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The Folate Status in Taiwanese Population from the NAHSIT 1993-1996

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Abstract

To investigate the folate status of the Chinese in Taiwan, the plasma and red blood cell (RBC) folate levels were measured in this study. The blood samples were collected from 1993 to 1996 when the Nutrition and Health Survey in Taiwan (NAHSIT) was carried out. Subjects of both sex, aged 4 years old and above representing the population in Taiwan were studied. Folate contents in plasma and RBC collected in the first year were measured by both microbiological assay and chemiluminescent enzyme immunoassay. Both the average plasma and RBC folate in male were significantly ($P < 0.05$) lower than those of female (8.2 ± 4.1 vs. 11.1 ± 4.6 ng/ml plasma; 504 ± 244 vs. 589 ± 234 ng/ml RBC). The population aged from 13 to 18 had the poorest folate status among all age groups. Only 58% male and 72% female adolescents had normal plasma folate levels. The percentage of marginal deficiency was higher in male group compared to female group and increased with age. The percentage of folate deficiency and marginal deficiency with low plasma folate was highest in the population of mountain area. People living in east coast and Penghu islands had higher percentages of RBC folate deficiency and marginal deficiency, respectively. The correlation between plasma and RBC folate levels and the dietary frequency of folate-rich food suggested that increased intakes of dark green vegetables, fresh vegetables, citric fruits, other fruits, squash, pickled vegetables and seaweed may have beneficial effect on folate status. In addition, plasma folate levels were reciprocally correlated with systolic pressure in this study. Therefore, whether dietary folate intake and folate status play a role in vascular disease in Taiwanese population remains of interest to be investigated and explored.

Key words: folate status, plasma folate, red blood cell folate, chemiluminescency, Taiwan

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Introduction

Folic acid (pteroylmonoglutamate, PteGlu) is a water-soluble B complex vitamin and the precursor to the coenzyme forms of folate. It occurs naturally as a group of conjugated pteroylpoly-γ-glutamate derivatives. Prior to absorption in the mammalian small intestine, folate is converted to a monoglutamate form in the lumen by a membrane-associated γ-glutamyl hydrolase (conjugase). Folates are widely distributed in tissues, predominantly as polyglutamate derivatives. Plants and lower animals can synthesize folates but mammals, and other organisms which are unable to synthesize folates, must obtain folates from dietary sources.

Folates are essential coenzymes for growth and proliferation of cells because they participate in the *de novo* biosynthesis of purines and thymidylate. Folate deficiency in man and other primates causes impaired cell division and macrocytic megaloblastic anemia with the characteristic morphologic changes of erythroid and myeloid cells, the signs of defective DNA synthesis (1). Folate deficiency may result from inadequate intake and absorption, increased folate requirements, such as in pregnancy, or may be secondary to vitamin B_{12} deficiency. The Recommended Dietary Allowances (RDA) for folate are 200 μg/day for adults and 400 μg/day for pregnant women (2). Folate nutrition has been much emphasized during this decade because low folate status may be also associated with disease such as neural tube defect, vascular disease, and cancer (3-6). Therefore, whether folate status is adequate or folate fortification is required are the main issues to be concerned.

However, most data concerning dietary folate intake come from populations consuming Western-type diet which supplies about 150 to 200 μg folate per day (7). Folate deficiency often occurs in pregnancy and alcoholic (8,9). The elderly and women using oral contraceptives were also risk groups for folate deficiency (10,11). More leafy vegetables, liver, yeast, legumes, and some fruits, which are rich sources of folate, were consumed in typical Chinese diets. However, folate in food may be destroyed by heat, oxidation, and ultraviolet light (12-14). Loss of folate due to cooking of foods should be considered because vegetables are consumed cooked rather than raw by Chinese people. Therefore, whether folate nutrition in Taiwanese population is adequate remains of interest. Folate status is reflected in the serum/plasma and RBC levels. Serum/plasma folate reflects recent dietary intake and falls rapidly when folate-depleted diets are consumed experimentally, whereas RBC folate levels are not decreased significantly until 16~18 weeks on folate-deficient diet (15). Therefore, in this study, plasma folate and RBC folate were measured to evaluate folate status in Taiwanese population using the blood samples collected in the first year of the Nutrition and Health Survey in Taiwan, NAHSIT 1993-1996. In addition, the relationships of dietary intakes and folate status and of blood pressure and folate status were also evaluated.

