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Association between Dietary Folate Intake and Blood Status of Folate and Homocysteine in Malaysian Adults

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Summary Folate is of prime interest among investigators in nutrition due to its multiple roles in maintaining health, especially in preventing neural tube defects and reducing the risk of cardiovascular diseases. We investigated the effect of dietary folate intake on blood folate, vitamin B_{12}, vitamin B_6, and homocysteine status. One hundred subjects consisting of Chinese and Malay subjects volunteered to participate in this cross-sectional study. Dietary folate intake was assessed by 24-h dietary recall and a food-frequency questionnaire (FFQ). Serum and red blood cell folate were analyzed using a microbiological assay, while serum vitamin B_{12} was determined by electrochemiluminescence immunoassay (ECLIA), and high-performance liquid chromatography (HPLC) was used for the determination of serum vitamin B_6 and homocysteine. The mean folate intake, serum folate, RBC folate, serum vitamin B_{12}, and B_6, were higher in female subjects, with the exception of serum homocysteine. The Chinese tended to have higher folate intake, serum folate, RBC folate, and vitamin B_{12}. A positive association was found between folate intake and serum folate while a negative association was found between folate intake and serum homocysteine. Stepwise linear regression of serum folate showed a significant positive coefficient for folate intake whilst a significant negative coefficient was found for serum homocysteine when controlling for age, gender, and ethnicity. In conclusion, high dietary folate intake helps to increase serum folate and to lower the homocysteine levels.

Key Words microbiological assay, folate, homocysteine, ethnicity

Folate is a water-soluble B vitamin. Its deficiency could lead to anemia, cardiovascular disease, neural tube defects, and other congenital defects, adverse pregnancy outcomes, neuropsychiatric and cognitive disorders, and cancer (*1*, *2*). The mechanisms by which folate deficiency may enhance the development of these disorders are in part related to the fundamental function of folate, which mediates the transfer of one carbon group (*3*). Folate is an essential cofactor for the de novo biosynthesis of purines and thymidylate, which is the building block in DNA synthesis, stability and integrity, and repair. Folate also serves as the substrate in the remethylation of homocysteine to methionine, which is the precursor of *S*-adenosylmethionine (SAM), the primary methyl group donor for most biological methylations, including that of DNA (Fig. 1).

Generally, the Recommended Nutrient Intakes (RNI) for folate differ among countries and range from 300 to 400 μg/d. In Malaysia, 400 μg/d is important to maintain an adequate folate intake. As humans are unable to synthesize folate in the body, they depend solely on adequate folate intake in order to maintain the folate status. Various ways can be taken to increase folate intake including consuming folate-rich food, supplementation, and fortification. However, Malaysia does not have a mandatory fortification scheme in place yet and this results in higher dependency on fruit and vegetables.

Currently, data is lacking concerning dietary folate intake in Malaysia due to the absence of a Malaysian food folate database. A study on Malaysian women of childbearing age by Khor and associates (*4*) reported that only around 50% of the Malaysian RNI level was achieved (202 μg/d). However this study only investigated the relationship between dietary folate and folate status in women. Furthermore little is known about the effect of dietary folate intake, and its relationship with B_{12}, B_6 and homocysteine status among Malaysians which comprise different ethnic groups. Hence, the aim of this study is to assess the effect of folate intake on B-vitamins and the homocysteine blood parameter status of the Malaysian population in Universiti Putra Malaysia (UPM).

MATERIALS AND METHODS

Sampling. This is a cross-sectional study where 100 subjects (54 Chinese and 46 Malays) consisting of staff and postgraduate students were recruited by using simple random sampling from five selected faculties (For-

