

## Original Article

# Co-existing micronutrient deficiencies among stunted Cambodian infants and toddlers

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The prevalence of malnutrition in Cambodia is among the highest in Southeast Asia, and diarrhea and pneumonia are the leading causes of death among children. Whether these adverse health outcomes are associated with co-existing micronutrient deficiencies is uncertain. We have determined the prevalence of anaemia, as well as iron, zinc, and vitamin A deficiency and their co-existence among stunted children (77 females; 110 males) aged 6–36 mos. Non-fasting morning venipuncture blood samples were taken and analyzed for haemoglobin (Hb), serum ferritin (via IMx system), retinol (via HPLC), and Zn (via AAS), C-reactive protein (CRP) (via turbidimetry) and Hb type (AA, AE, or EE) (via Hb gel electrophoresis). Children with CRP  $\geq 5.0$  mg/L (n=34) were excluded. Zinc deficiency defined as serum Zn  $< 9.9$   $\mu\text{mol/L}$  had the highest prevalence (73.2%), followed by anaemia (71%) (Hb  $< 110$  g/L), and then vitamin A deficiency (28.4%) (serum retinol  $< 0.70$   $\mu\text{mol/L}$ ). Of the anaemic children, only 21% had iron deficiency anaemia, and 6% had depleted iron stores. Age, log serum ferritin, and Hb type were significant predictors of Hb in the AA and AE children. Serum retinol was unrelated to haemoglobin or serum zinc. The prevalence of two or more micronutrient deficiencies (low Hb, serum retinol, and/or serum zinc) was 44%. Nearly 10% had low values for all three indices, and 18% had just one low value. In conclusion, anaemia, and deficiencies of iron, zinc, and vitamin A are severe public health problems among these stunted Cambodian children. Intervention strategies addressing multiple micronutrient deficiencies are needed.

**Key Words:** anaemia, Cambodia, children, stunted, haemoglobinopathies, iron, zinc, vitamin A

## INTRODUCTION

Cambodia is one of the most disadvantaged countries in Southeast Asia, with a higher prevalence of stunting, underweight, and wasting among infants and children than in most other countries in the region.<sup>1,2</sup> Diarrhea and pneumonia are the leading causes of death among young children.<sup>3,4</sup> Whether these adverse health outcomes in Cambodian children are associated with co-existing micronutrient deficiencies has not been established. Micronutrient surveys to date in Cambodia have only included serum retinol as an indicator of vitamin A deficiency and haemoglobin as an indicator of iron deficiency anaemia,<sup>1,5,6</sup> with no measurements of serum zinc concentrations. However, although iron deficiency is a major cause of anaemia, other factors may also be involved. These include vitamin A deficiency and non-nutritional factors such as genetic haemoglobinopathies, chronic inflammatory disorders, and parasitic infections.<sup>7</sup> The relative importance of these factors in Cambodia is unknown.

The diets of infants and young children in Cambodia are rice-based,<sup>8</sup> with low intakes of animal-source foods, a pattern comparable to that reported for children elsewhere in Southeast Asia, e.g. Thailand<sup>9,10</sup> and the Philippines.<sup>11</sup> In these SE Asian countries, multiple micronutrient deficiencies have been reported among young children, specifically with regard to iron, zinc, vitamin A, iodine, and sometimes riboflavin.<sup>10,12-16</sup> Clearly, such micronutri-

ent deficiencies may also exist in Cambodia, especially among stunted children, as noted for young children in Honduras.<sup>17</sup> However, biochemical data on the prevalence of iron, zinc, or vitamin A deficiency are very limited. Hence, in this study we have investigated the prevalence of biochemical iron, zinc, and vitamin A deficiency and anaemia among stunted Cambodian infants and toddlers — a population group that we consider to be particularly at-risk. Anaemia is defined here by haemoglobin and mean cell volume, iron deficiency by serum ferritin concentrations, vitamin A deficiency by serum retinol concentrations, and zinc deficiency by serum zinc concentrations. We have also examined associations between micronutrient status and haemoglobin type, breast-feeding practices, and socio-demographic and anthropometric status.

## SUBJECTS AND METHODS

### Subjects

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This cross-sectional study was conducted in May 2003 in the Mean Chey District of Phnom Penh, Cambodia, home to some of the poorest families in the city. The participants were children (173 males; 149 females) aged 6 to 36 months who had no evidence of chronic disease (e.g., active tuberculosis, symptomatic HIV) but were stunted (i.e., length-for-age Z scores < -2.0). Ethical approval of the study protocol was obtained from the National Ethics Committee for Health Research of the Ministry of Health, Cambodia and the Human Ethics Committee of the University of Otago, New Zealand. Written informed consent was obtained from the parents or guardians of each child after the purpose and methods of the study were clearly explained to them.

#### ***Socio-economic, health status, and breastfeeding practices questionnaire***

Trained Cambodian research assistants administered a pre-tested questionnaire on socio-demographic and health status, and breastfeeding practices to the mother or guardian of the child from each participating household. Socio-demographic variables assessed included ethnicity, household size, income, employment of the head of the household, and level of education of the mother or caregiver. Health status indicators included infection status (diarrhea, respiratory, and parasites), and use of vitamin and/or mineral supplements as reported by the mother or caregiver. Birth dates of the children were obtained by questioning the parents or from immunization cards.

