THE JOURNAL OF
NUTRITION OFFICIAL PUBLICATION OF THE AMERICAN INSTITUTE OF NUTRITION
April 1995
SUPPLEMENT
VOLUME 125 Number 4S

THE EFFECTS OF IMPROVED NUTRITION IN EARLY CHILDHOOD: THE INSTITUTE OF NUTRITION OF CENTRAL AMERICA AND PANAMA (INCAP) FOLLOW-UP STUDY

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FOREWORD

The articles in this supplement deal with the effects of improving the nutrition of preschool Guatemalan children on status and function measured in the same individuals as adolescents and young adults. This collective effort is a unique scientific contribution and represents the first, comprehensive, long-term evaluation of a nutrition intervention aimed at mothers and children in a developing country.

Many institutions and people have contributed to making possible the research included in this supplement to the *Journal of Nutrition*. Foremost is the Institute of Nutrition of Central America and Panama (INCAP), which successfully implemented and conducted the two related studies reviewed: the INCAP longitudinal study on the effects of nutrition supplementation on child growth and development (1969–1977) and the follow-up study (1988–1989) of former participants.

We are grateful to the people of the four villages in eastern Guatemala in which the studies took place. The overwhelming majority gave willingly of their time and cheerfully tolerated the many examinations and interviews. Though we made efforts to compensate them directly, particularly through the provision of medical care, we doubt we matched their generosity. Through analysis and dissemination of the results, we hope to impact positively on policies and programs aimed at preventing childhood malnutrition and in this manner, justify the many demands we made on the peoples of San Miguel de Conacaste, Santo Domingo los Ocotes, San Juan de las Flores and Espíritu Santo.

The longitudinal study of 1969–1977 is the work of many outstanding scientists in the mid-sixties, first laying the groundwork and later carrying out the study. Much is owed to the leadership of the two directors of the former Division of Human Development at INCAP, Drs. Cipriano Canosa (1969–1970) and Robert E. Klein (1970–1977), who guided the study throughout its initial history.

The U.S. National Institute of Child Health and Human Development (NICHD) supported the initial longitudinal study (Contract No. PH 43-65-640). Through timely monitoring, including periodic site visits, NICHD helped the project remain scientifically rigorous. Two smaller but important contributions to the financing of the study were a grant from the U.S. Agency for International Development (USAID), which supported the collection of birthweight data in the later stages of the project, and a grant from the Rockefeller Foundation to the RAND Corporation and INCAP, which financed the collection of wealth, fertility and other social data starting in 1974.

The follow-up study was a natural extension of the longitudinal study. Seed money (from the Alfred Sloan Foundation) from Stanford University to R. Martorell made possible the submission of a proposal to the U.S. National Institutes of Health (NIH), which was eventually approved and funded (RO1-HD-2240) with R. Martorell as principal investigator. Funding from NIH (RO1-HD-24684) to J. Himes permitted the collection of bone mineral data. Other support included a grant from the Pew Charitable Trusts (No. 99G0070–0001), which funded the analyses of educational data, with E. Pollitt and R. Martorell as principal investigators, and a grant from the Thrasher Research Fund to J. A. Rivera, R. Martorell, and J.-P. Habicht for a pilot study of the newborns of follow-up females (No. 2805–5). Assistance has also been obtained for analyses of the growth and maturation of adolescents through a grant from the International Center for Research on Women (LIC-75/03), made to R. Martorell and J. A. Rivera, and for analyses of the functional consequences of growth failure through a grant from UNICEF to R. Martorell. A grant from the United Nations University helped to obtain additional social data on the communities during the follow-up study. The Pew Charitable Trusts is funding an effort to apply the lessons of the INCAP studies to nutrition and educational programs in Central America, through a grant (No. 93–03448–000) to R. Martorell, E. Pollitt and M. Ruel.

The present set of articles is an outgrowth of a workshop held at the Rockefeller Conference Center in Bellagio, Italy, in July 1990, under the aegis of the International Dietary Energy Consultative Group (IDECG). Travel funds and per diem were generously provided by the Office of Nutrition of USAID and the United Nations University. Articles emphasizing the results of reanalysis of data from the longitudinal study of 1969–77 were published in a supplement of the Food and Nutrition Bulletin (Vol. 14, No. 3, 1992) with the financial assistance of UNICEF and the World Food Program. This second volume emphasizes the results of the 1988–89 follow-up study; a preliminary version of some of these papers, including the contribution of Lindsay Allen, were presented at the 1992 FASEB meetings held in Anaheim, California. We appreciate the contribution of Laura Caulfield and colleagues on the subject of bone mineralization; this was an analysis carried out at the University of Minnesota, independent of efforts at Emory and Cornell, a fact which explains the different analytic approaches undertaken.

While the emphasis is on the follow-up study, the collection also includes three papers which summarize the history and design of the longitudinal study, the analytic strategies and the types of inferences which can be made from the study and the effects of nutritional supplementation on physical growth in children less than seven years of age. This is important information for understanding the papers on the follow-up study which form the major part of the set. All of the follow-up papers with one exception contain unpublished, original results. They are published as a set rather than individually in various journals to enable readers to locate them easily in a single source. An important aspect of the research is the results on intellectual performance, and any presentation of the follow-up would be incomplete without it. This is the reason for including in this volume a brief paper by Pollitt et al. summarizing previously published results.

All papers were subjected to rigorous peer review. Six external reviewers read about four papers each and one reviewed the entire set. All reviewers were well known experts in nutrition, growth or development.

We are grateful to the American Institute of Nutrition for encouraging publication of the research as a unit in the *Journal of Nutrition* and for its financial contribution.

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History and Design of the INCAP Longitudinal Study (1969–77) and its Follow-Up (1988–89)[1,2]

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ABSTRACT This is an overview of the design and methods of the INCAP longitudinal study (1969–77) and its follow-up study (1988–89). The first study had the objective of assessing the effects of intrauterine and preschool malnutrition on growth and mental development. To achieve this, food supplements were provided to pregnant women and young children residing in four Guatemalan villages. Two villages were given a high-protein, high-energy drink and two were provided a no-protein, low-energy drink. Both supplements contained vitamins and minerals. Longitudinal information was collected during the first seven years of life on physical growth, mental development, attendance and consumption of supplement, home diet, morbidity and on characteristics of the family. Health and nutrition data on mothers also were collected. The INCAP follow-up study was a cross-sectional evaluation of former participants of the first study and was carried out when the subjects were 11–27 y old. The hypothesis of the INCAP follow-up study was that improved nutrition in early childhood leads to enhanced human capital formation in adolescents and adults. Data were collected on physical growth and body composition, maturation, work capacity, intellectual performance and school achievement. J. Nutr. 125: 1027S–1041S, 1995.

INDEXING KEY WORDS:

• *malnutrition* • *supplementation* • *field methods* • *growth and development*

The INCAP longitudinal study (1969–1977) continues to be one of the richest sources of information about the importance of nutrition for growth and development in children from developing countries. One of two key objectives of this paper is to provide an overview of the objectives, design and methods of this study, drawing heavily on a review by Habicht and Martorell (1992). In 1988–89, the children of the study were revisited when they were adolescents and young adults in what has come to be known as the "INCAP follow-up study". The second objective is to review the characteristics of the follow-up study, this time using material presented in Martorell and Rivera (1992) and in Rivera, Martorell and Castro (1992).

The INCAP longitudinal study (1969–77)

Objectives and design. The design eventually implemented in the INCAP longitudinal study called for comparison of outcomes in pregnant and lactating women and in their children between two pairs of villages exposed to different supplements. Read and Habicht (1992) state that the major motivation for the INCAP longitudinal study was to assess the impact of intrauterine and preschool malnutrition on behavior. Thus, food supplements were provided and their consumption measured in pregnant women and in children younger than 7 y of age.

Nutritional supplementation was to produce an unambiguous contrast between well and poorly nourished children. Because protein deficiency was perceived to be the major cause of malnutrition at the time the study was being planned (Scrimshaw and Behar 1965), major emphasis was placed on improving protein malnutrition while assuring enough extra energy to allow for protein utilization. The feeding in-

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-Term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to the Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Supported by National Institute of Health grant HD-22440.

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tervention took advantage of INCAP's extensive experience with Incaparina (Bressani and Elias 1968), a nutritious gruel with a high-protein and moderate-energy content that was widely accepted in Guatemala. Thus, the pregnant mothers' and childrens' diets were improved with an Incaparina-based drink, referred to as *"Atole"*, the Guatemalan name for a hot, maize gruel. This drink had to be prepared at the time of ingestion and therefore required a central kitchen and feeding hall.

The centrally located feeding stations precluded random allocation of treatment to individuals within a village; instead, allocation to treatment was by village within pairs chosen to be as similar as possible. One pair was large (~900 people per village) and one was small (~500 people per village).

The comparison beverage to this gruel was a sweet, cool, colored and fruit-flavored drink called *"Fresco"*. Drinks of this type were much appreciated in the area. The Fresco was intended originally to be devoid of any nutritional value, in effect to be a placebo. It was envisaged as a control for the social stimulus and other factors associated with supplementation. The use of cyclamates for sweetening was considered but concern about carcinogenicity led to sugar being used instead, which of course introduced energy. Finally, other nutrients were introduced as discussed below in an attempt to narrow the contrast between the Atole and Fresco groups to differences in energy and above all in protein. Consequently, the Fresco should not be viewed as a placebo control to the Atole because it contained some energy and important concentrations of micronutrients. Instead, both drinks are referred to as "supplements".

Originally, three pairs of supplementation villages were specified, but budgetary constraints reduced the number of pairs to two. A less accessible pair of "large" villages was dropped early in the study with dire consequences for statistical power. As implemented, the effects of improved protein nutrition were to be ascertained through comparisons of results before and after the intervention in the two Atole and two Fresco villages. The before-after comparison was, however, only possible for selected variables collected with adequate sample sizes in 1968, before the intervention began, or for those variables collected in the first months of the study that could not be immediately affected by supplementation (e.g., height of 7-y-old children). Issues of statistical power and analytic strategies in the study are discussed extensively by Habicht, Martorell and Rivera (1995).

The effect due to the study activities per se on behavior was to have been estimated by contrasting outcomes before and after the study in the villages not receiving protein (i.e., Fresco villages) to those in villages not visited in the interim, termed "Supercontrol" villages in project documents. However, budgetary constraints curtailed baseline data collection in these "Supercontrol" villages, nor were data collected in these communities in 1977, at the end of the INCAP longitudinal study.

Although the allocation of treatment across villages was random, ingestion of the supplements was voluntary and therefore subject to self-selection. The implication of this combination of random error and self-selection bias for data analysis and interpretation is discussed by Habicht, Martorell and Rivera (1995).

Finally, it is important to consider that conducting the study involved intensive contact between data collectors and villagers. The data-collection activities were designed and implemented to affect all villages equally and therefore cannot be a source of bias in the Fresco versus Atole contrasts. However, the study setting does affect the validity of extrapolations to other populations if its activities impacted on outcomes synergistically with the supplements. These same concerns apply to the medical care that was provided to the study villages; it too may have potentiated or diminished the impact of the supplements.

Village selection and description. Several needs were considered in selecting villages: population size, relatively compact settlements to allow easy access to the centrally located feeding station and stability and homogeneity among villages. A further limitation was imposed by the psychometric testing. To find a large enough sample of mutually isolated villages speaking the same language, the study had to be located in the Spanish-speaking (i.e., Ladino) region of Guatemala instead of in the more picturesque Maya-speaking area where most previous INCAP research had been done. The selection and matching criteria that were used are presented in **Table 1**.

Ultimately 10 villages were found to fulfill best these selection criteria from among 300 Ladino villages identified from maps as being within the selection radius and from the Guatemalan Census as having population sizes of 500–1000 inhabitants (Canosa et al. 1972). This was a time-consuming process entailing data collection in as many as 45 villages. Most of these data are lost except for some of the dietary, anthropometric, census and socioeconomic data collected in the four villages finally selected for the study. Some useful baseline information about the four study villages is given by Mejía-Pivaral (1972).

