

Does zinc deficiency play a role in stunting among primary school children in NE Thailand?

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Stunting in school-age years may result in a decrease in adult size, and thus reduced work capacity and adverse reproductive outcomes. We have compared the mean intakes of energy, protein and selected growth-limiting nutrients in fifty-eight stunted children and 172 non-stunted controls drawn from 567 children aged 6–13 years attending ten rural schools in NE Thailand. Control children were selected randomly after stratifying children by age in each school. Dietary data were calculated from 24-h recalls using nutrient values from Thai food composition data and chemical analysis. Inter-relationships between stunting and sociodemographic, anthropometric and biochemical variables were also examined. Biochemical variables investigated were serum albumin, zinc, ferritin, transferrin receptor and retinol, and iodine in casual urine samples. Significantly more males than females were stunted (males, n 38, 65.5% *v.* females, n 20, 34.5%; $P=0.025$). Stunted males had lower mean intakes of energy, protein, calcium, phosphorus and zinc, and a lower mean (95% CI) serum zinc (9.19 (8.53, 9.84) *v.* 9.70 (8.53, 9.29) $\mu\text{mol/l}$) than non-stunted males; no other biochemical differences were noted. Stunted males also had a lower mean arm muscle area ($P=0.015$), after adjusting for age, than non-stunted males. In conclusion, the lower dietary intakes of the stunted males compared to their non-stunted counterparts may be associated with anorexia and hypogeusia induced by zinc deficiency. Hence, zinc deficiency may be a factor limiting linear growth, especially among boys in NE Thailand, but more research is needed to establish whether other factors also play a role.

Dietary intakes: Dietary quality: Serum zinc: Anthropometry

Stunting is defined by the WHO as the gaining of insufficient height relative to age (World Health Organization, 1995). It is an indicator of chronic undernutrition, and is the result of extended periods of inadequate food intake, poor dietary quality, increased morbidity, or a combination of these factors (Gopalan, 1988). The processes that lead to stunting can occur both pre- and post-natally (Rivera & Ruel, 1997). The prevalence of stunting is generally highest during the second and third years of life (World Health Organization, 1986), but it often persists into school-age years. Understanding stunting among this older age-group is important because, if still untreated, stunting results in a reduction in adult size. This, in turn, has been associated with reduced work capacity (Spurr, 1988) and, in women, adverse reproductive outcomes (Adair & Guilkey, 1997).

The northeast region of Thailand is the poorest in the country. Rainfall is low and erratic, so that the region is very susceptible to drought and hence poor rice yields. The capita income and education level in this region are among the lowest in the country, whereas the family size is generally larger compared to the national average (5.7 *v.* 4.0 members; Ministry of Public Health, 1995). A large family size has been shown to be a risk

factor for malnutrition in young children in several studies (e.g. Pelto *et al.* 1991). Hence, it is not surprising that in NE Thailand, low intakes of energy and several growth-limiting micronutrients (e.g. calcium, phosphorus, iron, vitamin A and zinc) have been reported among preschool (Chusilp *et al.* 1992; Ministry of Public Health, 1995) and school-aged children (Bloem *et al.* 1989; Egger *et al.* 1991; Udomkesmalee *et al.* 1992). Deficits in some of these micronutrients in school-aged children have been associated with reduced linear growth, as well as impaired immune competence, cognition and school performance (Soemantri *et al.* 1985; Kramer *et al.* 1993; Sandstead *et al.* 1998; Van Stuijvenberg *et al.* 1999). If these micronutrient deficits persist, the likelihood of catch-up growth is diminished.

Of the growth-limiting micronutrients, zinc is likely to be most limiting in developing countries in which children consume plant-based diets with a low content of total or absorbable zinc (Gibson, 1993; Yeudall *et al.* 2005). In NE Thailand, glutinous rice (*Oryza glutinosa*) or noodles prepared from rice flour often contribute as much as 70% of the total energy of the diets of children (Winichagoon, 2002). In much of this area, the soil is low in zinc (Alloway, 2004), and as a result, rice grown locally has a low zinc content (Umar Khan *et al.* 2002; Welch &

Abbreviations: WHO, World Health Organization; RCT, randomized controlled trial; HAZ, height-for-age z-score; NCHS, National Centre for Health Statistics; CRP, C-reactive protein; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score; HOH, Head of household; BMI, Body mass index; AMA, arm muscle area; MJ, megajoules; FAO/WHO/UNU, Food & Agriculture Organization/World Health Organization/United Nations University.

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Graham, 2004). Hence, NE Thai children may be especially at risk to low intakes of total dietary zinc (Udomkesmalee *et al.* 1992; Krittaphol *et al.* in press).