#### ***Anthropometry***

Measurements of weight and length for all subjects were taken in triplicate using calibrated equipment and standardized techniques, with children wearing light clothing and no shoes.<sup>18</sup> Length was measured to the nearest mm using a locally made wooden pediatric length board placed on the floor. Each measurement was conducted by the same anthropometrist to eliminate inter-examiner error. Z-scores for length-for-age (LAZ), weight-for-age (WAZ), and weight-for-length (WLZ) were calculated using the WHO 2006 multicenter growth reference data and the computer program WHO Anthro 2005. A small number of extreme anthropometric values and associated Z-scores were assumed to result from operator error and excluded from the analyses of the anthropometric data.

#### ***Biochemical assessment***

A non-fasting peripheral venipuncture blood sample was taken between 7.00 to 11 am from each child in the sitting position. One blood sample was drawn into a trace element (TE)-free evacuated tube (Beckton Dickinson, Franklin Lakes, NJ), and a second into an evacuated tube containing EDTA as an anticoagulant (Becton Dickinson, Franklin Lakes, NJ). All blood samples were refrigerated immediately after collection,<sup>19</sup> and the serum from the TE-free tubes separated within two hours using TE-free techniques<sup>20</sup> and protected from ultra-violet light. Aliquots of serum for the analyses of zinc, ferritin, retinol, and C-reactive protein (CRP) were frozen in TE-free polyethylene vials at -20°C initially, and later at -70°C.

Aliquots of anticoagulated whole blood were analysed at the Pasteur Institute in Phnom Penh for a complete blood count (CBC) by using an electronic Coulter counter and for haemoglobinopathies by cellulose acetate haemoglobin electrophoresis.<sup>21</sup> Based on the latter results, children were classified as normal haemoglobin type (AA) if homozygous for normal haemoglobin, haemoglobin E trait (AE) if heterozygous for the E variant, or haemoglobin E disease (EE) if homozygous for the EE variant. A small number of children were identified as having other haemoglobin variants.

Serum zinc, ferritin, retinol, and C-reactive protein were analysed in the Department of Human Nutrition, University of Otago, New Zealand. Analyses of serum zinc was undertaken by flame atomic absorption spectrophotometry (Perkin Elmer 2690, Ebos Group Ltd, Auckland, New Zealand) using a modification of the method suggested by Smith et al.,<sup>22</sup> serum ferritin concentration by the IMx system (Abbott Laboratories, IL, USA), serum retinol by high pressure liquid chromatography,<sup>23</sup> and serum C-reactive protein (CRP) by using turbidimetry using the COBAS MIRA system. A serum CRP value of  $\geq 5$  mg/L was used to indicate the presence of acute inflammation or infection.<sup>24</sup>

A pooled serum sample and manufacturer's controls were used to check the precision and accuracy of all the analytical methods. The certified values of the manufacturer's controls for serum ferritin, supplied for the IMx analyzer system, had been calibrated by the manufacturer with the WHO International Reference Material. The between assay coefficients of variation (CV) for serum ferritin, serum retinol, serum zinc, and serum CRP were 7.5, 4.6, 4.1, and 3.9%, respectively. Values for the controls fell within the certified ranges for serum ferritin, zinc, and CRP.

The following interpretive criteria were used to define risk of micronutrient deficiencies: serum zinc <9.9  $\mu$ mol/L,<sup>25</sup> serum retinol <0.70  $\mu$ mol/L.<sup>26</sup> Anaemia was defined as a haemoglobin concentration of <110 g/L<sup>27</sup> and microcytic hypochromic anaemia as a mean cell volume of <77 fL.<sup>28</sup> Anaemia accompanied by a serum ferritin concentration of <10  $\mu$ g/L (in the absence of infection) was considered to reflect iron deficiency anaemia, whereas a low serum ferritin (i.e., <10  $\mu$ g/L) in the absence of anaemia or infection was taken to indicate depleted iron stores.<sup>29</sup>

#### ***Statistical analysis***

Data were checked for normality by using the Kolmogorov-Smirnov test. Only the serum ferritin values showed significant departures from normality, and hence were log-transformed prior to statistical analyses. One-way analysis of variance (ANOVA) with post hoc analysis (Tukey's honestly significant difference) was used to determine the source of differences for the hematological and biochemical indicators among the three haemoglobin types. ANOVA was also used to show the effect of anaemia on selected hematological and iron status indices, and ANCOVA used to examine predictors of haemoglobin, serum retinol, and serum zinc concentrations. A *P* value of <0.05 indicated statistical significance.

## RESULTS

### Socio-economic, health status, and breastfeeding practices questionnaire

Table 1 presents the socio-demographic and health status of the children aged 6 to 36 months of age. Of the children, 54% were males and 46% were females. Only about half (i.e., 57%) of the children were fully immunized, 33% were partially immunized; 10% had received no immunizations at the time of the survey. Seventeen percent of the children had elevated serum CRP concentration (ie, CRP  $\geq$  5 mg/L), which indicated inflammation or acute infection. The primary source of drinking water for the households was the river or canal (59%). However, 60% of the households boiled their water before drinking, although 40% only boiled their drinking water sometimes or not at all. Less than 50% of the households had a home latrine (45.5%) and 32% used a garden, field, or river for sewage disposal.

