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Intake of antioxidant nutrients and the risk of skin cancer

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ABSTRACT

To investigate the associations between intake of antioxidant nutrients and risk of basal cell (BCC) and squamous cell carcinomas (SCC) of the skin, we carried out a prospective study among 1001 randomly selected adults living in an Australian community. Intake of antioxidants was estimated in 1996. Incident, histologically-confirmed BCC and SCC were recorded between 1996 and 2004. High dietary intake of lutein and zeaxanthin was associated with a reduced incidence of SCC in persons who had a history of skin cancer at baseline (highest versus lowest tertile, multivariable adjusted relative risk (RR) = 0.47, 95% confidence interval (CI): 0.25–0.89; *P* for trend = 0.02). In persons without a history of skin cancer at baseline, development of BCC was positively associated with intake of vitamins C and E from foods plus supplements (RR = 3.1, 95% CI: 1.1–8.6; *P* for trend = 0.03 and RR = 2.6, 95% CI: 1.1–6.3; *P* for trend = 0.02, respectively). In those with a skin cancer history at baseline, dietary intake in the second tertile for β-carotene (multivariable adjusted RR = 2.2, 95% CI: 1.2–4.1) and for vitamin E (multivariable adjusted RR = 2.1, 95% CI: 1.1–3.9) was associated with increased BCC risk, with no trend, and similar results were seen in those with a specific history of BCC. These data suggest quite different associations between antioxidant intake and SCC compared with BCC, consistent with other evidence of their different causal pathways.

1. Introduction

Basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most commonly occurring skin cancers in white populations and incidence rates have increased in Europe, USA and Australia in recent years.[1–3] While mortality from these cancers may be low, the disease burden is high with the large numbers of patients requiring careful treatment, often surgical excision, and follow-up. Costs to health systems are considerable compared to costs of other cancers.[4]

Ultraviolet (UV) radiation from sunlight, the main environmental cause of skin cancer, causes direct damage to DNA and the immune system[5,6] and indirect damage through formation of free radicals.[7] There is evidence that antioxidants may help prevent UV damage in the skin. *In vitro* and animal studies suggest that selenium (an important component of the antioxidant glutathione peroxidase),[8] vitamins C and E and β-carotene may help protect the skin against oxidative damage by neutralising reactive oxygen species and other free radicals.[9]

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In a recent review of epidemiological studies, McNaughton and colleagues[10] concluded that there was little evidence for an association between β-carotene intake and BCC or SCC risk; the findings for retinol were inconsistent, and there was insufficient evidence from high quality studies about intake of carotenoids, vitamins E and C, and selenium and keratinocyte cancer risk. Many of the epidemiological studies published to date have been limited by small numbers of cases, reliance on health records and cancer registries for case ascertainment, poor dietary assessment methods, combined analysis of BCC and SCC, or a lack of adjustment for potential confounding factors.

We carried out a prospective cohort study to investigate the relationship between intake of antioxidant nutrients and the occurrence of BCC and SCC in a high-risk population whose sun exposure and skin cancer histories have been fully characterised.

2. Materials and methods

2.1. Study population

We conducted an 8-year prospective study among randomly selected adults who had participated in a skin cancer prevention field trial as part of the ongoing Nambour Skin Cancer Study. Detailed descriptions of the community sample, the field trial and its outcomes have been reported previously.[11,12] In summary, the Nambour Skin Cancer Prevention Trial was a randomised controlled trial with a 2 by 2 factorial design that evaluated the effectiveness of daily consumption of beta-carotene tablets and application of sunscreen in preventing skin cancer. The Nambour Trial was conducted between 1992 and 1996 among 1621 adult residents of Nambour, a subtropical community in Queensland, Australia. Information on skin colour, tanning ability of the skin and other phenotypic characteristics were ascertained in 1992. At the end of the trial in 1996, participants completed a food frequency questionnaire (FFQ) and questionnaires on sun exposure and other personal characteristics. The current analyses included all participants who completed the FFQ and provided a blood sample in 1996. This study was approved by the ethics committee of the Queensland Institute of Medical Research and all participants provided written informed consent.

2.2. Data collection

Study participants were further followed-up from 1996 until the end of 2004 to ascertain all occurrences of BCC and SCC. Skin cancers were ascertained through an intensive surveillance system such that questionnaires were mailed twice-yearly to participants and any reported skin cancers were confirmed through histological reports. Independent pathology laboratories throughout Queensland provided pathology reports for all skin cancers diagnosed among study participants. In 2000, a full-body skin examination of available study participants was conducted by a dermatology specialist trainee. These methods ensured virtually 100% ascertainment of histologically-confirmed skin cancers in the study population.[13]

Information on skin cancer history prior to 1996 was based on skin cancers identified during skin examinations and surveys conducted in the participants between 1986 and 1996,[11,14–16] and on self-reports of any type of skin cancer prior to 1986[14] with pathology verification of the subset of cancers reported in 1985–1986.[15]

Standardised self-administered questionnaires completed by participants throughout the follow-up period provided information on smoking habits and time spent outdoors on weekends and weekdays.[12] During a physical examination in 1992, degree of elastosis of the neck was recorded as an objective measure of long-term sun exposure.

2.3. Dietary intake estimates

Habitual diet during the past 6 months was assessed using a self-administered, semi-quantitative FFQ consisting of 129 food or food group items in 1996. The FFQ was originally developed for the US Nurses' Health Study, adapted for the Australian setting and validated against weighed food records.[17,18] For each food, a commonly used unit or portion size was specified and participants were asked to estimate how often, on average, they had eaten the given amount of food over the past 6 months. The nine response options ranged from 'never' to '4+ times per day'. Information on cooking methods, specific types of fats, oils, margarines, breakfast cereals and takeaway foods was also collected and subjects were asked to provide detailed information regarding consumption of nutritional supplements.

