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British Journal of Nutrition / Volume 105 / Issue 01 / January 2011, pp 123 - 132

DOI: 10.1017/S0007114510003053, Published online: 01 September 2010

Link to this article: http://journals.cambridge.org/abstract_S0007114510003053

How to cite this article:

Christopher J. Bates, Mark Hamer and Gita D. Mishra (2011). Redox-modulatory vitamins and minerals that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over. British Journal of Nutrition, 105, pp 123-132 doi:10.1017/S0007114510003053

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Redox-modulatory vitamins and minerals that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of people aged 65 years and over

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(Received 19 January 2010 – Revised 11 June 2010 – Accepted 5 July 2010 – First published online 1 September 2010)

Abstract

The predictive power, for total, vascular, cancer and respiratory mortality, of selected redox-modulatory (vitamin and mineral nutrient) indices measured at baseline, was studied in the British National Diet and Nutrition Survey (community-living subset) of people aged 65 years and over. Mortality status and its primary and underlying causes were recorded for 1054 (mean age 76.6 (SD 7.4) years and 49.0% female) participants, from the baseline survey in 1994–5 until September 2008. During this interval, 74% of the male and 62% of the female participants died. Total mortality was significantly predicted by baseline plasma concentrations (per SD) of vitamin C (hazard ratio (HR) 0.81; 95% CI 0.74, 0.88), α -carotene (HR 0.90; 95% CI 0.81, 0.99), Se (HR 0.76; 95% CI 0.69, 0.84), Zn (HR 0.79; 95% CI 0.72, 0.87), Cu (HR 1.27; 95% CI 1.14, 1.42) and Fe (HR 0.81; 95% CI 0.74, 0.89). Total mortality was also significantly predicted by baseline dietary intakes (per SD) of food energy (HR 0.86; 95% CI 0.79, 0.94), vitamin C (HR 0.88; 95% CI 0.80, 0.94), carotenoids (HR 0.89; 95% CI 0.83, 0.96), Zn (HR 0.89; 95% CI 0.82, 0.96) and Cu (HR 0.91; 95% CI 0.84, 1.00). Prediction patterns and significance for primary vascular, cancer and respiratory mortality differed in certain respects, but not fundamentally. Model adjustment for known disease or mortality risk predictors resulted in loss of significance for some of the indices; however, plasma Se and Zn, and food energy remained significant predictors. We conclude that total and primary vascular, cancer and respiratory mortality in older British people of both sexes is predicted by several biochemical indices of redox-modulatory nutrients, some of which may reflect the respondents' acute-phase status at baseline, whereas others may reflect the healthiness of their lifestyle.

Key words: British National Survey of Older Adults: Mortality prediction: Intakes and biochemical indices: Plasma vitamins C and E, carotenoids, selenium, zinc, copper and iron

Relationships between biochemical status indices and later morbidity and mortality experience can help to predict causal relationships, and thereby to clarify certain physiological and pathological mechanisms that may be related to important disease risk factors in ageing humans. In the present study, we have focused on mortality outcomes of the community-living participants from the countrywide British National Diet and Nutrition Survey of people aged 65 years and over, for which the fieldwork was performed in 1994–5⁽¹⁾. Subsequent mortality outcomes were available from the National Health Service register of deaths, up to September 2008. The purpose of the present paper is to explore the predictive significance of a selection of biochemical indices for nutrients that are believed to mediate redox-modulatory (antioxidant or pro-oxidant) functions in living tissues, all of which were measured as part of the original

population surveillance protocol. Evidence that subsequent all-cause mortality may be predicted by vitamin C intakes and/or status has been obtained in several previous studies^(2–10), and similarly for carotene^(11,12) and Se^(12–14).

In addition to the modulation of redox status, several nutrient indices are known to be modified by, and hence to reflect, acute-phase status, and hence, potentially, to reflect mortality risk (since chronic inflammatory states frequently underlie those disease processes that lead ultimately to death⁽¹⁵⁾). Also, the same vitamins and minerals that modulate redox status may, in many cases, also modulate key immune functions^(16–18). As well as the practical usefulness of status indices as predictors of future mortality, a key question is whether the observed links between baseline nutrient status and future mortality are likely to be driven by (potentially correctable) nutritional

Abbreviation: ICD, International Classification of Diseases.

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imbalances, or by the more intractable processes of inflammatory response to chronic disease?

Subjects and methods

The survey procedures have been described in detail elsewhere⁽¹⁾; therefore only a brief summary is given here. At baseline, in 1994–5, two separate population samples were randomly selected: one from community-living people aged 65 years and over and the other from long-stay institutions. Only the community-living sample has been included in the present study. Participants were drawn from eighty randomly selected postcode sectors in mainland Britain and allocated to four sequential 3-month fieldwork ‘waves’ corresponding to the four seasons, beginning in October 1994. Demographic, socio-economic and other information was obtained by a trained interviewer in the participant’s home. A 4 d weighed dietary record was also obtained by the interviewer, and anthropometric indices, blood pressure and pulse rate, and after separate consent, a fasting early morning venous blood sample were taken by a trained nurse. The blood sample was subdivided and used for a wide range of analyses. Of these, the assays that are relevant to the present study were as follows: (a) fluorometric assay based on the reaction of dehydroascorbic acid with *ortho*-phenylenediamine (checked for validity against a HPLC assay and against quality assurance standards with assigned values, provided by the US National Institute of Standards and Technology) for plasma (total) vitamin C; (b) liquid chromatographic assay for plasma retinol, α - and γ -tocopherols and carotenoids; (c) colorimetric assays for plasma Zn, Cu, Fe and Fe% saturation of transferrin; (d) an antibody-based nephelometric assay for plasma α_1 -antichymotrypsin; (e) an inductively coupled plasma MS assay for plasma Se. In-house quality assessments and inter-laboratory exchanges were undertaken in order to monitor the accuracy and stability of the assays. Plasma α_1 -antichymotrypsin was selected as a medium-duration plasma acute-phase indicator, which tends to remain raised during chronic inflammatory states. Between-run quality-control sample CV were all $\leq 11\%$, and the mean quality-control CV was 5.7%.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Local Research Ethics Committees representing each of the eighty postcode sectors used. The protocol was also approved by the Ethical Committee of the MRC Dunn Nutrition Unit (of which the Micronutrient Status Laboratory is now part of MRC Human Nutrition Research), in Cambridge. Written informed consent was obtained from all the subjects.

