Vitamin C intake from diary recordings and risk of breast cancer in the UK Dietary Cohort Consortium

Running title: Diary vitamin C intake and breast cancer risk

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Abstract

Background/objectives: Vitamin C intake has been inversely associated with breast cancer risk in case-control studies, but not in meta-analyses of cohort studies using Food Frequency Questionnaires, which can over-report fruit and vegetable intake, the main source of vitamin C. This is the first study to investigate associations between vitamin C intake and breast cancer risk using food diaries.

Subjects/Methods: Estimated dietary vitamin C intake was derived from four to seven day food diaries pooled from five prospective studies in the UK Dietary Cohort Consortium. This nested case-control study of 707 incident breast cancer cases and 2144 matched controls examined breast cancer risk in relation to dietary vitamin C intake using conditional logistic regression adjusting for relevant covariates. Additionally, total vitamin C intake from supplements and diet was analysed in three cohorts.

Results: No evidence of associations were observed between breast cancer risk and vitamin C intake analysed for dietary vitamin C intake (OR = 0.98 per 60mg/d, 95%CI: 0.88 to 1.09, $P_{trend} = 0.7$), dietary vitamin C density (OR = 0.97 per 60mg/d, 95% CI: 0.87 to 1.07) or total vitamin C intake (OR = 1.01 per 60mg/d, 95%CI: 0.99 to 1.03, $P_{trend} = 0.3$). Additionally, there was no significant association for post-menopausal women (OR = 1.02 per 60mg/d, 95%CI: 0.99 to 1.05, $P_{trend} = 0.3$).

Conclusions: This pooled analysis of individual UK women found no evidence of significant associations between breast cancer incidence and dietary or total vitamin C intake derived uniquely from detailed diary recordings.

Key Words: Breast cancer, Vitamin C, cohort studies, food diaries.

Introduction

In the UK a woman's cumulative risk of being diagnosed with breast cancer is 6% by the age of 65, and 11% over a lifetime (Office for National Statistics 2000). It has been hypothesised that antioxidant properties of vitamin C can reduce cancer risk by decreasing reactive oxygen species (ROS) that may cause DNA damage (Willcox et al 2004). ROS, nevertheless, are involved in apoptosis, the beneficial death of tumour cells (Valko et al 2006).

Initial findings from retrospective case-control studies showed that fruit and vegetable intake, the main source of vitamin C, and also vitamin C intake were inversely associated with breast cancer risk (Gandini et al 2000, WCRF/AICR 1997, WCRF/AICR 2007). However, no conclusive evidence of a protective effect from fruit and vegetables has been produced prospectively from cohort studies (Key 2010, Michels et al 2007, Smith-Warner et al 2001, van Gils et al 2005, WCRF/AICR 2007). Similarly, the meta-analyses of prospective cohorts using Food Frequency Questionnaires (FFQs) in the 2007 World Cancer Research Fund (WCRF) report showed no significant associations with dietary or supplement vitamin C intake, nor in subgroup analyses by menopausal status (WCRF/AICR 2007). Only four prospective studies in this report included vitamin C from supplements as well as diet (Cho et al 2003, Kushi et al 1996, Nissen et al 2003, Zhang et al 1999), one of which showed an increased risk with increased total vitamin C intake (Nissen et al 2003). Only two studies since the WCRF report was published have assessed total vitamin C intake and breast cancer risk,(Cui et al 2008, Roswall et al 2010), one of which found a weak positive association (Cui et al 2008).

FFQs tend to encourage the over-reporting of fruit and vegetable consumption (Bingham et al 1997, Cade et al 2002, Calvert et al 1997), leading to the over-estimation of vitamin C intake (Bingham et al 1997). Alternatively, diaries may more

accurately record numbers of fruit and vegetable portions consumed individually or in mixed dishes, (Bingham et al 1997) over a period of days, though they are limited by their short-term nature.

Our pooled analysis of the UK Dietary Cohort Consortium is the first study to investigate the relationship between breast cancer risk and vitamin C intake using food diaries; an alternative tool to FFQs used in previous analyses. Additionally, the current analysis is one of a small number of prospective studies assessing the relationship of breast cancer risk with total vitamin C intake, which includes intake from supplements as well as from diet.

Methods

Subjects

Individual participant data were pooled from five established cohort studies within the UK Dietary Cohort Consortium: EPIC-Norfolk (Bingham et al 2001) the UK Women's Cohort Study (UKWCS) (Cade et al 2004), EPIC-Oxford (Davey et al 2003), Whitehall II (Marmot and Brunner 2005), and the MRC National Survey of Health and Development (NSHD) (Wadsworth et al 2006). Methods used were similar to those previously described for colorectal case-control analyses nested within this UK consortium(Dahm et al 2010).