Average daily intake was calculated by expressing the response to the food item as a proportion of daily use, which was then multiplied by the gram amounts of the specified portion sizes and by the nutrient content of the food. Seasonal foods were weighted according to the proportion of the year that each food was available. Supplement intake was analysed using a specially designed supplement database.[19] Dietary intakes of the carotenoids and vitamin E were calculated using data from international food composition tables.[20,21] Recipes were used to calculate food composition, and where necessary we used values for different forms of the same food or values for similar foods. Adjustments were made for differences in water, protein, sugar or fat content or for weight changes on cooking. Intakes of retinol and vitamin C were estimated using Australian food composition tables.[22] A specially developed food composition database for the selenium content of Australian foods was used to estimate selenium intakes. This database was developed by using standard principles, based on published food composition data.[23]

2.4. Statistical analysis

Two outcomes were used in the analysis: (a) tumour-based incidence: number of newly developed histologically confirmed BCC or SCC after the 1996 skin examination through to 31 December 2004, divided by the person-years of follow-up accumulated between these dates, and (b) person-based incidence: number of persons newly affected by BCC or SCC during the same person-years of follow-up time as calculated for the tumour-based analysis. Person-based analyses were

carried out to investigate whether the results of lesion-based analyses were influenced by persons with multiple lesions. Tumours diagnosed during the 1996 skin survey were not included in the analyses in order to exclude disease that already existed during the baseline nutritional assessment. Tumours and person-years of follow-up were counted until date of withdrawal from the study, date of death, or 31 December 2004, whichever came first.

Intake was calculated based on diet alone; supplements alone; and then on diet plus supplement intake. We present results of the combined diet and supplement intake only when the results were different from those of intake from diet only, or when a significant association was found for both estimates. Compliance with the trial supplement, assessed by self-reported consumption at the end of the intervention period, was used to calculate each participant's intake of β-carotene from the trial supplement. Intake from the trial supplement was added to intake of β-carotene from other supplements.

For linear modelling, intake estimates of dietary antioxidants were adjusted for total energy intake using the nutrient residual method as described by Willett.[24] Distributions of the intake of dietary antioxidants were identified as skewed and variables were log-transformed to improve normality prior to calculation of the residuals.

Tertiles were calculated for each dietary intake variable and used as cut-points for grouping. For tumour-based analyses, relative risks (RRs) with 95% confidence intervals (CIs) for increasing levels of dietary intake compared to the lowest tertile were derived using generalised linear models with negative binomial distribution and person-years of follow-up as offset. The negative binomial distribution has been recommended for analysing non-negative integer data with variance greater than the mean[25] and provided the best fit to our tumour-count data. Risk estimates for person-based analysis (absence or presence of a new BCC or SCC in the follow-up period) were estimated by generalised linear models specifying Poisson distribution with a robust error variance.[26]

Intake from supplements was divided into two equal groups, above and below the median intake from supplements for each nutrient. These groups were compared to subjects without intake from supplements for that nutrient.

Analyses first used a basic model, which controlled for age (continuous), sex and trial treatment allocation (sunscreen treatment and β-carotene treatment between 1992 and 1996, yes/no). Secondly, in a multivariable model additional confounders were selected based on examination of their associations with exposure and outcome variables. Covariates were retained in the model if they changed the risk estimates by more than 10%, while age, sex, trial treatment and skin colour were retained in all multivariable models. The multivariable model for BCC included age, sex, trial treatment allocation, total energy intake (in kJ/day), skin colour (fair, medium, olive), degree of elastosis of the neck, pack-years of smoking until 1996, use of dietary supplements (yes/no) and history of skin cancer before 1996. In addition to these covariates, the multivariable model for SCC included propensity to burn or tan after acute sun exposure and number of painful sunburns ever experienced. Other variables were considered as confounders but did not change risk estimates, including eye colour, self-reported sunscreen use, education level, and usual time spent outdoors (weekdays, weekends).

To test for linear trends, we assigned an ordinal number ranging from one (for the lowest tertile of intake) to three (highest tertile) for each participant's consumption level and modelled this as a continuous variable.

People who have a history of skin cancer have an increased risk of developing subsequent skin cancers[27] and may be more prone to risk modification by dietary factors. We therefore carried out a formal test of interaction for all nutrients by including a multiplicative interaction term in the linear models. If interactions were present we repeated the above analyses in two strata based on presence or absence of a history of any type of skin cancer prior to the 1996 skin examination. In addition, we investigated whether such interaction was specific to past history of either BCC or SCC.

Stata statistical package version 9.0 (StataCorp LP USA) was used in all analyses. All statistical tests were two-sided using a significance level of $P < 0.05$.

3. Results

Of the 1027 eligible participants, 26 (3%) were excluded: eight participants did not indicate consumption frequencies for 10% or more of the FFQ food items and 18 subjects reported energy intakes outside the normal ranges.[24] The 1001 participants included in the present study were not different from the original 1621 trial participants in terms of trial treatment allocation, age, sex, education, occupation, smoking status, use of dietary supplements and skin cancer risk factors such as skin colour, lifetime number of sunburns and other measures of sun exposure, and skin cancer history prior to 1996.

In the 8-year follow-up period a total of 221 histologically-confirmed SCC tumours were diagnosed in 116 participants during 7547 person-years of follow-up (tumour-based incidence: 2928/100,000; person-based incidence: 1537/100,000). For BCC, a total of 321 tumours were diagnosed in 149 participants during the same person-years of follow-up (tumour-based incidence: 4253/100,000; person-based incidence: 1974/100,000). 279 participants had a history of skin cancer before the study, of whom 191 were known to have had a past BCC, 90 a past SCC, while for 46 persons type of past skin cancer was unknown.

Participants who had an SCC in the follow-up period were more likely to be male, older, have a tendency to sunburn, to have smoked and to have a history of skin cancer than those who did not develop an SCC (Table 1). Similarly those who developed a BCC were more likely to be male, older, have fair skin and a tendency to sunburn, to have smoked and to have a past history of skin cancer compared to those without BCC.

After basic adjustment, intake of lutein and zeaxanthin showed an inverse trend with SCC tumour risk (highest versus lowest tertile, basic adjusted RR = 0.54, 95% CI: 0.31–0.92; $P = 0.02$; Table 2), but this association disappeared after full covariate adjustment. Overall SCC risk was not associated with intake of any of the other anti-oxidant nutrients.