Materials and Methods

Subjects

The subjects in this study were obtained from the first year samples of the project of Nutrition and Health Survey in Taiwan (NAHSIT) from 1993 to 1996. This includes 1,929 samples from 916 males and 1013 female, aged from 4 years. Plasma and RBC were separated right after sampling and frozen at -80 ℃ until measuring. For detailed information on the design of NAHSIT and the assessments made, see Pan et al. (16). Since NAHSIT project did not plan on assessing the folate status in the beginning, ascorbic acid was not added to blood samples before storage.

Folate Measurement

Plasma folate concentration was measured using both microbiological assay and chemiluminescent enzyme immunoassay. The microbiological assay is a conventional method using *Lactobacillus casei* which requires folate for growth. The growth density of *Lactobacillus casei* correlates to the folate contents in medium and thus can be detected by spectrophotometer. The 96-well microtiter plates method was used as described by Horne and Patterson (17). In brief, *Lactobacillus casei* (subsp. *Rhamnoosus* ATCC 7469) purchased from Food Industry Research and Development Institute (Hsinchu, Taiwan) were cultivated in Lactobacillus broth (Difco, Detroit, MI). To prepare glycerol-cryoprotected *L. casei*, cells were washed twice with sterile 0.9% NaCl solution to remove folate residue. An equal volume of sterile glycerol was added and mixed, and stored at -70 ℃ . Plasma was 50~70-fold diluted in 0.5 M K-P buffer containing 0.5% ascorbic acid prior to assay. To each well of 96-well plates, 140 μl diluted sample, 150 μl folic acid casei medium (Difco) and 10 μl glycerol-protected *L. casei* were added. After incubation at 37 ℃ for 24 hrs, plates were read at OD 630 nm (Microplate, Bio-Tek Instrument, Inc. Winooski, VT). A pooled plasma sample provided by Dr. Ning-Sing Shaw, Department of Agricultural Chemistry at National Taiwan University, was used as positive control to evaluate the precision of the assay.

The chemiluminescent enzyme immunoassay was also used for plasma and RBC folate measurement using IMMULITE Folic Acid kit (Diagnotic Products Corporation, LA, CA). The procedure was performed according to the instructions of kit manual with the IMMULITE Automated Analyzer. The IMMULITE System automatically handles sample and reagent additions, the incubations and separation step, and measurement of the photon output via the temperature-controlled luminometer. A series of quality control tests were performed to evaluate the precision of the kits. These tests confirmed both the between- and within-run consistency of this method.

Statistical analysis

Two-sided non paired Student's t-test of the SAS program system was used to analyze the data to investigate the difference between male and female. Pearson correlation analysis was used to examine the associations between dietary folate intake and folate status. Food frequency of folate-rich foods was used as the indicator of dietary folate intake. Folate status was evaluated by plasma and RBC folate levels. All statistical procedures were adjusted by sample weight (16).

Results

Plasma and red blood cell folate levels

The chemiluminescent immunoassay is a recently developed and potentially easier approach for clinical analysis. Since the automated chemiluminescent immunoassay system was first applied for folate determination in this study and no other folate survey data by this method was available, data validation is necessary for this new method. Therefore, *Lactobacillus casei* assay using an automatic 96-well plate reader were also used for quantitative measurement of folate in plasma of 1,926 people. The results showed that plasma folate concentration measured by chemiluminescent immunoassay correlated with those data measured by microbiological assay ($r = 0.81$, $P = 0.0001$). Thus, RBC folate was determined by automated chemiluminescent immunoassay only.

Table 1 demonstrated average plasma folate concentrations by both methods. The average folate concentration of all age groups was significantly higher in female than those in male population by both methods. Females aged over 19 years old had better folate status than males. Teenagers (13~18 yrs.) had the lowest plasma folate concentration among various age groups. In addition, male teenagers had lower plasma folate than females. Table 2 displays plasma folate concentrations in various districts. The people living in mountainous areas showed lower plasma folate levels in both male and female populations.

The RBC folate concentrations which may indicate body store of folate are shown in Table 3. Red blood cell folate levels were positively correlated with plasma folate levels ($r = 0.46$, $P = 0.0001$) in this study. Teenagers (13~18 yrs.) also had the lowest RBC folate level among all age groups. Male teenagers and aldults had significantly lower value than females. Table 4 demonstrated RBC folate levels in various districts. The people living in Penghu islands and males living in mountainous areas showed lower RBC folate levels.

