

## Only a small proportion of anemia in northeast Thai schoolchildren is associated with iron deficiency<sup>1-3</sup>

Rosanne A Thurlow, Pattanee Winichagoon, Timothy Green, Emorn Wasantwisut, Tippawan Pongcharoen, Karl B Bailey, and Rosalind S Gibson

### ABSTRACT

**Background:** Iron deficiency is assumed to be the major cause of anemia in northeast Thailand, but other factors may be involved.

**Objective:** We determined the prevalence of anemia among schoolchildren in northeast Thailand and the role of hemoglobinopathies, selected micronutrient deficiencies, and other factors in hemoglobin status.

**Design:** Blood samples were collected from 567 children aged 6–12.9 y attending 10 primary schools for the determination of a complete blood count and hemoglobin type [Hb AA (normal hemoglobin), Hb AE (heterozygous for Hb type E), and Hb EE (homozygous for Hb type E)] and the measurement of serum ferritin, transferrin receptor, retinol, vitamin B-12, and plasma and erythrocyte folate concentrations. Children with a C-reactive protein concentration  $\geq 10$  mg/L ( $n = 12$ ), which indicated infection, were excluded.

**Results:** The prevalence of anemia was 31%. Age, hemoglobin type, and serum retinol were the major predictors of hemoglobin concentration. Hb AA and Hb AE children with anemia had lower ( $P < 0.01$ ) hematocrit, mean cell volume, and serum retinol values than did their nonanemic counterparts; no significant differences in serum ferritin were found by hemoglobin type. Only 16% ( $n = 22$ ) of the anemic Hb AA and Hb AE children were iron deficient. Hb AA and Hb AE children with a serum retinol concentration  $< 0.70$   $\mu\text{mol/L}$  ( $n = 14$ ) had a significantly higher geometric mean serum ferritin concentration than did those with a retinol concentration  $\geq 0.70$   $\mu\text{mol/L}$  ( $P = 0.009$ ); no significant difference in transferrin receptor concentrations was found between these 2 groups.

**Conclusions:** Hemoglobinopathies, suboptimal vitamin A status, and age were the major predictors of hemoglobin concentration. The contribution of iron deficiency to anemia was low, and its detection was complicated by coexisting suboptimal vitamin A status. *Am J Clin Nutr* 2005;82:380–7.

**KEY WORDS** Anemia, northeast Thailand, school-aged children, serum retinol, hemoglobin type E, iron

### INTRODUCTION

Based on the most recent 1995 Thai National Nutrition Survey, the prevalence of anemia among school-aged children in northeast Thailand is 24% (1). Nutritional iron deficiency is assumed to be a major etiologic factor for anemia (2, 3), which is induced by rice-based diets and low intakes of readily available heme iron from cellular animal protein. The etiology of anemia, however, is multifactorial; genetic hemoglobinopathies, other

micronutrient deficiencies, chronic inflammatory disorders, and parasitic infections may also play a role (4), although their relative importance in northeast Thailand is uncertain.

In many countries in Southeast Asia, including Thailand, hereditary disorders affecting the production of hemoglobin are widespread (5–8). There are 2 genetically distinct groups: hemoglobinopathies and thalassemias, each of which results in a slower rate of hemoglobin synthesis and hypochromic, microcytic anemia of varying severity and pathophysiology (6). Among populations in northeast Thailand, the most common hemoglobinopathy is hemoglobin type E (7, 8)—a genetically caused amino acid substitution in the  $\beta$  chain of hemoglobin A that changes the structure of the hemoglobin tetramer. Hemoglobin type E is relatively benign and has few clinical symptoms; therefore, those individuals who are homozygous for hemoglobin type E (Hb EE) or heterozygous for hemoglobin type E (Hb AE) are often not diagnosed (6). Unfortunately, it is difficult to discriminate between the hypochromic, microcytic anemia that is due to these hereditary disorders and iron deficiency anemia (IDA). In both cases, hemoglobin and mean cell volume (MCV) are reduced.

Several other micronutrients besides iron, notably vitamin A, riboflavin, folic acid, and vitamin B-12, are also required for normal hematopoiesis (9). Vitamin A deficiency is well documented among children in northeast Thailand (10–12), but data on deficiencies of the other vitamins are more limited.

Clearly, to design effective intervention strategies, the major predictors of anemia among schoolchildren in northeast Thailand must be identified. Therefore, in this cross-sectional study, we determined the prevalence of anemia among a selected sample of rural primary school children in northeast Thailand and investigated the role of hemoglobinopathies, selected micronutrient deficiencies (iron, vitamin A, folic acid, and vitamin B-12), and sociodemographic factors on hemoglobin concentrations.

