Published in final edited form as:

Cancer Epidemiol. 2018 August ; 55: 176–183. doi:10.1016/j.canep.2018.06.006.

Intake of folate and other nutrients related to one-carbon metabolism and risk of cutaneous melanoma among US women and men

Ashar Dhana^{1,2}, Hsi Yen^{1,3}, Tricia Li⁴, Michelle D. Holmes^{1,4}, Abrar A. Qureshi^{4,5,6}, and Eunyoung Cho^{4,5,6}

¹Harvard T.H. Chan School of Public Health, Boston, MA, USA

²Division of Dermatology, Groote Schuur Hospital and University of Cape Town, Cape Town, South Africa

³Department of Dermatology, Chang Gung Memorial Hospital, Taoyuan, Taiwan

⁴Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

⁵Department of Dermatology, The Warren Alpert Medical School, Brown University, Providence, RI, USA

⁶Department of Epidemiology, Brown University School of Public Health, Providence, RI, USA

Abstract

Background: Nutrients involved in one-carbon metabolism - folate, vitamins B6 and B12, methionine, choline, and betaine - have been inversely associated with multiple cancer sites and may be related to skin cancer. However, there is a lack of research on the association between intake of these nutrients and cutaneous melanoma risk. The aim of this study was to examine the associations between intake of one-carbon metabolism nutrients and cutaneous melanoma risk in two large prospective cohorts.

Corresponding author: Eunyoung Cho, Box G-D, Department of Dermatology, Warren Alpert Medical School, Brown University, Providence, RI 02912, Tel: 401-863-5895, Fax: 401-863-5799, eunyoung_cho@brown.edu.

AUTHORS' CONTRIBUTIONS

Conception and design: A. Dhana, H. Yen, T. Li, M.D. Holmes, E. Cho

Development of methodology: A. Dhana, H. Yen, T. Li, M.D. Holmes, E. Cho

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E. Cho

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Dhana, H. Yen, T. Li, M.D. Holmes, E. Cho

Writing, review, and/or revision of the manuscript: A. Dhana, H. Yen, T. Li, M.D. Holmes, A. Qureshi, E. Cho

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A. Qureshi, E. Cho
Study supervision: E. Cho.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conflict of interest statement: The authors declare no personal or financial conflict of interest.

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Methods: The cohorts included 75,311 white women and 48,523 white men. Nutrient intake was assessed repeatedly by food frequency questionnaires and self-reported supplement use. We used Cox proportional hazards regression to estimate multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs) and then pooled HRs using a random-effects model.

Results: Over 24 to 26 years of follow-up, we documented 1,328 melanoma cases (648 men and 680 women). Higher intake of folate from food only, but not total folate, was associated with increased melanoma risk (pooled HR for top versus bottom quintile: 1.36; 95 % CI: 1.13–1.64; P for trend = 0.001). The association was significant in men, but attenuated in women. Higher intake of vitamins B6 and B12, choline, betaine, and methionine were not associated with melanoma risk, although there was modest increasing trend of risk for vitamin B6 from food only (pooled HR for top versus bottom quintile: 1.18; 95% CI: 0.99–1.41; P for trend = 0.03).

Conclusions: We found some evidence that higher intake of folate from food only was associated with a modest increased risk of cutaneous melanoma. However, since other factors related to dietary folate intake may account for the observed association, our findings warrant further investigation.

Keywords

melanoma; skin neoplasms; prospective study; one-carbon metabolism; folate; vitamin B6; vitamin B12; methionine; choline; betaine

INTRODUCTION

Melanoma is the most lethal form of skin cancer [1]. In 2017, there will be an estimated 87,110 new cases and 9,730 deaths due to melanoma in the United States alone [2]. Unlike those of other cancers, the mortality rate of melanoma has not decreased [3]. The incidence rate has also tripled in recent years, increasing from 6 per 100,000 people in the 1970s to 20 per 100,000 people in the 2000s [4].

One-carbon metabolism - a network of pathways that transfer methyl groups from one compound to another - is a key process in cancer biology [5]. Disruption of these pathways may result in carcinogenesis by adversely influencing DNA synthesis, repair, and methylation [6].

Folate, a water-soluble B vitamin naturally present in foods, is a methyl donor in one-carbon metabolism, where it participates in DNA synthesis and methylation. Folate may play a dual role in carcinogenesis [7]. Low intake may result in alteration of DNA repair mechanisms, DNA hypo-methylation, and chromosomal breaks [8]. Epidemiologic evidence has shown that lower intake of folate is associated with increased risk of cancer of the breast, colon, and pancreas [9–11]. Conversely, higher intake may contribute to increased tumor growth [12, 13]. In addition, experimental studies have shown that folic acid - a synthetic form of folate that is found in fortified foods and supplements and that constitutes a large portion of total folate intake - results in nuclear DNA damage in the presence of ultraviolet A radiation in keratinocyte and melanoma cell lines of the skin [14–16]. To our knowledge, however, no previous epidemiological studies have examined the association between folate intake and melanoma risk. A recent prospective cohort study in France found that higher intake of

epidermis, were censored at the time of diagnosis, since the risk of developing into an invasive form is unknown. As a result, we included a total 123,834 participants (75,311 women and 48,523 men) in the present analysis (Supplemental Figure F1).

2.2 Assessment of One-carbon Metabolism Nutrient Intake

Participants in the NHS responded to an ~130 item semi-quantitative FFQ in 1984, 1986, and then every four years thereafter, while those in the HPFS responded to a similar FFQ in 1986 and then every four years thereafter. The FFQ collected information on how often each type of food was consumed on average during the past year. It also specified a common serving size for each item. Participants could select from one of nine intake frequency choices, ranging from less than once per month to six or more times per day.

Participants also provided information on current use and dose of multivitamins and use of other vitamin supplements. We used data from the U.S Department of Agriculture to calculate nutrient intake [37, 38]. The nutrient database is specific to a given year, since fortification and composition of food changes. Total intake included both dietary and supplemental intake. Dietary intake included intake from food only (both natural and fortified). Folic acid included the synthetic form of folate found in fortified foods and supplements, while natural folate included folate occurring naturally in food. We used the regression-residual method to adjust nutrient intakes for total energy intake [39].

Intakes one-carbon metabolism nutrients measured by FFQ have been validated in several separate studies between 1985 and 2006. The Pearson correlation coefficients between the FFQ and multiple diet records for the HPFS were 0.77 for total folate, 0.85 for vitamin B6, and 0.56 for vitamin B12 [40]. The Pearson correlation coefficients for the NHS were 0.77 for folate and 0.58 for total vitamin B6 [41, 42]. The correlation coefficient between red blood cell folate and total folate was 0.55 in the NHS and 0.56 in the HPFS. [43]. The validity of choline and betaine intakes using our FFQ was previously assessed in the Framingham Offspring Study, which showed that choline and betaine intake predicted plasma homocysteine levels [44]; for the lowest and highest quintiles of intake, the multivariate geometric means for choline intake were 10.6 and 9.8 $\mu\text{mol/L}$ (P for trend < 0.0001) and for betaine intake were 10.4 and 10.1 $\mu\text{mol/L}$ (P for trend = 0.05).

