

## Original Article

# Thiamin and riboflavin status of Taiwanese elementary schoolchildren

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In The Nutrition and Health Survey of Taiwan Elementary School Children (NAHSIT Children 2001~2002), erythrocyte activity coefficients of transketolase (ETKAC) and glutathione reductase (EGRAC) were chosen as indices for assessing the functional status of thiamin and riboflavin nutriture. Mean values of the ETKAC for boys and girls, both of which were in a normal range, were  $1.07\pm 0.00$  and  $1.06\pm 0.01$ , respectively. The respective prevalence rates of marginal and deficient thiamin states were 10.4% and 7.8% for boys and 9.3% and 7.3% for girls. Mean values of the EGRAC were  $1.18\pm 0.00$  for boys and  $1.19\pm 0.01$  for girls, both of which showed an increasing trend with age. The respective rates of marginal and deficient riboflavin states were as high as 32.5% and 3.5% for boys and 35.9% and 4.5% for girls. The marginal and deficient riboflavin statuses of Taiwanese schoolchildren were associated with a low frequency of dairy food consumption and an elevated risk of anemia.

**Key Words:** thiamin status, riboflavin status, elementary schoolchildren, biochemical assessment, Nutrition and Health Survey in Taiwan Elementary School Children (2001-2002)

## INTRODUCTION

Thiamin is involved in two categories of biochemical reactions in the coenzyme form of thiamin pyrophosphate (TPP): (1) decarboxylation of  $\alpha$ -ketoacids, such as pyruvate,  $\alpha$ -ketoglutarate, and branched-chain ketoacids (BCKAs), and (2) transketolation between hexose and pentose phosphates in the hexose monophosphate shunt, which supplies ribose for nucleic acid synthesis and NADPH for fatty acid synthesis.<sup>1,2</sup> In addition to being an important cofactor in fuel metabolism, TPP also significantly contributes to the nervous system by aiding in neurotransmitter and nerve conduction.<sup>2</sup> Although thiamin-deficient beriberi is now rarely seen in developed countries, the presence of marginal deficiency is sometimes overlooked in an apparently healthy population with adequate intakes. The study of Bailey *et al.*<sup>3</sup> on British adolescents reported the analyzed thiamin intakes of  $1.52\pm 0.09$  mg/d for girls and  $1.95\pm 0.19$  mg/d for boys. However there were 35.3% of the girls and 22.3% of the boys who were determined as marginal or deficient thiamin status using ETKAC as the biochemical index.<sup>3</sup>

In the coenzyme forms of FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide), riboflavin catalyzes numerous redox reactions in fuel and intermediary metabolism,<sup>5,6</sup> including activation of vitamin B6 and folate to their respective coenzyme forms of pyridoxal 5'-phosphate (PLP) and 5'-methyl tetrahydrofolate.<sup>4,5</sup> Clinical

signs of riboflavin deficiency include cheilosis, angular stomatitis, glossitis, and seborrheic dermatitis. Severe stage riboflavin deficiency is usually accompanied by poor nutriture of other nutrients such as vitamin B6 due to insufficient activation of PLP.<sup>4,5</sup>

When reviewing the results of previous nationwide nutritional surveys in Taiwan, we found that erythrocyte activity coefficients of transketolase and glutathione reductase (ETKAC and EGRAC) were used for the first time as biomarkers of the long-term functional status of thiamin and riboflavin, respectively, for Taiwanese aged 4 years and above in the Nutrition and Health Survey in Taiwan 1993~1996 (NAHSIT 1993~1996). The prevalence rates of thiamin insufficiency, mainly a stage of marginal deficiency, were 29.3% for boys and 21.8% for girls of school age (7~12 years old).<sup>6</sup> The NAHSIT 1993~1996 did not assess the dietary intake of schoolchildren; the mean dietary thiamin intake of the nearest age group, 13~15-year-old adolescents, reached 96% of the Recommended Daily Nutrient Allowance (RDNA) for Taiwan.<sup>6</sup>

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Accepted 28 June 2007

The riboflavin status has long been a problem as reported in our previous surveys. Although the mean dietary riboflavin intake had gradually increased to near 100% of the RDNA in the NAHSIT 1993~1996, the prevalence rates of riboflavin deficiency were 24.7% for boys and 35% for girls of school age.<sup>7</sup> Riboflavin deficiency was more severe in mountainous areas and offshore areas like the PengHu islands.<sup>7</sup> The high prevalence of riboflavin deficiency was thought to be related to the gradual decrease in dairy product consumption with increasing age in children 4~6 years old and older.