Breastfeeding was started immediately after birth for most of the children, although 15 mothers waited until colostrum secretion had ended before initiating breastfeeding. The majority of the children were given fluids other than breast milk in the first few days after birth (e.g., unboiled water, sugary water, and honey). By six months of age, 54% of the breast-fed children were also receiving complementary foods, mainly rice porridge (*borbor*), followed by soups. Of the children aged between 12 to 24 months and 24 to 36 months, 35% and 20%, respectively, were receiving complementary foods with continued breast feeding.

**Table 1.** Socio-demographic and health status characteristics of the study population

Variables	Number of participants	Percentage (%)
<b>Age</b>		
6-11 months	48	14.9
12-23 months	156	48.4
24-36 months	118	36.6
<b>Gender</b>		
Male	173	53.7
Female	149	46.3
<b>Source of drinking water</b>		
River / Canal	141	57.1
Well	27	10.9
Hand/treadle pump	36	14.6
Water traders	43	17.4
<b>Boiling of drinking water.</b>		
Yes	147	60.2
No	33	13.5
Sometimes	64	26.2
<b>Sewerage</b>		
Home latrine	112	45.5
Public latrine	5	2.0
Garden	45	18.3
Field	63	25.6
River	21	8.5
<b>Immunisations</b>		
No Immunisations	25	10.1
Partly Immunised	80	32.3
Fully Immunised	142	57.5

**Table 2.** Mean (SD) growth measurements and indices by sex and age

	6–11 months n=25	12–23 months n=65	24–36 months n=57
<b>Females</b>			
Length (cm)	63.3 (2.0)	71.5 (3.2)	79.9 (3.8)
Weight (kg)	7.0 (1.4)	8.5 (1.3)	10.2 (1.3)
LAZ	-2.80 (0.56)	-2.92 (0.62)	-3.1 (0.72)
WAZ	-1.43 (1.30)	-1.45 (1.20)	-1.92 (0.94)
WLZ	0.18 (1.54)	0.01 (1.61)	0.24 (1.10)
BAZ	0.15 (1.62)	0.50 (1.67)	0.13 (1.15)
<b>Males</b>			
Length (cm)	63.7 (3.5)	73.8 (4.0)	80.4 (3.8)
Weight (kg)	7.5 (1.4)	8.9 (1.2)	10.4 (1.4)
LAZ	-3.58 (1.14)	-3.19 (0.85)	-3.4 (0.77)
WAZ	-1.58 (1.51)	-2.02 (1.07)	-2.19 (0.96)
WLZ	0.82 (1.79)	-0.61 (1.52)	-0.49 (1.27)
BAZ	0.70 (1.94)	0.05 (1.63)	0.07 (1.32)

**Table 3.** Mean (SD) Z scores and prevalence of low anthropometric indices by sex

	Male	Female	Sig <sup>1</sup>
LAZ	-3.32 (0.87)	-2.97 (0.66)	0.001
WAZ	-2.02 (1.11)	-1.63 (1.14)	0.002
WLZ	0.38 (1.54)	-0.06 (1.42)	0.052
Severely stunted <sup>2</sup> (% of Total)	27.8	17.4	0.006
Underweight <sup>3</sup> (% of Total)	32.4	24.4	0.132
Severely underweight <sup>4</sup> (% of Total)	11.7	4.4	0.003
Wasted <sup>5</sup> (% of Total)	8.0	2.2	0.003
Severely wasted <sup>6</sup> (% of Total)	1.9	0.6	0.205

<sup>1</sup> Significance of difference from independent samples t-test (equal variances not assumed), and Fisher's exact test.

<sup>2</sup>(LAZ<-3SD), <sup>3</sup>(WAZ<-2SD), <sup>4</sup>(WAZ<-3SD), <sup>5</sup>(WLZ<-2SD), <sup>6</sup>(WLZ<-3SD)

### Anthropometry

Table 2 shows selected growth variables by gender and age. Stunted children were selected (length-for-age (LAZ) <-2SD). However, the results show that the children were also underweight with low weight-for-age (WAZ) scores, but not particularly wasted with WHZ scores of near zero. There is some indication that the anthropometric status of the older children was worse than that of their younger counterparts. Differences in anthropometric status between the male and female children are marked (Table 3): male children were more stunted ( $p=0.001$ ) and more underweight ( $p=0.002$ ) than females. Disproportionately more male children were severely stunted (LAZ<-3SD;  $p=0.006$ ) and severely underweight (WAZ<-3SD;  $p=0.003$ ) than females.