Table 1 Plasma folate concentrations in various age groups measured by two different methods[1,2]

Age (yr)	Male			Female		
	n	Chemilumi	L. casei	n	Chemilumi	L. casei
			(ng/ml plasma)			
All age	915	8.1±3.7	8.4±4.3	1011	11.0±4.7*	11.2±4.3*
4~6	62	12.6±4.7	11.9±5.0	80	11.5±4.5	11.5±4.3
7~12	228	9.4±3.1	9.9±3.8	219	10.9±7.7*	10.3±4.1
13~18	166	6.8±2.7	7.3±3.4	198	7.7±2.9*	7.8±3.4
19~44	161	7.5±2.9	8.0±3.5	217	11.5±4.0*	11.7±4.9*
45~64	209	8.6±4.2	8.7±4.9	212	12.3±4.3*	13.0±5.5*
65~	89	8.7±5.4	8.4±6.3	85	11.4±4.7*	11.1±4.8*

[1] Chemilumi: data from chemiluminescent immunoassay; L. casei: data from *Lactobacillus casei* assay.
[2] Data are shown as mean ± SD. * Significantly different from the corresponding male values by the same method ($P < 0.05$).

Table 2 Plasma folate concentrations of adults (≥ 19 years) in various districts in Taiwan by two different methods[1,2]

Strata	Male			Female		
	n	Chemilumi	L. casei	n	Chemilumi	L. casei
			(ng/ml plasma)			
Hakka area	64	8.4±3.4	9.2±5.4	77	11.4±3.6	13.7±5.3
Mountainous area	64	6.4±3.0	6.7±4.6	75	9.0±3.6	8.9±4.6
East coast area	73	8.2±3.0	8.0±3.7	68	10.9±4.4	10.6±4.5
Penghu islands	56	8.5±3.6	7.8±3.8	70	11.1±4.6	11.2±5.2
Metropolitan areas	75	7.8±3.9	7.6±4.1	86	11.0±4.2	11.0±4.7
Provincial cities and urbanization class I townships	63	7.8±3.3	8.5±4.0	64	12.1±4.1	12.6±4.8
Urbanization class II townships	64	8.0±3.9	8.6±4.5	74	11.6±4.0	11.7±5.5

[1] Chemilumi: data from chemiluminescent immunoassay; L. casei : data from Lactobacillus casei assay.
[2] Data are shown as mean ± SD.

Table 3 The red blood cell folate concentrations in various age groups[1,2]

Age (yr)	Red blood cell folate (ng/ml red blood cells)			
	n	Male	n	Female
		(ng/ml red blood cells)		
All age	905	504±244	993	589±234*
4~6	69	569±223	79	500±204
7~12	224	478±229	218	492±214
13~18	166	338±195	192	393±198*
19~44	161	505±228	212	630±214*
45~64	193	563±252	207	659±217*
65~	92	588±291	85	678±271*

[1] The average folate concentrations in total population are age-standardized.
[2] Data are shown as mean ± SD. The values were measured by chemiluminescent enzyme immunoassay. * Significantly different from the male values ($P < 0.05$).

Table 4 The red blood cell folate of adults (≥ 19 years) concentrations in various strata in Taiwan[1]

Strata	n	Male	n	Female
		(ng/ml red blood cells)		
Hakka area	55	529±227	67	588±222
Mountainous area	55	460±223	71	637±234
East coast area	72	508±216	69	555±240
Penghu islands	59	469±205	73	521±204
Metropolitan areas	78	567±237	86	661±220
Provincial cities and urbanization class I townships	64	500±253	63	611±220
Urbanization class II townships	63	541±234	75	670±234

[1] Data are shown as mean ± SD. The values were measured by chemiluminescent enzyme immunoassay.

Folate status

The criteria currently considered to define folate deficiency are either serum/plasma folate < 3 ng/ml or RBC folate as below < 140 ng/ml or < 160 ng/ml (18,19). Percent of persons with low plasma folate levels by age and sex is shown in Table 5. Sightly different values of deficiency ratio were obtained by the two assessment methods. The deficiency of males aged over 65 showed different percentages by both methods may be due to lower sensitivity of chemiluminescent enzyme immunoassay to those samples with folate levels below 3 ng/ml. The higher percentage obtained by microbiological method than those by chemiluminescent enzyme immunoassay indicated that there were many samples with plasma folate near cutoff point value 3 ng/ml, which cannot be discriminated by the latter method.