Analyses of baseline data indicated that three pairs of villages were most similar to each other: two pairs of villages within an hour's jeep ride from each other and a third pair of villages much further away. As noted earlier, the study was never fully implemented in the third pair of villages, Tapalapa and Santa Gertrudis. The final two pairs of villages selected were San Juan de las Flores and Espíritu Santo with ~500 inhabitants each and San Miguel de Conacaste and Santo Domingo los Ocotes with ~900 inhabitants each. All were far enough apart from each other to make intervillage contact unlikely.

TABLE 1

Criteria for selecting and matching villages in the INCAP Longitudinal Study[1]

Area	Criteria
Ethnicity	100% Spanish-speaking, Ladino culture (i.e., not Indian)
Population	500–1000 inhabitants
Birth rate	35–45/1000 live births annually
Death rate	14–18/1000 population annually
Age distribution	Birth–6 ys: 24–30% Birth–15 ys: 35–50% 16–45 y: 40–45% ≥55 ys: 5–10%
Family composition	Average of five family members per nuclear family
Population mobility	80% or more born in area; 2% annual migration, with little likelihood of change
Social isolation	50–150 km from Guatemala City; any village included in the study should be ≥10 km from other selected villages and under the jurisdiction of a different municipality (i.e., county)
Transportation	Accessible by four-wheel drive vehicles
Compact nuclear settlement	80% of homes within 1-km radius from the community research center
Housing and community services	60% similarity across villages
Annual income	$200 ± $50 per family unit
Education level	30% literacy among population ≥8 y
Basic foods	Corn and beans
Health and nutrition	High levels of malnutrition and of gastrointestinal and respiratory disorders. Anthropometric, dietary and morbidity information of 10 villages which satisfied best the above criteria were considered in selecting the most similar pairs of large and small villages.

[1] Adapted from Canosa et al. 1972.

The four villages selected are located in the Department of El Progreso, a dry, mountainous area northeast of Guatemala City. The large Fresco village (Santo Domingo) is closest to Guatemala City, at 36 km, whereas the small Fresco village (Espíritu Santo) is furthest, at 102 km. The elevation of the large Fresco village is 1250 m above sea level, whereas both Atole villages are at 860 meters, and the small Fresco village is at 275 meters. The average temperature range for the small Fresco village is 24–38°C, and for the other three villages it is ~14–32°C with the rainy season occurring from June to October. Two of the major crops in each village were corn and beans, with tomatoes also being a major crop in the large Atole (Conacaste) and small Fresco villages, sorghum in the large Fresco village, and manioc, locally known as "yuca," in the small Atole village (San Juan).

In 1967, <10% of the families in the four villages had a source of water in their homes. Almost everyone obtained water from open, unprotected hand-dug wells, and in the small Fresco village from a nearby river. Few households had a latrine, and no one had a sewage or drainage system.

In three of the four villages, the typical house had one to two rooms with adobe walls, dirt floors and a tile or metal roof. However, poorer families lived in houses with reed walls and thatched roofs. In the small Fresco village, where the climate is warmest, most houses, even those of better-off families, had thatched roofs, walls made of reeds and mud, and dirt floors. Families usually prepared food in either a separate room or in a separate area located just outside the house. Most people owned their homes, as well as at least some of the land around their homes. About one-third of the families had radios; only a few (<5%) owned a television, record player, refrigerator or bicycle. No homes were equipped with electricity.

The primary income for most villagers was from agricultural production. Almost all were tenant farmers or small-land owners. No one in any of the villages reported being a large landholder, and very few reported being merchants. Wage labor was reported as a principal occupation by 21% of the men in the small Fresco village and by 15% of the men in the small Atole village. Wage labor was not a significant source of income in the large villages. Very few women reported having occupations outside the household except in the small Fresco village, where they had the opportunity to make money independently through basket weaving.

Literacy was self-reported, usually by the mother of the family, for all family members. The percent of mothers at least partially literate in each village ranged from 25 to 40%. The large Fresco village had the highest literacy rate for mothers (40%). Literacy levels of the fathers ranged from 38 to 60% with those in the small Fresco village having the highest literacy rate. Additional data about the social, economic and demographic development of these villages are given by Bergeron (1992) and Engle et al (1992a).

TABLE 2

***Formula and nutrient content of beverages per cup serving (180 mL)*[1]**

	Atole		Fresco	
	For subjects >4 mo in age	For older subjects	From 69–71	From 71–77[1]
Ingredients (g/180 mL)				
Incaparina (g)	—	13.5	—	—
Dry skim milk (g)	28.8	21.6	—	—
Sugar (g)	3.6	9.0	13.3	13.3
Flavoring (g)	—	—	2.1	2.1
Nutrients (per 180 mL)				
Energy (kJ)	498	682	245	247
Energy (kcal)	119	163	59	59
Protein (g)	10.3	11.5	—	—
Carbohydrates (g)	15.3	27.8	13.3	13.3
Fats (g)	0.2	0.8	—	—
Calcium (g)	0.4	0.4	—	—
Phosphorus (g)	0.3	0.3	—	—
Iron (mg)	0.2	1.2, 5.0[2]	—	5.0
Fluoride (mg)	—	0.0, 0.2[2]	—	0.2
Thiamin (mg)	0.1	0.4, 1.1[2]	—	1.1
Riboflavin (mg)	0.5	0.5, 1.5[2]	—	1.5
Niacin (mg)	0.3	1.3, 18.5[2]	—	18.5
Ascorbic acid (mg)	—	0.0, 4.0[2]	—	4.0
Vitamin A (mg)	0.4	0.5	—	0.5

[1] Higher values as of October 1, 1971.

[2] Values differ slightly for some ingredients and nutrients in project documents. Those given here are from the Manual de Operaciones issued by the División de Desarrollo Humano (1971).

The interventions: supplementation and medical care. *Supplementation.* The intervention design calls for comparisons of villages in which pregnant and lactating mothers and their children up to 7 y of age received verified and recorded amounts of either Atole or Fresco. **Table 2** presents the ingredients and the energy and nutrient concentration of the supplements per one-cup serving (i.e. 180 mL) as given elsewhere (División de Desarrollo Humano 1971; Martorell et al. 1982). The Atole contained a high-quality protein mixture whereas the Fresco contained none. The energy concentration of Atole for children older than 4 mo was 2.8 times greater than for Fresco.

There were two formulations of Atole depending on age: one for children <4 mo of age made up of powdered skim milk and sugar (28.8 and 3.6 g, respectively, per 180 mL) and one for older children and mothers containing Incaparina, skim milk and sugar (13.5, 21.6 and 9.0 g, respectively, per 180 mL). Very little of the milk supplement was consumed by children <4 mo of age. The Atole for older children was pale gray-green, and tasted smooth but slightly gritty and sweet; it was served hot. The energy, protein and micronutrient contents of the Atole are shown in Table 2.

In October of 1971, the riboflavin content of the Atole for children >4 mon was raised from 0.5 mg to 1.5 mg per serving after noting that the biochemical indices of riboflavin were not satisfactory in 2-y-old children consuming Atole (Habicht et al. 1973). Iron and a small amount of ascorbic acid to facilitate iron absorption also were added because anemia was common in pregnant mothers; however, anemia was not found in unsupplemented 2-y-old children (Habicht et al. 1973). The thiamin and niacin contents increased because they were present in the iron-vitamin mix. On the basis of calculations from knowledge about water fluoridation (Infante 1975), fluoride was also added at this time because the drinking water had a low fluoride content at the end of the dry season (0.166–0.384 ppm compared with the recommended 1 ppm) when the concentrations should be highest and because of the high incidence of dental caries in the communities (Infante and Gillespie 1976; Infante and Gillespie 1977).

The comparison beverage, Fresco, was a low-calorie supplement containing 247 kJ (59 kcal) and no protein per serving. It was a cool, clear-colored, fruit-flavored drink similar to KoolAid™ sold in the USA. The ingredients and nutrient content of the Fresco are given in Table 2. Previous to 1971, it contained only flavoring, color and sugar. In October 1, 1971 other nutrients

TABLE 3

Children with anthropometry*[1] *by birth cohort in Atole and Fresco villages

Cohort number	Birth cohorts	Exposure period	Atole	Fresco	Total
I	≥1 March 1974	Gestation, partial birth to 3 y	260	280	540
II	1 March 1969–28 February 1974	Partial during gestation[2]; all birth to 3 y	374	395	769
III	1 January 1966–28 February 1969	Partial birth to 3 y	185	194	379
IV	≤1 January 1966	No exposure during gestation or birth to 3 y	151	153	304
	Total		970	1022	1992

[1] With data for birthweight or for any of the anthropometric postnatal examinations.

[2] Some cases in the early part of the study may not have had full exposure to supplement during pregnancy.

were added to make it more similar to the Atole. Many of these nutrients had been found to be marginal after review of biochemical indicators in blood and urine in children who drank the Fresco regularly (Habicht et al. 1973).

In all villages, the supplements were distributed and consumed in a centrally located, feeding hall for 2–3 h during midmorning and midafternoon, including weekends. These times were chosen because they were easiest for mothers and children to attend and because they did not interfere with usual meal times.

Medical care. Curative medical care was available on week days and free of charge throughout the duration of the study at a clinic adjacent to the beverage feeding halls. These services were available for all residents and were not tied to participation in any aspect of the study. The medical care program was justified not only on ethical grounds but on design considerations as well. For example, immunizations would prevent an epidemic from striking one village and not another as had happened in a previous INCAP nutrition field trial, with dire consequences for data interpretation.

The new system of curative care was implemented in the fall of 1969 (Working Group 1973). Effective but affordable medical care was provided through auxiliary nurses instead of physicians and by streamlining the purchasing and use of medicines. It featured continuous supervision of adequacy of the quality of history taking, diagnosis and treatment (Habicht 1979). Cases that could not be diagnosed by the auxiliary nurses were referred to the supervisory physician (<1% of cases). Furthermore, arrangements were made with Hospital Roosevelt, a teaching hospital in Guatemala City, to honor referrals (0.4% of cases) and keep INCAP informed of patients' progress and discharge. By early 1971, the quality of care had stabilized and >99% of cases were managed correctly (Working Group 1973).

The local traditional midwives were funded to attend midwifery courses given by the Guatemalan public health authorities. Their care complemented that given on demand by the nurses.

All children were examined 15 d after birth by a well-trained pediatrician for diagnosis and treatment of any neonatal ills. The pediatrician also examined the children at 3 mo and at 1, 3 and 7 y to detect any remediable pathology that had escaped the clinic's attention. Pregnant mothers were immunized against tetanus and children against tuberculosis, diphtheria, whooping cough, tetanus, measles and poliomyelitis (Habicht et al. 1979). Deworming medicines were offered twice a year, but the medical program did not give health or nutrition education, except to encourage attendance to the supplementation feeding stations and to participate in immunization campaigns. The curative and preventive health care services were the same in all the villages, and any preventive campaigns such as deworming or immunization were done simultaneously in all the villages.

Compared with rates for the period previous to 1969, infant mortality had declined by 1970–72 from 139 to 55 deaths per 1000 births and preschool mortality had decreased from 28 to 6 deaths per 1000 children at risk, at a total cost for primary health care of < $5 per villager per year. National death rates in Guatemala remained constant during this comparison period. Data collected in 1988–89 confirmed these declines in mortality rates (Rose et al. 1992).

Other influences of the study team. The intensity of data collection and the supplementation and medical interventions required the continuous presence of four to eight well-educated persons in each village. Although they did not live in the villages, at least one of them visited each family twice a month. The influence of these contacts could be variable depending upon the personalities of the personnel and therefore could have affected the outcomes of the study differentially across villages. Therefore all personnel were rotated through all the villages for equal durations of time.

Sample persons, data collection and data availability. All women who were pregnant or lactating and all children from birth to 7 y of age were included in the original design of the INCAP longitudinal study if they lived in the study villages from January 1, 1969 to February 28, 1977. Absence from the village was the most common cause for missing data. Refusal to participate in the study was rare; <2% of all families declined participation. Anthropometric data are available for 517 different women for one or more of their pregnancies and corresponding lactation periods. The distribution by birth date cohort of the children with one anthropometric examination or more ($n = 1992$) is presented in **Table 3** by village type. The cohorts identify children with different exposure to supplementation during the critical periods of gestation and the first 3 y of life.

