Original Article

Association of B vitamins status and homocysteine levels in elderly Taiwanese

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To investigate the relationship between homocysteine (Hcy) and B vitamins status in the Taiwanese elderly population, an analysis was made of the plasma Hcy levels in elderly persons. The study sample was taken from the Elderly Nutrition and Health Survey in Taiwan (1999-2000) (Elderly NAHSIT) and included 1094 males and 1135 females aged 65-90 years. The results showed that average plasma Hcy was 13.3 ± 0.6 μmol/L for males and 10.6 ± 0.7 μmol/L for females. The average plasma Hcy levels of males from all age groups were significantly higher than those of females, and significantly increased with age (*P* trend <0.0001). The overall prevalence of hyperhomocysteinemia (Hcy >15 μmol/L) was 23.4% for elderly males and 11.2% for elderly females, and this also increased with age (*P* trend <0.0001). In subjects with normal renal function, folate, vitamin B_2, B_6, and B_{12} status were significantly lower in males with hyperhomocysteinemia, while only folate and vitamin B_{12} were significantly lower in females with hyperhomocysteinemia. Further analysis suggested that folate, vitamin B_6 or B_{12} insufficiency were associated with hyperhomocysteinemia in both sexes, while vitamin B_2 insufficiency was significantly associated only in males. In elderly persons with adequate folate, vitamin B_6, and B_{12} status, there was no significant association between vitamin B_2 and hyperhomocysteinemia. This association occurred only in those who had concurrent poor folate, vitamin B_6, or B_{12} status. The strength of the association between vitamin B_{12} insufficiency and hyperhomocysteinemia was not affected by simultaneous vitamin B_2 or B_6 insufficiency, but increased about 3-fold when combined with folate. This suggests that poor folate and vitamin B_{12} status has a synergistic effect on the risk of hyperhomocysteinema in the elderly, as did a poor folate and vitamin B_6 status. Therefore, maintaining adequate vitamin B_{12} status and avoiding multiple B vitamin insufficiency, especially that of folate and vitamin B_{12} or B_6, should be emphasized as an important measure for reducing plasma Hcy levels among elderly Taiwanese.

Key Words: folate, vitamin B_2, vitamin B_6, vitamin B_{12}, homocysteine, elderly, Elderly Nutrition and Health Survey in Taiwan (1999-2000)

Introduction

Homocysteine (Hcy) is a sulphur-containing amino acid derived from the metabolism of methionine, which is the only dietary precursor of Hcy. Several B vitamins are involved in Hcy metabolism. Vitamin B_6 is the coenzyme for cystathionine β-synthase, which irreversibly converts Hcy to cystathionine. Vitamin B_{12} is the coenzyme for 5-methyltetrahydrofolatehomocysteine *S*-methyltransferase, which remethylates Hcy to methionine. The 5-methyltetrahydrofolate form of folate donates the methyl group in this reaction. Afterwards, 5-methyltetrahydrofolate is re-formed through the enzyme methylenetetrahydrofolate reductase (MTHFR), for which riboflavin (vitamin B_2) is required as the coenzyme.[1,2] Therefore, Hcy metabolism may be affected by the status of these B vitamins.

Several studies have shown that hyperhomocysteinemia has been associated with CVD and other age-related diseases in elderly people.[3-5] Such findings have led to increased attention to Hcy concentrations in the elderly population. Studies have shown that blood Hcy concentration exhibits an inverse association with concentrations of vitamin B_2, B_6 and B_{12} and in particular blood folate, in subjects in the lower quartile of blood B vitamin levels.[10,11] Prior investigations have found that elderly persons are at higher risk than younger persons for deficiencies of B vitamins, leading to elevated plasma Hcy concentrations.[7,12] As a result, maintaining good B vitamin status is important for elderly persons.

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A previous survey, the Nutrition and Health Survey in Taiwan 1993-1996 (NAHSIT 1993-1996), indicated that elderly people had a high prevalence of folate insufficiency.[13] This has led to increased attention to Hcy levels in the Taiwanese elderly population. Therefore, the aim of this study was to determine plasma Hcy concentrations in the Taiwanese elderly population, and to assess the associated status of B vitamins such as B_2, B_6, B_{12} and folate.

