Original Article

Dietary diversity no longer offsets the mortality risk of hyperhomocysteinaemia in older adults with diabetes: a prospective cohort study

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Background and Objective: The increased mortality risk of hyperhomocysteinaemia in diabetes may be mitigated by dietary quality. **Methods and Study Design:** The Nutrition and Health Survey in Taiwan of 1999-2000 for elders formed this prospective cohort. Baseline health status, diet and anthropometry were documented and plasma homocysteine and biomarkers for B vitamins measured. Participants without diabetes (n=985) were referent for those who had diabetes or developed diabetes until 2006 (n=427). The effect of homocysteine on mortality risk during 1999-2008 was evaluated. **Results:** Men, smokers and those with poorer physical function had higher homocysteine, but less so with diabetes. Diabetes incidence was unrelated to homocysteine. In hyperhomocysteinaemia (≥15 vs <15 μmol/L), those with diabetes had an adjusted hazard ratio (HR) (95% CI) for mortality of 1.71 (1.18-2.46); *p* for interaction between homocysteine and diabetes was 0.005. Without diabetes, but with hyperhomocysteinaemia and a low dietary diversity score (DDS ≤4 of 6), where the joint mortality hazard for the greater DDS, (>4) and lower homocysteine (<15) was referent, the HR was 1.80 (1.27-2.54) with significant interaction (p=0.008); by contrast, there was no joint effect with diabetes. The contribution of DDS to mortality mitigation in hyperhomocysteinaemia could not be explained by B group vitamins, even though plasma folate was low in hyperhomocysteinaemic participants. With hyperhomocysteinaemia, heart failure was a major cause of death. **Conclusions***:* In non-diabetic hyperhomocysteinaemia, a more diverse diet increases survival prospects independent of B group vitamins, but not in hyperhomocysteinaemic diabetes where the cardiomyopathy may be less responsive.

Key Words: homocysteine, type 2 diabetes, elderly, mortality, dietary diversity

INTRODUCTION

The prevalence of type 2 diabetes continues to increase, reaching prevalences of about 25% among those of Chinese ethnicity beyond the age of 65 years.[1] Homocysteine is a risk factor for a number of diseases which overlap with the complications of diabetes.[2-4] This makes their interaction a candidate for joint pathogenesis. Hyperhomocysteinaemia is a risk factor for diabetes itself[5] and there have been attempts to prevent diabetes by reducing homocysteine.[6] With advancing age, homocysteine increases[7] and may partially account for various age-related pathologies including cardiovascular disease,[8] insulin resistance and diabetes complications,[9] and mood disorders.[10] But people who have diabetes do not always have high plasma homocysteine.[11] It may be lowered with the advent of diabetes[12] or found to be high.[3] Hyperhomocysteinaemia confers a higher mortality risk in people with diabetes.[13]

Homocysteine metabolism has many determinants being genetic and epigenetic, related to personal behaviours like diet (B-group vitamins and n-3 fatty acids), exercise, smoking and alcohol.[14,15] Its toxicity could be related to its conversion to thiolactone which is toxic to endothelial

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cells, with both thiolactone and homocysteine formation dependent on folic acid.[16] Dietary factors are associated with both the development of diabetes and hyperhomocysteinaemia and their consequences. Therefore, we investigated whether homocysteine and diabetes might jointly increase mortality in Taiwanese elders and that this might be mitigated by dietary quality.

METHODS

Research design and participants

The Elderly Nutrition and Health Survey in Taiwan (NAHSIT Elderly) of 1999-2000 provided a nationally representative sample of free-living elders aged 65 and over who formed this prospective cohort (Figure 1).[17] Among those 1442 participants who had a face-to-face interview and health examination, 3 had an incorrect death record, 26 did not have homocysteine measurements and 1 had 2 discordant baseline readings for homocysteine and these were excluded. Some 1412 participants (724 men and 688 women) were eligible for analysis. Informed consent was signed by all participants. The ethics committees of both the National Health Research Institutes and Academia Sinica approved the study protocol (EC0961004).

At baseline, 167 participants (73 men and 94 women) were known to have diabetes. All but 10 (5 men and 5 women) had the diagnosis of type 2 diabetes confirmed from the National Health Insurance (NHI) database at least twice a year so that we studied the 157 who were confirmed (see below). We linked the NAHSIT to the 1999-2006 NHI datasets to ascertain type 2 diabetes incidences during follow-up and found 270 participants (129 men and 141 women) had developed diabetes. Thus the total diabetes population studied was 427. In this paper, diabetes means type 2 diabetes and included those prevalent at baseline and incident cases. In order to determine survival status, we also linked the NAHSIT to the 1999-2008 National Death Registration datasets.