**Table 4.** Mean (SD) of selected hematological variables, and serum ferritin, serum zinc, and serum retinol concentrations by haemoglobin type<sup>1</sup>

	AA n = 117	AE n = 37	EE and other n = 11
Haemoglobin (g/L)	105.4 (11.3) <sup>a</sup>	99.4 (11.7) <sup>b</sup>	101.9 (6.2) <sup>ab</sup>
Hematocrit (%)	33.6 (3.2)	32.6 (2.5)	33.0 (2.0)
Mean cell volume (fL)	70.8 (6.5) <sup>a</sup>	65.2 (5.6) <sup>b</sup>	66.9 (6.2) <sup>ab</sup>
Serum ferritin (µg/L)	20.0 (8.1, 49.5) <sup>2</sup>	16.9 (7.5, 38.2) <sup>2</sup>	21.2 (10.7, 42.3) <sup>2</sup>
Serum zinc (µmol/L)	9.0 (1.9)	9.0 (1.6)	8.7 (2.7)
Serum retinol (µmol/L)	1.0 (0.4)	0.9 (0.4)	1.0 (0.2)

<sup>1</sup> Subjects with CRP  $\geq 5$  mg/L were excluded. <sup>2</sup> Values are geometric mean ( $\pm$  1SD). Different letters indicate significant differences between haemoglobin types (ANOVA with Tukey's post hoc analysis)

**Table 5.** Prevalence (as %) of low haematological indices and serum ferritin by haemoglobin type<sup>1</sup>

	AA n = 118	AE n = 37	EE and other n = 11	<i>p</i> <sup>2</sup>
Low haemoglobin (%) <sup>3</sup>	64.4	81.1	90.9	0.03
Low haematocrit (%) <sup>4</sup>	42.7	61.0	54.5	0.02
Low mean cell volume (%) <sup>5</sup>	78.8	97.3	90.9	0.05
Low serum ferritin (%) <sup>6</sup>	28.2	24.3	16.7	ns
Iron deficiency anaemia (%) <sup>7</sup>	20.5	24.3	16.7	ns

<sup>1</sup> Mean (95% CI); those with CRP  $\geq 5$  mg/L were excluded, n=34 <sup>2</sup>Significance of differences with haemoglobin type. <sup>3</sup>Haemoglobin <110g/L, <sup>4</sup>Haematocrit <33%, <sup>5</sup>Mean cell volume <77fL, <sup>6</sup>Serum ferritin <10µg/L, <sup>7</sup>Low serum ferritin and low haemoglobin.

### Biochemical assessment

**Inflammation and infection.** Of the children, 17% showed evidence of concurrent inflammation or infection based on an elevated serum CRP concentration ( $\geq 5$  mg/L). These subjects were not included in the data summaries presented in Tables 4 and 5. They had a higher geometric mean serum ferritin concentration (34.4 vs. 19.3 µg/L ( $p < 0.05$ )) and tended to have lower mean serum zinc (8.5 vs. 9.0 µmol/L) and serum retinol (0.87 vs. 0.99 µmol/L) concentrations than those with a CRP <5 mg/L, although these latter differences were not significant.

**Haemoglobinopathies.** Of the children, approximately 71% had a normal haemoglobin type (AA), and 22% were heterozygous for the haemoglobin variant type E (AE). The remaining 7% of children were either homozygous for type E haemoglobinopathy (EE), or had other undefined haemoglobin abnormalities. Not surprisingly, the mean haemoglobin concentration was highest in those children with a normal haemoglobin type (i.e., AA) compared to those with haemoglobin type AE and EE (Table 4). Similarly, mean values for haematocrit and mean cell volume (MCV) for children with haemoglobin type AA were also higher, although differences were only significant for MCV.

Overall, the prevalence of anaemia was 71%; hypochromic microcytic (MCV <77fL) anaemia predominated. The prevalence of anaemia, based on low haemoglobin, was of course, lowest in the Hb type AA group (64%) (Table 5).

**Biochemical iron status indicators.** In contrast to haemoglobin and mean cell volume, there were no significant differences in the geometric mean serum ferritin by haemoglobin type (Table 4). The overall prevalence of iron

deficiency anaemia (IDA), based on both a low haemoglobin and low serum ferritin (in the absence of infection) was 21.1%, and 6% for storage iron depletion (i.e., serum ferritin <10µg/L). Prevalence estimates for IDA among the haemoglobin types were 20.5% (n=24) in the Hb AA group, 24.3% (n=9) in the Hb AE group, and 16.7% (n=2) in the Hb EE group; these differences were not significant (Table 5). There were no significant differences in the prevalence estimates for depleted iron stores or IDA among the children in the Hb AA, AE, and EE groups. Table 6 shows that age was the most important predictor of haemoglobin, followed by log serum ferritin, and haemoglobin type (AA or AE). None of the measured breastfeeding practices, anthropometric, or socio-demographic status variables were significant predictors of haemoglobin.

**Biochemical vitamin A and zinc status indicators.** Both serum zinc and serum retinol were independent of haemoglobin type (Table 4) and sex, although serum retinol,

**Table 6.** ANCOVA table showing the predictors of haemoglobin concentration in a sample of stunted Cambodian children<sup>1</sup>

	$\beta$	95% CI	Partial $\eta^2$	<i>p</i>
Age	0.41	0.19, 0.63	0.088	0.001
Log serum ferritin	2.55	0.60, 4.49	0.044	0.011
Hemoglobin type	4.40	0.53, 8.27	0.033	0.026

<sup>1</sup> n=151; only children with haemoglobin type AA or AE and with C-reactive protein concentration <5 mg/L are included.

unlike serum zinc, decreased significantly with increasing age ( $p < 0.05$ ). There was no association between serum retinol and serum zinc concentrations. Likewise, no significant relationships could be demonstrated between serum zinc or serum retinol and any of the measured breastfeeding practices, anthropometry, and socio-demographic status variables.

