Original Article

Association between dietary folate-rich food intake and folate status of elderly Taiwanese

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To investigate the relationship between folate status and dietary folate intake in the Taiwanese elderly, we analyzed plasma folate levels and dietary folate intake in 725 males and 705 females aged 65-90 years, sampled from the Elderly Nutrition and Health Survey in Taiwan (1999-2000) (Elderly NAHSIT). Results showed that the mean plasma folate levels were 22.9 ± 1.4 nmol/L (10.1 ± 0.6 ng/ml) for males and 29.5 ± 1.6 nmol/L (13.0 ± 0.7 ng/ml) for females. The average plasma folate concentrations of males from all age groups were significantly lower than those of females (P<0.0001). None of the study subjects had a plasma folate below 7 nmol/L (3 ng/ml). However, 18.6% of males and 12.1% of females had marginal folate deficiency, with plasma folate between 7-14 nmol/L (3-6 ng/ml). This suggests that elderly males have a poorer folate status than elderly females in the Taiwanese population. The percentage of marginal folate deficiency tended to increase with age among females (P trend = 0.0137). The average estimated folate intakes were 379 ± 18 μg/d in males and 351 ± 27 μg/d in females. However, 45.5% of males and 48.8% of females had a dietary folate intake below 2/3 of the RDA of 400 μg/d. Our results indicated that dietary folate intake is positively correlated with plasma folate levels (r = 0.10, P <0.05). In addition, dietary folate intake increased with increased intakes of vegetables, mushrooms and fruit. A lower intake of fruit appeared to be responsible for the higher prevalence of marginal folate deficiency among females over the age of 80 years.

Key Words: folate intake, folate status, plasma folate, elderly, Elderly Nutrition and Health Survey in Taiwan (1999-2000)

Introduction

Folate is a water-soluble vitamin which occurs in nature as derivatives of pteroylglutamic acid. These derivatives are differentiated by their state of oxidation, one carbon substitution of the pteridine ring, and by the number of glutamate residues in gamma linkage with the *p*-aminobenzoyl glutamate moiety. Folate-rich foods include orange juice, leafy vegetables, liver, yeast, legumes and citrus fruit. Emphasis on adequate folate nutrition has received attention in recent years because of the association of low folate status with neural tube defects, vascular disease, and certain cancers.[1-4] Several studies have shown that folate deficiency may be particularly common in the elderly.[5,6] Such findings have led to increased attention to the folate status of the elderly population.

Folate status may be evaluated from serum/plasma, RBC levels and dietary folate intake.[7] Early reports indicated that the average daily folate intake was approximately 200-300 μg with a Western-type diet. The daily folate intake in elderly populations was reported to be even lower, 150 to 250 μg, in part because of reduced calorie intake.[5,8] One study from Spain has shown that 6% of an elderly population were folate deficient and 35% were marginally deficient.[9]

Recommended dietary intakes for folate differ between countries and range from 300 to 400 μg/day.[10,11] To reduce the frequency of neural tube defect affected births, a number of countries have adapted a folate fortification program. Recent data indicates that folate status in the US population has improved significantly due to fortification of grain and cereal products with folic acid. [12]

In Taiwan, the Recommended Dietary Allowance (RDA) for folate is 400 μg/day for adults,[13] but limited data were available for the elderly. A previous survey, the Nutrition and Health Survey in Taiwan 1993-1996 (NAHSIT 1993-1996), revealed that 41% of elderly males and 15% of elderly females had marginal folate deficiency, but few were folate deficient.[14] The NAHSIT 1993-1996 however, included only 200 elderly persons, and the database on folate contents for most vegetables and food produced in Taiwan were not fully available.

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In this Elderly NAHSIT study, we have improved the methods for evaluation of dietary folate intakes. In addition, we have also obtained data on plasma folate which has allowed determination of the association between folate status and folate-rich food sources or folate intake in the Taiwanese elderly population.

Subjects and Methods

Subjects

The subjects in this study were sampled from the Taiwanese population as part of the Elderly NAHSIT project by a multistage, stratified sampling method. Detailed description of sampling design can be found in Pan *et al's* report.[15] The sample included 1430 subjects, comprising 725 males and 705 females aged 65 to 90 years.