In the present study, we have compared the intakes of energy, protein and selected growth-limiting micronutrients in stunted and non-stunted primary school children living in rural NE Thailand. Inter-relationships between stunting and sociodemographic, anthropometric and biochemical variables were also examined. Biochemical zinc status was based on serum zinc, iron status on serum ferritin and transferrin receptor, vitamin A status on serum retinol, iodine status on iodine in casual urine samples, and protein status on serum albumin concentrations.

Subjects and methods

Subjects

The present study was undertaken in Ubon Ratchathani province, NE Thailand. The subjects were a subsample drawn from primary school children (284 males, 285 females) who were participating in the baseline data collection of a randomized controlled trial (RCT) conducted between June 2002 and March 2003. All the children for the RCT were attending ten primary schools situated in poor rural subdistricts of the Trakarn Phutphon district. For the RCT, children were stratified in each school into four strata: females grades 1–3; males grades 1–3; females grades 4–6; males grades 4–6. Fifteen children were selected randomly from each stratum, although in strata with less than fifteen children, all children were selected. To minimize correlation between participants, only one child per family was selected.

For the subsample of children reported in the present study, a representative sample of stunted children (n 58) from the larger sample (n 567) were included in the subsample and compared with a non-stunted control group consisting of up to five randomly selected non-stunted children from each stratum within each school. This yielded a total sample size of fifty-eight stunted and 172 non-stunted children. This sample size permits a difference in the mean zinc intakes of 0.8 mg Zn/d to be detected between the stunted and non-stunted children, based on a standard deviation in zinc intake of 1.8 mg/d (Krittaphol, personal communication) assuming α (two-tailed) = 0.05 and β = 0.10 (Hulley & Cummings, 1988). Stunted children were defined as those with height-for-age Z-scores (HAZ) < -2 calculated from the 1977 National Center for Health Statistics (NCHS) growth reference data using Epi-Info version 6.0 (USD Inc. Stone Mountain, GA, USA).

The Human Ethics Committees from the University of Otago (New Zealand) and Mahidol University (Thailand) approved the study protocol. Permission was also obtained from local school boards, Thai health workers, and the parents or guardians of the children, as noted previously (Thurlow *et al.* 2005).

Method

Details of the methods used to collect information on the sociodemographic, health, anthropometric and biochemical micronutrient status of the children who participated in the RCT at baseline have been reported elsewhere (Thurlow

et al. 2005, 2006). Onset of infection was assessed by measurement of serum C-reactive protein (CRP), as described previously (Thurlow *et al.* 2005). A CRP value of ≥ 10 mg/l was used to indicate the presence of infection or inflammation. Children with elevated serum CRP were excluded from the statistical analyses.

We collected dietary data from the stunted and non-stunted control group using the interactive 24-h recall method, validated earlier (Ferguson *et al.* 1989, 1995). Details of this method are given in Gibson & Ferguson (1999). Children were randomly allocated into either weekend or weekday groups within each stratum to ensure that both weekday and weekend days were represented in the final sample of recall days. Parents were instructed not to change the dietary patterns of their children on the recall day. Trained research assistants interviewed the children at school, along with their primary caregiver. If a child could not attend school on the day of the scheduled interview, the interviewer visited the child at home. To assist in the estimation of portion sizes, measurement aids such as graduated measuring cups and spoons, household spoons, tape measures, plastic food models of common foods, portions of cooked rice, and weighing scales (Soehnle, CMS Weighing Equipment, London, UK) were used (Gibson, 2005).

Average daily intakes and major food sources of energy, protein, selected growth-limiting micronutrients and phytate were calculated from the 24-h recalls. Indicators of dietary quality were also determined based on the proportion of energy from animal protein (as a percentage), nutrient intakes per MJ (i.e. nutrient densities), and intakes of animal protein, haem iron, and phytate:iron, phytate:calcium and phytate:zinc molar ratios. Intakes were calculated using nutrient and phytate values from food composition databases, with the exception of ten food items frequently consumed by the children, including steamed glutinous rice (*Oryza glutinosa*). For these ten foods, values for calcium, iron, zinc and phytate were obtained by chemical analyses, as described by Krittaphol *et al.* (in press). Most of the food composition values used were taken from the Thai Food Composition Database for the INMUCAL program (Institute of Nutrition, Mahidol University, 2002). However, for any food item with missing nutrient values, alternative food composition databases were used (Bunch & Murphy, 1994; Siong *et al.* 1997; Puwastien *et al.* 2000; United States Department of Agriculture, 2003).

Adjustments were made to the food composition values obtained by chemical analyses or from the alternative databases to take into account any discrepancies in their moisture content compared to that stated in the INMUCAL database. If a food could not be found in any of the additional sources, values for a similar Thai food were used based on advice from a Thai nutritionist (T.P.). When data were not available in the cooked form, nutrient values were derived from dried or raw foods using adjustments for changes in yield and nutrient retention, where appropriate, as described by Gibson & Ferguson (1999). The yield factors used for cooked NE Thai foods were compiled by Banjong *et al.* (2001).

