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Biochemical risk indices, including plasma homocysteine, that prospectively predict mortality in older British people: the National Diet and Nutrition Survey of People Aged 65 Years and Over

Christopher J. Bates[1]*, Mohammed A. Mansoor[2], Kristina D. Pentieva[3], Mark Hamer[4] and Gita D. Mishra[4]

[1]*MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK*
[2]*Department of Natural Sciences, University of Agder, Kristiansand, Norway*
[3]*Northern Ireland Centre for Food and Health, School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK*
[4]*Department of Epidemiology and Public Health, University College London, London WC1E 6BT, UK*

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Predictive power, for total and vascular mortality, of selected indices measured at baseline in the British National Diet and Nutrition Survey (community-living subset) of People Aged 65 Years and Over was tested. Mortality status and its primary and underlying causes were recorded for 1100 (mean age 76·7 (SD 7·5) years, 50·2 % females) respondents from the baseline survey in 1994–5 until September 2008. Follow-up data analyses focussed especially on known predictors of vascular disease risk, together with intakes and status indices of selected nutrients known to affect, or to be affected by, these predictors. Total mortality was significantly predicted by hazard ratios of baseline plasma concentrations (per SD) of total homocysteine (tHcy) (95 % CI) 1·19 (1·11, 1·27), pyridoxal phosphate 0·90 (0·81, 1·00), pyridoxic acid 1·10 (1·03, 1·19), α_1-antichymotrypsin 1·21 (1·13, 1·29), fibrinogen 1·14 (1·05, 1·23), creatinine 1·20 (1·10, 1·31) and glycosylated Hb 1·23 (1·14, 1·32), and by dietary intakes of energy 0·87 (0·80, 0·96) and protein 0·86 (0·77, 0·97). Prediction patterns and significance were similar for primary-cause vascular mortality. The traditional risk predictors plasma total and HDL cholesterol were not significant mortality predictors in this age group, nor were the known tHcy-regulating nutrients, folate and vitamin B_{12} (intakes and status indices). Model adjustment for known risk predictors resulted in the loss of significance for some of the afore-mentioned indices; however, tHcy 1·34 (1·04, 1·73) remained a significant predictor for vascular mortality. Thus, total and primary vascular mortality is predicted by energy and protein intakes, and by biochemical indices including tHcy, independent of serum folate or vitamin B_{12}.

British National Survey of older adults: Mortality prediction: Intakes and biochemical indices: Plasma homocysteine: B-vitamins and inflammatory indices

Relationships between biochemical status indices and later morbidity and mortality experience can help to predict causal relationships, and thereby to clarify physiological and pathological mechanisms that may be related to important disease risk factors in human subjects. Some indices and risk factors can be modified through lifestyle adjustment, and are thus amenable to public health intervention. Here, we have focussed on mortality outcomes of the community-living participants of 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. Because the predictive value of conventional risk factors appears to diminish with advancing age[(2)], recently, attention has been focused on the discriminative ability of novel risk markers in elderly cohorts[(3)]. The purpose of the present paper is to explore the predictive significance of a subset of the novel biochemical indices, measured as part of the original population surveillance protocol, with specific focus on plasma total homocysteine (tHcy), which has been associated with vascular disease incidence and mortality, and certain other disease risks[(3–23)], and the functionally related nutrients folate, riboflavin, vitamin B_{12} and vitamin B_6, together with biochemical indicators of inflammatory status, renal function and blood glucose exposure.

Subjects and methods

The survey procedures have been described in detail elsewhere[(1)]; therefore, only a brief summary is given here. Originally, 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 is included herein. Participants were drawn from eighty randomly selected postcode sectors in mainland Britain, allocated to four sequential 3-month fieldwork 'waves' corresponding to the four seasons, beginning in October 1994. Demographic, socio-economic

Abbreviations: HbA1c, glycosylated Hb; ICD, International Classification of Diseases; tHcy, total homocysteine.

