73.3)		--	8339 (59.9)	

HRT				
Current	--	1764 (21.2)	--	2824 (20.3)
Former	--	1490 (17.9)	--	1582 (11.4)
Never	--	5059 (60.9)	--	9523 (68.4)

[a]Ultrasound group characteristics at 2nd health-check (time of ultrasound). [b]Fracture group characteristics at 1st health-check or time of consent. [c]Differences between men and women using t-test for continuous or chi-square for categorical variables. [d]Retinol as pre-formed intake only. [e]n=2362. [f]n=2208. [g]n=3817. [h]n=3657. Values are mean ± SD or frequency (percentage).

Table 2 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population at follow-up *versus* baseline, stratified by sex and dietary intake quintiles of specific carotenoids or retinol (Prentice-weighted Cox proportional hazard ratio and 95% CI, quintile 1 as reference).

Men		**Fracture incidence and risk**							
		Total fractures		**Hip fracture**		**Spine fracture**		**Wrist fracture**	
(μg/day)		Incidence	Hazard ratio	Incidence	Hazard ratio	Incidence	Hazard ratio	Incidence	Hazard ratio
Alpha-carotene intake	Q1	*111/2302*	*1.00 (ref)*	*57/2302*	*1.00 (ref)*	*33/2302*	*1.00 (ref)*	*28/2302*	*1.00 (ref)*
(μg/day)	Q5	*85/2302*	0.71 (0.53-0.95)*	*44/2302*	0.71 (0.47-1.06)	*22/2302*	0.61 (0.35-1.07)	*23/2302*	0.79 (0.45-1.40)
	Total	*467/11510*	*P trend = 0.040*	*228/11510*	*P trend = 0.111*	*149/11510*	*P trend = 0.096*	*115/11510*	*P trend = 0.730*
Beta-carotene intake	Q1	*103/2302*	*1.00 (ref)*	*55/2302*	*1.00 (ref)*	*31/2302*	*1.00 (ref)*	*23/2302*	*1.00 (ref)*
(μg/day)	Q5	*85/2302*	0.77 (0.57-1.03)	*39/2302*	0.70 (0.46-1.07)	*27/2302*	0.78 (0.46-1.33)	*25/2302*	0.93 (0.52-1.68)
	Total	*467/11510*	*P trend = 0.044*	*228/11510*	*P trend = 0.181*	*149/11510*	*P trend = 0.132*	*115/11510*	*P trend = 0.540*
Beta-cryptoxanthin intake	Q1	*102/2302*	*1.00 (ref)*	*59/2302*	*1.00 (ref)*	*22/2302*	*1.00 (ref)*	*25/2302*	*1.00 (ref)*
(μg/day)	Q5	*79/2302*	0.80 (0.59-1.08)	*36/2302*	0.65 (0.42-0.99)*	*29/2302*	1.38 (0.78-2.44)	*18/2302*	0.69 (0.37-1.28)
	Total	*467/11510*	*P trend = 0.115*	*228/11510*	*P trend = 0.190*	*149/11510*	*P trend = 0.846*	*115/11510*	*P trend = 0.088*
Lutein & zeaxanthin	Q1	*96/2302*	*1.00 (ref)*	*48/2302*	*1.00 (ref)*	*31/2302*	*1.00 (ref)*	*26/2302*	*1.00 (ref)*
(μg/day)	Q5	*81/2302*	0.82 (0.61-1.12)	*41/2302*	0.90 (0.56-1.38)	*25/2302*	0.74 (0.43-1.27)	*20/2302*	0.70 (0.39-1.27)
	Total	*467/11510*	*P trend = 0.143*	*228/11510*	*P trend = 0.929*	*149/11510*	*P trend = 0.131*	*115/2302*	*P trend = 0.230*
Lycopene	Q1	*109/2303*	*1.00 (ref)*	*61/2303*	*1.00 (ref)*	*33/2303*	*1.00 (ref)*	*23/2303*	*1.00 (ref)*
(μg/day)	Q5	*69/2302*	0.79 (0.58-1.07)	*35/2302*	0.85 (0.56-1.31)	*19/2302*	0.67 (0.38-1.20)	*19/2302*	0.83 (0.44-1.56)
	Total	*467/11510*	*P trend = 0.137*	*228/11510*	*P trend = 0.386*	*149/11510*	*P trend = 0.298*	*115/11510*	*P trend = 0.552*
Retinol	Q1	*105/2302*	*1.00 (ref)*	*41/2302*	*1.00 (ref)*	*40/2302*	*1.00 (ref)*	*29/2302*	*1.00 (ref)*
(μg/day)	Q5	*467/11510*	0.71 (0.52-0.97)*	*44/2302*	1.11 (0.70-1.77)	*28/2302*	0.61 (0.36-1.05)	*16/2302*	0.33 (0.17-0.65)**
	Total	*260/6538*	*P trend = 0.106*	*228/11510*	*P trend = 0.966*	*149/11510*	*P trend = 0.404*	*115/11510*	*P trend = 0.005*
Women									

