Early Nutritional Supplementation and Skeletal Maturation in Guatemalan Adolescents[1,2]

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ABSTRACT The effect of early childhood nutritional supplementation on skeletal maturation at adolescence was investigated in 663 rural Guatemalans, aged 11–18 y. Skeletal maturation was assessed by the Tanner-Whitehouse-2 method. The subjects were former participants in the Institute of Nutrition of Central America and Panama longitudinal study of growth and development (1969–77) residing in four villages (two large and two small) in eastern Guatemala. The villages were randomized within pairs to receive either a high energy, high protein supplement (Atole) or a low energy supplement with no protein (Fresco). Skeletal maturity was observed across all villages to be delayed significantly relative to a British reference for boys < 14 y of age, but not for older boys or for girls < 14 y of age. Delays in girls > 14 years could not be determined reliably because many had reached maturity. Girls < 14 years from Atole villages were more advanced in skeletal maturity than similar age girls from Fresco villages but these differences were found only in comparisons of the large villages. The relationship between early nutrition and biological maturation at adolescence may be obscured in this sample by the advanced age at which the subjects were examined in adolescence. J. Nutr. 125: 1097S–1103S, 1995.

INDEXING KEY WORDS:

- *protein-energy malnutrition* • *skeletal age*
- *adolescence* • *childhood supplementation*
- *Guatemala*

More is known about the effects of early childhood protein-energy malnutrition on growth than on biological maturation. Some studies have shown, however, that early undernutrition delays maturation in both experimental animals (Schrader and Zeman 1973) and in humans (Alvear et al. 1986, Bailey et al. 1984, Himes 1978). In the Institute of Nutrition of Central America and Panama (INCAP) longitudinal study, maturation was assessed in preschool children by counting the number of ossification centers present in a hand-wrist X-ray (Yarbrough et al. 1973). Effects of nutritional supplementation on maturation were found in both sexes but the differences were of lesser magnitude than those seen on linear growth (Martorell et al. 1979). Approximately 20% of the effects on linear growth could be attributed to accelerated maturation.

The long-term effects of malnutrition in early childhood on growth and development at adolescence are less well known. Associations between stunting in early childhood with patterns of growth in adolescence and with attained adult height have been demonstrated (Billewicz and MacGregor 1982, Hauspie et al. 1980, Satyanarayana et al. 1980, Satyanarayana et al. 1989). Martorell et al. (1990) found that stunting in early childhood in rural Guatemalans persisted into adolescence and that height gain between 5 and 18 y of age was independent of height status at 5 y.

Satyanarayana et al. (1989) found that timing and duration of peak height velocity in an Indian sample was dependent on the degree of stunting at 5 y of age. Cross-sectional studies from developing countries suggest that nutritional status assessed by anthropometry during adolescence is related to maturity indicators such as age at menarche, skeletal age (SA) and the development of secondary sex characteristics (Spurr et al. 1983). However, it is not known whether preschool-age delays in biological maturation related

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to protein-energy malnutrition persists into adolescence. Biological maturation may be an important mediating factor for the effects of early malnutrition on growth, body composition, work capacity, activity and social development during adolescence—all important outcomes examined in the INCAP follow-up study reported in this volume.

The objective of this research is to assess the long-term effects of early childhood nutritional supplementation on biological maturation at adolescence, indicated by SA. The effects of both supplement type (protein-plus-energy vs. energy) and quantity on maturation at adolescence are investigated in a follow-up sample of the INCAP longitudinal study. Effects on menarche are considered elsewhere (Khan et al. 1995).

MATERIALS AND METHODS

From 1969–77 INCAP conducted a longitudinal study of growth and development with a nutrition intervention in four rural Guatemalan villages (Martorell et al. 1995). The population was Ladino, or mixed Spanish and Amerindian, heritage. Four villages were selected, stratified by size (i.e., two had 900 people each and two had 500 people each) and randomly assigned to receive one of two types of supplement. Two received Atole, which is a high energy drink with a high protein content (163 kcal or 682 kJ plus 11.5 g protein per cup or 180 mL) and two received Fresco, a low-energy drink with no protein content (59 kcal or 247 kJ plus 0 g protein/180 mL). Consumption was ad libitum but recording, daily to the nearest 10 mL, ceased when children reached 7 y of age. As reported by Martorell et al. (1995), both supplements contained equal amounts of selected micronutrients (iron, fluoride, riboflavin, niacin, thiamin, ascorbic acid and vitamin A), while the Atole also contained calcium and phosphorus. Extensive growth and development data were collected in children ≤ 7 y, including skeletal maturation, assessed by counting the number of ossification centers present in a hand-wrist X-ray. At follow-up, during 1988 and 1989, researchers returned to the four study villages. All residents of the four villages were considered potential subjects for the maturation study if they had participated in the original INCAP study, were between 11 and 18 y of age at follow-up and were nonpregnant. Coverage was 80% and the radiographic sample for the four villages consisted of 883 subjects.