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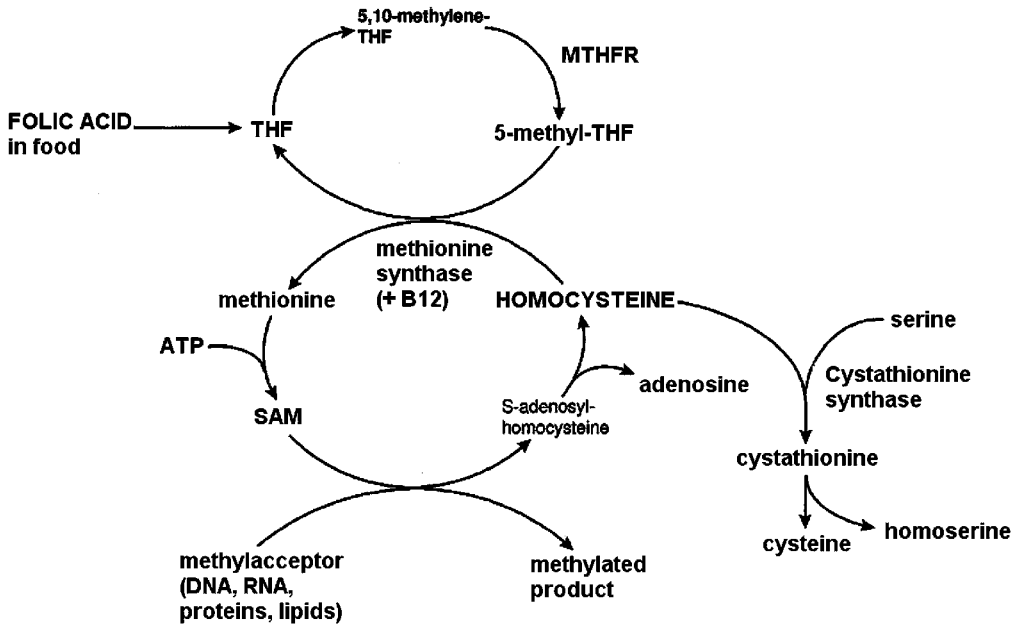

Fig. 1. Metabolic pathways of folic acid and homocysteine (THF: tetrahydrofolate, SAM: *S*-adenosylmethionine) (*18*).

estry, Engineering, Economy, Language, and Medicine and Health Sciences) in UPM, Serdang. The sample size was calculated by using the *t*-test comparing for two groups of mean (wild and variant genotype which affect the folate status) in a Singapore study on Chinese and Malay subjects (*5*). The α value set was 0.05 and β value was 0.10. Approval from the Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, UPM, was obtained for the study. Subjects were given an information sheet explaining the purpose and methodology of the study. Those who agreed to participate were asked to sign an informed consent form prior to their participation. The inclusion criteria were as follows: 20–45 y old male and female, Chinese or Malay, non pregnant or breastfeeding, not consuming alcohol habitually, and not a regular smoker. The exclusion criteria were: use of any type of B-vitamin supplement in the past 3 mo or oral contraceptives.

Dietary assessment. The 24-h dietary recall method was used by the interviewer to record the dietary folate intake. The food quantities were assessed by the use of household measurements. Dietary folate intakes were then tabulated based on the USDA Nutrient Database for Standard Reference. The Nutritionist Pro, version 2.5 food database (First Databank, California, USA) was modified by adding food recipes and local processed food items using nutrient data provided by the industry. The frequency of food consumption was classified according to the score method using a five-point scale.

Biochemical assessment. Subjects were required to fast (10–12 h), and from each subject 10 mL of blood was drawn in order to test for complete blood count, folate, homocysteine, vitamin B_{12} and vitamin B_6. The obtained serum was stored at -20°C until the analysis of folate, vitamin B_{12}, vitamin B_6, and homocysteine took place. Determination of serum folate and red blood cell folate was based on the microbiological assay method (MBA) with *Lactobacillus casei* as the test microorganism (*6*). *L. casei* was cryopreserved by the methods of Wilson and Horne (*7*). Serum vitamin B_{12} was determined by using the electrochemiluminescence immunoassay (ECLIA) with an Elecsys vitamin B_{12} reagent kit on an Elecsys 2010 (Roche Diagnostics GmbH, Germany). Pyridoxal-5-phosphate (PLP) was measured with a reagent kit for high-performance liquid chromatography (HPLC) with fluorescence detection for vitamin B_6 (Chromsystems, GmbH, Germany). A commercial kit (Chromsystems, GmbH) for HPLC was used to determine total homocysteine.