Although the percentage of deficiency with low plasma folate was in the range of 2~12% for various age-sex groups, it is notable that over 20% males over 13 years of age are marginally deficient. Females had lower percentage of deficiency than males. However, there were still over 25% female teenagers and over 15% females aged over 65 showing marginal folate deficiency.

Percent of persons with low plasma folate levels living in various districts is showed in Table 6. Higher percent of deficiency was found in mountainous areas than in other strata. Males living in metropolitan areas may have risk for deficiency. Overall, folate deficiency was not noticeable in Chinese population in Taiwan. However, marginal deficiency was up to 41% for males living in mountainous areas, 34% in Penghu islands and over 25% in other areas for male population. The women living in Penghu islands also had higher percentage of marginal folate deficiency. Although it may not cause clinical alarm for megaloblastic anemia, the high percentage of marginal folate deficiency should not be ignored.

Table 5 Percent of persons with low plasma folate levels by sex and age using two different methods for folate assay[1,2]

Age (yr)	Male					Female				
	n	Deficiency		Marginal deficiency		n	Deficiency		Marginal deficiency	
		Chemilumi	L. casei	Chemilumi	L. casei		Chemilumi	L. casei	Chemilumi	L. casei
		%	%	%	%		%	%	%	%
Total	915	2.2	5.2	28.7	25.5	1011	0	2.6	8.7	11.7
4~6	62	0	4.8	2.8	2.5	80	0.2	0.2	4.3	14.4
7~12	228	0	2.5	13.2	13.0	219	0	0.9	9.4	14.0
13~18	166	2.9	7.9	38.6	34.5	198	0	2.9	27.8	25.4
19~44	161	3.0	3.5	29.0	24.4	217	0	3.2	4.7	8.0
45~64	209	2.2	6.5	29.7	28.9	212	0.1	2.7	4.6	8.7
65~	89	0.2	12.1	41.3	36.7	85	0	0.4	15.0	18.2

[1] Criteria are plasma folate less than 3.0 ng/ml as deficiency and between 3~6 ng/ml as marginal deficiency.

[2] Chemilumi: data from chemiluminescent immunoassay; L. casei: data from *Lactobacillus casei* assay.

Table 6 Percent of adults (≥ 19 years) with low plasma folate levels by sex and strata using two different folate assays[1,2]

Strata	Male					Female				
	n	Deficiency		Marginal deficiency		n	Deficiency		Marginal deficiency	
		Chemilu	L. casei	Chemilu	L. casei		Chemilu	L. casei	Chemilu	L. casei
		%	%	%	%		%	%	%	%
Hakka area	64	0	1.5	32.5	32.8	77	0	0	7.0	8.5
Mountainous area	64	6.8	20.8	45.8	41.1	75	1.8	13.1	18.1	17.8
East coast area	73	4.4	4.7	19.1	25.2	68	0	5.6	14.2	10.6
Penghu islands	56	0	1.2	24.3	34.6	70	0	0	12.8	26.0
Metropolitan areas	75	2.4	11.7	31.8	25.9	86	0	0.8	8.4	12.6
Provincial cities and urbanization class I townships	63	0.8	3.9	31.6	26.5	64	0	4.0	4.8	2.3
Urbanization class II townships	64	4.3	2.4	28.5	26.8	74	0	2.9	3.3	14.2

[1] Criteria are plasma folate less than 3 ng/ml as deficiency and between 3~6 ng/ml as marginal deficiency.
[2] Chemilumi: data from chemiluminescent immunoassay; L. casei: data from *Lactobacillus casei* assay.

The cutoff value for low RBC folate has been set at 140 ng/ml or 160 ng/ml RBC (18,19). According to these criteria, folate deficiency and marginal deficiency for various age groups are illustrated in Table 7. For male population, folate deficiency counted at 3.5~4.1%. Children aged 4~6 years had no folate deficiency in this study. However, over 10% adolescents aged 13~18 years old had higher risk of folate deficiency. For female population, only 1.9~2.8% were at risk of folate deficiency. The population over 45 years old had lower deficiency percentage. The worst body folate status for females were the adolescence groups and children aged 7~12. Overall, the deficiency of RBC folate was higher in male group. Table 8 indicates the folate status of body stores in various districts. Folate deficiency was higher in people living in east coast area for both sexes, and in provincial cities and urbanization class I townships for male. However, it is noticeable that about 10% males living in Penghu islands showed marginal folate deficiency.