Details of data collection are shown in **Table 4**. Most data collection began January 1, 1969 in the large villages and somewhat later (range March to May) in the small villages. All data collection ceased in September 1977 but cessation occurred as early as March 1977 for some types of information. Data-collection methods are described in detail in a manual of standard operating procedures in which the forms used are also presented (División de Desarrollo Humano 1971). Table 4 also lists data collected in cross-sectional surveys conducted in collaboration with the Rand Corporation in 1974–76; additional details are given elsewhere (Corona 1980). Short descriptions of the type of data collected in key areas are presented below.

Census and socioeconomic data. A census was conducted in the four villages at the end of 1968. This was updated whenever the dietary-morbidity interviewers in their fortnightly home visits identified

TABLE 4

Data collected in the INCAP longitudinal study, 1969–1977

Type of data	For whom (Target)	When collected	Where collected	By whom	Collection dates
Supplement intake	Children 0–7 y and pregnant and lactating women	Everyday	Feeding Centers	Supplementation supervisors	1969–77
Morbidity, breastfeeding and menstruation recall	Children 0–7 y and pregnant and lactating women	every 15 days	Home	Dietary and Morbidity Interviewers	1969–77
Diet: 24-h recall in large villages and 72-h recall in small villages	Children 0–12 mo	Monthly	Home	Dietary	1973–77
	Children 15–36 mo	Every 3 mo	Home	Morbidity	1969–77
	Children 42–60 mo	Every 6 mo	Home	Interviewers	1969–77
	All pregnant women	Every trimester	Home	Dietary and morbidity interviewers	1969–77
	Lactating mothers				
	0–12 mo	Every 3 mo	Home	Dietary	1969–77
	15–36 mo	Every 3 mo	Home	Morbidity interviewers	1973–77
	Other mothers	3, 6, 9 & 18 mo post partum	Home	Dietary and morbidity interviewers	1973–77
Birth weight, birth process and Apgar anthropometric indicators of nutritional status	Children 0–7 y	At birth	Home	Perinatologist	1969–77
	Children 15 d–24 mo	Every 3 mo	Clinic	Child Anthrop.	1969–77
	Children 30–48 mo	Every 6 mo	Clinic	Child Anthrop.	1969–77
	Children 60–84 mo	Every year	Clinic	Child Anthrop.	1969–77
	Pregnant and lactating women	During each trimester of pregnancy or lactation	Clinic	Maternal anthropometrist	1971–77
Hand-wrist x-rays	All children	At 3 mo. From 6 to 48 mo every 6 mo. At 60, 72 and 84 mo	Clinic	Child anthropometrist	1969–77
Mental development					
Cognitive infant scale	Children 6–24 mo	At 6, 15 and 24 months	Test room in village	Psychometrist	1969–77
Preschool battery	Children 36–84 mo	Every year	Test room in village	Psychometrist	1969–77
Physical examination	Children 0–7 y	15 d; 3 mo; 1, 3 and 7 y	Clinic	Pediatrician	1971–77
Prenatal examinations	Pregnant women	Each trimester of pregnancy	Clinic	Auxiliary nurses	1969–77
Reproductive histories	Pregnant women	First prenatal examination	Clinic	Auxiliary nurses	1969–77
Records of visits to outpatient clinics	Children 0–7 y and pregnant and lactating women attending the medical clinic for any reason	For all visits for therapeutic care; Monday through Friday	Medical clinic	Auxiliary nurses	1971–77
Census and socioeconomic information	Families in community	Twice	Home	Census interviewers	1968–1969, 1974
Changes in household composition through census updates	Families in community	Every 15 days	Home	Dietary and morbidity interviewers	1968–1969
Father's anthropometry	Fathers of children in study	Once (cross-sectional)	Home	Child anthropometrist	1973–75
Retrospective life history of women (fertility, infant mortality and maternal employment)	Women 15–49 y ever in a marriage/union or ever a mother	Once (cross-sectional)	Home	Interviewer	1974–75
Income and wealth	Heads of household	Once (cross-sectional)	Home	Interviewer	1974–75
Attitudes and expectations of women	Women 15–49 y ever in a marriage/union or ever a mother	Once (cross-sectional)	Home	Interviewer	1975–76
Attitudes and expectations of men and retropective life history of men	One-half of the husbands of respondents to the "retrospective life history of women" and one-half of all single men	Once (cross-sectional)	Home	Interviewer	1975–76

changes in household composition, new families (new marriages/unions or in-migrants), changes in residency within the village and out-migration. This updating of the census was verified by a cross-sectional census in 1974.

The informant was the mother or other primary caretaker. Data collected included information about family structure, marital status, religion, number of pregnancies, number of children alive and relation of the nuclear family to the head of the extended family, and about ownership of items such as radios, sewing machines, refrigerators, bicycles and motor vehicles. Information about parental literacy was obtained through interviews and also through tests. Status (e.g., alive, dead, immigrated), birth order, kinship (e.g., father, son, adopted), parity (for mothers), schooling and occupation (for those older than 10 y) were recorded for each household member. Observations were noted about the house such as the types of walls, floor and roof, availability of electricity, type of water source and of grey water and feces disposal. Whenever a change occurred in the location of the home or in the status or kinship of a family member, the date of the change was noted.

Psychometric data. Full descriptions of the psychometric tests are given by Klein et al. (1977) and by Engle et al. (1992b). Neonates were tested within 10 d of birth with the Brazelton Neonatal Scale and then at 6, 15 and 24 mo with an infant scale composed of items compiled from the Bayley, Cattell, Gesell and Merrill-Palmer Infant Scales. From 3–7 y of age the children were tested annually on a battery of 24 tests chosen to tap memory, language, perceptual reasoning, learning and abstract reasoning ability.

Supplementation. Supplementation and measurement of attendance and consumption began on January 1, 1969 in the large villages and on May 1, 1969 in the small villages. Attendance at the supplementation feeding station was recorded for all sample persons. The supplement was poured into cups calibrated to 180 mL. Cups were filled as often as requested. Individual intake was measured by recording the number of cups given and subtracting any leftover supplement measured to the nearest 10 mL.

All leftovers were poured into a large vessel. At the end of the serving period, the total amount recorded as ingested and the total amount served minus the volume of leftovers were compared. Calculations based on these data showed that reliability for supplement ingested was better than 99% per cup served. The reliability of concern in this study is of intakes during a week or longer periods; that reliability is almost perfect.

Home diet. Information on the home diets of children, pregnant and lactating women was collected according to the schedule indicated in Table 4. Daily home diet was estimated from 24-h recall surveys in the large villages and from 3-d recall surveys in the small villages. The amounts of food ingested were recorded in grams according to the usual INCAP recall method (Flores et al. 1970) and then converted to energy and nutrients using the INCAP food composition tables (Flores et al. 1960; Flores et al. 1971). The reliability of the dietary data was, however, found to be equally poor for both recall methods (División de Desarrollo Humano 1971; Habicht et al. 1974; Klein et al. 1973; Lechtig et al. 1976). Energy and protein had the highest reliabilities of all nutrients but these were only of the order of 0.15–0.30.

Medical care. Records of the presenting complaints, diagnoses and treatments were kept for all visits for use in the quality control system but, unfortunately, are no longer available. As of 1971, the symptomatology was recorded for each visit on the same form as the fortnightly morbidity data collected in the home and those data are available.

Morbidity, breast feeding and menstruation. Morbidity data were gathered every 14 d through retrospective interviews of mothers in the home by four home visitors, one for each village. The home visitors were rotated periodically among the four villages to offset interviewer bias. Interviews took place Monday through Friday, the families being so divided that routinely the entire population of each village was interviewed every 2 wk. During the interview, the mother was asked to recall any symptoms that she and any of her children younger than 7 years might have had in the previous 2 wk. Each subject's information was recorded in a separate questionnaire. The beginning and ending dates of a symptom were always noted. A routine quality-control system was applied allowing the method to be standardized, using a supervisor, and validated, using a physician. Information on menstruation in the mother (beginning and ending dates) were recorded or noted as absent in the morbidity questionnaire for the youngest child. Also noted was whether or not the child was breast fed.

To validate the morbidity survey, a physician examined children half a day after the morbidity visit without previously informing the morbidity interviewer. This study generally showed satisfactory sensitivities and specificities for the symptoms recorded. Sensitivity and specificity were 66% and 99%, respectively for diarrhea and 75% and 99% for fever (Martorell et al. 1975b).

A study of the prevalence of recalled symptoms over the 14 d between periodic surveys showed a fall in prevalence with respect to the day of interview indicating memory loss over the 2-wk period (Martorell et al. 1976). In spite of an average underreporting of 22%, diarrhea was nevertheless reported reliably enough to reveal statistically significant associations between percent of time ill with diarrhea and growth (Martorell et al. 1975b; Schroeder et al. 1995).

Anthropometry. Body measurements were taken at specific ages by trained and standardized anthropometrists. A single person measured mothers throughout the study but three persons measured children at different times. All changes in personnel were preceded by rigorous standardization sessions.

The techniques of measurement are given in Martorell et al. (1982) and the quality control procedures used are described in Martorell et al. (1975a). Each week the data collected in the field were brought to the INCAP headquarters, computerized and analyzed. All children with values beyond two standard deviations from the age-specific means were remeasured for all variables to determine whether or not there had been an error in measurement, recording or punching. There was a weekly calibration of instruments, frequent standardization exercises for the anthropometrist at an urban orphanage and field replications. These exercises permitted the monitoring of precision and reliability; results of these exercises are given in Martorell et al. (1975a).

The anthropometry standardization method (Habicht 1974) is widely used today. Comparison of the reliability and precision achieved during the longitudinal study to results from others (Lohman et al. 1988; Marks et al. 1989;) speak favorably of the quality of the INCAP data.

Hand-wrist roentgenograms. The anthropometrist also took an X-ray of the left hand and wrist of children using a General Electric X-ray machine (model 100-15) set at 15 mA and 65 kV and using power from a gasoline generator. The X-ray head was set at 76 cm above the third metacarpal of the left hand with the fingers moderately splayed and the forearm placed at a right angle to the X-ray beam. Exposure was 48/60 of a second for children <2 y, and one second for older children. X-rays were taken at the ages specified in Table 4 concurrently with anthropometry.

Great care was taken to protect the children from stray X-rays. The X-ray film was placed in a lead lined box attached to the head of the X-ray machine. The seated child placed the hand into the box through a lead curtain. A film was placed on the child's seat and developed every month to be sure that there was no stray radiation.

The films were read for the number of ossification centers and the thickness of compact bone. Initially bone age also was determined according to the Tanner-Whitehouse and the Greulich and Pyle methods. These detailed assessments were discontinued when analyses showed that the simple counting of ossification centers provided as much information (Yarbrough et al. 1973).

Physical examination. A pediatrician examined children at 15 d and at 3, 12, 36 and 84 mo of age to identify developmental and other problems and gave special attention to neurological function and minor anomalies indicative of congenital mental retardation or neurological impairment. This information would permit one to identify children whose association between less adequate nutrition and impaired mental development was probably due to the latter—impaired behavior leading to inadequate bonding and poor coping, both of which might result in malnutrition. It was also thought that the neurological data might reflect improved nutrition from the supplement. None of these data have been analyzed and published.

The INCAP follow-up study (1988–89)

Objectives and design. The INCAP follow-up study of 1988–89 was a cross-sectional evaluation of former participants of the INCAP longitudinal study of 1969–77. At the time of the measurement, former participants of the longitudinal study ranged in age from 11 to 27 y. Also included in the follow-up study were subjects of the same age living in three nearby communities that INCAP had identified as potential study sites in the 1960s but which were not chosen in the end (referred to as "comparison" villages).

The main hypothesis of the follow-up study was as follows: *Nutritional improvements in the critical period of gestation and the first three years of life ultimately produce adolescents with a greater potential for leading healthy, productive lives.* An equivalent, but briefer statement of the central hypothesis is that "*improved nutrition in early childhood leads to enhanced human capital formation*".