Alpha-carotene intake	Q1	*233/2786*	*1.00 (ref)*	*142/2786*	*1.00 (ref)*	*42/2786*	*1.00 (ref)*	*73/2786*	*1.00 (ref)*
(μg/day)	Q5	*223/2785*	0.97 (0.80-1.16)	*127/2785*	0.89 (0.69-1.13)	*53/2785*	1.42 (0.94-2.15)	*72/2785*	0.98 (0.70-1.37)
	Total	*1165/13920*	*P trend = 0.372*	*665/13920*	*P trend = 0.172*	*249/13920*	*P trend = 0.129*	*398/13929*	*P trend = 0.777*
Beta-carotene intake	Q1	*254/2786*	*1.00 (ref)*	*153/2786*	*1.00 (ref)*	*48/2786*	*1.00 (ref)*	*84/2786*	*1.00 (ref)*
(μg/day)	Q5	*218/2785*	0.88 (0.73-1.07)	*121/2785*	0.81 (0.63-1.04)	*54/2785*	1.29 (0.86-1.92)	*73/2785*	0.87 (0.63-1.20)
	Total	*1165/13920*	*P trend = 0.340*	*665/13920*	*P trend = 0.203*	*249/13920*	*P trend = 0.224*	*398/13929*	*P trend = 0.558*
Beta-cryptoxanthin intake	Q1	*260/2786*	*1.00 (ref)*	*154/2786*	*1.00 (ref)*	*60/2786*	*1.00 (ref)*	*86/2786*	*1.00 (ref)*
(μg/day)	Q5	*223/2785*	0.89 (0.74-1.07)	*120/2785*	0.82 (0.64-1.04)	*45/2785*	0.85 (0.57-1.26)	*84/2785*	1.00 (0.73-1.36)
	Total	*1165/13920*	*P trend = 0.646*	*665/13920*	*P trend = 0.293*	*249/13920*	*P trend = 0.831*	*398/13929*	*P trend = 0.708*
Lutein & zeaxanthin	Q1	*246/2786*	*1.00 (ref)*	*141/2786*	*1.00 (ref)*	*52/2786*	*1.00 (ref)*	*88/2786*	*1.00 (ref)*
(μg/day)	Q5	*221/2785*	0.93 (0.78-1.13)	*134/2785*	1.01 (0.79-1.29)	*46/2785*	1.00 (0.66-1.50)	*64/2785*	0.72 (0.52-1.00)
	Total	*1165/13920*	*P trend = 0.123*	*665/13920*	*P trend = 0.545*	*249/13920*	*P trend = 0.884*	*398/13929*	*P trend = 0.022*
Lycopene	Q1	*267/2787*	*1.00 (ref)*	*151/2787*	*1.00 (ref)*	*58/2787*	*1.00 (ref)*	*87/2787*	*1.00 (ref)*
(μg/day)	Q5	*185/2785*	0.92 (0.76-1.12)	*92/2785*	0.89 (0.68-1.16)	*43/2785*	1.08 (0.72-1.61)	*72/2785*	0.99 (0.71-1.36)
	Total	*1165/13920*	*P trend = 0.150*	*665/13920*	*P trend = 0.097*	*249/13920*	*P trend = 0.475*	*398/13929*	*P trend = 0.700*
Retinol	Q1	*222/2786*	*1.00 (ref)*	*120/2786*	*1.00 (ref)*	*57/2786*	*1.00 (ref)*	*84/2786*	*1.00 (ref)*
(μg/day)	Q5	*240/2785*	0.93 (0.76-1.14)	*149/2785*	1.00 (0.77-1.31)	*44/2785*	0.68 (0.44-1.05)	*72/2785*	0.81 (0.57-1.15)
	Total	*1165/13920*	*P trend = 0.449*	*665/13920*	*P trend = 0.864*	*249/13920*	*P trend = 0.171*	*398/13929*	*P trend = 0.194*

Total risk is for the first occurrence of one of these fractures and therefore the sum of the specific-site fracture incidences do not sum to the total.

Full model: age, BMI, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, smoking status, physical activity, calcium intake, total energy intake, calcium and vitamin D containing supplement use, days of food diary completed, and the ratio of energy intake to estimated energy requirement.

Retinol as pre-formed intake only.