<sup>1</sup> From the Institute of Nutrition, Mahidol University, Salaya, Thailand (PW, EW, TP), and Department of Human Nutrition, University of Otago, Dunedin New Zealand (RAT, KBB, and RSG).

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<sup>3</sup> Address reprint requests to RS Gibson, Department of Human Nutrition, University of Otago, PO Box 56, Dunedin 9001, New Zealand. E-mail: rosalind.gibson@stonebow.otago.ac.nz.

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Anemia was assessed by hemoglobin and MCV and iron deficiency by using various biochemical iron indexes. Vitamin A status was based on serum retinol, folate status on plasma and red blood cell folate, and vitamin B-12 status on serum vitamin B-12 concentrations.

## SUBJECTS AND METHODS

### Subjects

This cross-sectional study was conducted in June and July 2002 in Ubon Ratchathani province, northeast Thailand. The participating children (281 boys and 286 girls) were aged 6.0–12.9 y and resided in 10 poor rural subdistricts that served a large rice growing area surrounding the town of Trakarn Phutphon. Ten primary schools with the largest student enrollment in each subdistrict were selected to participate in this study. Eligible children from each school were stratified by age and sex, and equal numbers of children were randomly selected from each strata.

Ethical approval of the study protocol was obtained from Human Ethics Committees of Mahidol University (Nakhonpathom, Thailand) and the University of Otago (New Zealand). Written informed consent was obtained from the parents or guardians of each child in the survey. In addition, permission was sought from local school boards and Thai health workers after meetings in which the purpose and methods of the study were clearly explained by one of the principal investigators (PW).

### Sociodemographic and health-status assessment

Trained Thai research assistants administered a pretested sociodemographic and health-status questionnaire to an adult member of each participating household. Sociodemographic variables assessed included ethnicity, household size, education, income, employment of the head of the household, and level of education for the mother or caregiver. Health-status indicators included self-reported infection status (diarrhea, respiratory, and parasite), and the use of vitamin or mineral supplements or both.

### Biochemical assessment

Morning, nonfasting peripheral venipuncture blood samples were drawn by trained nurses from children in the recumbent position into either a trace element-free evacuated tube protected from exposure to light or an evacuated tube containing EDTA as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ). All blood samples were refrigerated immediately after collection. Aliquots of serum for ferritin, transferrin receptor, retinol, vitamin B-12, and C-reactive protein (CRP) measurement were frozen in trace element-free polyethylene vials at  $-20^{\circ}\text{C}$  initially and later at  $-70^{\circ}\text{C}$ . The remaining serum was frozen in light-resistant amber-colored polyethylene vials for retinol analyses.

An aliquot of anticoagulated whole blood was hemolyzed by a 10-fold dilution in 1% ascorbic acid and stored for erythrocyte folate analyses. Additional aliquots of whole blood were analyzed for a complete blood count and hemoglobinopathies. The remainder of the whole blood was then centrifuged ( $2500 \times g$ , 10 min, room temperature), and the plasma was separated as quickly as possible. For the folate analysis, an aliquot of plasma was diluted 1 in 20 with 0.5% sodium ascorbate solution before being frozen. A complete blood count was determined by using an electronic Coulter Counter (Beckman, Fullerton, CA) in Trakarn Phutphon District Hospital. Hemoglobinopathy analysis was

performed by using the Variant  $\beta$ -Thalassemia Short Program (Bio-Rad Laboratories Inc, Hercules, CA) in the Thalassemia Research Centre (Institute of Science and Technology for Research and Development, Mahidol University). Hemoglobin type was determined in each subject on the basis of hematologic indexes: Hb AA (normal hemoglobin type), Hb AE (trait for hemoglobin E disease), or Hb EE (hemoglobin E disease).

Serum retinol was analyzed by using HPLC according to the method of the International Vitamin A Consultative Group (13). Serum ferritin and vitamin B-12 concentrations were determined by using an IMx analyzer that uses Microparticle Enzyme Immunoassays technology sera (Abbott Laboratories, Abbott Park, IL). Serum transferrin receptor was analyzed with an enzyme immunoassay by using commercial kits (Ramco Laboratories Inc, Houston, TX). Serum folate and whole-blood folate were measured by using the microtiter technique described by O'Broin and Kelleher (14) with chloramphenicol-resistant *Lactobacillus casei* as the test microorganism. Erythrocyte folate was calculated from whole-blood folate by subtracting serum folate and correcting for hematocrit. An external whole-blood standard (National Institute for Biological Standards and Control, South Mimms, United Kingdom) with a certified folate concentration of 29.4 nmol/L was used to generate the standard curve. Infection was determined on the basis of the CRP concentration by using the Behring Turbitimer System (Behringwerke AG Diagnostica, Germany); a cutoff of  $\geq 10$  mg/L was used to indicate the presence of inflammation or infection as recommended by the manufacturer.