Type 2 diabetes ascertainment

Incident type 2 diabetes was defined by at least two records in NHI within one year of the diagnostic codes A181 (pre-ICD-9 used in Taiwan before 2000), or ICD-9 Code CM-250, without evidence of insulin dependency (e.g., ketoacidosis) or use of any oral antihyperglycaemic agent for more than 3 months. Unless otherwise indicated, we mean type 2 diabetes when we refer to diabetes.

Dietary Diversity Score (DDS)

From a 24-hour recall, a DDS (range 1-6) was derived and previously shown to be predictive of population health outcomes.[17] It is in accordance with the Taiwanese Food Guides where a half serving per day for any one of the six food groups ('dairy', 'egg/soy/fish/meat', 'grain', 'fruit', 'vegetable' and 'fat and oil') is the minimal intake required for a score of 1. Higher scores indicate a better diet. This older population is known to have relatively monotonous dietary patterns over a longer period of time.[17] The intra-class correlation coefficient (ICC) for energy and nutrients for three repeated 24 h dietary recalls ranged from 0.13 to 0.84 with an average of 0.48.[18]

Plasma homocysteine and B group vitamins

Plasma homocysteine was measured as previously reported[19] and other analytes as in the NAHSIT protocols.[20] Plasma was separated and frozen at −80°C until analysis. Homocysteine was measured by automated analyzer (IMMULITE 2000 analyzer and IMMULITE Homocysteine Kit, Diagnostic Products Corporation, LA, USA). Hyper-homocysteinaemia was defined as ≥15 μmol/L.[19]

Biomarkers for B-group vitamin assays were erythrocyte transketolase activation coefficient (TEKAC), erythrocyte glutathione reductase activity coefficient (EGRAC), plasma pyridoxal phosphate (PLP), plasma folate and serum B-12 which have been described elsewhere for this population.[20]

The putative determinants of plasma homocysteine were assessed by multivariable regression.

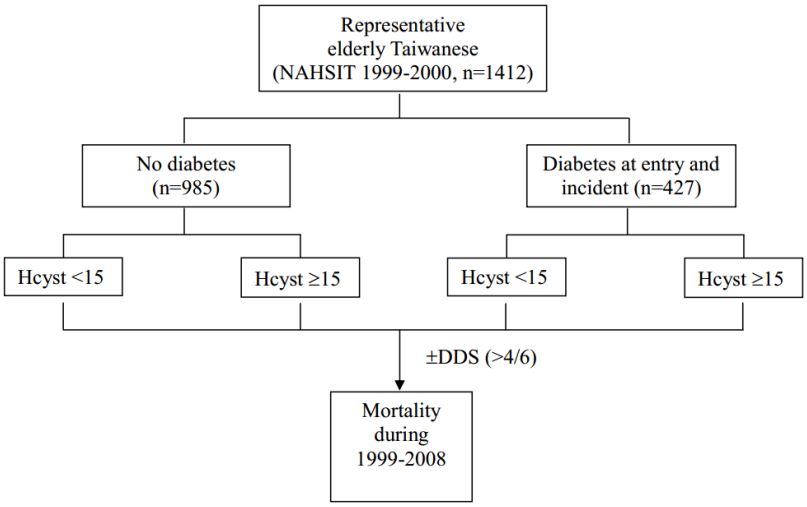


Figure 1. Study design. The association of dietary quality, judged by a dietary diversity score (DDS) (range 1-6), with mortality in representative free-living older people with hyperhomocysteinaemia (≥15 μmol/L) has been assessed in people without and with diabetes (at entry and incident until December 31st 2006). Previous studies have indicated that a DDS ≥4 is associated with better survival in this population and which is the basis of the categorical measurement of dietary quality used. Baseline observations were made in 1999-2000 and mortality ascertained until December 31st 2008.

Other information

Physical functioning and general health was assessed by the Chinese version 36-item Short Form (SF-36).[21] Subjects' responses were grouped into eight subscales following the norm-based scoring system (μ=50, σ=10). We used the physical functioning (PF) score as our measure of physical function and also the perceived general health (GH) score. Co-morbidities were estimated as the sum of 12 chronic diseases at interview.

Statistical analysis

All data were weighted to represent the elderly population in Taiwan during 1999-2000. Chi-square tests and ANOVA were used for categorical variables and continuous variables by homocysteine status (<15 and ≥15 μmol/L), respectively. Cox proportional-hazards models were used to appraise the effect of homocysteine status on risk of mortality during 1999-2008 with covariate adjustments, including age (continuous variable), gender, smoking status (never, former and current), alcohol drinking (never, former and current), body mass index (BMI) (<18.5, 18.5-23.9, 24.0-26.9, ≥27.0 kg/m^2), physical functioning (PF) (quartiles, <45, 45-53.9, 54-57.9, ≥58), general health (GH) (tertiles, <46, 46-56, >56). Missing values for each variable were assigned to a discrete group for model adjustment. The models were further adjusted on this background for DDS and for B-group nutrient intakes and biomarkers. To test the joint effect of variables of interest (diabetes status, homocysteine status and DDS), a zero-order interaction term (the product of the regressors for the two variables) was added to the regression model. All data analyses were performed using SAS statistical software (version 9.1.3) and SUDAAN (version 10.0).