* $p<0.05$; ** $p<0.01$ *versus* quintile 1, according to ANCOVA.

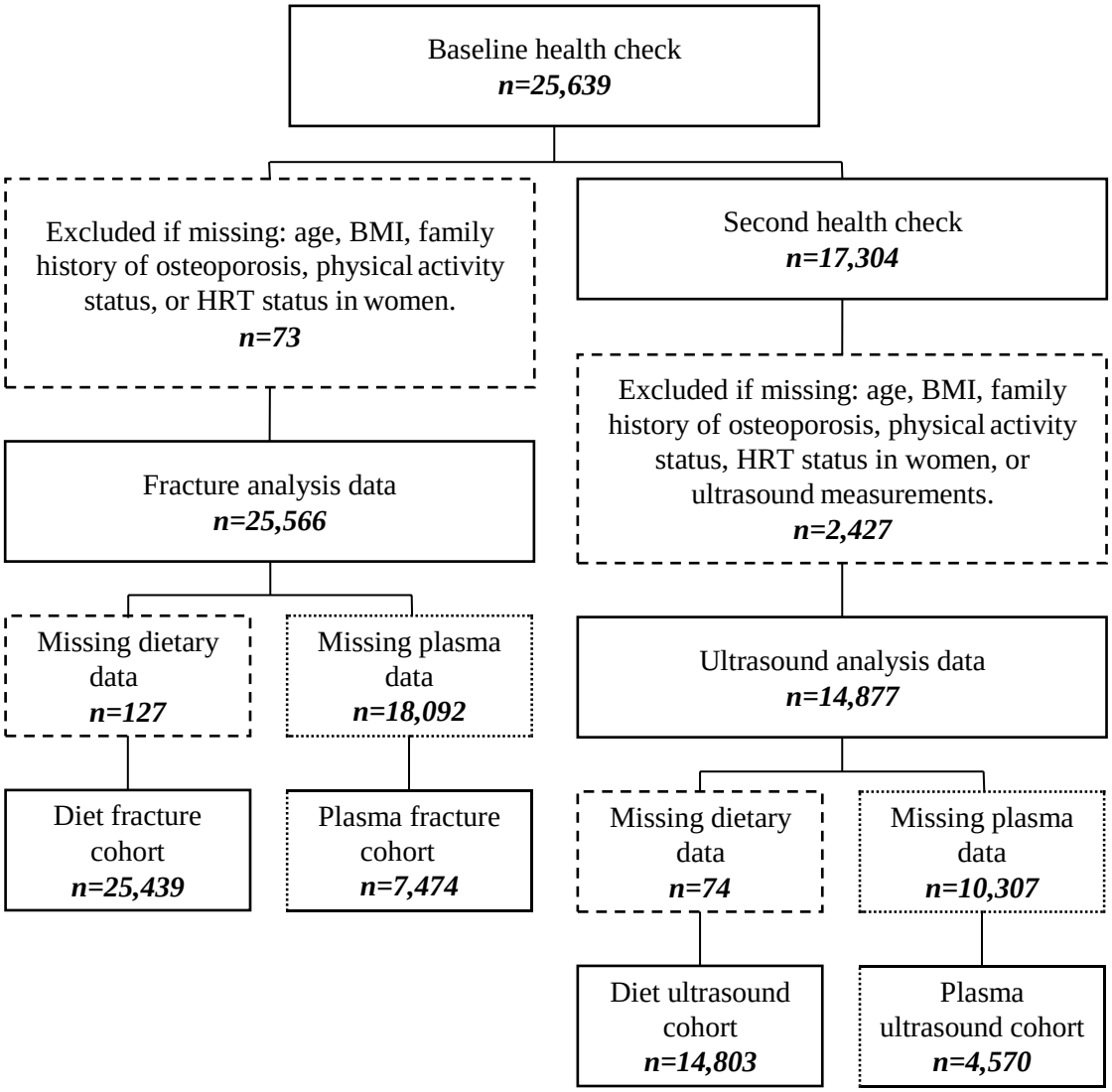
Table 3 – Risk of hip, spine, and wrist fractures in the EPIC-Norfolk cohort population at follow-up *versus* baseline, stratified by sex and serum concentration quintiles of specific carotenoids or retinol (Prentice-weighted Cox proportional hazard ratio and 95% CI, quintile 1 as reference).