Subjects and Methods

Subjects

The subjects in this study were sampled from the Taiwanese elderly population as part of the Elderly NAHSIT project by a multistage, stratified sampling method. A detailed description of sampling design can be found in Pan *et al's* report.[14] The sample consisted of 2229 subjects aged 65 to 90 years, including 1094 males and 1135 females. In order to determine the relationship between plasma Hcy levels and various B vitamins, subjects with serum creatinine ≥1.5 mg/dL were excluded to eliminate the effect of renal function on Hcy clearance. This resulted in 1350 subjects, including 656 males and 694 females, who had complete data from physical examinations and interviewer-administered questionnaires.

Biochemical analyses, data collection and definition of vitamin B insufficiency

Plasma was separated immediately after sampling and frozen at -80°C until analysis. Plasma Hcy was measured by automated analyzer (IMMULITE 2000 analyzer and IMMULITE Hcy Kit, Diagnostic Products Corporation, LA, USA). A series of quality control tests were performed to evaluate the precision of this assay. These tests +confirmed both the between- and within-run consistency of this method. The Coefficient of Variation (CV) for the Hcy assay was 7.1 %.

Data collection included physical examinations, biochemical measurements of blood and interviewer-administered questionnaires. The data items included height, weight, systolic blood pressure, diastolic blood pressure, sex, age, smoking history, alcohol intake, plasma folate, erythrocyte glutathione reductase activity coefficient (EGRAC), pyridoxal phosphate (PLP) and serum vitamin B_{12}. Height and weight were used to calculate body mass index (weight in kg/height^2 in meters).

Although there is considerable debate about the definition of hyperhomocysteinemia, with suggested cut-offs ranging from 9 to over 20 μmol/L, hyperhomocysteinemia was defined as a Hcy plasma level >15 μmol/L in our study as this is the cut-off used most fre-quently.[7,15,16] Insufficient vitamin B_2 status was defined as an EGRAC value of ≥1.2, a functional test for vitamin B_2.[17] Insufficient folate was defined as a plasma folate ≤14 nmol/L (6 ng/ml).[18,19] Insufficient vitamin B_6 status was defined as a plasma pyridoxal phosphate <30nmol/L,[20] and insufficient vitamin B_{12} status was defined as a serum cobalamin ≤ 258 pmol/L.[21]

Statistical analyses

Statistical analysis was carried out using the SAS program (SAS/STAT Version 8.0, SAS Institute, Cary, NC). As the Elderly NAHSIT was conducted in a stratified, multistage probability design, sample weighting by SUDAAN, SAS-callable version 8.0 was used to account for the complex survey design in the variance estimates. The data was analyzed by gender, and age was grouped into the age groups 65-69, 70-74, 75-79, and 80 and older. The Student's *t* test was used for analysis of differences in continuous variables between the groups with and without hyperhomocysteinemia. Differences were considered significant if $P < 0.05$. Multiple logistic regression was used to obtain adjusted odds ratios (AOR) and 95 percent confidence intervals (95% CIs). Further analyses were done in which the data were stratified to investigate the AOR and 95% CIs of insufficiency in one or two B vitamins in relation to hyperhomocysteinemia. All estimates were adjusted for potential confounders including age, systolic blood pressure, diastolic blood pressure, smoking and alcohol intake.

Results

The frequency distribution of various plasma Hcy levels in the Taiwanese elderly population is shown in Fig 1. The average plasma Hcy levels and prevalence of hyperhomocysteinemia are shown in Table 1. The average plasma Hcy level was 13.3 ± 0.6 μmol/L for males and 10.6 ± 0.7 μmol/L for females aged 65-90 years. The age-specific total plasma Hcy concentrations were significantly higher in males than in females for each age group, and significantly increased with age (*P* trend <0.0001). Furthermore, using the cut-off of 15μmol/L for hyper-homocysteinemia,[15,16] our results showed that the prevalence of hyperhomocysteinemia was 23.4% for males and 11.2% for females. A higher percentage of hyperhomocysteinemia in males compared to females was noted in all age groups. In addition, a significant age