2.3 Assessment of Other Covariates

We repeatedly collected and updated information on several anthropometric and lifestyle factors: weight, smoking status, alcohol use, coffee intake, citrus consumption, and physical activity level. We calculated body mass index (BMI) using self-reported height and weight. Participants were asked several questions about various types of physical activity, and quintiles of metabolic equivalent task-hours per week were calculated. This type of assessment of physical activity level has been validated against past-week activity recalls in a similar cohort ($r=0.79$).[45] We also collected information on several major melanoma risk factors: family history of melanoma, number of arm moles, natural hair color, sunburn susceptibility as a child or adolescent, number of lifetime blistering sunburns, type of tan after repeated sun exposure as a child or adolescent, and cumulative ultraviolet flux since baseline. Ultraviolet flux is considered an estimate of ultraviolet B radiation and part of

ultraviolet A radiation that reaches the earth's surface and accounts for several factors such as cloud cover, altitude, and latitude [46]. A network of ultraviolet radiation meters exists across the United States and ultraviolet flux in the present study was estimated based on methods described in detail previously [46–48]. Cumulative ultraviolet flux for each subject was based on residential history and estimated by summing annual ultraviolet flux data over follow up.

2.4 Assessment of Melanoma Cases

Study subjects reported new diagnoses of melanoma biennially. After obtaining permission from participants, we acquired their medical and pathological records. Study physicians blinded to questionnaire information reviewed these records to confirm diagnoses using International Classification of Diseases, 9th revision codes. The confirmation rate among subsets of the reported melanoma cases within the NHS was 93% [49]. In addition, given that the populations consist of health professionals who regularly undergo physical examinations, a large proportion of melanoma cases would likely be captured by self-report. We also further classified melanomas into two groups according to their location: melanomas on higher sun exposure sites (head, neck, and extremities) and those on lower sun exposure sites (trunk) [50].

2.5 Statistical Analysis

We used cumulative average nutrient intakes to best estimate long-term intake and minimize measurement error [51]. We calculated nutrient intakes as the mean of all reported intakes up until the beginning of every 2-year follow-up interval. For a questionnaire cycle when FFQ was not administered, the intake from previous cycle was carried over. Participants contributed person-time from the date of return of the baseline questionnaire to the date of first report of cancer (excluding non-melanoma skin cancer), death, or end of follow-up (June 1, 2010 for NHS and January 1, 2010 for HPFS), whichever came first.

We separated participants into quintiles of nutrient intake and selected the lowest quintile as reference. We used a Cox proportional hazard model to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) of melanoma associated with total and dietary one-carbon metabolism nutrient intakes. To control as finely as possible for confounding by age, calendar time, and any possible two-way interactions between these two time scales, we stratified the analysis jointly by age in months at start of follow-up and calendar year of the current questionnaire cycle. We tested the proportional-hazards assumption separately in the NHS and HPFS by modelling the interaction of total folate intake with follow-up time, and we observed no significant violation of the assumption ($P > 0.05$ for both tests). In multivariable analyses, we adjusted for several known and emerging melanoma risk factors and/or potential confounders: age; family history of melanoma (yes v no); natural hair color (red, blonde, light brown, dark brown, or black); number of arm moles (0, 1–2, 3–5, or 6); sunburn susceptibility as a child or adolescent (none/some redness, burn, or painful burn/blisters); number of lifetime blistering sunburns (0, 1–2, 3–5, or 6); cumulative ultraviolet flux since baseline (quintiles), type of tan after repeated sun exposure as a child or adolescent (none, light, average, or deep), BMI (<18.5, 18.5 to 24.9, 25.0 to 29.9, 30.0 to 34.9, or 35.0 kg/m²), physical activity level (quintiles; metabolic-equivalents hours/week),

smoking status (never; past <10, 10–19, 20–39, or ≥40 pack years; or current <25, 25–44, or ≥45 pack years); alcohol intake (0, 0.1 to 4.9, 5.0 to 9.9, 10.0 to 19.9, or ≥20.0 g per day), coffee intake (quintiles; cups/day), and citrus consumption (quintiles; servings/day). For the HPFS, data were unavailable for type of tan after repeated sun exposure as a child or adolescent, and categories of smoking status differed slightly (never; past <10, 10–19, 20–39, or ≥40 pack years; or current). We used the Anderson–Gill data structure to handle time-varying covariates efficiently [52]. We conducted trend tests using the median values for each category of intake as a continuous variable. We then pooled hazard ratios for the NHS and HPFS groups using a random effects model and tested for heterogeneity between studies using the *Q* statistics.

We performed several additional analyses. First, folate intake was additionally analyzed as folic acid (from supplements and fortified foods) and natural folate (folate from food only excluding folic acid from fortified foods). We also conducted separate analyses for pre-fortification (before 1998) and post-fortification (after 1998) periods and examined the association between the ten major food sources of dietary folate and melanoma risk. Second, we performed stratified analyses according to citrus consumption, alcohol intake, number of arm moles, and annual ultraviolet flux at residence. Finally, we performed analyses according to location of tumor, comparing sites with higher sun exposure to sites with lower sun exposure.

All statistical tests were two-sided and set at a significance level of 0.05. We used SAS software version 9.4 (SAS Institute Inc., North Carolina) to conduct all statistical analyses.

3. RESULTS

During 26 years of follow up for 75,311 women (1,719,764 person-years) in the NHS and 24 years of follow up for 48,523 men (958,400 person-years) in the HPFS, we documented 1,328 cases of incident invasive cutaneous melanoma (648 men and 680 women).

Table 1 shows the baseline characteristics according to total folate intake. In both NHS and HPFS, participants with higher total folate intake were more likely to be older, and have a higher physical activity level and total citrus intake. They were also generally more likely to have higher intakes of vitamins B6 and B12, choline, and betaine. They were less likely to smoke, drink coffee, and consume alcohol. In contrast, there was no appreciable difference in sun exposure–related variables and other host risk factors over the intake categories.

Higher intake of folate from food only, but not total folate, compared with lowest intake was associated with an increased risk of melanoma (Table 2). The pooled multivariable HRs for the top versus bottom quintiles were 1.16 (95 % CI: 0.96–1.40; *P* for trend = 0.21) for total folate and 1.36 (95 % CI: 1.13–1.64; *P* for trend = 0.001) for folate from food only. The latter association was significant in men (HR for the top versus bottom quintile: 1.43; 95% CI: 1.09–1.87; *P* for trend = 0.02), but less so in women (HR for the top versus bottom quintile: 1.30; 95% CI: 1.00–1.69; *P* for trend = 0.03) (Supplementary Table S1). Higher intake of vitamins B6 and B12, choline, betaine, and methionine were not associated with risk of melanoma, although the trend test for vitamin B6 from food only was significant (HR

for the top versus bottom quintile: 1.18; 95% CI: 0.99–1.41; P for trend = 0.03) (Table 2). No between-study heterogeneity was evident; the results were similar for both cohorts of men and women (Supplementary Table S1) and when history of non-melanoma skin cancer was adjusted for in the multivariate analysis (data not shown).

Citrus fruit is one of the major sources of dietary folate. Higher citrus intake was found to be associated with increased risk of melanoma in NHS and HPFS [53]. Thus, we explored the associations between intakes of these nutrients and risk of melanoma additionally adjusting for citrus fruit intake to assess the effect of these nutrients independent of citrus fruits (Table 2). Higher intake of folate from food only was still significantly associated with an increased risk of melanoma (HR for the top versus bottom quintile: 1.29; 95% CI: 1.05–1.58; P for trend = 0.02) even after adjusting for citrus intake. However, the p value for the trend test of vitamin B6 from food only after adjustment for citrus intake was borderline at 0.07. Other results remained essentially similar, and none of the other covariates substantially impacted risk estimates.