Hand-wrist X-rays were taken by trained INCAP field workers after gaining informed parental consent and screening females of postmenarcheal status for pregnancy. The radiographs were 8 × 10-inch anterior-posterior images of the hand-wrist area, exposed through an intensifying screen using a portable X-ray machine at a distance of 1 m. An adjustable columnator was used to reduce scatter radiation. The radiation dosage received was 0.015 REM; allowable annual exposure for the U.S. population is 0.5 REM beyond background radiation. The research protocol was approved by the University Committees on Human Subjects Research of Stanford and Cornell Universities and INCAP.

All radiographs were assessed in the field by one investigator (K.E.P.). Skeletal maturation was assessed by the Tanner-Whitehouse-2 (TW2) method (Tanner et al. 1983). The TW2 method involves the examination of 20 bones of the hand and wrist and the assignment of a letter grade to each bone dependent on the attainment of clearly described bone-specific maturity indicators. The letter grade is then converted to a numeric score in accordance with the tables given by Tanner et al. (1983) and the scores are summed for each individual to give a maturity score on a scale of 0 to 1000. Maturity scores can be converted to SA for each individual by comparison to British standards given by Tanner et al. (1983) and the relative maturation can be assessed by comparison of the SA to the individual's chronological age (CA). In this study the radius-ulna-short bones (RUS) option of the TW2 method, using only the radius, ulna and 11 short bones of the hand, was used to determine maturity scores, SA and relative maturation. The carpal bones of the hand contribute little to the assessment of skeletal maturation after the onset of puberty (Johnston and Jahina 1965) and the RUS scheme therefore was more appropriate for the assessment of variation in our sample of 11–18-y-old subjects. The accuracy of the TW2 method is discussed by Tanner et al. (1983). Reliability, composed of both precision and dependability, was assessed for the single observer in this study as the coefficient of reliability (R^2) in a test-retest assessment of a random 10% of the sample radiographs (Marks et al. 1989). The coefficient of reliability was very high at 0.858. Of the 14.2% unreliability estimate, 3.33% could be explained by the age of the subject, 1.1% by the sex of the subject and 0.1% by the supplement group (Atole, Fresco) to which the subject belonged.

For statistical analysis, the sample was divided into cohorts corresponding to differential exposure to the nutrition intervention study in early childhood (Martorell et al. 1995). The older cohort, Cohort 2, aged ~14–18 y at follow-up, was exposed to the nutrition intervention in utero and from birth to 3 y of age, the period considered most sensitive to the effects of the nutrition intervention (Schroeder et al. 1995). After the age of 3 y these subjects had variable exposure to the intervention, dependent on their age at the end of the study in 1977. The subjects of the younger cohort, Cohort 1, aged 11–14 y at follow-up, had complete exposure in utero but variable age-dependent exposure from birth to 3 y of age.

The outcome variable in all analyses was the SA deviation, or relative maturation computed as SA − CA. Approximately 68% of the females in Cohort 2 had reached skeletal maturity (SA = 15.9 y); in cases where CA was ≥15.9 y, SA was set equal to CA and the SA deviation score was 0. A large number of zero values accumulated at the higher ages of Cohort 2 and this created analytical problems when testing for group differences in SA deviation scores. Therefore, Cohort 2 females were excluded from the analysis reported in this paper. In contrast, only 20% of the males in Cohort 2 had reached skeletal maturity (SA = 18.0 y), while none had reached a CA of 18 y. Therefore, this entire cohort was retained for analysis.

All statistical analyses were sex- and cohort-specific, with age controlled within cohort. Sex specificity was essential due to sexual dimorphism in skeletal maturation. CA was controlled in all within-cohort analyses because SA deviation will regress naturally toward zero with advancing age.