Folate status and dietary intakes

Since the database for folate contents of food produced in Taiwan are not available, the relationship between folate status and dietary folate intake is hard to evaluate. However, the dietary survey evaluated as the food frequency was available in this NAHSIT survey. Therefore, an attempt was made to evaluate the relationship between dietary folate intake and folate status by intake frequency of folate-rich food. Folate status evaluated as plasma folate levels was the average of folate concentrations determined by both microbiological and chemiluminescent enzyme immunoassay methods. Body store folate status evaluated as RBC folate levels was determined by chemilumi-

nescent enzyme immunoassay methods. Table 9 provides correlation coefficients between folate status and folate-rich foods consumed by male and female groups. For 13~64 year-old population, plasma folate levels were significantly positively correlated with both dark green vegetables and citrus fruits intakes for male and with both fresh and dark green vegetables for females, respectively. In elderly aged over 65 years old, plasma folate levels were positively correlated with fruits intake. Both plasma and RBC folate in female elderly population also positively correlated with pickled vegetables intakes. The RBC folate levels were significantly positively correlated with intake frequency of various food such as dark green vegetables, citrus fruits, squash, pickled vegetables and seaweed in 13~64 years old male population. For females aged 13~64 year-old, RBC folate level was significantly positively correlated with intake frequencies of dark green vegetable and squash. The significantly negative correlation between RBC folate level and citrus fruit intake frequency is hard to explain so far due to limited information. For people over 65 years old, body folate status was positively correlated with fruit intake frequency for males.

Table 7 Percent of persons with low red blood cell folate levels by sex and age using two different criteria[1]

Age (yr)	n	Deficiency[2]		Marginal deficiency[3]	
		< 140	< 160	140~200	160~200
		%	%	%	%
Male					
All age		3.5	4.1	3.0	2.5
4~6	62	0	0	0.1	0.1
7~12	228	1.6	3.2	6.2	4.6
13~18	166	10.7	14.0	7.6	4.4
19~44	161	3.5	3.5	2.7	2.7
45~64	209	1.2	1.2	1.0	0.9
65~	89	3.3	3.8	0.8	0.3
Female					
		1.9	2.8	1.9	0.9
All age	80	1.8	1.8	0	0
4~6	219	1.1	2.1	5.4	4.4
7~12	198	5.2	9.3	8.0	3.8
13~18	217	1.7	2.3	0.6	0
19~44	212	0.8	0.8	0	0
45~64	85	0.3	0.5	0.5	0.3

[1] The red blood cell folate levels were measured by chemiluminescent enzyme immunoassay.

[2] Folate deficiency was defined as RBC folate less than 140 ng or less than 160 ng per ml red blood cells.

[3] Marginal folate deficiency was defined as RBC folate at 140~200 ng or 160~200 ng per ml red blood cells.

Table 8 Percent of adults (≥ 19 years) with low red blood cell folate levels by sex and strata using two different criteria[1]

Strata	n	Deficiency[2]		Marginal deficiency[3]	
		< 140	< 160	140~200	160~200
		%	%	%	%
Male					
Hakka area	64	1.7	3.5	1.8	0
Mountainous area	64	2.3	2.3	0	0
East coast area	73	4.9	5.7	3.1	2.3
Penghu islands	56	0	1.4	11.1	9.8
Metropolitan areas	75	0	0	2.7	2.7
Provincial cities and urbanization class I townships	63	6.3	6.3	0	0
Urbanization class II townships	64	0.8	0.8	3.9	3.9
Female					
Hakka area	77	0.7	0.7	0.8	0.8
Mountainous area	75	1.8	1.8	0.8	0.8
East coast area 68	68	2.8	5.2	4.2	1.7
Penghu islands 70	70	0	1.8	4.7	2.9
Metropolitan areas	86	0.6	2.3	1.7	0
Provincial cities and urbanization class I townships	64	3.0	3.0	0	0
Urbanization class II townships	74	0	0	0	0

1 The red blood cell folate levels were measured by chemiluminescent enzyme immunoassay.

2 Criteria for red blood cell folate deficiency are folate less than 140 ng/ml or less than 160 ng/ml red blood cells.

3 Criteria for marginal folate deficiency are RBC folate at 140~200 ng/ml or 160~200 ng/ml red blood cells.

Table 9 Pearson correlation coefficient between plasma or red blood cell folate levels and dietary folate intakes evaluated as folate-rich food intake frequency[1]