Men		**Fracture incidence and risk**							
		Total fractures		**Hip fracture**		**Spine fracture**		**Wrist fracture**	
		Incidence	Hazard ratio	Incidence	Hazard ratio	Incidence	Hazard ratio	Incidence	Hazard ratio
Alpha-carotene	Q1	*32/764*	*1.00 (ref)*	*18/764*	*1.00 (ref)*	*9/764*	*1.00 (ref)*	*8/764*	*1.00 (ref)*
(μg/dL)	Q5	*28/763*	0.69 (0.41-1.16)	*12/763*	0.52 (0.25-1.11)	*9/763*	0.92 (0.35-2.39)	*7/763*	0.60 (0.21-1.73)
	Total	*175/3817*	*P trend = 0.062*	*88/3817*	*P trend = 0.026*	*63/3817*	*P trend = 0.594*	*33/3817*	*P trend = 0.474*
Beta-carotene	Q1	*33/764*	*1.00 (ref)*	*18/764*	*1.00 (ref)*	*10/764*	*1.00 (ref)*	*7/764*	*1.00 (ref)*
(μg/dL)	Q5	*41/763*	1.00 (0.62-1.63)	*13/763*	0.52 (0.25-1.09)	*16/763*	1.65 (0.72-3.82)	*13/763*	1.46 (0.54-3.90)
	Total	*175/3817*	*P trend = 0.744*	*88/3817*	*P trend = 0.027*	*63/3817*	*P trend = 0.151*	*33/3817*	*P trend = 0.360*
Beta-cryptoxanthin	Q1	*29/764*	*1.00 (ref)*	*16/764*	*1.00 (ref)*	*10/764*	*1.00 (ref)*	*6/764*	*1.00 (ref)*
(μg/dL)	Q5	*35/763*	1.12 (0.68-1.85)	*16/763*	0.91 (0.45-1.85)	*15/763*	1.53 (0.67-3.48)	*4/763*	0.58 (0.16-2.09)
	Total	*175/3817*	*P trend = 0.655*	*88/3817*	*P trend = 0.282*	*63/3817*	*P trend = 0.360*	*33/3817*	*P trend = 0.239*
Lutein & zeaxanthin	Q1	*29/764*	*1.00 (ref)*	*12/764*	*1.00 (ref)*	*16/764*	*1.00 (ref)*	*1/764*	*1.00 (ref)*
(μg/dL)	Q5	*37/763*	1.07 (0.65-1.75)	*13/763*	0.85 (0.39-1.90)	*18/763*	1.04 (0.52-2.09)	*6/763*	5.15 (0.61-43.4)
	Total	*175/3817*	*P trend = 0.970*	*88/3817*	*P trend = 0.809*	*63/3817*	*P trend = 0.840*	*33/3817*	*P trend = 0.947*
Lycopene	Q1	*44/764*	*1.00 (ref)*	*27/764*	*1.00 (ref)*	*12/764*	*1.00 (ref)*	*7/764*	*1.00 (ref)*
(μg/dL)	Q5	*29/763*	0.79 (0.49-1.29)	*10/763*	0.54 (0.26-1.13)	*15/763*	1.40 (0.64-3.08)	*6/763*	0.82 (0.26-2.57)
	Total	*175/3817*	*P trend = 0.339*	*88/3817*	*P trend = 0.107*	*63/3817*	*P trend = 0.529*	*33/3817*	*P trend = 0.659*
Retinol	Q1	*42/764*	*1.00 (ref)*	*23/764*	*1.00 (ref)*	*16/764*	*1.00 (ref)*	*5/764*	*1.00 (ref)*
(μg/dL)	Q5	*34/763*	0.76 (0.49-1.20)	*16/763*	0.67 (0.35-1.27)	*14/763*	0.84 (0.41-1.72)	*5/763*	0.93 (0.27-3.23)
	Total	*175/3817*	*P trend = 0.293*	*88/3817*	*P trend = 0.475*	*63/3817*	*P trend = 0.482*	*33/3817*	*P trend = 0.723*
Women									