A pooled serum sample and manufacturer's controls were used to check the precision and accuracy of all the analytic methods. The certified values for the manufacturer's controls for serum ferritin supplied for the IMx analyzer system had been calibrated by the manufacturer with the World Health Organization International Reference Material. The between-assay CVs for serum ferritin, transferrin receptor, serum retinol, serum vitamin B-12, and serum and red blood cell folate were 5.1%, 3.3%, 5.4%, 7.2%, and 11.8%, respectively. Values for the controls fell within the certified ranges for serum ferritin, transferrin receptor, vitamin B-12, and CRP.

Anemia was defined as a hemoglobin concentration  $< 115$  g/L for children aged 6–11 y and  $< 120$  g/L for those aged  $\geq 12$  y (15). Iron deficiency was defined on the basis of an elevated serum transferrin receptor concentration ( $> 8.5$  mg/L) (16) or an elevated ratio of transferrin receptor to ferritin ( $500 \mu\text{g/L}$ ) (17). For storage iron depletion, the commonly used cutoff for serum ferritin of  $< 12 \mu\text{g/L}$  was used. IDA was defined as iron deficiency concurrent with anemia.

### Statistical analyses

All data were analyzed by using the STATISTICAL PACKAGE FOR SOCIAL SCIENCES (SPSS for WINDOWS, version 10.0; SPSS, Chicago, IL). Data were checked for normality by using the Kolmogorov-Smirnov test. One-way analysis of variance with post hoc analysis (Tukey's honestly significant difference) was used to determine the source of differences in hematologic and biochemical indicators between the 3 hemoglobin types. ANOVA was also used to show the effect of anemia and vitamin A deficiency on selected hematologic and iron-status indexes and to examine the predictors of hemoglobin concentration. Statistically significant differences are indicated by  $P < 0.05$ .

**TABLE 1**  
Sociodemographic characteristics of the participants<sup>1</sup>

Variable	Value
	<i>n</i> (%)
Sex	
Female	286 (50.4)
Male	281 (49.6)
Age	
6.00–7.99 y	175 (30.9)
8.00–9.99 y	180 (31.7)
10.00–12.99 y	212 (37.4)
Male head of household	491 (86.6)
Estimated annual yearly income ≤ 30000 baht <sup>2</sup>	299 (52.7)
Occupation of head of household	
Farming	503 (88.7)
Education level of head of household	
Primary school	473 (83.4)
High school or beyond	87 (15.2)
Education level of caregiver or mother	
Primary school	501 (88.4)
High school or beyond	15 (10.2)

<sup>1</sup> There was no relation between the 3 hemoglobin types (Hb AA, Hb AE, and Hb EE) and the sex or age of the children or with the estimated annual yearly income of the head of household.

<sup>2</sup> US\$1.0 was equivalent to ≈40 Thai baht in 2002.

## RESULTS

### Sociodemographic and health status

Selected sociodemographic results are presented in **Table 1**. A total of 567 children (286 girls and 281 boys), all of Thai ethnicity, participated in the survey. The participants were predominantly of low socioeconomic status. Farming, primarily rice growing, was the occupation of 89% of the households, and the median estimated annual household income was 28 400 baht. More than 80% of those identified as either head of household or the primary caregiver had received only primary school education; <3% of the children (*n* = 12) had an elevated serum CRP concentration (ie, CRP ≥ 10 mg/L), which indicated inflammation or infection; >90% (*n* = 531) of the sample had never used a nutritional supplement; and <2% (*n* = 5) of the girls reported that menstruation had commenced.

Of the adults responding to the health-status questionnaire, 35% (*n* = 201) reported that their child currently had a parasitic

infection, and 60% (*n* = 340) reported that their child had received the appropriate treatment for helminth infections.

### Assessment of hemoglobinopathies and hematologic indexes

Of the 548 children examined for hemoglobin type, 57% (*n* = 321) had Hb AA, 33% (*n* = 186) had Hb AE, and 5% (*n* = 28) had Hb EE. One child had evidence of Hb EE and β thalassemia disease, and the remaining 12 children had either an ambiguous result or a rare hemoglobin variant. These 13 children were excluded from any further statistical analyses. There was no relation between the hemoglobin type and the age or sex of the children or the socioeconomic status of the family as measured by the estimated annual yearly income of the head of household.