The richness of the longitudinal study data set allows for several ways of operationalizing improved nutrition in early childhood. Foremost, this can be done relative to the nutrition intervention by classifying subjects as belonging to Atole, Fresco or comparison villages. Also, information about daily attendance and intake of supplement permit estimates to be made of energy and nutrient intakes from supplement over any time period for all individuals. Finally, the study permits many other alternative definitions of childhood nutritional status which are not based on the nutrition intervention but that rely instead on the longitudinal information available for children. Although not anchored in an experimental design, measures such as growth rates or degree of stunting, by virtue of being responsive to the full range of factors which influence child health (i.e. diet, infection and their determinants), provide a wider range in nutritional status than measures of supplement. An advantage of anthropometric measures is that they are widely used as indicators of nutritional status and hence are familiar to the international nutrition community.

The central hypothesis of the follow-up study refers to "*greater potential for leading healthy, productive lives*" in recognition of the fact that *productivity*, particularly in an economic sense, was not measured as well as *potential*. Greater *potential* was operationalized as improved status in terms of measures of physical growth and body composition, maturation, work capacity, information processing, intelligence, functional competence (reading, numeracy, general knowledge) and educational achievement (Rivera et al. 1992). Some *productivity* data were collected; labor participation and earnings were obtained for all subjects for the previous year. Nonetheless, many of the follow-up study subjects, particularly those exposed to supplement during pregnancy and the first three years of life, were too young in 1988–89 to allow for a meaningful exploration of the links between early nutrition and productivity. These aspects can be more adequately studied as the subjects become older and settled into an occupation.

A range of impact was predicted depending upon age at exposure to the nutrition intervention. Maximum effects at follow-up were predicted for subjects born from 1969–1974 (Table 3). These subjects were exposed to supplement during "critical phases" of growth and development, namely pregnancy and the first 3 y of life. The basis for this claim was the greater degree of growth retardation observed at these ages and the lack of demonstrated effect of the supplement on physical growth rates after 3 y of age (Martorell and Klein 1980; Schroeder et al. 1995).

The investigators argued to NIH, the agency that funded the research, that the follow-up study was unique because it was the first long-term, comprehensive follow-up of a nutrition intervention. Specifically, the follow-up study was expected to address whether the benefits of nutrition interventions on growth and development in early childhood persist into adolescence and beyond, to inform about effects and functions that can only be measured later in life and to contribute to understanding the importance of early growth and development for future status. Also, the investigators argued that the policy implications were clear and compelling. The following statement was included in the abstract to the first proposal submitted to NIH:

If valid, it will demonstrate that there are strong linkages among malnutrition, human capital formation and poverty which justify investments in health and nutrition as components of economic development strategies.

In designing the study, the investigators were troubled by two questions: should the study be longitudinal or cross-sectional and should the study be carried out sometime in the future when subjects reach maturity or should the study take place as soon as possible? The limitations of a cross-sectional evaluation were recognized readily. It is well known that the dynamic process of growth and maturation during adolescence can not be adequately studied through a cross-sectional design. Also, important areas such as fertility, employment history and wage earnings are difficult to collect accurately from single interviews. Although a longitudinal design would have provided better data for many outcomes, it would have increased dramatically the complexity and the cost of the study. Cost estimates, even for a cross-sectional survey, were already very high. For these reasons, a cross-sectional approach was selected.

The next question had to do with the timing of the study, when to do it? The study could proceed immediately while the subjects were adolescents or young adults or it could be postponed till most subjects were adults. The advantages of studying adults were recognized. One would be the elimination of the need to control for maturity in the analyses. By virtue of studying adults, information about truly long-term outcomes would be collected and the assessment of effects on fertility, occupation and earnings would be more definitive. Nonetheless, it was decided to proceed as soon as possible even though many subjects were still adolescents, partly to learn about effects on maturity and adolescence but more importantly, because the opportunity to carry out the study existed and it was feared that suitable conditions may not present themselves later. At this time, there was a team of interested researchers in place, enthusiasm was high and funding prospects looked reasonable.

The follow-up study, as noted, included data collection in three comparison villages chosen from six villages considered but not selected for in the longitudinal study in 1969. These villages were of interest because they were presumably similar to the Atole and Fresco and offered the theoretical possibility of shedding light on what might have occurred in the study villages in the absence of INCAP. However, it was not clear why they were not ultimately selected for the longitudinal study. Clearly, they were not felt to be the ideal choices. Still, the decision was made to include three of the previously unselected communities in the follow-up study. To reduce costs and travel time, three of the villages that were closest in distance to the four longitudinal study villages were included for study (i.e., Subinal, Las Ovejas and El Caulote).

Another issue that was carefully considered was that of migration. It was feared that to leave migrants out of the study would leave open the possibility of selection bias. On the other hand, the inclusion of migrants would complicate the logistics of data collection and increase costs. A compromise was reached. Migrants were included but only those who migrated to Guatemala City, Sanarate and El Jícaro (the last two being the provincial towns nearest to the study villages). Tracking down migrants to more dispersed areas, it was felt, would have been too costly.

Subjects studied. Census data were collected between October 1 and December 1, 1987 and included general information about the family (e.g., religion, number of family members, characteristics of the dwelling) and about each family member (e.g., birth date, place of birth, migration history, education and occupation). Follow-up data collection took place between May 1988 and June 1989; the census was continuously updated throughout this period. The principal targets of the follow-up study were adolescents and young adults but other groups were studied as well as noted below.

Adolescents and young adults (follow-up cohorts). These subjects were born between January 1962 and February 1977. Records for 2393 children meeting these criteria were generated from a computer master file prepared jointly by Cornell University and INCAP in 1983. Of the 2393 children included in the master file 224 (9.4%) had died when the follow-up study began, for the most part in early childhood. The 2169 subjects alive in 1988 were the target sample in the supplemented villages, a number greater than that given in Table 3 (i.e., 1992) because it includes subjects with no anthropometric measures available from the longitudinal study. The target sample in the three comparison villages (929 individuals) were subjects who had lived in these villages between January 1969 and February 1977, according to information obtained in the 1987 census. The total target sample therefore, including subjects from supplemented and comparison villages, consisted of 3098 subjects. For simplicity, these subjects are referred to hereafter as the "follow-up cohorts". The functional domains measured in the entire follow-up cohorts included physical growth and body composition, maturation, strength, physical health and retrospective life history.

Special sample for the behavioral component. Measures of information processing, intelligence, functional competence and educational achievement were collected on all subjects born in 1966 and later. A large number of outcome variables in the behavioral area were of interest; therefore, the time demands for testing each subject were high. To reduce costs and the duration of the study, the cohorts born between 1962 and 1965 were excluded. These cohorts had received supplementation at noncritical developmental periods (ages 4–7) and thus, minimal effects were expected. Their exclusion was unfortunate because they would have been a useful contrast group to that selected.

Special work capacity sample. This is a sample of ~25% of the follow-up cohorts, excluding migrants, (539 subjects) for whom measurements of work capacity, bioelectrical impedance, bone density and physical activity were obtained. Based on the 1987 census, a stratified random sample of 25% of the follow-up cohorts was selected. Details about the selection of these subjects are provided by Haas et al. (1995).

Parents of follow-up cohorts. Anthropometric measurements of *resident* parents of the follow-up cohorts were obtained to better interpret growth patterns in the follow-up study cohorts.

A retrospective life history was conducted through interviews of mothers, including those who had migrated to Guatemala City and to the two provincial cities closest to the study villages (Sanarate and El Jícaro). This information was required to complete the reproductive histories of women for the entire reproductive period and to obtain information about events that took place in the families of the follow-up cohorts between 1977 and 1988, and that could have influenced the outcomes of interest at adolescence. All parents were included in the study, irrespective of whether their follow-up cohort child (or children) still lived with them or had formed an independent family.

Wives of the follow-up cohorts. All wives of the follow-up cohort males, whether or not they were former participants of the INCAP longitudinal study, were interviewed for the collection of retrospective life history information.

Heads of households. All heads of households in which at least one of the spouses or dependents was a follow-up cohort subject were interviewed to obtain information on income and wealth of the family. The sample of households included the newly formed households as well as the parents' households.

Children <5 y of age. Anthropometric measurements were obtained on all children <5 y of age in the seven villages to assess their nutritional status. These data were seen as useful for estimating the degree of secular change in the study villages through comparisons of the results with those collected earlier in children in the longitudinal study.

Tests, exams, measurements and interviews. The tests, exams, measurements and interviews made on the different groups are described briefly below. For a more detailed description of the methods and a presentation of the data forms used see Rivera (1989) and Castro and Rivera (1992).

Follow-up cohorts. The following measurements and examinations were made in the follow-up cohorts:

1) Anthropometric measurements, including height, sitting height, and weight; five body breadths: biacromial, bicristal, knee, elbow and wrist; six circumferences: head, arm, waist, hip, thigh and calf; and seven skinfolds: biceps, triceps, subscapular, midaxillary, suprailiac, anterior thigh and medial calf. Indirect estimates of body composition (fat-free mass, percent body fat) were obtained using predictive equations from a validation study conducted at INCAP as part of the follow-up study (Conlisk et al. 1992).
2) A clinical examination by a physician, including a medical history, a detailed physical examination, the measurement of blood pressure, heart and respiratory rates, body temperature and the assessment of age at menarche (status quo and history). In addition, a vision test was performed and examination for signs of vitamin A deficiency and goiter were made. Abnormalities and diagnoses of diseases were recorded and treatment was provided when needed.
3) A blood sample was collected, that was further used to determine anemia and iron status.

4) A hand-wrist X-ray was obtained in males and in nonpregnant females 18 y and younger. The X-rays were later used to assess skeletal age by the Tanner and Whitehouse-2 method of rating. A gravindex test on urine was performed in females 18 y and younger who had reached menarche to identify early pregnancies. Pregnant women were not exposed to X-rays.
5) Hand strength was measured for right and left hands using a dynamometer.
6) Follow-up cohorts were interviewed for the collection of retrospective life history information. Spouses of follow-up subjects also were interviewed. The female retrospective life history included information about current reproductive status, parity and gravidity; a detailed reproductive history (for every pregnancy: pregnancy outcome, newborn birth date, mortality, feeding mode at time of death, prenatal care and delivery care); breast feeding and weaning practices for any child in the last 5 y, contraceptive use, marital/union status and history; education, occupation and migration history; and the characteristics of the dwelling where the women had lived. The male life history included income, occupation, education and migration history as well as some information about accumulated wealth. In addition it contained information about marital/union status, dependents and their ages and sexes.
7) School performance. Information from school records was recorded including: age at first enrollment, grades attended, attendance rate and test scores.

Sample for the behavioral component. Full details about the behavioral data collected are given by Pollitt et al (1993). The following tests were included:

1) Information processing. This test was applied using a microcomputer. Computer programs were designed specifically for the purposes of this study. Test of simple and choice reaction time, a short memory task, and a paired associates test comprised the computerized battery.
2) Tests of functional performance. The battery included tests of literacy, numeracy and general knowledge, which were developed locally, and two standardized educational achievement tests of reading and vocabulary. The achievement tests were part of the Interamerican Series used extensively in Guatemala by faculty from the Universidad del Valle.
3) Intelligence. Intelligence was assessed with the Raven's Standard Progressive Matrices.

Work capacity sample. Physical work capacity was determined as the oxygen consumption at maximum physical exertion (VO_2 max) on a motorized treadmill.

Besides the work capacity test, measurements of bioelectrical impedance and bone density, using photon absorptiometry, were made in the work capacity sample. In addition, physical activity was investigated through a questionnaire.

Parents of follow-up cohorts.

1) Anthropometric data were collected in men and women. Men: height, sitting height, weight, six circumferences (head, arm, waist, hip, thigh and calf) and five skinfolds (biceps, triceps, subscapular, anterior thigh and medial calf). Women: as detailed above for follow-up cohorts.
2) A retrospective life history was completed, identical to the one obtained by interview in follow-up subjects.
3) Heads of households. A detailed questionnaire regarding the family's income during the previous year, as well as accumulated wealth over the years was applied to heads of households. Information obtained included land tenure, crops produced, agricultural inputs (including labor, production and operation costs), time spent in agricultural jobs by crop produced and family member, production, revenue and income from agriculture and other sources and inventory of livestock ownership.