Case ascertainment and matching

Incident cases of breast cancer were identified from data provided by UK cancer registries based on the International Classification of Diseases (ICD) version 9 (174) or 10 (C50). Diagnoses within six months of food diary completion were excluded to ensure that latent disease without formal diagnosis was not present; otherwise disease suspected by participants could have influenced their dietary habits. Across the cohorts 707 incident cases and 2144 controls were used in the dietary vitamin C analysis. Only

three cohorts (EPIC-Oxford, EPIC-Cambridge and UKWCS) were used in the total vitamin C analysis which involved 601 incident cases and 1725controls (85% of the consortium participants); the remaining two cohorts did not have adequate supplement use data to determine the vitamin C content of supplements consumed at diary date. Within each cohort, each case was matched to randomly selected controls based on age at recruitment (± 3 years) and date of diary completion (± 3 months or as close as possible). The number of controls matched to cases was four for EPIC-Norfolk, Whitehall and NSHD, and up to five for UKWCS. In EPIC-Oxford one control was matched to each case, to within six months of case diary completion. Controls had no registry-reported cancer diagnosis at recruitment (except non-melanoma skin cancer) and were free from breast cancer at the end of the follow-up period. The mean length of follow-up for cases in the cohorts ranged from 2.4 years to 10.8 years as detailed in Table 1; these were not adjusted for in the analyses.

Insert Table 1

Dietary methods

All cohorts collected dietary information using semi-weighed food diaries or photographs to aid the estimation of portion size. The number of days intake recorded for each cohort is shown in table 1.

Food diary details were input by trained food diary analysts; the majority were entered into Data into Nutrients for Epidemiological Research (DINER), and checked and calculated using DINERMO to derive nutrient data (Welch et al 2001). Diaries from UKWCS were entered using an in-house Microsoft Access-based dietary analysis program (DANTE), which had previously been validated against DINER on a subsample of 100 randomly selected diaries, with acceptable agreement (Dahm et al 2010). Diaries from the NSHD were entered into DIDO (Price et al 1995); which, after

validation proved to use portion sizes and recipes that were more concurrent with the time of NSHD diary completion. All estimated dietary vitamin C intake was based on standard tables of food composition and daily averages were calculated (Holland et al 1991).

In separate sections of the diaries, participants were asked to record supplement brand, name and amount per day for any supplement taken. In three cohorts databases were created to match this information against manufacturers' information: EPIC-Norfolk (Lentjes et al 2011) and EPIC-Oxford and UKWCS (Hutchison *et al 2011)*. The two databases included supplement descriptions and ingredient composition from product labels directly obtained from manufacturers or the participants' descriptions and/or labels. Where participants were unclear in their description, a weighted average of vitamin C from similar supplements was calculated from the database and applied (Lentjes et al 2011). For instance, separate generic averages were calculated for multivitamins, antioxidant ACE supplements and high dose vitamin C supplements. For each participant the average daily vitamin C amount consumed from all supplement types was calculated.

Statistical methods

Separate quintile cut points were determined for dietary intake (mg per day), dietary vitamin C intake density (mg per megajoule per day) and total vitamin C intake including supplements (mg per day). Dietary vitamin C intake density was analysed as a separate method of controlling for potential confounding by total energy intake. Conditional logistic regression was used to model the associations between fifths of vitamin C intake and breast cancer incidence. To test for linear trends we used continuous intake variables per increment of approximately one standard deviation of mean intake (being 60mg/day for dietary intake and 8mg/MJ/day for intake density). No

supplement intakes were implausible. However, in sensitivity analyses women with extreme intakes, defined as more than 1.5 times the inter-quartile range above the 75th percentile, were excluded in tests for linear trends. These upper thresholds were 224.1 mg/d for dietary intake, 30.6 mg/MJ/day for intake density and 262.4 mg/d for total vitamin C intake, which excluded 77, 91 and 206 women respectively.

Due to the process of matching cases and controls the conditional logistic regression model automatically adjusted for date of diary completion, age (in years) and cohort. The multivariate model adjusted for exact age, parity (0, 1, 2, 3, 4+, missing), hormone replacement therapy use (current, non-current, missing), alcohol intake, total energy intake, weight (<60kg, 60-, 66-, >72kg, missing), height (<158cm, 158-, 163-, >168cm, missing), physical activity (low, low-medium, medium-high, high, missing), and menopausal status (pre, peri or post-menopausal, missing). The level of missing data ranged from 0% for alcohol and total energy intake, to 0.4% for parity to 3.6% for physical activity. Alcohol and total energy intake were ascertained from the diaries. All other covariates were collected by standard questionnaires, either self-administered or by trained researchers at or close to time of diary completion. Sensitivity analysis was performed to adjust for variables which have weaker associations with breast cancer risk; smoking status and level of education, and also to adjust for important risk variables which had moderate levels of missing data; age at menarche (16%) and cumulative duration of breastfeeding (weeks) (18%); which restricted the sensitivity analysis to 2150 participants. To investigate robustness of results to missing data, analyses were repeated using multiple imputation by chained equations (Royston 2009), with imputations based on exposure, covariates and outcome. Additional sensitivity analyses also controlled for dietary vitamin E and iron which affect vitamin C bioavailability. Finally, we formally tested our assumption of no heterogeneity across the different cohorts by including an exposure by centre interaction term in the models.