Overall, 31% (*n* = 175) of the children were anemic. At the 95% confidence level, the sample size for this cross-sectional study (*n* = 567) was sufficient to detect a 30% prevalence of anemia with an absolute precision of ≈0.04% of the proportion. The prevalence of anemia decreased with increasing age (*P* < 0.001) but was independent of sex. The medians (1st and 3rd quartiles) for selected hematologic indexes of the children by hemoglobin type are shown in **Table 2**. Marked differences in all these indexes occurred by hemoglobin type. Hb AA children had significantly higher median values for hemoglobin, hematocrit, and MCV than did Hb EE children. Likewise, Hb AE children had significantly higher median hemoglobin, hematocrit, and MCV values than did the Hb EE children (Table 2). Consequently, the prevalence of anemia, based on low hemoglobin, was lowest in the Hb AA group (21%; *n* = 68) and second lowest in the Hb AE group (37%; *n* = 68). Eighty-six percent (*n* = 24) of the Hb EE group had hemoglobin concentrations indicative of anemia.

The anemia observed in these children was predominantly microcytic hypochromic, as indicated by an MCV < 80 fL in the 6–11 y age group or <82 fL in the >12 y age group (18). The prevalence of a low MCV was dependent on hemoglobin type as shown in **Figure 1** and was negatively associated with age. Specifically, 40% (*n* = 126), 99% (*n* = 184), and 100% (*n* = 28) of the Hb AA, Hb AE, and Hb EE children, respectively, had a low MCV. Within each of the specific hemoglobin types, we observed various degrees of microcytic anemia, as indicated by the following correlations between hemoglobin and MCV within each specific hemoglobin type: Hb AA (*n* = 310; *r* = 0.420, *P* =

**TABLE 2**  
Selected hematologic and biochemical variables of the children by hemoglobin type (Hb AA, Hb AE, and Hb EE)<sup>1</sup>

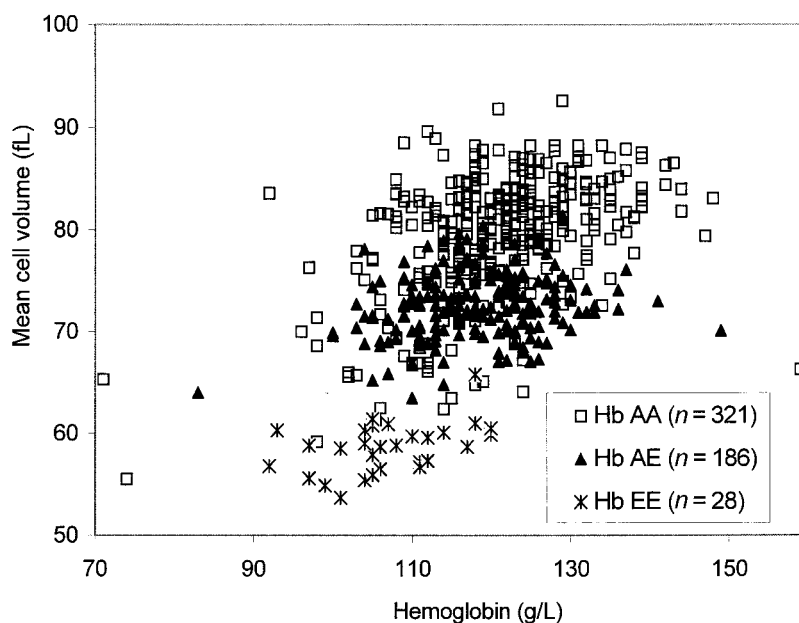
	Hb AA ( <i>n</i> = 321)	Hb AE ( <i>n</i> = 186)	Hb EE ( <i>n</i> = 28)
Hemoglobin (g/L) <sup>2</sup>	121.6 ± 9.8 <sup>a</sup>	118.4 ± 9.4 <sup>b</sup>	106.6 ± 7.4 <sup>c</sup>
Hematocrit <sup>2</sup>	36.6 ± 2.8 <sup>a</sup>	35.8 ± 2.7 <sup>b</sup>	32.8 ± 2.2 <sup>c</sup>
Mean cell volume (fL) <sup>2</sup>	80.1 ± 5.8 <sup>a</sup>	72.6 ± 3.4 <sup>b</sup>	58.4 ± 2.1 <sup>c</sup>
Serum ferritin (μg/L) <sup>3</sup>	37.5 (19.6, 71.8) <sup>a</sup>	37.4 (20.3, 68.9) <sup>a</sup>	59.3 (35.5, 99.1) <sup>b</sup>
Serum transferrin receptor (mg/L) <sup>2</sup>	6.8 ± 2.4 <sup>a</sup>	6.5 ± 1.9 <sup>a</sup>	8.8 ± 2.1 <sup>b</sup>
Serum retinol (μmol/L) <sup>4</sup>	1.31 ± 0.38 [312] <sup>a</sup>	1.25 ± 0.30 [178] <sup>a</sup>	1.27 ± 0.28 [28] <sup>a</sup>

<sup>1</sup> Means in a row with different superscript letters are significantly different, *P* < 0.01 (Tukey's post hoc test for differences between means).