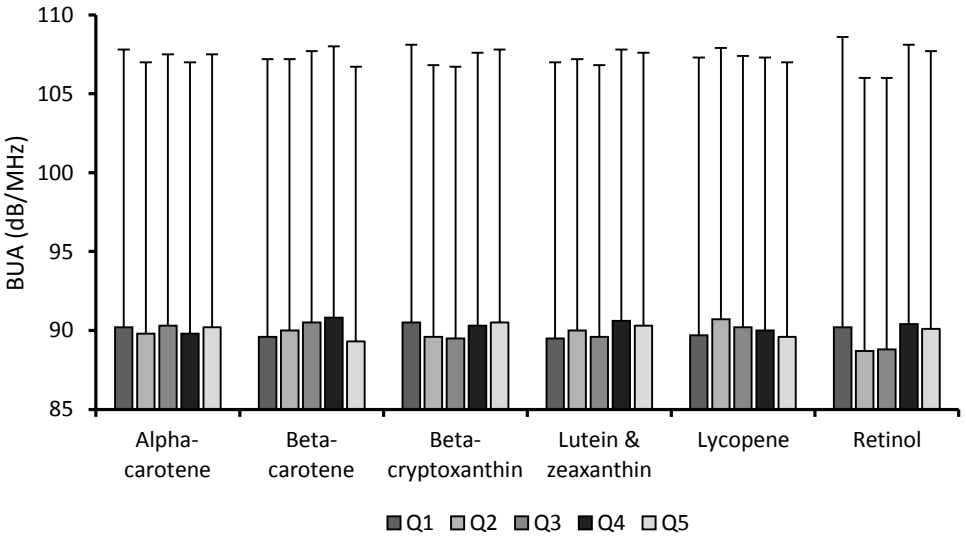
Alpha-carotene	Q1	*84/732*	*1.00 (ref)*	*50/732*	*1.00 (ref)*	*22/732*	*1.00 (ref)*	*24/732*	*1.00 (ref)*
(μg/dL)	Q5	*81/731*	0.79 (0.57-1.08)	*47/731*	0.74 (0.49-1.12)	*15/731*	0.60 (0.30-1.20)	*32/731*	1.15 (0.66-2.01)
	Total	*386/3657*	*P trend = 0.422*	*232/3657*	*P trend = 0.265*	*89/3657*	*P trend = 0.278*	*121/3657*	*P trend = 0.241*
Beta-carotene	Q1	*56/732*	*1.00 (ref)*	*29/732*	*1.00 (ref)*	*20/732*	*1.00 (ref)*	*16/732*	*1.00 (ref)*
(μg/dL)	Q5	*78/731*	0.96 (0.67-1.38)	*48/731*	1.00 (0.62-1.63)	*15/731*	0.50 (0.25-1.02)	*26/731*	1.27 (0.66-2.45)
	Total	*386/3657*	*P trend = 0.378*	*232/3657*	*P trend = 0.249*	*89/3657*	*P trend = 0.160*	*121/3657*	*P trend = 0.563*
Beta-cryptoxanthin	Q1	*73/732*	*1.00 (ref)*	*42/732*	*1.00 (ref)*	*17/732*	*1.00 (ref)*	*22/732*	*1.00 (ref)*
(μg/dL)	Q5	*85/731*	0.98 (0.72-1.35)	*51/731*	0.97 (0.64-1.46)	*15/731*	0.75 (0.37-1.53)	*29/731*	1.16 (0.66-2.04)
	Total	*386/3657*	*P trend = 0.873*	*232/3657*	*P trend = 0.651*	*89/3657*	*P trend = 0.245*	*121/3657*	*P trend = 0.180*
Lutein & zeaxanthin	Q1	*58/732*	*1.00 (ref)*	*31/732*	*1.00 (ref)*	*17/732*	*1.00 (ref)*	*20/732*	*1.00 (ref)*
(μg/dL)	Q5	*81/731*	1.02 (0.72-1.44)	*55/731*	1.20 (0.77-1.90)	*16/731*	0.69 (0.34-1.40)	*22/731*	0.86 (0.46-1.60)
	Total	*386/3657*	*P trend = 0.862*	*232/3657*	*P trend = 0.752*	*89/3657*	*P trend = 0.420*	*121/3657*	*P trend = 0.589*
Lycopene	Q1	*99/732*	*1.00 (ref)*	*61/732*	*1.00 (ref)*	*20/732*	*1.00 (ref)*	*30/732*	*1.00 (ref)*
(μg/dL)	Q5	*63/731*	0.85 (0.62-1.18)	*38/731*	0.96 (0.63-1.46)	*14/731*	0.97 (0.48-1.96)	*22/731*	0.87 (0.49-1.54)
	Total	*386/3657*	*P trend = 0.722*	*232/3657*	*P trend = 0.971*	*89/3657*	*P trend = 0.974*	*121/3657*	*P trend = 0.575*
Retinol	Q1	*71/732*	*1.00 (ref)*	*46/732*	*1.00 (ref)*	*12/732*	*1.00 (ref)*	*22/732*	*1.00 (ref)*
(μg/dL)	Q5	*79/731*	1.00 (0.72-1.39)	*51/731*	0.98 (0.65-1.46)	*21/731*	1.51 (0.74-3.10)	*19/731*	0.81 (0.43-1.50)
	Total	*386/3657*	*P trend = 0.695*	*232/3657*	*P trend = 0.473*	*89/3657*	*P trend = 0.279*	*121/3657*	*P trend = 0.495*

Total risk is for the first occurrence of one of these fractures and therefore the sum of the specific-site fracture incidences do not sum to the total.