Statistical analysis

Statistical analyses were carried out using SPSS version 12 (USD Inc., Stone Mountain, GA, USA). All continuous

variables were examined for normality using the Kolmogorov–Smirnov test. Dietary intake results are expressed as median (interquartile range) for consistency because of non-normal distributions of some nutrients. The binomial test, with a two-tailed significance based on the Z-approximation, was used to test for sex differences among the stunted group; Fisher's exact test was used for examining sex differences among children with low serum zinc concentrations indicative of zinc deficiency (Hotz & Brown, 2004).

Analysis of covariance (ANCOVA) was used to calculate the means of the stunted and non-stunted groups for each sex while simultaneously adjusting for age. Two sets of variables were examined: first, intakes of energy, protein, animal protein, calcium, phosphorus, zinc and iron; second, biochemical indices of protein, iron, vitamin A, iodine and zinc status; each dietary and biochemical variable was tested separately. Sex and stunting [$<$ or \geq HAZ -2 SD] were treated as independent classificatory variables and age as a covariate. In addition, the two-way interaction effects for each model were assessed for significance. All models conformed to the assumptions of the General Linear Model. A P value <0.05 was taken to indicate statistical significance.

Results

Of the 230 children selected for this study, 226 children (124 males, 102 females) participated in the 24-h recalls, giving a response rate of 98%. All the children who participated were of Thai ethnicity.

Socio-economic and anthropometric status indicators

The results shown in Table 1 indicate that the socio-economic status of the households of stunted and non-stunted children was comparable. More than 80% of both groups of children came from male-headed households in which the head of household or the primary caregiver had received only primary-school education. The major occupation for more than 90% of the households in both groups was farming, primarily rice-growing. The annual income of nearly 60% of the households was less than 30 000 Baht, or approximately US\$750.00 per year. The median family size was five in both groups.

Table 1. Sociodemographic characteristics of non-stunted (n 169) and stunted (n 58) children

| | Non-stunted | | Stunted | |
|-------------------------------|-------------|------|---------|------|
| | n | % | n | % |
| Head of household (HOH) | | | | |
| Male | 143 | 84.6 | 52 | 89.7 |
| Education of HOH | | | | |
| Primary school | 146 | 86.4 | 48 | 82.8 |
| Occupation of HOH | | | | |
| Agriculture | 152 | 89.9 | 55 | 94.8 |
| Education of caregiver/mother | | | | |
| Primary school | 151 | 89.3 | 49 | 84.5 |
| Estimated yearly income | | | | |
| \leq 30000 (baht) | 98 | 58.0 | 33 | 56.9 |
| Land ownership | | | | |
| Own family land | 141 | 83.4 | 53 | 91.4 |

Of the stunted children (n 58), a significantly higher proportion (65.5%) were male (Table 2). The mean ages of the two groups studied were similar and regression analysis (data not shown) suggests that the degree of stunting as indicated by the HAZ is independent of age. As might be expected, the mean weight-for-age Z-score (WAZ) for the stunted children was also lower than for the non-stunted children. The mean weight-for-height Z-score (WHZ) and the mean BMI for the stunted children were significantly lower than for the non-stunted children, as were other related variables including mid-arm circumference, arm muscle area, arm fat area, triceps skinfold and subscapular skinfold. There were no significant sex differences for any of the anthropometric variables except for arm muscle area (AMA; Table 2); males had a significantly higher AMA than females (2001 v. 1869 mm²; $P=0.015$).

Intakes of energy, nutrients and dietary quality indicators

The median daily intakes (first and third quartiles) for energy, selected growth-limiting nutrients, dietary fibre and phytate for the non-stunted and stunted children are shown in Table 3. For both groups, median intakes of energy, and most of the nutrients examined, with the exception of protein, are low when compared to the Thai Recommended Daily Dietary Allowances (Ministry of Public Health, 1989).

Median intakes of fat and total protein (g/day) and phosphorus were significantly lower ($P<0.05$) in the stunted group, with a tendency for lower intakes of energy ($P=0.062$) and zinc ($P=0.086$). By contrast, intakes of the other micronutrients were not significantly different in the stunted compared with the non-stunted children.

Dietary quality indicators are shown in Table 4. Stunted children had significantly lower median daily intakes of both energy and protein from animal sources, predominantly flesh foods ($P<0.01$). However, other indicators of dietary quality, including haem iron, and the [Phy]:[Ca], [Phy]:[Fe] and [Phy]:[Zn] molar ratios, were similar in the two groups.