Analyses were carried out using Stata version 10 and results were based on a significance level of $p<0.05$.

Results

Dietary vitamin C intake

On average the total women (2851) in the five cohorts were 56 years old and consumed 346g/d fruit and vegetables; 65% were post-menopausal, 58% had never smoked, 17% were educated to degree, HNC or HND level, and only 18% took HRT at the date of diary completion.

As observed in table 2 total cases (707) had similar characteristics to the 2144 controls and their mean (sd) dietary vitamin C intakes were 98mg/d (56) and 95mg/d (52) respectively. Women with a higher dietary vitamin C intake tended to have a higher energy intake, consume more alcohol, dietary vitamin E and iron as well as more fruit and vegetables. Additionally they had fewer children, were more active, had attained higher levels of education, or were more likely to be of higher socio-economic status or to have never smoked (table 2)

Insert Table 2

The odds ratios for breast cancer according to dietary intake of vitamin C in the five cohorts are shown in table 3 for the unadjusted and multivariable model. There was no evidence of any significant association between dietary vitamin C intake and incidence of breast cancer for total women in the five cohorts. In the adjusted analysis for total women the odds ratio of breast cancer per 60mg/day increments was 0.98 (95%CI: 0.88 to 1.09, $P_{trend} = 0.7$) Similarly, there was no evidence of any linear trends or significant associations between dietary vitamin C intake groups and incidence of breast cancer in the sub-analysis by post-menopausal status (OR=0.98 per 60mg/day,

95%CI: 0.85 to 1.13, P_{trend} = 0.8). The results remained non-significant in sensitivity analyses after further adjustment for smoking status, age at menarche, cumulative duration of breastfeeding (weeks), and level of education. Odds ratios did not alter substantially. There was no evidence of any linear trends or significant association between the incidence of breast cancer and dietary vitamin C expressed as intake density (Table 4). In the sensitivity analyses, which excluded women with extreme dietary vitamin C intakes, the odds ratios for linear trends relating to absolute dietary intake and intake density were reduced to between 0.91 and 0.95 but none were statistically significant.

Insert table 3 and 4

In tests for heterogeneity there was evidence of differences between the five study centres when a study centre by dietary vitamin C intake group interaction term was included (p=0.10 total women; p=0.05 post-menopausal).

The mean (sd) dietary intake by cohort are shown in Table 1 The lower intake for the younger, nationally representative NSHD women (mean age 43 vs 50s in other cohorts) reflected previous findings from households with similar aged adults (Defra 2004).

Total vitamin C intake

In the analyses of total vitamin C cases had a somewhat higher total vitamin C intake than controls: 174mg/d (sd 374) vs 143mg/d (sd 213). The average vitamin C intake from supplements for cases was 1.5 times higher than controls: 73mg/d (sd 364) vs 48mg/d (sd 201). Total intakes by cohort are shown in table 1. The mean vitamin C supplement intake per day for EPIC-Norfolk was significantly less than for UKWCS and EPIC-Oxford Based on diary completion date, mean total intake in autumn and winter

compared to spring and summer was not significantly different (151.7. (sd 312) vs 151.4 (sd 218) mg/d); comprising respectively of 46.4.1% and 53.6% of these women. The relationships between total vitamin C intake split by fifths and lifestyle characteristics were similar to those for dietary only intake shown in table 2. The highest intake group had the highest vitamin C intake from both diet and supplements (mean (sd) 159 (69) mg/d) and 256 (519) mg/d respectively); in this group 62% took supplements containing vitamin C and 84% of these women took them every day.

In pooling the three cohorts which recorded vitamin C intake from supplements there was also no evidence of any significant associations between total vitamin C intake and incidence of breast cancer for the continuous estimate for all women (OR = 1.01 per 60mg/d, 95%CI: 0.99 to 1.03, $P_{trend} = 0.3$), or for post-menopausal women (OR = 1.02 per 60mg/d, 95%CI: 0.99 to 1.05, $P_{trend} = 0.3$) or by fifths of total vitamin C intake (table 5). There was no evidence of significant differences between the three study centres when formally tested using a study centre by fifths of total vitamin C intake interaction term, for total and for post-menopausal women (p=0.7 and p=0.7 respectively).

For both dietary and total intake no substantial differences in the estimates were found in sensitivity analyses controlling for dietary vitamin E and iron.

Finally, a total of 73 matched case-control sets in the main analyses had some missing covariate information, mostly in HRT exposure, however the strength of associations were almost identical whether these matched sets were included by using a category for missing, or included with additional information using multiple imputation.