RESULTS

Demographic considerations

The cumulative prevalence of diabetes was 30.2% over 6 years. Women (33.4%) were more likely to develop diabetes than men (27.2%), but women (11.1±0.3 μmol/L) had a lower homocysteine than men (14.2±0.4 μmol/L). The prevalence of hyperhomocysteinaemia (≥15 μmol/L) among participants without diabetes was 24.3% and with diabetes 21.6% (p=0.219) (Table 1). In those without diabetes, homocysteine status was associated with gender (lower in women), age (higher when older), cohabitation (lower), smoking (higher in smokers), drinking alcohol (higher with consumption) and physical function (lower when more active). However, in those with diabetes only gender (lower in women), being indigenous (higher where indigenous), smoking (higher in smokers) and PF (lower when more active) were associated.

Food, B-group vitamins and homocysteine

We found DDS not to be associated with homocysteine regardless of diabetes status (Table 2). However, of individual food categories, soybean product intakes were significantly higher when there was hyperhomocysteinaemia in those with diabetes.

There were no significant associations between B-group vitamin intakes and homocysteine except for vitamin B-2 in those without diabetes where it was lower with hyperhomocysteinaemia (Table 2). With B-group vitamin biomarkers, plasma folate was lower in those with hyperhomocysteinaemia regardless of diabetes status. Other significant biomarkers were lower plasma PLP (vitamin B-6) in those without and a lower serum vitamin B-12, lower in those with diabetes.

The relative contributions of nutritionally-related factors in hyperhomocysteinaemia were considered by multivariable regression analysis and presented as regression coefficients (Table 3). Without diabetes, being a woman and having a better vitamin B-2 status were the strongest determinants, with age and plasma folate also being contributors. However, in diabetes, while gender remained important, higher BMI (≥27.0 kg/m^2) was associated with lower homocysteine and, together with lower plasma folate, PLP and serum vitamin B-12 were associated with higher homocysteine.

Homocysteine, diabetes and mortality: taking account of food and nutrients

A total of 483 participants died during ten years follow-up (median follow-up times for hyper- and normohomocysteinaemic participants were 8.7 and 8.9 years, respectively; these amounted to 2167 and 8474 person-years). In diabetes, compared to those with normal homocysteine (<15 μmol/L) and diabetes-free over six years, hyperhomocysteinaemia was associated with a higher risk of mortality where the hazard ratio (HR) was 1.79 (95% confidence interval (CI), 1.26-2.56) after adjustment for potential covariates (Figure 2). By contrast, in those without diabetes, the corresponding HR was 1.18 (95% CI, 0.92-1.52). With diabetes, but normal plasma homocysteine, the mortality HR was still elevated at 1.42 (95% CI, 1.09-1.86), but less so than with hyperhomocysteinemia. With further adjustments by DDS, candidate foods (soybean products and fish as a source of n-3 fatty acids) and B-group vitamin status (B-1, B-2, niacin, B-6 intakes and ETKAC, EGRAC, PLP, plasma folate and serum B-12), one-by one and altogether, these findings were unchanged in those with diabetes (data not shown).

DDS and the risk of elder mortality related to homocysteine status in diabetes

We considered the contribution of dietary quality to homocysteine-related mortality as a possible joint effect with normal plasma homocysteine and DDS >4 as the referent group. We made the same adjustments described for the risk of mortality with diabetes and homocysteine, except for foods and B-group vitamins. In those without diabetes, when DDS was ≤4 and hyperhomocysteinaemia, the HR was 1.76 (95% CI, 1.29-2.41) with a significant interaction (p=0.004) (Figure 3a). In addition, without hyperhomocysteinaemia, but a low DDS of ≤4, the HR was 1.54 (95% CI, 1.05-2.27). This signifies a joint effect of DDS and homocysteine on mortality risk in those without diabetes. However, the joint effect is not apparent in those with diabetes (Figure 3b).