The relative risk of BCC tumours was increased in persons who had an intake in the second tertile for dietary β-carotene (multivariable adjusted RR = 1.6, 95% CI: 1.0–2.7; Table 3), but the estimate for the third tertile was weaker and there was

Table 1 – Characteristics of participants by skin cancer status

	SCC in 1996–2004			BCC in 1996–2004		
	Yes (n = 116)	No (n = 885)	P-value[a]	Yes (n = 149)	No (n = 852)	P-value[a]
Sex						
Male	70 (60%)	384 (43%)	0.0006	87 (58%)	367 (43%)	0.0005
Female	46 (40%)	501 (57%)		62 (42%)	485 (57%)	
Age						
Age (mean) in 1996	65 yrs	53 yrs	<0.0001	61 yrs	53 yrs	<0.0001
Skin colour						
Fair	72 (62%)	480 (54%)	0.20	98 (66%)	454 (53%)	0.02
Medium	39 (34%)	336 (38%)		44 (29%)	331 (39%)	
Olive	5 (4%)	68 (8%)		7 (5%)	66 (8%)	
Propensity to burn/tan after acute sun exposure						
Always burn	38 (33%)	167 (19%)	0.002	41 (28%)	164 (19%)	0.05
Burn then tan	68 (58%)	619 (70%)		96 (64%)	591 (69%)	
Tan only	10 (9%)	98 (11%)		12 (8%)	96 (11%)	
Painful sunburns during life						
None	20 (18%)	139 (16%)	0.02	30 (21%)	129 (16%)	0.27
1 Burn	26 (23%)	117 (14%)		19 (13%)	124 (15%)	
$\geqslant$2 Burns	66 (59%)	603 (70%)		94 (66%)	575 (69%)	
Smoking status in 1996						
Never	48 (41%)	497 (56%)	0.0005	73 (49%)	472 (55%)	0.01
Current	9 (8%)	100 (11%)		10 (7%)	99 (12%)	
Ex	59 (51%)	288 (33%)		66 (44%)	281 (33%)	
History of skin cancer before 1996						
No	27 (23%)	647 (73%)	<0.0001	41 (28%)	633 (74%)	<0.0001
Yes	89 (77%)	238 (27%)		108 (72%)	219 (26%)	

a P-value from chi-square test (categorical data) or ANOVA (continuous data).

no linear trend (P for trend = 0.22). Overall BCC risk was not associated with any other nutrients.

All nutrient intake estimates showed a significant statistical interaction with skin cancer history and specific history of BCC ($P < 0.001$) or of SCC ($P < 0.05$). Stratified analyses showed that dietary intake of lutein and zeaxanthin combined was associated with an inverse trend of SCC risk in persons with a skin-cancer history (highest versus lowest tertile, multivariable adjusted RR = 0.47, 95% CI: 0.25–0.89; P for trend = 0.02; Table 4) but not in those without a skin-cancer history. This inverse association was also seen when lutein intake was considered as a continuous variable. Analysis according to specific past history of SCC was not possible due to the small number of persons (n = 90) known to have past SCC before the 1996 skin examination.

After stratification by past history of any skin cancer, there was an apparent doubling of BCC tumour risk in persons with intake levels in the second tertile for β-carotene (multivariable adjusted RR = 2.2, 95% CI: 1.2–4.1; Table 5) and vitamin E (multivariable adjusted RR = 2.1, 95% CI: 1.1–3.9) in persons with a history of skin cancer, with no trend. These associations were of a similar magnitude in the 191 participants with a specific history of BCC (multivariable adjusted second tertile estimates for β-carotene: RR = 1.9, 95% CI: 0.97–3.6, P for trend = 0.75, and vitamin E: RR = 2.4, 95% CI: 1.3–4.7, P for trend = 0.20). Dietary intake of antioxidants was not associated with risk of BCC in participants without a history of skin cancer at baseline (Table 5).

Intake from supplements was not associated with skin cancer risk for any of the nutrients (results not shown). When intake from supplements was added to dietary intake, intake of vitamin C (highest versus lowest tertile RR = 3.1, 95% CI: 1.1–8.6; P for trend = 0.03) and vitamin E (highest versus lowest tertile RR = 2.6, 95% CI: 1.1–6.3; P for trend = 0.02) showed a significant positive trend with BCC risk in people without a history of skin cancer at baseline. The positive association between dietary intake of β-carotene in the second tertile and BCC risk disappeared after adding intake of β-carotene from supplements. Two participants had very high intakes of vitamin C from supplements, but excluding them from the analysis did not change the risk estimates.

Analyses of person-based incidence data showed similar results to those presented for tumour-based analyses (results not shown). The direction of associations was similar to the tumour-based analyses, but the magnitude of effects was weaker, and no significant associations were found.

4. Discussion

In this prospective community-based study of Australian adults, dietary intake of lutein and zeaxanthin was associated with a more than 50% reduction in risk of SCC in persons with a prior history of skin cancer. In a previous study carried out in this population, we reported an inverse association between green leafy vegetables (which are important food sources of lutein and zeaxanthin) and risk of SCC (highest

Table 2 – Relative risk (RR; 95% confidence interval) of squamous cell carcinoma (1996–2004) by tertile of dietary intake of antioxidants in 1996, tumour-based analyses