Prevalence estimates for low and deficient vitamin A status on the basis of a serum retinol  $< 0.7 \mu\text{mol/L}$  and  $< 0.35 \mu\text{mol/L}$  were 28.4% and 5.4%, respectively. In contrast, the prevalence of zinc deficiency, as indicated by a serum zinc  $< 9.9 \mu\text{mol/L}$ , was much higher (i.e., 73.2%) than that of low or deficient vitamin A status.

## DISCUSSION

One of the most striking findings of this study was the very high prevalence of co-existing micronutrient deficiencies among these disadvantaged stunted Cambodian children. Nearly half of the children (i.e., 44%) had two or more co-existing micronutrient deficiencies, on the basis of low values for haemoglobin, serum retinol, and/or serum zinc, and nearly 10% had all three micronutrient deficiencies.

### Zinc status

Of the micronutrients, zinc deficiency was most prevalent (i.e., 73%), followed closely by anaemia (71%). The high prevalence of zinc deficiency among these stunted Cambodian children is not unexpected. Impaired linear growth is a prominent feature of zinc deficiency among young children in both developed and developing countries.<sup>30-32</sup> Indeed, the International Zinc Nutrition Consultative Group (IZINCG) suggest<sup>20</sup> that the prevalence of stunting can be used as an indirect indicator for the risk of zinc deficiency. Hence, the high prevalence of stunting reported in the most recent national survey (i.e., 45%)<sup>5</sup> combined with the high prevalence of low serum zinc values among the stunted children reported here suggest that Cambodia is a country at high risk of zinc deficiency. Certainly, to our knowledge, the mean serum zinc concentration for these stunted Cambodian children is one of the lowest documented among apparently healthy but stunted young children to date. Nevertheless, serum zinc concentrations should be measured in a nationally representative sample to establish the prevalence of zinc deficiency among children at a population level in Cambodia.

In this study we observed no relationship between serum zinc and LAZ-scores, probably because all the children recruited for this study were stunted. Likewise, no association between serum zinc and sex was noted, despite some earlier reports that male children are more vulnerable to zinc deficiency than females<sup>33-35</sup> and in some cases have shown a greater response in linear growth than their female counterparts after zinc supplementation.<sup>36-40</sup> Nevertheless, the greater prevalence of severe stunting in male versus female children and the lower mean LAZ score of the male children (Table 3), suggest a relationship between stunting and inadequate zinc nutrition for the reasons cited above. Serum zinc concentrations were also independent of age and haemoglobin type, consistent with results of an earlier study on Northeast Thai primary school children.<sup>15</sup>

### Anaemia and iron status

The high prevalence of anaemia in this study was associated in part with the existence of genetic haemoglobinopathies; almost one third of the children carried the beta chain variant haemoglobin type E. However, the frequency of haemoglobin type E is much higher (i.e., about 60%) among the Khmer population residing in the borders of Cambodia, Thailand, and Lao PDR.<sup>41</sup> Haemoglobin E is characterized by a reduction in the synthesis of the beta-globin chain of haemoglobin A. This in turn results in an unstable haemoglobin tetramer, and ultimately hypochromic microcytic anaemia. Hence, it is not surprising that of the children homozygous or heterozygous for haemoglobin type E, more than 90% had a mean cell volume indicative of hypochromic microcytic anaemia.

Serum ferritin was a predictor of haemoglobin concentration among these Cambodian children (Table 6). This finding is in contrast to our earlier report of older children in NE Thailand in whom serum ferritin was not a significant predictor of haemoglobin concentration.<sup>14</sup> The reason for this discrepancy is uncertain but is probably associated at least in part with the younger age of the Cambodian children studied here. Certainly, in a recent study of six-month old Cambodian infants, the rate of iron deficiency increased over a twelve month period in the group receiving a placebo but remained unchanged in those receiving a daily supplement of iron plus folate sprinkles.<sup>42</sup> Moreover, iron deficiency is often reported among infants after about six months of age, when foetal iron stores are depleted and infants can no longer meet their high iron requirements from breast milk alone.<sup>43</sup> Instead, they require an adequate supply of readily available iron from non-milk foods. In this study, the major sources of energy for the children were snacks and rice porridge (borbor). However, borbor for older children was often mixed with pork blood (VP Anderson: pers. com), a practice that may have led to the positive effect of age on haemoglobin concentrations noted in this study.