Food item	Male		Female	
	Frequency (times/week)	r value	Frequency (times/week)	r value
	Plasma folate status			
13~64 years old	n=408		n=518	
fresh vegetables	16.3±12.9	0.0493	18.2±14.1	0.1787*
dark green vegetables	9.2±6.2	0.2388*	10.2±6.3	0.2272*
citrus fruits	2.0±3.2	0.2166*	3.1±10.5	0.0223
>65 years old	n=75		n=71	
fruits	5.2±4.8	0.4353*	5.9±9.1	0.4059*
pickled vegetables	3.1±6.0	-0.0140	3.0±3.0	0.2465*
	Red blood cell folate status			
13~64 years old	n=507		n=583	
dark green vegetables	9.1±6.2	0.1112*	10.2±6.3	0.1312*
citrus fruits	2.1±3.5	0.1237*	3.1±10.5	-0.0879
squash	1.5±2.2	0.1536*	1.6±2.1	0.1861*
pickled vegetables	1.1±2.0	0.1088*	1.6±5.6	0.0508
seaweed	0.8±1.1	0.1080*	0.8±1.2	0.0604
>65 years old	n=92		n=82	
fruits	5.2±5.1	0.3084*	5.4±8.3	0.0935
pickled vegetables	2.7±5.6	0.1697	2.7±3.0	0.2481*

[1] Data are shown as mean ± SD. The plasma folate values used are the average values of the two methods for folate assays.

* The correlation between food frequency and folate status is significant ($P < 0.05$).

Table 10 showed the negative correlation between blood pressure and folate status. Both systolic pressure and diastolic pressure were found to significantly negatively correlated with plasma folate for both male and female population. When correlation analysis was carried out in different age groups, male age 13~18 and aged over 65 showed a significantly negative correlation between plasma folate and systolic pressure. Females aged 7~12 and 45~64 also had a significantly negative correlation between plasma folate and systolic pressure. The diastolic pressure also negatively correlated with plasma folate at $P < 0.1$ levels.

Table 10 The negative Pearson correlation coefficient between plasma folate levels and blood pressure

	n	Systolic pressure		Diastolic pressure	
		mmHg[1]	r value	mmHg	r value
Male					
All age	899	122±16	-0.1397*[2]	74.3±9.4	-0.0565#[2]
13~18 years old	164	120±12	-0.1600*	71.7±11.9	-0.0673
>65 years old	88	143±23	-0.2781*	81.2±13.8	-0.1861#
Female					
All age	991	119±18	-0.1218*	72.6±9.1	-0.0787*
7~12 years old	217	109±11	-0.1499*	66.8±12.6	-0.1143#
45~64 years old	208	134±22	-0.1377*	83.8±13.2	-0.1246#

[1] Data are shown as mean ± SD.
[2] The correlation is significant (*$P < 0.05$; #$0.05 < P < 0.1$)

Discussion

This study is the first to investigate the folate status of Chinese population in Taiwan. Senti (18) pointed out that data for prevalence of low folate value may not be taken as definitive assessments of population risk of folate deficiency because of the methodological and sample treatment problems encountered in NHANES studies. But they concluded those data could show relative differences among population. In order to avoid these problems, both microbiological assays and chemiluminescent enzyme immunoassay were used to validate the folate level in this study. The results indicated that the differences between two methods for plasma folate data were in general about 1~2 ng/ml. The difference were over 5 ng/ml for 3% samples which had been rechecked. For sample treatment problem, addition of ascorbic acid during blood collection did not significantly affect folate value (20). Therefore, RBC folate was determined only by chemiluminescent enzyme immunoassay, and it was positively correlated with plasma folate level. Despite the fact that different percentages of subjects with deficiency value might be due to analytic methodology and cutoff values used, the prevalence of low folate value could be taken as the population at risk of folate deficiency in this study.