ANCOVA (Table 5) provides further insight on the dietary data presented in Tables 3 and 4. Each of the dietary intake variables shown in Table 5 was analysed separately with sex and stunting as categorical independent variables and age as a covariate. The results reveal that for the intakes of energy, protein and phosphorus, there is a significant interaction between sex and stunting, sometimes with age as a significant covariate; the intakes of the male stunted group are anomalously low. The same pattern can be observed for the zinc intakes, although in this case the interaction term is not significant ($P=0.067$). For other nutrients (not shown) the pattern is less obvious, but even for iron, where the significance of the interaction is 0.581, the stunted males have a lower intake than the non-stunted males (Table 5), emphasizing the expected coherence of the dietary intake data.

Biochemical indices

Of the biochemical indices treated as dependent variables and shown in Table 6, only serum zinc was significantly influenced by stunting, with a significant two-way interaction between stunting and sex; stunted males had a significantly lower mean serum zinc than either stunted females or non-stunted

Table 2. Age, proportion of males, and the mean (95% CI) of selected anthropometric variables for non-stunted (*n* 169) and stunted (*n* 58) children

| | Non-stunted | | Stunted | | <i>P</i> * |
|------------------------------------|-------------|--------------|---------|--------------|------------|
| | Mean | 95% CI | Mean | 95% CI | |
| Males <i>n</i> | 86 | | 38 | | |
| % | 50.9† | | 65.5 | | 0.025‡ |
| Age (months) | 110.7 | 107.6, 113.7 | 114.7 | 109.5, 119.9 | 0.174§ |
| HAZ | -0.98 | -1.07, -0.88 | -2.44 | -2.60, -2.27 | |
| WAZ | -1.16 | -1.27, -1.05 | -2.17 | -2.41, -2.03 | |
| WHZ (<i>n</i> 147) | -0.74 | -0.89, -0.60 | -1.03 | -1.26, -0.80 | 0.038 |
| BMI | 15.1 | 14.8, 15.3 | 14.1 | 13.7, 14.6 | < 0.001 |
| Mid-arm circumference (cm) | 18.4 | 18.1, 18.6 | 16.8 | 16.3, 17.2 | < 0.001 |
| Arm muscle area (mm ²) | 2086 | 2035, 2137 | 1780 | 1691, 1869 | < 0.001 |
| Arm fat area (mm ²) | 640 | 593, 686 | 467 | 387, 548 | < 0.001 |
| Triceps skinfold (mm) | 7.2 | 6.8, 7.6 | 5.9 | 5.2, 6.6 | 0.001 |
| Subscapular skinfold (mm) | 5.5 | 5.2, 5.8 | 4.4 | 3.9, 4.9 | < 0.001 |

HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.

* Significance, except where indicated, from univariate analysis of covariance (ANCOVA), of the difference between the non-stunted and stunted children, with sex and stunting treated as categorical variables and age as a covariate. The interaction term sex × stunting was not significant in any of the ANCOVA models. Means for each model were evaluated at age 111.7 months.

† The stratified sampling scheme adopted ensured that the two sexes were approximately equally represented in the non-stunted group.

‡ From the binomial test on the stunted group, two-tailed significance based on the Z-approximation.

§ Significance for non-paired *t* test.

|| Significance for sex = 0.015.

males and females. The prevalence of low serum zinc concentrations indicative of zinc deficiency was higher among the male children (69%, *n* 72) than the female children (55%, *n* 47; *P* = 0.050, Fisher's exact test).

Discussion

The present results indicate that, of the rural NE Thai children in the present study, it was the stunted males who had the lowest mean serum zinc concentration (Table 6). The prevalence of low serum zinc concentrations, indicative of zinc deficiency, was also higher among the male children. Male children also formed a disproportionately high percentage of the stunted group; that is, nearly two-thirds of the group (Table 2). Several other studies have reported that males are more vulnerable to zinc deficiency than females (Smit-Vanderkooy & Gibson,

1987; Gibson *et al.* 1991; Cavan *et al.* 1993; Parnell *et al.* 2003), presumably because of a higher requirement for zinc. This may be partly due to a higher growth rate and greater proportion of muscle per kilogram body weight as muscle contains a higher content of zinc than fat (Hotz & Brown, 2004). Indeed, in several zinc supplementation trials, the zinc-supplemented males have shown a greater response in linear growth than their female counterparts (Walravens & Hambidge, 1976; Walravens *et al.* 1983; Hambidge *et al.* 1985; Castillo-Duran *et al.* 1994; Ruz *et al.* 1997).