### Vitamin A status

It is of interest that 28% of the children were still vitamin A deficient even though more than half (i.e., 54%) of the mothers claimed that their child had received a vitamin A capsule at six months of age. This suggests that vitamin A deficiency is still a severe public health problem among these stunted Cambodian children.<sup>44</sup> Certainly, coverage levels for vitamin A supplementation among children in Cambodia are reportedly especially low among the poorest households.<sup>5,45</sup>

Failure to improve vitamin A status, despite receiving a vitamin A capsule could be related in part to the existence of concomitant zinc deficiency in these vitamin A-deficient children. Zinc is known to be essential for both the intra- and intercellular transport of vitamin A, as well as in the absorption of vitamin A in the intestine.<sup>46-48</sup> In this study, however, we found no relationship between co-existing deficiencies of vitamin A and zinc in contrast to our earlier finding for NE Thai school children,<sup>15</sup> and those observed in children from Mexico and Bangladesh.<sup>49,50</sup>

Similarly, we found no interrelationship between low serum retinol and reduced haemoglobin concentrations in

this study. Indeed, mean serum retinol levels of the anaemic and non-anaemic children with haemoglobin type AA or AE were similar, whereas in the NE Thai school children studied earlier, low haemoglobin concentrations were a risk factor for reduced serum retinol concentrations,<sup>15</sup> a trend in accordance with some<sup>51,52</sup>, but not all,<sup>53</sup> of the earlier studies. The reasons for these discordant findings, although unknown, may be due, in part, to differences in the ages of the children studied, the prevalence and severity of micronutrient deficiencies, and, most importantly, the existence of other potentially confounding factors such as breastfeeding status and/or infections.

We recognize that our results are not based on a representative sample of Cambodian infants and toddlers aged 6 to 36 months. Indeed, because all the children were stunted, they are more likely to be at risk of suboptimal micronutrient status than their non-stunted counterparts. Certainly, there is abundant evidence that stunted children are likely to be zinc deficient and are also at high risk to nutritional iron deficiency anemia because the nutritional factors associated with zinc deficiency are similar to those for iron deficiency.<sup>20</sup> Finally, because all the children were stunted and from some of the poorest families in Phnom Penh, we did not observe any relationships between micronutrient status and anthropometric or socio-demographic variables.

In conclusion, our findings emphasize the co-existence of multiple micronutrient deficiencies among stunted Cambodian children living in the urban slums of Phnom Penh, and the important role of age, storage iron depletion, and haemoglobinopathies in the etiology of anaemia. Care must be taken when designing intervention programmes to co-ordinate efforts to alleviate coexisting micronutrient deficiencies. Such programmes should be initiated early in the life cycle to achieve optimal micronutrient status and thus prevent any adverse health consequences associated with micronutrient deficiencies.

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#### AUTHOR DISCLOSURES

Victoria P Anderson, Susan Jack, Didier Monchy, Neang Hem, Phearom Hok, Karl B Bailey and Rosalind S Gibson, no conflicts of interest.

#### REFERENCES

- National Institute of Statistics (2001) Directorate General for Health [Cambodia], and ORC Macro, Cambodia Demographic and Health Survey 2000. Phnom Penh, Cambodia and Calverton, MD, USA: National Institute of Statistics, Directorate General for Health, and ORC Macro.
- de Onis M, Blössner M. The WHO Global Database on Child Growth and Malnutrition: methodology and applications. *Int J Epidemiol.* 2003;32:518–526.
- Reltjen LLS, Pumratana K, Seflow P. A population-based health survey in Kon Dieng District, Cambodia. *Trans R Soc Trop Med Hyg.* 1993;87:421.
- Caulfield LE, de Onis M, Blössner M, Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhea, pneumonia, malaria, and measles. *Am J Clin Nutr.* 2004;80:193–198.
- Cambodia Demographic and Health Survey 2000, National Institute of Statistics, Directorate General for Health (Cambodia) and ORC Macro 2001.
- Helen Keller International. The need for increasing coverage of vitamin A capsule program to reduce vitamin A deficiency among young children in Cambodia. *Cambodia Nutr Bull.* 2000;2(2):1–4.
- Stoltzfus RJ. Defining iron-deficiency anemia in public health terms: a time for reflection. *J Nutr.* 2001;131:565S–567S.
- Stone T, Senemaud B, Thai E. Return to normalcy: nutrition and feeding practices in Phnom Penh, Cambodia. *Ecol Food Nutr.* 1989;23:249–259.
- Jackson DA, Imaong SM, Wongsawasdi L, Silprasert A, Preunglampoo S, Leelapat P, Drewett RF, Amatayakul K, Baum JD. Weaning practices and breastfeeding duration in Northern Thailand. *Br J Nutr.* 1992;67:149–164.
- Udomkesmalee E, Dhanamitta S, Yhoung-Aree J, Rjroongwasinkul N, Smith JC Jr. Biochemical evidence suggestive of suboptimal zinc and vitamin A status in schoolchildren in northeast Thailand. *Am J Clin Nutr.* 1990;52:564–567.
- Perlas LA, Gibson RS, Adair LS. Macronutrient and selected vitamin intakes from complementary foods of infants and toddlers from Cebu, Philippines. *Int J Food Sci Nutr.* 2004;55:1–15.
- Thurnham DI, Migasena P, Vidhivai N. Angular stomatitis and biochemical ariboflavinosis in village preschool children in NE Thailand. *Southeast Asian J Trop Med Public Health.* 1971;2:259–260.
- Charoenlarp P, Pholpothi T, Chatpunyaporn P, Schelp FP. The effect of riboflavin on the hematologic changes in iron supplementation of schoolchildren. *Southeast Asian J Trop Med Public Health.* 1980;11:97–103.
- Thurlow RA, Winichagoon P, Green T, Wasantwisut E, Bailey KB, Gibson RS. Only a small proportion of anemia in North East Thai children is associated with iron deficiency. *Am J Clin Nutr.* 2005;82:380–387.
- Thurlow RA, Winichagoon P, Pongcharoen T, Gowachirapant S, Boonpradern A, Manger MS, Bailey KB, Wasantwisut E, Gibson RS. Risk of zinc, iodine, and other micronutrient deficiencies among school children in N.E. Thailand. *Eur J Clin Nutr.* 2006;60:623–632.
- FNRI (Food and Nutrition Research Institute). Fourth national nutrition survey, Philippines, 1993. Manila, Philippines: FNRI Department of Science and Technology, 1995.
- Albalak R, Ramakrishnan U, Stein AD, Van der Harr F, Haber MJ, Schroeder D, Martorell R. Co-occurrence of nutrition problems in Honduran children. *J Nutr.* 2000;130:2271–2273.
- Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champagne, IL, USA, Human Kinetics Books, 1988.
- Tamura T, Johnston KE, Freeburg LE, Perkins LL, Goldenberg RL. Refrigeration of blood samples prior to separation is essential for the accurate determination of plasma or serum zinc concentrations. *Biol Trace Elem Res.* 1994;41:65–173.
- Hotz C, Brown KM. Assessment of the risk of deficiency in populations and options for its control. *Food Nutr Bull.* 2004;25:S99–S199.
- Shihabi ZK, Hinsdale ME. Simplified hemoglobin chain detection by capillary electrophoresis. *Electrophoresis.* 1999;26:581–585.

