FOOD BEHAVIOR AND FOLATE STATUS OF HILL-TRIBE SCHOOLCHILDREN AND WOMEN OF CHILDBEARING AGE ON THE NORTHERN BORDER OF THAILAND

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Abstract. An insight into the folate nutritional status of the population is important from a public health perspective. The protective effect of folate against neural tube defects (NTDs) is widely recognized. To assess the health and nutritional status, especially folate status, of vulnerable hill-tribe groups, a cross-sectional study was conducted on 197 schoolchildren and 136 women of childbearing age in Chaloem Phra Kiat District, Nan Province, Thailand. The nutritional status of the study group was investigated by dietary survey, and blood samples were taken to determine hematocrit, protein, and serum and red blood cell folate. Anthropometric measurements were taken to assess body size, composition and nutritional indexes. The health and nutritional status of the hill-tribe schoolchildren and women of childbearing age were found to be unacceptable, particularly inregard to folate status, which was indicated by low folate levels found in the blood samples, and in the intake of this micronutrient.

INTRODUCTION

Chaloem Phra Kiat District, Nan Province is located in northern Thailand. The district is a mountainous area of 518.7 km^2, 144 km from Nan, the provincial capital, and borders Lao PDR in the north and east. Residents of Chaloem Phra Kiat District are members of hilltribe ethnic groups who inhabit the hill and plains areas, and are engaged in highland agriculture. Tropical environmental conditions, clustered housing, low socio-economic status and traditional lifestyle intensify the transmission of parasitic diseases (Maipanich ***et al,*** 2002). Because the district is remote from the city, food supplies must be transported, and are expensive. The community largely depends on its own cultivation and hunting for sources of food. However, modern telecommunications technology, media, and information transmission systems are available. Hence, they acquire information from other parts of the world; clearly, this includes the influences of food-company advertising. Dietary changes through acculturation take place within the ethnic groups and many projects have been introduced to improve their quality of life. School lunches and school milk are provided for schoolchildren (http://www. lp.ago.go.th/law/law20/L20-02.htm). Interestingly, the food habits of these people ensure appropriate nutrition, especially with micronutrients. Folate

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is of micronutrient essential for health, growth and development. There is an increased demand for folate in pregnancy on account of marked placental growth and during the fast growing period of children. Principle folate sources include liver, dark green leafly vegetables, dry beans and peas. Most fruits and vegetables contain small amounts of the vitamin. Food derived folate is generally sensitive to changes in pH, the presence of an oxidizing agent and exposure to heat and light (Basu and Dickerson, 1996).

It has become increasingly evident that folate plays a vital role in human health. Folate is an important cofactor for one-carbon metabolism and for DNA base synthesis involved in neurotransmitter synthesis and aminoacid metabolism. Adequate folate intake is required for normal metabolism, cell division, neural function, and growth. Humans are unable to synthesize folate and depend on an adequate and constant intake. Mothers have an increased risk for giving birth to infants with spina bifida and other neural tube defects if their folate status is inadequate during early pregnancy (Pitkin, 2007).

Optimal folate nutritional status is important for minimizing developmental and degenerative disease. Therefore, constant monitoring of folate intake and of biomarkers of folate nutritional status are essential. This study aimed to survey the health and nutritional status, especially folate, of schoolchildren and women of childbearing age in a remote area of Thailand.

MATERIALS AND METHODS

Subjects

The study participants were comprised of 197 schoolchildren and 136 women of childbearing age recruited from Chaloem Phra Kiat District, Nan Province, Thailand. The schoolchildren were 99 boys and 98 girls age 5-16 years from Huay Sai Khoa, Huay Krone, and Chaloem Phra Kiat schools. Women age 15-45 years were recruited from housewives in each household in the district. Healthy childbearing age women free from serious or chronic diseases and non-oral contraceptive users or multivitamin user were selected. Pregnant and lactating women were excluded from the study. All participants provided informed consent and the research protocol was reviewed and approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University.