The onset of the pubertal growth spurt was probably not a significant confounder in the present study. Menarche had not commenced in any of the girls studied, and peak height velocity occurs later for boys than girls. Hence, it is likely that at the time of the present cross-sectional study, most of the boys had not experienced their pubescent growth spurt and associated

Table 3. Median daily intakes (first and third quartiles) for energy, selected growth-limiting nutrients, phytate and dietary fibre for the non-stunted (*n* 169) and stunted (*n* 58) children

| | Non-stunted | | | Stunted | | |
|-----------------------------|-------------|----------------|----------------|---------|----------------|----------------|
| | Median | First quartile | Third quartile | Median | First quartile | Third quartile |
| Energy (MJ) | 5.25 | 4.37 | 6.63 | 4.90 | 3.95 | 5.95 |
| Carbohydrate (g) | 223 | 178 | 274 | 214 | 164 | 270 |
| Fat (g) | 23.1 | 15.0 | 30.5 | 19.6 | 15.0 | 25.1 |
| Protein (g) | 40.2 | 32.9 | 49.2 | 37.8 | 27.1 | 43.8 |
| Calcium (mg) | 224 | 105 | 304 | 222 | 101 | 278 |
| Phosphorus (mg) | 390 | 279 | 485 | 332 | 273 | 398 |
| Iron (mg) | 5.2 | 4.0 | 7.2 | 4.9 | 3.6 | 7.1 |
| Zinc (mg) | 4.5 | 3.5 | 5.5 | 4.3 | 3.1 | 5.1 |
| Vitamin A (RAE) | 206 | 105 | 301 | 183 | 95 | 292 |
| Vitamin B ₁ (mg) | 0.39 | 0.29 | 0.57 | 0.38 | 0.25 | 0.60 |
| Vitamin B ₂ (mg) | 0.94 | 0.72 | 1.20 | 0.88 | 0.66 | 1.08 |
| Vitamin C (mg) | 22.2 | 6.5 | 44.5 | 21.4 | 4.6 | 57.5 |
| Niacin (mg) | 7.21 | 5.50 | 9.94 | 6.60 | 4.89 | 9.02 |
| Dietary fibre (g) | 3.5 | 1.9 | 5.3 | 3.2 | 2.0 | 6.0 |
| Phytate (mg) | 80 | 58 | 113 | 74 | 52 | 105 |

RAE, retinol activity equivalents.

Table 4. Median indicators (first and third quartiles) of dietary quality for the non-stunted (*n* 169) and stunted (*n* 58) children

| | Non-stunted | | | Stunted | | |
|------------------------------------|-------------|----------------|----------------|---------|----------------|----------------|
| | Median | First quartile | Third quartile | Median | First quartile | Third quartile |
| Energy from animal protein (MJ) | 0.36 | 0.25 | 0.51 | 0.31 | 0.23 | 0.38 |
| Protein density (mg/MJ) | 7.6 | 6.8 | 8.6 | 7.4 | 6.7 | 8.1 |
| Animal protein (g) | 21.3 | 14.5 | 30.0 | 18.0 | 13.6 | 22.1 |
| Iron from animal products (mg) | 2.35 | 1.47 | 3.60 | 2.04 | 1.43 | 3.93 |
| Iron from meat, poultry, fish (mg) | 1.44 | 0.78 | 2.48 | 1.28 | 0.79 | 2.11 |
| Iron from vegetables (mg) | 2.31 | 1.77 | 3.55 | 2.32 | 1.62 | 3.31 |
| Haem iron (mg) | 0.94 | 0.59 | 1.44 | 0.82 | 0.57 | 1.57 |
| [Phy]:[Ca] molar ratio | 0.02 | 0.01 | 0.05 | 0.03 | 0.01 | 0.04 |
| [Phy]:[Fe] molar ratio | 1.31 | 0.96 | 1.84 | 1.22 | 0.82 | 1.70 |
| [Phy]:[Zn] molar ratio | 1.70 | 1.34 | 2.36 | 1.71 | 1.32 | 2.25 |
| Calcium density (mg/MJ) | 36.5 | 18.7 | 58.5 | 38.1 | 19.3 | 68.3 |
| Phosphorous density (mg/MJ) | 73.9 | 53.9 | 91.7 | 68.9 | 56.9 | 83.4 |
| Iron density (mg/MJ) | 0.99 | 0.79 | 1.20 | 0.97 | 0.80 | 1.26 |
| Zinc density (mg/MJ) | 0.82 | 0.75 | 0.91 | 0.83 | 0.76 | 0.92 |
| Energy per kg body weight (MJ/kg) | 0.21 | 0.17 | 0.27 | 0.24 | 0.19 | 0.28 |
| Protein per kg body weight (g/kg) | 1.68 | 1.26 | 2.17 | 1.78 | 1.45 | 2.05 |

increases in muscle mass (World Health Organization, 1995), especially as zinc deficiency delays secondary sexual maturation in boys (Hotz & Brown, 2004). The significant interaction observed in the covariance model between stunting and sex was independent of age (Table 5), suggesting that the relationships observed applied across the age range studied.