Many B vitamins including thiamin and riboflavin participate in energy metabolic pathways which may be stressed by increased physical activity.<sup>8</sup> Because of rapid growth rates and physically active lifestyles, school-aged children may have enhanced requirements for thiamin and riboflavin per unit of body weight compared to older populations. Without sufficient dietary supply of these water-soluble vitamins, exercise may further decrease the nutritional status of physically active individuals with preexisting marginal status.<sup>8</sup> Since the nutritional status of these two water-soluble vitamins has not yet improved based on previous Nationwide Nutrition Surveys, it is critical to continually monitor the thiamin and riboflavin statuses of schoolchildren and determine possible contributing factors, in order to design effective strategies to improve this long-existing problem.

## MATERIALS AND METHODS

### Study design

This survey adopted a multi-stage, stratified, clustered probability sampling scheme as in previous NAHSIT series. The rationale and details of the study design and sampling procedures have been described in an article by Tu *et al.*<sup>9</sup> in this issue. Taiwanese school-aged children aged 6 to < 13 years old (with a date of birth between September 1, 1988 and August 31, 1995) and registered in all public or private elementary schools were the target population of the Nutrition and Health Survey in Taiwan Elementary School Children 2001-2002 (NAHSIT Children 2001-2002). Informed consent was signed by one of the parents of all of the schoolchildren. The study was approved by reviewers from the Department of Health in Taiwan.

### Blood sample preparation

As previously described, fasting venous blood samples were collected into vacutainers containing heparin<sup>10</sup>. A small fraction of each specimen was used for comprehensive blood chemistry tests including hemoglobin and mean corpuscular volume (MCV), and the remaining was immediately separated into plasma and erythrocytes in the field by centrifugation at 2500  $\times$ g and 4 °C. Packed erythrocytes were divided into aliquots which were immersed in liquid nitrogen, then kept and shipped on dry ice within 24 h, and finally stored at -80 °C until analysis. We randomly split, prepared, and blind-coded 5% of the samples in the field during sample preparation. These were analyzed along with all of the specimens in order to monitor the accuracy and consistency of the laboratory analyses. The standardized assay procedures of ETKAC and EGRAC gave a percentage of variation (%CV) of < 5%.

In total, 2031 erythrocyte specimens were collected, 1087 from boys and 944 from girls.

### ETKAC and EGRAC analyses

In order to perform sequential comparisons among the NAHSIT series of nutritional surveys,<sup>7,8,10</sup> we used ETKAC and EGRAC as the biochemical indicators to evaluate the long-term thiamin and riboflavin statuses of Taiwanese elementary schoolchildren. The activity coefficient was defined as the ratio of ETK or EGR activity measured by stimulating the respective enzymes with an added cofactor (TPP or FAD) to that without the addition of the respective cofactor. The semiautomatic analytical protocols developed by Mount *et al.*<sup>11</sup> were standardized, and the analyses were conducted on a Cobas Fara II (Roche).<sup>10</sup> Both the thiamin and riboflavin statuses were also classified into normal, marginal, or deficient based on the same cutoff points used in the previous NAHSITs.<sup>10</sup> An ETKAC of < 1.15 indicated a normal thiamin status,  $1.15 \leq \text{ETKAC} < 1.20$  for marginal thiamin deficiency, and  $\text{ETKAC} \geq 1.20$  for thiamin deficiency.<sup>11,12</sup> For riboflavin, an EGRAC of < 1.20 was considered a normal riboflavin status,  $1.20 \leq \text{EGRAC} < 1.40$  for marginal riboflavin deficiency, and  $\text{EGRAC} \geq 1.40$  for riboflavin deficiency.<sup>11,13</sup>

### Dietary intake

Information on the dietary intake of food and nutrients was collected through a 24-h recall and food frequency questionnaire conducted by trained surveyors.<sup>9</sup> Details of the dietary food and nutrient intake data were collected and tabulated as described by Wu *et al.* in this issue.<sup>14</sup> Complete dietary intake data for thiamin and riboflavin were collected from 1227 boys and 1109 girls.

### Statistical analysis

Descriptive statistics and hypothesis testing were analyzed by SAS 8.2<sup>15</sup> and SUDAAN 9.0, SAS-callableWindow.<sup>16</sup> Data expressed as the mean $\pm$ SE or prevalence rates were weighted for the unequal sampling probability in order to validate the representative results for schoolchildren in the entire country. The significance of variation between genders or various statuses was evaluated by analysis of variance (ANOVA) and appropriate post hoc testing; the trend of changes in specific variables among age groups was analyzed by contrast testing.