Preschool children. The following anthropometric measurements were obtained: length, crown-rump length, weight, knee breadth, three circumferences (head, arm and calf) and five skinfolds (biceps, triceps, subscapular, midaxillary and medial calf).

Organization and logistics of data collection. Six working teams conducted the tests, exams, measurements and interviews: two focused on the follow-up cohorts, one on parents of the follow-up cohorts, one on children's anthropometry, one on work capacity and one on migrants. Details about the composition of these teams and about coordination and supervision of their work are given in Rivera, Martorell and Castro (1992).

Logistics of data collection. *Rotation of teams.* The data collection teams were rotated among villages. Also, data collection in each village was staggered over the study to include both rainy and dry seasons.

Program of appointments for follow-up cohorts. The order in which subjects were measured was random. Subjects were scheduled to complete all tests, measurements and interviews in three visits but most subjects chose to complete them in 2 d and in some cases, in 1 d.

Informed consent was followed. When possible, the behavioral tests were done before the medical examination, which involved blood collection, because this procedure caused anxiety in some subjects. When possible, the behavioral tests were spread across visits to avoid fatigue.

Average durations of testing were as follows: Anthropometric measurements, hand-wrist X-rays and hand strength tests (25 min), medical examination and collection of urine and blood samples (25 min), functional competence and intelligence tests (60 min) and life history interview (20 min). In general, subjects tolerated the time involvement in the study better than expected.

Some subjects (3.6% of participants) refused to visit the testing center but were willing to be measured at home where all but X-rays and information processing tests could be completed. In contrast, most of the income and wealth interviews of heads of households and a large number of life history interviews of mothers and anthropometric measurements of parents of follow-up cohorts were made at home, because these subjects had less time to attend the centers.

The teams worked ~8 h/d but schedules were flexible to accommodate the preferred hours of participation of the subjects in each village and season. In all the villages, the teams had to work some weekends to allow for participation of subjects who worked in the fields during weekdays until late in the afternoon, subjects who worked out of the villages and returned during the weekends and migrants who visited their families during the weekends.

Training, supervision and data flow. *Training.* Training took ~2 mo and rigorous standardization exercises were held in all areas. Technical errors of measurement in anthropometry were equal or better than those reported in the literature (Pareja et al. 1989). For life history and income and wealth questionnaires as well as for functional performance tests, the percent agreement among interviewers usually exceeded 95%. Detailed results of the standardization procedures are given elsewhere (Rivera 1989; Castro and Rivera 1992).

Supervision and quality control. Supervision was continuous. All supervisors spent ≥2 or 3 d/wk in the field, providing direct supervision to field workers and examining and correcting data collection forms.

Ranges of permissible values in anthropometry were used to detect outliers; then, either obvious errors were corrected or subjects were reexamined. Errors in the behavioral area, the life history and the income and wealth interviews were detected through the review of data forms. In the work capacity area, the supervisors participated directly in data collection. After each test, the team reviewed the results and in this manner detected and corrected obvious errors.

Repeated measurements were made in ~10% of cases in anthropometry and in ~4% of cases in other areas. Analyses of these data are reported in the methods sections of papers in this volume.

Data flow, entry, verification and cleaning. Data were key punched twice at the INCAP computer center. The data then were cleaned using valid ranges of values and consistency checks across variables to detect errors and outliers. Values suspected to be incorrect were sent back to the field where the supervisor of each area corrected coding errors. For anthropometry and the life history questionnaires, subjects were reexamined whenever errors other than coding were found.

Primary health care activities. Efforts to strengthen the primary health care in each of the villages were implemented in coordination with the Ministry of Health.

A physician, hired by INCAP, instructed the nurses in several areas, especially the government's child survival program. He visited each village once a week to examine cases referred to him by the nurses as well as to attend to anyone in the village wishing to see him. Drugs and medicines were donated to the clinics to make up for the unpredictability of government supplies. In the two villages lacking clinics, INCAP established clinics in buildings donated by the community and hired nurses to staff them. At the end of the study, all medical equipment in the new clinics was donated to the communities.

Minimal dental services were provided through an arrangement with the University of San Carlos' dental school.

Coverage rates. Coverage is defined as the rate "participants/target sample." Participants being subjects for whom data were available for at least one study area (Tables 5–7).

Table 5 shows rates of coverage by village and type of village. Overall coverage was 71.7% with rates being slightly greater for supplemented villages (72.6%) than for comparison villages (69.5%). Coverage rates did not differ significantly between Atole and Fresco villages.

Overall coverage rates were greater for females (74.5%) than for males (68.9%); this pattern was similar in supplemented (females: 799/1060 = 75.4% and males: 775/1109 = 69.9%) and comparison villages

TABLE 5

Overall coverage in the follow-up cohorts

Village type	T	P	%
Fresco[1]			
Santo Domingo	594	411	69.2
Espíritu Santo	423	322	76.1
Atole[2]			
Conacaste	675	488	72.3
San Juan	477	353	74.0
Supplemented Combined	2169	1574	72.6
Comparison			
Subinal	238	165	69.3
Las Ovejas	386	280	72.5
El Caulote	305	201	65.9
Comparison Combined	929	646	69.5
ALL VILLAGES	3098	2220	71.7

[1] Large Fresco village, Santo Domingo; small Fresco village, Espíritu Santo.

[2] Large Atole village, Conacaste; small atole village, San Juan; T, target sample; P, participants; % = coverage.

TABLE 6

Coverage in the follow-up cohorts by migration status

Village type	Migrants			Nonmigrants		
	T	P	%	T	P	%
Fresco[1]						
Santo Domingo	212	79	37.3	382	332	86.9
Espíritu Santo	138	66	47.8	285	256	89.8
Atole[2]						
Conacaste	201	80	39.8	474	408	86.1
San Juan	176	71	40.3	301	282	93.7
Supplemented Combined	727	296	40.7	1442	1278	88.6
Comparison						
Subinal	61	28	45.9	177	137	77.4
Las Ovejas	98	44	44.9	288	236	81.9
El Caulote	76	33	43.4	229	168	73.4
Comparison Combined	235	105	44.7	694	541	78.0
ALL VILLAGES	962	401	41.7	2136	1819	85.2

[1] Large Fresco village, Santo Domingo; small Fresco village, Espíritu Santo.

[2] Large Atole village, Conacaste; small Atole village, San Juan; T, target sample; P, participants; %, coverage.

(females: 343/473 = 72.5% and males: 303/456 = 66.4%).

Table 6 presents coverage rates by migration status. Coverage rates differed between supplemented and comparison villages; among nonmigrant subjects, coverage rates were ~10% greater in supplemented (88.6%) than in comparison villages (78.0%). This is probably the result of the good rapport built by INCAP during the 9 y of the longitudinal study. On the other hand, coverage for migrants was slightly less in supplemented (40.7%) than comparison villages (44.7%). This may be due to differences in how the target sample was defined in supplemented and comparison villages. The target sample of migrants in the comparison villages was identified using information available in the 1987 census. Therefore, only adolescent migrants whose families were still living in the villages at the time of the follow-up census were selected. In contrast, in the supplemented villages, follow-up cohorts whose entire families had migrated before the beginning of the follow-up study also were selected, using records from the longitudinal study. Some of these migrant families were located using information provided by neighbors and relatives; however, as a result of the absence of parents or close relatives, follow-up cohorts belonging to these families were much more difficult to locate than migrants whose families were still living in the villages.

Coverage rates for migrants were overall much lower than those for nonmigrants because of the difficulty of locating migrants and because data collection in migrants was restricted to those living in Guatemala City and two provincial cities. The decision to focus on these locations was based on resource restrictions and the fact that information available at the beginning of the study indicated that ~64% of the subjects for whom locations were known lived in one of these three cities. Coverage for migrants known to have moved to these three cities was 62%. For the entire migrant sample, coverage was 42% (Table 6).

Coverage rates for females were greater in both migrants and nonmigrants. In migrants, coverage rates were 45.6% and 36.9% for females and males respectively; these patterns were similar in supplemented

TABLE 7

Percent coverage in the follow-up cohorts by birth cohorts and gender

Cohorts[1] Village type	Females				Males			
	I	II	III	IV	I	II	III	IV
Fresco[2]								
Santo Domingo	84.1	73.0	56.4	68.8	82.3	70.9	61.5	50.0
Espíritu Santo	90.4	74.4	65.9	69.2	87.5	77.3	55.8	62.1
Atole[3]								
Conacaste	84.8	74.1	79.4	81.4	87.5	65.7	55.8	53.8
San Juan	76.3	79.0	67.9	67.9	90.8	74.0	58.5	57.1
Supplemented Combined	83.8	75.1	67.9	73.1	87.1	71.2	60.3	54.5
Comparison								
Subinal	89.2	83.3	54.2	58.3	90.9	67.4	46.7	42.9
Las Ovejas	83.8	81.2	64.7	66.0	85.7	81.4	61.9	49.0
El Caulote	87.8	76.6	55.2	47.6	91.7	70.6	38.5	42.4
Comparison Combined	87.0	80.4	58.6	57.5	88.8	73.7	51.8	45.5
ALL VILLAGES	84.8	76.6	65.2	67.5	87.6	71.9	57.9	51.2

[1] See Table 3 for cohort definitions. Values are percentages.

[2] Large Fresco village, Santo Domingo; small Fresco village, Espíritu Santo.

[3] Large Atole village, Conacaste; small Atole village, San Juan.

(females: 176/394 = 44.7% and males: 120/333 = 36.0%) and comparison villages (females: 65/134 = 48.5% and males: 40/101 = 39.6%). In nonmigrants, coverage for females was 89.7% and for males 81.2%. Coverage rates were greater in females in supplemented villages (females: 623/666 = 93.5% and males: 655/776 = 84.4%) as well as in comparison villages (females:278/339=82.0% and males:263/255=74.1%).

Subjects were classified into four birth cohorts according to ages of exposure to supplementation (Table 3). **Table 7** presents coverage rates by cohort and village. In general, Cohort I has the highest coverage rates, followed by Cohort II and finally by Cohorts III and IV. Younger subjects may have had more time to participate in the various tests and interviews than older subjects.

Table 8 presents coverage rates for the different study domains by village type. Coverage rates were ~70% for most domains. The low coverage for blood collection deserves comment. Interviews of subjects who had refused to participate and of their families revealed that anxiety related to blood collection was one of the principal reasons for nonparticipation. Some subjects felt that the very small amount of blood collected (5 mL) was very large relative to the total blood volume in an adult. In one village, there were rumors that the blood was being sold. To remedy the situation, subjects were informed that blood collection was not essential for participation in the rest of the tests, measurements and interviews. In addition, subjects were reminded that blood samples also were used for the diagnosis of anemia, with treatment provided when necessary. Subsequently the refusal rate declined, though refusals to provide a blood sample among participants in the study remained high.

Table 8 also presents coverage rates for the different study domains in the comparison villages. Coverage rates were slightly less than found in the supplemented villages, but follow the same patterns. For the work capacity test, more subjects than originally planned were examined in comparison villages (see Haas et al. 1995).

Coverage rates for anthropometric measurements of parents of the follow-up sample was 82.4%, with no difference between supplemented (809/979 = 82.6%) and comparison villages (386/472 = 81.8%). Similar coverage rates were obtained for the life history of mothers of the follow-up samples (82.7%), with similar coverage rates for supplemented (452/543 = 83.2%) and comparison villages (207/253 = 81.8%). In contrast, coverage of the income and wealth questionnaire applied to heads of households was lower (62.0%), with rates being similar in supplemented (62.5%) and comparison villages (60.7%). The low coverage rates for the income and wealth interviews were due in part to the long time required to obtain the information and the fact that most of the heads of households worked in agriculture and were away during most of the day.