In additional analyses, higher intake of natural folate and folic acid were not related to risk of melanoma (Supplementary Table S2). We assessed whether folate fortification of grain products had any impact on our findings. Total folate and folate from food only were not associated with melanoma risk in both pre- and post-fortification periods. The results for the pre- and post-fortification periods were similar. For the ten major food sources of dietary folate, higher intake of orange juice and romaine lettuce was associated with increased risk of melanoma, while other food items also had a positive direction of association (Supplementary Table S3). The pooled multivariable HRs for the top versus bottom quintiles were 1.26 (95 % CI: 1.06–1.49; P for trend = 0.003) for orange juice and 1.31 (95 % CI: 1.10–1.56; P for trend = 0.001) for romaine lettuce.

In stratified analyses, number of arm moles (0, or 1), citrus consumption (<0.78, or 0.78 servings/day), alcohol intake (<10, or 10 g/day), and annual ultraviolet flux at residence (<113, or 113×10^{-4} Robertson-Berger units) did not significantly modify the association between intake of one-carbon metabolism nutrients and melanoma risk (P for interaction > 0.05) (Supplementary Table S4).

We also evaluated location of melanoma, comparing sites with higher sun exposure to sites with lower sun exposure. However, there was no evidence that the association between intake of one-carbon metabolism nutrients and risk of melanoma differed by body location of tumours.

DISCUSSION

We found that higher intake of folate from food only, but not total folate, was associated with an increased risk of cutaneous melanoma in these two large prospective cohorts of women and men. Study subjects in the highest category of intake had a 36% increased risk of melanoma than did those in the lowest category of intake. In contrast, intakes of vitamins B6 and B12, choline, betaine, and methionine were not associated with risk of melanoma,

although there was a suggestion that higher intake of vitamin B6 from food only was associated with an increased risk of melanoma.

To our knowledge, no previous epidemiologic study has specifically examined dietary intake of folate and other one-carbon metabolism nutrients in relation to melanoma risk. The positive association of folate from food only but not total folate is unexpected and difficult to interpret. Our findings are in line with that of a recent prospective study in France by *Donnenfeld et al (2015)*, which found a positive association between dietary folate intake and overall skin cancer risk (HR for the top versus bottom tertile was 1.79; 95% CI: 1.07–2.99; P for trend = 0.03) [17]. However, basal cell carcinoma (BCC) accounted for 106 cases out of the 144 skin cancer cases in the study, and only 20 cases of melanoma were included. Thus, BCC cases may have driven the observed association. Indeed, positive associations between total and dietary folate intake and BCC risk were found in the NHS and HPFS [54, 55]. In addition, the study did not evaluate whether the association was independent of citrus consumption. Citrus fruits - along with green leafy vegetables and legumes - are a major food source of dietary folate [56], and have been positively correlated with plasma folate concentrations [57–59]. They are also rich in furocoumarins [60–62], compounds known to play a role in skin carcinogenesis from previous experimental studies [63, 64]. In fact, higher citrus consumption was recently shown to be associated with an increased risk of melanoma, BCC, and squamous cell carcinoma of the skin in NHS and HPFS [53, 65]. When we adjusted for citrus consumption in our multivariable analysis, the positive association with folate from food only was slightly attenuated, but still present. Since total folate intake was not associated with melanoma risk, our finding with folate from food only is unlikely due to folate *per se*, but may be due to other components that occur in folate rich foods. Moreover, given that we evaluated multiple different nutrients, the positive association may simply be due to chance.

Folate has long been thought to reduce the risk of cancer – by influencing DNA synthesis, repair, and methylation [8, 66]. Very high intakes of folate, however, may also increase the risk of cancer. For instance, folate plays a crucial role in nucleotide synthesis, which is needed to support DNA synthesis and cell growth among rapidly proliferating cells such as those seen in pre-neoplastic or malignant lesions [7, 67]. In fact, several mouse models have shown that once pre-neoplastic lesions are present, higher intakes of folate promote progression of these lesions [68, 69]. However, it should be noted that folate supplementation in these studies was very high (four to ten times the basal dietary requirements of mice) and that we found a positive association not with total folate (which included supplemental folate with higher bioavailability) but with dietary folate, which comprises a lower folate intake level. The recommended dietary intake (RDI) of folate is 400ug/d and the upper limit of folate intake is 1000ug/d. In the cohorts, median total folate levels in the 4th and 5th quintiles were over RDI but below the upper limit. In terms of dietary folate, the top quintile of intake was close to RDI. Furthermore, in the presence of ultraviolet radiation folic acid degrades to 6-formylpterin and pterin-6-carboxylic acid – compounds that act as photosensitizers and generate reactive oxygen species [15, 70, 71]. Experimental studies using melanocytes and keratinocytes from the human skin have found that these metabolites of folic acid induce DNA damage and may possibly lead to skin photocarcinogenesis [14, 72, 73]. However, we did not find any association with total folate,

which included supplemental folic acid. Furthermore, a recent meta-analysis of 13 randomised trials by *Vollset et al (2013)* found no association between folic acid supplementation and overall and site-specific cancer, including melanoma [74]. The study, however, was limited by few melanoma cases (n=126) and some trials with short follow-up periods (average treatment duration was 5.2 years).

This study had several strengths. First, we collected and adjusted for a number of potential confounders. Second, our study benefitted from a large sample size and long duration of follow up. Third, the prospective nature of this study likely minimized potential biases such as recall or selection bias. Our study also had a few limitations. First, the generalizability of our findings may be limited – both cohorts comprised white educated U.S. health professionals. This lack of diversity, however, may help to reduce possible residual confounding by socioeconomic status, ethnicity, and healthcare access. Second, dietary data was self-reported and may have been subject to non-differential misclassification. To minimize this error, we used cumulatively averaged intake from multiple time points. Nevertheless, nutritional biomarkers are not affected by bias associated with self-reporting, and serum and red blood cell folate biomarkers, for example, have been shown to be robust indicators of folate status [75]. Thus, future studies could consider investigating the association between these nutrients and melanoma risk using nutritional biomarkers. Third, although we adjusted for several known melanoma risk factors such as history of sunburns and cumulative ultraviolet flux, [76] we did not have information on outdoor sun exposure hours. Finally, a potential limitation is residual confounding.

In conclusion, we found that higher intake of folate from food only, but not total folate, was associated with a modest increased risk of cutaneous melanoma. However, since other factors related to dietary folate intake may account for the observed association, our findings warrant further investigation. Our findings also suggest that intake of other nutrients involved in one-carbon metabolism is not associated with risk of melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We would like to thank the participants and staff of the Nurses' Health Study and Health Professionals Follow-Up Study for their valuable contributions as well as the following several state cancer registries for their assistance: Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Minnesota, Nebraska, New Hampshire, New Jersey, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Virginia, Washington, and Wyoming.

FINANCIAL SUPPORT: Supported by the National Institutes of Health (CA186107, CA167552, and CA198216) and by the Research Career Development Award of Dermatology Foundation.