DISCUSSION

Ethnicity and increased mortality with hyperhomocysteinaemia in diabetes

We have confirmed in a representative, dominantly Chinese population that hyperhomocysteinaemia exacerbates

Table 1. Distributions of demographics and study variables by diabetes and homocysteine status among NAHSIT 1999-2000 participants tracked through the NHI until 2006 (n =1,412)[†‡]

Descriptor	Plasma homocysteine (μmol/L)					
	No diabetes (n=985)			Diabetes (n=427)		
	<15	≥15	*p* value[§]	<15	≥15	*p* value[§]
Proportion	75.7	24.3		78.4	21.6	
Mean (SE) of plasma homocysteine (μmol/L)	12.9 (0.3)			12.5 (0.4)		
	10.5 (0.1)	20.0 (0.5)		10.4 (0.1)	20.2 (0.9)	
Gender (%)			<0.001			0.004
Men	49.8	74.8		41.6	64.9	
Age at baseline, years			<0.001			0.568
<75	62.4	45.4		71.0	66.4	
Education (%)			0.779			0.189
Illiterate	37.5	34.8		26.5	35.9	
Some up to primary School	40.3	43.2		45.5	35.6	
High school and above	22.2	22.0		28.1	28.5	
Ethnicity (%)			0.511			0.050
Non indigenous	97.6	98.1		98.4	99.6	
Marital status (%)			0.232			0.441
Never	2.4	4.3		1.9	5.0	
Yes and live together	67.9	62.5		66.0	60.6	
Separated[¶]	29.7	33.2		32.1	34.4	
DDS (%)			0.478			0.339
≤4	45.9	48.9		45.3	37.9	
>4	54.1	51.1		54.7	62.1	
Smoker (%)			<0.001			0.026
Never	66.0	48.6		70.5	49.1	
Former	13.4	20.5		15.7	28.8	
Current	20.6	30.9		13.8	22.0	
Alcohol drinker (%)			0.107			0.255
No	75.7	66.7		79.0	65.3	
Former	6.4	11.9		5.1	10.1	
Current	17.9	21.4		15.9	24.6	
Physical functioning (%)			0.004			0.048
<45	21.7	31.8		23.8	37.7	
45.0-53.9	31.2	35.6		31.8	19.3	
54.0-57.9	27.2	22.3		27.2	36.3	
≥58.0	19.9	10.3		17.2	6.81	
General health (perceived) (%)			0.466			0.101
<46	32.8	36.0		38.3	44.4	
46-56	38.2	38.6		28.7	32.4	
>56	29.0	25.5		33.1	23.2	
Number of co-morbidities (%)			0.972			0.119
0	22.0	22.0		11.5	16.4	
1-2	45.5	43.7		44.0	31.4	
3-5	27.9	29.2		35.0	42.9	
≥6	4.56	5.19		9.54	9.33	
Body mass index (kg/m^2)			0.434			0.135
<18.5	8.1	8.5		2.4	9.9	
18.5-23.9	51.1	50.8		41.5	43.5	
24.0-26.9	27.5	24.1		31.0	28.7	
≥27.0	13.3	16.6		25.0	18.0	

[†]Percentages are weighted to reflect their representativeness in the population.
[‡]Total sample size is 1412; cases with missing values were not included for the relevant variable.
[§]Chi-square test by SUDAAN program.
[¶]Including spouse deceased, divorced or widowed.

independently the mortality risk in those with diabetes by 1.7-fold. It has been of health concern that hyperhomocysteinaemia in European populations also about doubles the risk of mortality in those with diabetes.[13] The demographics of our study population, in regard to age and ethnicity could be expected to have a range of homocysteine concentrations; we found that the prevalences of hyperhomocysteinaemia were marginally lower than those reported for Europeans which have been found to exhibit the diabetes-homocysteine increased mortality.[13] Yet, whether plasma homocysteine is higher or not in diabetes has been controversial. It has been found, however, that homocysteine is correlated with insulin and its resistance (albeit in obesity)[22] and that hyperhomocysteinaemia can induce impairment of insulin secretion (at least from clonal beta cells).[23] Moreover, there is genetic evidence that homocysteinaemia may be a risk factor for diabetes.[5] It could be apparent that similarities in fasting homocysteine between people with and without type 2 diabetes may be a feature of anti-hyperglycaemic therapy whether

Table 2. Mean energy-adjusted food and nutrient intakes and of B-group vitamin biomarkers, by diabetes and homocysteine status in the NAHSIT elderly (n=1,412)[†]