TABLE 8

Coverage rates by study domain and village type for the follow-up cohorts

	Supplemented villages			Comparison villages		
Study area	T	P	%	T	P	%
Anthropometry	2169	1554	71.7	929	633	68.1
Medical Exam	2169	1543	71.1	929	630	67.8
Hand-wrist x-rays	1149	920	80.1	459	337	73.4
Blood sample	2169	1196	55.1	929	425	45.7
Psychology tests						
Functional competence and intelligence	1897	1367	72.1	766	532	69.5
Information processing	1897	1331	70.2	766	521	68.0
Life history						
Males	1109	742	66.9	456	282	61.8
Females	1060	730	68.9	473	311	65.8
Work capacity subsample	388	361	93.0	152	178	100.0[1]

T, target sample; P, participants, %, coverage.
[1] More subjects were examined than originally planned.

Concluding remarks

Full details about design and methods, such as contained in this article, are often not readily available in the literature. The INCAP longitudinal and follow-up studies are among the most important sources of information from developing countries about child growth, development and nutrition and it is likely that there will be continued analyses of these data for years to come. It is important to have a faithful record of the design, objectives, methods and procedures, particularly for the benefit of analysts who may not have been directly involved with the studies.

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Nutritional Impact of Supplementation in the INCAP Longitudinal Study: Analytic Strategies and Inferences[1,2]

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ABSTRACT From 1969 to 1977 a supplementation trial was conducted in Guatemala to ascertain the effects on physical and behavioral outcomes of improved nutrition in pregnant women and in preschool children. This paper reviews different strategies to analyze the effect of the intervention on physical growth. One strategy compares outcomes in two villages that were randomly allocated to receive Atole, a supplement containing high amounts of protein and energy, with values in two other villages that received Fresco, a beverage containing no protein and little energy. Both supplements contained micronutrients. This comparison of village means gives a probability significance statement ($P < 0.005$) that the difference in growth was because of the supplement intervention, although it does not specify the aspect of the intervention that caused the effect. Complementary strategies increase the credibility that the effect of the supplement was nutritional. Thus, analysis of the dose response with increasing supplement intake within the villages excludes the possibility that the above findings were the result of knowing which villages received which supplement (i.e., measuring biases). A greater effect in those most likely to respond nutritionally also increases the credribility that the mechanism was nutritional. In studying other behavioral and biomedical impacts of this supplementation intervention, analyses for credibility should always be included. J. Nutr. 125: 1042S–1050S, 1995.

INDEXING KEY WORDS:

- *analytic methods* • *pregnancy*
- *supplementation* • *growth* • *Guatemala*

The study population, the experimental design and the methods used in the INCAP longitudinal study (1969–77) are described elsewhere (Martorell et al. 1995). In summary, two kinds of supplements were distributed in a central refectory in four villages, in midmorning and midafternoon, to any villager who attended. Supplement consumption was recorded for infants, children <7 y of age and pregnant and lactating women. From 1969–1977, two of the villages received a high-protein, high-energy supplement called "Atole" and the other two were given a no-protein, low-energy supplement called "Fresco." The villages were paired by size (i.e., large and small) and allocation within pairs to the supplements was random. From 1971 to 1977, the supplements had the same concentrations of specified micronutrients.

Growth and behavior, the key outcomes in children, were assessed periodically during the preschool period, as were measurements of potential modifiers or confounders such as morbidity, home diet and socioeconomic status. Maternal nutrition and health information also was collected periodically during pregnancy and lactation.

This paper presents different approaches to the analysis of the impact of supplementation on physical growth. These approaches may be divided into those that took advantage of the randomized design and

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-Term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] The INCAP longitudinal study was supported by contract No. HD-5-0640 from the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland. Additional sources of support were from the Agency for International Development, Washington, DC (AID-TA-C/1224) and a grant from Rockefeller Foundation (73030-E7352). The follow-up study was supported by RO1 grant HD-22440.

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those that sought other ways to control for confounding. The analyses selected as examples are tests of the effect of the supplement type on growth in length by three years of age, the effect of different amounts of maternal supplementation during pregnancy on birth weight and the effect of supplementation on improving weight in wasted children.

Except for the effect on growth presented in Table 1, all the findings discussed have been published elsewhere and are appropriately cited.

Using the randomized design

The analysis. To take advantage of the randomized design, one must use village as the unit of analysis because villages and not individuals were randomized. Most publications describing differences in outcomes between Atole and Fresco villages use the child or mother as the unit of analysis. Thus, the error term used to test these differences has many-fold more degrees of freedom and therefore will result in greater statistical significance than in analyses using village as the unit of analysis. These analyses do not give the statistical significance relating the treatment to the difference and instead only provide the statistical significance that the difference is not due to chance. The difference might be due to intrinsic village differences and not due to the treatments themselves. For example, the children of Espíritu Santo, the small Fresco village, had smaller head circumferences than the children in the other villages. When all the children from the Fresco villages are compared with all the children in the Atole villages, the systematically lower values of Espíritu Santo exaggerate the statistical significance of the difference between groups compared with using village as the units of comparison.

Martorell et al. (1982) developed an approach using the consistency of the response to supplementation across the two village sizes and two genders, (i.e., four sex-size groups for each treatment). This paper showed that the lengths of 3-y olds who had lived in Fresco villages their entire lives after the supplementation program began did not differ significantly ($P \geq 0.05$) in any of the four sex-village size groups compared with 3-y-old children measured before the study in the same groups. The range of change was −0.7 cm to 1.1 cm with a mean of 0.45 cm. In contrast, the change in the Atole villages relative to baseline values was statistically significant for all four sex-size groups ($P < 0.05$). The range of this secular change was 2.5–3.6 cm with a mean of 2.90 cm. Of course, the statistical probability of the change within each of the Atole sex-village size groups is not the probability that this was caused by Atole. However, the consistency of the changes across the Atole groups compared with the negligible changes observed in the Fresco groups over time makes the inference that the Atole improved growth credible.

TABLE 1

Length[1] of 3-y-old children before and after supplementation by village size and type of supplement

	Large villages		Small villages	
	Atole	Fresco	Atole	Fresco
After[2]	86.70	84.00	85.95	84.35
Before[3]	83.45	83.30	83.40	84.15
Change	3.25	0.70	2.55	0.20
Difference in change	2.55		2.35	

Overall difference in change: mean = 2.45 ± 0.10, *t*-test = 24.50, $P < 0.005$ (Two-tailed probability; df = 2).

[1] Means of sex-specific data calculated from Table 3 in Martorell et al. (1982).

[2] Born between 1969 and 1973.

[3] Measured in 1968.

A more rigorous statistical test can be made of the above-mentioned changes in Atole (A) and Fresco (F) villages by using village as the unit of analysis (**Table 1**). This analysis is true to the randomized design and deals with potential intrinsic differences between villages within each pair of similar sized villages by incorporating them into the statistical probability statement.

According to this analysis, the difference in net change (Atole minus Fresco) in the large villages was 2.55 cm and in the small villages it was 2.35 cm (Table 1). The mean of these differences is 2.45 ± 0.10 cm (mean ± SD). Even though the standard deviation only has 2 deg of freedom, the *t*-test is 24.5 with a two-tailed probability of $P < 0.005$. It is well known that the probability statement, $P < 0.005$, means that there is only one chance in a thousand that this difference could be due to chance. What is less well understood is that such a probability statement, except in a randomized design, does not exclude the likelihood, often strong, that the difference is due to something other than the intervention. Only a randomized design incorporates the potential effects of confounding factors into the probability statement. Thus, one can infer, with very little chance of being wrong ($P < 0.005$), that the difference in growth between Atole and Fresco villages was due to difference in the interventions and not to chance or to confounding.

The probability of the *t*-test shown above is for a two-tailed test. However, there is such a clear expectation that the effect of Atole will be beneficial compared with Fresco that it may be more appropriate to use a one-tailed test. In this case, P would be <0.0025.

Potential biases. It is generally well understood that the statistical significance of the above impact cannot be due to initial village differences because these are included in the error term of the test statistic. Similarly, differential changes that occurred among the villages during the period of supplementation also are included in the error term so long as these are not associated with the supplementation.

Also, the effect of the intervention on growth cannot be explained by self-selection to ingest the supplement. A repeated criticism of the study is that children who came for supplementation may have had parents who were more concerned about child health and nutrition and thus, would have grown and performed better anyway. However, this self-selection hypothesis also would predict that the village mean growth would remain unchanged. This, as seen above, was not the case. Therefore, these and similar factors associated with ingestion of supplementation within a village could not affect the comparison across Atole and Fresco groups as presented above. Even differential self-selection where, for instance, the better off children in the Atole villages and the worst off children in the Fresco villages ingested the supplement, would not bias the results in Table 1. Thus, self-selection for ingestion of the supplement within the villages cannot introduce, by itself, biases into the analyses performed appropriately for the randomized design.

The causal statistical significance for an effect of the intervention is impressive, both in its statistical significance and in its exclusion of other factors related to the villages and to those who ingested the supplement. It is important, however, to remember that it does not specify what aspect of the intervention is responsible for the effect. Anything done in the villages that was associated with the supplement could have caused the effect seen. This is why care was taken to spread the INCAP presence equally across the villages through designing and implementing all interventions similarly in all villages, and through rotation of all personnel (Martorell et al. 1995).

One effect associated with supplementation across villages that could not be excluded is the effect of knowing the kind of supplementation a village received. The villagers were, for all practical purposes, "blinded" to this fact because of the distances and the lack of communication among the villages. However, the measurers could not be "blinded". All field workers knew that both supplements were good for mothers and children, so one might expect them not to have been biased. Nevertheless, this possibility must be excluded as described below when discussing the dose response to supplementation.

Another measurement effect that could be associated with supplementation across villages is differential participation in the measurement of outcomes. This could happen, for instance, if better off and worse off participants to Atole and Fresco, respectively, came to be measured. This has been investigated and no evidence of this kind of bias has been found, but this must be kept in mind and verified in each analysis.

Another way that other interventions could have been associated with ingestion of the supplements is if attendance rates were different between Atole and Fresco villages. This is, indeed, the case. Attendance rates were much higher from birth to 3 y in Atole than in Fresco villages (Schroeder et al. 1992). As noted above, this presents no problem if this was because of self- selection within a village. However, differential attendance can result in differential exposure to programmatic influences other than the supplements. For instance, it could have been that those who came to the feeding centers also received better medical care because the clinic and the feeding centers were in the same building. Or, maybe, the socialization experienced in the feeding centers fostered better scores in the behavioral tests. Fortunately, these influences due to differential attendance rates can be taken into account because, in this data set, it is possible to differentiate between nutritional ingestion from the supplements and differential attendance rates. All analyses carried out to date on various outcomes indicate ingestion remains significant after controlling for attendance (see below).

Summary. The randomized design permits a strong inference ($P < 0.005$) that the intervention caused improvements in the outcomes. The component of the intervention that caused the impact must be elucidated by other analyses.

Dose response to supplementation

The analysis. One component of the intervention is, for instance, the knowledge gained by the measurers about the kind of supplement each village received. As discussed above, this could have biased the measurers. A dose-response analysis can be used to exclude the possibility because even though the measurers knew which villages received which supplement they did not know how much supplement each villager ingested. Therefore, any relationship between the amount of supplement (dose) and growth (response) could not be due to measuring biases. All the behavioral and most of the biomedical outcomes published to date have been examined by this method.

One reason for this approach is that sometimes village level analyses cannot identify a statistically significant impact of supplementation, even though it is present. This may be because of lack of statistical power because there were only two villages in each treatment. Had there been four villages per treatment, the *t*-test values would in all likelihood have been doubled with a large improvement in significance probabilities. There is no single appropriate number of replicates (villages) within a treatment. Instead, the number of replicates depends upon power analyses (Cohen 1988) of two-stage sampling designs (Snedecor and Cochran 1980) for each outcome of concern. Nevertheless, it is obvious that analyses of some outcomes will have less power than has growth because they are less reliably measured and because they are affected to a greater extent by nonnutritional factors than is the case for growth.

Power also may be reduced if baseline values differed by supplement type, as was the case for length in the small village (Table 1), and these data were not available or taken into account. In that case, the impact of Atole relative to Fresco would be obscured. If one does not correct length after supplementation (Table 1) by the baseline data before supplementation, the difference between Atole and Fresco villages at the end of the study would be 2.15 ± 0.78 cm with a *t*-test of only 3.91 with 2 deg of freedom and a two-tailed probability of only $P < 0.10$. This compares to the much greater statistical significance of $P < 0.005$ when the baseline data are taken into account.