REFERENCES

- [1]. Narayanan DL , Saladi RN , Fox JL , Ultraviolet radiation and skin cancer, *International journal of dermatology* 49(9) (2010) 978–86. [PubMed: 20883261]

- [2]. Siegel RL , Miller KD , Jemal A , Cancer Statistics, 2017, CA Cancer J Clin 67(1) (2017) 7–30. [PubMed: 28055103]
- [3]. Whiteman DC , Green AC , Olsen CM , The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031, The Journal of investigative dermatology 136(6) (2016) 1161–71. [PubMed: 26902923]
- [4]. Tuong W , Cheng LS , Armstrong AW , Melanoma: epidemiology, diagnosis, treatment, and outcomes, Dermatol Clin 30(1) (2012) 113–24, ix. [PubMed: 22117873]
- [5]. Selhub J , Folate, vitamin B12 and vitamin B6 and one carbon metabolism, J Nutr Health Aging 6(1) (2002) 39–42. [PubMed: 11813080]
- [6]. Friso S , Choi SW , Gene-nutrient interactions in one-carbon metabolism, Curr Drug Metab 6(1) (2005) 37–46. [PubMed: 15720206]
- [7]. Ulrich CM , Folate and cancer prevention: a closer look at a complex picture, The American journal of clinical nutrition 86(2) (2007) 271–3. [PubMed: 17684194]
- [8]. Choi SW , Mason JB , Folate and carcinogenesis: an integrated scheme, The Journal of nutrition 130(2) (2000) 129–32. [PubMed: 10720158]
- [9]. Chen P , Li C , Li X , Li J , Chu R , Wang H , Higher dietary folate intake reduces the breast cancer risk: a systematic review and meta-analysis, British journal of cancer 110(9) (2014) 2327–38. [PubMed: 24667649]
- [10]. Lin HL , An QZ , Wang QZ , Liu CX , Folate intake and pancreatic cancer risk: an overall and dose-response meta-analysis, Public Health 127(7) (2013) 607–13. [PubMed: 23769243]
- [11]. Sanjoaquin MA , Allen N , Couto E , Roddam AW , Key TJ , Folate intake and colorectal cancer risk: a meta-analytical approach, International journal of cancer. Journal international du cancer 113(5) (2005) 825–8. [PubMed: 15499620]
- [12]. Chen RZ , Pettersson U , Beard C , Jackson-Grusby L , Jaenisch R , DNA hypomethylation leads to elevated mutation rates, Nature 395(6697) (1998) 89–93. [PubMed: 9738504]
- [13]. Kim YI , Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies, Environ Mol Mutagen 44(1) (2004) 10–25. [PubMed: 15199543]
- [14]. Butzbach K , Epe B , Photogenotoxicity of folic acid, Free radical biology & medicine 65 (2013) 821–7. [PubMed: 23973753]
- [15]. Juzeniene A , Grigalavicius M , Ma LW , Juraleviciute M , Folic acid and its photoproducts, 6-formylpterin and pterin-6-carboxylic acid, as generators of reactive oxygen species in skin cells during UVA exposure, J Photochem Photobiol B 155 (2016) 116–21. [PubMed: 26780587]
- [16]. Bentley TG , Willett WC , Weinstein MC , Kuntz KM , Population-level changes in folate intake by age, gender, and race/ethnicity after folic acid fortification, Am J Public Health 96(11) (2006) 2040–7. [PubMed: 17018833]
- [17]. Donnenfeld M , Deschasesaux M , Latino-Martel P , Diallo A , Galan P , Hercberg S , Ezzedine K , Touvier M , Prospective association between dietary folate intake and skin cancer risk: results from the Supplementation en Vitamines et Mineraux Antioxydants cohort, The American journal of clinical nutrition 102(2) (2015) 471–8. [PubMed: 26156743]
- [18]. Mason JB , Biomarkers of nutrient exposure and status in one-carbon (methyl) metabolism, The Journal of nutrition 133 Suppl 3 (2003) 941S–947S. [PubMed: 12612180]
- [19]. Bailey LB , Gregory JF , Folate metabolism and requirements, The Journal of nutrition 129(4) (1999) 779–82. [PubMed: 10203550]
- [20]. Craig SA , Betaine in human nutrition, The American journal of clinical nutrition 80(3) (2004) 539–49. [PubMed: 15321791]
- [21]. Fanidi A , Muller DC , Yuan JM , Stevens VL , Weinstein SJ , Albanes D , Prentice R , Thomsen CA , Pettinger M , Cai Q , Blot WJ , Wu J , Arslan AA , Zeleniuch-Jacquotte A , McCullough ML , Le Marchand L , Wilkens LR , Haiman CA , Zhang X , Han J , Stampfer MJ , Smith-Warner SA , Giovannucci E , Giles GG , Hodge AM , Severi G , Johansson M , Grankvist K , Langhammer A , Krokstad S , Naess M , Wang R , Gao YT , Butler LM , Koh WP , Shu XO , Xiang YB , Li H , Zheng W , Lan Q , Visvanathan K , Bolton JH , Ueland PM , Midttun O , Ulvik A , Caporaso NE , Purdue M , Ziegler RG , Freedman ND , Buring JE , Lee IM , Sesso HD , Gaziano JM , Manjer J , Ericson U , Relton C , Brennan P , Johansson M , Circulating

Folate, Vitamin B6, and Methionine in Relation to Lung Cancer Risk in the Lung Cancer Cohort Consortium (LC3), *J Natl Cancer Inst* 110(1) (2018).