Descriptor	Plasma homocysteine (μmol/L)					
	No diabetes (n=985)			Diabetes (n=427)		
	<15	≥15	*p value*[‡]	<15	≥15	*p value*[‡]
Dietary diversity score	4.58	4.45	0.187	4.51	4.59	0.650
Daily food intakes (/1000 Kcal)						
Rice and grains (g)	146	151	0.507	143	169	0.285
Fat and oil (g)	9.16	7.60	0.216	9.88	8.4	0.577
Meat (g)	44.2	50.4	0.139	50.1	39.2	0.104
Fish and shellfish (g)	43.1	38.5	0.483	36.1	40.1	0.571
Egg (g)	10.2	10.2	0.980	8.70	12.4	0.157
Dairy (g)	29.0	23.2	0.360	39.2	22.7	0.069
Soybean products (g)	27.5	34.0	0.332	18.7	47.1	0.015
Vegetable (g)	224	181	0.074	226	205	0.298
Fruit (g)	106	106	0.962	112	107	0.823
Daily energy intake (kcal)	1679	1777	0.287	1679	1816	0.408
Daily nutrient densities (/1000 Kcal)						
Protein (g)	42.9	42.0	0.556	42.7	39.4	0.076
Fat (g)	28.6	28.6	0.987	30.1	30.3	0.942
Cholesterol (mg)	129	136	0.641	127	124	0.790
Carbohydrate (g)	142	143	0.726	140	143	0.717
Dietary fibre (g)	13.0	12.2	0.326	13.0	12.2	0.402
Vitamin B-1 (mg)	0.65	0.71	0.182	0.78	0.76	0.780
Vitamin B-2 (mg)	0.90	0.76	0.023	0.91	0.85	0.619
Niacin (mg)	8.74	9.12	0.554	8.42	9.52	0.087
Vitamin B-6 (mg)	0.71	0.77	0.390	0.71	0.72	0.794
Biomarkers (nmol/L)						
ETKAC	1.09	1.10	0.239	1.09	1.08	0.570
EGRAC	1.16	1.19	0.088	1.14	1.12	0.327
Plasma PLP	57.5	42.4	0.004	64.4	49.2	0.067
Plasma folate (ng/mL)	12.3	8.46	<0.001	12.6	9.10	<0.001
Serum B-12	2.46	0.75	0.068	1.63	0.55	0.033

ETKAC: erythrocytetransketolase activation coefficient; EGRAC: erythrocyte glutathione reductase activity coefficient; PLP: pyridoxal phosphate.
Mean, weighted to reflect their representativeness in the population by SUDAAN.
[†]Cases with missing values were not included for the relevant variable.
[‡]ANOVA by SUDAAN program.

oral agents or insulin.[24]

Aside from its more general public health relevance there is reason to be particularly concerned about the implications of these findings for Chinese since there is evidence that the combination of diabetes and hyperhomocysteinaemia may be an ethnically peculiar risk for nephropathy. Taiwanese have among the highest incidence of chronic renal failure in the world, partly related to diabetes.[25] It is conceivable that this might be worsened by hyperhomocysteinaemia. Since hyperhomocysteinaemia is more common in chronic renal failure, a vicious cycle might obtain. Such a phenomenon may not be easily amenable or responsive to a more nutritious diet.

Mechanisms for increased mortality with hyperhomocysteinaemia in diabetes

Given that the most recognized link between hyperhomocysteinaemia and mortality is cardiovascular disease, it would be plausible that this, whose risk is also increased in diabetes, might account for synergy or additive effects of the two factors on mortality. The findings in Chinese with diabetes for an increased risk of nephropathy in the presence of hyperhomocysteinaemia would add to the recognized problem with cardiovascular disease.

The greater adverse effects of hyperhomocysteinaemia in diabetes extend to a number of organs beyond the cardiovasculature including the nervous system[2] and retina.[3] This suggests that there is likely to be an underlying adverse perturbation of homocysteine metabolism or perhaps a pathogenesis common to both hyperhomocysteinaemia and diabetes.[12] In our study, the mortality profiles for those with different combinations of diabetes and hyperhomocysteinaemia differed. Diabetes was the leading cause of death where it was present. Where hyperhomocysteinaemia was present, cardiovascular disease accounted for three (ischemic heart disease (IHD); heart failure; and cerebrovascular disease (CVD)) of the top five causes of death, but only one (IHD) where neither abnormalities was present or two (IHD and CVD) where only diabetes was present (data not shown). It would appear that the way hyperhomocysteinaemia increases mortality risk in those with diabetes is by shifting the mortality profile towards cardiovascular disease. In particular, heart failure features in the top five causes of death only where there is hyperhomocysteinaemia. The most likely pathology to account for this finding would be the presence of a combined diabetic and homocysteinemic cardiomyopathy. Such an eventuality maybe difficult to reverse by nutritional means as our findings would suggest.