Impaired linear growth is a prominent feature of zinc deficiency among children in both developed and developing countries (Aggett, 1995; Hambidge, 2000; Brown *et al.* 2002). The present results demonstrate a significant and positive relationship between stunting among the male children of the study and serum zinc concentrations (Table 6). By contrast, no relationships were found between stunting among the male children and other biochemical indices of growth-limiting nutrients, such as serum albumin, log serum ferritin, serum transferrin receptor, serum retinol or urinary iodine concentrations (Table 6), suggesting that neither protein, iron, vitamin A nor iodine status was associated with the higher prevalence of stunting among the males, at the time period of the present study.

The existence of zinc deficiency among the NE Thai children was confirmed by a significant increase in serum zinc concentration among those children who participated in the RCT and received a school lunch containing a seasoning powder fortified with zinc (and vitamin A, iron and iodine) for 31 weeks, compared to those consuming an unfortified lunch (Winichagoon *et al.* 2006).

We also observed a striking feature of the dietary intakes of the male stunted children; their intakes of energy, protein and phosphorus were lower compared to the female stunted and the non-stunted children of both sexes, whereas their intakes of calcium and zinc were lower than those of their non-stunted counterparts (Table 5). It is possible that the low biochemical zinc status of these stunted males was responsible, at least in part, for the low dietary intakes. In some studies, poor appetite (Krebs *et al.* 1984; Umata *et al.* 2002) and impaired taste acuity (Hambidge *et al.* 1972; Buzina *et al.* 1983; Gibson *et al.* 1989; Cavan *et al.* 1993) have been associated with mild zinc deficiency in children, which together, if present, could lead to

Table 5. Analysis of covariance (ANCOVA) of energy and other nutrient intakes with sex and stunting treated as categorical variables and age as a covariate for the non-stunted (*n* 169) and stunted (*n* 58) children

| Dependent variable | Sex | Non-stunted | | Stunted | | <i>P</i> | |
|--------------------|-----|-------------|------------|---------|------------|-----------------------------|------------------|
| | | Mean | 95% CI | Mean | 95% CI | Sex × stunting ^a | Age ^b |
| Energy (MJ) | F | 5.05 | 4.71, 5.39 | 5.04 | 4.35, 5.73 | 0.027 | 0.001 |
| | M | 6.01 | 5.67, 6.34 | 4.88 | 4.38, 5.39 | | |
| Protein (g) | F | 38.8 | 35.9, 41.7 | 37.8 | 31.9, 43.8 | 0.042 | 0.024 |
| | M | 45.8 | 42.9, 48.6 | 36.1 | 31.7, 40.4 | | |
| Animal protein (g) | F | 21.4 | 19.2, 23.7 | 19.7 | 15.2, 24.3 | 0.190 | 0.660 |
| | M | 24.2 | 22.0, 26.4 | 18.1 | 14.8, 21.4 | | |
| Calcium (mg) | F | 206 | 169, 244 | 239 | 162, 316 | 0.214 | 0.010 |
| | M | 242 | 205, 279 | 206 | 150, 262 | | |
| Phosphorus (mg) | F | 368 | 337, 399 | 380 | 316, 444 | 0.042 | 0.472 |
| | M | 421 | 390, 451 | 338 | 292, 385 | | |
| Zinc (mg) | F | 4.2 | 3.9, 4.6 | 4.2 | 3.5, 4.9 | 0.067 | 0.017 |
| | M | 5.2 | 4.8, 5.5 | 4.2 | 3.7, 4.7 | | |
| Iron (mg) | F | 5.4 | 4.7, 6.1 | 5.4 | 3.9, 6.8 | 0.581 | 0.279 |
| | M | 6.4 | 5.7, 7.1 | 5.7 | 4.6, 6.8 | | |

^a The significance indicated is that of the interaction term, sex × stunting.

^b Each of the seven ANCOVA models was evaluated at age 112.4 months. In four of the seven cases the covariate age term is significant.