* **Corresponding author:** Dr Christopher J. Bates, email chris.bates@mrc-hnr.cam.ac.uk

and other information were obtained by a trained interviewer at the participants' 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[1,24,25] that are relevant to the present study are (a) the reverse-phase high-performance liquid chromatographic assay used for measuring plasma tHcy, in which the samples were pre-treated with sodium borohydride, sulphosalicylic acid, dithioerythritol and monobromobimane[24]; (b) the microbiological assay used for measuring serum folate, which was based on the growth of chloramphenicol-resistant *Lactobacillus casei* and developed in-house by the Haematology Laboratory of Addenbrookes Hospital, Cambridge, UK, in 1994–5; (c) the commercial competitive protein-binding radioassay (Bio-Rad (Hercules, CA, USA) Quantaphase II Folate Radioassay kit, based on a milk protein folate binder bound to microbeads) used for measuring red cell folate; (d) the intrinsic factor-based competitive binding radioassay used for measuring vitamin B_{12}, which used purified gastric intrinsic factor as the selective binder, and was developed in-house by the Haematology Laboratory of Addenbrookes Hospital, Cambridge, UK, in 1994–5; (e) the chromatographic assay with fluorescence detection for measuring vitamin B_6[25]; (f) the assay used for the determination of the activation coefficient of erythrocyte glutathione reductase, index of riboflavin status, which is the ratio of the enzyme activity with added FAD cofactor to that without added cofactor *in vitro*, which was developed at the MRC Dunn Nutrition Unit, Cambridge, UK, in 1994–5; (g) the specific nephelometric immunoassays used for measuring plasma α_1-antichymotrypsin and fibrinogen, with the α_1-antichymotrypsin assay being based on its binding to a specific commercial (Dako, Glostrup, Denmark) antibody to α_1-antichymotrypsin raised in rabbits; (h) the commercial colorimetric assays used for the determination of the indices of lipid status (plasma total and HDL cholesterol and fasting TAG; Roche (Basel, Switzerland) Unimate kits); (i) the HPLC (Department of Clinical Chemistry, Addenbrookes Hospital, in-house method) for the determination of blood glucose exposure (glycosylated Hb (HbA1c)). Participation in the available UK external quality assessment schemes for lipid, folate and vitamin B_{12} assays was undertaken; however, analogous quality assessment schemes were not available for most of the other indices; therefore, in-house quality assessments and inter-laboratory exchanges were undertaken in order to monitor the accuracy and stability of the assays. Where available (i.e. for plasma tHcy, cysteine, cysteinylglycine, pyridoxal phosphate, pyridoxic acid, α_1-antichymotrypsin, TAG, total and HDL cholesterol, creatinine, serum folate and vitamin B_{12}, red cell folate and erythrocyte glutathione reductase activation coefficient), all the between-run quality control sample CV were $\leq 11\,\%$, and the mean quality control CV was $5{\cdot}7\,\%$.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving the human subjects were approved by each of 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 1100 participants, comprising 547 men and 553 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.

Statistical analysis

Cox proportional hazards models were used, with years 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 participants who survived, and were age adjusted, thereby including both the participants' post-survey duration of survival (up to September 2008) and their age at baseline, in the mortality risk calculations. Vascular disease mortality was defined according to the International Classification of Diseases (ICD) – version 9 (ICD-9) and version 10 (ICD-10), (ICD-9: 390–459; ICD-10: I01–I99), and it was limited to the primary cause of death, i.e. by excluding those with only secondary or underlying vascular causes.

In the basic multivariate model, we adjusted for potential confounders including age and sex in all the models, and then by adding other known (BMI, physical activity, alcohol consumption, receipt of welfare benefit and cigarette smoking) or observed correlates of mortality risk or disease and the nutrients that are known to influence tHcy. We adjusted for each risk factor separately in a sequential fashion.

The proportional hazards assumption was evaluated by testing the null hypothesis that the proportional hazards assumption holds for all variables simultaneously (the global test) and for each individual covariate. These were obtained from the statistical package STATA 10.0 command stphtest. The predictive power of tHcy for all-cause and vascular mortality was also tested by calculating the areas under the receiver operating characteristic curves. The areas were compared with the neutral value of 0·5 indicating risk prediction by chance alone, and 95 % CI were calculated using all-cause mortality and vascular mortality *v.* survival. All the tests of statistical significance were based on two-sided probability.

Results

Of the (free-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 in terms of known deaths plus known survivors, and it is their data that are included in this report.

Table 1 provides mean and median baseline values, subdivided by sex, for the indices explored in this report. The original survey report[1] provided baseline index values for all the original survey participants, together with further details about the aims of the selection procedures and the methodologies used.