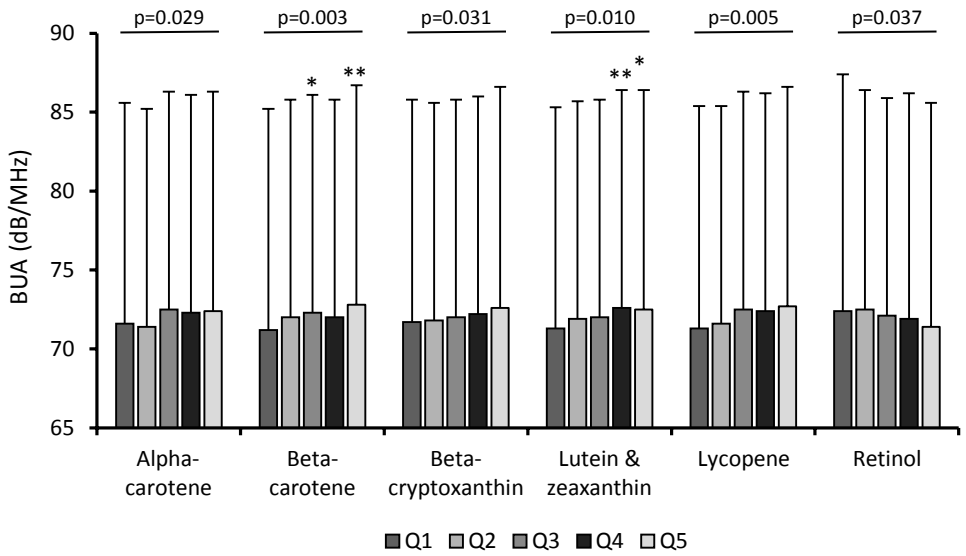
Full model: age, BMI, family history of osteoporosis, menopausal and HRT status in women, corticosteroid use, smoking status, physical activity.


Baseline health check
n=25,639
Excluded if missing: age, BMI, family history of osteoporosis, physical activity status, or HRT status in women.
n=73
Second health check
n=17,304
Fracture analysis data
n=25,566
Excluded if missing: age, BMI, family history of osteoporosis, physical activity status, HRT status in women, or ultrasound measurements.
n=2,427
Missing dietary data
n=127
Missing plasma data
n=18,092
Ultrasound analysis data
n=14,877
Diet fracture cohort
n=25,439
Plasma fracture cohort
n=7,474
Missing dietary data
n=74
Missing plasma data
n=10,307
Diet ultrasound cohort
n=14,803
Plasma ultrasound cohort
n=4,570

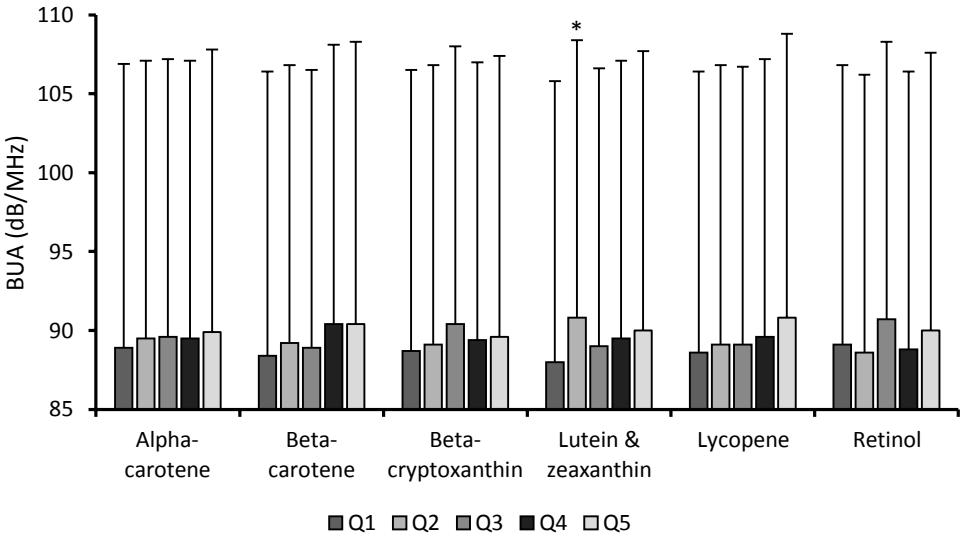
Men


110
105
100
95
90
85
BUA (dB/MHz)
Alpha-carotene
Beta-carotene
Beta-cryptoxanthin
Lutein & zeaxanthin
Lycopene
Retinol
Q1 Q2 Q3 Q4 Q5