The analysis was conducted in two stages. First, the effects of early nutritional supplementation were assessed by comparing supplementation groups (Atole vs. Fresco) using the GLM procedure of the SAS statistical package (SAS Institute Inc. 1985) for analysis of covariance (ANCOVA). Analysis of individual supplement intake followed, to establish the dose-response to early supplementation in Atole male subjects in Cohort 2. This analysis was restricted to Cohort 2 because most of the subjects in the younger cohort had <3 y of exposure to the intervention and that exposure was confounded by age. Cohort 2, on the other hand, was exposed to the intervention throughout gestation and the first 3 y of life, with variable exposure thereafter, again depending on age. Previous analysis (Schroeder et al. 1995) of anthropometry from this study has shown that supplementation effects on linear growth are seen until 3 y of age and not thereafter. Only Atole villages were used in analyses of dose-response because the mean amount (110 kcal/d or 460 kJ/d) and range (0–366 kcal/d or 0–1531 kJ/d) of supplemental energy ingested was greater than in Fresco villages (mean = 17 kcal/d or 71 kJ/d, range = 0–90 kcal/d or 0–377 kJ/d). Individual supplemental energy intake was used as a continuous independent variable in a multiple regression model using the REG procedure, after controlling for socioeconomic status and village size in an ANCOVA model using the GLM procedure. Several indicators of household socioeconomic status relating to quality of house construction and household material possessions were measured in 1975 and included in a factor analysis (see Rivera et al. 1995) to create a general socioeconomic index. The resulting factor score (SES) was evaluated as a possible confounder in all analyses. Statistical interactions between supplementation group and village size were tested also.

RESULTS

Descriptive statistics of biological maturation measures for the study sample are presented in **Table 1.** For comparison, both the 20-bone and RUS SA are reported. The RUS ages are somewhat greater and more variable than the 20-bone ages; however, the differences are not statistically significant (paired *t* test). Deviation of the RUS SA from CA suggests a significant ($P < 0.001$) delay in skeletal maturity in the younger cohort of males of 1.20 y, with a lesser delay ($P > 0.05$) of 0.16 y in older males, and no significant delay in females from Cohort 1.

Age-adjusted mean and SE for the difference between SA and CA (SA deviation) are presented for each sex and cohort for the Atole and Fresco groups in **Figure 1.** The results of regression analysis that support this figure are seen in **Table 2.** The Atole-Fresco differences were tested after controlling for age and village size (large vs. small). Among males, there are no significant Atole-Fresco differences in SA deviation for either cohort. Among females from Cohort 1, the expected trend of Atole subjects being more mature than Fresco subjects is statistically significant (Difference between Atole and Fresco means = 0.39 y, $t = 2.13$, $P = 0.035$).

The analysis was expanded to include socioeconomic status (SES) as a potential confounder of the supplementation effect on SA deviation. Cohort 1 boys and girls from Atole villages had a tendency towards higher SES scores (i.e., higher socioeconomic status) than their age mates from Fresco villages ($P < 0.10$). Moreover, SES scores tended to be associated posi-

TABLE 1

***Descriptive statistics of skeletal maturity indicators and other variables among male adolescents in cohorts 1 and 2 and female adolescents in cohort 1*[1]**

	Males		Females
Variable	Cohort 1	Cohort 2	Cohort 1
CA *y*	12.79 ± 0.99	16.26 ± 1.01	12.81 ± 1.04
Height, *cm*	139.3 ± 8.6	156.9 ± 8.8	141.3 ± 7.6
Weight, *kg*	33.1 ± 5.9	47.3 ± 7.3	36.1 ± 7.6
RUS maturity score	379 ± 124	762 ± 201	689 ± 172
RUS SA *y*	11.58 ± 2.27	16.09 ± 1.71	13.04 ± 1.78
20-bone SA *y*	11.59 ± 2.11	15.87 ± 1.62	12.55 ± 1.70
SA deviation *y*[2]	−1.20 ± 1.80	−0.16 ± 1.32	0.23 ± 1.41
SES	−0.11 ± 0.89[3]	−0.11 ± 0.90[4]	−0.03 ± 0.91[5]
Percent atole	58.0	54.2	52.7
n	220	223	220

[1] Values are means ± SD. Abbreviations used: CA = chronological age; RUS = radius-ulna-short bones; SA = skeletal age; SES = socioeconomic status.

[2] RUS age − CA.

[3] $n = 185$.

[4] $n = 198$.

[5] $n = 187$.