## RESULTS

There were 2031 eligible records for ETKAC and EGRAC (1087 for boys and 944 for girls) and 2236 for the dietary intake of thiamin and riboflavin (1127 for boys and 1109 for girls) analyzed in the NAHSIT Children 2001-2002. The mean ETKAC of school-aged boys was  $1.07 \pm 0.00$  and was significantly higher than that of girls ( $1.06 \pm 0.01$ ) ( $p < 0.05$ ). The mean ETKAC of all age categories were within a normal range, and those of 6~6.9- and 8~8.9-year-old boys were significantly higher than those of girls ( $p < 0.01$ ) (Table 1). On average, the respective prevalence rates for marginal and deficient thiamin states were 10.4% and 7.8% for boys and 9.3% and 7.3% for girls (Table 1). Both boys and girls of 8~8.9

**Table 1.** Prevalence of thiamin and riboflavin deficiencies in Taiwanese schoolchildren across age and gender strata<sup>1</sup>

Gender		Boys				Girls				
Age (yr)	<i>n</i>	ETKAC <sup>2,3</sup>	Normal	Marginal %	Deficient	<i>n</i>	ETKAC <sup>2,3</sup>	Normal	Marginal %	Deficient
6~6.9	84	1.08±0.01 <sup>†</sup>	81.6	12.5	5.9	75	1.04±0.01 <sup>†</sup>	89.2	10.5	0.3
7~7.9	187	1.05±0.01	90.6	2.5	7.9	150	1.06±0.01	84.4	11.4	4.2
8~8.9	178	1.09±0.01 <sup>†</sup>	69.5	19.1	11.5	151	1.07±0.01 <sup>†</sup>	79.6	8.4	11.9
9~9.9	180	1.07±0.01	83.8	10.9	5.3	153	1.05±0.01	85.0	6.9	8.1
10~10.9	184	1.07±0.01	82.3	10.3	7.4	172	1.06±0.01	80.5	12.9	6.7
11~11.9	197	1.08±0.01	79.2	11.0	9.8	158	1.06±0.01	83.4	8.5	8.1
12~12.9	74	1.06±0.01	87.6	7.5	4.9	85	1.06±0.01	85.1	5.3	9.6
Total	1084	1.07±0.00 <sup>†</sup>	81.9	10.4	7.8	944	1.06±0.01 <sup>†</sup>	83.3	9.3	7.3
		EGRAC <sup>2,4</sup>	Normal	Marginal	Deficient	<i>n</i>	EGRAC <sup>2,4</sup>	Normal	Marginal	Deficient
6~6.9	84	1.15±0.01	74.6	24.9	0.4	75	1.17±0.01	68.9	29.1	2.0
7~7.9	189	1.16±0.01 <sup>†</sup>	67.6	31.2	1.2	150	1.19±0.01 <sup>†</sup>	62.6	32.3	5.1
8~8.9	178	1.18±0.01	66.6	31.2	2.2	151	1.17±0.01	66.2	31.7	2.1
9~9.9	180	1.16±0.01 <sup>†</sup>	65.4	33.0	1.6	153	1.20±0.01 <sup>†</sup>	63.9	27.0	9.1
10~10.9	184	1.18±0.01	61.7	33.7	4.6	172	1.20±0.01	54.7	41.2	4.2
11~11.9	197	1.19±0.01	61.0	32.3	6.7	158	1.21±0.01	54.8	40.9	4.3
12~12.9	74	1.23±0.01	52.8	39.5	7.7	85	1.21±0.01	43.5	53.4	3.1
Total	1087	1.18±0.01 <sup>†</sup>	64.2	32.4	3.5	944	1.19±0.01 <sup>†</sup>	59.6	35.9	4.5

<sup>1</sup>Data were weighted and analyzed by the SUDAAN program. <sup>2</sup>Mean±SE analyzed by two-way ANOVA. <sup>3</sup>Cutoff points of thiamin status were erythrocyte activity coefficient of transketolase (ETKAC) < 1.15 for a normal status, 1.15 ≤ ETKAC < 1.20 for a marginal status, and ETKAC ≥ 1.20 for a deficient status. <sup>4</sup>Cutoff points of riboflavin status were erythrocyte activity coefficient of glutathione reductase (EGRAC) < 1.20 for a normal status, 1.20 ≤ EGRAC < 1.40 for a marginal status, and EGRAC ≥ 1.40 for a deficient status. <sup>†</sup>Significantly different between boys and girls (*p* < 0.05).