Table 6. Analysis of covariance (ANCOVA) of some growth-limiting biochemical indices of micronutrient status as the dependent variable with sex and stunting treated as categorical variables and age as a covariate

| Dependent variable | Sex | Non-stunted | | | Stunted | | | P* |
|-----------------------------------|-----|-------------|-------------|-----|---------|-------------|----|---|
| | | Mean | 95% CI | n | Mean | 95% CI | n | |
| Serum ferritin† (μg/l) | F | 37.7 | 31.8, 45.2 | 92 | | | | Sex NS; stunting NS, sex × stunting NS, age < 0.001 |
| | M | 38.1 | 33.1, 43.8 | 113 | | | | |
| Serum transferrin receptor (mg/l) | F | 6.94 | 6.37, 7.51 | 97 | | | | Sex NS; stunting NS, sex × stunting NS, age < 0.001 |
| | M | 6.72 | 6.27, 7.18 | 120 | | | | |
| Serum retinol (μmol/l) | F | 1.34 | 1.25, 1.42 | 97 | | | | Sex NS; stunting NS, sex × stunting NS, age < 0.001 |
| | M | 1.25 | 1.19, 1.32 | 122 | | | | |
| Serum albumin (g/l) | F | 47.5 | 46.5, 48.4 | 92 | | | | Sex = 0.008; stunting NS, sex × stunting NS, age NS |
| | M | 45.9 | 45.2, 46.6 | 118 | | | | |
| Urinary iodine† (μg/l) | F | 49.4 | 42.1, 58.0 | 102 | | | | Sex = 0.012; stunting NS, sex × stunting NS, age NS |
| | M | 66.7 | 58.0, 74.4 | 122 | | | | |
| Serum zinc (μmol/l) | F | 9.64 | 9.23, 10.06 | 71 | 10.58 | 9.67, 11.49 | 15 | Sex × stunting = 0.026, age NS |
| | M | 9.70 | 9.29, 8.53 | 75 | 9.19 | 8.53, 9.84 | 29 | |

* Each of the six ANCOVA models was evaluated at approximately age 112 months, the exact age depending on the sample. In five of the six cases the interaction term sex × stunting is non-significant and there are no significant biochemical difference between the stunted and non-stunted children. Only in the case of serum zinc is the interaction term sex × stunting significant.

† Data for serum ferritin and urinary iodine based on log-transformed values.

a concomitant decrease in food intake, and thus deficits in energy and associated nutrients. An alternative hypothesis is to view the non-stunted male children as representing boys who are taller, with more muscle, larger appetites, and therefore higher food intakes. We prefer to view these non-stunted male children as 'normal', but their much higher energy and nutrient intakes are certainly a striking feature of the data presented in Table 5.

The present dietary data also confirm that the low biochemical zinc status of the stunted male children was associated with low intakes of zinc *per se* (Table 5), and not with low intakes of absorbable zinc arising from the inhibitory effect of phytate on zinc absorption (Lönnerdal, 2000). Such low intakes of zinc arise in part because the diets of the children were based on glutinous rice (*Oryza glutinosa*) grown on the low-zinc soils of NE Thailand (Umar Khan *et al.* 2002; Alloway, 2004). Glutinous rice also has a low content of phytate, even when raw (Krittiphol *et al.* in press), that is further reduced by washing and soaking overnight in water prior to cooking. Both of these practices leach water-soluble potassium and magnesium phytates into the water, which is then discarded (Perlas & Gibson, 2002). Therefore, it is not surprising that, unlike the unrefined maize-based diets of school children in many Sub-Saharan African countries (Gibson, 1993; Murphy *et al.* 1995; Yeudall *et al.* 2005), the phytate intakes of all the NE Thai school children of the present study were very low (Table 3).

The lower protein intakes observed in the male stunted children of the present study were not associated with correspondingly lower serum albumin concentrations; no significant interaction existed between sex and stunting for serum albumin (Table 6). This suggests that protein status was probably not associated with stunting among these male children at the time of the study. Certainly, both stunted and non-stunted children (Table 2) had higher mean plasma albumen concentrations than non-stunted, healthy middle-class Nepali children of a comparable age (Panter-Brick *et al.* 2001). Allen (1994) noted that linear growth faltering, or subsequent failure to 'catch up', can occur in the presence of adequate intakes of energy and/or protein. In addition, protein intakes reported in this study probably met the FAO/WHO/UNU requirements (Food & Agriculture

Organization/World Health Organization/United Nations University, 1985), even when a 50% increment in the growth component of the requirement is applied to allow for catch-up growth for the stunted children, and the protein intake is not adjusted for amino acid composition. Limited data are available on the amino acid composition of Thai foods. However, protein utilization is unlikely to be compromised by poor digestibility because the dietary fibre content of these NE Thai diets was very low (Table 3).

Unlike protein, no biochemical indices of phosphorus status were measured in the present study. Hence, whether the lower phosphorus intake in the male stunted children (Table 5) was associated with a compromised biochemical phosphorus status is unknown. Low phosphorus intakes among NE Thai children consuming diets based on glutinous rice have been reported by others (Valyasevi *et al.* 1969; Chusilp *et al.* 1992). Indeed, Chusilp *et al.* (1992) in a study of NE Thai pre-school children reported very low phosphorus intakes among those in the lowest tercile for length attained at 2 years, but whether the poor linear growth was sex-related was not reported.