Table1. Plasma homocysteine levels and prevalence of hyperhomocysteinemia in the Taiwanese elderly, by age

		Males	
Age group	*N*	Homocysteine [1] (μmol/L)	Hyperhomocysteinemia[2] % (*N*)
65-69	421	12.6 ± 0.5*	17.6 (74)
70-74	377	13.1 ± 0.6*	21.8 (82)
75-80	203	14.1 ± 0.6*	32.0 (65)
80~	93	15.6 ± 1.5*	37.7 (35)
Total	1094	13.3 ± 0.6*	23.4 (256)
P trend		<0.0001	<0.0001
		Females	
Age group	*N*	Homocysteine [1] (μmol/L)	Hyperhomocysteinemia[2] % (*N*)
65-69	467	9.7 ± 0.6	6.2 (29)
70-74	350	10.8 ± 0.6	10.0 (35)
75-80	198	11.3 ± 0.6	14.7 (29)
80~	120	12.7 ± 1.0	28.3 (34)
Total	1135	10.6 ± 0.7	11.2 (127)
P trend		<0.0001	<0.0001

[1]All values are shown as mean±SE or number of participants.
[2]Hyperhomocysteinemia: plasma homocysteine levels >15 μmol/L.
*Significantly different from the female values ($P<0.0001$).

trend (P trend<0.0001) in the prevalence of hyperhomocysteinemia was noted in both males and females.

Epidemiological studies have shown that the level of Hcy is increased in chronic renal insufficiency.[22] Decreased renal function with reduced clearance of Hcy results in elevated plasma Hcy levels which are inversely related to serum creatinine levels.[23] Therefore, to investigate the association between B vitamin status and Hcy levels, subjects with a serum creatinine ≥ 1.5 mg/dL were excluded. The clinical characteristics of the elderly subjects with normal renal function by plasma Hcy level are shown in Table 2. Folate, vitamin B_2, B_6, and B_{12} status were significantly better in males with normal Hcy levels as compared to those with hyperhomocysteinemia. Female subjects with normal Hcy levels, also had significantly better folate and vitamin B_{12} status, and lower systolic blood pressure, suggesting that folate and vitamin B_{12} status might have a more important impact on Hcy levels in the elderly population.

Table 3 shows the association between folate, vitamin B_2, B_6 or B_{12} insufficiency and hyperhomocysteinemia and confirms the importance of adequate vitamin B status in maintaining a normal Hcy level in the elderly. After adjustment for age, systolic pressure, diastolic pressure, smoking and alcohol intake, the status of vitamin B_6 (AOR =1.7, 95% CI 1.14-2.38 for males, AOR =2.1, 95% CI 1.28-3.47 for females), folate (AOR =3.0, 95% CI 1.94-4.60 for males, AOR =4.1, 95% CI 2.25-7.33 for females) and vitamin B_{12} (AOR =3.4, 95% CI 2.23-5.32 for males, AOR =3.3, 95% CI 1.72-6.32 for females) were significantly associated with hyperhomocysteinemia in both sexes. Vitamin B_2 insufficiency was only significantly associated with hyperhomocysteinemia in males (AOR =1.7, 95% CI 1.19-2.51). We investigated whether the relationship between vitamin B_2 and plasma Hcy in elderly Taiwanese was influenced by the status of other B vitamins. The association between insufficiency in one or two B vitamins and hyperhomocysteinemia is illustrated in Table 4, with subjects stratified on the basis of their B vitamin status. After adjustment for age, systolic pressure, diastolic pressure, smoking and alcohol intake, vitamin B_2 insufficiency was not significantly associated with hyperhomocysteinemia in elderly persons with adequate folate, vitamin B_6 and B_{12} status. However, vitamin B_2 insufficiency combined with poor vitamin B_6 status (AOR=3.5 for B_2 plus B_6 vs. AOR=2.1 for B_6 only), or poor vitamin B_2 combined with poor folate status (AOR=4.5 for B_2 plus folate vs. AOR=2.1 for folate only), were significantly associated with hyperhomocysteinemia. Insufficiency in both vitamin B_2 and B_{12} was not associated with hyperhomocysteinemia (AOR =6.4 for B_2 plus B_{12} vs AOR =6.3, 6.5 for B_{12} only). These results suggest that the association between vitamin B_2 insufficiency and hyperhomocysteinemia is influenced by folate and vitamin B_6 status.