**Table 2.** Prevalence of a riboflavin deficiency over various gender and regional stratum of elementary schoolchildren<sup>1,2</sup>

Region	Male			Female		
	Normal	Marginal	Deficient	Normal	Marginal	Deficient
Hakka	62.6	32.6	4.8	54.1	34.1	11.8
Mountainous	50.7	39.9	9.5	44.9	43.1	12.0
Eastern area	55.0	35.2	9.8	45.8	42.7	11.5
PengHu	66.3	28.1	5.6	48.8	36.5	14.8
North-stratum 1	67.8	28.4	3.9	54.7	42.4	3.0
North-stratum 2	61.6	33.3	5.1	66.0	27.4	6.7
North-stratum 3	50.6	44.6	4.8	59.7	38.2	2.1
Central-stratum 1	54.5	39.3	6.2	52.6	43.3	4.1
Central-stratum 2	73.7	24.7	1.6	62.3	36.6	1.1
Central-stratum 3	72.0	25.7	2.3	67.3	30.4	2.4
South-stratum 1	69.4	29.4	1.2	68.0	30.1	2.0
South-stratum 2	70.5	29.6	0.0	70.5	25.2	4.3
South-stratum 3	64.2	34.9	0.9	56.5	40.5	3.0

<sup>1</sup>Data (%) were analyzed by the SUDAAN program. <sup>2</sup>Normal: erythrocyte activity coefficient of glutathione reductase (EGRAC) < 1.20; Marginal: 1.20 ≤ EGRAC < 1.40; Deficient: EGRAC ≥ 1.40.

years old had the highest deficient rates of all age strata.

The mean values of the EGRAC of Taiwanese school-aged boys was 1.18±0.01, which was significantly lower than that of girls (1.19±0.01, *p*<0.05). It seemed that schoolchildren of a younger age tended to have a better riboflavin status. The mean values of EGRAC of boys in the 12~12.9-year-old group and of girls older than 9 years were in the range of marginal deficiency (1.20 ≤ EGRAC < 1.40) (Table 1). There was a tendency for the mean EGRAC to increase with age in both genders.

The prevalence rate of riboflavin insufficiency showed a remarkable variation by region. The riboflavin deficiency rates of school-aged boys in mountainous and eastern areas were 9.5% and 9.8%, respectively; the defi-

ciency rates of girls in Hakka, mountainous, east coast areas and the PengHu islands were even as high as 11.8%, 12.0%, 11.5%, and 14.8%, respectively (Table 2). The riboflavin deficiency rates of the rest of the areas ranged from 0% to 6.7% (Table 2).

Since dairy products are significant sources of dietary riboflavin, we analyzed the influence of the frequency of dairy product consumption on schoolchildren's EGRAC of various age groups. In boys, the frequency of dairy product consumption had little influence on their riboflavin status. However, the EGRAC of girls significantly decreased with an increased frequency of dairy product consumption (*p*<0.001), especially for girls in the 1<sup>st</sup> and 2<sup>nd</sup> grades (*p* = 0.024) and the 3<sup>rd</sup> and 4<sup>th</sup> grades (*p* =

**Table 3.** Effects of dairy product consumption frequency on the erythrocyte activity coefficient of glutathione reductase (EGRAC) of elementary schoolchildren in Taiwan<sup>1,2</sup>

Gender	Boys						Girls							
	Consumption frequency (servings/wk)		Consumption frequency (servings/wk)		Consumption frequency (servings/wk)		<i>p</i> trend <sup>4</sup>	Consumption frequency (servings/wk)		Consumption frequency (servings/wk)		Consumption frequency (servings/wk)		<i>p</i> trend <sup>4</sup>
	≤ 2	3~7	≥ 8		≤ 2	3~7		≥ 8		≤ 2	3~7	≥ 8		
Grade	<i>n</i>	mean±SE	<i>n</i>	mean±SE	<i>n</i>	mean±SE		<i>n</i>	mean±SE	<i>n</i>	mean±SE	<i>n</i>	mean±SE	
Grades 1 and 2	81	1.18±0.01	137	1.16±0.01	133	1.15±0.01	0.068	63	1.19±0.01 <sup>†</sup>	135	1.19±0.01 <sup>†</sup>	102	1.16±0.01 <sup>‡</sup>	0.024
Grades 3 and 4	93	1.18±0.01	148	1.17±0.01	123	1.18±0.02	0.794	86	1.21±0.02 <sup>†</sup>	125	1.20±0.01 <sup>†</sup>	97	1.17±0.01 <sup>‡</sup>	0.022
Grades 5 and 6	109	1.19±0.01	152	1.20±0.01	111	1.18±0.01	0.474	83	1.22±0.01	152	1.21±0.01	106	1.18±0.01	0.054
Total	283	1.19±0.01	437	1.18±0.01	367	1.17±0.01	0.145	232	1.21±0.01 <sup>†</sup>	412	1.20±0.01 <sup>†</sup>	305	1.17±0.01 <sup>‡</sup>	<0.001
<i>p</i> trend <sup>3</sup>	0.556		0.003		0.011			0.109		0.259		0.034		