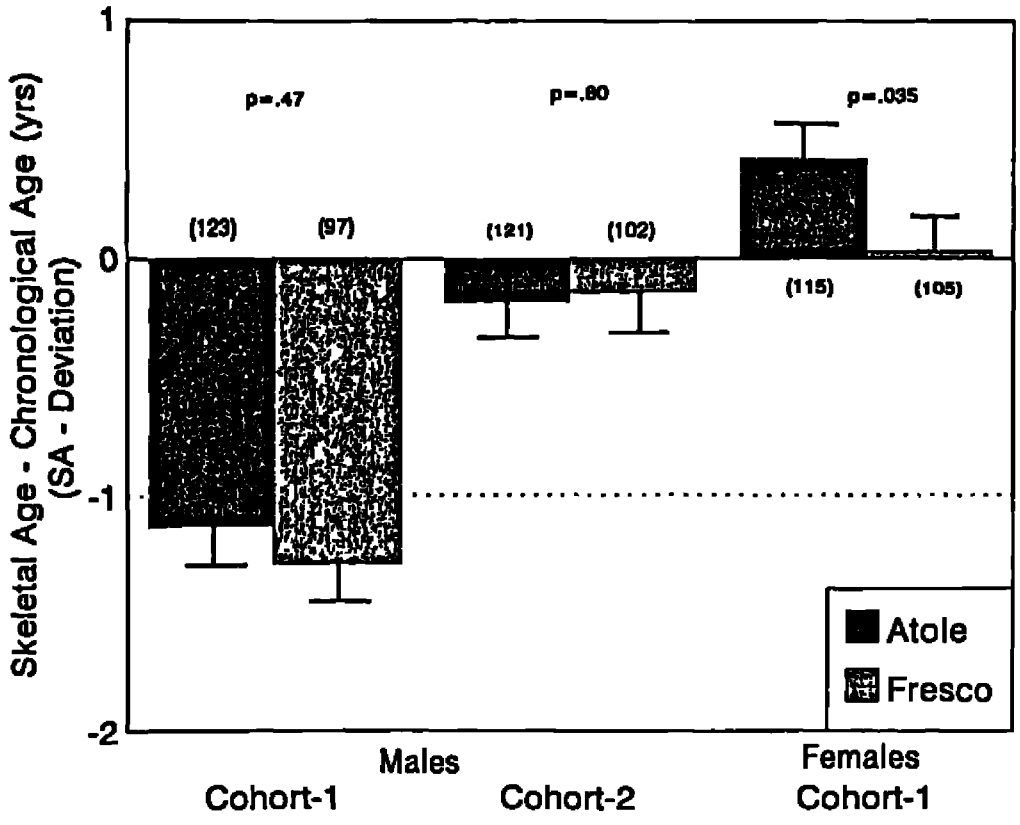

FIGURE 1 Atole and Fresco differences in the deviation of SA from CA among male and female Guatemalan adolescents. Height of the bars represent means after adjusting for age and village size (see Table 2). Brackets represent SE. Sample size for each sample in parenthesis. *P* values are for test of Atole-Fresco differences in SA deviation on a two-sided test after controlling for age and village size.

tively with SA deviation ($P < 0.10$), thus fulfilling the two criteria for confounding. When SES was entered as a covariate into the basic models for Cohort 1 presented in Table 2, the Atole-Fresco main effects were not changed for males but were reduced slightly from 0.39 to 0.33 y for females, with the statistical significance changing from $P = 0.035$ to 0.096. In Cohort 2 males the SES scores were similar in Atole and Fresco subjects and tended to be related to the SA deviation ($P = 0.065$). Inclusion of SES in the models for this older male cohort did not affect the Atole-Fresco differences reported in Table 2.

The interaction between village size and supplementation was tested for each sex and cohort group with SES also included in the models. **Table 3** summarizes the results of the regression analysis that includes this interaction. Atole exposure was associated with earlier maturation only in the larger villages. The interaction was statistically significant for females in Cohort 1 and males in Cohort 2. Among Cohort 1 females, who also had the only significant main effect (Table 2) of supplement, Atole subjects were 0.94 y more advanced than the Fresco subjects in the large villages but were 0.39 y behind in the small villages.

To measure the dose-response of adolescent maturation, we investigated the relationship between SA deviation and total supplemental energy intake from birth to 3 y of age in Cohort 2 males from Atole villages (**Table 4**). After controlling for age, village size and SES, the effects of supplemental energy intake was not significant.