Table 2. Clinical characteristics of elderly persons with a serum creatinine below 1.5 mg/dL by plasma homocysteine level[1]

Characteristics	Males	
	Plasma homocysteine level	
	≤15 μmol/L (N = 491)	>15 μmol/L (N =165)
Age (year)	71.3 ± 0.4*	73.2 ± 0.3
Body Mass Index (kg/m^2)	23.6 ± 0.9	23.5 ± 0.9
Systolic blood pressure (mmHg)	132 ± 2	134 ± 1
Diastolic blood Pressure (mmHg)	76.6 ± 0.9	77.0 ± 0.5
Plasma folate (nmol/L)	24.5 ± 1.1*	18.6 ± 0.9
EGRAC[2]	1.15 ± 0.02*	1.18 ± 0.01
PLP (nmol/L)[3]	54.3 ± 4.5*	39.7 ± 2.5
Serum vitamin B_{12} (pmol/L)	781 ± 94*	454 ± 190
	Females	
Characteristics	Plasma homocysteine levels	
	≤ 15 μmol/L (N = 615)	>15 μmol/L (N =79)
Age (year)	71.1 ± 0.7*	72.5 ± 0.2
Body Mass Index (kg/m^2)	24.2 ± 0.9	24.0 ± 0.9
Systolic blood pressure (mmHg)	138 ± 2*	144 ± 1
Diastolic blood pressure (mmHg)	75.7 ± 1.3	75.3 ± 0.6
Plasma folate (nmol/L)	29.5 ± 1.1*	21.9 ± 0.9
EGRAC[2]	1.14 ± 0.02	1.16 ± 0.01
PLP (nmol/L)[3]	62.2 ± 6.1	55.4 ± 2.3
Serum vitamin B_{12} (pmol/L)	1791 ± 320*	529 ± 105

[1]All data excludes subjects with serum creatinine ≥1.5 mg/dL, and are shown as mean±SE. [2]Erythrocyte glutathione reductase activity (EGRAC) was an indicator of vitB_2 status. [3]Plasma pyridoxal phosphate (PLP) was an indicator of vitB_6 status. *Significantly different between subjects with normal plasma homocysteine concentration and hyperhomocysteinemia (P<0.05).

Table 3. Adjusted Odds ratio (AOR) of hyperhomocysteinemia in elderly persons with a serum creatinine below 1.5 mg/dL[1,2]

		Males (N = 656)		Females (N = 694)	
	B vitamins status[3]	Case/ normal (N)	AOR[4] 95 % CIs	Case/ normal (N)	AOR[4] 95 % CIs
Vit B_2	Adequacy	96/344	1.0	55/458	1.0
	Insufficiency	69/147	1.7 (1.19-2.51)	24/157	1.1 (0.64-1.84)
Vit B_6	Adequacy	84/316	1.0	43/462	1.0
	Insufficiency	81/175	1.7 (1.14-2.38)	36/153	2.1 (1.28-3.47)
Vit B_{12}	Adequacy	112/432	1.0	63/571	1.0
	Insufficiency	53/59	3.4 (2.23-5.32)	16/44	3.3 (1.72-6.32)
Folate	Adequacy	113/424	1.0	57/565	1.0
	Insufficiency	52/67	3.0 (1.94-4.60)	22/50	4.1 (2.25-7.33)

[1]All data excludes subjects with a serum creatinine ≥1.5 mg/dL. [2]Hyperhomocysteinemia: plasma homocysteine levels > 15 μmol/L. [3]The criteria for various B vitamins status was defined as following: insufficient vitamin B_2 status was defined as EGRAC value ≥1.2, insufficient folate was defined as plasma folate ≤ 14 nmol/L (6 ng/ml), insufficient vitamin B_6 status was defined as plasma pyridoxal phosphate <30 nmol/L, and insufficient vitamin B_{12} status was defined as serum cobalamin ≤ 258 pmol/L.
[4] Adjusted for age (65-69, 70-74, 75-80 and >80), systolic pressure, diastolic pressure, smoking and alcohol drinking status (never, previous and everyday) by multiple logistical regression.