<sup>1</sup> Values are the mean±SE analyzed and weighted with SUDAAN. <sup>2</sup> Dairy products included milk, cheese, yogurt, yogurt drink, and ice cream. <sup>3</sup> The *p* trend was analyzed by the grade of each category of dairy food consumption frequency. <sup>4</sup> The *p* trend was analyzed for various frequencies of dairy product consumption. <sup>5</sup> Different superscript symbols indicate a significant difference between the various frequencies of dairy product consumption within an age group.

**Table 4.** Association between the riboflavin status and risk of anemia of schoolchildren stratified by the erythrocyte activity coefficient of glutathione reductase (EGRAC)<sup>1</sup>

Riboflavin status	Hemoglobin concentration			MCV		
	< 12 g/dL	≥ 12 g/dL	Odds ratio (95%CI) <sup>1</sup>	< 80/fL	≥ 80/fL	Odds ratio (95%CI) <sup>1</sup>
Normal (n)	61	503		18	546	
EGRAC < 1.2	10.8%	89.2%	1.00	3.2%	96.8%	1.00
Marginal (n)	60	281		30	311	
1.2 ≤ EGRAC < 1.4	17.6%	82.4%	1.8 (1.2-2.7)	8.8%	91.2%	3.0 (1.6-5.5)
Deficient (n)	16	48		17	47	
EGRAC ≥ 1.4	25.0%	75.0%	3.1 (1.6-5.8)	26.6%	73.4%	11.5 (5.5-24.2)

<sup>1</sup>Data were analyzed by SUDAAN software, and were adjusted for gender and age.

0.022) (Table 3). A significant and interesting trend of EGRAC increasing with age was observed in boys consuming dairy products 3~7 times/week ( $p = 0.003$ ) and  $\geq 8$  times/week ( $p = 0.011$ ) as well as in girls consuming dairy food  $\geq 8$  times/week ( $p = 0.038$ ) (Table 3).

Because riboflavin is required for activating pyridoxine to its coenzyme form, pyridoxal phosphate (PLP), which participates in the biosynthesis of heme, we analyzed the relationship between the riboflavin status and respective iron status indicators. EGRAC was significantly and negatively correlated with the hemoglobin concentration ( $r = -0.19$ ,  $p < 0.01$ ), mean corpuscular volume (MCV,  $r = -0.20$ ,  $p < 0.0001$ ), mean corpuscular hemoglobin (MCH,  $r = -0.23$ ,  $p < 0.01$ ), and mean corpuscular hemoglobin concentration (MCHC,  $r = -0.23$ ,  $p < 0.01$ ). Our data showed that riboflavin deficiency increased the risk of anemia. The risk of schoolchildren with a marginal riboflavin status having anemia (hemoglobin concentration  $< 12$  g/dL) was significantly elevated by 80% ( $p < 0.05$ ), and the risk for riboflavin-deficient children was as high as 3.1 times that for normal children (Table 4). In addition, marginal and deficient riboflavin statuses also raised the risk of microcytic anemia (MCV  $< 80$  fL) of schoolchildren to 3 and 11.5 times that of children with a normal riboflavin status ( $p < 0.05$ ) (Table 4).