Study methods

The nutritional status of the study groups was investigated. Five milliliters of fasting venous blood was taken from all subjects. EDTA was used and one milliliter whole blood was centrifuged for 3 minutes at 14,000*g* in cipillary tubes (Clay Adams, USA) and for the hematrocrit measurement. Four milliliters blood was left to clot, and the serum was aliquoted. Both the whole blood and serum were kept at 4ºC until use. Total protein and albumin (automatic chemistry analyzer, Hitachi 917), prealbumin (rocket immunoelectrophoresis; Laurell, 1972), serum and whole blood folate levels were measured in duplicate by microbiologic assay (Waters and Mollin, 1961) using *Lactobacillus casei*, ATCC No. 7469. RBC folate was calculated from measurements of folate and hematocrit. Anthropometry was comprised of weight and height measurements. Waist and hip circumference measurements were included for the women of childbearing age.

Trained staff conducted interviews with structured questionnaires to obtain detailed demographic characteristics and information about alcohol use, smoking, and diet. Twenty-four-hour recalls were used to estimate energy, macronutrients and folate intake for the women's groups. With the advice of the research investigators, the schoolchildren were asked to record their dietary intake for three days. Young schoolchildren kept records of their food intakes with the help of their

teachers and parents. The amount of food consumed was estimated by equivalent household measuring utensils. Energy, macronutrients, and folate intake were calculated using the computer program NutriSurvey 2006; the food composition database was provided by the Nutrition Division, Department of Health, Ministry of Public Health (2001) and the folate food table of Areekul (1986).

Statistical analysis

The resultant information was analyzed using descriptive statistics with Stata version 5.0 for Windows (Stata Corp LP, College Station, TX).

RESULTS

All the study participants belonged to different hill tribes, including Lour, Kamu, Thin, and Loeh, and lived in Chaloem Phra Kiat District, an area of highland agriculture. The area is mountainous and wooded, with gullies and small waterfalls. The products of the district are corn, sticky rice, and lychees. The children went to public schools supported by the government. Generally, the children looked well, although were somewhat small. The anthropometric indices – weight-for-age and height-for-age – indicated a higher percentage of substandard physical development among schoolchildren age 5-8 years. None of the children had overnutrition, as their anthropometric indices did not exceed the standard level (+2SD) (Table 1). This under-developed proportion reduced in adolescence (9-16 years). However, blood parameters showed only a few children to be abnormal; only one little boy had a low hematocrit. None had abnormal serum total-protein or albumin levels. Nevertheless, the findings of the more sensitive protein indicator, serum prealbumin, indicated a small percentage of inadequate protein status among the boys and girls in both age groups. While blood protein indicators and hematocrit were not considered a serious problem among the children, serum folate, which indicated present folate status, was unsatisfactory. More than half the children had a low serum folate level, and a rather high percentage of those exhibited folate deficiency. Fortunately, red blood cell folate, which reflects folate storage, was normal (Table 2).

Some women were illiterate; 32.3% of them had not gone to school at all. Only 3.2% of women had finished high school. Their income came mostly from agriculture and ranged between 100-20,000 Baht per month. More than half the women (59%) earned their living as farmers, 19% as employees, 15% as cotton weavers, and 7% conducted small businesses. Some of them drank a small amount of alcohol on occasion (9.6%) and 3.2% smoked cigarettes. Table 3 shows that 21.4% of the younger women (15-29 years) and 8.8% of the older group (30-45 years) were underweight (BMI<18.5), whereas 10% of the younger and 21.0% of the older group were overweight (BMI>25). Obesity was not found among the women in this study. However, a Waist-Hip Ratio (WHR) over 0.8, which indicates risk for chronic disease, was found

Table 1
Average percentage below the normal levels (-2SD) (NCHS, 1977) for children various anthropometric indices. Weight-for-age (W/A), height-for-age (H/A) and weight-for-height (W/H).