Dietary factor	Tertile of intake			*P* for trend
	T1	T2	T3	
α-Carotene				
Median intake (μg; min-max)	2445 (94–3393)	4194 (3393–5229)	6800 (5229–23716)	
Number of tumours	75	74	72	
Age-sex-treatment adjusted RR[a,b]	1.0	0.92 (0.54–1.6)	0.68 (0.40–1.2)	0.16
Multivariable adjusted RR[c,d]	1.0	1.2 (0.70–2.1)	0.76 (0.42–1.4)	0.38
β-Carotene				
Median intake (mg; min-max)	6.2 (0.52–8.4)	10.2 (8.4–12.7)	16.0 (12.7–50.7)	
Number of tumours	66	73	82	
Age-sex-treatment adjusted RR[a,b]	1.0	0.80 (0.46–1.4)	0.67 (0.39–1.2)	0.16
Multivariable adjusted RR[c,d]	1.0	0.89 (0.50–1.6)	0.70 (0.39–1.3)	0.24
β-Cryptoxanthin				
Median intake (μg; min-max)	41 (0.01–83)	143 (83–207)	323 (208–1309)	
Number of tumours	55	56	110	
Age-sex-treatment adjusted RR[a,b]	1.0	0.80 (0.45–1.4)	1.0 (0.57–1.7)	0.96
Multivariable adjusted RR[c,d]	1.0	0.86 (0.47–1.6)	1.1 (0.58–1.9)	0.82
Lutein and zeaxanthin				
Median intake (μg; min-max)	974 (108–1347)	1681 (1348–2130)	2945 (2144–9922)	
Number of tumours	83	80	58	
Age-sex-treatment adjusted RR[a,b]	1.0	0.62 (0.37–1.1)	0.54 (0.31–0.92)	0.02
Multivariable adjusted RR[c,d]	1.0	0.79 (0.46–1.4)	0.65 (0.38–1.1)	0.13
Lycopene				
Median intake (μg; min-max)	1945 (6–2998)	3880 (3000–5033)	6744 (5043–30502)	
Number of tumours	82	69	70	
Age-sex-treatment adjusted RR[a,b]	1.0	1.1 (0.69–2.0)	0.75 (0.44–1.3)	0.31
Multivariable adjusted RR[c,d]	1.0	1.0 (0.60–1.8)	0.84 (0.48–1.5)	0.56
Total carotenoids				
Median intake (mg; min-max)	13.6 (2.8–17.6)	21.0 (17.7–25.1)	31.5 (25.1–84.4)	
Number of tumours	66	78	77	
Age-sex-treatment adjusted RR[a,b]	1.0	1.1 (0.66–1.9)	0.81 (0.47–1.4)	0.43
Multivariable adjusted RR[c,d]	1.0	1.2 (0.66–2.1)	0.96 (0.54–1.7)	0.85
Retinol				
Median intake (μg; min-max)	247 (38–317)	416 (317–668)	1066 (669–17394)	
Number of tumours	76	57	88	
Age-sex-treatment adjusted RR[a,b]	1.0	0.86 (0.50–1.5)	1.2 (0.68–1.9)	0.56
Multivariable adjusted RR[c,d]	1.0	0.86 (0.48–1.5)	1.2 (0.70–2.1)	0.47
Vitamin C				
Median intake (mg; min-max)	119 (24–150)	184 (151–218)	275 (218–592)	
Number of tumours	46	74	101	
Age-sex-treatment adjusted RR[a,b]	1.0	1.4 (0.80–2.4)	1.2 (0.72–2.2)	0.49
Multivariable adjusted RR[c,d]	1.0	1.4 (0.78–2.5)	1.2 (0.66–2.2)	0.63
Vitamin E				
Median intake (mg; min-max)	7.3 (2.8–8.1)	8.9 (8.1–9.7)	10.9 (9.7–18.6)	
Number of tumours	60	78	83	
Age-sex-treatment adjusted RR[a,b]	1.0	0.96 (0.55–1.7)	0.84 (0.48–1.5)	0.53
Multivariable adjusted RR[c,d]	1.0	1.2 (0.65–2.1)	1.2 (0.67–2.2)	0.54
Selenium				
Median intake (μg; min-max)	70.1 (34.3–76.2)	82.2 (76.2–89.3)	99.1 (89.3–168.9)	
Number of tumours	61	63	97	
Age-sex-treatment adjusted RR[a,b]	1.0	0.75 (0.43–1.3)	1.1 (0.63–1.8)	0.73
Multivariable adjusted RR[c,d]	1.0	1.1 (0.59–1.9)	1.3 (0.77–2.3)	0.28

a Treatment refers to Nambour Trial treatment allocation of β-carotene and/or sunscreen.

b $n = 1001$.

c $n = 940$.

d Multivariable negative binomial regression controlling for age, sex, energy intake (kJ/day), skin colour, tanning ability of skin, elastosis of the neck, number of painful sunburns, smoking, treatment allocation, use of dietary supplements (yes/no), history of skin cancer before 1996.

Table 3 – Relative risk (RR; 95% confidence interval) of basal cell carcinoma (1996–2004) by tertile of dietary intake of antioxidants in 1996, tumour-based analyses

Dietary factor	Tertile of intake			*P* for trend
	T1	T2	T3	
α-Carotene				
Median intake (μg; min-max)	2445 (94–3393)	4194 (3393–5229)	6800 (5229–23716)	
Number of tumours	82	97	142	
Age-sex-treatment adjusted RR[a,b]	1.0	1.2 (0.71–1.9)	1.4 (0.84–2.3)	0.19
Multivariable adjusted RR[c,d]	1.0	1.3 (0.82–2.2)	1.4 (0.87–2.3)	0.17
β-Carotene				
Median intake (mg; min-max)	6.2 (0.52–8.4)	10.2 (8.4–12.7)	16.0 (12.7–50.7)	
Number of tumours	64	135	122	
Age-sex-treatment adjusted RR[a,b]	1.0	1.6 (1.0–2.7)	1.4 (0.81–2.4)	0.27
Multivariable adjusted RR[c,d]	1.0	1.6 (1.0–2.7)	1.4 (0.85–2.4)	0.22
β-Cryptoxanthin				
Median intake (μg; min-max)	41 (0.01–83)	143 (83–207)	323 (208–1309)	
Number of tumours	82	100	139	
Age-sex-treatment adjusted RR[a,b]	1.0	1.0 (0.62–1.7)	1.4 (0.81–2.3)	0.24
Multivariable adjusted RR[c,d]	1.0	0.82 (0.50–1.3)	1.1 (0.65–1.8)	0.76
Lutein and zeaxanthin				
Median intake (μg; min-max)	974 (108–1347)	1681 (1348–2130)	2945 (2144–9922)	
Number of tumours	118	101	102	
Age-sex-treatment adjusted RR[a,b]	1.0	0.80 (0.49–1.3)	1.0 (0.64–1.7)	0.91
Multivariable adjusted RR[c,d]	1.0	0.74 (0.46–1.2)	1.1 (0.71–1.8)	0.61
Lycopene				
Median intake (μg; min-max)	1945 (6–2998)	3880 (3000–5033)	6744 (5043–30502)	
Number of tumours	98	121	102	
Age-sex-treatment adjusted RR[a,b]	1.0	1.2 (0.74–1.9)	1.1 (0.66–1.8)	0.77
Multivariable adjusted RR[c,d]	1.0	1.1 (0.71–1.8)	0.98 (0.61–1.6)	0.94
Total carotenoids				
Median intake (mg; min-max)	13.6 (2.8–17.6)	21.0 (17.7–25.1)	31.5 (25.1–84.4)	
Number of tumours	75	127	119	
Age-sex-treatment adjusted RR[a,b]	1.0	1.6 (0.95–2.5)	1.4 (0.82–2.3)	0.25
Multivariable adjusted RR[c,d]	1.0	1.7 (1.0–2.7)	1.4 (0.87–2.4)	0.21
Retinol				
Median intake (μg; min-max)	247 (38–317)	416 (317–668)	1066 (669–17394)	
Number of tumours	123	102	96	
Age-sex-treatment adjusted RR[a,b]	1.0	0.93 (0.58–1.5)	0.76 (0.47–1.2)	0.27
Multivariable adjusted RR[c,d]	1.0	1.0 (0.65–1.6)	0.79 (0.49–1.3)	0.33
Vitamin C				
Median intake (mg; min-max)	119 (24–150)	184 (151–218)	275 (218–592)	
Number of tumours	84	83	154	
Age-sex-treatment adjusted RR[a,b]	1.0	0.87 (0.53–1.4)	1.3 (0.76–2.1)	0.35
Multivariable adjusted RR[c,d]	1.0	0.82 (0.50–1.3)	1.1 (0.65–1.7)	0.75
Vitamin E				
Median intake (mg; min-max)	7.3 (2.8–8.1)	8.9 (8.1–9.7)	10.9 (9.7–18.6)	
Number of tumours	65	137	119	
Age-sex-treatment adjusted RR[a,b]	1.0	1.7 (1.0–2.8)	1.6 (0.94–2.7)	0.11
Multivariable adjusted RR[c,d]	1.0	1.5 (0.92–2.5)	1.5 (0.91–2.5)	0.14
Selenium				
Median intake (μg; min-max)	70.1 (34.3–76.2)	82.2 (76.2–89.3)	99.1 (89.3–168.9)	
Number of tumours	84	122	115	
Age-sex-treatment adjusted RR[a,b]	1.0	1.2 (0.71–1.9)	1.0 (0.62–1.7)	0.90
Multivariable adjusted RR[c,d]	1.0	1.2 (0.73–1.9)	0.95 (0.59–1.5)	0.81