High risk of folate deficiency has been reported in pregnant women, the alcoholic, the elderly, adolescents, and infants fed sterilized milk (9,10,21-23). Low dietary folate intakes, higher folate requirement, low folate bioavailability due to heat destruction, malabsorption or drug interaction, etc. may be considered as reasons causing folate deficiency (25,26). It has been reported that the highest percent with low folate values was in females aged 20~44 years, using 10% of the second National Health and

Nutrition Examination Survey (NHANES II) samples(18). The percentage of adult subjects aged 20~39 years with low folate level determined by either unadjusted data or by new cutoff value appeared to be higher in female population, though the folate data in NHANES III still need to be validated due to the problems of reference standard and cutoff value (19). However, our results indicated that female adults had higher plasma folate concentrations than male adults in this study. Interestingly, the difference of folate status between sexes was observed in this study. Plasma folate value was the highest in children aged 4~6 years for male group and in adults aged 45~64 years for female group, respectively. The adolescents aged 13~18 years appear to be the group at greatest risk for developing folate deficiency for both sexes, though female teenagers may had higher folate values than that of males. One study investigated the dietary folate intakes and plasma concentrations of folate in healthy adolescents and found that plasma folate concentration were reported to be subnormal in 9.4% of boys and 4.7% of girls from low-income families. Although boys did have higher intakes of dietary folate than girls, girls had higher concentrations of plasma folate than did boys. It has been proposed that higher intake of dietary folate for boys than for girls and decreasing plasma folate concentrations with increasing maturity may represent greater tissue and cellular demands for folate in boys than in girls (24). Thus, male teenagers in Taiwan should be especially aware of their folate nutritional status.

The percentages of males with low plasma folate, RBC folate and both were only 5.2%, 4.1% and 0%, respectively. The percentages of females with low of plasma folate, RBC folate and both were only 2.6%, 2.8% and 0%, respectively. Therefore, folate deficiency may not be a clinical problem in Taiwanese population. However, up to about 29% of males and 12% of females were in the marginal deficiency range with plasma folate at 3~6 ng/ml. So, 31% of males and 14% of females were not in good folate status. In addition, 42% of males and 28% of females adolescents with inadequate folate status should be noticed. Since this may be due to either the increased needs for growth or the inadequate folate intakes, more folate rich foods should be consumed by adolescents.

In HANES, 6% or fewer of elderly subjects had serum folate below 3 ng/ml (27). The percentages of males aged 45~74 with low plasma folate, RBC folate and both were 10%, 8% and 3%, respectively, and 9%, 4% and 2% for female aged 45~74, respectively (18). In our study, the percentages of elderly males with low plasma folate, RBC folate and both were only 0.2%, 3.8% and 0%, respectively. The percentages of elderly females with low plasma folate, RBC folate and both were only 0.4%, 0.5% and 0%, respectively. Overall, the folate status of the elderly is not a problem because folate rich foods such as various fresh and pickled vegetables are still commonly consumed by the elderly in Taiwan. However, 41% male elderly are marginally deficient which is twice that of the female elderly. Whether higher percentage of marginal folate deficiency in males is correlated with lower folate intake or more alcohol consumption remains to be investigated.

Studies in different locales suggested that low-income elderly persons or people in lower socioeconomic group may be at higher risk of folate deficiency (10,28). The folate status in different regions of Taiwan was also investigated in this study. The results showed that people living in mountainous areas and Penghu islands had the lowest plasma and RBC folate values especially for males. The highest percentage of folate deficiency with low plasma folate values were 7~21% for males and 2~13% for females living in mountainous areas. The people living in east coast had relatively higher percentage of deficiency with low RBC folate values for both sexes. However, relatively higher percent of marginal deficiency was found for people living on Penghu island. Since folate-rich food are relatively inexpensive, whether the income, food availability, dietary pattern, or alcohol consumption may affect the folate status in various districts remains to be investigated.

The dietary habits of people in Taiwan may represent a typical Chinese dietary pattern which is different from Western diet. Rice is the staple food in many Asian countries. Chinese cuisine usually includes more vegetables and beans compared to those of the other countries. There are a lot of varieties of vegetables and fruits, especially in Taiwan because of the warm climate and agricultural technology. In addition, fermented food and pickled vegetables, which may supply folate from original material or microorganism, are commonly consumed by Chinese people. Therefore, folate status in Taiwan is different from that in Western countries. However, the dietary habits may have changed for some population recently because instant food has become more popular due to industrialization and internationalization. Westernized dietary habits may affect the vegetable and fruit consumption in Taiwan, especially for young generations. Daily folate intake has been reported to be about 200 μg in Western countries in Europe and America (7). Although estimates of dietary folate intake is important to investigate the folate nutritional status in Taiwan, folate contents of food are not currently available for calculation. In this study, several folate-rich food items were selected to evaluate whether there is a correlation between these food intake frequency and folate status. Although it is likely that measurement error exists in these values, significant carrelation of the intake frequency of folate-rich food with folate status suggests good food sources for people with adequate folate status. The results suggested that, under Taiwanese dietary pattern, increasing intakes of dark green vegetables and citrus fruits was helpful for improving folate status for males aged 13~64. Females aged 13~64 who consumed fresh vegetables and dark green vegetables more often had higher plasma folate levels. In addition, both males and females who consumed squash more often had higher RBC folate levels, suggesting that squash may be a good food source for folate in Taiwanese dietary pattern. Increasing intake of other foods such as pickled vegetables and seaweed also increased body store folate in males aged 13~64. For elderly, males who consumed more fruits had higher plasma and RBC folate levels. Females with increasing intakes of fruits and pickled vegetables had increasing plasma or RBC folate levels. Dietary data from 24-hour recalls collected in NHANES II reported that orange juice is the major source, contributing 9.7% of dietary folate intake. White bread, cooked dried beans, green salad, cold