Insert table 5

Discussion

This pooled analysis of individual participant data from five UK cohorts found no evidence of an association between incidence of breast cancer and dietary vitamin C intake recorded by food diaries. Neither was there any evidence of an association with total vitamin C intake when vitamin C from supplements was included. Our non-significant results for post-menopausal women relating to dietary vitamin C intake support results of the 2007 WCRF meta-analyses of three cohort studies (HR=1.15 per 100mg/d, 95% CI: 0.92-1.43) (Graham et al 1992, Nissen et al 2003, Verhoeven et al 1997, WCRF/AICR 2007), also the high versus low intake results of two US studies (Kushi et al 1996, Zhang et al 1999), and the recent European Prospective Investigation into Cancer (EPIC) involving the pooling of data from 10 European countries (highest vs. lowest quintile HR = 0.98, 95% CI: 0.87–1.11) (Nagel et al 2010); all of which used FFQs. Our results for dietary vitamin C are in conflict with significant evidence of a 12-14% reduced risk found in the meta-analysis of retrospective case-control studies (WCRF/AICR 2007) which, unlike our study, are prone to recall bias.

In contrast to our results and other studies (Cho et al 2003, Kushi et al 1996, Roswall et al 2010, Zhang et al 1999), the large Women's Health Initiative study (Cui et al 2008) found significant but weak evidence of increased breast cancer risk for total intake. The advanced age of the participants in this cohort (average 64 years) might suggest that high vitamin C intake may promote the progression of cancer in older people or at later stages of the disease. Similarly positive associations with post-menopausal breast cancer for both dietary and total vitamin C intake (OR= 2.06 per 100mg/d, 95% CI: 1.45-2.91; and OR=1.08 per 100mg/d, 95% CI: 1.02-0.1.15 respectively) were found in a small Danish nested case-control study (Nissen et al 2003), but not in the recent full analysis of this Danish cohort (Roswall et al 2010); selection bias of controls or exclusion of non-supplement users may have possibly influenced the earlier results.

Pooling individual participant data in this consortium had three advantages. Firstly, it ensured that vitamin C intake over the whole consortium could be categorised into fifths; secondly the variations in intake across the cohorts increase the power to detect smaller effect sizes (Schatzkin et al 2001), i.e. many women in EPIC-Oxford and UKWCS were vegetarians and/ or consumed supplements containing vitamin C compared to the other cohorts; thirdly, analysis and adjustment by covariates could be done in a uniform way.

Our study had a few caveats. Whilst the use of missing covariate categories may have grouped dissimilar individuals and introduced some bias, its affect on the adjusted results may be considered acceptable since the level of missing data was small, confounding was judged to be weak and multiple imputation results were almost identical. To account for the possible modulation of vitamin C on cancer development due to its the role in the regeneration of vitamin E, in the absorption of iron and in the Fenton reaction, (Valko et al 2006) sensitivity analysis adjustments were made for these dietary nutrients. Supplement intake data for these nutrients, however, was not available. The Danish studies, one of which found a positive association, controlled for both dietary and supplement intake of vitamin A and E (Nissen et al 2003, Roswall et al 2010). In the current study data were unavailable to adjust for family history of breast cancer which has been associated with high-dose vitamin C supplement use in the UK (Hutchinson et al 2011). Data were unavailable from all cohorts to exclude general supplement users from the dietary analysis; the different health behaviours of users may have influenced the results (Kirk et al 1999). There was inadequate power to sub-analyse by HRT users, oestrogen receptor-negative or pre-menopausal breast cancers.

This is the first time the relationship between breast cancer risk and vitamin C intake has been analysed using prospective data from food diaries. Diaries can capture detailed and accurate intake over a narrow period of days due to their open format, whereas FFQs aim to reflect intake over a much longer period, normally an estimated average of the previous 12 months. Repeated diary data collections may reduce their short-term limitations but were not undertaken for the whole consortium due to expense and time taken to administer, complete and analyse. The required commitment and awareness of intake may have also influence participants' consumption during diary recording. When compared to FFQs, food diaries have shown stronger correlations with plasma vitamin C biomarkers in validity tests when collected in close temporal proximity. However this may reflect the short-term nature of both plasma vitamin C and diary data, particularly since correlations with biomarker levels re-measured several years later were similar for diaries and FFQs (Bingham et al 2008, Bingham et al 1997, Willett 2008) Furthermore, other UK validation studies have shown similar associations between biomarkers and vitamin C estimated from FFQs and diaries (Brunner et al 2001, Michels et al 2005). Overall correlations between biomarkers and FFQs or diaries are generally weak to moderate (Cade et al 2002, Henríquez-Sánchez et al 2009). Since the absorption and storage of vitamin C is limited, particularly above 400mg/d (Levine et al 2001), biomarkers are unlikely to reflect dietary vitamin C intake well. Therefore it is difficult to objectively assess whether diaries or FFQs can rank individual intake sufficiently well in order to find associations between vitamin C and cancer risk. Given the limitations, vitamin C results from both FFQs and diaries need to be treated with some caution.

To conclude, the evidence to date from this and other prospective studies does not indicate either a beneficial or a detrimental effect of vitamin C intake on breast cancer risk, whether this intake is from diet only or also from supplements.

Conflict of interest

The authors declare no conflict of interest

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References

Bingham S, Luben R, Welch A, Low YL, Khaw KT, Wareham N *et al*. (2008). Associations between dietary methods and biomarkers, and between fruits and vegetables and risk of ischaemic heart disease, in the EPIC Norfolk Cohort Study. *Int J Epidemiol* **37,** 978-987.