Table 4. Adjusted Odds ratio (AOR) of hyperhomocysteinemia and insufficiency in one or two B vitamins in elderly persons with a serum creatinine below 1.5 mg/dL[1,2]

B vitamins status[3,4]				Male (N = 656)		Female (N = 694)	
Vitamin B_2	Vitamin B_{12}	Vitamin B_6	Folate	Case/normal (N)	AOR[5] 95 % CI	Case/ normal (N)	AOR[5] 95 % CI
+	+	+	+	24/194	1.0	19/316	1.0
-	+	+	+	12/53	1.9 (0.89-4.14)	7/87	1.3 (0.54-3.34)
-	+	-	+	20/47	3.5 (1.75-6.90)	8/46	2.7 (0.92-5.62)
-	+	+	-	5/11	4.5 (1.41-14.60)	1/4	4.2 (0.46-39.2)
-	-	+	+	6/8	6.4 (1.50-16.62)	-	-
+	-	+	+	14/18	6.3 (3.54-11.95)	6/15	6.5 (3.18-23.28)
+	-	-	+	8/10	6.5 (1.20-10.43)	4/10	6.4 (1.79-22.66)
+	+	-	+	20/78	2.1 (1.05-3.87)	11/79	2.4 (1.06-5.25)
+	+	+	-	10/29	3.1 (1.17-6.59)	7/28	3.9 (1.49-9.56)
+	+	-	-	12/8	12.5 (4.56-34.63)	6/8	12.4 (3.71-41.50)
+	-	+	-	6/3	16.4 (3.76-71.78)	1/5	4.2 (0.46-39.2)

[1]All data excludes subjects with a serum creatinine ≥1.5 mg/dL. [2]Hyperhomocysteinemia: plasma homocysteine levels > 15 μmol/L. [3]The criteria for various B vitamins status was defined as following: insufficient vitamin B_2 status was defined as EGRAC value ≥1.2, insufficient folate was defined as plasma folate ≤14 nmol/L (6 ng/ml), insufficient vitamin B_6 status was defined as plasma pyridoxal phosphate <30 nmol/L, and insufficient vitamin B_{12} status was defined as serum cobalamin ≤ 258 pmol/L. [4] '+' indicates B vitamin adequacy; '-' indicates B vitamin insufficiency. [5]Adjusted for age (65-69, 70-74, 75-80 and >80), systolic pressure, diastolic pressure, smoking and alcohol drinking status (never, previous and every day) by multiple logistical regression.

As shown in Table 4, our data indicate that in elderly men and women, insufficiency in vitamin B_{12} is the one most strongly associated with hyperhomocysteinemia in those who have adequate levels of the three other B vitamins (AOR =6.3, 95% CI 3.54-11.95 for males, AOR =6.5, 95% CI 3.18-23.28 for females). In addition, the presence of vitamin B_2 or B_6 insufficiency did not increase the odds of hyperhomocysteinemia in elderly males and females who had poor vitamin B_{12} status. As the association between vitamin B_{12} insufficiency and hyperhomocysteinemia does not seem to be greatly influenced by vitamin B_2 or B_6 status, greater attention should be paid to maintaining adequate vitamin B_{12} status in the elderly.