Mishra and colleagues have found a synergism between hyperhomocysteinaemia and the attenuation of PPAR gamma which might play a role in diabetic cardio-

Table 3. Multivariable regression model for homocysteine in NAHSIT 1999-2000 participants tracked through the NHI until 2006 (n=1,412)

Descriptor	Regression coefficients (β) for prediction of plasma homocysteine			
	Crude model		Multivariable model	
	No diabetes	Diabetes	No diabetes	Diabetes
Gender (ref: Men)				
Women	-3.25***	-2.88***	-3.08***	2.31**
Age at baseline	0.21***	0.25	0.25***	0.21
Smoker (ref: Never)				
Current	3.20***	2.69**	0.4	-0.38
Former	1.77***	2.02*	-0.89	-0.83
Alcohol drinker (ref: No)				
Former	1.82*	2.73	0.46	0.92
Current	1.01	1.15	-0.16	0.44
Perceived general health (SF-36) (ref: <46)				
46-56	0.29	0.02	-0.38	0.51
>56	-0.31	-1.03	-0.27	-0.92
Body mass index (kg/m^2) (ref: <18.5)				
18.5-23.9	-0.37	-1.28	0.11	-1.36
24.0-26.9	-0.73	-1.95	0.63	-2.19
≥27.0	0.64	-2.72*	1.94	-2.40[a]
Physical functioning (ref: <45)				
45.0-53.9	-0.32	-2.84*	0.41	-2.36*
54.0-57.9	-1.15	-1.38	-0.40	-0.84
≥58.0	-1.56	-2.56	-0.89	-2.23
No. of co-morbidities (ref: 0)				
1-2	0.31	-0.98	0.24	-1.10
3-5	0	0.40	0.10	-0.77
≥6	-0.08	0.14	-0.23	1.40
DDS (ref: ≤4)				
>4	-0.75	-0.38	0.07	0.19
Soy bean (ref: ≤1)				
≥1	-0.50	0.89	-0.24	1.20
Nutrient intakes & biomarker status				
Vitamin B-1	0.33	-0.06	0.19	0.04
Vitamin B-2	-0.28	-0.38	-0.24	-0.26
Niacin	0.05	0.04	0.01	0.04
Vitamin B-6	0.34	-0.54	0.00	0.02
ETKAC	5.15	1.30	0.99	3.37
EGRAC	8.79***	-2.63	4.23*	-4.81
PLP	-0.02***	-0.01*	-0.01	-0.01*
Folate	-0.28***	-0.26***	-0.20***	-0.23***
Vitamin B-12	-0.00	-0.00*	-0.00	-0.00*

ETKAC: erythrocyte transketolase activation coefficient; EGRAC: erythrocyte glutathione reductase activity coefficient; PLP: pyridoxal phosphate.
Significance is shown by $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$.

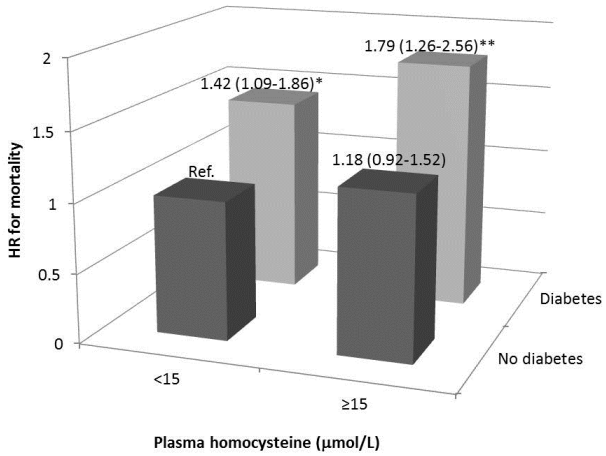
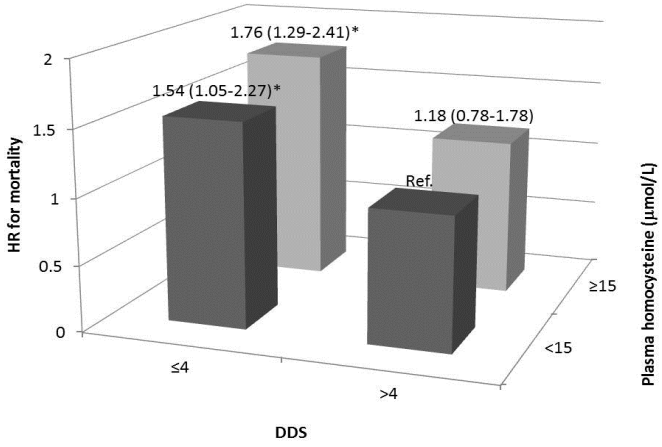
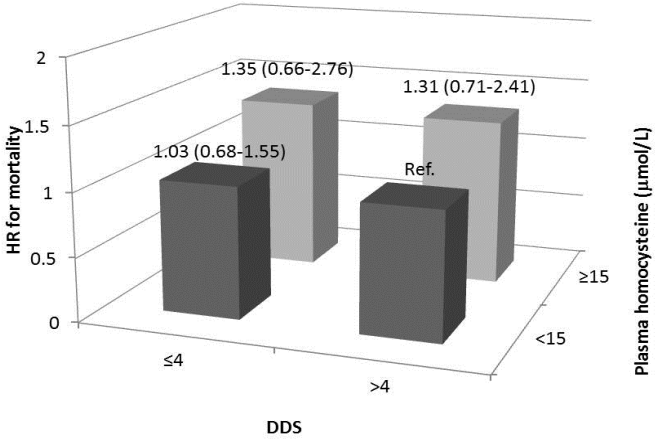