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

Bingham SA, Welch AA, McTaggart A, Mulligan AA, Runswick SA, Luben R *et al*. (2001). Nutritional methods in the European Prospective Investigation of Cancer in Norfolk. *Public Health Nutr* **4,** 847-858.

Brunner E, Stallone D, Juneja M, Bingham S, Marmot M. (2001). Dietary assessment in Whitehall II: comparison of 7 d diet diary and food-frequency questionnaire and validity against biomarkers. *Br J Nutr* **86,** 405-414.

Cade J, Thompson R, Burley V, Warm D. (2002). Development, validation and utilisation of food-frequency questionnaires - a review. *Public Health Nutr* **5,** 567-587.

Cade JE, Burley VJ, Greenwood DC, UK Women's Cohort Study Steering Group. (2004). The UK Women's Cohort Study: comparison of vegetarians, fish-eaters and meat-eaters. *Public Health Nutr* **7,** 871-878.

Calvert C, Cade J, Barrett JH, Woodhouse A, UKWCS Steering Group. (1997). Using cross-check questions to address the problem of mis-reporting of specific food groups on Food Frequency Questionnaires. *Eur J Clin Nutr* **51,** 708-712.

Cho E, Spiegelman D, Hunter DJ, Chen WY, Zhang SM, Colditz GA *et al*. (2003). Premenopausal Intakes of Vitamins A, C, and E, Folate, and Carotenoids, and Risk of Breast Cancer. *Cancer Epidemiol Biomarkers Prev* **12,** 713-720.

Cui Y, Shikany JM, Liu S, Shagufta Y, Rohan TE. (2008). Selected antioxidants and risk of hormone receptor-defined invasive breast cancers among postmenopausal women in the Women's Health Initiative Observational Study. *Am J Clin Nutr* **87,** 1009-1018.

Dahm CC, Keogh RH, Spencer EA, Greenwood DC, Key TJ, Fentiman IS *et al*. (2010). Dietary Fiber and Colorectal Cancer Risk: A Nested Case Control Study Using Food Diaries. *J Natl Cancer Inst* **102,** 614-626.

Davey GK, Spencer EA, Appleby PN, Allen NE, Knox KH, Key TJ. (2003). EPIC-Oxford:lifestyle characteristics and nutrient intakes in a cohort of 33 883 meat-eaters and 31 546 non meat-eaters in the UK. *Public Health Nutr* **6,** 259-268.

Defra (2004). *Family Food. A Report on the 2002-2003 Expenditure and Food Survey*. The Stationary Office: London

Gandini S, Merzenich H, Robertson C, Boyle P. (2000). Meta-analysis of studies on breast cancer risk and diet: the role of fruit and vegetable consumption and the intake of associated micronutrients. *Eur J Cancer* **36,** 636-646.

Graham S, Zielezny M, Marshall J, Priore R, Freudenheim J, Brasure J *et al*. (1992). Diet in the Epidemiology of Postmenopausal Breast Cancer in the New York State Cohort. *Am J Epidemiol* **136,** 1327-1337.

Henríquez-Sánchez P, Sánchez-Villegas A, Doreste-Alonso J, Ortiz-Andrellucchi A, Pfrimer K, Serra-Majem L. (2009). Dietary assessment methods for micronutrient intake: a systematic review on vitamins. *Br J Nutri* **102,** S10-S37

Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT (1991). *McCance and Widdowson's the Composition of Foods*, 5th edn. Royal Society of Chemisty: Cambridge, UK.

Hutchinson J, Burley VJ, Greenwood DC, Thomas JD, Cade JE. (2011). High-dose vitamin C supplement use is associated with self-reported histories of breast cancer and other illnesses in the UK Women's Cohort Study. *Public Health Nutr* **14,** 768-777.

Key TJ. (2010). Fruit and vegetables and cancer risk. *Br J Cancer* **104,** 6-11.

Kirk SFL, Cade JE, Barrett JH, Conner M. (1999). Diet and lifestyle characteristics associated with dietary supplement use in women. *Public Health Nutr* **2,** 69-73.

Kushi LH, Fee RM, Sellers TA, Zheng W, Folsom AR. (1996). Intake of Vitamins A, C, and E and Postmenopausal Breast Cancer: The Iowa Women's Health Study. *Am J Epidemiol* **144,** 165-174.

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

Levine M, Wang Y, Padayatty SJ, Morrow J. (2001). A new recommended dietary allowance of vitamin C for healthy young women. *Proc Natl Acad Sci USA* **98,** 9842-9846.

Marmot M, Brunner E. (2005). Cohort Profile: The Whitehall II study. *Int J Epidemiol* **34,** 251-256.

Michels KB, Welch AA, Luben R, Bingham SA, Day NE. (2005). Measurement of Fruit and Vegetable Consumption with Diet Questionnaires and Implications for Analyses and Interpretation. *Am J Epidemiol* **161,** 987-994.