The present study included 1054 participants, comprising 538 men and 516 women, with partial or complete data available for the analyses of interest here, all of

whom agreed to be flagged on the National Register of Births and Deaths, and whose status (i.e. as still alive or registered as having died) was known in September 2008. No exclusions, other than those resulting from willingness to participate or the availability of blood samples, were imposed, and there was no evidence for sampling bias. Because of missing values (principally due to incomplete consent availability for the blood sampling), the analyses of the blood biomarker variables are typically based on a subset of approximately 800 participants.

Statistical analysis

Cox proportional hazards models were used, with years of survival as the time scale, to estimate the risk of mortality (all-cause and vascular disease) according to each biochemical and nutritional index. The data were censored to September 2008 in those participants who had survived. The proportional hazards assumption was examined by comparing the cumulative hazard plots, grouped as exposure, wherein no appreciable violations were observed. We used standardised values (*z*-scores) for each of the explanatory variables examined, which have the advantage of expressing the hazard ratios per standard deviation, rather than per measurement unit, thereby achieving an enhanced conformity between the disparate indices. Vascular disease mortality was defined according to International Classification of Diseases – versions 9 (ICD-9: 390–459) and 10 (ICD-10: I01–I99). Cancer mortality was defined according to ICD-9: 140–239 and ICD-10: C00–D48. Respiratory disease mortality was defined according to ICD-9: 460–519 and ICD-10: J00–J99, and all of the above were limited to the primary cause of death.

In the multivariate models, adjustment was made for potential confounders, including age and sex in all models. Since we were only interested in relationships between indices, rather than estimates of prevalence, we did not apply the weighting factors that were used in the data analyses for the Survey Report⁽¹⁾. All tests of statistical significance were based on two-sided probability, and $P < 0.05$ was deemed significant throughout.

Results

Of the community-living survey participants who gave consent for follow-up flagging of the National Health Service Register of deaths, and who had provided at least one index value reported in this follow-up study, 94.5% could be accounted for by known deaths and known survivors. As noted previously in the Subjects and methods section, the blood biomarker analyses are confined to the subset of the participants who provided a blood sample, comprising approximately 800 participants.

Table 1 provides mean and median baseline values, subdivided by sex, for the indices explored in this report.

Table 1. Summary of selected status indices and nutrient intakes in the survey respondents who are included in the present study (*n* 1054) (Mean values and standard deviations; median and range values)

	Male					Female				
	<i>n</i> *	Mean	SD	Median	Range	<i>n</i> *	Mean	SD	Median	Range
Age (years)	538	75.8	6.9	75.0	65–96	516	77.3	7.9	76.0	65–99
Body wt (kg)	532	75.2	12.2	74.6	38.7–121	509	64.0	12.7	63.3	32.5–112.9
Ht (m)	528	1.69	0.07	1.69	1.49–1.98	503	1.55	0.07	1.55	1.20–1.75
BMI (kg/m ²)	527	26.3	3.7	26.1	16.3–43.2	502	26.6	4.8	26.2	14.4–44.6
Biochemical indices										
Plasma vitamin C (μmol/l)	442	38.2	22.6	38.6	<3–101.5	410	45.8	26.1	48.2	<3–116.5
Plasma vitamin A (μmol/l)	409	2.21	0.58	2.15	0.85–5.55	390	2.18	0.66	2.09	0.42–6.8
Plasma α-tocopherol (μmol/l)	409	35.0	9.9	34.2	0.45–7.49	390	39.1	12.4	37.5	10.3–128.0
Plasma γ-tocopherol (μmol/l)	407	2.24	1.06	2.07	0.45–7.49	385	2.53	1.20	2.31	0.57–8.65
Plasma α-carotene (nmol/l)	403	64.0	64.0	51.0	5.0–84.8	385	79.5	84.4	55.0	4.0–88.8
Plasma β-carotene (nmol/l)	408	323	225	273	8–1674	390	405	261	349	37–1960
Plasma β-cryptoxanthin (nmol/l)	399	117	121	77	3–866	389	151	165	98	4–1265
Plasma lutein + zeaxanthin (nmol/l)	409	378	179	340	99–1583	390	389	212	352	66–2189
Plasma lycopene (nmol/l)	408	268	184	238	12–1015	389	274	209	225	8–1262
Plasma Se (μmol/l)	428	95	218	962	375–2376	398	924	211	925	461–1786
Blood glutathione peroxidase (nmol NADPH/mg Hb per min)	412	140	34	133	59–359	369	145	37	13	85–353
Plasma Zn (μmol/l)	377	14.2	2.1	14.1	7.2–20.5	364	14.2	2.4	14.0	8.2–24.2
Plasma Cu (μmol/l)	376	17.4	3.0	17.0	10.4–31.5	362	19.5	3.9	19.3	8.4–38.0
Plasma Fe (μmol/l)	438	13.7	4.9	13.0	2.8–37.4	412	12.0	4.4	11.9	2.2–32.3
Plasma Fe % saturation (%)	437	28.1	11.1	26.7	4.1–91.2	412	24.2	10.3	23.5	4.0–82.7
Plasma α ₁ -antichymotrypsin (g/l)	430	0.38	0.094	0.365	0.16–1.14	408	0.39	0.089	0.385	0.22–1.01
Estimated average daily dietary intakes										
Energy (MJ)	538	7.95	1.94	7.95	3.44–17.3	516	5.95	1.42	5.88	1.91–9.77
Vitamin C (mg)	538	71.1	70.5	59.0	4.9–1023	516	65.4	57.1	49.0	1.0–601
Vitamin A (retinol) (mg)	538	0.94	1.76	0.45	<0.01–20.4	516	0.85	1.56	0.39	0.06–18.8
Vitamin E (mg)	538	9.51	8.18	7.80	0.8–114	516	10.69	39.4	6.09	0.06–18.8
Total carotenoids (mg)	538	1.97	1.55	1.62	0.10–12.0	516	1.62	1.40	1.12	0.06–9.97
Zn (mg)	538	8.81	2.86	8.50	1.86–27.1	516	6.96	2.56	6.52	1.65–23.3
Cu (mg)	538	1.10	0.68	0.97	0.29–6.72	516	0.88	0.55	0.77	0.19–5.87
Non-haem Fe (mg)	538	11.4	10.3	10.0	1.7–174.2	516	10.9	18.4	7.9	2.3–201.5
Haem Fe (mg)	538	0.72	0.57	0.59	0.00–4.83	516	0.53	0.45	0.42	0.00–4.80