Figure 2. Joint mortality hazards ratios for plasma homocysteine and diabetes status. Cox proportional-hazards regression adjusted for age (year), gender, smoking status (never, former and current), alcohol drinking status (no, former, current), BMI (<18.5, 18.5-23.9, 24.0-26.9, ≥27.0), physical functioning (<45, 45-53.9, 54-57.9, ≥58), perceived general health (<46, 46-56, >56) and number of co-morbidities (0, 1-2, 3-5, ≥6). The interaction of incident diabetes status and homocysteine was $p=0.001$ (n=1412). $^{*}p<0.05$, $^{**}p<0.01$.



(a) Non-diabetic participants



(b) Diabetic participants

Figure 3. Joint mortality hazards ratios for DDS and plasma homocysteine by diabetes status. (a) In non-diabetic participants (n=970), the interaction of DDS and homocysteine was p=0.004. $^{*}p$<0.05. (b) In diabetic participants (n=427), the interaction of DDS and homocysteine was p=0.52. All models used Cox proportional-hazards regression adjusted for age (year), gender, smoking status (never, former and current), alcohol drinking status (no, former, current), BMI (<18.5, 18.5-23.9, 24.0-26.9, ≥27.0), physical functioning (<45, 45-53.9, 54-57.9, ≥58), perceived general health (<46, 46-56, >56) and number of co-morbidities (0, 1-2, 3-5, ≥6).

myopathy and unify several possibilities.[26] It would be useful to know whether protein N-homocysteinylation, which seems to be a common pathway by which homocysteine effects tissue damage, as described by Jakobowski, was exaggerated in diabetes with its own adverse effects on proteins throughout the body by the generation of AGEs.[16] In this case it may be difficult to alter the course of established tissue damage and its contribution to life expectancy with a more diverse diet (see below).

Homocysteine has been suggested to be a cause of type 2 diabetes[5] and may subsequently increase mortality risk in those without diabetes at baseline in our study. We have considered whether time of diabetes diagnosis modifies the mortality risk of hyper-homocysteinaemia. We have performed stratified analyses according to diabetes status, namely prevalent at baseline or incident during follow-up. For those without diabetes at baseline, a higher but not significant HR for mortality of hyper-homocysteineamic diabetes was 1.49 (95% CI, 0.87-2.53) compared with apparently healthy participants. However, diabetes prevalent at baseline increased the mortality risk at least two-fold, but this relationship was not altered by homocysteine status, although there was a significant interaction between diabetes and homocysteine for mortality. Thus, diabetes is a more potent predictor of mortality than homocysteine in this situation (data not shown). Overall, when homocysteine is higher, the presence of diabetes increases the risk of mortality further, irrespective of whether the diabetes is established at baseline or incident in follow-up.

Nutritional factors and hyperhomocysteinaemic-diabetes related mortality

The question then arises as to what might be done to ameliorate these joint effects of hyperhomocysteinaemia and diabetes.

Elevated homocysteine's association with mortality has been demonstrated in many studies.[7,27] Yet despite the ability to reduce homocysteine concentrations by increased intakes of B-group vitamins, there is little evidence that this can change the course of associated cardiovascular disease[28] or other pathology. Folate may be an exception, on account of pathways to macrovascular disease other than via homocysteine.[27] We could not account for the joint homocysteine-diabetes association with mortality by any of the B-group vitamins, including folate, although it and others had lower status. This might be partly because the nutrients involved in homocysteine

metabolism have been incompletely recognized; n-3 fatty acids are an example with implications for the potential of foods which are good sources of them being candidate preventive or management agents.[13] We know as well that the population we have studied is characterized by an increased survival when consuming a varied diet, so improving overall nutritional quality.[17] In the present study, we have found that there was a joint effect of increased dietary variety (DDS) and plasma homocysteine such that greater variety could protect against some of the risk of hyperhomocysteinaemic-mortality in those without diabetes. Unfortunately, this was not evident in those with diabetes and the challenge is to know why.

Gender differences in susceptibility to hyperhomocysteinaemia, which we have confirmed, and in its consequences have suggested hormonal pathways, including net estrogenicity.[29] In that event, a Chinese diet, replete with phytoestrogens from soy-bean products, might be of interest. As it turns out, we found these products to be associated with hyperhomocysteinaemia in diabetes, but not to account for plasma homocysteine in a multivariable regression model. Nor did soy have any evident interaction with homocysteine in determining mortality risk.