It is important to note that the sample sizes reported in Tables 1 and 2 are reduced by ~10% for any analysis that includes SES (Tables 3 and 4) because of missing values. The descriptive statistics reported in Table 1 are essentially the same when repeated on the reduced sample of subjects with SES data.

DISCUSSION

We hypothesized that nutritional supplementation in early childhood would affect positively skeletal maturation status at adolescence. Specifically, we hypothesized that subjects supplemented with the high

TABLE 2

Regression analysis of supplementation effects on SA deviation by sex and cohort[1]

	Cohort 1				Cohort 2	
	Males		Females		Males	
Independent variables	Estimate	*t*	Estimate	*t*	Estimate	*t*
Intercept	−7.325	−5.11***	−3.525	−3.08**	−1.925	−1.34[2]
Age, y	0.486	4.43***	0.285	3.27**	0.112	1.28[2]
Village size (0 = small, 1 = large)	−0.377	−1.72[2]	−0.191	−1.06[2]	−0.067	−0.38[2]
Supplement type (0 = Fresco, 1 = Atole)	0.161	0.73[2]	0.387	2.13*	−0.044	−0.25[2]
R^2	0.10		0.06		0.01	
Residual mean square error	1.62		1.33		1.30	
df	3,216		3,216		3,219	

[1] SA deviation = SA − CA. Abbreviations used: SA = skeletal age; CA = chronological age.
[2] Not significant ($P > 0.05$).
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$ on two-sided test.

energy, high protein Atole would be advanced in maturation at adolescence compared with subjects who received the low energy, no protein Fresco supplement. As further support of the supplementation effect, we hypothesized that maturation at adolescence would show a dose-response to the amount of supplemental energy ingested in the first 3 y of life. We found only minimal support for these hypotheses. Atole-Fresco differences in maturation status were small (0.4 y) and restricted to the youngest cohort of girls between 11 and 14 y of age; also, these weak effects were attenuated after controlling for SES. No linear dose-response to individual supplemental energy intake was observed in the only group suitable for testing this relationship: Cohort 2 males from the Atole villages. These findings differ to some extent from those of Khan et al. (1995) who found that the mean age at menarche was similar in Atole (13.75 ± 1.22 y) and Fresco (13.74 ± 1.75 y) villages. Thus, taken together, the studies of skeletal maturation and of menarche suggest that the effect of improved nutrition in childhood on maturation in adolescence is weak to absent.

As the results differ in some respects from our expectations, it is necessary to seek an explanation of our findings by examining both our original hypotheses and the various factors that may have caused the nonsignificant results of this study: sample size, study design and the influence of negative confounders.

The indicator of biological maturation chosen for this study was skeletal maturation, which is sensitive to the effects of early undernutrition. Martorell et al. (1979) reported a significant impact of the INCAP nutrition intervention (of both type and amount of supplementation) on skeletal maturation, measured by the number of hand-wrist ossification centers present in early childhood (12–36 mo). We measured skeletal maturation by the RUS option of the TW2 method,

TABLE 4

Regression analysis of amount of energy consumed per day from Atole and SA deviation in Cohort 2 males[1]

Independent variables	Estimate	t
Intercept	−2.993	−1.47[2]
Age, *y*	0.161	1.29[2]
Village size (0 = small, 1 = large)	0.370	1.43[2]
SES	0.318	2.25*
Supplement (kcal × 100/d)[3]	0.004	0.03[2]
R^2	0.06	
RMSE	1.31	
df	4,101	

[1] SA deviation = SA − CA. Abbreviations used: SA = skeletal age; CA = chronological age; SES = socioeconomic status.
[2] Not significant ($P > 0.05$).
[3] To obtain kilojoules multiply kilocalories by 4.184.
* $P \leq 0.05$ on two-sided test.

which can be used over the entire developmental period and is a more accurate and precise measure of variation in maturation at adolescence than alternative atlas methods, such as that of Greulich and Pyle (1959). The TW2 system allows for population variation in the pattern of maturation (Shakir and Zaini 1974), is robust to minor assessment problems (Van Venrooij and Van Ipenburg 1978) and assessment is neither age- nor sex-dependent (Wenzel et al. 1984). In addition, the use of the RUS option allows the exclusion of the carpal bones, problematic in the adolescent age range corresponding to this sample (Johnston and Jahina 1965).