a Treatment refers to Nambour Trial treatment allocation of β-carotene and/or sunscreen.

b $n = 1001$.

c $n = 969$.

d Multivariable negative binomial regression controlling for age, sex, energy intake (kJ/day), skin colour, elastosis of the neck, smoking, treatment allocation, use of dietary supplements (yes/no), history of skin cancer before 1996.

Table 4 – Relative risk (RR; 95% confidence interval) of squamous cell carcinoma (1996–2004) by tertile of dietary intake of antioxidants in 1996, stratified by history of skin cancer prior to 1996, tumour-based analyses (n = 940)

Dietary factor	No skin cancer prior to 1996 (n = 646)				With skin cancer prior to study (n = 294)			
	Tertile of intake			P for trend	Tertile of intake			P for trend
	T1	T2	T3		T1	T2	T3	
α-Carotene								
Multivariable adjusted RR[a]	1.0	1.1 (0.35–3.6)	0.69 (0.19–2.4)	0.60	1.0	1.1 (0.57–2.0)	0.72 (0.37–1.4)	0.32
β-Carotene								
Multivariable adjusted RR[a]	1.0	0.49 (0.13–1.9)	0.76 (0.24–2.4)	0.55	1.0	1.1 (0.59–2.1)	0.65 (0.32–1.3)	0.20
β-Cryptoxanthin								
Multivariable adjusted RR[a]	1.0	0.54 (0.14–2.1)	1.2 (0.36–4.1)	0.78	1.0	0.79 (0.39–1.6)	0.87 (0.44–1.7)	0.75
Lutein and zeaxanthin								
Multivariable adjusted RR[a]	1.0	3.0 (0.87–10.3)	0.94 (0.24–3.6)	0.99	1.0	0.52 (0.27–0.97)	0.47 (0.25–0.89)	0.02
Lycopene								
Multivariable adjusted RR[a]	1.0	0.69 (0.19–2.4)	1.1 (0.35–3.6)	0.87	1.0	1.0 (0.56–1.9)	0.78 (0.42–1.5)	0.45
Total carotenoids								
Multivariable adjusted RR[a]	1.0	1.0 (0.29–3.7)	1.7 (0.53–5.2)	0.38	1.0	1.2 (0.60–2.2)	0.68 (0.35–1.3)	0.19
Retinol								
Multivariable adjusted RR[a]	1.0	1.4 (0.34–5.3)	2.1 (0.60–7.3)	0.23	1.0	0.61 (0.32–1.2)	1.1 (0.60–2.0)	0.74
Vitamin C								
Multivariable adjusted RR[a]	1.0	1.5 (0.41–5.3)	1.6 (0.46–5.7)	0.45	1.0	1.1 (0.52–2.2)	0.81 (0.41–1.6)	0.48
Vitamin E								
Multivariable adjusted RR[a]	1.0	0.77 (0.20–2.9)	0.88 (0.22–3.6)	0.86	1.0	1.4 (0.69–2.6)	1.1 (0.58–2.1)	0.80
Selenium								
Multivariable adjusted RR[a]	1.0	2.4 (0.68–8.5)	1.2 (0.34–4.5)	0.76	1.0	0.81 (0.42–1.6)	1.3 (0.71–2.4)	0.32

a Multivariable negative binomial regression controlling for age, sex, energy intake (kJ/day), skin colour, tanning ability of skin, elastosis of the neck, number of painful sunburns, smoking, treatment allocation, use of dietary supplements (yes/no).

versus lowest tertile of intake RR = 0.45, 95% CI: 0.22–0.91, P for trend = 0.02),[28] an association that was independent of a diet pattern characterised by high vegetable and fruit intake.[29] Thus the results of the current study suggest that lutein and zeaxanthin may be active components of green-leafy vegetables that underlie this protective association. Animal studies have shown that the carotenoids lutein and zeaxanthin intake may protect the skin from UV-induced inflammation and photodamage.[30,31] The specific mechanisms that are underlying this protective effect are yet to be determined, but these nutrients may quench peroxyl radicals and demonstrate antioxidant properties against oxidative damage *in vitro*.[32] However, it remains unclear why this association was seen for lutein and zeaxanthin but not for other antioxidant nutrients.