* The values for *n* in Table 1 and the maximum values for *n* in Tables 2 and 3 are limited to the numbers definitely known to have died or to have been still alive at the time of the follow-up analysis, i.e. they excluded those (approximately 5% of the original participants) who were lost to follow-up. Where individual index values of *n* are lower in Tables 2 and 3, it was because of missing values, since not all of the respondents provided blood (or sufficient blood) for every one of the assays or calculations⁽¹⁾.

The original Survey Report⁽¹⁾ provided baseline index values for all of the original survey participants, together with further details about the aims of the selection procedures and the methodologies used.

Table 2 shows the age- and sex-adjusted hazard ratios for all-cause and primary vascular disease-cause mortality. Significant predictors of all-cause mortality were as follows: plasma vitamin C, α-carotene, lutein + zeaxanthin, Se, Zn, Cu, Fe (and the % saturation of its carrier, Fe-binding protein or transferrin); plasma α₁-antichymotrypsin, and dietary intakes of energy, vitamin C, carotenoids, Zn and Cu. With the exception of plasma Cu and α₁-antichymotrypsin, all of the significant mortality predictors had hazard ratios below 1.0, which signifies reduced risk. α₁-Antichymotrypsin concentrations are known to increase during inflammatory states, and hence the association of increased concentrations with increased risk, i.e. hazard ratios greater than 1.0. The major Cu-containing plasma protein, caeruloplasmin, like α₁-antichymotrypsin, also increases during inflammatory states, which probably explains the observed association of increased concentrations with increased risk. Serum ferritin (not shown)

was not a significant predictor of mortality, either for all-cause or for the three subcategories of mortality risk considered here. Likewise, neither α-tocopherol nor γ-tocopherol became significant predictors, for all-cause, vascular disease or cancer mortality, even when adjusted for plasma (total) cholesterol (not shown). Intakes of food energy, vitamin C, total carotenoids, Zn and Cu were significant predictors of mortality, with increased intakes being associated with longer survival for each of these nutrients (Table 2). If food energy was included in the model (not shown), then vitamin C and carotenoid intakes still retained their prediction significance ($P=0.003$ and 0.046 , respectively), whereas the other nutrient intakes shown in Table 2 lost their significance ($P>0.05$).

When subdivided by sex (not shown), the predictive power of the biochemical status indices was similar for both men and women, with the exception of plasma Cu, which was significant only for men (hazard ratio 1.60 (95% CI 1.35, 1.90) ($P\leq 0.001$)) compared with women (hazard ratio 1.06 (95% CI 0.93, 1.22) ($P=0.4$)). The predictive power of nutrient intakes was significant ($P<0.05$)

for men for food energy, vitamins C and E, carotenoids, Zn and Cu, but was NS for women, for any of these nutrient intakes. After adjustment for food energy, intakes of vitamin C and carotenoids remained significant for men ($P=0.03$ for both).

For primary vascular disease mortality, comprising approximately 26% of all mortality, the significant predictors were similar to those for all-cause mortality; however, plasma α -carotene, lutein + zeaxanthin and dietary energy, and intakes of carotenoids and Cu failed to achieve significance at the 5% probability level here.

For primary cancer mortality, comprising approximately 20% of all mortality, the significant biochemical index predictors were similar to those for all-cause mortality, with the exceptions that plasma lutein + zeaxanthin, Cu and α_1 -antichymotrypsin were NS. Here, the only dietary intakes that were significant were those of vitamins C and E.

For primary respiratory disease mortality, comprising approximately 16% of all mortality, the significant biochemical index predictors were similar to those for all-cause mortality, with the exception that plasma α -carotene, Cu, Fe and α_1 -antichymotrypsin were NS, but lycopene became marginally significant. The only dietary intake to achieve significance here was that of total carotenoids.

When subdivided both by sex and by primary cause of mortality (not shown), the most striking sex differences

again arose with respect to plasma Cu, which was a significant predictor of primary vascular disease or cancer or respiratory disease mortality, for males only, but not for females. Dietary intake of vitamin E was a significant predictor of cancer and respiratory disease mortality in males but not in females, and dietary intake of carotenoids was a significant predictor of respiratory disease mortality in males but not in females. However, in contrast to previous studies which have described significant mortality prediction by low serum or plasma vitamin C concentrations in men but not in women^(2,6-8), the present study found similar strengths of mortality prediction by plasma vitamin C in both men and women.