Sample sizes for Cohorts 1 and 2 of males were 220 and 223, respectively, and 220 for Cohort 1 females. SD for the mean deviation in maturation (SA deviation) of the three groups were 1.80, 1.32, and 1.41 y, respectively. Using a statistical power ($1 - \beta$) of 0.90

TABLE 3

Summary of regression analysis of supplementation effects on SA deviation by cohort, sex and village size controlling for age and SES[1]

	Cohort 1				Cohort 2	
	Males		Females		Males	
	Large[2]	Small	Large	Small	Large	Small
Sample size	100	85	99	88	109	89
Atole-Fresco difference in SA deviation, *y*[3]	0.47	−0.17	0.94	−0.39	0.31	−0.62
Significance of village size by supplement group interaction (*P*)	0.22		0.001		0.016	
Significance of SES (*P*)	0.039		<0.001		0.015	
Mean SA deviation	−1.19		0.30		−0.20	

[1] Abbreviations used: SA = skeletal age; SES = socioeconomic status.
[2] Village size.
[3] Number represents value for Atole minus value for Fresco.

and a P value (α) of 0.05 to estimate $z = 6.6$ for a two-tailed test (Snedecor and Cochran 1980), we can calculate the minimum difference (δ) in SA deviation that could have been detected in this study. Solving the equation

$$\delta = [2(Z)SD^2/N]^{1/2}$$

separately for males and females of each cohort shows that sample size was sufficient to detect significant differences in the delay of maturation between the Atole and Fresco groups as small as 0.43 and 0.32 y in males of each cohort and 0.30 y in females of Cohort 1. This suggests that sample sizes within each cohort by sex group were marginally adequate to detect differences of biological significance (0.3–0.5 y).

It is probable that CA is acting as a negative confounder of the effects of early supplementation on adolescent skeletal maturation at the time of follow-up, as the age range of the subjects is 11–18 y. In well-nourished populations, skeletal maturity is reached around the age of 16 in females and 18 in males. As the skeleton approaches full maturity, SA converges with CA to reach zero difference at the completion of maturation. The variation in relative maturation status (SA deviation) used as an outcome in this study therefore decreases with age. This may explain the failure to show any dose-response in the older cohort of males. For the test of Atole-Fresco differences in each cohort, CA, along with other potential confounding factors such as village size and sex, are controlled in each analysis. But lack of variation in SA deviation in the older cohort cannot be corrected by controlling for confounders.

Cohort 1, the youngest group, had shorter, although variable, exposure to the nutrition intervention than Cohort 2 and was not expected to provide strong evidence of supplement effects on maturation at adolescence. Therefore, it is somewhat surprising that the only significant supplementation effect was seen in this younger cohort. However, it is also likely that even a limited exposure to the Atole supplement has a significant effect on skeletal maturation. Martorell et al. (1979) found a significant difference in the number of hand-wrist ossification centers between Atole and Fresco male infants as young as 12 mo of age and female infants as young as 24 mo. Considering that the better test of any supplementation effect is likely to be in younger rather than older adolescents because of the age confounding effect discussed above, the carryover into early adolescence of the early supplementation effects seen in the first 2 y is plausible.

The interaction between village size and supplement group is statistically significant ($P = 0.016$) for Cohort 2 boys but weaker in Cohort 1 boys ($P = 0.22$). A significant interaction also is seen in Cohort 1 girls ($P < 0.001$). We observed a greater positive effect of Atole supplementation in large compared with small villages. Large villages were more delayed in skeletal maturity than small villages (Table 2). This might be interpreted as a greater potential for the intervention to have been effective in groups where the maturation process was more delayed. We have no explanation for the apparent negative effect of Atole in the small villages, especially in Cohort 2 males.

The possible persistence into adolescence of a small and selective effect of supplementation on skeletal maturity while the supplementation effects on height remain about the same as seen at 3 y (Rivera et al. 1995) suggests that the effect of delaying maturity to allow more time for catch-up growth is minimal in this population.

In conclusion, we found that type of early nutritional supplementation significantly affected skeletal maturation at adolescence, but its effect was restricted to females < 14 y of age and in large villages. It is probable that the interpretation of results of this study are obscured by the advanced age of most of the subjects at the time of the measurement of skeletal maturation in adolescence. A study with a similar research design and that follows up youth and adolescents at a somewhat younger age would help to establish whether early nutritional supplementation affects maturation at adolescence. Given the central importance of maturation status in explaining variation in physical growth and performance, particularly at adolescence, and the possibility that maturation acts as a mediator of long-term nutritional effects on such outcomes, the relationship of early nutrition to later biological maturation is worthy of further attention.

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