Table 1. Summary of selected status indices and nutrient intakes in those survey respondents who are included in the present study

(Mean values and standard deviations; medians and ranges, *n* 1100)

	Male					Female				
	*n**	Mean	SD	Median	Range	*n**	Mean	SD	Median	Range
Age (years)		75·9		75	65–96	543	77·6		77	65–99
Body weight (kg)	551	75	12	75	39–121	536	64	13	63	33–113
BMI (kg/m^2)	546	26·3	3·7	26·1	16–43	529	26·6	4·8	26·2	14–45
Waist (cm)	550	97·7	10·9	98·1	47·5–129	537	87·8	11·6	86·5	27–131
Blood pressure (mmHg)										
Systolic	536	150	22	149	92–222	518	156	24	153	94–224
Diastolic	536	80	13	80	47–123	518	78	15	77	40–137
Pulse rate (per min)	536	68	13	66	41–134	518	72	11	71	46–110
Biochemical indices										
Plasma tHcy (μmol/l)	361	16·3	7·5	15·2	5·8–95·6	355	15·2	6·4	13·8	4·4–54·9
Plasma Cys (μmol/l)	359	262	30	259	167–393	353	262	30	261	129–366
Plasma cysteinylglycine (μmol/l)	359	36·6	8·5	35·3	17·2–64·1	352	36·0	8·6	34·7	14–66
Serum folate (nmol/l)	461	15·4	10·3	12·5	0·9–40·8	442	16·6	10·6	13·4	2–40·8
Red cell folate (nmol/l)	460	496	292	424	60–2216	426	507	297	425	78–2357
Serum vitamin B_{12} (pmol/l)	457	226	103	207	49–737	438	238	124	214	48–737
Plasma pyridoxal phosphate (nmol/l)	456	40·2	26·8	36·8	5–319	431	43·7	41·7	34	6·5–397·5
Plasma pyridoxic acid (nmol/l)	457	17·7	10·6	15·3	4–103	431	19·6	25·0	14·8	4·5–372·5
EGRAC (ratio)	448	1·31	0·18	1·27	1·0–2·2	427	1·30	0·16	1·27	1·02–2·41
Plasma α_1-antichymotrypsin (g/l)	445	0·38	0·10	0·37	0·16–1·15	421	0·40	0·09	0·39	0·23–1·01
Plasma fibrinogen (g/l)	428	5·8	1·8	5·5	1·3–11	401	5·8	1·5	5·6	2–11
Plasma TAG (mmol/l)	443	1·57	0·93	1·33	0·43–7·06	414	1·62	0·77	1·45	0·29–5·93
Plasma total cholesterol (mmol/l)	443	5·45	1·2	5·5	2·1–9·4	414	6·18	1·5	6·1	2·4–11·5
Plasma HDL cholesterol (mmol/l)	443	1·17	0·45	1·1	0·49–4·09	414	1·39	0·45	1·3	0·5–2·95
Blood HbA1c (%)	451	5·3	1·2	5·0	3–12·4	425	5·10	0·93	5·0	3·3–11·9
Estimated average daily dietary intakes										
Energy (kJ)	540	7935	1942	7949	3437–17 301	521	5924	1414	5864	1908–9769
Fat (g)	540	74·0	23	72	20–221	521	57	18	56	14–221
Protein (g)	540	70·3	17	70	17–124	521	55·0	14	55	15–105
Folate (μg)	540	263·4	95	253	75–728	521	204	75	193	27–535
Vitamin B_{12} (μg)	540	5·9	6·4	4·3	0·55–87·2	521	4·3	4·4	3·2	0·66–42·8
Vitamin B_6 (mg)	540	2·1	0·7	2·0	0·4–6·0	521	1·6	0·5	1·5	0·14–3·93
Vitamin B_2 (riboflavin; mg)	540	1·7	0·7	1·7	0·2–7·29	521	1·4	0·6	1·3	0·3–3·9

tHcy, Total (plasma) homocysteine; EGRAC, erythrocyte glutathione reductase activation coefficient (index of riboflavin status; ratio of two enzyme activities); HbA1c, glycosylated Hb (expressed as the percentage of total Hb).

* Maximum values for *n* in Tables 1 and 2 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 (about 5 % of the original participants) who were lost to follow-up. Where individual index values of *n* are lower in Tables 1 and 2, it was because of missing values, since not all the respondents provided blood (or sufficient blood) or intake data for every one of the assays or calculations[1].