The mean calcium intake of the male stunted children was lower than the intake of the non-stunted males. Indeed, the mean calcium intakes of all four groups of children studied were very low and below those reported for NE Thai pre-school children in the Thai National Nutrition Survey (Ministry of Public Health, 1995). Children consuming plant-based diets characteristically have such low calcium intakes (Murphy *et al.* 1995), but these have not been associated with linear growth failure. Indeed, there is some evidence that populations in Asia may have increased calcium retention and thus adequate bone mineralization at lower intakes of calcium (Food & Agriculture Organization/World Health Organization, 2002).

In the present study, the mean dietary zinc intake of these stunted NE Thai male children was very low compared to that of their non-stunted counterparts (Table 5). Furthermore, the stunted males also had a lower intake of animal protein (Table 5), although the interaction term sex × stunting was not significant. Animal protein is a rich source of readily available zinc (Lönnerdal, 2000). Several studies have observed

significant and positive correlations between intakes of animal protein and attained height, even after controlling for sociodemographic variables (Allen *et al.* 1991, 1992; Paik *et al.* 1992).

Earlier research suggests that deficits in stature among children in NE Thailand commence within the first 3 months of life and increase steadily up to about the second (Chusilp *et al.* 1992) or third year of life (Ministry of Public Health, 1995), remaining relatively constant thereafter. This trend is consistent with that noted in many other developing countries (Rivera & Ruel, 1997). Moreover, there was a tendency for the deficits in stature to be greater among NE Thai boys than girls, based on the most recent Thai National Nutrition Survey (Ministry of Public Health, 1995). Hence, linear growth failure for the NE Thai children in the present study probably began during early childhood, a time when the adverse effects of poor dietary intakes and frequent infections on growth play a crucial role (World Health Organization, 1998). We recognize that the present 24-h recall data only provide information on recent dietary intakes and not on intakes at the likely onset of linear growth failure in these children (i.e. during the first 3 years of life). However, in this region rice and rice products provide the major proportion of the dietary energy throughout childhood (Ministry of Public Health, 1995; Winichagoon, 2002), so it is likely that the food consumption patterns of these NE Thai children were probably comparable during their earlier childhood. Whether zinc was the crucial limiting micronutrient in relation to dietary adequacy and stunting among the male children of this study during these earlier phases of growth is unknown.

Genetic differences are unlikely to be responsible for stunting among these primary school children in NE Thailand because there is no evidence that growth potential among population groups in Thailand is influenced by genetic differences (Chusilp *et al.* 1992). Indeed, there is little difference between the growth curves for healthy Thai children during the first 5 years of life (Ministry of Public Health, 1987) and those of US children of similar age, compiled by the NCHS (Hamill *et al.* 1979). Likewise, ethnic differences were not a factor because all the participating children were of Thai ethnicity. Several investigators have provided convincing evidence that genetic and ethnic differences are less important in linear growth during childhood than the influences of food intake, dietary quality and morbidity (Martorell *et al.* 1994; Mei *et al.* 1998).

The stunted children of the present study were underweight and thinner than their non-stunted counterparts, as indicated by significantly lower mean weight-for-height Z-scores (WHZ) and BMI, respectively (Table 2).

Even the non-stunted children of this study had a mean energy intake well below the FAO/WHO/UNU average requirement for light physical activity (Food & Agriculture Organization/World Health Organization/United Nations University, 2004). The low WHZ and BMI were accompanied by significantly lower anthropometric indices of body fat and muscle mass in the stunted children compared to their non-stunted counterparts (Table 2).

Low WHZ and BMI have also been noted among stunted school children in some countries including Nepal and Zanzibar (Stoltzfus, 2001), although in most Latin American countries, the WHZ and BMI of stunted children are generally not low (Post & Victora, 2001). Reasons for the differences

that distinguish the stunted, thinner Thai children from their stunted but fatter Latin American counterparts are unclear. Post and Victora (2001) have argued that differences in body proportions may account for the apparently adequate WHZ among stunted Latin American children.

In summary, the present results suggest that zinc may have a crucial role in stunting in these children from NE Thailand. Support for this suggestion stems from the greater prevalence of stunting among the males, coupled with their lower serum zinc concentrations, and lower intakes of zinc, as well as energy and other growth-limiting nutrients compared to their non-stunted male counterparts. This conclusion is further supported by the significant improvement in serum zinc concentrations observed among some of these NE Thai school children after they consumed a school lunch containing a seasoning powder fortified with micronutrients, including zinc, for 31 weeks compared to their counterparts consuming an unfortified lunch. Nevertheless, further research is required to identify all the nutritional and health factors responsible for limiting the linear growth of these NE Thai children. Sustainable interventions are needed that permit full catch-up growth during childhood so that the adverse effects associated with stunting in both school-age years and adulthood can be avoided.

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