- [22]. Agnoli C , Grioni S , Krogh V , Pala V , Allione A , Matullo G , Di Gaetano C , Tagliabue G , Pedraglio S , Garrone G , Cancarini I , Cavalleri A , Sieri S , Plasma Riboflavin and Vitamin B-6, but Not Homocysteine, Folate, or Vitamin B-12, Are Inversely Associated with Breast Cancer Risk in the European Prospective Investigation into Cancer and Nutrition-Varese Cohort, *The Journal of nutrition* 146(6) (2016) 1227–34. [PubMed: 27121532]
- [23]. Huang JY , Butler LM , Wang R , Jin A , Koh WP , Yuan JM , Dietary Intake of One-Carbon Metabolism-Related Nutrients and Pancreatic Cancer Risk: The Singapore Chinese Health Study, *Cancer Epidemiol Biomarkers Prev* 25(2) (2016) 417–24. [PubMed: 26711329]
- [24]. Cancarini I , Krogh V , Agnoli C , Grioni S , Matullo G , Pala V , Pedraglio S , Contiero P , Riva C , Muti P , Sieri S , Micronutrients Involved in One-Carbon Metabolism and Risk of Breast Cancer Subtypes, *PloS one* 10(9) (2015) e0138318. [PubMed: 26376452]
- [25]. Johansson M , Fanidi A , Muller DC , Bassett JK , Midttun O , Vollset SE , Travis RC , Palli D , Mattiello A , Sieri S , Trichopoulou A , Lagiou P , Trichopoulos D , Ljungberg B , Hallmans G , Weiderpass E , Skeie G , Gonzalez CA , Dorronsoro M , Peeters PH , Bueno-de-Mesquita HB , Ros MM , Boutron Ruault MC , Fagherazzi G , Clavel F , Sanchez MJ , Gurra AB , Navarro C , Quiros JR , Overvad K , Tjonneland A , Aleksandrova K , Vineis P , Gunter MJ , Kaaks R , Giles G , Relton C , Riboli E , Boeing H , Ueland PM , Severi G , Brennan P , Circulating biomarkers of one-carbon metabolism in relation to renal cell carcinoma incidence and survival, *J Natl Cancer Inst* 106(12) (2014).
- [26]. Zschabitz S , Cheng TY , Neuhauser ML , Zheng Y , Ray RM , Miller JW , Song X , Maneval DR , Beresford SA , Lane D , Shikany JM , Ulrich CM , B vitamin intakes and incidence of colorectal cancer: results from the Women's Health Initiative Observational Study cohort, *The American journal of clinical nutrition* 97(2) (2013) 332–43. [PubMed: 23255571]
- [27]. Le Marchand L , White KK , Nomura AM , Wilkens LR , Selhub JS , Tiirikainen M , Goodman MT , Murphy SP , Henderson BE , Kolonel LN , Plasma levels of B vitamins and colorectal cancer risk: the multiethnic cohort study, *Cancer Epidemiol Biomarkers Prev* 18(8) (2009) 2195–201. [PubMed: 19661077]
- [28]. Weinstein SJ , Albanes D , Selhub J , Graubard B , Lim U , Taylor PR , Virtamo J , Stolzenberg-Solomon R , One-carbon metabolism biomarkers and risk of colon and rectal cancers, *Cancer Epidemiol Biomarkers Prev* 17(11) (2008) 3233–40. [PubMed: 18990766]
- [29]. Mocellin S , Briarava M , Pilati P , Vitamin B6 and Cancer Risk: A Field Synopsis and Meta-Analysis, *J Natl Cancer Inst* 109(3) (2017) 1–9.
- [30]. Liu Y , Wang X , Sun X , Lu S , Liu S , Vitamin intake and pancreatic cancer risk reduction: A meta-analysis of observational studies, *Medicine (Baltimore)* 97(13) (2018) e0114. [PubMed: 29595633]
- [31]. Sun NH , Huang XZ , Wang SB , Li Y , Wang LY , Wang HC , Zhang CW , Zhang C , Liu HP , Wang ZN , A dose-response meta-analysis reveals an association between vitamin B12 and colorectal cancer risk, *Public Health Nutr* 19(8) (2016) 1446–56. [PubMed: 26373257]
- [32]. Collin SM , Metcalfe C , Refsum H , Lewis SJ , Zuccolo L , Smith GD , Chen L , Harris R , Davis M , Marsden G , Johnston C , Lane JA , Ebbing M , Bona KH , Nygard O , Ueland PM , Grau MV , Baron JA , Donovan JL , Neal DE , Hamdy FC , Smith AD , Martin RM , Circulating folate, vitamin B12, homocysteine, vitamin B12 transport proteins, and risk of prostate cancer: a case-control study, systematic review, and meta-analysis, *Cancer Epidemiol Biomarkers Prev* 19(6) (2010) 1632–42. [PubMed: 20501771]
- [33]. Youn J , Cho E , Lee JE , Association of choline and betaine levels with cancer incidence and survival: A meta-analysis, *Clin Nutr* (2018).
- [34]. Zhou ZY , Wan XY , Cao JW , Dietary methionine intake and risk of incident colorectal cancer: a meta-analysis of 8 prospective studies involving 431,029 participants, *PloS one* 8(12) (2013) e83588. [PubMed: 24340103]
- [35]. Colditz GA , Hankinson SE , The Nurses' Health Study: lifestyle and health among women, *Nat Rev Cancer* 5(5) (2005) 388–96. [PubMed: 15864280]
- [36]. Smith-Warner SA , Spiegelman D , Ritz J , Albanes D , Beeson WL , Bernstein L , Berrino F , van den Brandt PA , Buring JE , Cho E , Colditz GA , Folsom AR , Freudenheim JL ,

Giovannucci E , Goldbohm RA , Graham S , Harnack L , Horn-Ross PL , Krogh V , Leitzmann MF , McCullough ML , Miller AB , Rodriguez C , Rohan TE , Schatzkin A , Shore R , Virtanen M , Willett WC , Wolk A , Zeleniuch-Jacquotte A , Zhang SM , Hunter DJ , Methods for pooling results of epidemiologic studies: the Pooling Project of Prospective Studies of Diet and Cancer, *Am J Epidemiol* 163(11) (2006) 1053–64. [PubMed: 16624970]

- [37]. Agriculture Research Service and U.S. Department of Agriculture, USDA Database for the Choline Content of Common Foods, Release 2, Beltsville, MD, USA, 2008.
- [38]. Agriculture Research Service and U.S. Department of Agriculture, USDA National Nutrient Database for Standard Reference, Release 10, Beltsville, MD, USA, 2001.
- [39]. Willett W , Stampfer MJ , Total energy intake: implications for epidemiologic analyses, *Am J Epidemiol* 124(1) (1986) 17–27. [PubMed: 3521261]
- [40]. Rimm EB , Giovannucci EL , Stampfer MJ , Colditz GA , Litin LB , Willett WC , Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals, *Am J Epidemiol* 135(10) (1992) 1114–26; discussion 1127–36. [PubMed: 1632423]
- [41]. Willett WC , Sampson L , Stampfer MJ , Rosner B , Bain C , Witschi J , Hennekens CH , Speizer FE , Reproducibility and validity of a semiquantitative food frequency questionnaire, *Am J Epidemiol* 122(1) (1985) 51–65. [PubMed: 4014201]
- [42]. Salvini S , Hunter DJ , Sampson L , Stampfer MJ , Colditz GA , Rosner B , Willett WC , Food-based validation of a dietary questionnaire: the effects of week-to-week variation in food consumption, *Int J Epidemiol* 18(4) (1989) 858–67. [PubMed: 2621022]
- [43]. Giovannucci E , Stampfer MJ , Colditz GA , Rimm EB , Trichopoulos D , Rosner BA , Speizer FE , Willett WC , Folate, methionine, and alcohol intake and risk of colorectal adenoma, *J Natl Cancer Inst* 85(11) (1993) 875–84. [PubMed: 8492316]
- [44]. Cho E , Zeisel SH , Jacques P , Selhub J , Dougherty L , Colditz GA , Willett WC , Dietary choline and betaine assessed by food-frequency questionnaire in relation to plasma total homocysteine concentration in the Framingham Offspring Study, *The American journal of clinical nutrition* 83(4) (2006) 905–11. [PubMed: 16600945]
- [45]. Wolf AM , Hunter DJ , Colditz GA , Manson JE , Stampfer MJ , Corsano KA , Rosner B , Kriska A , Willett WC , Reproducibility and validity of a self-administered physical activity questionnaire, *Int J Epidemiol* 23(5) (1994) 991–9. [PubMed: 7860180]
- [46]. F. T Scotto J , Fraumeni JF , Cancer epidemiology and prevention, Oxford University Press, New York, 1996, pp. pp. 355–72.
- [47]. Scotto J , Cotton G , Urbach F , Berger D , Fears T , Biologically effective ultraviolet radiation: surface measurements in the United States, 1974 to 1985, *Science* 239(4841 Pt 1) (1988) 762–4. [PubMed: 3340857]
- [48]. Fears TR , Bird CC , Guerry D.t. , Sagebiel RW , Gail MH , Elder DE , Halpern A , Holly EA , Hartge P , Tucker MA , Average midrange ultraviolet radiation flux and time outdoors predict melanoma risk, *Cancer research* 62(14) (2002) 3992–6. [PubMed: 12124332]
- [49]. Li WQ , Cho E , Weinstock MA , Mashfiq H , Qureshi AA , Epidemiological Assessments of Skin Outcomes in the Nurses' Health Studies, *Am J Public Health* 106(9) (2016) 1677–83. [PubMed: 27459457]
- [50]. Whiteman DC , Stickley M , Watt P , Hughes MC , Davis MB , Green AC , Anatomic site, sun exposure, and risk of cutaneous melanoma, *J Clin Oncol* 24(19) (2006) 3172–7. [PubMed: 16809740]
- [51]. Cho E , Spiegelman D , Hunter DJ , Chen WY , Stampfer MJ , Colditz GA , Willett WC , Premenopausal fat intake and risk of breast cancer, *J Natl Cancer Inst* 95(14) (2003) 1079–85. [PubMed: 12865454]
- [52]. Therneau TM , Proceedings of the First Seattle Symposium in Biostatistics: Survival Analysis, Springer, New York, 1997.
- [53]. Wu S , Han J , Feskanich D , Cho E , Stampfer MJ , Willett WC , Qureshi AA , Citrus Consumption and Risk of Cutaneous Malignant Melanoma, *J Clin Oncol* 33(23) (2015) 2500–8. [PubMed: 26124488]