22. Smith Jr JC, Butrimovitz GP, Purdy WC. Direct measurement of zinc in plasma by atomic absorption spectroscopy. *Clin Chem.* 1979;25:1487–1491.
23. Thurnham DI, Smith E, Flora PS. Concurrent liquid-chromatographic assay of retinol, alpha-tocopherol, beta-carotene, alpha-carotene, lycopene, and beta-cryptoxanthin in plasma with tocopherol acetate as an internal standard. *Clin Chem.* 1988;34:377–381.
24. Thurnham DI, Mburu ASW, Mwaniki DL, De Wagt A. Micronutrients in childhood and the influence of subclinical inflammation. *Proc Nutr Soc.* 2005;64:502–509.
25. Hotz C, Peerson JM, Brown KH. Suggested lower cutoffs of serum zinc concentrations for assessing zinc status: reanalysis of the second National Health and Nutrition Examination Survey data (1976–1980). *Am J Clin Nutr.* 2003;78:756–764.
26. de Pee S, Dary O. Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. *J Nutr.* 2002;132:2895S–28901S.
27. Stoltzfus RJ, Dreyfuss ML. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Washington, DC: INACG/WHO/UNICEF, 1998.
28. Dallman PR, Looker AC, Johnson CL, Carroll M. Influence of age on laboratory criteria for the diagnosis of iron deficiency anaemia and iron deficiency in infants and children. In: Hallberg L, Asp N-G, eds. *Iron nutrition in health and disease.* London: John Libby & Co, 1996:65–74.
29. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. *JAMA* 1997;277:973–976.
30. Aggett PJ. Zinc and human health. *Nutr Rev.* 1995;53:S16–S22.
31. Hambidge KM. Human zinc deficiency. *J Nutr.* 2000;130:1344S–1349S.
32. Brown KH, Peerson JM, Rivera J, Allen LH. Effect of supplemental zinc on the growth and serum zinc concentrations of prepubertal children: a meta-analysis of randomized controlled trials. *Am J Clin Nutr.* 2002;75: 1062–1071.
33. Smit-Vanderkooy, P. and Gibson, R.S. Food consumption patterns of Canadian pre-school children in relation to zinc and growth status. *Am J Clin Nutr.* 1987;42:165–169.
34. Cavan KR, Gibson RS, Grazioso CF, Isalgue AM, Ruz M, Solomons NW. Growth and body composition of periurban Guatemalan children in relation to zinc status: a cross-sectional study. *Am J Clin Nutr.* 1993;57:334–343.
35. Gibson RS, Manger MS, Krittaphol W, Pongcharoen T, Gowachirapant S, Bailey KB, Winichagoon P. Does zinc deficiency play a role in stunting among primary school children in NE Thailand? *Br J Nutr.* 2007;97:167–175.
36. Walravens PA, Hambidge KM. Growth of infants fed a zinc supplemented formula. *Am J Clin Nutr.* 1976;26:1114–1120.
37. Walravens PA, Krebs NF, Hambidge KM. Linear growth of low income preschool children receiving a zinc supplement. *Am J Clin Nutr.* 1983;38:195–201.
38. Hambidge KM, Krebs NF, Walravens PA. Growth velocity of young children receiving a dietary zinc supplement. *Nutr Res.* 1985;Suppl 1:306–316.
39. Castillo-Duran C, Garcia H, Venegas P, Torrealba I, Pantheon E, Concha N, Perez P. Zinc supplementation increases growth velocity of male children and adolescents with short stature. *Acta Paediatr.* 1994;83:833–837.
40. Ruz M, Castillon-Duran, C, Lara X, Codoceo J, Rebolledo A, Atalah E. A 24-mo zinc-supplementation trial in apparently healthy Chilean preschool children. *Am J Clin Nutr.* 1997;66:1406–1413.
41. Bains BJ. *Haemoglobinopathy. Diagnosis.* Oxford, United Kingdom: Blackwell Science Ltd, 2001.
42. Giovannini M, Sala D, Usuelli M, Livio L, Francescato G, Braga M, Radaelli G, Riva E. Double-blind, placebo-controlled trial comparing effects of supplementation with two different combinations of micronutrients delivered as sprinkles on growth, anemia, and iron deficiency in Cambodian infants. *J Pediatr Gastroenterol Nutr.* 2006;42:306–312.
43. Booth IW, Aukett M. Iron deficiency anaemia in infancy and early childhood. *Arch Dis Child.* 1997;76:549–554.
44. WHO (World Health Organization). Indicators for assessing vitamin A deficiency and their application for monitoring and evaluating intervention programmes. WHO, Geneva 1996.
45. Victora CG, Fenn B, Bryce J, Kirkwood BR. Co-coverage of preventive interventions and implications for child-survival strategies: evidence from national surveys. *Lancet.* 2005;366: 1460–1466.
46. Christian P, West KP Jr. Interactions between zinc and vitamin A: an update. *Am J Clin Nutr.* 1998;68:435S–441S.
47. Ahn J, Koo SI. Effects of zinc and essential fatty acids deficiencies on the lymphatic absorption of vitamin A and secretion of phospholipids. *J Nutr Biochem.* 1995;6:595–603.
48. Ahn J, Koo SI. Intraduodenal phosphatidylcholine infusion restores the lymphatic absorption of vitamin A and oleic acid in zinc-deficient rats. *J Nutr Biochem.* 1995;6:604–612.
49. Muñoz EC, Rosado JL, López P, Furr HC, Allen LH. Iron and zinc supplementation improves indicators of vitamin A status of Mexican preschoolers. *Am J Clin Nutr.* 2000;71: 789–794.
50. Rahman MM, Wahed, MA, Fuchs, GJ, Baqui, AH and Alvarez, OJ. Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. *Am J Clin Nutr.* 2002;75:92–98.
51. Bloem MW, Wedel M, Egger RJ, Speek AJ, Schrijver J, Saowakontha S, Schreurs WH. Iron metabolism and vitamin A deficiency in children in northeast Thailand. *Am J Clin Nutr.* 1989;50:332–338.
52. Ahmed F, Barua S, Mohiduzzaman M, Shaheen N, Bhuyan MA, Margetts BM, Jackson AA. Interactions between growth and nutrient status in school-age children of urban Bangladesh. *Am J Clin Nutr.* 1993;58:334–338.
53. Semba RD, Bloem MW. The anemia of vitamin A deficiency: epidemiology and pathogenesis. *Eur J Clin Nutr.* 2002;56:271–281.