<sup>2</sup> All values are  $\bar{x} \pm SD$ .

<sup>3</sup> All values are geometric  $\bar{x}$  (−1SD, +1SD).

<sup>4</sup> *n* in brackets.



**FIGURE 1.** Relation between mean cell volume and hemoglobin concentration in children by hemoglobin type: homozygous for hemoglobin type E (Hb EE), heterozygous for hemoglobin type E (Hb AE), and normal hemoglobin (Hb AA).

0.001), Hb AE ( $n = 177$ ;  $r = 0.273$ ,  $P = 0.001$ ), and Hb EE ( $n = 27$ ;  $r = 0.329$ ,  $P = 0.094$ ).

#### Assessment of iron deficiency and iron deficiency anemia

Children with an elevated serum CRP indicative of inflammation or infection ( $\geq 10$  mg/L;  $n = 12$ ) had a significantly higher ( $P < 0.001$ ) geometric mean ( $-SD$ ,  $+SD$ ) serum ferritin concentration (109.4; 20.3, 72.6  $\mu\text{g/L}$ ) than did those with a serum CRP concentration  $< 10$  mg/L (38.4; 62.5, 191.3  $\mu\text{g/L}$ ) and were thus excluded from any further statistical analyses. There was no significant difference between the mean ( $\pm SD$ ) serum transferrin receptor concentrations of those children with a serum CRP concentration  $\geq 10$  mg/L and those with a concentration  $< 10$  mg/L ( $6.9 \pm 2.6$  compared with  $6.8 \pm 1.8$  mg/L, respectively).

In children with Hb AA and Hb AE, there was an increase in serum ferritin ( $P = 0.001$ ) and a decrease in serum transferrin receptor ( $P < 0.001$ ) with increasing age. After age was controlled for in the Hb AA and Hb AE groups, there were no significant differences in serum ferritin or transferrin receptor concentrations between boys and girls.

Geometric mean serum ferritin and mean transferrin receptor concentrations by hemoglobin type are shown in Table 2. There were no significant differences in these indexes between the Hb AA and Hb AE groups (Table 2). However, Hb EE children had significantly higher ( $P < 0.001$ ) serum ferritin and transferrin receptor concentrations than did the Hb AA and Hb AE children.

Very little of the anemia in the northeast Thai schoolchildren with Hb AA and Hb AE was associated with iron deficiency. Indeed, the prevalence of IDA on the basis of a low hemoglobin concentration and an elevated transferrin receptor was only 4.4% ( $n = 14$ ) in the AA group and 4.3% ( $n = 8$ ) in the AE group. The prevalence of iron deficiency (without anemia) was also low in both groups: 12.1% ( $n = 39$ ) in the Hb AA group and 5.4% ( $n = 10$ ) in the Hb AE group, and these differences were significant ( $P = 0.008$ ; Fisher's exact test). Even fewer children with Hb AA and Hb AE had evidence of depleted iron stores, on the basis of

low serum ferritin concentrations ( $< 12$   $\mu\text{g/L}$ ); the corresponding prevalences were 0.6% ( $n = 2$ ) and 2.2% ( $n = 4$ ) in the Hb AA and Hb AE groups, respectively, and these differences were not significant. When IDA was assessed in these 2 groups on the basis of an elevated ratio of transferrin receptor to serum ferritin (500  $\mu\text{g/L}$ ) and coexisting anemia, the prevalence of IDA was only 1.6% in both the Hb AA ( $n = 5$ ) and Hb AE ( $n = 3$ ) groups, respectively; the prevalence of iron deficiency alone was 3.7% ( $n = 12$ ), and 3.2% ( $n = 6$ ) in the Hb AA and Hb AE groups, respectively. Note that the prevalence of IDA was slightly higher in both the Hb AA and Hb AE groups when defined as anemia plus an elevated serum transferrin receptor concentration.

It is noteworthy that of the children with Hb EE, 54% ( $n = 15$ ) had both an elevated serum transferrin receptor and a low hemoglobin concentration, whereas only 3.6% ( $n = 1$ ) had an elevated serum transferrin receptor concentration but no evidence of anemia. Ineffective erythropoiesis is known to accompany the hemoglobin type E variant and was thus responsible for the elevated transferrin receptor concentrations observed (19, 20). None of the Hb EE children had a serum ferritin concentration  $< 12$   $\mu\text{g/L}$ , and only one child in the Hb EE group had a ratio of serum transferrin to serum ferritin  $> 500$   $\mu\text{g/L}$ . High serum ferritin concentrations may occur in persons who are homozygous for hemoglobin E because they have erythrocytes with a reduced survival time (19). Hence, the iron released from the erythrocytes may accumulate as storage iron because of the slow rate of synthesis of hemoglobin E.