Table 2 shows the age-and sex-adjusted hazard ratios for all-cause and primary vascular disease-cause mortality for the indices of interest here. Significant predictors of all-cause mortality were plasma total tHcy, pyridoxal phosphate, α_1-antichymotrypsin, fibrinogen and creatinine; blood HbA1c and dietary intakes of energy and protein. For primary vascular disease mortality, comprising about 27 % of all mortality, the significant predictors were similar, with only plasma pyridoxal phosphate and energy intake failing to achieve significance at the 5 % probability level. Importantly, the traditional lipid biochemical risk factors plasma total and HDL cholesterol were not significant mortality predictors either for all-cause or for primary vascular mortality.

In order to test whether all-cause mortality prediction was heavily dependent on pre-existing disease (not shown), the all-cause mortality hazard ratio analyses were repeated after elimination of each of the three subgroups of respondents with evidence of pre-existing disease: model a, those who reported 'very poor' self-assessed health at baseline (159 of 1200 eliminated); model b, those who survived less than 2 years after baseline (263 eliminated); and model c, those regularly taking any prescribed medication for vascular disease (617 eliminated). The only substantive change in prediction significance compared with the results shown in Table 2 was that pyridoxic acid lost significance in model c. We concluded from this evidence that all-cause mortality prediction probably was not heavily influenced by pre-existing disease.

Elimination of the ninety-nine subjects who were taking either folic acid or vitamin B_{12} or vitamin B_6 (or a combination of these) as dietary supplements made essentially no difference to any of the index predictions of mortality, except for pyridoxic acid, which lost significance as a result of this elimination.

Model adjustment for a number of known risk factors for all-cause mortality (not shown) did not, in most instances, seriously affect the prediction power of the indices studied. Thus, model 1 was adjusted for age, sex, BMI, physical activity, alcohol consumption, receipt of welfare benefit, cigarette smoking, energy intake, folate, vitamin B_{12}, vitamin B_6 intakes and plasma creatinine; model 2 was adjusted for age, sex, BMI, physical activity, alcohol consumption, receipt of welfare benefit, cigarette smoking, energy intake, folate, vitamin B_{12}, vitamin B_6 intakes, plasma creatinine, plasma pyridoxal phosphate, plasma pyridoxic acid, plasma α_1-antichymotrypsin, plasma fibrinogen and blood HbA1c. In model 3 but not in model 2, plasma fibrinogen lost significance, and tHcy became marginally significant, but energy intake, plasma creatinine, α_1-antichymotrypsin and blood HbA1c all retained their significance.

Similar model adjustments were tested for primary vascular mortality. Here, plasma tHcy and energy intake remained significant predictors after the same multiple adjustments as are listed above for all-cause mortality, but plasma creatinine, fibrinogen, α_1-antichymotrypsin and blood HbA1c did not.

The predictive value of tHcy alone assessed by the area under the receiver operating characteristic curve was 0·66 (95 % CI 0·43, 0·88) for all-cause mortality, and it was 0·58 (0·31, 0·86) for primary vascular mortality (receiver operating characteristic curve not shown). In the sex, age and tHcy

Table 2. Age- and sex-adjusted hazard ratios for biochemical and nutritional indices*

(Hazard ratios and 95 % confidence intervals)