The lack of such a protective association for BCC is consistent with the fact that SCC more than BCC shows a monotonic dose-response relationship with sun-exposure[33] and is amenable to short- and long-term prevention by application of sunscreen,[12,34] thus suggesting a more central role for UV-induced oxidative damage in SCC than BCC aetiology. This may also explain why this protective association is particularly seen in persons who were previously affected by skin cancer. We propose that far more oxidative damage has occurred in susceptible, highly sun-exposed individuals who have already had skin cancer, giving these carotenoids ample opportunity to act as free-radical quenchers.

The intermittent sun exposure pattern has been shown to be a risk factor for BCC in some studies.[35,36] In our analyses there was no confounding by the two variables that indicate sun exposure on week days and weekends respectively, whether included in the model separately or combined. Thus residual confounding by intermittent sun exposure patterns is considered to be unlikely. Furthermore, in previous analyses we have shown that past sun exposure per se, whether long-term or intermittent, is a main risk factor for BCC in our study population.[37]

It has long been accepted that SCCs arise in committed keratinocytes in the epidermis. On the other hand, the histogenesis of BCC has been debated for almost a century but it appears that defects in molecular pathways such as the Hedgehog pathway play an important role in the development of these cancers.[38] Thus the different findings between SCC and BCC in our study are consistent with their likely divergent causal pathways.

Medium level (8–13 mg/day) intake of β-carotene from foods was associated with an estimated doubling of BCC risk in persons who had a history of skin cancer at baseline. β-carotene has anti-carcinogenic properties and is capable of quenching singlet oxygen and scavenging peroxide radicals.[9] A possible

Table 5 – Relative risk (RR; 95% confidence interval) of basal cell carcinoma (1996–2004) by tertile of dietary intake of antioxidants in 1996, stratified by history of skin cancer prior to 1996, tumour-based analyses (n = 969)

Dietary factor	No skin cancer prior to 1996 (*n* = 658)				With skin cancer prior to study (*n* = 311)			
	Tertile of intake			*P* for trend	Tertile of intake			*P* for trend
	T1	T2	T3		T1	T2	T3	
α-Carotene								
Multivariable adjusted RR[a] (95% CI)	1.0	1.3 (0.59–3.0)	1.3 (0.57–3.1)	0.51	1.0	1.4 (0.77–2.6)	1.5 (0.80–2.7)	0.24
β-Carotene								
Multivariable adjusted RR[a] (95% CI)	1.0	0.85 (0.35–2.1)	1.5 (0.68–3.3)	0.33	1.0	2.2 (1.2–4.1)	1.4 (0.73–2.8)	0.50
β-Cryptoxanthin								
Multivariable adjusted RR[a] (95% CI)	1.0	0.58 (0.25–1.4)	1.3 (0.61–2.9)	0.52	1.0	0.97 (0.51–1.8)	0.93 (0.48–1.8)	0.82
Lutein and zeaxanthin								
Multivariable adjusted RR[a] (95% CI)	1.0	0.53 (0.21–1.3)	1.4 (0.65–2.9)	0.40	1.0	0.77 (0.43–1.4)	0.87 (0.48–1.6)	0.67
Lycopene								
Multivariable adjusted RR[a] (95% CI)	1.0	1.0 (0.43–2.3)	1.2 (0.53–2.8)	0.64	1.0	1.1 (0.63–2.0)	0.82 (0.45–1.5)	0.52
Total carotenoids								
Multivariable adjusted RR[a] (95% CI)	1.0	1.0 (0.42–2.4)	1.8 (0.78–4.0)	0.17	1.0	2.2 (1.2–4.0)	1.3 (0.69–2.4)	0.74
Retinol								
Multivariable adjusted RR[a] (95% CI)	1.0	1.5 (0.67–2.3)	1.1 (0.47–2.5)	0.86	1.0	0.83 (0.47–1.5)	0.69 (0.39–1.2)	0.21
Vitamin C								
Multivariable adjusted RR[a] (95% CI)	1.0	0.85 (0.37–1.9)	1.3 (0.58–2.9)	0.54	1.0	0.84 (0.44–1.6)	0.89 (0.48–1.7)	0.76
Vitamin E								
Multivariable adjusted RR[a] (95% CI)	1.0	0.70 (0.28–1.7)	1.8 (0.81–4.0)	0.11	1.0	2.1 (1.1–3.9)	1.6 (0.85–3.1)	0.28
Selenium								
Multivariable adjusted RR[a] (95% CI)	1.0	0.98 (0.46–2.1)	0.49 (0.20–1.2)	0.11	1.0	1.2 (0.64–2.1)	1.1 (0.59–1.9)	0.84

a Multivariable negative binomial regression controlling for age, sex, energy intake (kJ/day), skin colour, elastosis of the neck, smoking, treatment allocation, use of dietary supplements (yes/no).

mutagenic effect of β-carotene is evidenced mainly at doses which exceed the dietary intake, when it results in a disruption of the natural balance of the body's antioxidant systems.[39] A large prospective cohort of women did not find an effect of dietary β-carotene on BCC incidence[40] and three clinical trials failed to show any harmful or beneficial effects after supplementation with 30 mg or 50 mg β-carotene for 5 to 12 years.[12,41,42] In our study, intake in the highest tertile of β-carotene was not associated with BCC risk and the association with intake in the second tertile disappeared after intake from supplements was added, thus evidence for a skin cancer promoting effect of β-carotene is lacking from this and other studies.

High intakes of vitamins C and E from foods plus supplements were associated with an estimated tripling of risk of BCC tumours in persons without a skin cancer history. In addition, medium level intake of dietary vitamin E was associated with an estimated doubling of BCC risk in persons who had a history of skin cancer at baseline. These findings disagree with the expected protective role for these nutrients, but are similar to the findings of the Nurses' Health Study, in which intake of vitamins C and E from diet as well as from supplements was positively associated with BCC risk after 12 years of follow-up.[43] Some evidence for a pro-oxidant effect of these nutrients exists from experimental studies.[44] Human studies show that high intake levels of vitamin E may reduce serum levels of other fat-soluble antioxidants and thus disrupts the natural balance of antioxidant systems.[45] However, small experimental studies in humans that included 6-months supplementation with 400 IU of vitamin E[46] or 8-weeks supplementation with 500 mg vitamin C[47] resulted in no meaningful photoprotection nor harm, thus evidence for a skin cancer-promoting effect of these nutrients is lacking.