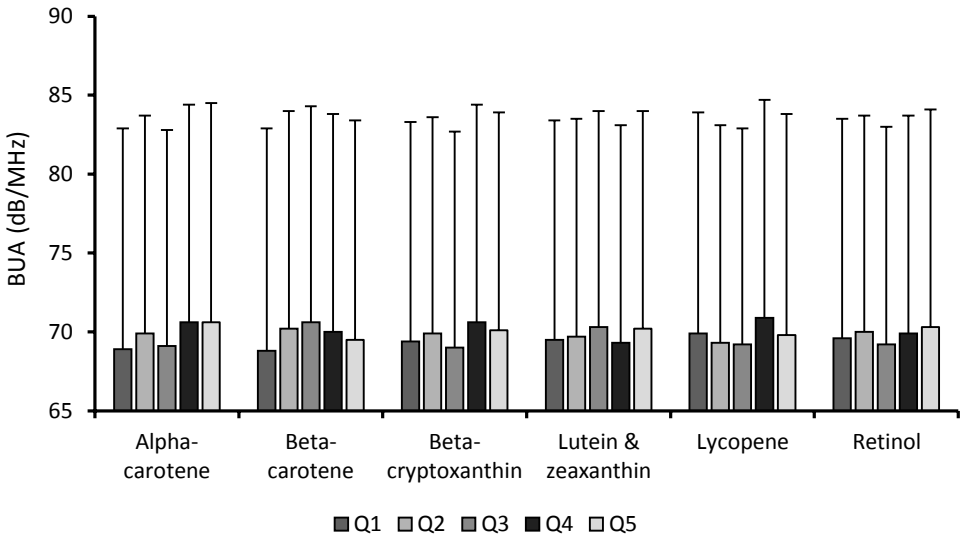
Women


p=0.029
p=0.003
p=0.031
p=0.010
p=0.005
p=0.037
90
85
80
75
70
65
BUA (dB/MHz)
*
**
**
*
Alpha-carotene
Beta-carotene
Beta-cryptoxanthin
Lutein & zeaxanthin
Lycopene
Retinol
Q1 Q2 Q3 Q4 Q5

Men


110
105
100
95
90
85
BUA (dB/MHz)
*
Alpha-carotene
Beta-carotene
Beta-cryptoxanthin
Lutein & zeaxanthin
Lycopene
Retinol
Q1 Q2 Q3 Q4 Q5

Women


90
85
80
75
70
65
BUA (dB/MHz)
Alpha-carotene
Beta-carotene
Beta-cryptoxanthin
Lutein & zeaxanthin
Lycopene
Retinol
Q1 Q2 Q3 Q4 Q5

SUPPLEMENTARY TABLES – Hayhoe *et al*, 2017

Table 1 – Unadjusted calcaneal BUA of 6490 men and 8313 women from the EPIC-Norfolk cohort, stratified by sex and dietary intake quintiles of specific carotenoids or retinol.

Men	**Dietary carotenoid intake**						
	Total	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
	n=6490	**n=1298**	**n=1298**	**n=1298**	**n=1298**	**n=1298**	**P for trend**
Alpha-carotene (μg/day)	90.1 ± 17.5	90.0 ± 17.2	89.7 ± 17.4	90.3 ± 17.7	89.8 ± 17.6	90.4 ± 17.7	0.587
Beta-carotene (μg/day)	90.1 ± 17.5	89.3 ± 17.2	89.9 ± 17.5	90.6 ± 17.7*	90.9 ± 17.3*	89.6 ± 17.9	0.520
Beta-cryptoxanthin (μg/day)	90.1 ± 17.5	89.9 ± 17.9	89.5 ± 17.5	89.4 ± 17.6	90.6 ± 17.0	90.9 ± 17.5	0.019
Lutein & zeaxanthin (μg/day)	90.1 ± 17.5	89.1 ± 17.0	89.9 ± 17.8	89.9 ± 17.5	90.8 ± 17.2*	90.6 ± 18.1*	0.027
Lycopene (μg/day)	90.1 ± 17.5	89.4 ± 18.2	90.3 ± 17.5	90.2 ± 16.9	90.2 ± 17.3	90.2 ± 17.7	0.468
Retinol (μg/day)	90.1 ± 17.5	90.0 ± 17.9	89.7 ± 18.0	90.3 ± 17.4	90.6 ± 17.3	89.7 ± 17.0	0.569
Women							
	n=8313	**n=1663**	**n=1663**	**n=1662**	**n=1663**	**n=1662**	**P for trend**
Alpha-carotene (μg/day)	72.1 ± 16.5	71.6 ± 17.0	71.5 ± 16.0	72.3 ± 16.3	72.2 ± 16.2	72.7 ± 16.8	0.032
Beta-carotene (μg/day)	72.1 ± 16.5	70.9 ± 16.2	72.0 ± 16.4	72.1 ± 16.4*	72.2 ± 16.5*	73.1 ± 16.9***	0.001
Beta-cryptoxanthin (μg/day)	72.1 ± 16.5	71.0 ± 16.5	71.6 ± 17.0	72.1 ± 16.4	72.6 ± 16.3**	73.0 ± 16.1***	<0.001
Lutein & zeaxanthin (μg/day)	72.1 ± 16.5	70.4 ± 16.4	71.8 ± 16.5*	72.2 ± 16.1**	73.5 ± 16.3***	72.4 ± 17.0**	0.001
Lycopene (μg/day)	72.1 ± 16.5	70.1 ± 16.2	70.5 ± 16.6	72.2 ± 16.4***	73.3 ± 16.1***	74.3 ± 16.8***	<0.001
Retinol (μg/day)	72.1 ± 16.5	72.6 ± 17.0	72.6 ± 16.6	71.9 ± 16.4	72.1 ± 16.1	71.0 ± 16.3**	0.002