Michels KB, Mohllajee AP, Roset-Bahmanyar E, Beehler GP, Moysich KB. (2007). Diet and breast cancer. *Cancer* **109,** 2712-2749.

Nagel G, Linseisen J, van Gils C, Peeters P, Boutron-Ruault M, Clavel-Chapelon F *et al*. (2010). Dietary β-carotene, vitamin C and E intake and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Breast Cancer Res Treat* **119,** 753-765.

Nissen SB, Tjønneland A, Stripp C, Olsen A, Christensen J, Overvad K *et al*. (2003). Intake of vitamins A, C, and E from diet and supplements and breast cancer in postmenopausal women. *Cancer Causes Control* **14,** 695-704.

Office for National Statistics. (2000). Registrations of cancer diagnosed in 1994-1997, England & Wales. *Health Stat Quart* **07,** 71-82.

Price GM, Paul AA, Key FB, Harter AC, Cole TJ, Day KC *et al*. (1995). Measurement of diet in a large national survey: comparison of computerized and manual coding of records in household measures. *J Hum Nutr Diet* **8,** 417-428.

Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjonneland A. (2010). Micronutrient intake and breast cancer characteristics among postmenopausal women. *Eur J Cancer Prev* **19,** 360-365.

Royston P. (2009). Multiple imputation of missing values: Further update of ice, with an emphasis on categorical variables. *Stata Journal* **9**.

Schatzkin A, Subar AF, Thompson FE, Harlan LC, Tangrea J, Hollenbeck AR *et al*. (2001). Design and Serendipity in Establishing a Large Cohort with Wide Dietary Intake Distributions. *Am J Epidemiol* **154,** 1119-1125.

Smith-Warner SA, Spiegelman D, Yaun S-S, Adami H-O, Beeson WL, van den Brandt PA *et al*. (2001). Intake of Fruits and Vegetables and Risk of Breast Cancer: A Pooled Analysis of Cohort Studies. *JAMA* **285,** 769-776.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biol Interact* **160,** 1-40.

van Gils CH, Peeters PHM, Bueno-de-Mesquita HB, Boshuizen HC, Lahmann PH, Clavel-Chapelon F *et al*. (2005). Consumption of Vegetables and Fruits and Risk of Breast Cancer. *JAMA* **293,** 183-193.

Verhoeven DTH, Assen N, Goldbohm RA, Dorant E, van 't Veer P, Sturmans F *et al*. (1997). Vitamins C and E, retinol, beta-carotene and dietary fibre in relation to breast cancer risk: a prospective cohort study. *Br J Cancer* **75,** 149-155.

Wadsworth M, Kuh D, Richards M, Hardy R. (2006). Cohort Profile: The 1946 National Birth Cohort (MRC National Survey of Health and Development). *Int J Epidemiol* **35,** 49-54.

WCRF/AICR (1997). *Food, Nutrition and the Prevention of Cancer: a Global Perspective*. AICR: Washington, DC.

WCRF/AICR (2007). *Food, nutrition, physical activity, and the prevention of cancer: a global perspective* AICR: Washington, DC.

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

Willcox JK, Ash SL, Catignani GL. (2004). Antioxidants and Prevention of Chronic Disease. *Crit Rev Food Sci Nutr* **44,** 275-295.

Willett WC. (2008). Commentary: Flawed study designs are not salvaged by large samples. *Int J Epidemiol* **37,** 987-989.

Zhang S, Hunter DJ, Forman MR, Rosner BA, Speizer FE, Colditz GA *et al*. (1999). Dietary Carotenoids and Vitamins A, C, and E and Risk of Breast Cancer. *J Natl Cancer Inst* **91,** 547-556.

Table 1: Characteristics of the 5 cohorts participating in analyses of vitamin C and breast cancer risk in the UK Dietary Cohort Consortium.

Cohort	Participants	Diary days	Years when food diary completed	Last follow up date	Mean time to diagnosis of cases	Cases	Controls	Mean(sd) dietary vit c intake	Mean(sd) total vit c intake
EPIC-Norfolk	General population in Norfolk	7 days	1993-1998	31.12.2006	6.0 yrs	365	1329	91 (50)	118 (167)
EPIC-Oxford	General population and vegetarians in the UK	7 days	1993-1998	31.12.2004	3.5 yrs	194	194	111 (61)	233 (436)
UK Women's Cohort Study (UKWCS)	Middle aged women in the UK	4 days	1999-2003	31.12.2006	2.4 yrs	42	202	118 (60)	251 (376)
Whitehall II	Civil servants in the UK	7 days	1991-1993	30.09.2005	7.8 yrs	70	275	101 (51)	_[a]
National Survey of Health and Development (NSHD)	Nationally reprehensive cohort of women who were born in one week in March 1946 in England, Wales and Scotland.	5 days	1989	31.12.2006	10.8 yrs	36	144	66 (37)	_[a]

[a]Whitehall and NSHD did not have detailed diary data of vitamin C intake from supplements

Table 2: Participant characteristics by fifth of dietary vitamin C intake derived from food diaries in the UK Dietary Cohort Consortium