## DISCUSSION

The thiamin status of Taiwanese schoolchildren surveyed in NAHSIT Children 2001-2002 showed limited progress compared to our previous Nationwide Nutrition Surveys.<sup>7</sup> The thiamin deficiency rate of schoolchildren was 7%, and the marginal deficiency rate was around 9%~15%. The highest thiamin deficiency rate occurred in the 8-8.9-year-old group and was scattered in the northern, central, and southern areas of Taiwan regardless of the degree of urbanization. According to the analyses of 24-h dietary recall of schoolchildren by Wu *et al.*,<sup>14</sup> their average dietary thiamin intakes were  $1.5 \pm 0.1$  mg/day for boys (ranging  $1.2 \pm 0.1$ ~ $1.9 \pm 0.3$  mg/day) and  $1.3 \pm 0.1$  mg/day for girls (ranging  $1.1 \pm 0.1$ ~ $1.6 \pm 0.2$  mg/day). These average dietary thiamin intake levels were 144% of the RDA for boys and 134% for girls. However, there were still 1.4% of boys and 9.7% of girls 6~9 years old and 2.2% of boys and 21.0% of girls 10~12 years old who did not consume adequate thiamin in the recommended amount.<sup>14</sup> Insufficient dietary consumption may partially explain why there were still 16%~22% of children with an abnormal thiamin status. Wu *et al.*<sup>14</sup> also calculated the contributions of various categories of food to dietary thiamin. In the diet of schoolchildren, pork and its products contributed 35% of thiamin, followed by rice and its products (12.1%) and soybeans and soy products (10.8%). Each of the remaining food categories provided less than 5% of the thiamin to the diet of schoolchildren.<sup>14</sup> Such a dietary pattern is very similar to that of Taiwanese adults<sup>17</sup> and elderly.<sup>18</sup> The similarities in dietary patterns illustrates how the presence of thiamin deficiency would be hard to resolve without nutritional intervention.

According to the results of the NHANES III, US children 4~13 years old received 1.60~1.84 mg of thiamin per day from their diets.<sup>19</sup> The policy of nutrient fortification of staple foods practiced in the US should signifi-

cantly contribute to ensuring adequate intake of dietary thiamin. This assumption could be confirmed by the fact that food groups containing fortified staples as the main ingredient (such as bread and its products, mixed foods with grain as the main ingredient, ready-to-eat cereals, pasta, rice, and cooked cereals) provided 51% of the dietary thiamin for adults, followed by pork and its products and soy and its products.<sup>19</sup> Without a nutrient fortification policy, we would support the strategy to encourage people including thiamin-rich foods in their diets and replacing polished rice with whole-grain or multigrain rice.

Due to the influence of traditional dietary habits, people in Taiwan usually consume less and less milk as they pass from infancy to childhood. Although there are a lot more varieties of dairy products appearing in the market compared to the past, progress in improving the riboflavin status of all surveyed populations<sup>7,10</sup> including schoolchildren is still very slow. There were 3.5% of school-aged boys and 4.5% of school-aged girls classified as riboflavin deficient in the current survey; the prevalence rates of marginal riboflavin deficiency in various age strata were as high as nearly 30% to over 50%. The average dietary riboflavin intake levels of Taiwanese schoolchildren were  $1.48 \pm 0.1$  mg/day for boys and  $1.31 \pm 0.1$  mg/day for girls, which are equivalent to 134% and 126% of their respective RDAs. However, the dietary riboflavin intake of 7.1% of 6~9-year-old boys, 20.1% of 10~12-year-old boys, 8.8% of 6~9-year-old girls, and 25.8% of 10~12-year-old girls failed to meet 100% of their RDAs.<sup>14</sup> Thus, it is not surprising to see such high prevalence rates of riboflavin insufficiency in our elementary schoolchildren.

Based on the report of the Continuing Survey of Food Intakes by Individuals (CSFII) in the US,<sup>20</sup> fortification of staple foods with B vitamins resulted in staples such as bread, grains, cereals, and related products containing them as the major ingredients providing 30% of the dietary riboflavin intake for American adults,<sup>20</sup> followed by milk and dairy products which provided 15%. In contrast, milk and dairy products contributed 0.33 mg (22.6%) to the dietary riboflavin intake of Taiwanese schoolchildren, followed by pork and its products (11%), eggs and related products (8.9%), soft drinks (6.8%), rice and its products (5.5%), and poultry and its products (5.5%).<sup>14</sup> The remaining food sources had limited contributions to the dietary riboflavin intake ( $< 5\%$  each). It is a characteristic of Taiwanese diet<sup>17</sup>, obtaining dietary riboflavin from small amounts of riboflavin-rich foods, (such as milk and dairy foods) and from numerous food groups containing low level of riboflavin.<sup>10,18</sup>

According to analyses of the 24-h recall of NAHSIT Children 2001~2002, 6~9-year-old boys and girls consumed 0.8 and 0.7 servings/day of milk or dairy products, respectively; while 10~12-year-old schoolchildren consumed only 0.6 servings/day,<sup>14</sup> which are far below the recommendation of two servings per day for school-aged children by the DOH.<sup>21</sup> Data in Table 4 clearly show a significant trend of improvement in riboflavin status with an increase in dairy product consumption, especially in girls. This finding further confirms the importance of including adequate dairy products to ensure a satisfactory riboflavin status of schoolchildren.