#### Assessment of folate and vitamin B-12 deficiency

Unlike the hematologic and biochemical iron variables, there were no significant differences in mean plasma or erythrocyte folate or serum vitamin B-12 concentrations by hemoglobin type. The geometric mean ( $-SD$ ,  $+SD$ ) plasma and erythrocyte folate concentrations were 21.4 (13.5, 33.9) and 842 (599, 1182) nmol/L, respectively. Mean serum vitamin B-12 was 514 (355,



744) pmol/L. Plasma folate was positively correlated with erythrocyte folate ( $r = 0.259$ ,  $P < 0.05$ ). There are no pediatric interpretive values for plasma and erythrocyte folate and serum vitamin B-12. Accordingly, the interpretive values for "at risk" adults were used for plasma folate and erythrocyte folate and serum vitamin B-12 concentrations, ie,  $<6.8$  and  $<317$  nmol/L (21) for plasma and erythrocyte folate, respectively, and  $<150$  pmol/L for serum vitamin B-12 (22). On the basis of these cut-offs, 4 and 5 of the children, respectively, had low blood folate concentrations, but none of the children had a serum vitamin B-12 concentration indicative of vitamin B12 deficiency (22).

### Assessment of vitamin A deficiency

Serum retinol concentrations were independent of hemoglobin type (Table 2) and sex but increased significantly with increasing age ( $P < 0.001$ ). Very few of the children (3%) had a low vitamin A status (serum retinol:  $<0.7$   $\mu\text{mol/L}$ ; 23), but 20% ( $n = 98$ ) had marginal vitamin A status (serum retinol:  $0.7$ – $1.05$   $\mu\text{mol/L}$ ; 24).

### Interrelations among laboratory, sociodemographic, and health-status variables

ANOVA showed that in children with Hb AA and Hb AE, age was the most important predictor of hemoglobin concentration, followed by hemoglobin type and serum retinol; serum ferritin was not a significant predictor (Table 3). Moreover, ANOVA (adjusted  $r^2 = 0.255$ ) showed that when serum transferrin receptor was treated as a dependent variable, it was inversely related to log serum ferritin ( $P < 0.001$ ) after adjustment for age and school (data not shown). ANOVA results also showed that, after adjustment for the significant effects of age, anemic children had significantly lower ( $P < 0.01$ ) mean hematocrit, MCV, and serum retinol values than did their nonanemic counterparts; no significant differences in the geometric mean for serum ferritin and the mean for transferrin receptor were found (Table 4). In contrast,

**TABLE 3**

ANOVA showing predictors of hemoglobin concentration in a sample of school-aged children from northeast Thailand<sup>1</sup>

	$\beta$	95% CI	Partial $\eta^2$
Age	0.096	0.054, 0.137	0.033
Hemoglobin type <sup>2</sup>	2.940	1.311, 4.569	0.020
Serum retinol ( $\mu\text{mol/L}$ )	4.276	1.952, 6.601	0.019
Log serum ferritin ( $\mu\text{g/L}$ )	1.024	-0.227, 2.275	0.006

<sup>1</sup>  $n = 450$ . Children with hemoglobin type EE and a C-reactive protein concentration  $\geq 10$  mg/L were excluded. The interaction terms hemoglobin type  $\times$  log serum ferritin and hemoglobin type  $\times$  serum retinol are not statistically significant.

<sup>2</sup> Hb AA or Hb AE.

children with low vitamin A status, as indicated by a serum retinol concentration  $<0.70$   $\mu\text{mol/L}$  ( $n = 14$ ), had a significantly higher geometric mean serum ferritin concentration than did those with serum retinol concentrations  $\geq 0.70$   $\mu\text{mol/L}$ , but no significant difference in mean serum transferrin receptor concentrations after adjustment for age (Table 5). Sex, estimated annual income, and self-reported parasitic infection status were not associated with any of the laboratory indexes investigated.

### DISCUSSION

Our results showed a high prevalence of anemia and emphasized the importance of hemoglobinopathies in the etiology of anemia in northeast Thai schoolchildren. Indeed, more than one-third of these children carried a genetic hemoglobinopathy, specifically the  $\beta$  chain variant hemoglobin type E. Of the children who were homozygous (Hb EE) for this variant, nearly all (86%) were anemic, compared with more than one-third (35%) who were heterozygous (Hb AE) and 21% who had normal hemoglobin (Hb AA).