Measurement of folate

Plasma was separated promptly after blood collection and frozen at -80°C until analysis. Plasma folate was measured by a combined system of competitive immunoassay and chemiluminesence (IMMULITE 2000 analyzer, Diagnostic Products Corporation, LA, USA). This procedure involved the use of monoclonal antibodies, paramagnetic particles, and a chemiluminesence substrate. The light emitted was inversely proportional to the concentration of folic acid. 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 folate assay was 11%. The plasma folate status was classified according to the cut-points by Waters *et al*., and Herbert.[16,17] A serum/plasma folate less than 7 nmol/L (3 ng/ml) was considered deficient and between 7-14 nmol/L (3-6 ng/ml) was considered marginally deficient.

Dietary assessment

Dietary folate intake was assessed using data from 24-hour dietary recalls. Dietary folate intake was estimated using the folate composition databank. This was prepared by Rwei-Fen S. Huang, Fu-Jen University[18] by integrating the information on food folate contents from:

1) USDA Nutrient database for standard reference,[19]
2) Bowes & Church's food values of portions commonly used[20]
3) folate contents for vegetables commonly used in Taiwan.[21]

This folate composition databank was designed to estimate folate intake based on a Chinese-style diet. We considered a daily folate intake below 2/3 of the RDA as an inadequate intake of folate. As the RDA in Taiwan for folate is 400 μg/day for people aged over 13,[13] subjects with a daily folate intake lower than 267 μg/d were considered to have inadequate folate intake.

Consumption of supplements was not included in this survey due to limited information about the types of supplements taken by subjects. In addition, folate-rich food sources and intake frequency were also estimated using the food frequency questionnaire (FFQ) with the past 1 month as the reference period. The FFQ listed 28 food items and provided 3 frequency choices from "times/month" to "times/day". The selected frequency of choice for folate-rich food was then converted to a weekly intake.

Statistical analyses

Statistical analysis was carried out by 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 was used to account for the complex survey design in the variance estimates using SUDAAN, SAS-callable version 8.0. The data was analyzed by gender, and age was grouped into 65-69, 70-74, 75-79 and 80 and above year age groups. Trends across quintiles were evaluated with linear regression. Differences in continuous variables between genders by various age groups were tested by the Student's *t*-test. Differences were considered significant if $P < 0.05$.

Results

Data presented in Table 1 show that elderly females of all age groups had significantly higher plasma folate levels than males. In neither males or females were there any cases of deficient folate status, i.e <7 nmol/L (3 ng/ml), however 18.6% of males and 12.1% of females were marginally folate deficient (7-14 nmol/L or 3-6ng /ml).[16,17]

The higher prevalence of marginal folate deficiency among males compared to females was observed in all age groups except those over 80 years. In females the prevalence of marginal folate deficiency increased with age (*P* trend = 0.0137). Females over the age of 80 years had the poorest folate status compared to the other groups.

Estimated dietary folate intake is shown in Table 2. The folate intake levels in the elderly population consuming a Chinese-style diet were 379 ± 18 μg/d for males and 351 ± 27 μg/d for females. There was no significant difference between males and females. The prevalence of low folate intake among the Taiwanese elderly was assessed and it was found that the percentage of subjects with a daily folate intake below 2/3 of the RDA (267 μg/d) was 45.5% for males and 48.8% for females. The contribution of the five basic food groups to folate is shown in Figure 1. Vegetables were the main source of folate in diets, and fruit was the second main folate food source. Data in Table 3 indicate that for both males and females, daily folate intake significantly increased as vegetables, mushrooms or fruit intake frequency increased. However, daily folate intake significantly decreased as intake frequency of pickled vegetables increased.

To further investigate whether a higher marginal folate deficiency in females aged 80 years and over (Table 1) was associated with their dietary habits, the intake frequency of vegetables, mushrooms and fruit by various age groups was examined (Table 4). Among the male population, there was a significant age trend for decreased vegetable intake frequency, which may explain the decrease in folate intake with increased age (Table 2). In females, there were no significant differences among the various age groups with respect to the frequency of vegetable and mushroom intakes. With fruit intake, however, there was a significant trend (*P*=0.0001) of age-associated decreased intake. In the 80 years and over age

Table1. Plasma folate levels and folate status of the Taiwanese elderly by age

Male			
Age group (yrs)	*N*	Plasma folate[1] (nmol/L)	Marginal deficiency[2] (%)
65-69	270	22.2 ± 1.1*	18.1
70-74	252	22.2 ± 1.4*	22.4
75-80	138	23.6 ± 1.4*	17.9
>80	65	25.4 ± 3.4*	11.8
Total	725	22.9 ± 1.4*	18.6
P trend		0.6600	0.6503
Female			
Age group (yrs)	*N*	Plasma folate[1] (nmol/L)	Marginal deficiency[2] (%)
65-69	299	28.6 ± 1.4	10.4
70-74	220	29.9 ± 1.4	10.1
75-80	111	29.9 ± 1.4	11.3
>80	75	29.0 ± 3.4	20.0
Total	705	29.5 ± 1.6	12.1
P trend		0.7858	0.0137

[1]All values are shown as mean ± SE or percentage of participants.
[2]Plasma folate concentrations of 7-14 nmol/L (3.0-6.0 ng/ml) indicate marginal folate deficiency.*Significantly different from the female values (P<0.0001).