Riboflavin deficiency has always been among one of the nutritional problems since Nationwide Nutritional Survey were initiated in Taiwan half a century ago. In 1950s<sup>22</sup> and 1970s,<sup>23</sup> riboflavin deficiency was manifested with the presence of clinical signs such as angular stomatitis and reddened tongue. The similar trend of older children (6<sup>th</sup> graders) having worse riboflavin status than younger children (1<sup>st</sup> graders) was also observed in these earlier surveys.<sup>22,23</sup> The prevalence rates of active angular stomatitis were 69% for 1<sup>st</sup> graders and 78% for 6<sup>th</sup> graders in 1950s,<sup>22</sup> and were 6.9% for 1<sup>st</sup> graders and 17.5% for 6<sup>th</sup> graders in 1970s.<sup>23</sup> Although the riboflavin insufficiency state of the current study is mainly as marginal deficiency, the trend is similar. In addition to rapid growth rate and more physically active lifestyle experienced by older children,<sup>8,22,23</sup> dieting may also have negative contribution to the riboflavin status of older children,<sup>8</sup> especially girls.

Another facet of problem concerning riboflavin insufficiency is the higher deficiency rates in certain regions of Taiwan, including mountainous, eastern, and Hakka areas, and the PengHu islands.<sup>7</sup> In NAHSIT Children 2001~2002, the riboflavin deficiency rates of these four areas were as high as 11%~15%, and less than 50% of the schoolchildren in these areas had a normal riboflavin status. The low frequency of dairy product consumption is one of the major dietary factors leading to the riboflavin insufficiency in these areas. The results of the 24-h recall showed that on average, schoolchildren in mountain area consumed only 0.2 servings of milk or dairy products a day;<sup>14</sup> this dairy food consumption frequency was the lowest among all regions surveyed. School-aged girls in areas of high riboflavin deficiency also consumed relatively less of other foods considered to be good riboflavin sources, such as eggs and their products, compared to other areas of Taiwan.<sup>14</sup> There may be many other factors which interweave together and result in an unsatisfactory riboflavin status. First of all, the physical environment limits transportation and socioeconomic development in mountainous and eastern areas that might further restrict the people living there from updating their nutritional knowledge and decrease the availability of milk and dairy products on the market. Second, a high percentage of residents in mountainous and eastern areas of Taiwan are indigenous peoples. Economic hardships and the inherited culture, traditions, and lifestyles including dietary habits experienced by indigenous people might hinder them from obtaining milk or recognizing the importance of dairy foods to their health.

Instead of drinking milk, schoolchildren of mountainous and eastern areas obtained 9.3%~10.5% of their total energy from sugar-flavored soft drinks;<sup>14</sup> the percentages were the highest among all regions for both boys and girls. This dietary behavior could be another factor which is further deteriorating the nutritional status of school-aged children in mountainous and eastern areas, in addition to the riboflavin deficiencies. Bowman assessed the dietary information of 732 young females 12~19 years old in the CSFII 1994~1996 to evaluate the influence of beverage choices on their nutrient intake,<sup>24</sup> and a significant trend of decreased milk intake with an increase in age was also observed.<sup>24</sup> Among those young females, non-milk drink-

ers had a significantly lower intake of riboflavin (1.2 mg/day) than milk drinkers (2.1-2.4 mg/day). In addition, milk drinkers also had significantly higher dietary intakes of vitamin A, vitamin B6, folate, calcium, and magnesium.<sup>24</sup>

Due to the broad influence of riboflavin on metabolism and the interactions of riboflavin with many other nutrients, a poor riboflavin status may be linked to the nutritional status of many other nutrients.<sup>25</sup> One example observed in the NAHSIT Children 2001~2002 is the significant association between a poor riboflavin status and an elevated risk of hypochromic and microcytic anemia (Table 5). Riboflavin deficiency has long been reported to result in anemia in animal studies (reviewed by Powers<sup>26</sup>). Riboflavin deficiency may reduce the efficiency of mobilizing ferritin and alters the morphology of the intestinal mucosa of rats.<sup>26</sup> Short-term riboflavin depletion in weanling rats caused an increase in the size and cellularity of intestinal crypts, and decreases in bifurcating crypts and the proliferation index.<sup>26</sup> Further depletion reduced the number of villi per unit area of intestinal mucosa, indicating a reduction in the nutrient absorption surface area.<sup>26</sup> Therefore a riboflavin deficiency in early age disrupts the normal development of the gastrointestinal mucosa, and may be an important factor contributing to poor handling and absorption of iron, thus resulting in iron-deficiency anemia.