		Breast cancer		Dietary vitamin C intake (diary fifths)					
Covariates (at diary date)		**Cases**	**Controls**	**1**	**2**	**3**	**4**	**5**	**P***
Cases/controls		707	2144	130/440	138/432	144/426	142/428	153/418	
Dietary vitamin C intake (mg/day)	mean (SD)	98 (56)	95 (52)	36.9 (9.9)	61.9 (6.4)	85.1 (7.2)	114.6 (10.0)	178.4(45.5)	
Fruit Intake g/d	mean (SD)	191 (138)	185 (135)	81 (74)	136 (84)	188 (105)	223 (123)	304 (159)	<0.001
Vegetable intake g/d	mean (SD)	165 (86)	158 (82)	101 (49)	141 (59)	161 (65)	182 (79)	214 (104)	<0.001
Age at diary completion (yr)	mean (SD)	55.7 (9.4)	56.3 (9.6)	55.5 (10.0)	55.7 (9.8)	56.9 (9.6)	56.8 (9.6)	56.0 (9.4)	0.3
Height (cm)	mean (SD)	163 (7)	162 (6)	160.7 (6.5)	161.5 (6.3)	161.8 (6.8)	162.3 (6.4)	162.9 (6.2)	<0.001
Weight (kg)	mean (SD)	67.8 (11.8)	67.2 (12.2)	67.9 (12.0)	67.5 (12.6)	67.8 (12.7)	66.6 (11.2)	66.8 (12.0)	0.1
Energy intake (diary, MJ/day)	mean (SD)	7.6 (1.7)	7.4 (1.7)	6.8 (1.8)	7.4 (1.6)	7.4 (1.6)	7.7 (1.7)	7.8 (1.7)	<0.001
Alcohol intake (diary, g/day)	mean (SD)	10.3 (13.6)	8.7 (12.8)	7.9 (12)	8.5 (13)	10.3 (14)	9.0 (13)	9.7 (13)	0.02
Total fat (g/d)	mean (SD)	68.9 (21.8)	67.2 (22.0)	64.9 (21.3)	69.5 (21.7)	66.2 (21.5)	70.3 (22.5)	67.6 (21.4)	0.07
Dietary vitamin E (mg/d)	mean (SD)	9.9 (4.1)	9.3 (4.0)	8.0 (3.8)	9.2 (3.7)	9.3 (3.7)	10.2 (4.2)	10.6 (4.1)	<0.001
Dietary Iron (mg/d)	mean (SD)	11.8 (3.5)	11.3 (3.4)	9.5 (3.1)	10.9 (2.9)	11.5 (3.4)	12.0 (3.5)	13.0 (3.5)	<0.001
Parity (number of children)	mean (SD)	1.8 (1.2)	1.9 (1.3)	2.1 (1.3)	1.9 (1.3)	1.9 (1.3)	1.8 (1.3)	1.6 (1.3)	<0.001
Exercise (medium - high)	n (%)	242 (37)	796 (38)	162 (30)	198 (36)	208 (37)	230 (42)	240 (44)	<0.001
HRT use (current user)	n (%)	122 (18)	373 (18)	89 (16)	94 (17)	106 (19)	110 (20)	96 (17)	0.4
Menopausal status (post-menopausal)	n (%)	436 (63)	1424 (67)	352 (63)	368 (66)	385 (68)	387 (69)	367 (65)	0.2
Never smoked	n (%)	413 (60)	1233 (58)	272 (49)	316 (56)	333 (59)	349 (62)	376 (67)	<0.001
Education level (degree, HNC, HND)	n (%)	136 (21)	313 (15)	38 (7)	68 (13)	77 (15)	113 (21)	151 (28)	<0.001
Social class (professional or intermediate)	n (%)	238 (47)	901 (47)	187 (37)	207 (42)	232 (48)	254 (53)	259 (58)	<0.001

*p is Ptrend over continuous variables, and p for $\chi 2$ tests for categorical variables

Table 3: Dietary vitamin C intake recorded by diaries and risk of breast cancer in the UK Dietary Cohort Consortium breast cancer study

Dietary vitamin C intake	Cases/	Unadjusted *	Multivariate †
Fifths: mean mg/day (sd)	**Controls**	**OR (95% CI)**	**OR (95% CI)**
Total women			
1 (lowest): 36.9 (9.9)	130/440	0.94 (0.71, 1.25)	0.95 (0.71, 1.28)
2 61.9 (6.4)	138/432	1	1
3 85.1 (7.2)	144/426	1.03 (0.78, 1.36)	1.00 (0.75, 1.33)
4 114.6 (10.0)	142/428	0.98 (0.74, 1.29)	0.92 (0.69, 1.22)
5 (highest): 178.4 (45.5)	153/418	1.02 (0.77, 1.35)	0.96 (0.72, 1.27)
P trend per 60mg/d		0.9	0.7
Continuous estimate/ 60mg/d		1.01 (0.91, 1.11)	0.98 (0.88, 1.09)
Post menopausal			
1 (lowest) 36.9 (9.7)	77/276	1.02 (0.70, 1.48)	1.05 (0.71, 1.55)
2 62.2 (6.5)	79/289	1	1
3 85.0 (7.2)	96/289	1.22 (0.85, 1.74)	1.19 (0.82, 1.71)
4 114.9 (10.0)	91/296	1.06 (0.74, 1.52)	0.99 (0.68, 1.44)
5 (highest) 179.0 (47.7)	93/274	1.12 (0.77, 1.61)	1.01 (0.69, 1.48)
P trend per 60mg/d		0.7	0.8
Continuous estimate/ 60mg/d		1.03 (0.90, 1.17)	0.98 (0.85,1.13)