**TABLE 4**

Adjusted mean (and 95% CI) values for selected biochemical indexes by the presence or absence of anemia in a sample of school-aged children from northeast Thailand by hemoglobin type (Hb AA and Hb AE)<sup>1</sup>

Variable and hemoglobin type	Anemic children ( $n = 122$ )	Nonanemic children ( $n = 340$ )	$P^2$	$P$ for interaction <sup>3</sup>
Hematocrit (%)				
AA + AE	33.6 (33.3, 33.9)	37.1 (36.9, 37.3)	0.001	NS
Mean cell volume (fL)			0.001	0.002
AA	76.2 (75.0, 77.4)	81.0 (80.4, 81.6)	—	—
AE	71.6 (70.4, 72.8)	73.3 (72.4, 74.2)	—	—
Serum ferritin ( $\mu\text{g/L}$ )				
AA + AE	37.3 <sup>4</sup> (33.2, 41.8)	37.4 <sup>4</sup> (34.8, 40.2)	0.945 <sup>5</sup>	NS
Serum transferrin receptor (mg/L)			0.123	0.042
AA	7.38 (6.83, 7.92)	6.55 (6.27, 6.82)	—	—
AE	6.40 (5.87, 6.94)	6.51 (6.11, 6.91)	—	—
Serum retinol ( $\mu\text{mol/L}$ )				
AA + AE	1.22 (1.16, 1.28)	1.31 (1.27, 1.35)	0.017	NS

<sup>1</sup> Children with Hb EE and a C-reactive protein concentration  $\geq 10$  mg/L were excluded from the analysis; anemia was defined as a hemoglobin concentration  $<115$  g/L for children aged 6–11 y and  $<120$  g/L for children aged  $\geq 12$  y. The mean values were adjusted for age by using a general linear model.

<sup>2</sup> Significance of the difference between the anemic and nonanemic children.

<sup>3</sup> Interaction of hemoglobin type and anemia.

<sup>4</sup> Geometric  $\bar{x}$ .

<sup>5</sup> Calculated from natural log values.

TABLE 5

Adjusted mean (and 95% CI) values for selected biochemical indexes by serum retinol concentration in a sample of school-aged children from northeast Thailand<sup>1</sup>

Variable	Serum retinol <0.7 $\mu\text{mol/L}$ ( <i>n</i> = 19)	Serum retinol $\geq 0.7 \mu\text{mol/L}$ ( <i>n</i> = 462)	<i>P</i>
Serum ferritin ( $\mu\text{g/L}$ )	56.9 <sup>2</sup> (41.4, 78.3)	37.0 <sup>2</sup> (34.9, 39.2)	0.009 <sup>3</sup>
Serum transferrin receptor (mg/L)	7.00 (6.02, 7.99)	6.61 (6.41, 6.81)	0.441

<sup>1</sup> Children with hemoglobin type EE and a C-reactive protein concentration  $\geq 10 \text{ mg/L}$  were excluded from the analysis. The mean values were adjusted for age by using a general linear model.

<sup>2</sup> Geometric  $\bar{x}$ .

<sup>3</sup> Calculated from natural log values.

The anemia reported in the Thai children was predominantly microcytic, hypochromic anemia (Figure 1), as indicated by a low MCV. Anemia of this type is known to be associated with hemoglobinopathies and thalassemias (6, 25) as well as with deficiencies of iron (26) and vitamin A (27, 28).

It is of interest that little of the observed anemia reported here appeared to be associated with storage iron depletion. Indeed, concentrations of serum ferritin in the anemic and nonanemic Hb AA and Hb AE children were not significantly different (Table 4) and were within the range reported by other Thai investigators (29, 30). This finding was unexpected in light of the assumed role of nutritional iron deficiency in the etiology of anemia in Thailand (2, 3) but was nevertheless consistent with earlier findings in school-aged children from northern Thailand (27, 29, 31).

Instead, ANOVA results confirmed that age, hemoglobin type, and serum retinol were the most important factors predicting hemoglobin concentration (Table 3). Furthermore, the increase in hemoglobin concentration with age was independent of hemoglobinopathies (ie, there was no significant interaction).

The positive association between serum retinol and hemoglobin observed was reported by others in studies of children in Southeast Asia and elsewhere (32–36). Specifically, the anemic children with Hb AA and Hb AE in our study had a significantly lower mean serum retinol concentration than did their nonanemic counterparts (Table 4). Nevertheless, the effect of suboptimal vitamin A status on the prevalence of anemia has received little attention by policy makers in Thailand, perhaps because the high prevalence of hemoglobinopathies has confounded the detection of a positive relation between hemoglobin and serum retinol in earlier studies in northeast Thailand (27). In addition, there has been a dramatic decrease in the prevalence of vitamin A deficiency in Thailand in recent years. Indeed, only 3% of the northeast Thai children studied had serum retinol concentrations indicative of low vitamin A status (23). Nevertheless, 20% had concentrations indicative of marginal vitamin A status (0.7–1.05  $\mu\text{mol/L}$ ) (24), which have also been shown to be positively associated with low hemoglobin concentrations (35–37).