About 19% of the study respondents were regularly taking dietary supplements that contained vitamin and/or mineral components, at baseline. However, subsequent mortality was not significantly predicted by the supplemental intakes of any of the above-mentioned vitamins or minerals, and the mortality prediction patterns were similar in the (81%) non-supplement users, to those of the entire cohort (not shown).

Exclusion of those respondents (approximately 7%) who died in less than 2 years after the baseline fieldwork made little difference to any of the index predictions of mortality (not shown); therefore the observed patterns of mortality prediction appear not to have been

Table 2. Age- and sex-adjusted risk for the biochemical and nutritional indices, for all-cause and primary vascular disease mortality* (Hazard ratios and 95% confidence intervals)

	All-cause mortality: died <i>n</i> 717, alive <i>n</i> 337			Vascular disease mortality: died <i>n</i> 189, alive <i>n</i> 337*		
	Age- and sex-adjusted hazard ratios	95% CI	<i>P</i>	Age- and sex-adjusted hazard ratios	95% CI	<i>P</i>
Biochemical indices (per SD)						
Plasma vitamin C ($\mu\text{mol/l}$)	0.81	0.74, 0.88	<0.001	0.83	0.71, 0.98	0.02
Plasma vitamin A ($\mu\text{mol/l}$)	0.96	0.87, 1.06	0.4	1.01	0.84, 1.21	0.9
Plasma α -tocopherol ($\mu\text{mol/l}$)	0.96	0.87, 1.06	0.4	1.07	0.90, 1.29	0.4
Plasma γ -tocopherol ($\mu\text{mol/l}$)	0.96	0.87, 1.05	0.3	0.91	0.75, 1.11	0.4
Plasma α -carotene (nmol/l)	0.90	0.81, 0.99	0.04	1.01	0.85, 1.20	0.9
Plasma β -carotene (nmol/l)	0.92	0.84, 1.01	0.08	0.96	0.81, 1.15	0.7
Plasma β -cryptoxanthin (nmol/l)	0.91	0.83, 1.01	0.07	1.04	0.86, 1.26	0.7
Plasma lutein + zeaxanthin (nmol/l)	0.91	0.83, 1.00	0.04	0.90	0.75, 1.08	0.3
Plasma lycopene (nmol/l)	0.92	0.84, 1.01	0.09	0.86	0.71, 1.05	0.15
Plasma Se ($\mu\text{mol/l}$)	0.76	0.69, 0.84	<0.001	0.73	0.61, 0.87	0.001
Blood glutathione peroxidase (nmol NADPH/mg Hb per min)	0.94	0.85, 1.03	0.2	1.00	0.84, 1.20	1.0
Plasma Zn ($\mu\text{mol/l}$)	0.79	0.72, 0.87	<0.001	0.73	0.61, 0.88	0.001
Plasma Cu ($\mu\text{mol/l}$)	1.27	1.14, 1.42	<0.001	1.35	1.12, 1.63	0.002
Plasma Fe ($\mu\text{mol/l}$)	0.81	0.74, 0.89	<0.001	0.79	0.67, 0.94	0.008
Plasma Fe % saturation (%)	0.84	0.76, 0.92	<0.001	0.79	0.67, 0.94	0.009
Plasma α_1 -antichymotrypsin (g/l)	1.22	1.14, 1.32	<0.001	1.12	1.05, 1.35	0.005
Daily dietary intakes (per SD)						
Energy (MJ)	0.86	0.79, 0.94	0.001	0.83	0.70, 1.00	0.05
Vitamin C (mg)	0.88	0.80, 0.97	0.008	0.93	0.81, 1.08	0.4
Vitamin A (mg)	1.00	0.93, 1.07	1.0	0.95	0.82, 1.10	0.5
Vitamin E (mg)	1.00	0.93, 1.08	0.9	1.06	0.90, 1.23	0.5
Total carotenoids (mg)	0.89	0.82, 0.96	0.003	0.95	0.83, 1.10	0.5
Zn (mg)	0.89	0.82, 0.96	0.003	0.84	0.71, 0.99	0.04
Cu (mg)	0.91	0.84, 1.00	0.04	0.92	0.78, 1.10	0.4
Non-haem Fe (mg)	1.03	0.94, 1.13	0.5	1.06	0.94, 1.20	0.4
Haem Fe (mg)	1.03	0.97, 1.10	0.3	0.93	0.82, 1.07	0.3

* As explained in the legend to Table 1, these were the study maximum values for *n*; the actual values for each index were the same as shown in Table 1.

Table 3. Age- and sex-adjusted risk for the biochemical and nutritional indices for primary cancer and primary respiratory disease mortality* (Hazard ratios and 95% confidence intervals)