Supplement use in our study population was higher in persons who had a history of skin cancer at baseline than in those without a prior history. It is possible that the persons without a prior history who did take vitamin E or vitamin C

supplements may have done so because they perceived themselves at higher risk of skin cancer. However, possible protective effects of nutrients on skin cancer risk were not widely known at the start of our study and our analyses included full adjustment for indicators of skin cancer risk, thus we believe that residual confounding is an unlikely explanation of this finding.

In a previous nested case-control study of BCC in this population, we found a weak overall positive association between intake of lutein and zeaxanthin and BCC risk,[48] which we did not find in this prospective study. The previous study included many fewer participants and shorter follow-up. Also, stratification by prior history of skin cancer was not carried out in the previous study, making it difficult to directly compare its results to the current results.

Major strengths of this study are its prospective nature and analysis of histologically-confirmed SCC and BCC only, as ascertained through an extensive surveillance system. We consider any bias due to misclassification of participants due to misdiagnosis or missed diagnosis of skin cancer very unlikely. Caution is warranted however because analyses were based on a single estimate of anti-oxidants from diet and supplements, thus we cannot exclude the effects of dietary or lifestyle changes from these results. Furthermore, misclassification of intake levels may have occurred. For example, we have shown that the validity of lutein intake estimates was relatively low (validity coefficient 0.19) compared to other nutrients.[49] Such misclassification, even if non-differential, can cause bias towards or away from the null.[50,51] Chance findings due to our investigation of multiple nutrients could also have occurred.

We conclude that high dietary intake of the carotenoids lutein and zeaxanthin is associated with a reduced incidence of SCC in persons at high risk of skin cancer. These results suggest that our previously reported finding of a reduced SCC risk associated with high intake of green leafy vegetables[28] may be due to these carotenoids. Medium level dietary intakes of vitamin E and β-carotene, and high intakes of vitamin E and vitamin C from foods plus supplements were associated with increased risk of BCC in persons with and without a prior history of this skin cancer, respectively. These data suggest that the associations between antioxidant intake and skin cancer risk are different for SCC and BCC, consistent with other evidence of divergent causal pathways for these common skin cancers. Our findings require replication in different populations before causality can be assumed.

Conflict of interest statement

None declared.

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REFERENCES

1. Christenson LJ, Borrowman TA, Vachon CM, et al. Incidence of basal cell and squamous cell carcinomas in a population younger than 40 years. *Jama* 2005;**294**:681–90.
2. de Vries E, Louwman M, Bastiaens M, de Gruijl F, Coebergh JW. Rapid and continuous increases in incidence rates of basal cell carcinoma in the southeast Netherlands since 1973. *J Invest Dermatol* 2004;**123**:634–8.
3. Staples MP, Elwood M, Burton RC, Williams JL, Marks R, Giles GG. Non-melanoma skin cancer in Australia: the 2002 national survey and trends since 1985. *Med J Aust* 2006;**184**:6–10.
4. Joseph AK, Mark TL, Mueller C. The period prevalence and costs of treating nonmelanoma skin cancers in patients over 65 years of age covered by medicare. *Dermatol Surg* 2001;**27**:955–9.
5. Ullrich SE. Modulation of immunity by ultraviolet radiation: key effects on antigen presentation. *J Invest Dermatol* 1995;**105**:30S–6S.
6. Camplejohn RS. DNA damage and repair in melanoma and non-melanoma skin cancer. *Cancer Surv* 1996;**26**: 193–206.
7. Takeshita K, Takajo T, Hirata H, Ono M, Utsumi H. In vivo oxygen radical generation in the skin of the protoporphyria model mouse with visible light exposure: an L-band ESR study. *J Invest Dermatol* 2004;**122**:1463–70.
8. Pence BC, Delver E, Dunn DM. Effects of dietary selenium on UVB-induced skin carcinogenesis and epidermal antioxidant status. *J Invest Dermatol* 1994;**102**:759–61.
9. Steenvoorden DP, van Henegouwen GM. The use of endogenous antioxidants to improve photoprotection. *J Photochem Photobiol B* 1997;**41**:1–10.
10. McNaughton SA, Marks GC, Green AC. Role of dietary factors in the development of basal cell cancer and squamous cell cancer of the skin. *Cancer Epidemiol Biomarkers Prev* 2005;**14**:1596–607.
11. Green A, Battistutta D, Hart V, et al. The Nambour Skin Cancer and Actinic Eye Disease Prevention Trial: design and baseline characteristics of participants. *Control Clin Trials* 1994;**15**:512–22.
12. Green A, Williams G, Neale R, et al. Daily sunscreen application and betacarotene supplementation in prevention of basal-cell and squamous-cell carcinomas of the skin: a randomised controlled trial. *Lancet* 1999;**354**:723–9.
13. Valery PC, Neale R, Williams G, Pandeya N, Siller G, Green A. The effect of skin examination surveys on the incidence of basal cell carcinoma in a Queensland community sample: a 10-year longitudinal study. *J Investig Dermatol Symp Proc* 2004;**9**:148–51.
14. Green A, Beardmore G, Hart V, Leslie D, Marks R, Staines D. Skin cancer in a Queensland population. *J Am Acad Dermatol* 1988;**19**:1045–52.
15. Green A, Battistutta D. Incidence and determinants of skin cancer in a high-risk Australian population. *Int J Cancer* 1990;**46**:356–61.