That we found a higher BMI in those with diabetes to be associated with a lower homocysteine on multivariable analysis merits comment. It is also known that malnutrition may be associated with hyperhomocysteinaemia so that we may be seeing a difference in accord with underlying nutritional status. BMI did not account detectably for any of the joint association of homocysteine and diabetes on mortality. In any case, in older people, survival is associated with higher rather than lower BMIs.[17]

It is of interest to note what current diabetic pharmacotherapy with metformin might do to the conjoint effects of homocysteine and diabetes, given its promise in the reduction of the complications of diabetes and on mortality.[4] To secure benefit with metformin, attention seems required to energy regulation, which might need to be addressed in parallel to the problem of hyperhomocysteinaemia itself. The apparent resistance to nutritional measures may or may not be entrenched and locked-in. This possibility is raised by the studies of Smith et al in regard to hyperhomocysteinaemia and cognitive impairment, where benefit is seen only with high homocysteine values and supra-physiological does of B group vitamins. This has suggested that some metabolic block (e.g., with n-3 fatty acid deficiency or polymorphism) is at play.[14] A plausible block might be that of insulin resistance in diabetes which has been shown to affect homocysteine metabolism.[30]

Limitations

We are unable to exclude residual confounding especially in relation to food intake and metabolic changes which may have taken place during the period of observation. This information was not available to us through the NHI administrative data base.

Extrapolation to populations other than Taiwanese must be circumspect. Nevertheless, since the majority of participants were of Han Chinese ancestry and a definable minority was indigenous Malayo-Polynesian, the findings are likely to be relevant to their counterparts in other countries. We have also noted gender differences as suggested elsewhere.[25,31] It remains possible that ethnicity and gender may reveal joint effects of diabetes and homocysteine which we have not fully ascertained.

One reason why dietary-homocysteine linkages may not have been recognizable in the present diabetes population is that Chinese diets tend to be skewed towards plant-derived food. Li and others have shown that such diets may be associated with elevated plasma homocysteine, sometimes due to inadequate intake not only of vitamin B-12, but also of n-3 fatty acids.[32] Insofar as our methodology allowed, we found no explanation in differences in fish (and, therefore, n-3 fatty acid sourced from fish) intake.

In conclusion, diabetes increases the mortality risk of elevated homocysteine in elderly Taiwanese of dominantly Chinese ancestry, particularly in those who have established diabetes at baseline rather than those with diabetes incident in follow-up. Dietary diversity reduces the mortality risk of homocysteinaemia in this population, but not in those who have diabetes. These findings are not predicated on B-group vitamin status. For those with hyperhomocysteinaemia or diabetes, avoidance of the other of the two is a priority.

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AUTHOR DISCLOSURES

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Original Article

Dietary diversity no longer offsets the mortality risk of hyperhomocysteinaemia in older adults with diabetes: a prospective cohort study

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飲食多樣性無法抵銷高同半胱氨酸血症老年糖尿病患者的死亡風險：一個前瞻性世代研究

背景與目的：飲食品質可能減輕糖尿病患因高同半胱氨酸血症增加的死亡風險。**方法與研究設計**：1999 - 2000 年臺灣老人營養健康狀況調查形成這個前瞻世代。基線健康狀況、飲食和體位測量被記錄，血漿同半胱氨酸和 B 群維生素生物標記被測量。非糖尿病的參與者（n=985）當作那些已經有糖尿病或至 2006 年期間發生糖尿病者（n=427）之參考組，評估參與者於 1999-2008 年間同半胱氨酸對死亡風險的影響。**結果**：男性、吸菸者和那些身體生理功能較差者，有較高的同半胱氨酸，但糖尿病患者並非如此。糖尿病發生率與同半胱氨酸無關。在高同半胱氨酸寫症患者中（≥15 vs <15 mol/ L），患有糖尿病者，其調整後之死亡危害比（HR）及 95％信賴區間為 1.71（1.18-2.46）；同半胱氨酸與糖尿病之間的交互作用 *p* 值 0.005。非糖尿病患者，以有較高的飲食多樣性得分（DDS >4）及較低的同半胱氨酸血症者為參考組，高同半胱氨酸血症者加上較低的 DDS（≤4）的聯合死亡風險更大 HR 為 1.80 （1.27-2.54）及顯著的交互作用（*p*=0.008）；相較之下，對糖尿病並無聯合作用。儘管有高同半胱氨酸血症的參與者其血漿葉酸濃度較低，DDS 減輕同半胱氨酸血症對死亡的貢獻並無法被維生素 B 群所解釋。心臟衰竭是高同半胱氨酸血症參與者的主要死因。**結論**：在非糖尿病的高同半胱氨酸血症患者，無論維生素 B 群的狀況，較多樣化的飲食可以增加存活率。但是，對高同半胱氨酸血症的糖尿病患者，其心肌病變對飲食的反應可能較差。

關鍵字：同半胱氨酸、2 型糖尿病患者、老年人、死亡率、飲食多樣性