Several mechanisms whereby vitamin A interacts with iron and thus affects hemoglobin concentrations have been proposed. Whether the reduction in hematopoiesis observed in vitamin A deficiency is due to decreased incorporation of iron into hemoglobin or to impaired mobilization of iron from spleen or liver stores into the circulation is still uncertain (38, 39). In our study, the significantly higher serum ferritin concentrations observed in the children classified with low vitamin A status (Table 5) lends support to the hypothesis that a decrease in the mobilization of iron may be involved. Certainly, in earlier studies of children in


Thailand in whom vitamin A deficiency anemia has been reported, the absence of low serum ferritin concentrations complicates the detection of storage iron depletion (27, 29, 31). Indeed, in many cases, the existence of relatively elevated serum ferritin concentrations similar to those reported here has often been assumed to result from inflammation or infection (40). This conclusion is not supported by our data; only 2% of the children studied had elevated CRP concentrations indicative of inflammation or infection. More studies involving the simultaneous determinations of serum retinol, iron-status indexes, hemoglobin, hemoglobin type, and inflammation and infection status in population groups in Thailand and other regions of Southeast Asia are needed to further elucidate these interrelations.

Our results indicated that some of the children in the current study had a biochemical iron deficiency; however, the prevalence was low. In 2 earlier studies of Thai school-aged children (30, 31), serum ferritin, hemoglobin, or both indicators showed a positive response to iron supplements, although the effect was lower than expected in some cases (31). It appears that the detection of iron deficiency or IDA in northeast Thai schoolchildren is complicated by the coexistence of concomitant hemoglobinopathies and suboptimal vitamin A status.

Additional nonnutritional factors implicated in the etiology of anemia include parasitic infections, such as malaria and hookworm. Malaria no longer exists in northeast Thailand, but hookworm infestation does (30). However, we observed no relation between hemoglobin and self-reported helminth status in these Thai schoolchildren, although no objective measure of hookworm infection was obtained. Unlike some other studies (41), we could not detect any effect of socioeconomic status on hemoglobin concentrations in this study, after controlling for age and hemoglobin type, probably because all the children were selected from schools in the poorer subdistricts in Ubon Ratchathani province.

Nutritional deficiencies of folate and vitamin B-12 may also cause anemia. However, deficiencies of folate and vitamin B-12 are associated with the development of macrocytic anemia, which is characterized by an elevated MCV (26), rather than the microcytic anemia noted here (Figure 1). Moreover, only  $\approx 1\%$  of the children had biochemical evidence of folate deficiency on the basis of plasma and erythrocyte folate concentrations, and none of the children had vitamin B-12 deficiency. The absence of vitamin B-12 deficiency was attributed to the consumption of fish sauce and possibly fermented tempeh by these Thai school children, both of which foods are known to provide significant amounts of vitamin B-12 in the diet (42).

It is possible that riboflavin deficiency contributed to the anemia reported in the present study (9), although, unfortunately, we did not measure any biomarker of riboflavin status. Riboflavin deficiency is known to occur among rice-eating populations (43, 44), and the riboflavin intake of a subsample of these children was low in relation to the Thai recommended dietary allowance, consistent with findings of the 1995 National Nutrition Survey for the northeast region of Thailand (1). Furthermore, in an earlier study in Thailand (45) supplementation with both iron and riboflavin was more efficient at restoring hematologic indexes than was an iron supplement alone.

In conclusion our results indicate that hemoglobinopathies and suboptimal vitamin A status, together with age, were the major predictors of hemoglobin concentration in the population of primary school children from northeast Thailand that we studied and should be taken into account in the planning of anemia control programs. Little of the anemia noted in the present study was associated with iron deficiency, which indicates that hemoglobin alone is not a reliable indicator of the prevalence of IDA in this setting. Some iron deficiency probably occurs but its detection is complicated by the coexistence of suboptimal vitamin A status in this region. 

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RAT participated in the collection, analyses, and interpretation of the laboratory and statistical data. RAT and RSG were the primary writers of the manuscript but received input from the other authors. PW and RSG were responsible for the study concept and design, for securing the funding for the study, and for the acquisition, statistical analysis, and interpretation of the data. TP was the overall project field coordinator and participated in the data acquisition, analysis, and interpretation. KB conducted most of the biochemical analysis in New Zealand and some of the statistical analysis. EW supervised the analysis and interpretation of the serum retinol values. None of the authors had any conflicts of interest in connection with this study.

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