Data as mean ± SD. * p<0.05; ** p<0.01; *** p<0.001 *vs.* Quintile 1, according to ANCOVA.

SUPPLEMENTARY TABLES – Hayhoe *et al*, 2017

Table 2 – Unadjusted calcaneal BUA of 2362 men and 2208 women from the EPIC-Norfolk cohort, stratified by sex and plasma concentration quintiles of specific carotenoids or retinol.

Men	**Plasma carotenoid concentration**						
	Total	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
	n=2362	**n=473**	**n=472**	**n=473**	**n=472**	**n=472**	**P for trend**
Alpha-carotene (µg/dL)	89.5 ± 17.8	88.9 ± 18.2	89.5 ± 17.1	89.7 ± 17.4	89.5 ± 18.0	89.8 ± 18.2	0.555
Beta-carotene (µg/dL)	89.5 ± 17.8	88.8 ± 17.7	89.4 ± 17.5	88.8 ± 16.8	90.3 ± 18.1	89.9 ± 18.8	0.247
Beta-cryptoxanthin (µg/dL)	89.5 ± 17.8	88.5 ± 18.1	89.2 ± 17.4	90.4 ± 17.5	89.4 ± 18.0	89.8 ± 17.9	0.407
Lutein & zeaxanthin (µg/dL)	89.5 ± 17.8	88.3 ± 17.5	90.8 ± 17.4*	89.2 ± 17.9	89.2 ± 18.1	89.8 ± 17.9	0.568
Lycopene (µg/dL)	89.5 ± 17.8	88.5 ± 17.8	89.0 ± 17.5	89.0 ± 17.3	89.7 ± 17.6	91.1 ± 18.5*	0.015
Retinol (µg/dL)	89.5 ± 17.8	88.8 ± 17.8	88.9 ± 17.7	90.8 ± 17.8	88.9 ± 17.8	89.9 ± 17.6	0.357
Women							
	n=2208	**n=442**	**n=442**	**n=441**	**n=442**	**n=441**	**P for trend**
Alpha-carotene (µg/dL)	69.8 ± 16.2	69.6 ± 16.8	70.3 ± 16.0	69.0 ± 15.8	70.5 ± 16.5	69.6 ± 16.0	0.939
Beta-carotene (µg/dL)	69.8 ± 16.2	71.4 ± 16.5	71.1 ± 15.8	70.4 ± 16.4	68.6 ± 16.0**	67.4 ± 16.1***	<0.001
Beta-cryptoxanthin (µg/dL)	69.8 ± 16.2	69.7 ± 16.8	70.1 ± 17.4	69.3 ± 16.5	70.1 ± 15.6	69.8 ± 14.7	0.919
Lutein & zeaxanthin (µg/dL)	69.8 ± 16.2	71.1 ± 16.2	70.1 ± 17.0	70.7 ± 16.0	68.5 ± 16.0*	68.6 ± 15.7*	0.008
Lycopene (µg/dL)	69.8 ± 16.2	67.2 ± 16.3	68.6 ± 16.4	69.3 ± 15.8*	72.3 ± 15.8***	71.6 ± 16.2***	<0.001
Retinol (µg/dL)	69.8 ± 16.2	70.7 ± 17.2	70.1 ± 16.4	68.2 ± 15.4*	69.7 ± 16.1	70.3 ± 15.9	0.781

Data as mean ± SD. * p<0.05; ** p<0.01; *** p<0.001 *vs.* Quintile 1, according to ANCOVA.