Table 2. Dietary folate intake in the Taiwanese elderly by age

Male			
Age group (yrs)	*N*	Folate intake[1] (μg/d)	Inadequate intake[2] (%)
65-69	270	415 ± 28	38.3
70-74	252	375 ± 24	48.2
75-80	138	355 ± 28	53.4
>80	65	326 ± 31	46.7
Total	725	379 ± 18	45.5
P trend		0.0234	0.1965
Female			
Age group (yrs)	*N*	Folate intake [1] (μg/d)	Inadequate intake [2] (%)
65-69	299	362 ± 22	45.2
70-74	220	349 ± 40	45.8
75-80	111	371 ± 48	56.1
>80	75	307 ± 31	53.6
Total	705	351 ± 27	48.8
P trend		0.2077	0.0796

[1]All values are shown as mean ± SE or percentage of participants.
[2]Inadequate folate intake is indicated by a dietary folate intake <2/3 DRIs in Taiwan.

Table 3. Average weekly frequency of folate-rich food intake by quartile of dietary folate intake

Male					
	Folate intake (μg/day)				
Food frequency (times/week)	<152 (*N* =170)	152-262 (*N* =186)	263-470 (*N* =174)	>470 (*N* =195)	*P* trend
Liver	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.9607
Vegetables	14.0 ± 1.1	15.2 ± 0.8	16.4 ± 0.8	17.4 ± 1.0	0.0074
Mushrooms	0.7 ± 0.1	1.3 ± 0.3	1.2 ± 0.2	1.3 ± 0.2	0.0189
Fruit	6.5 ± 0.5	6.4 ± 0.5	8.6 ± 0.6	8.5 ± 0.5	0.0138
Fruit juice	0.5 ± 0.2	0.5 ± 0.2	0.6 ± 0.2	0.9 ± 0.4	0.2992
Pickled vegetables	2.7 ± 0.5	2.9 ± 0.3	1.9 ± 0.3	1.8 ± 0.2	0.0460
Fermented foods	1.2 ± 0.2	1.1 ± 0.2	1.5 ± 0.3	1.2 ± 0.2	0.6848
Female					
	Folate intake (μg/day)				
Food frequency (times/week)	<152 (*N* =190)	152-262 (*N* =170)	263-470 (*N* =184)	>470 (*N* =161)	*P* trend
Liver	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.7792
Vegetables	13.9 ± 0.9	15.2 ± 1.0	16.3 ± 0.8	19.4 ± 1.7	0.0029
Mushrooms	0.9 ± 0.1	1.1 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	0.0192
Fruit	6.6 ± 0.5	6.8 ± 0.5	7.7 ± 0.6	8.0 ± 0.7	0.0166
Fruit juice	0.6 ± 0.2	0.3 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.3127
Pickled vegetables	2.9 ± 0.4	4.2 ± 0.8	2.3 ± 0.2	2.2 ± 0.3	0.0820
Fermented foods	1.0 ± 0.2	1.8 ± 0.4	1.1 ± 0.1	1.2 ± 0.3	0.8909

[1] All values are shown as mean ± SE.

range the consumption of fruit was significantly lower in females than in males (P = 0.004).

Discussion

Several studies have indicated that folate status is associated with age related diseases such as cardiovascular disease and some cancers.[1,2,22] In addition, a high risk of folate deficiency has been reported in the elderly.[5,6] Our results showed that the average plasma folate concentration was 23-30 nmol/L (10-13 ng/ml) among the elderly. This mean value was higher than the levels found in free-living elderly reported among Spanish (6.9-7.4 ng/ml), Canadian (3.7-4.7 ng/ml), and South African (5.0-6.0 ng/ml) elderly populations.[8,9,23] The present study has also shown that elderly Taiwanese females had significantly higher plasma folate than males, implying that the difference in folate status between sexes is more noticeable in Taiwan than has been reported in other countries. The prevalence of folate deficiency in the elderly has varied substantially in different countries, with frequencies of 0–19% being reported. It was reported that among the elderly in the Spanish population, 36% of males and 34% of females had marginal folate deficiency.[9] The plasma folate concentration in all members of the Taiwanese elderly population was determined to be higher than 7 nmol/L (3 ng/ml), indicating that there is no folate deficiency. Nevertheless, the possibility of overestimation of an actual value under 7nmol/L (3 ng/ml) cannot be