16. Green A, Battistutta D, Hart V, Leslie D, Weedon D. Skin cancer in a subtropical Australian population: incidence and lack of association with occupation. The Nambour Study Group. *Am J Epidemiol* 1996;**144**:1034–40.
17. Marks GC, Hughes MC, van der Pols JC. Relative validity of food intake estimates using a food frequency questionnaire is associated with sex, age, and other personal characteristics. *J Nutr* 2006;**136**:459–65.
18. Marks GC, Hughes MC, van der Pols JC. The effect of personal characteristics on the validity of nutrient intake estimates using a food-frequency questionnaire. *Public Health Nutr* 2006;**9**:394–402.
19. Ashton BA, Ambrosini GL, Marks GC, Harvey PW, Bain C. Development of a dietary supplement database. *Aust N Z J Public Health* 1997;**21**:699–702.
20. United States Department of Agriculture. USDA-NCC Carotenoid Database for US Foods, 1998.
21. United States Department of Agriculture. USDA Nutrient Database for Standard Reference, Release 13. Nutrient Data Laboratory Homepage, 1999.
22. National Food Authority. NUTTAB95. Nutrient Data Table for use in Australia. Canberra: Australian Government Publishing Service, 1995.
23. McNaughton SA, Marks GC. Selenium content of Australian foods: A review of literature values. *J Food Comp Anal* 2002;**15**:169–82.
24. Willett WC. *Nutritional epidemiology*. Oxford University Press; 1998.
25. Gardner W, Mulvey EP, Shaw EC. Regression analyses of counts and rates: Poisson, overdispersed Poisson, and negative binomial models. *Psychol Bull* 1995;**118**:392–404.
26. Zou G. A modified poisson regression approach to prospective studies with binary data. *Am J Epidemiol* 2004;**159**:702–6.
27. Marcil I, Stern RS. Risk of developing a subsequent nonmelanoma skin cancer in patients with a history of nonmelanoma skin cancer: a critical review of the literature and meta-analysis. *Arch Dermatol* 2000;**136**:1524–30.
28. Hughes MC, van der Pols JC, Marks GC, Green AC. Food intake and risk of squamous cell carcinoma of the skin in a community: The Nambour skin cancer cohort study. *Int J Cancer* 2006;**119**:1953–60.
29. Ibiebele TI, van der Pols JC, Hughes MC, Marks GC, Williams AC, Green AC. Dietary pattern in association with squamous cell carcinoma of the skin: a prospective study. *Am J Clin Nutr* 2007;**85**:1401–8.
30. Gonzalez S, Astner S, An W, Goukassian D, Pathak MA. Dietary lutein/zeaxanthin decreases ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice. *J Invest Dermatol* 2003;**121**:399–405.
31. Lee EH, Faulhaber D, Hanson KM, et al. Dietary lutein reduces ultraviolet radiation-induced inflammation and immunosuppression. *J Invest Dermatol* 2004;**122**:510–7.
32. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys* 1999;**371**:301–7.
33. Green A, van der Pols JC, Hunter D. Skin cancer. In: Adami HO, Hunter D, Trichopoulos D, editors. *Textbook of cancer epidemiology*. 2nd ed. New York: Oxford University Press; In Press.
34. Van der Pols JC, Williams GM, Pandeya N, Logan V, Green AC. Prolonged prevention of squamous cell carcinoma of the skin by regular sunscreen use. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:2546–8.
35. Kricker A, Armstrong BK, English DR, Heenan PJ. Does intermittent sun exposure cause basal cell carcinoma? a case-control study in Western Australia. *Int J Cancer* 1995;**60**:489–94.
36. Pelucchi C, Di Landro A, Naldi L, La Vecchia C. Risk factors for histological types and anatomic sites of cutaneous basal-cell carcinoma: an italian case-control study. *J Invest Dermatol* 2007;**127**:935–44.
37. Neale RE, Davis M, Pandeya N, Whiteman DC, Green AC. Basal cell carcinoma on the trunk is associated with excessive sun exposure. *J Am Acad Dermatol* 2007;**56**:380–6.
38. Tilli CM, Van Steensel MA, Krekels GA, Neumann HA, Ramaekers FC. Molecular aetiology and pathogenesis of basal cell carcinoma. *Br J Dermatol* 2005;**152**:1108–24.
39. Black HS. Pro-oxidant and anti-oxidant mechanism(s) of BHT and beta-carotene in photocarcinogenesis. *Front Biosci* 2002;**7**:d1044–55.
40. Hunter DJ, Colditz GA, Stampfer MJ, Rosner B, Willett WC, Speizer FE. Diet and risk of basal cell carcinoma of the skin in a prospective cohort of women. *Ann Epidemiol* 1992;**2**:231–9.
41. Greenberg ER, Baron JA, Stukel TA, et al. A clinical trial of beta carotene to prevent basal-cell and squamous-cell cancers of the skin. The Skin Cancer Prevention Study Group. *N Engl J Med* 1990;**323**:789–95.
42. Frieling UM, Schaumberg DA, Kupper TS, Muntwyler J, Hennekens CH. A randomized, 12-year primary-prevention trial of beta carotene supplementation for nonmelanoma skin cancer in the physician's health study. *Arch Dermatol* 2000;**136**:179–84.
43. Fung TT, Hunter DJ, Spiegelman D, Colditz GA, Speizer FE, Willett WC. Vitamins and carotenoids intake and the risk of basal cell carcinoma of the skin in women (United States). *Cancer Causes Control* 2002;**13**:221–30.
44. Abudu N, Miller JJ, Attaelmannan M, Levinson SS. Vitamins in human arteriosclerosis with emphasis on vitamin C and vitamin E. *Clin Chim Acta* 2004;**339**:11–25.
45. Huang HY, Appel LJ. Supplementation of diets with alpha-tocopherol reduces serum concentrations of gamma- and delta-tocopherol in humans. *J Nutr* 2003;**133**:3137–40.
46. Werninghaus K, Meydani M, Bhawan J, Margolis R, Blumberg BA, Gilchrest BA. Evaluation of the photoprotective effect of oral vitamin E supplementation. *Arch Dermatol* 1994;**130**:1257–61.
47. McArdle F, Rhodes LE, Parslew R, Jack CI, Friedmann PS, Jackson MJ. UVR-induced oxidative stress in human skin in vivo: effects of oral vitamin C supplementation. *Free Radic Biol Med* 2002;**33**:1355–62.
48. McNaughton SA, Marks GC, Gaffney P, Williams G, Green AC. Antioxidants and basal cell carcinoma of the skin: a nested case-control study. *Cancer Causes Control* 2005;**16**:609–18.
49. McNaughton SA, Marks GC, Gaffney P, Williams G, Green A. Validation of a food-frequency questionnaire assessment of carotenoid and vitamin E intake using weighed food records and plasma biomarkers: the method of triads model. *Eur J Clin Nutr* 2005;**59**:211–8.
50. Greenland S. The effect of misclssification in the presence of covariates. *Am J Epidemiol* 1980;**112**:564–9.
51. Greenland S, Gustafson P. Accounting for independent nondifferential misclassification does not increase certainty that an observed association is in the correct direction. *Am J Epidemiol* 2006;**164**:63–8.