## Original Article

## Co-existing micronutrient deficiencies among stunted Cambodian infants and toddlers

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### 發育不良柬埔寨嬰幼兒之微量營養素缺乏共存情形

柬埔寨的營養不良盛行率為東南亞之冠，而腹瀉及肺炎是孩童的主要死因。是否這些不利於健康的結果與微量營養素缺乏共存現象有關並不確定。我們評估 6-36 個月大發育不良的兒童(77 名女童；110 名男童)他們的貧血以及鐵、鋅和維生素 A 缺乏的盛行率，以及之間共存現象。收集早上非禁食靜脈血液樣本，分析血紅素(Hb)、血清運鐵蛋白(透過 IMx 系統)、視網醇(透過高效液相層析儀)、C 反應蛋白(CRP)(透過比濁法)及 Hb 類型(AA, AE 或 EE)(透過 Hb 膠體電泳法)。排除 CRP  $\geq 5.0$  mg/L (n=34)的兒童。鋅缺乏(定義為血清鋅 $<9.9$   $\mu\text{mol/L}$ )的盛行率最高(73.2%)，續為貧血(71%) (Hb $<110$  g/L)，及維生素 A 缺乏(28.4%)(血清視網醇 $<0.70$   $\mu\text{mol/L}$ )。貧血的兒童中只有 21%為鐵缺乏貧血，而 6%為鐵儲存量耗盡。年齡、血清運鐵蛋白取對數及 Hb 類型，為 AA 及 AE 型孩童 Hb 的顯著預測因子。血清視網醇與血紅素或血清鋅無相關性。2 個或以上的微量營養素缺乏盛行率(低 Hb、血清視網醇及/或血清鋅)為 44%。接近 10%的人有這三個指標值較低的現象，18%的人只有一個值較低。綜合上述，在發育不良的柬埔寨兒童中，貧血與鐵、鋅及維生素 A 的缺乏是嚴重的公共衛生問題，需要有針對多重微量營養素缺乏的介入政策。

關鍵字：貧血、柬埔寨、兒童、發育不良、血色素病變、鐵、鋅、維生素 A。