	Cancer mortality: died <i>n</i> 140, alive <i>n</i> 337			Respiratory disease mortality: died <i>n</i> 112, alive <i>n</i> 337*		
	Age- and sex-adjusted hazard ratios	95% CI	<i>P</i>	Age- and sex-adjusted hazard ratios	95% CI	<i>P</i>
Biochemical indices (per SD)						
Plasma vitamin C ($\mu\text{mol/l}$)	0.81	0.66, 0.99	0.035	0.78	0.63, 0.96	0.02
Plasma vitamin A ($\mu\text{mol/l}$)	0.90	0.73, 1.12	0.4	0.88	0.70, 1.11	0.3
Plasma α -tocopherol ($\mu\text{mol/l}$)	0.96	0.87, 1.06	0.4	0.76	0.60, 0.96	0.02
Plasma γ -tocopherol ($\mu\text{mol/l}$)	0.89	0.71, 1.13	0.3	0.84	0.65, 1.08	0.2
Plasma α -carotene (nmol/l)	0.67	0.47, 0.96	0.03	0.94	0.75, 1.18	0.6
Plasma β -carotene (nmol/l)	0.87	0.69, 1.10	0.25	0.84	0.67, 1.05	0.13
Plasma β -cryptoxanthin (nmol/l)	0.86	0.66, 1.13	0.3	0.80	0.58, 1.09	0.16
Plasma lutein+zeaxanthin (nmol/l)	1.06	0.87, 1.30	0.5	0.77	0.60, 0.99	0.04
Plasma lycopene (nmol/l)	1.06	0.87, 1.30	0.5	0.77	0.59, 1.00	0.05
Plasma Se ($\mu\text{mol/l}$)	0.72	0.58, 0.89	0.002	0.65	0.51, 0.82	<0.001
Blood glutathione peroxidase (nmol NADPH/mg Hb per min)	1.00	0.81, 1.23	1.0	0.83	0.65, 1.05	0.12
Plasma Zn ($\mu\text{mol/l}$)	0.69	0.55, 0.86	0.001	0.79	0.62, 0.99	0.04
Plasma Cu ($\mu\text{mol/l}$)	1.18	0.92, 1.49	0.2	1.12	0.89, 1.41	0.3
Plasma Fe ($\mu\text{mol/l}$)	0.73	0.59, 0.89	0.002	0.83	0.66, 1.04	0.11
Plasma Fe % saturation (%)	0.72	0.58, 0.89	0.002	0.93	0.74, 1.16	0.5
Plasma α_1 -antichymotrypsin (g/l)	1.08	0.91, 1.27	0.4	1.15	0.98, 1.36	0.09
Daily dietary intakes (per SD)						
Energy (kJ)	0.82	0.67, 1.01	0.06	0.85	0.66, 1.08	0.2
Vitamin C (mg)	0.73	0.57, 0.94	0.015	0.81	0.62, 1.05	0.11
Vitamin A (mg)	1.03	0.88, 1.019	0.7	0.87	0.70, 1.09	0.2
Vitamin E (mg)	0.13	0.04, 0.42	0.001	0.32	0.10, 1.08	0.07
Total carotenoids (mg)	0.92	0.77, 1.10	0.3	0.74	0.59, 0.93	0.01
Zn (mg)	0.86	0.71, 1.04	0.11	0.88	0.70, 1.09	0.2
Cu (mg)	0.87	0.71, 1.04	0.2	0.96	0.78, 1.18	0.7
Non-haem Fe (mg)	1.05	0.93, 1.18	0.4	0.97	0.79, 1.27	1.0
Haem Fe (mg)	1.18	1.03, 1.35	0.014	0.96	0.80, 1.15	0.7

* As explained in the legend to Table 1, these were the study maximum values for *n*; the actual values for each index were the same as shown in Table 1.

disproportionately affected by early deaths, and hence by the effects of serious illness on appetite or status indices at baseline.

Tables 4–7 show which of the nutrient indices survive into multivariate models: part (a) in each table contains just age, sex, and each single status and dietary indices that were found to be significant as shown in Tables 2 and 3; part (b) contains all of the significantly predictive nutrient variables plus age and sex, followed by removal of those which then became non-significant in the multivariate model; part (c) contains these same indices, plus five further 'risk' indices that were measured at baseline, namely: α_1 -antichymotrypsin (acute-phase status indicator), plasma creatinine (renal status indicator), plasma total and HDL-cholesterol concentrations (traditional vascular disease risk indicators), plasma albumin concentration (frailty indicator); and finally part (d) also contains BMI, systolic blood pressure, current smoking index, number of prescribed drugs being taken, self-reported health score, physical activity score and receipt (or not) of certain state benefits (a potential index of relative poverty). For all-cause mortality (Table 4), only the mineral status indices (for Se, Zn, Cu and Fe) plus dietary energy remained significant in the partly adjusted multivariate model (b), with only plasma Se, Zn and dietary energy surviving to the fully adjusted model (d). In Tables 5

and 6, somewhat similar patterns were observed for primary vascular and primary cancer mortality; however, for the latter (alone), haem Fe was a significant predictor, and indeed, became progressively more significant in the more fully adjusted multivariate models. Dietary vitamin E (but not dietary energy) was significant for cancer mortality. From Table 7, it can be observed that only plasma α -tocopherol and dietary carotenoids were significant predictors for respiratory disease mortality. It can be observed from Tables 4–7 that for some of the nutritional variables and mortality types, there was a steady progression towards decreasing significance of mortality prediction in the more fully adjusted models, whereas for other nutritional variables and mortality types, the prediction significance either stayed essentially the same or even increased in the more fully adjusted models.

Discussion

Because the predictive value of conventional risk factors for disease and mortality appears to diminish with advancing age⁽¹⁹⁾, recent attention has focused on the discriminative ability of novel risk markers in elderly cohorts⁽²⁰⁾. The purpose of the present paper is to explore the predictive significance of a subset of the biochemical status indices and nutrient intakes that were measured at baseline as

Table 4. Multivariate hazard ratios for nutritional indices and intakes for all-cause mortality (Hazard ratios and 95 % confidence intervals)

	Hazard ratios (per SD)	95 % CI	P	Hazard ratios (per SD)	95 % CI	P
	Model (a): died <i>n</i> 717, alive <i>n</i> 357*			Model (b): died <i>n</i> 482, alive <i>n</i> 242		
Plasma Se ($\mu\text{mol/l}$)	0.76	0.69, 0.84	<0.001	0.82	0.73, 0.91	<0.001
Plasma Zn ($\mu\text{mol/l}$)	0.79	0.72, 0.87	<0.001	0.84	0.76, 0.93	0.001
Plasma Cu ($\mu\text{mol/l}$)	1.27	1.14, 1.42	<0.001	1.20	1.07, 1.34	0.002
Plasma Fe ($\mu\text{mol/l}$)	0.81	0.74, 0.89	<0.001	0.87	0.78, 0.96	0.005
Dietary energy (MJ/d)	0.86	0.79, 0.94	0.001	0.86	0.77, 0.96	0.007
	Model (c): died <i>n</i> 472, alive <i>n</i> 241			Model (d): died <i>n</i> 403, alive <i>n</i> 226		
Plasma Se ($\mu\text{mol/l}$)	0.82	0.74, 0.92	0.001	0.83	0.73, 0.94	0.004
Plasma Zn ($\mu\text{mol/l}$)	0.88	0.79, 0.98	0.017	0.86	0.76, 0.97	0.017
Plasma Cu ($\mu\text{mol/l}$)	1.08	0.94, 1.22	0.3	1.02	0.88, 1.18	0.8
Plasma Fe ($\mu\text{mol/l}$)	0.87	0.78, 0.96	0.005	0.94	0.84, 1.05	0.3
Dietary energy (MJ/d)	0.87	0.77, 0.98	0.027	0.87	0.77, 0.98	0.03