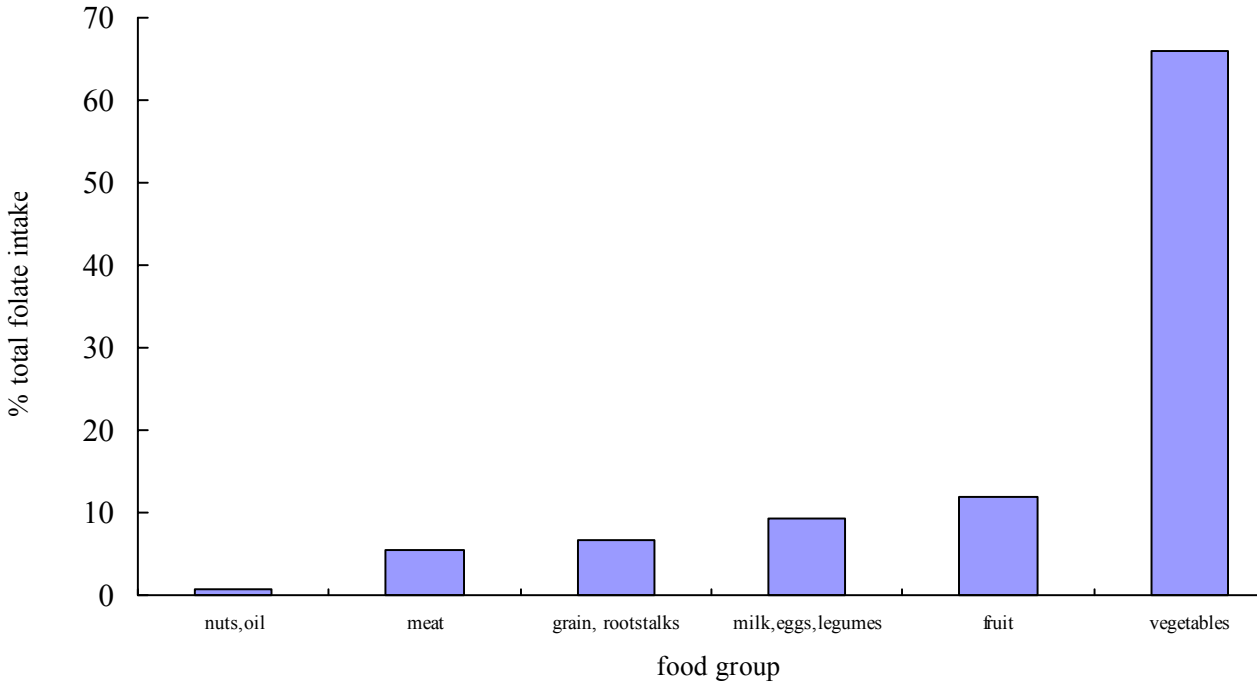

Figure 1. Mean contribution of food groups to total folate intake

Table 4. Average weekly frequency of major folate-rich food intake by age

Age group (yrs)	Frequency (times/week)[1]					
	Male			Female		
	Vegetables	Mushrooms	Fruit	Vegetables	Mushrooms	Fruit
65-69	17.0 ± 0.9	1.2 ± 0.2	8.0 ± 0.4	16.8 ± 0.9	1.1 ± 0.2	8.7 ± 0.7
70-74	16.4 ± 0.9	1.2 ± 0.2	7.8 ± 0.6	16.1 ± 1.2	1.1 ± 0.1	7.9 ± 0.5
75-79	15.3 ± 1.0	0.9 ± 0.2	6.6 ± 0.5	15.9 ± 1.2	1.0 ± 0.2	5.6 ± 0.5
>80	14.1 ± 1.0	1.4 ± 0.3	8.8 ± 1.0*	15.1 ± 1.0	1.1 ± 0.2	4.3 ± 0.7
P trend	0.0228	0.9257	0.6268	0.1471	0.6734	0.0001

[1]All values are shown as mean ± SE. *Significantly different from the female values ($P = 0.004$).