Statistical analysis. Results were expressed as mean± SD. Data was analyzed by using Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) version 17. Independent sample *t*-tests and ANOVA were done to examine the mean differences for categorical independent variables with continuous variables, while Pearson's correlation was used to examine the relationship between two normally distributed continuous variables. Stepwise linear regression analysis with blood parameters as independent variables was used to elucidate the variables that better predict folate intake.

RESULTS

Demographic and socio-economic background

Baseline characteristics of the subjects are shown in Table 1. Out of 100 subjects, 58% were female and 42% were male with a mean age of 28 y. The majority of the subjects were in the age group of 20–24 y (42%), followed by 25–29 y (28%), 35–39 y (15%), 30–34 y (11%), and finally 40–45 y (4%). Chinese subjects comprised 54% whilst 46% were Malay subjects. Predominantly, the income earned by subjects was in the range of Ringgit Malaysia (RM) 1,001–2,000 (41%), followed by RM 1,000 or less (26%), RM 3,001 and above (23%) and finally 10% was in the range of RM 2,001–3,000.

Table 1. Demographic and socioeconomic characteristics of the subjects (n=100).

Variables	Frequency	Percent	Mean±SD
Gender			
Male	42	42%	
Female	58	58%	
Ethnic			
Chinese	54	54%	
Malay	46	46%	
Marital status			
Single	27	27%	
Married	68	68%	
Engaged	5	5%	
Occupation			
Staff	52	52%	
Student	48	48%	
Monthly income (RM)			
<1,000	26	26%	
1,000–2,000	41	41%	
2,001–3,000	10	10%	
>3,001	23	23%	
Age group (y)			27.970±6.196
20–24	42	42%	
25–29	28	28%	
30–34	11	11%	
35–39	15	15%	
40–45	4	4%	

The subjects in this study were predominantly married (68%), while only 27% were single and 5% were engaged.

Folate intake

Overall, the mean total intake of folates from diet was 260.281 μg/d for males and 321.934 μg/d for females (p=0.035) (Table 2). Chinese subjects tended to have significantly higher folate intakes (325.451 μg/d) compared to the Malay subjects (261.514 μg/d). Analysis of food consumption showed that the folate intake was below the Recommended Nutrient Intake (RNI) for Malaysia (400 μg/d). The ranges of scoring for FFQ (food-frequency questionnaire) were divided into three categories: highly consumed foods (score= 80.0–100.0), moderately consumed foods (score= 60.0–79.9), and less consumed foods (score=≤59.9). Cereals as well as egg and dairy products showed the highest consumption with 22% for each, followed by vegetables (21%), legumes (18%) and finally fruits (17%). The most commonly consumed foods, in decreasing order, were rice (96.6%), followed by eggs (84.4%), noodles (72.6%), cabbage (62.4%), and tofu (62.2%).

Red blood cell (RBC) folate, serum folate, vitamin B_{12}, B_6 and homocysteine status

Most of the subjects had normal levels of serum folate, vitamin B_{12} and B_6 (Table 2). However, the majority of the subjects were deficient in RBC folate and slightly more than half had elevated serum homocysteine levels. The serum folate, vitamin B_{12}, and B_6 levels were significantly different between the males and

Table 2. Folate intake and clinical characteristics of the subjects.