* As explained in the legend to Table 1, these were the study maximum values for *n* (i.e. those for the dietary variables); the values for the biochemical indices were lower, see Table 1. These models, for all-cause mortality, follow on from the left-hand data column in Table 2. For the calculations in part (a), each single (nutrient) variable was entered into the Cox proportional hazards model, together with age and sex (as in Tables 2 and 3). For those in part (b), all of the significant ($P < 0.05$) vitamin or mineral predictor variables from Table 3, plus age and sex, were initially included together in the model; then those which fell below the assigned $P < 0.05$ significance cut-off were successively removed, yielding just the significantly predictive nutrient variables in this multivariate model. For the calculations for the models in part (c), the significantly predictive vitamin or mineral indices or intakes from model (b) were further adjusted by the inclusion of α_1 -antichymotrypsin (an acute-phase indicator), plasma creatinine (a renal status indicator), plasma total and HDL-cholesterol concentrations and plasma albumin concentration. For the calculations in model (d), BMI, systolic blood pressure, current smoking index, number of prescribed drugs being taken, self-reported health score, physical activity score and receipt (or not) of certain state benefits (as an index of poverty) were further added to the variables in model (c).

part of the original population surveillance protocol of the National Diet and Nutrition Survey of People Aged 65 Years and Over, with a specific focus on those nutrients that are known to modulate redox status in living tissues.

Important strengths of the present study are that, as far as possible, the population sample was chosen as being statistically representative of the community-living people of mainland Britain in 1994–5. A wide range of nutrition-related factors was measured at baseline, including questionnaire-derived socio-demographic information, a 4 d weighed diet estimate, anthropometric measurements, haematology, blood and urine biochemistry (including a large number of nutritional indices), dental assessment, etc., and the follow-up period for mortality outcomes was substantial, i.e. 13–14 years. One inevitable weakness, invariably associated with any cross-sectional national survey, was the fact that the baseline measures were sampled at a single time-point only. It was thus, in

principle, unable to address the issues of long-term causal pathways, or of intervening events occurring after the baseline measures. Another weakness was that cost considerations and database limitations precluded the inclusion of some potentially desirable indices: for instance, Se intakes could not be calculated at baseline because British food Se contents were not sufficiently well established. Nevertheless, the National Diet and Nutrition Survey series has probably included more relevant biochemical and nutrient intake indices than most population surveys except the National Health and Nutrition Examination Surveys (NHANES) of North America.

In the present study, both the status indices and the nutrient intakes which significantly predicted early mortality appeared to be driven by basic dietary nutrient concentrations rather than by dietary supplements. Our conclusions from Tables 2–5, that plasma concentrations of vitamin C, certain carotenoids and Se can be significant

Table 5. Multivariate hazard ratios for nutritional indices and intakes for vascular mortality* (Hazard ratios and 95 % confidence intervals)

	Hazard ratios (per SD)	95 % CI	P	Hazard ratios (per SD)	95 % CI	P
	Model (a): died <i>n</i> 189, alive <i>n</i> 337			Model (b): died <i>n</i> 129, alive <i>n</i> 242		
Plasma Se ($\mu\text{mol/l}$)	0.73	0.61, 0.87	0.001	0.77	0.64, 0.94	0.01
Plasma Zn ($\mu\text{mol/l}$)	0.73	0.61, 0.88	0.001	0.77	0.63, 0.93	0.008
Plasma Cu ($\mu\text{mol/l}$)	1.35	1.12, 1.63	0.002	1.33	1.10, 1.60	0.002
Dietary energy (MJ/d)	0.83	0.70, 1.00	0.05	0.79	0.64, 0.98	0.03
	Model (c): died <i>n</i> 125, alive <i>n</i> 241			Model (d): died <i>n</i> 105, alive <i>n</i> 226		
Plasma Se ($\mu\text{mol/l}$)	0.81	0.66, 0.99	0.04	0.84	0.67, 1.06	0.14
Plasma Zn ($\mu\text{mol/l}$)	0.81	0.66, 1.00	0.05	0.83	0.65, 1.07	0.15
Plasma Cu ($\mu\text{mol/l}$)	1.19	0.96, 1.47	0.12	1.02	0.80, 1.30	0.9
Dietary energy (MJ/d)	0.75	0.60, 0.94	0.014	0.83	0.64, 1.07	0.16

* Please see the legend to Table 4 for an explanation of the four models, which follow on from the data, for primary vascular disease mortality, in the right-hand data column in Table 2.