Sex/Age	W/A	H/A	W/H
Boys			
5-8 years (*n*=36)	22.2%	22.2%	13.9%
9-16 years (*n*=63)	19.0%	19.0%	11.1%
Girls			
5-8 years (*n*=41)	17.1%	17.1%	17.1%
9-16 years (*n*=57)	14.0%	14.0%	3.5%

Table 2
Hematocrit, total protein, albumin, prealbumin, serum folate and red blood cell folate in children studied.

	Boys 5-8 years			Girls 5-8 years			Boys 9-16 years			Girls 9-16 years		
	Mean±SD	*n*	%	Mean±SD	*n*	%	Mean±SD	*n*	%	Mean±SD	*n*	%
Hematocrit	38.2±2.8			38.5±2.1			41.8±3.0			42.4±2.6		
< cut-off point		1	2.8		0	0		0	0		0	0
≥ cut-off point		35	97.2		39	100		62	100		54	100
Total protein	7.6±0.4			7.6±0.4			7.9±0.4			8.0±0.4		
< 6.5 g/dl		0	0		0	0		0	0		0	0
≥ 6.5 g/dl		35	100		37	100		60	100		53	100
Albumin	4.3±0.2			4.5±0.2			4.5±0.2			4.5±0.2		
< 3.5 g/dl		0	0		0	0		0	0		0	0
≥ 3.5 g/dl		35	100		37	100		60	100		53	100
Prealbumin	20.2±5.2			21.0±4.0			25.1±6.0			24.8±5.4		
< 10 mg/dl		2	5.7		1	2.6		1	1.7		2	3.7
≥ 10 mg/dl		33	94.3		37	97.4		59	98.3		52	96.3
Serum folate	4.7±3.5			6.3±4.4			3.8±2.2			5.1±4.8		
< 3 ng/ml		11	34.4		8	22.2		27	47.4		14	26.4
3.0-6.0 ng/ml		15	46.9		15	41.7		20	35.1		27	50.9
≥ 6.0 ng/ml		6	18.8		13	36.1		10	17.5		12	22.6
RBC folate	277.8±70.6			308.2±95.5			268.6±74.6			264.4±72.4		
< 140 ng/ml		0	0		0	0		0	0		0	0
140-160 ng/ml		0	0		0	0		0	0		0	0
≥ 160 ng/ml		33	100		36	100		60	100		53	100

Reference value: cut-off point for hematocrit: boys and girls 5-8 years = 34%; boys and girls 9-16 years = 34-39% (WHO, 1972), serum and red blood cell folate (Garcia-Casal *et al*, 2005).

Table 3
Body mass index (BMI), waist-hip ratio (WHR), hematocrit, serum folate, and red blood cell (RBC) folate in women of childbearing age.

	Women age 15-29 years			Women age 30-45 years		
	Mean±SD	*n*	%	Mean±SD	*n*	%
BMI	20.7±2.9			22.3±2.9		
< 18.5		15	21.4		5	8.8
18.5-24.99		48	68.6		40	70.2
25-25.99		7	10		12	21
WHR	0.77±0.05			0.79±0.04		
<0.8		51	79.7		30	62.5
>0.8		13	20.3		18	37.5
Hematocrit	42.6±2.9			41.6±3.2		
< 36		2	2.9		4	7
≥ 36		68	97.1		53	93
Serum folate	6.2±4.0			8.5±5.5		
< 3 ng/ml		13	20		3	5.5
3.0-6.0 ng/ml		26	40		20	36.4
≥ 6.0 ng/ml		26	40		32	58.2
RBC folate	264.3±88.2			200.9±104.7		
< 140 ng/ml		8	11.9		23	41.1
140-160 ng/ml		1	1.5		5	8.9
≥ 160 ng/ml		58	86.6		28	50

Reference value: BMI, WHR (WHO, 1998; Deshmukh *et al*, 2005), Hematocrit (WHO, 1972), serum and red blood cell folate (Garcia-Casal *et al*, 2005)

in 20.3% of the younger age group and in 37.5% in the older age group. Anemia was seen in 2.9% of the younger and 7% of the older group, with hematrocrit levels < 36%. None of the women subjects were found to have an abnormal protein status, since all the blood parameters – serum total protein, albumin, and prealbumin – were normal. Compared with the children, the folate status of the women's groups in the study were worse. Eleven point nine percent of the younger and 41.1% of the older women's groups were found to have depleted stored folate (RBC folate < 140 ng/ml), though a higher percentage of children had folate levels below the cut-off point (serum folate <3 ng/ml).