Our results also showed that vitamin B_6 insufficiency alone (AOR = 2.1, 95% CI 1.05 -3.87 for males, AOR = 2.4, 95% CI 1.06-5.25 for females), or folate insufficiency alone (AOR = 3.1, 95% CI 1.17-6.59 for males, AOR = 3.9, 95% CI 1.49-9.56 for females) were also associated with hyperhomocysteinemia. Moreover, the odds ratio of hyperhomocysteinemia in elderly males and females with both poor folate and vitamin B_6 status (AOR = 12.5, 95% CI 4.56-34.63 for males; AOR = 12.4, 95% CI 3.71-41.50 for females) increased 3~4-fold compared to subjects with either poor folate alone or poor vitamin B_6 status alone. In addition, the odds ratio of hyperhomocysteinemia in elderly males with both poor folate and vitamin B_{12} status (AOR = 16.4, 95% CI 3.76-71.78) increased 3~5-fold as compared with that in subjects with either poor vitamin B_{12} alone (AOR = 6.3, 95% CI 3.54-11.95) or poor folate status alone (AOR = 3.1, 95% CI 1.17-6.59). These data suggest that poor vitamin B_6 status and poor vitamin B_{12} status each have a synergistic effect on the risk of hyperhomocysteinemia in elderly people with folate insufficiency.

Discussion

The average plasma Hcy concentration in Taiwanese elderly persons aged 65 and over was 13.3 μmol/L for males and 10.6 μmol/L for females. Our study showed that the plasma Hcy level was higher in males than in females and significantly increased with age, which is consistent with other studies.[24,25] To compare our Hcy levels with other studies, we stratified age according to the age groups used in these studies and then reanalyzed our data.

The mean Hcy level in Taiwanese elderly females was lower than figures from the USA (10.4 vs 10.7 μmol/L, aged 67-74yrs; 11.3 vs 11.9 μmol/L, aged 75-79yrs; 12.7 vs 13.2 μmol/L, aged >80yrs, Framingham study),[24] Norway (9.4 vs 11.0 μmol/L, aged 65-67 yrs),[25] Finland (10.1 vs 11.2 μmol/L, aged 65-74 yrs),[26] England (10.6 vs 13.9 μmol/L, aged >65 yrs),[27] Spain (10.6 vs 12.2 μmol/L, aged >65yrs),[28] and New Zealand (11.0 vs 11.6 μmol/L, aged 70-80 yrs).[29] However, Taiwanese elderly females had a higher mean value than Mexicans in the USA (9.7 vs 9.3 μmol/L, aged 60-69yrs; 11.0 vs 9.5 μmol/L, aged 70-79yrs; 12.8 vs 11.0 μmol/L, aged >80yrs, NHANES III).[30] In other words, Taiwanese elderly women had lower Hcy levels than the elderly in countries with meat-dominated diets as the major dietary pattern, but did not have a lower value than Mexican Americans. This suggests that a vegetable-rich diet is beneficial, although racial or ethnic differences may not be excluded.[30] In addition, higher folate intake by Taiwanese elderly women may contribute to their lower Hcy levels.[31]

The mean Hcy level in Taiwanese elderly males was lower than that in a British study (13.3 vs 16.0 μmol/L, aged >65),[27] and similar to levels in Norway (12.3 μmol/L, aged 65-67 yrs) and Finland (12.8 vs 13.0 μmol/L, aged 65-74yrs).[25,26] However, Taiwanese elderly males had a higher mean value than those reported in the

USA (13.0 vs 11.8μmol/L, aged 67-74yrs; 14.1 vs 11.9 μmol/L, aged 75-79yrs; 15.6 vs 14.1 μmol/L, aged >80yrs, Framingham study),[24] Australia (14.6 vs 13.5μmol/L, aged 75yrs, Perth),[32] and Spain (13.3 vs 12.6μmol/L, aged >65yrs).[28] It is of note that Taiwanese elderly men had comparatively higher Hcy levels than men from the other countries, which is in contrast to the comparatively lower Hcy levels in Taiwanese elderly women.