Table 6. Multivariate hazard ratios for nutritional indices and intakes for cancer mortality* (Hazard ratios and 95 % confidence intervals)

	Hazard ratios (per sd)	95 % CI	P	Hazard ratios (per sd)	95 % CI	P
	Model (a): died <i>n</i> 140, alive <i>n</i> 337			Model (b): died <i>n</i> 92, alive <i>n</i> 243		
Plasma Se ($\mu\text{mol/l}$)	0.72	0.58, 0.89	0.002	0.79	0.63, 0.99	0.04
Plasma Zn ($\mu\text{mol/l}$)	0.69	0.55, 0.86	0.001	0.70	0.56, 0.88	0.002
Dietary vitamin E (mg/d)	0.13	0.04, 0.42	0.001	0.23	0.06, 0.84	0.026
	Model (c): died <i>n</i> 90, alive <i>n</i> 242			Model (d): died <i>n</i> 87, alive <i>n</i> 227		
Plasma Se ($\mu\text{mol/l}$)	0.76	0.60, 0.96	0.024	0.78	0.60, 1.01	0.06
Plasma Zn ($\mu\text{mol/l}$)	0.74	0.58, 0.95	0.019	0.74	0.57, 0.96	0.03
Dietary vitamin E (mg/d)	0.16	0.04, 0.65	0.011	0.19	0.04, 0.81	0.025

*If haem Fe is added to these models of cancer mortality, it is a significant predictor, higher values being associated with greater cancer mortality, as follows: Model 1, 1.18 (1.03, 1.35) (0.014); Model 2, 1.15 (0.99, 1.34) (0.06); Model 3: 1.27 (1.08, 1.49) (0.003); Model 4: 1.26 (1.06, 1.50) (0.008). Please see the legend to Table 4 for an explanation of the four models, which follow on from the data, for primary cancer mortality, in the left-hand data column in Table 3.

predictors of subsequent all-cause or cardiovascular or cancer mortality in older adults, are in agreement with several other studies in Western countries during the past two decades^(2-14,21-24). Both plasma vitamin C and Se also predicted mortality from primary vascular, cancer and respiratory mortality; however, the predictive power of individual plasma carotenoids was more variable, between these subcategories of mortality risk. Se prediction (but not that of vitamin C) persisted into six of the eight multivariate models as shown in Tables 4 and 5, thus appearing to be, perhaps surprisingly, robust. (The fact that blood glutathione peroxidase, another putative index of Se status, failed to predict mortality in any of the models tested may be due to the fact that this enzyme only reflects Se status in situations of relative Se deficiency⁽²⁵⁾, and indeed, the correlation between plasma Se and blood glutathione peroxidase was comparatively weak in the population studied⁽²⁶⁾.) Recent research on Se in human nutrition has suggested a wide range of possible interactions with disease processes, including some important protective effects^(16,27). A study based on the Third National Health and Nutrition Examination Survey in the USA⁽²⁸⁾ found a non-linear association between serum Se concentration and all-cause or cancer mortality. The range of plasma Se concentrations found in the British National Diet and Nutrition Survey was generally lower than that of the US study, and included only very few (approximately 1%) that fell into the

region of high Se concentrations that predicted shorter survival in the US study; therefore our conclusion that higher baseline Se concentrations are predictive of longer survival in Britain is entirely consistent with the observations of the US study.

Plasma α -tocopherol, which was not a significant predictor for all-cause mortality, became significant for respiratory mortality as shown in Tables 3 and 5, and somewhat surprisingly, vitamin E intake became significant (only) for cancer mortality as shown in these same two tables; carotenoid intake becoming significant (only) for respiratory disease mortality as shown in these two tables. The observation that plasma vitamin E differed fundamentally from the estimated vitamin E intake in its mortality prediction capacity seems unsurprising in view of the relative weakness of the inter-index correlation between these two indices (only approximately 1.6% of the variance of plasma α -tocopherol was explained by the variation in vitamin E intake, based on Pearson's correlation). All of the vitamins studied plus Se (with regard to its role at the active catalytic centre of the glutathione peroxidase enzymes) are considered to be primarily anti-oxidant (and hence protective) *in vivo*, although several of them can also exhibit pro-oxidant (i.e. possibly deleterious) properties⁽²⁹⁻³²⁾. Dietary supplements of some carotenoids, in particular, have been associated with an increased risk of some degenerative diseases^(33,34), and a recent meta-analysis⁽³⁵⁾ has found consistent evidence

Table 7. Multivariate hazard ratios for nutritional indices and intakes for respiratory disease mortality* (Hazard ratios and 95 % confidence intervals)

	Hazard ratios (per sd)	95 % CI	P	Hazard ratios (per sd)	95 % CI	P
	Model (a): died <i>n</i> 482, alive <i>n</i> 242			Model (b): died <i>n</i> 129, alive <i>n</i> 242		
Plasma α -tocopherol ($\mu\text{mol/l}$)	0.76	0.60, 0.96	0.02	0.77	0.60, 0.98	0.03
Carotenoids intake (mg/d)	0.74	0.59, 0.96	0.01	0.72	0.56, 0.91	0.008
	Model (c): died <i>n</i> 407, alive <i>n</i> 227			Model (d): died <i>n</i> 123, alive <i>n</i> 259		
Plasma α -tocopherol ($\mu\text{mol/l}$)	0.80	0.63, 1.01	0.06	0.87	0.67, 1.14	0.3
Carotenoids intake (mg/d)	0.77	0.57, 1.04	0.09	0.86	0.62, 1.19	0.4

*Please see the legend to Table 4 for an explanation of the four models, which follow on from the data, for primary respiratory disease mortality, in the right-hand data column in Table 3.

for deleterious effects, on all-cause mortality, for dietary supplements containing vitamins A, E and/or β -carotene.