Variables	n	Mean±SD	p-value
Folate intake (μg/d)			
Gender			0.035
Male	42	260.281±28.108	
Female	58	321.934±67.747	
Ethnic			0.032
Chinese	54	325.451±78.330	
Malay	46	261.514±49.234	
Serum folate (nmol/L)[1]			
Normal (>6.8)	73		
Deficient (≤6.8)	27		
Gender			0.007
Male	42	8.231±3.198	
Female	58	10.289±4.293	
Ethnic			0.020
Chinese	54	10.276±3.947	
Malay	46	8.425±3.836	
RBC folate (nmol/L)[1]			
Normal (>363)	9		
Deficient (≤363)	91		
Gender			0.393
Male	42	196.855±27.423	
Female	58	219.046±36.557	
Ethnic			0.642
Chinese	54	220.661±30.824	
Malay	46	200.411±21.997	
Serum vitamin B_{12} (pmol/L)[2]			
Deficient (<148)	13		
Normal (148–664)	84		
High (>664)	3		
Gender			0.02
Male	42	397.950±13.923	
Female	58	505.567±42.506	
Ethnic			0.049
Chinese	54	492.539±47.796	
Malay	46	432.963±23.996	
Serum vitamin B_6 (nmol/L)[3]			
Normal (>20)	63		
Deficient (≤20)	37		
Gender			0.045
Male	42	21.151±7.841	
Female	58	22.849±14.513	
Ethnic			0.029
Chinese	54	19.599±9.022	
Malay	46	25.114±14.551	
Serum homocysteine (μmol/L)[3]			
Normal (<14)	46		
Elevated (≥14)	54		
Gender			0.046
Male	42	15.088±2.314	
Female	58	14.141±2.312	
Ethnic			0.815
Chinese	54	14.487±2.259	
Malay	46	14.599±2.472	

[1]The cutoff point for folate (*4*).
[2]The cutoff point for vitamin B_{12} (*19*).
[3]The cutoff point for vitamin B_6 (*20*).
[3]The cutoff point for homocysteine (*20*).

Table 3. Correlation of folate intake and serum homocysteine to blood parameters concentration.

Variables	r	p
Folate intake		
Serum folate (nmol/L)	0.315	0.001
RBC folate (nmol/L)	0.066	0.513
Serum vitamin B_{12} (pmol/L)	0.018	0.861
Serum vitamin B_6 (nmol/L)	−0.043	0.675
Serum homocysteine (μmol/L)	−0.143	0.016
Serum homocysteine (μmol/L)		
Serum vitamin B_{12} (pmol/L)	−0.851	0.019
Serum vitamin B_6 (nmol/L)	−0.411	0.033

Table 4. Stepwise linear regression model predicting blood parameters (folate, vitamin B_{12}, B_6, and homocysteine) using folate intake.

Model		Unstandardized coefficients		Sig
		B	Std. error	
1	(Constant)	−94.971	129.105	0.464
	serum_fol	41.49	12.627	0.001
	serum_homo	−8.35	−0.852	0.040

Adjuster R^2=0.09. serum_fol: serum folate, serum_homo: serum homocysteine.
B, Beta; Sig, significant.

females as well as between the Chinese and Malay subjects ($p<0.05$) (Table 2). The level of homocysteine was significantly higher in male subjects but no significant difference was found between the two ethnic groups. The RBC folate level was not significantly different between genders and the same could be observed when comparing ethnicity ($p>0.05$). Besides folate, serum homocysteine also strongly negatively associated with serum vitamin B_{12} ($p=0.019$) and moderately negatively associated with vitamin B_6 ($p=0.033$) (Table 3), which are the two cofactors in folate metabolism.

Folate intake and folate, vitamin B_{12}, B_6, and homocysteine status

From the correlation table, there was a significant negative correlation of folate intake with serum homocysteine ($p<0.05$) (Table 3). As for serum folate, a positive correlation was seen with folate intake. In contrast, there was no correlation between folate intake and RBC folate, serum vitamin B_{12} or B_6. In Table 4, the stepwise linear regression model using folate intake showed a significant positive coefficient for serum folate whilst a significant negative coefficient was noted for serum homocysteine.

DISCUSSION

A study among Taiwanese by Chen and associates (*8*) stated that the level to be considered as constituting an adequate intake of dietary folate is when the consumption is above 2/3 of the Recommended Dietary Allowance (RDA) recommendation (400 μg/d), which is 267 μg /d and above. Surprisingly, from our findings, the Chinese showed an intake above 2/3 and had higher folate intake than the Malay subjects. The differences in folate intake in terms of ethnicity might be due to the cultural differences in food preparation in terms of cooking practices. According to Hughes and Ong (*9*) in their study on Indians, Malays and Chinese in Singapore, the Malay dishes such as curries are often cooked for a long duration at high temperature whereas the Chinese dishes are often stir-fried, thus, causing less destruction in dietary folate. Kasiman and associates (*5*) in their study on Singaporeans also reported the same opinion concerning the prolonged cooking which is a feature of Malay cuisine. Due to the physical characteristic of folate, which is heat labile, prolonged cooking at high temperature causes up to 90% destruction of the folate content of the foods (*10*). In addition, consumption of folate-rich food, such as fortified breakfast cereals, is inadequate, and might not be customary among the Malay subjects, as can be seen from the FFQ. As for the Chinese, they consume more green leafy vegetables and legumes than Malays, which are a good source of folate based on the FFQ in this study.