ruled out as the competitive immunoassay may have a lower sensitivity than the microbiological assay.[14] In addition, it is notable that 18.6% of elderly males and 12.1% of elderly females had marginal folate deficiency. A poorer folate status in elderly males was also reported in Spain, South Africa and Holland, but not in Canada.[8,9,23,24] Therefore, the folate status of the male population still warrants attention. Daily folate intake has been reported to be about 150-250 μg/d in Western countries.[8] For Eastern countries, several small-scale surveys of Japanese adult women have reported an average dietary folate intake of 293 μg/day and that only 34% of female students had sufficient folate intake.[25,26] A survey in Vietnam estimated daily folate intake was 251μg.[27] The Shanghai Breast Cancer Study reported 294 μg/d folate intake in female subjects aged 25-64 years.[28] The daily folate intake of 204 urban Indonesian elderly persons was even lower at 187 μg.[29] In our study, the estimated average dietary folate intake in a nation-wide survey of 1430 elderly subjects was 379μg/d for males and 351μg/d for females using the folate composition databank established by Professor Rwei-Fen S Huang.[18] The data suggests that the Taiwanese elderly have higher folate intake compared to other countries without folate fortification. To verify this data, folate contents of daily meals served in hospitals which had menus planned by dietitians, were measured and shown to be 417 ± 60 μg/d (unpublished data). It is possible that a higher folate intake is achievable due to the abundance of a diverse variety of fruit and vegetables in Taiwan. However, our data is still lower than the US folate intake of 389 μg/d from diets including supplements and fortified foods.[30] In addition, 46% of males and 49% of females had a dietary folate intake below 2/3 the RDA values (267 μg/d). This suggests that half of the Taiwanese elderly population is at risk of inadequate dietary folate intake and that greater attention should be paid to this health issue.

Our study had some limitations. Firstly, data in this study came from a cross-sectional study. Therefore, even though our study showed that dietary folate intake is positively correlated with plasma folate levels ($r = 0.10$, $P < 0.05$), the causal effect of dietary folate intake on plasma folate cannot be certain. In this study of elderly people whose diets were not westernized, vegetables were the main source of folate, contributing up to 66% of folate intake. Fruit (11.8%) followed by milk, eggs and beans (9.4%) were also major contributors to folate intake among the Taiwanese elderly. In contrast, vegetables in the US and Dutch diets contributed only 18% and 22%, respectively to folate intake. Cold breakfast cereals

(13.3%), multivitamins (12.8%) and orange juice (12.4%) in the US diet, and bread (26%) and potatoes (8%) in the Dutch diet were also major contributors and are not regularly consumed in a Chinese diet.[30, 31]

The increase in dietary folate intake associated with vegetable, mushroom and fruit intake frequency in males and females suggests that a higher intake of vegetables, mushrooms and fruit should be emphasized as a means of increasing dietary folate intake in the Taiwanese elderly. Our results also showed that the association between increased frequency of pickled vegetable intake with lower daily folate intake for elderly males might be due to their replacement of vegetable portions, and the low folate content in pickled vegetables compared to fresh vegetables.[18,21] These results also suggest that intake of fresh vegetables is important for elderly people.

The significant age trend for vegetable intake frequency and folate intake in males indicates that the fewer vegetables consumed by older males might reduce their daily folate intake. Although Taiwanese females had significantly better folate status than males overall, in those aged over 80, higher marginal folate deficiency was found only in females and not in males of a similar age. A significant age trend for fruit intake frequency was only found in females. In particular, women over the age of 80 consumed fruit significantly less often than men of the same age, probably due to chewing problems. This finding agrees with the higher percentage of marginal folate deficiency in females aged over 80 years. The cross-sectional analyses from the Framingham Heart Study indicated that frequent consumption of certain foods, particularly fruit and vegetables, is correlated with low plasma levels of homocysteine, perhaps as a result of the high folate content of this food.[30] Therefore, these data strongly suggest that fruit intake is an important dietary component to maintain adequate folate status in women aged over 80 years.

In summary, this study provides information on the folate status of the Taiwanese elderly population and their dietary folate intake pattern. Folate status seems adequate, with a low percentage of folate deficiency. However, it should be noted that up to 18.6% of males and 12.1% of females had marginal folate deficiency. In addition, half of the Taiwanese elderly still had inadequate folate intake. Vegetables, mushrooms and fruit may be suggested as good sources of increasing folate intake for Taiwanese elderly on a Chinese-style diet. In addition, consuming fruit every day should be especially promoted for those over the age of 80.

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