cereal, and eggs each contributed from 8.6% to 4.6% (29). Since orange juice and cereal are not commonly consumed by most people in Taiwan, these items were not specified for questionnaire and their contribution to folate status is hard to evaluate. The intake frequencies of squash, pickled vegetables and seaweed by adult males were low and varied, suggesting promoting these food intakes may be helpful for those with low folate status. However, the contribution of food items to folate status remains to be confirmed when folate contents of Chinese food data are available in the future.

Several studies indicated that high levels of plasma homocysteine may associated with risk of vascular disease such as myocardial infarction, stroke, premature coronary artery disease, carotid artery atherosclerosis (30-33). Folate deficiency was associated with marked elevation of homocysteine, which is negatively correlated with serum folate level (34-36). Folic acid supplement was shown to significantly reduce plasma homocysteine concentrations by 41.7% (37). Plasma concentration of folate and folate intake values were inversely associated with extracranial carotid stenosis after adjustments for age, sex and other risks (38). In our study, the correlation between plasma folate and systolic pressure and diastolic pressure were examined to investigate if folate may play a role in vascular disease. The significantly negative correlation between plasma folate and systolic pressure suggests folate status may be considered an important factor for vascular disease in Taiwan.

In conclusion, folate status of Chinese in Taiwan is thought to be in a good condition overall from the view of the percentage of folate deficiency. However, up to 30% for males and 12% for females are noted to have marginal deficiency with low plasma folate, suggesting folate intake should be emphasized to avoid the risk of developing folate deficiency. Adolescents are the group at the greatest risk in Taiwan and their dietary patterns should be adjusted. The consumption of vegetables, fruits, squash, seaweed, pickled vegetables may be helpful for improving folate status. The negative correlation between plasma folate level and blood pressure suggests that folate status may play a role in vascular disease.

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國民營養健康狀況變遷調查－葉酸之營養狀況

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摘 要

本研究對象爲民國八十二年七月至八十五年六月進行之「國民營養健康狀況變遷調查」之第一年血漿與紅血球的血樣，包括 4 歲以上人數 1929 人（男 916 人，女 1013 人）。採用微生物法和化學冷光測定法，測定血漿葉酸與紅血球葉酸濃度，對國人葉酸營養狀況作一評估。結果顯示，男性瀕臨缺乏的盛行率以 13 歲年齡層最高。男性只有約 58% 和女性約 72% 血漿葉酸在正常值範圍，瀕臨缺乏率也以男性較女性爲高，並隨年齡增而瀕臨缺乏率增加。若以台灣地區不同行政區來區分，山地地區的血漿葉酸缺乏與瀕臨缺乏之盛行率最高，但以紅血球葉酸含量評估，則葉酸缺乏率以東部地區最高，瀕臨缺乏率以澎湖的最高。爲探討與飲食的相關性，以富含葉酸的食物種類的每週攝食頻率與血漿或紅血球葉酸濃度作相關性分析。結果顯示，深綠色蔬菜、新鮮蔬菜、柑橘、水果類、瓜類、醃漬蔬菜、海藻等的攝取可能有益於葉酸的營養狀況。此外，在本研究中，血漿葉酸濃度與血壓有負相關性，尤其是收縮壓，因此，葉酸的營養狀況與其血管相關疾病之關係是值得繼續探討和注意的問題。

關鍵字：葉酸營養狀況、血漿葉酸、紅血球葉酸、微生物法、化學冷光法、國民營養調查