Generally, females had a higher folate intake than males due to the role played by folate, especially for pregnancy, since adequate folate intake during the periconceptual period helps in protecting against the incidence of neural tube defects. In addition, better consumption abilities in females enable them to access more high-folate food, especially fruit and vegetables. The present folate intake among females (321.934 μg/d) is slightly higher compared with the data from the study of Khor et al. (*4*) in Malaysia with 227.9 μg/d and 219 μg/d in Malay and Chinese females, respectively. By referring to the RNI, the present results in females seem to be sufficient as it was above the recommended level. The variation in the folate intake reported when using the 24-h dietary recall might be due to the possibility of under or over reporting since it relies heavily on the precision of memory and the honesty of the subjects as well as differences in the instruments used (pictures, household measurement) while assisting the recording process. Compared with other countries, such as females from Denmark, 283 μg/d (25–30 y old) (*11*), and Vietnam, 251 μg/d (19–60 y old) (*12*) the results in this study showed a slightly higher folate intake. These observations indirectly support the fact that ethnicity and lifestyle could influence the folate intake. In this study, males had lower folate status than females. This may be due to the fact that biologically, males who have a larger body size tend to have a lower response to folic acid since the dose distributes over a larger volume compared to females (*13*). Hence, this explains why men need more folic acid than women to increase their folate status by the same extent.

The only source of folate consumption among the subjects in the present study was natural foods. Hence, it is important to clarify the association between the intake of natural food folate in Malaysian food and

blood folate concentrations. A weak but statistically significant association was seen between folate intake and serum folate concentration. This was supported in other studies as well (*14*). In contrast, Jacob et al. (*15*) reported that dietary folate intake showed no association with red blood cell folate concentration, which is similar to this study. This was probably due to low folate intake, which might not be sufficient for a long period of storage in the body. Among the nutritional factors, deficiency in folate is associated with an elevation in homocysteine concentration. Theoretically, when the folate intake is high, it helps lower the homocysteine levels by converting it to methionine, with the help of vitamin B_{12}, or converts it to cysteine with the help of vitamin B_6. This is further supported by Boushey et al. (*16*) who reported that a folate intake of ≈200 μg/d resulted in 4 μmol/L or 12% reduction in the homocysteine concentration. Bronstrup and associates (*17*) showed that in order for serum homocysteine concentration to be reduced about 11%, at least 400 μg/d of folate intake is required. Hence, this provides evidence of the protective effect played by folate in lowering the homocysteine concentration and the concurrent risk of vascular diseases. In the present study, there was no association between the folate intakes and serum vitamin B_6 which might be due to the slow response of serum B_6 to the changes in the intake of folate as a result from the sustained release from tissue store.

Serum vitamin B_6 was higher in Malay than in Chinese subjects as shown in Table 2. We did not measure their dietary B_6 intake but it could be due to the differences in their dietary intake of B_6 or the higher prevalence of the thermolabile MTHFR allele found among the Chinese population compared to the Malay (5). People possessing thermolabile MTHFR would have their transmethylation pathway impaired, leading to the takeover by transsulfuration pathway. It might therefore be expected that serum vitamin B_6 concentrations would be lower in those possessing the thermolabile allele.

In conclusion, our data illustrate the importance of folate intake in influencing the serum folate and homocysteine levels. Although the intake was above the recommended cutoff level of 2/3 for sufficient folate, it was still below the recommendation by RNI Malaysia. Nevertheless, high folate intake helps increase serum folate but not RBC folate and lowers the homocysteine levels. Considering the importance of folate in health maintenance it is important to increase the public's awareness of diet, which may be associated with improved folate status.

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