	All-cause mortality died (*n* 749, alive *n* 351)*		Vascular disease mortality died (*n* 199, alive *n* 351)*	
	Age- and sex-adjusted hazard ratios	95 % CI	Age- and sex-adjusted hazard ratios	95 % CI
Biochemical indices (per SD)				
Plasma tHcy (μmol/l)	1·19	1·11, 1·27	1·36	1·13, 1·63
Plasma Cys (μmol/l)	1·06	0·97, 1·16	1·09	0·91, 1·30
Plasma cysteinylglycine (μmol/l)	0·96	0·87, 1·06	0·91	0·75, 1·11
Serum folate (nmol/l)	0·93	0·86, 1·01	0·96	0·82, 1·12
Red cell folate (nmol/l)	0·97	0·90, 1·05	1·02	0·89, 1·17
Serum vitamin B_{12} (pmol/l)	0·94	0·86, 1·02	0·95	0·80, 1·12
Plasma pyridoxal phosphate (nmol/l)	0·90	0·81, 1·00	0·99	0·85, 1·17
Plasma pyridoxic acid (nmol/l)	1·10	1·03, 1·19	1·17	1·07, 1·29
EGRAC (ratio)	1·05	0·97, 1·14	0·91	0·77, 1·08
Plasma α_1-antichymotrypsin (g/l)	1·21	1·13, 1·29	1·21	1·06, 1·40
Plasma fibrinogen (g/l)	1·14	1·05, 1·23	1·16	1·00, 1·36
Plasma TAG (mm)	0·96	0·88, 1·05	0·96	0·80, 1·15
Plasma total cholesterol (mmol/l)	0·90	0·83, 0·99	0·89	0·73, 1·08
Plasma HDL cholesterol (mmol/l)	1·02	0·94, 1·11	1·03	0·85, 1·25
Blood HbA1c (%)	1·23	1·14, 1·32	1·32	1·11, 1·57
Plasma creatinine	1·20	1·10, 1·31	1·25	1·05, 1·49
Dietary data (per SD)				
Energy (kJ)	0·87	0·80, 0·96	0·86	0·72, 1·02
Fat (g)	1·10	0·94, 1·29	0·92	0·79, 1·08
Protein (g)	0·86	0·77, 0·97	0·79	0·67, 0·94
Folate (μg)	0·95	0·86, 1·05	0·88	0·75, 1·04
Vitamin B_{12} (μg)	1·01	0·94, 1·10	0·91	0·74, 1·11
Vitamin B_6 (mg)	0·91	0·82, 1·01	0·85	0·72, 1·01
Vitamin B_2 (riboflavin; mg)	0·98	0·90, 1·06	0·87	0·73, 1·03

tHcy, Total (plasma) homocysteine; EGRAC, erythrocyte glutathione reductase activation coefficient (index of riboflavin status; ratio of two enzyme activities); HbA1c, glycosylated Hb (expressed as the percentage of total Hb).

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

model for all-cause mortality, the areas under the receiver operating characteristic curve were significantly greater than those in age–sex model only (0·73 (0·69, 0·77) *v.* 0·69 (0·65, 0·73)). Similar results were obtained for vascular mortality (0·71 (0·65, 0·76) *v.* 0·74 (0·68, 0·79).

The relationships between tHcy and all-cause or vascular mortality exhibited no significant deviations from linearity, suggesting that no specific tHcy threshold effects were detectable.

In view (a) of the strength of mortality prediction by both tHcy and dietary protein intake here, and (b) of a previous report[26] that tHcy and protein intake may be inversely correlated with each other in older adults in the USA, this inter-relationship was investigated. There was a strong inverse linear cross-sectional relationship between ln(tHcy) and protein intake ($t = -6{\cdot}3$, $P < 0{\cdot}0001$) after adjustment for age and sex, which was not substantially altered by inclusion of energy intake, or by folate, vitamin B_{12}, vitamin B_6 or riboflavin in the model. There was a significant, but less strong, direct relationship between protein intake and vitamin B_{12} intake, and there were similar relationships (all essentially unaffected by adjustment for energy intake) between protein intake and intakes of the other three B-vitamins.

Discussion

Important strengths of this 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 were 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), and dental assessment, and the follow-up period for mortality outcomes was substantial, i.e. about 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, although most biomarkers are thought to be sufficiently stable for use in long-term risk prediction[27].

Our conclusion from Table 2 that plasma tHcy, whose concentration was much higher in older community-living British adults than in younger people living in the same community[28], is also a robust and independent predictor of subsequent mortality in these older adults, both for all-cause mortality and especially for primary-cause vascular mortality, is in agreement with a considerable number of studies in Western countries during the past decade[3,6,8,9,12,14,17,19–23,29]. In agreement with certain other studies[3,23,30], we observed no significant mortality prediction from the available blood status indices of folate or vitamin B_{12} (i.e. serum folate, serum vitamin B_{12} and red cell folate) or with the estimates of their intakes, even though these two vitamins are well known to be powerful modulators of plasma tHcy concentrations, including in this population sample[28,31].

Another putative modulator of plasma tHcy concentrations, plasma pyridoxal phosphate, was a modest predictor of mortality in the present study; however, the available evidence suggests that it may reflect acute phase status more strongly than tHcy status[32,33], and that it failed to survive into the multivariate-adjusted models described in the Results section. The same was true for the other measured vitamins[32,33], which also failed to survive in our highly adjusted models.