- [54]. 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 : CCC* 13(3) (2002) 221–30. [PubMed: 12020103]
- [55]. van Dam RM , Huang Z , Giovannucci E , Rimm EB , Hunter DJ , Colditz GA , Stampfer MJ , Willett WC , Diet and basal cell carcinoma of the skin in a prospective cohort of men, *The American journal of clinical nutrition* 71(1) (2000) 135–41. [PubMed: 10617958]
- [56]. de Bree A , van Dusseldorp M , Brouwer IA , van het Hof KH , Steegers-Theunissen RP , Folate intake in Europe: recommended, actual and desired intake, *Eur J Clin Nutr* 51(10) (1997) 643–60. [PubMed: 9347284]
- [57]. Ohrvik VE , Witthoft CM , Human folate bioavailability, *Nutrients* 3(4) (2011) 475–90. [PubMed: 22254106]
- [58]. Brouwer IA , van Dusseldorp M , West CE , Meyboom S , Thomas CM , Duran M , van het Hof KH , Eskes TK , Hautvast JG , Steegers-Theunissen RP , Dietary folate from vegetables and citrus fruit decreases plasma homocysteine concentrations in humans in a dietary controlled trial, *The Journal of nutrition* 129(6) (1999) 1135–9. [PubMed: 10356077]
- [59]. Streiff RR , Folate levels in citrus and other juices, *The American journal of clinical nutrition* 24(12) (1971) 1390–2. [PubMed: 5118010]
- [60]. Dugo P , Piperno A , Romeo R , Cambria M , Russo M , Carnovale C , Mondello L , Determination of oxygen heterocyclic components in citrus products by HPLC with UV detection, *J Agric Food Chem* 57(15) (2009) 6543–51. [PubMed: 19722564]
- [61]. Dugrand A , Olry A , Duval T , Hehn A , Froelicher Y , Bourgaud F , Coumarin and furanocoumarin quantitation in citrus peel via ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS), *J Agric Food Chem* 61(45) (2013) 10677–84. [PubMed: 24117278]
- [62]. Sayre RM , Dowdy JC , The increase in melanoma: are dietary furocoumarins responsible?, *Med Hypotheses* 70(4) (2008) 855–9. [PubMed: 17881138]
- [63]. Ashwood-Smith MJ , Poulton GA , Barker M , Mildenerberger M , 5-Methoxypsoralen, an ingredient in several suntan preparations, has lethal, mutagenic and clastogenic properties, *Nature* 285(5764) (1980) 407–9. [PubMed: 6991953]
- [64]. Cartwright LE , Walter JF , Psoralen-containing sunscreen is tumorigenic in hairless mice, *Journal of the American Academy of Dermatology* 8(6) (1983) 830–6. [PubMed: 6863646]
- [65]. Wu S , Cho E , Feskanich D , Li WQ , Sun Q , Han J , Qureshi AA , Citrus consumption and risk of basal cell carcinoma and squamous cell carcinoma of the skin, *Carcinogenesis* 36(10) (2015) 1162–8. [PubMed: 26224304]
- [66]. Williams JD , Jacobson EL , Kim H , Kim M , Jacobson MK , Folate in skin cancer prevention, *Subcell Biochem* 56 (2012) 181–97. [PubMed: 22116700]
- [67]. Mason JB , Unraveling the complex relationship between folate and cancer risk, *Biofactors* 37(4) (2011) 253–60. [PubMed: 21915934]
- [68]. Song J , Medline A , Mason JB , Gallinger S , Kim YI , Effects of dietary folate on intestinal tumorigenesis in the *apcMin* mouse, *Cancer research* 60(19) (2000) 5434–40. [PubMed: 11034085]
- [69]. Song J , Sohn KJ , Medline A , Ash C , Gallinger S , Kim YI , Chemopreventive effects of dietary folate on intestinal polyps in *Apc+/-Msh2-/-* mice, *Cancer research* 60(12) (2000) 3191–9. [PubMed: 10866310]
- [70]. Dantola ML , Denofrio MP , Zurbano B , Gimenez CS , Ogilby PR , Lorente C , Thomas AH , Mechanism of photooxidation of folic acid sensitized by unconjugated pterins, *Photochem Photobiol Sci* 9(12) (2010) 1604–12. [PubMed: 20922252]
- [71]. Off MK , Steindal AE , Porojnicu AC , Juzeniene A , Vorobey A , Johnsson A , Moan J , Ultraviolet photodegradation of folic acid, *J Photochem Photobiol B* 80(1) (2005) 47–55. [PubMed: 15963436]
- [72]. Hirakawa K , Suzuki H , Oikawa S , Kawanishi S , Sequence-specific DNA damage induced by ultraviolet A-irradiated folic acid via its photolysis product, *Arch Biochem Biophys* 410(2) (2003) 261–8. [PubMed: 12573286]

- [73]. Ito K , Kawanishi S , Photoinduced hydroxylation of deoxyguanosine in DNA by pterins: sequence specificity and mechanism, *Biochemistry* 36(7) (1997) 1774–81. [PubMed: 9048561]
- [74]. Vollset SE , Clarke R , Lewington S , Ebbing M , Halsey J , Lonn E , Armitage J , Manson JE , Hankey GJ , Spence JD , Galan P , Bonna KH , Jamison R , Gaziano JM , Guarino P , Baron JA , Logan RF , Giovannucci EL , den Heijer M , Ueland PM , Bennett D , Collins R , Peto R , Collaboration B.V.T.T., Effects of folic acid supplementation on overall and site-specific cancer incidence during the randomised trials: meta-analyses of data on 50,000 individuals, *Lancet* 381(9871) (2013) 1029–36. [PubMed: 23352552]
- [75]. Duffy ME , Hoey L , Hughes CF , Strain JJ , Rankin A , Souverein OW , Dullemeijer C , Collings R , Hooper L , McNulty H , Biomarker responses to folic acid intervention in healthy adults: a meta-analysis of randomized controlled trials, *The American journal of clinical nutrition* 99(1) (2014) 96–106. [PubMed: 24225357]
- [76]. Oliveria SA , Saraiya M , Geller AC , Heneghan MK , Jorgensen C , Sun exposure and risk of melanoma, *Arch Dis Child* 91(2) (2006) 131–8. [PubMed: 16326797]

Highlights

- Intake of nutrients involved in one-carbon metabolism were prospectively evaluated in relation to melanoma risk.
- Higher intake of folate from food only was associated with an increased risk of melanoma.
- No association was found between intake of other nutrients involved in one-carbon metabolism and melanoma risk.