The estimated dietary patterns of the children's groups are shown in Table 4. The daily energy intake of more than half the children met the recommended levels, except for the group of older boys (age 9-16 years). The carbohydrate intake for all children's groups was relatively high, exceeding the upper limits of the recommended levels. In contrast, the percentage of fat intake was below the lower limits. Daily protein intake among the younger boys and girls was satisfactory, while the older boys and girls had low-protein diets. The folate intake for all the children's groups was unacceptable. Table 5 shows the dietary patterns of the women's groups. Like the older boys and girls, both women's groups consumed high levels of carbohydrates, but low intakes of energy, fat, and protein, as well as folate.

Table 4
Daily intake of energy, macronutrients and folate in children studied.

	Boys 5-8 years			Girls 5-8 years			Boys 9-16 years			Girls 9-16 years		
	Mean±SD	*n*	%	Mean±SD	*n*	%	Mean±SD	*n*	%	Mean±SD	*n*	%
Energy (kcal/d)	1,390.0±258.0			1,376.4±257.1			1,783.8±413.4			1,702.7±415.0		
Intake <DRI		16	44.4		19	47.5		38	60.3		25	43.8
Carbohydrate	67.0±5.3			65.5±5.4			72.6±8.0			73.0±6.5		
(% of energy intake)												
Intake < lower limit DRI		0	0		0	0		1	1.6		0	0
Intake >upper limit DRI		27	75		19	47.5		53	84.1		50	87.7
Protein	12.1±2.0			13.0±1.8			11.3±1.8			11.0±1.4		
(% of energy intake)												
Intake <DRI		0	0		0	0		19	30.2		25	43.8
Fat	20.2±4.7			21.5±4.5			16.1±7.2			15.9±6.0		
(% of energy intake)												
Intake < lower limit DRI		29	80.6		31	77.7		55	87.3		54	94.7
Intake >upper limit DRI		0	0		0	0		1	1.6		0	0
Folate (μg/d)	77.2±27.8			88.1±34.7			75.5±25.5			75.5±27.0		
Intake <DRI		36	100		40	100		63			57	100

Dietary reference intake for Thais (Nutrition Division, 2003):
Energy: boys and girls 5-8 years=1,250-1,400 kcal/d; boys 9-16 years 1,700-2,300 kcal/d; girls 9-16 years 1,600-1,850 kcal/d
Carbohydrate: lower limit = 45%; upper limit = 65% for all group
Protein: boys and girls 5-8 years=7.04-8.0%; boys 9-16 years 9.4-11.2%; girls 9-16 years 10.3-11.5%
Fat: lower limit = 25%; upper limit = 35% for all group
Folate: boys and girls 5-8 years=200 μg/d; boys and girls 9-16 years 300-400 μg/d

Table 5
Daily intake of energy, macronutrients, and folate in women of childbearing age.

	Women 15-29			Women 30-45 years		
	Mean±SD	*n*	%	Mean±SD	*n*	%
Energy (kcal/d)	1,879.3±543.8			1,874.9±562.4	22	
Intake <DRI		28	40			39.3
Carbohydrate	74.9±8.8			80.2±6.9		
(% of energy intake)						
Intake < lower limit DRI		0	0		0	0
Intake >upper limit DRI		62	88.6		38	96.4
Protein	10.7±2.3			10.6±2.5		
(% of energy intake)						
Intake <DRI		49	70		42	75
Fat	14.4±7.9			9.1±6.3		
(% of energy intake)						
Intake < lower limit DRI		53	75.7		51	91.1
Intake >upper limit DRI		0	0		0	0
Folate (μg/d)	74.2±40.8			80.6±47.8		
Intake <DRI		70	100		56	100