It has been claimed that tHcy may predict the risk of stroke more powerfully than it predicts that of IHD[34,35]; however, we were unable to test this particular claim in our study, because the ICD information available to us was not sufficiently cause specific. The absence of any detectable non-linearity, or threshold effect, for tHcy with respect to its prediction of all-cause and vascular mortality in our study is consistent with the view[8] that tHcy is a graded risk factor, whereby even a modest reduction from moderate concentrations may be beneficial.

An important question for the interpretation of the observed associations is whether the (biochemical) indices mainly reflect pre-existing disease, or acute phase status, or whether they reflect other frailty causes and their indices, such as impaired renal function. With respect to pre-existing disease, we have shown that the elimination of those respondents who were with very poor self-reported health at baseline, or of those who survived less than 2 years from baseline, or of those who were taking medication for vascular disease at baseline all made essentially no difference to the patterns and strength of prediction by the indices, including tHcy, that are described in the present paper. (It is still possible, of course, that long-term pre-existing disease, especially vascular disease, may have resulted in both raised tHcy and enhanced risk of mortality, and it is difficult, in most studies of this relationship, to determine whether observed increases in tHcy have preceded and thus truly predicted future disease risk, or whether they have been consequent upon them.) With respect to acute phase status, plasma α_1-antichymotrypsin, a medium-term acute phase indicator, proved to be a robust predictor of all-cause mortality in the present study, as (to a lesser degree) did plasma fibrinogen, another index of acute phase status. A recent Dutch study[3], in which tHcy was strongly predictive for vascular mortality in people aged 85 + years, did not observe significant mortality predictive power for two acute phase indicators: C-reactive protein and IL-6. In contrast, Cesari *et al.*[36] and two other studies quoted by them found a significant mortality prediction for C-reactive protein in older adults. In the Uppsala Longitudinal Study[37], although none of the novel biomarkers investigated by themselves had significant impact, the addition of several biomarkers together, including C-reactive protein, substantially improved risk stratification for CVD death beyond that of conventional risk factors. Thus, the putative role for acute phase indicators in subsequent mortality prediction appears to remain uncertain. The same appears to be true for HbA1c and for other indicators of blood sugar exposure and diabetic- or metabolic syndrome-status that are known to be linked to vascular risk. Our study indicated a strong and robust mortality prediction efficacy of HbA1c (Table 2); however, this was greatly attenuated if those subjects who were already, at baseline, taking drugs for the treatment of diabetes[38] were omitted from the analysis (data not shown), and likewise, a recent study in the USA[39] reported that HbA1c failed to predict mortality in non-diabetic older adults.

In our study, three other robust predictors of all-cause mortality (Table 2) proved to be plasma creatinine, energy intake and total dietary protein intake. In older people, kidney function is a known and powerful factor with respect

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to the onset of age-related debility, and likewise, such debility is well known to lead to reductions in appetite and physical activity[40], which is linked in turn to reduced energy expenditure, and thus probably helps to account for the observed associations between all-cause mortality and baseline food energy and protein intakes. A prediction of acute coronary events by relatively low protein intakes was described previously[41]. A study of retired school teachers in the USA has reported an independent inverse relationship between tHcy and protein intake[26], and a similar relationship was observed in the present study, suggesting that dietary protein may have an important modulating effect on tHcy in older people. In our study, there was a moderate direct relationship between protein intake and intakes of vitamin B_{12} and of the other three B-vitamins studied here.

In conclusion, our present study has clearly demonstrated that, in agreement with other recent studies of older adults in Western societies, total plasma tHcy was a strong and independent predictor of early all-cause and vascular mortality in older adults living in the British community. Moreover, this property of tHcy appeared to be independent of its established modulatory relationships with folate and vitamin B_{12} intakes, and their status indices. Other robust predictors of all-cause mortality in the present study included plasma α_1-antichymotrypsin (an acute phase indicator), plasma creatinine (an index of renal status), HbA1c (an index of blood glucose exposure), and energy intake and protein intakes, which were all measured at baseline. Future intervention priorities in ageing populations will be based on an improved understanding of critical mortality risk factors, and of their response to individual- and community-based interventions.

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