Table 1.

Baseline characteristics of study population according to quintile of total folate intake in the NHS (1984) and HPFS (1986)^a.

	Quintile of total folate intake				
	1	2	3	4	5
Women in NHS (1984)					
Number of participants	15009	15241	14898	15114	15049
Age (years) ^b	48.8 (7.1)	49.9 (7.1)	50.8 (7.1)	50.9 (7.2)	51.4 (7.1)
Total folate (µg/day)	179.3 (27.7)	243.3 (15.5)	303.1 (20.8)	429.2 (62.3)	765.7 (214.4)
Folate from food only (µg/day)	179.6 (28.2)	239.7 (21.1)	294.2 (31.0)	330.6 (94.5)	323.0 (131.0)
Total vitamin B6 (mg/day)	4.4 (18.3)	5.8 (21.2)	6.6 (21.8)	9.6 (26.2)	22.0 (44.1)
Vitamin B6 from food only (mg/day)	1.4 (0.3)	1.6 (0.3)	1.8 (0.3)	1.9 (0.5)	1.9 (0.6)
Total vitamin B12 (µg/day)	6.9 (4.6)	8.4 (5.8)	9.6 (7.7)	12.4 (15.8)	20.9 (42.5)
Vitamin B12 from food only (µg/day)	6.5 (4.5)	7.8 (5.0)	8.6 (5.4)	8.9 (5.8)	8.9 (6.6)
Total methionine (g/day)	1.6 (0.4)	1.7 (0.3)	1.7 (0.3)	1.7 (0.4)	1.8 (0.4)
Total choline (mg/day)	290.0 (61.9)	320.3 (62.9)	338.0 (67.3)	345.7 (80.1)	360.3 (98.1)
Total betaine (mg/day)	86.6 (35.0)	97.1 (29.9)	108.0 (36.7)	115.7 (48.1)	112.4 (50.3)
Total citrus intake (servings/day)	0.4 (0.4)	0.8 (0.5)	1.0 (0.7)	1.1 (0.9)	1.0 (0.9)
Coffee intake (cups/day)	2.0 (1.9)	1.9 (1.8)	1.7 (1.7)	1.7 (1.7)	1.5 (1.7)
Total energy intake (kcal/day)	1630.3 (528.9)	1761.1 (523.4)	1809.3 (522.7)	1855.3 (543.9)	1660.1 (498.3)
Body mass index (kg/m ²)	25.3 (5.0)	25.3 (4.8)	25.2 (4.8)	24.8 (4.6)	24.7 (4.5)
Physical activity level (metabolic-equivalents hours/week)	10.1 (17.0)	12.72 (18.1)	14.7 (19.2)	16.1 (24.3)	16.9 (24.3)
Alcohol intake (g/day)	7.5 (13.0)	7.3 (11.7)	6.7 (10.5)	6.7 (10.6)	6.5 (10.6)
Current smoking (%)	34.1	25.4	21.2	21.3	20.4
Annual UV flux at residence (×10 ⁻⁴ Robertson-Berger units)	121.2 (24.3)	120.8 (23.8)	121.2 (23.8)	121.7 (24.2)	123.5 (25.3)
Family history of melanoma (%)	2.8	2.6	2.7	2.8	2.8
Red or blonde hair (%)	15.3	15.7	16.0	15.6	16.3
Deep tan after sun exposure as child or adolescent (%)	23.5	23.4	23.2	23.5	23.7
Painful burn or blistering skin reaction as a child or adolescent (%)	34.6	34.3	34.5	33.9	34.9
Lifetime blistering sunburns 6 (%)	7.3	7.4	7.2	7.1	7.7

	Quintile of total folate intake				
	1	2	3	4	5
Number of arm moles 6 (%)	4.5	4.4	5.0	4.7	4.8
Men in HPFS (1986)					
Number of participants	9748	9671	9698	9703	9703
Age (years) ^b	52.9 (9.6)	54.0 (9.8)	54.6 (9.9)	54.5 (10.0)	55.5 (9.8)
Total folate (µg/day)	238.2 (36.0)	318.5 (18.9)	390.5 (24.4)	529.2 (65.6)	935.8 (275.0)
Folate from food only (µg/day)	239.2 (37.3)	315.0 (27.8)	380.3 (40.6)	428.4 (112.6)	424.3 (166.2)
Total vitamin B6 (mg/day)	3.6 (12.2)	4.3 (13.2)	5.6 (17.4)	8.0 (20.2)	22.2 (43.2)
Vitamin B6 from food only (mg/day)	1.8 (0.4)	2.1 (0.4)	2.3 (0.4)	2.5 (0.6)	2.5 (0.8)
Total vitamin B12 (µg/day)	8.6 (5.5)	9.4 (5.9)	10.4 (7.0)	13.2 (14.4)	21.9 (34.4)
Vitamin B12 from food only (µg/day)	8.1 (4.6)	8.8 (5.2)	9.4 (6.0)	9.4 (6.0)	9.2 (6.0)
Total methionine (g/day)	2.1 (0.5)	2.2 (0.4)	2.2 (0.5)	2.2 (0.5)	2.2 (0.5)
Total choline (mg/day)	372.4 (80.6)	392.6 (80.2)	401.7 (82.8)	404.6 (88.1)	430.3 (119.9)
Total betaine (mg/day)	109.0 (36.8)	123.3 (43.4)	136.5 (61.2)	142.8 (68.2)	142.5 (73.0)
Total citrus intake (servings/day)	0.5 (0.5)	0.8 (0.6)	1.1 (0.7)	1.3 (1.1)	1.2 (1.1)
Coffee intake (cups/day)	1.6 (1.7)	1.4 (1.6)	1.2 (1.5)	1.2 (1.5)	1.1 (1.5)
Body mass index (kg/m ²)	25.3 (5.4)	25.1 (5.0)	25.0 (4.9)	24.7 (5.1)	24.6 (4.9)
Physical activity level (metabolic-equivalents hours/week)	16.1 (24.8)	19.1 (24.4)	22.2 (33.0)	23.2 (31.3)	24.3 (32.2)
Alcohol intake (g/day)	14.0 (19.3)	12.5 (16.5)	11.2 (14.9)	11.1 (15.5)	11.0 (14.9)
Current smoking (%)	15.6	10.7	7.4	7.7	8.4
Annual UV flux at residence (×10 ⁻⁴ Robertson-Berger units)	129.6 (27.3)	129.2 (27.1)	129.0 (27.2)	130.1 (27.6)	132.1 (28.3)
Family history of melanoma (%)	2.9	3.3	2.9	3.3	3.0
Red or blonde hair (%)	13.9	14.4	13.2	14.3	14.1
Painful burn or blistering skin reaction as a child or adolescent (%)	52.7	56.0	55.1	55.8	54.7
Lifetime blistering sunburns 6 (%)	13.4	14.3	13.5	13.8	13.5
Number of arm moles 6 (%)	5.1	5.4	5.5	5.5	5.4

^aValues are means (SD) or percentages and are standardized to the age distribution of the study population.

^bValues are not age adjusted.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Abbreviations: HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study; SD, Standard deviation; UV, Ultraviolet.

Table 2.

Pooled hazard ratios and 95% confidence intervals of melanoma according to quintile of intake of cumulatively averaged folate, vitamins B6 and B12, methionine, choline, and betaine.