Dietary reference intake for Thais (Nutrition Division, 2003): women in both age groups
Energy: 1,750 kcal/d
Carbohydrate: lower limit = 45%; upper limit = 65% for all group
Protein: 20-35%
Fat: lower limit = 20%; upper limit = 35% for all group
Folate: 400 μg/d

DISCUSSION

Health and good nutrition rely on adequate food intake. The food consumption patterns of the study group were evaluated, based on the Thai recommended intake levels, which have been established for each nutrient based on the amount needed to maintain adequate stores and circulating levels of each nutrient in the healthy Thai population (Nutrition Division, 2003). The calorie intake in the study group was mainly derived from sticky rice, resulting in a relatively high carbohydrate intake. Snacks, products from corn flour or wheat flour, and sugar were popular among the children. This behavior may have been influenced by the marketing of food companies. These snacks delivered relatively high levels of carbohydrates. Milk was the main protein source in the younger children, whereas older children drank less milk and gained less protein from other sources, which corresponds to the findings in both women's groups. However, their protein blood parameters showed no serious problems, since fish was a common source of protein for them. The low level of fat intake was obtained from vegetable oils, pork, meat, poultry and eggs, and less commonly, coconut milk. The serum folate levels for all the study groups revealed low folate intake. Although the storage of folate in the children's groups was in the normal range, their folate intake needed to be increased.

Anthropometry is the most portable, universally applicable, inexpensive, and noninvasive method available to assess the proportion,

size, and composition of the human body. It reflects both health and nutrition and predicts performance, health, and survival (NCHS, 1977; WHO, 1998). It indicated the improper development in some children in this study, resulting from inadequate energy and macronutrient intake, which corresponded to being underweight in some women. Growth and development were impaired in some children, which may be a consequence of parasitic infection in this area (Maipanich *et al*, 2002). However, being overweight in some women may have been a consequence of excessive intake of carbohydrates.

All nutrients are important for neuronal cell growth and development, but some appear to have greater effects during the late-fetal and neonatal periods; these include protein, iron, zinc, selenium, iodine, folate, vitamin A, choline, and long-chain polyunsaturated fatty acids. Consistent evidence shows that micronutrients are essential for the chemical processes that assure the survival, growth, and functioning of human systems. All age groups benefit from micronutrients, but deficiencies are particularly damaging and difficult to reverse when they occur during fetal development and in early childhood (Georgieff, 2007). A RBC folate level is a good biomarker for folate status because of its correlation with the liver, and it reflects long-term intake (>3 months), whereas plasma and serum folate values are commonly accepted to reflect recent dietary intake (Jacob *et al,* 1998). It is not surprising that the estimated folate intake in these children was unsatisfactory. When they regularly consumed low levels of folate over prolonged periods, their storage levels become depleted, which affects their health and development. During pregnancy, low concentrations of dietary and circulating folate are associated with increased risk for pre-term delivery, low infant birthweight, and retarded fetal growth (Scholl and Johnson, 2000). Most women of childbearing age in this study were found to be at risk for adverse pregnancy outcomes. In addition, women with insufficient folate intake are at risk of other chronic diseases, such as cervical, breast, and lung cancers, in combination with other risk factors (Rohan *et al,* 2000; Kwanbunjan *et al,* 2005; Shen *et al,* 2005). The chance of pregnancy is higher in the younger group, because fertility progressively declines in women over 30 years of age (American Society for Reproductive Medicine, 2003). Besides the probability of adverse pregnancy outcomes, both groups of women were at risk for other degenerative diseases. Due to the poor socioeconomic conditions of the study participants, health and nutritional education, including health promotion, should be implemented.

In conclusion, these survey findings provided evidence of the unsatisfactory health and nutritional status of hill-tribe schoolchildren and women of childbearing age in Chaloem Pra Kiat District, especially in folate status. This micronutrient deficiency exists in Thailand. To improve quality of life, relevant health and nutrition intervention programs should be developed.

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