Less well studied, however, is the relationship of the mineral nutrient indices plasma Zn and Fe, as potential predictors of future mortality. Tables 2–5 suggest that, in the present study, these were all comparatively robust predictors, not only of all-cause-mortality but also of two of the three subcategories of primary mortality from vascular diseases and cancer, while plasma Zn also predicted mortality from primary respiratory disease. Whereas Zn is generally considered to be ‘antioxidant’ and therefore protective *in vivo*^(36,37), which may, in turn, be linked to its well-established immunoprotective functions^(17,38), in redox-modulatory terms, the opposite is true for Fe, which often exerts pro-oxidant potential. However, Fe is, of course, an essential nutrient, and Fe deficiency is one of the commonest recognised mineral deficiency disease in human populations. Since in the present study, both Zn and Fe were associated with reduced mortality risk, it appears unlikely that their redox-modulatory properties are dominant here. Deficiency effects (on mortality) seem likewise unlikely, although possible. More probable, however, is their relationship with debility and risk of mortality through their relationship with chronic inflammation and the acute-phase reaction. Both plasma Zn and plasma Fe (or the Fe % saturation of Fe-binding transferrin) are negative acute-phase reactants and were associated with reduced mortality risk here. Plasma Cu, like plasma α_1 -antichymotrypsin, is a positive acute-phase reactant, and both of these indices were associated with increased risk of all-cause and primary vascular disease mortality (Table 2). Neither index, however, significantly predicted primary cancer or respiratory disease mortality (Table 3), suggesting that these two status indices may tend to change in parallel with each other. In this context, vascular disease is usually considered to be a long-term chronic condition, possibly characterised by an inflammatory state at baseline, whereas death from respiratory disease may more often arise from acute infections near the end of life (e.g. bronchitis, pleurisy, etc.), which might be one of the reasons why plasma α_1 -antichymotrypsin and Cu were significant predictors of vascular disease mortality but not of respiratory disease (or cancer) mortality here. As observed from Table 4, when α_1 -antichymotrypsin was introduced as part of a suite of adjuster indices (i.e. Table 4 part (b) *v.* part (a)), plasma Cu lost its significance for the prediction for all-cause and vascular disease mortality; however, this was not necessarily true for plasma Zn or Se (Tables 4 and 5).

The pattern of significant nutrient intake predictors in the present study seems, in some respects, to be predictable, but in others it is somewhat surprising. The fact that (increased) energy intake predicted (reduced) mortality in three of the models as shown in Tables 2 and 4 may simply reflect the fact that a robust appetite reflects relatively sound health at baseline. Prediction by intakes

of vitamins C and E and carotenoids as shown in Tables 2, 3 and 5 may reflect ‘healthy lifestyles’, including ‘healthy’ food choices, and factors such as healthy dental status and ability to chew fruit and other rich sources of these and related nutrients, including vitamin E and certain minerals⁽³⁹⁾. The observed relationship between higher haem Fe intakes and higher risk of mortality attributed to cancer (in Table 3) seems consistent with the prevalent view that certain categories of meat intake may represent a risk factor for incidence of some cancers, especially bowel cancer.

The fact that only dietary energy survived as a significant predictor in the fully adjusted models as shown in Tables 4 and 5 may reflect the fact that many nutrient intakes are strongly correlated with energy intakes; however, the survival of dietary vitamin E (cancer mortality) and carotenoids (respiratory disease) in the absence of significance for dietary energy may imply a special significance for these nutrients in these diseases, and thus deserves future investigation. It is well known that variable misreporting of dietary intakes is a major unresolved problem for the interpretation of all surveys that include the estimation of nutrient intakes. Our survey sought to minimise this problem by the use of robust 4 d diet estimates based on weighed food intakes; furthermore, any measurement error present would result in attenuation of the observed relationships rather than the strengthening of relationships. However, we acknowledge that some uncertainty remains in this respect. In the present study, energy intake was found to be a significant predictor of mortality when standardised values were used, but it failed to reach conventional significance when its values were not standardised. In contrast, the key biochemical (status) indices all remained significant predictors of mortality, whether or not they were standardised.

From Tables 4–7, it is clear that whereas the prediction significance of some of the nutritional indices was progressively attenuated after additional variables were included in the multivariate models, in other instances there was a little change, or even an increase in significance. Such variations may help to assess the relative robustness of mortality prediction, by the different (e.g. nutrient) variables, and for the different mortality categories.

In conclusion, a number of baseline nutrient status indices with ‘redox-modulatory’ connotations appear to predict all-cause, primary vascular disease, cancer or respiratory disease mortality in older British adults. Of these, plasma Se, Zn and Fe appeared to be especially robust, with plasma vitamins C, E and carotenoids being predictive in some, but not all, of the models examined. Some of the status indices (especially plasma Cu) appeared to reflect acute-phase status; others (including dietary intakes of certain nutrients) may have reflected the robustness of appetites and dentition, and ‘healthiness’ of lifestyles (see Khaw *et al.*⁽⁴⁰⁾). Future studies should attempt to determine, first, which nutrients are the most frequent predictors

of all-cause and specific-cause mortality in different populations, and second, whether these predictions can imply causal relationships, such that dietary or other interventions might promote disease-free longevity.

Acknowledgements

The survey was commissioned jointly by the Department of Health and the Ministry of Agriculture, Fisheries and Food, whose survey responsibility has since been transferred to the Food Standards Agency. It was carried out by the National Centre for Social Research, formerly Social and Community Planning Research in conjunction with the Micronutrient Status Laboratory of the MRC Dunn Nutrition Unit, now part of MRC Human Nutrition Research. The survey datasets were obtained from the survey commissioners, the University of Essex Data Archive and the Social Survey Division of the Office for National Statistics. We are indebted to Dr H. Trevor Delves and staff of the Trace Element Unit of the University of Southampton for the Se analyses and to Claire Deverill and Marie Sanchez (National Centre for Social Research) for their assistance in obtaining the mortality data. All the three authors were active in the data analysis, its interpretation and in preparing the manuscript, and in addition, G. D. M. advised on, and interpreted, the statistical analyses. There were no conflicts of interest. Funding was provided by the Medical Research Council. C. J. B. coordinated the dataset, drafted and submitted the manuscript and contributed to the data analysis; M. H. and G. D. M. contributed to the data analysis and helped to revise the manuscript.

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