The elevated risk of anemia associated with riboflavin insufficiency may also be related to the interaction between riboflavin and vitamin B6. One of the biochemical functions of riboflavin concerns the FMN-dependent limiting enzyme, pyridoxal phosphate oxidase, which catalyzes the activation of vitamin B6 to the coenzyme, pyridoxal-5'-phosphate (PLP),<sup>5</sup> and the PLP-dependent enzyme,  $\delta$ -aminolevulinic synthase, which catalyzes the first step in the biosynthesis of heme,<sup>6</sup> the prosthetic group of hemoglobin. Whether a poor riboflavin status is involved in the progression of microcytic anemia through its interaction with PLP remains to be confirmed by further research.

In conclusion, the current NAHSIT Children 2001 ~ 2002 revealed slight progress in the long-term thiamin and riboflavin statuses of schoolchildren. One of the major factors hindering progress with the thiamin and riboflavin status may be inherited dietary patterns characterized by the inadequate consumption of nutrient-rich food sources such as whole grains for thiamin and dairy products for riboflavin, and obtaining thiamin and riboflavin from numerous categories of food containing very limited amounts of these vitamins. Improvements in the riboflavin status of schoolchildren (especially girls) in mountain, eastern, and Hakka areas and the PengHu islands were further restricted by their poor geographic and socioeconomic conditions. Excluding important food sources may be associated with the preference for less nutrient-dense foods, such as refined staples and sugar-flavored beverages, thus further deteriorating the nutritional status of metabolically related nutrients. One example confirmed in our study was the elevated risk of anemia associated with riboflavin insufficiency. Nationwide nutritional interventions, such as nutritional education for targeted populations which focus on prudent food choices and

meal planning, nutrient supplementation in school lunch programs, and nutrient fortification of certain food products, are strongly recommended to the Taiwanese government so that steps are taken to improve the long-existing problems of thiamin and especially riboflavin insufficiency.

#### ACKNOWLEDGEMENTS

We sincerely thank Dr. JT Kao and Dr. SB Lin from the Department of Clinical Laboratory and Medical Biotechnology, College of Medicine, National Taiwan University, for kindly providing the Cobas Fara II and supplies, which allowed us to carry out the laboratory analyses. Data analyzed in this article were collected by the research project "Nutrition and Health Survey in Taiwan (NAHSIT)" sponsored by the Department of Health in Taiwan (DOH-88-FS, DOH89-88shu717, DOH90-FS-5-4, and DOH91-FS-5-4). This research project was carried out by the Institute of Biomedical Sciences, Academia Sinica and the Research Center for Humanities and Social Sciences, Center for Survey Research, Academia Sinica, directed by Dr. Wen-Harn Pan and Dr. Su-Hao Tu. The Center for Survey Research, Academia Sinica was responsible for data distribution. The assistance provided by the institutes and aforementioned individuals is greatly appreciated. The views expressed herein are solely those of the authors.

#### AUTHOR DISCLOSURES

Ning-Sing Shaw, Jui-Line Wang, Wen-Harn Pan, Pei-Chun Liao, and Feili Lo Yang, no conflicts of interest.

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## Original Article

**Thiamin and riboflavin status of Taiwanese elementary schoolchildren**

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**臺灣國小學童之維生素 B1 與維生素 B2 營養狀況**

在「臺灣國小兒童營養與健康狀況變遷調查」(NAHSIT Children 2001~2002)中，我們選用紅血球轉酮酶活性係數(ETKAC)與穀胱甘肽還原酶活性係數(EGRAC)，為評估長期維生素 B1 與維生素 B2 生化營養狀況的功能性指標。男、女學童的 ETKAC 平均值分別為  $1.07 \pm 0.00$  與  $1.06 \pm 0.01$ ，二者皆在正常範圍。男孩的維生素 B1 臨界缺乏率與缺乏率分別為 10.4% 與 7.8%，女孩則分別為 9.3% 與 7.3%。男、女學童的 EGRAC 平均值分別為  $1.18 \pm 0.00$  與  $1.19 \pm 0.01$ ，二者皆有隨年齡增加而上升的趨勢。男孩的維生素 B2 臨界缺乏率與缺乏率分別為 32.5% 與 3.5%，女孩則分別為 35.9% 與 4.5%。臺灣學童的維生素 B2 臨界缺乏與缺乏現象與飲食乳製品攝取頻率低有關，並與貧血的危險率增加顯著相關。

**關鍵字：**維生素 B1 營養狀況、維生素 B2 營養狀況、國小學童、生化營養評估、臺灣國小學童營養與健康狀況變遷調查(2001~2002)。