* Conditional logistic regression on cases and controls matched by cohort, age and date of diary completion

† As for the unadjusted model * with additional adjustment for exact age, height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, diary-derived alcohol consumption and total energy intake. Missing data added as a category.

Table 4: Dietary vitamin C intake densities recorded by diaries and risk of breast cancer in the UK Dietary Cohort Consortium breast cancer study

Vitamin C nutrient density Fifths: mean mg/MJ/d (sd)	Cases/ Controls	Unadjusted* OR (95% CI)	Multivariate † OR (95% CI)
Total women			
1 (lowest): 5.2 (1.3)	140/430	0.98 (0.75, 1.29)	0.98 (0.74, 1.30)
2 8.5 (0.8)	143/427	1	1
3 11.6 (1.0)	139/431	0.90 (0.68, 1.18)	0.89 (0.67, 1.19)
4 15.8 (1.4)	152/418	1.05 (0.81, 1.39)	1.05 (0.79, 1.39)
5 (highest): 25.0 (7.1)	133/438	0.80 (0.60, 1.06)	0.80 (0.60, 1.08)
P trend per 8 mg/MJ/d		0.4	0.5
Continuous estimate/ 8 units		0.96 (0.87, 1.06)	0.97 (0.87, 1.07)
Post menopausal			
1 (lowest): 5.3 (1.3)	76/261	0.90 (0.62, 1.30)	0.89 (0.61, 1.31)
2 8.5 (0.9)	81/272	1	1
3 11.6 (1.0)	89/293	0.95 (0.66, 1.35)	0.95 (0.66, 1.37)
4 15.7 (1.4)	106/297	1.10 (0.77, 1.56)	1.11 (0.77, 1.61)
5 (highest): 25.4 (7.5)	84/301	0.80 (0.55, 1.15)	0.80 (0.54, 1.19)
P trend per 8 mg/MJ/d		0.6	0.7
Continuous estimate/ 8 units		0.97 (0.86, 1.09)	0.97 (0.86,1.10)

* Conditional logistic regression on cases and controls matched by cohort, age and date of diary completion

† As for the unadjusted model * with additional adjustment for exact age, height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, alcohol consumption and total energy intake. Missing data added as a category.

Table 5: Total vitamin C intake from diet and supplements recorded by diaries and risk of breast cancer in EPIC-Oxford, EPIC-Norfolk and UKWCS cohorts

Total vitamin C intake	**Cases/**	**Unadjusted***	**Multivariate** †
Fifths: mean mg/day (sd)	**Controls**	**OR (95% CI)**	**OR (95% CI)**
Total women			
1 (lowest): 39.3 (10.9)	101/364	0.88 (0.64, 1.21)	0.86 (0.62, 1.20)
2 69.0 (8.0)	112/353	1	1
3 97.7 (8.9)	133/332	1.21 (0.90, 1.64)	1.22 (0.89, 1.65)
4 136.9 (14.6)	130/335	1.08 (0.79, 1.48)	1.02 (0.74, 1.40)
5 (highest): 414.2 (507.3)	125/341	0.98 (0.71, 1.34)	0.93 (0.67, 1.28)
P for trend per 60mg/d		0.3	0.3
Continuous estimate/ 60mg/d		1.01 (0.99, 1.03)	1.01 (0.99, 1.03)
Post menopausal			
1 (lowest) 39.7 (10.7)	72/275	0.97 (0.67, 1.41)	0.99 (0.67, 1.47)
2 68.9 (8.0)	82/292	1	1
3 97.8 (8.9)	91/257	1.29 (0.90, 1.84)	1.38 (0.95, 1.99)
4 136.2 (14.5)	83/253	1.06 (0.72, 1.55)	0.99 (0.66, 1.47)
5 (highest) 395.3 (466.7)	78/228	1.15 (0.78, 1.67)	1.08 (0.72, 1.59)
P for trend per 60mg/d		0.2	0.3
Continuous estimate/ 60mg/d		1.02 (0.99, 1.05)	1.02 (0.99,1.05)

* Conditional logistic regression on cases and controls matched by cohort, age and date of diary completion

† As for the unadjusted model * with additional adjustment for height (<158cm, 158-, 163-, 168+), weight (<60kg, 60-, 66-, 72+), physical activity, parity (0,1,2,3,4+), current HRT use, menopausal status, diary-derived alcohol consumption and total energy intake. Missing data added as a category.