The prevalence of hyperhomocysteinemia in the elderly has varied substantially due to different cut-offs used by different studies. In our study, 23.4% of elderly men and 11.2% of elderly women had elevated Hcy levels (>15μmol/L). The overall prevalence was lower than that in the Framingham Study, USA (25.7 vs 29.3% >14μmol/L, aged 70~80yrs).[24] The prevalence of high Hcy levels in Taiwanese elderly females was lower than that in elderly women in New Zealand (18.0% >15μmol/L) and in NHANES III (44.0 vs 46.7% >10.4μmol/L, aged >60y),[29,33] and similar to that in Australia (24.0% >13 μmol/L, aged >70y).[32] In contrast, the prevalence of high Hcy levels in Taiwanese elderly men was higher (~1.4 fold) than that in Australia (33.8 vs 24.0% >15μmol/L, aged >75y),[32] and in NHANES III (60.0 vs 43.2%>11.4 μmol/L, aged >60y).[33] This indicates that the higher prevalence of hyperhomocysteinemia in Taiwanese elderly males still needs to be monitored and improved upon.

The average age of subjects with hyperhomocysteinemia was significantly higher than that of subjects with normal Hcy levels, which agreed with the trend of an increased plasma Hcy level with age.[24,25] Our study showed that females with hyperhomocysteinemia had higher systolic pressures than those with normal Hcy levels. This result supports the findings of the Hordaland Study and the paper by Brattstrom *et al*,[25,34] although other studies have reported no association between systolic pressure and Hcy.[8]

The four B vitamins – vitamin B_2, B_6, B_{12} and folate – which are involved in Hcy metabolism have been reported to be important determinants of plasma Hcy.[6-11] Our data indicates that folate, vitamin B_6 and B_{12} are independent nutritional factors associated with hyperhomocysteinemia. In addition, elderly persons who have poor folate and vitamin B_6 status, or poor folate and vitamin B_{12} status had a 3-5 fold increased risk of hyperhomocysteinemia compared to those with only one B vitamin insufficiency. These results agreed with the report that elevated Hcy concentration was associated with a combination of low plasma levels of vitamin B_6, B_{12} and folate.[24]

Vitamin B_2 insufficiency was only significantly associated with hyperhomocysteinemia in males. However, vitamin B_2 insufficiency was not significantly associated with hyperhomocysteinemia in elderly persons who had adequate folate, vitamin B_6 and B_{12} status. The combination of vitamin B_2 insufficiency with either vitamin B_6 insufficiency or folate insufficiency increased dramatically the adjusted odds ratio of hyperhomocysteinemia in elderly males as compared to deficiency in only one B vitamin. This suggests that the association between vitamin B_2 insufficiency and hyperhomocysteinemia is influenced by folate and vitamin B_6 status, and insufficiency of two B vitamins has a synergistic effect on the risk of elevated Hcy. Therefore, adequate levels of vitamin B_2, B_6 , B_{12} and folate may be important for an optimal Hcy concentration in the elderly population.

Although the importance of vitamin B_2 in Hcy metabolism is recognized,[9,35] little attention has been paid to the relationship between Hcy and vitamin B_2 levels in human studies, and the interactions among vitamin B_2 and folate, or vitamin B_6.[36,37] The reason why this association has not been found in other studies might be due to good vitamin B_2 status in the subjects investigated.[38] The inadequate intake of milk and its products, which are a good food source of vitamin B_2, has been reported in several surveys in Taiwan.[39,40] Therefore, our results revealing the association between vitamin B_2 and plasma Hcy levels may provide the rationale for a trial of the effect of Hcy lowering vitamin therapy for hyperhomocysteinemia in elderly persons in Taiwan.

In summary, this study provides information on plasma Hcy levels and the relationship between levels of Hcy and B vitamins in the Taiwanese elderly population. It is of note that the prevalence of plasma Hcy above 15 μmol/L was particularly high in elderly males at 23.4% but was lower at 11.2% in females. Vitamin B_6, B_{12} and folate were independent nutritional factors associated with elevated Hcy levels in the Taiwanese elderly. A combination of insufficiency in two B vitamins had an additive effect on the risk of hyperhomocysteinemia. Although cause and effect was unable to be clarified in this cross-sectional study, good B vitamin status, particularly that of folate and B_{12}, should be emphasized as important for reducing Hcy concentrations of Taiwanese elderly persons, especially that of males .[31]

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