Another and unexpected obstacle to using the randomized design analyses occurred when it was found that mothers in Atole and Fresco villages consumed similar mean amounts of energy despite the much greater energy density of the Atole. The greater volume consumption of supplement in Fresco villages was unexpected. At the time the study was designed, birth weight was expected to respond to maternal protein supplementation. But what if birth weight responded to energy supplementation instead? Mean energy intakes in Atole and Fresco villages were so similar that differences in birth weight between supplement types would not be expected and indeed, none was found. A further complication is that baseline data on birth weight were not collected precluding analyses according to the randomized design. However, dose-response analyses revealed a 29-g increment in birth weight for every 10,000 kcal (41,840 kJ) of supplement ingested during pregnancy (Lechtig et al. 1975a). This birth weight increment was similar for women living in Atole (23 g/10,000 kcal or 41,840 kJ) and Fresco (30 g/10,000 kcal or 41,840 kJ) villages even though there was no protein in the Fresco. This corresponded to the lower end of the range (25–84 g of birth weight per 10,000 kcal or 41,840 kJ) expected from factorial calculations of the anticipated response (Lechtig et al. 1975b). As discussed below, the actual dose response is higher after controlling for data reliability. The congruence between the finding and its theoretical expectation is important in deciding the credibility of the inference that energy from the supplement affected birth weight.

Potential biases. Dose-response analyses are amenable to statistical significance testing. However, in contrast to analyses appropriate for the randomized treatment design, the statistical significance does not relate to the causality of the association, only to the evidence for an association between supplement and the outcome of interest. Unmeasured or poorly measured confounding still remains a possible explanation for the findings. However, if due attention is paid to critical issues, it is still possible to credibly infer causality.

For example, mothers with short durations of pregnancy had less time to partake of the supplements and consumed less than other mothers. They also bore smaller children because of shorter gestational ages. Therefore, the association between gestational age and supplement intake could explain the dose response between birth weight and supplementation. Also, attendance to the feeding centers was voluntary, as was consumption. Those who came to the centers differed in many characteristics from those who did not and these differences may account for the variation in birth weight (Johnson 1988). However, when these confounding measures and gestational age were included in multivariate analysis, the dose response between birth weight and supplementation was not reduced (Lechtig et al. 1975a). The dose response actually tended to increase indicating that those who would otherwise have borne smaller babies tended to ingest more energy from the supplement and subsequently had larger babies. Thus, the inference that the increase in birth weight was due to the supplementation was strengthened. Multivariate analyses of this type are the conventional means of demonstrating that a relationship is not due to confounding factors (Snedecor and Cochran 1980). Almost all evidence of economic impact (Judge et al. 1980), and most of the evidence about public health impact, come from these kinds of analyses.

However, it may have been that factors associated with both supplementation and growth were inaccurately and unreliably measured (Habicht et al. 1979) or not measured at all. Therefore, controlling statistically for confounding is always open to question (Kupper 1984), unless the factors controlled for are perfectly measured and are a complete proxy for all confounding variables.

It is most likely that attendance to the supplementation centers, or that amount of supplement ingested, is more directly related to potential confounders than is nutrient ingestion from the supplement, and, equally important, that all confounding associated with nutrient ingestion is mediated through attendance or amount ingested. If this is so, attendance and amount ingested are complete proxies for confounding related to nutrient ingestion. Both of these variables are almost perfectly measured. If these proxies are statistically controlled for in the analyses, and the nutrient ingestion continues to be associated with the outcome, one can be reasonably sure that this is not because of some confounding associated with self-selection for supplementation. Fortunately, this is the case for supplementation during pregnancy and birth weight. The association between birth weight and energy remains statistically significant even when attendance or amount ingested is controlled for, while the converse is not true; the association of amount ingested or of attendance is not statistically significant when energy is controlled for.

There is one situation in which the above analysis is not completely convincing: if the correlates of ingestion differ between Atole and Fresco villages. This is because there is no power to differentiate between attendance or amount ingested from nutrients ingested within a treatment group; there is too much colinearity. The power comes from comparing women with identical nutrient intake, but different volumetric intakes (and vice versa), across the treatments. In pregnant women, no such difference in correlates is seen (Johnson 1988).

The above differentiation between nutritional impact and confounding associated with amount ingested is not possible for the micronutrients added to the supplements. They were added in equal concentrations to both Atole and Fresco. Therefore, it is more difficult to exclude the possibility that a dose response to the micronutrients is due to the effect of socialization in the supplementation centers or to self-selection rather than to any nutritional effect. For birth weight and growth, this is not an issue because no dose response with these micronutrients was observed once energy was taken into account.

There is another, quite different way to control for confounding that does not rely on statistical control of differences among women. Constant differences among mothers were controlled for by relating differences in birth weight to differences in supplement intake across consecutive pregnancies (Lechtig et al. 1975a). This excludes all unvarying characteristics of the mother as a source of confounding (e.g., early childhood nutritional history, genetics, etc.), although it does not exclude factors that may change across pregnancies.

Summary. The above dose-response analyses depend upon demonstration of a statistical association between supplement intake and the outcome of interest. This association could be causal, but it also could be because of other factors, maybe unknown or poorly measured.

Credibility that the association is causal depends, therefore, on a constellation of findings and other evidence from the literature supporting the causal inference, and rejecting other explanations. Credibility is ultimately a qualitative judgement call in contrast to the quantitative assignment of probability that the randomized design permits.

Combining randomized and dose-response analyses

Rationale. Dose-response analyses are useful for revealing the underlying patterns relating supplementation to the outcomes. They also permit analyses that test alternative hypotheses about the cause of the relationship and that take these confounding factors into account. Finally, as discussed below, they permit estimates of the dose response and corrections for those estimates. The statistical significance tests, however, only relate to the associations and not to the causality of the relationships. Inferences must therefore depend on how effectively one has dealt with confounding factors.

The importance of the statistical significance of a causal relationship between supplementation and outcome was emphasized earlier in analyses based on the randomized design. Analyses that combine both dose response and randomized approaches are more persuasive than either alone. Of course, this can only be done for outcomes that can be analyzed on the basis of the randomized design. Finally, credibility is further increased if the pattern of dose response across different kinds of children is as expected. Such analyses have been done (Rivera et al. 1991) to assess the impact of the Atole on the recovery from moderate wasting [<90% weight-for-length according to WHO's reference data (1983)] in children 6–24 mo old. Recovery is defined as recuperation of weight to >90% weight-for-length after 3 mo.

Analysis according to randomized design. The apparent recovery rate from moderate wasting was 50% in the Atole villages and 38% in the Fresco villages (**Table 2**). According to the randomized design analysis the statistical significance of the difference was $P = 0.07$ (two-tailed test) or $P = 0.035$ (one-tailed test). This statistical significance relates to the causal relationship between the intervention and the recovery rates. It fully takes into account the effects due to nonintervention factors that may cluster within a village.

Dose-response analysis. For these analyses high Atole ingestors were compared with high Fresco and low Atole and Fresco ingestors (Rivera 1988; Rivera et al. 1991). High Atole ingestion was defined as an ingestion of ≥10% of the recommended dietary intake of energy (RDI) from the supplement. Forty-five percent of the wasted children in Atole villages were high ingestors, and 55% were low ingestors. High and low Fresco ingestors were defined as being, respectively, above and below the 55th percentile of Fresco volume ingested. For these analyses, those with high Atole

TABLE 2

Recovery rates from moderate wasting (<90% weight-for-length) 3 mo after the diagnosis in children 6–24 mo in age

Supplement type	Recovery rates: By village	Recovery rates: Means of two villages
Atole	0.49, 0.52	0.50
Fresco	0.42, 0.35	0.38
Supplement effect (Atole-Fresco)		0.12[1]

[1] $P = 0.035$, one-tailed t-test; $P = 0.07$, two-tailed t-test.

ingestion were compared with those with high Fresco and with low Atole and Fresco ingestion. Of those high ingestors in the Atole villages, 59% recovered while only 44% of low ingestors recovered. Among those in the Fresco villages with high and low ingestion, only 41% and 36% recovered, respectively. The difference between 59% and each of the three other rates of recovery was statistically significant ($P < 0.05$; two-tailed test). None of the three other rates were statistically different one from the other, as expected because none of these three groups received enough nutritional supplementation for one to expect an impact.

There is evidence of true supplementation. The high Atole ingestors consumed larger amounts of supplement (17.3% of RDI) than did the high Fresco ingestors (2.5% of RDI) whereas the home dietary intakes did not differ between the two groups (61.4 and 63.5% RDI, respectively).

Examination for potential confounding factors (parents' education and height, maternal modernity and parity, household size and sanitation, child birth weight, breastfeeding, home diet and illnesses) revealed that high ingestors in the Atole and Fresco villages were more similar to each other than to low ingestors.

Only three measures were different between high ingestors in Atole and Fresco villages. These were proportion of time ill with respiratory symptoms, birthweight and duration of breast feeding. The first two were higher in the Atole villages and the third was lower. When these were taken into account in the above comparison between high Atole and high Fresco ingestors, the difference in recovery rates rose from 18 to 20% with a corresponding rise in statistical significance. Thus, these potential confounders were not the cause of the association between Atole and recovery from wasting.

As discussed previously, another potential bias is the measurer's knowledge about which village received which supplement. For example, an anthropometrist might unconsciously increase all the measurements in the Atole villages. The result would be a difference between Atole and Fresco villages that is "due" to the intervention, but that is not nutritional. However, the anthropometrist did not know levels of ingestion within a village and biases on the part of the measurer cannot explain the dose response. Furthermore, the dose response within the Atole and Fresco groups fully explains the difference between the Atole and Fresco villages found according to the randomized design. Thus, the difference in recovery from wasting between Atole and Fresco villages revealed by the analysis according to the randomized design was certainly not due to measurer bias. Only adding the analysis for dose response to that of the analyses based on the randomized design can exclude this kind of bias.

Credibility for a nutritional impact of the Atole is further improved if one has evidence that malnutrition is prevalent. In fact, some children with low weight-for-height are thin but not malnourished. When the proportion of these thin nonmalnourished children in the study villages is subtracted (i.e., that proportion found below the criterion in the reference population used) from the denominators of the recovery rates from wasting, all the malnourished children who were good attenders in the Atole villages recovered. This represented a range in the absolute increase of recovery rate of 29–52% in the Atole villages, more than in the Fresco villages, rather than the 18% found in the uncorrected analyses.

Credibility for a nutritional impact is also improved if those children respond most whom one would expect to do so on the basis of knowledge about malnutrition. The sample sizes are too small to use the randomized design for subgroups of children to investigate this issue. However, introducing the appropriate interactions with supplementation into dose-response equations permitted the demonstration that more wasted children, younger children, children who had diarrhea and those who were supplemented for longer periods responded most to the Atole compared with those who received the Fresco (Rivera 1988). These results all correspond to long held expectations, except for the finding in children with diarrhea. The diarrheal findings correspond to new knowledge that the better the nutrition the less diarrhea affects growth (Lutter et al. 1992). In conclusion, all the results from the dose-response analyses give credible evidence of an effect of supplementation on recovery from malnutrition.

Combined analyses. Combining the dose-response analyses with the results of the randomized design eliminates the possibility that the results about growth could have been due to measurer bias or to self-selection for the supplement. The dose-response analyses also contribute importantly to the inference that the impact of the intervention is not only causal but is also nutritional.

Differentiating among the contributions of energy and other nutrients

Some analysts have attempted to separate the specific contributions of energy from those of protein and other nutrients in the supplements.

Pregnant women. This has been done most successfully in pregnant women who ingested similar quantities of energy from the Atole and the Fresco by ingesting much greater quantities of the latter. The dose-response on energy was the same in both the Atole and Fresco groups. This would not have been the case if protein or other nutrients present in Atole but not in Fresco (e.g., calcium, phosphorous) had been limiting in which case the dose response of birth weight to energy would have been higher (steeper slope) in the Atole group. Nor would it have been the case if micronutrients added to the supplements had been limiting. These measured micronutrients (Table 2 in Martorell et al. 1995) were added in equivalent concentrations per volume to both supplements in 1971. Therefore, the dose-response would have been higher in the Fresco group if the measured micronutrients had been limiting because the micronutrient to energy concentration was higher in the Fresco group. Furthermore, there would have been no dose response in the Fresco group previous to 1971. This indicates that neither protein nor the other nutrients were limiting factors for fetal growth in the home diets of these mothers, but total energy was.