	Quintile of intake					P for trend
	1	2	3	4	5	
Total Folate						
No. of cases	215	257	272	289	295	
Age-adjusted	1.00	1.17 (1.07, 1.40)	1.21 (1.02, 1.45)	1.29 (1.08, 1.54)	1.30 (1.08, 1.55)	0.04
Multivariable-adjusted ^a	1.00	1.07 (0.89, 1.29)	1.08 (0.90, 1.30)	1.14 (0.95, 1.36)	1.16 (0.96, 1.40)	0.21
Multivariable-adjusted+citrus ^b	1.00	1.03 (0.85, 1.24)	1.03 (0.86, 1.24)	1.08 (0.90, 1.30)	1.09 (0.91, 1.32)	0.36
Folate from food only						
No. of cases	191	262	263	311	301	
Age-adjusted	1.00	1.34 (1.11, 1.61)	1.33 (1.10, 1.60)	1.55 (1.30, 1.86)	1.50 (1.25, 1.80)	<0.0001
Multivariable-adjusted	1.00	1.24 (1.03, 1.50)	1.20 (0.99, 1.45)	1.40 (1.16, 1.68)	1.36 (1.13, 1.64)	0.001
Multivariable-adjusted+citrus	1.00	1.20 (0.99, 1.46)	1.14 (0.94, 1.39)	1.33 (1.09, 1.62)	1.29 (1.05, 1.58)	0.02
Total Vitamin B6						
No. of cases	239	252	260	281	296	
Age-adjusted	1.00	1.06 (0.89, 1.27)	1.05 (0.83, 1.34)	1.13 (0.95, 1.34)	1.20 (1.01, 1.42)	0.05
Multivariable-adjusted	1.00	0.98 (0.82, 1.17)	0.95 (0.76, 1.18)	1.03 (0.86, 1.23)	1.08 (0.91, 1.29)	0.16
Multivariable-adjusted+citrus	1.00	0.96 (0.80, 1.14)	0.92 (0.73, 1.17)	1.00 (0.84, 1.19)	1.06 (0.89, 1.26)	0.18
Vitamin B6 from food only						
No. of cases	219	249	259	301	300	
Age-adjusted	1.00	1.11 (0.93, 1.34)	1.14 (0.95, 1.36)	1.29 (1.08, 1.54)	1.27 (1.07, 1.52)	0.002
Multivariable-adjusted	1.00	1.05 (0.87, 1.26)	1.05 (0.88, 1.26)	1.18 (0.99, 1.41)	1.18 (0.99, 1.41)	0.03
Multivariable-adjusted+citrus	1.00	1.03 (0.86, 1.23)	1.02 (0.85, 1.23)	1.14 (0.95, 1.37)	1.14 (0.95, 1.37)	0.07
Total Vitamin B12						
No. of cases	252	262	243	274	297	
Age-adjusted	1.00	1.03 (0.87, 1.23)	0.94 (0.79, 1.13)	1.05 (0.88, 1.24)	1.13 (0.96, 1.34)	0.01
Multivariable-adjusted	1.00	0.99 (0.84, 1.18)	0.90 (0.75, 1.08)	1.00 (0.84, 1.19)	1.08 (0.91, 1.28)	0.05

	Quintile of intake					P for trend
	1	2	3	4	5	
Multivariable-adjusted+citrus	1.00	0.99 (0.83, 1.18)	0.89 (0.75, 1.07)	0.99 (0.83, 1.18)	1.07 (0.90, 1.27)	0.06
Vitamin B12 from food only						
No. of cases	233	274	311	251	259	
Age-adjusted	1.00	1.16 (0.73, 1.84)	1.31 (1.11, 1.56)	1.04 (0.64, 1.69)	1.06 (0.89, 1.27)	0.79
Multivariable-adjusted	1.00	1.15 (0.73, 1.81)	1.30 (1.10, 1.55)	1.03 (0.65, 1.65)	1.10 (0.92, 1.31)	0.88
Multivariable-adjusted+citrus	1.00	1.15 (0.73, 1.79)	1.30 (1.10, 1.55)	1.03 (0.66, 1.62)	1.10 (0.92, 1.32)	0.87
Total methionine						
No. of cases	239	271	293	254	271	
Age-adjusted	1.00	1.13 (0.89, 1.44)	1.22 (1.03, 1.45)	1.07 (0.90, 1.28)	1.13 (0.95, 1.35)	0.37
Multivariable-adjusted	1.00	1.11 (0.89, 1.39)	1.20 (1.01, 1.42)	1.06 (0.88, 1.26)	1.15 (0.96, 1.37)	0.29
Multivariable-adjusted+citrus	1.00	1.11 (0.90, 1.37)	1.19 (1.00, 1.42)	1.05 (0.88, 1.26)	1.15 (0.96, 1.37)	0.27
Total choline						
No. of cases	242	277	266	287	256	
Age-adjusted	1.00	1.13 (0.87, 1.46)	1.07 (0.90, 1.28)	1.15 (0.95, 1.38)	1.01 (0.77, 1.33)	0.99
Multivariable-adjusted	1.00	1.10 (0.87, 1.39)	1.05 (0.88, 1.25)	1.12 (0.94, 1.33)	1.02 (0.85, 1.23)	0.89
Multivariable-adjusted+citrus	1.00	1.09 (0.87, 1.36)	1.03 (0.86, 1.22)	1.10 (0.92, 1.31)	1.00 (0.84, 1.20)	0.93
Total betaine						
No. of cases	216	251	314	265	282	
Age-adjusted	1.00	1.16 (0.97, 1.39)	1.45 (1.22, 1.73)	1.22 (1.02, 1.46)	1.29 (1.08, 1.54)	0.02
Multivariable-adjusted	1.00	1.11 (0.92, 1.33)	1.36 (1.14, 1.62)	1.13 (0.94, 1.36)	1.20 (1.00, 1.43)	0.15
Multivariable-adjusted+citrus	1.00	1.10 (0.92, 1.33)	1.35 (1.13, 1.60)	1.12 (0.94, 1.34)	1.19 (1.00, 1.43)	0.15

^aMultivariable hazard ratios were adjusted for age; family history of melanoma (yes v no); natural hair color (red, blonde, light brown, dark brown, or black); no. of arm moles (0, 1–2, 3–5, or 6); sunburn susceptibility as child or adolescent (none/some redness, burn, or painful burn/blisters); no. of lifetime blistering sunburns (0, 1–2, 3–5, or 6); cumulative ultraviolet flux since baseline (quintiles), type of tan after repeated sun exposure as a child or adolescent (none, light, average, or deep), body mass index (<18.5, 18.5 to 24.9, 25.0 to 29.9, 30.0 to 34.9, or 35.0 kg/m²), physical activity (quintiles), smoking status (never; past <10, 10–19, 20–39, or 40 pack years; or current <25, 25–44, or 45 pack years); alcohol intake (0, 0.1 to 4.9, 5.0 to 9.9, 10.0 to 19.9, or 20.0 g per day); and coffee intake (quintiles). Analysis of men did not adjust for type of tan after repeated sun exposure as child or adolescent (data not available) and used a different categorization for smoking status (never, past with <10, 10–19, 20–39, or current). Results among NHS and HPFS were pooled using a random effects model. Separate results according to NHS and HPFS are shown in Supplementary Table S1.

^bMultivariable hazard ratios were additionally adjusted for citrus intake (quintiles).

Abbreviations: CI, confidence interval; HR, hazard ratio; HPFS, Health Professionals Follow-up Study; NHS, Nurses' Health Study.