As discussed previously, the same conclusion results from step-wise multiple-regression analysis that reveals that energy is still statistically associated with birth weight, even when protein and the other measured nutrients are taken into account, whereas the converse is not true. Protein and the other nutrients are not statistically associated with birth weight when energy is taken into account. The above inference that energy is more deficient than protein in these diets is borne out by direct examination of the home diets. The mean of the home diet intakes for utilizable protein was slightly above the recommended daily allowances (7.4%) in contrast to the mean energy intake which was 39% less than the recommended daily intake (Lechtig et al. 1975b). Although the reliability of the individual measures of home diet is poor, that of the means is good (Habicht and Martorell 1992). Given this fact, plus some assumptions about the distribution of intakes around these means and about the dietary requirements, and above all, given the results about the relative effects of supplemental energy and protein, we conclude that energy was likely to be much more limiting than protein for these women.

Children. In children there was much less overlap between Atole and Fresco groups in energy supplement consumption (Schroeder et al. 1992). Atole children consumed much greater amounts of energy in the first three years of life. Analyses in Fresco children found similar or larger growth responses to energy intake than in Atole children, resulting in the conclusion that energy and not protein was limiting (Yarbrough et al. 1978). However, the results do not exclude the possibility that energy is limiting at lower levels of supplement intake as seen in the Fresco villages, but that protein may be limiting at higher levels of intake as seen in the Atole group.

The difference in slopes could not be because of a protein effect at lower levels of supplementation. They might be due to the flattening of the dose-response curve (Yarbrough et al. 1978) as energy intake approached adequacy. The higher response in Fresco also could be interpreted as evidence that another nutrient was limiting because their concentrations in relation to energy were much greater in the Fresco compared with the Atole.

In conclusion, none of these competing hypotheses have found resolution to date, possibly because resolution may not be possible in this data set. This lack of clarity about the exact nutrients that were responsible for the supplements' impact does not affect in any way the inference about a causal effect of the supplementation program on child growth to 3 y of age.

One claim that the effect of the supplement on growth was solely due to protein in the supplement (Balderston et al. 1981) is based on incorrect interpretations, as discussed below, from analyses using the home diet.

Taking home diet into account in dose-response analyses. The objective of the INCAP longitudinal study was to improve nutrition. It is therefore important to know whether the energy and nutrients from the supplements were supplemental or simply displaced home diet consumption. A direct approach would appear to be the use of total dietary intake (home diet + supplement) in the analyses. Alternatively, home diet and supplementation may be used as separate variables in a multivariate equation explaining the outcome. Both approaches would capture the net improvement in energy or nutrient intakes when comparing children from Atole and Fresco villages. Unfortunately, the low reliability of the home diet data usually precludes finding associations between the home diet and the outcomes. For example, Schroeder et al. (1995) found home diet energy to be much less related to growth increments than supplement energy. Rivera et al. (1995) increased the reliability of home diet energy by combining as many as eight separate surveys per subject and by creating a dummy variable, above or below the median; even though the diet variable was statistically significant and in the expected direction, analyses showed its inclusion in the model did not affect the regression coefficient for supplement. This indicates that the range in energy and protein intake from the home diets is too small to be important in the analyses. For this reason, home diet is also usually a de facto constant when included in analyses involving supplement because of the imprecision of its regression coefficients with the outcomes. Nonetheless, some analyses (Rivera et al. 1995, Schroeder et al. 1995) include home diet to increase persuasiveness as many readers would be troubled by the omission of this variable.

Inappropriate inclusion of home diet in the analyses and poor interpretation of the results can lead to false inferences as exemplified by the analyses that led to the claim that it was protein and not energy supplementation that produced better growth in children consuming Atole (Balderston et al. 1981). This claim was made on the basis of two findings: The first was: "The large effect [on growth] of increments of Atole supplementation for children eating the same home diet—contrasted with the small effect of increment in home diet for children consuming the same amount of supplement—is not consistent with the hypothesis that the total energy value of the supplement is what accounts for the gains of children in Atole villages" (1981:59). In fact, this contrast was even stronger for protein (not reported) and was solely because of the poorer reliability of home diet compared with that of supplement intake. When the independent variable is very poorly measured, the estimates of magnitude (regression coefficients) are biased so they approximate 0 (Habicht et al. 1979). The correlation coefficients are also small, but this does not depend upon whether or not home diet is an independent or dependent variable.

The second finding in Balderston et al. (1981) was that in multiple regression analysis of the total diet (i.e., sum of home diet and supplement) there was a strong association between total protein ingestion and growth and none between total calorie ingestion and growth. This was entirely because of the fact that the Atole had a greater impact on total protein intake (on the average 35%) than on total energy intake (on the average 17%; estimates from Martorell et al. 1982 and from WHO 1985). As noted above, the variability in supplement ingestion is much better correlated with growth than is variability in home diet because of home diet's poor reliability. Therefore, for equal variability of supplement intake, supplemental nutrients that contribute a greater proportion of total dietary intakes will be more highly correlated with growth. This is particularly the case in children under 3 y of age because energy and protein intakes from the supplements are highly correlated with each other; energy ingestion from the Fresco was very low in relationship to Atole.

Home diet data are important, however, in investigating the degree to which the supplement replaced rather than supplemented the home diet. One cannot estimate the amount of supplement used to replace the diet at different levels of diet because this requires using the total diet as the independent variable. On the other hand, the estimates of amount of home diet replaced by the supplement at different levels of supplementation can be estimated without bias because in this analysis supplementation is the independent variable and it is measured almost perfectly. The estimated level of replacement for supplemental energy was 22% for pregnant mothers (calculation from Table V in Lechtig et al. 1975a), but negligible for children (Martorell et al. 1982).

These figures give the apparent proportion of supplement that substitutes for the diet. It might be that high ingestors of supplement would not have had enough food at home to bring their home diets to the same levels of intake as low ingestors, even if they had not consumed the supplement. This is indeed likely because high-energy ingestion from the supplement is related to lower socioeconomic status (Johnson 1988, Schroeder et al. 1992).

These magnitudes still will be somewhat underestimated because the outcomes are affected by variations in nutrition, not because of the supplement. The most important variation is home diet. This variation reduces the power to find associations between measures of supplementation and outcomes so that both the statistical significance and the regression coefficients relating a nutritional component of the supplement to the outcome understate this nutritional relationship (Habicht et al. 1979). In principle, this effect of home diet is no different from the influence of other factors that affect an outcome and that are randomly distributed across different levels of supplementation. The omission of home diet from the analysis of the impact of supplement on outcome measures is no different from the omission of other variables that affect growth but that are not related to the supplement, if one has taken the confounding because of home diet into account. Such omissions are inevitable. The confounding because of home diet is dealt with by correcting for the apparent substitution of supplement for home diet. For instance, the magnitude of the effect of supplement on birth weight is ~20% higher than the figures published when this correction is made. This means that the response of birth weight to actual energy supplementation is ~35 g per 10,000 kcal (41,840 kJ).

Conclusions

This paper presents the statistical significance of causality for an effect of the supplementation of the growth of 3-y-old children. It presents credible evidence for an effect of the supplement during pregnancy on the birth weight of infants. Finally, it presents evidence and the significance of causality for the effect of the supplement on the recuperation of malnourished children. The combination of the probability tests for causality with tests of association for credibility is necessary to make the most persuasive argument that the supplement had a nutritional effect on the outcome of concern. Credibility analyses are always possible and should always be done. Where the probability analysis for causality cannot be done, more analyses for credibility are necessary.

ACKNOWLEDGMENTS

We thank Catherine Geissler for comments on an early draft of this paper.

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Age Differences in the Impact of Nutritional Supplementation on Growth[1,2]

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ABSTRACT Supplementary feeding programs are common in developing countries. These programs often cannot demonstrate an impact on child growth, however, possibly because they tend to reach older children. This study examines the impact of nutritional supplementation on annual growth rates in length and weight from birth to 7 y of age in 1208 rural Guatemalan children. A series of multiple linear regression models is used to control for initial body size, diarrheal disease, home diet, socioeconomic status and gender. During the first year of life, each 100 kcal/d (418 kJ) of supplement was associated with ~9 mm in additional length gain and 350 g in additional weight gain; the benefit decreased to ~5 mm in length gain and 250 g in weight gain during the 2nd y of life. Between 24 and 36 mo of age, supplement only had a significant impact on length. There was no impact of nutritional supplementation on growth between 3 and 7 y of age. Patterns were the same if supplement intakes were expressed as a percent of recommended allowances or growth was expressed as a percent of the expected rate. These impacts of nutritional supplementation on growth coincide with the ages when growth velocities, as well as growth deficits, are greatest in this population. J. Nutr. 125: 1051S–1059S, 1995.

INDEXING KEY WORDS:

• *nutritional supplementation* • *growth* • *child* • *age* • *Guatemala*

Supplementary feeding programs to improve nutritional status in children who are malnourished or at high risk of malnutrition are in wide use in developing countries. The ability of these programs to demonstrate an impact on growth, however, has been inconsistent (Beaton and Ghassemi 1982). This failure has been attributed to the use of inappropriate indicators in measuring impact as well as poor targeting of the intervention (Rivera et al. 1991).

A child's age often is used as a means of targeting interventions because it is a rough measure of nutritional needs, vulnerability (i.e., to infections, inappropriate care, etc.) and growth potential. A more precise understanding of the ages at which infants and children benefit most from nutritional supplementation may aid in the improved effectiveness of such interventions (Beaton 1993).

Very few published analyses have investigated age-specific responsiveness to supplementation (Burger 1992, Gopalan et al. 1973, Lutter et al. 1990, Martorell et al. 1980, Rivera et al. 1991). In one of these reports (Gopalan et al. 1973), results were presented by age of the child, but lack of detailed information on supplement intake makes interpretation difficult. The study by Lutter et al. (1990) restricted analyses to children <3 y of age. Using the same dataset employed in the current analysis, Burger (1992) examined the impact of supplement on growth by 3 mo age intervals (e.g., 0–3, 3–6, etc) but restricted the analysis to chil-

[1] Presented in the symposium on Nutrition, Growth, and Development, FASEB, March 28 to April 1, 1993, New Orleans, Louisiana. Published as a supplement to The Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Supported by NIH grant HD-22440 and by a grant from the Pew Charitable Trusts.

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dren <2 y of age. Examination of older children is of interest because a large number of on-going programs target children ≤5 y of age. The paper by Rivera et al. (1991) analyzed the same data set used in the current study, but focused on the impact of supplementation on the recovery from wasting (i.e., low weight-for-height).

An analysis with similar objectives to the current paper based on the same study was published previously but used only a subset of cases (Martorell and Klein 1980). Additional covariates, such as a well-constructed proxy for socioeconomic status, are now available. The current analysis will examine the research question using a variety of approaches not employed in the previous work.

The objective of the current paper is to examine the age-specific impact of nutritional supplementation on length and weight during the first 7 y of life.

MATERIALS AND METHODS

Study population and research design. The data for these analyses are derived from a supplementation trial conducted in Eastern Guatemala between 1969 and 1977. The overall objective of the study was to test the impact of food supplementation on physical growth and mental development in young children. Details of the study design, sample, and methods have been published elsewhere (Martorell et al. 1995a).

Briefly, four rural villages of similar ethnicity and development were randomized to receive either a high-energy, high-protein gruel-like beverage (Atole) or a low-energy, no-protein drink (Fresco). Atole contained 163 kcal (682 kJ) and 11.5 g of protein per cup (180 mL) whereas Fresco contained only 59 kcal (247 kJ) per cup. Both supplements were fortified with vitamins and minerals in equal amounts (Martorell et al. 1995a).

Supplements were offered ad libitum in the morning and afternoon at fixed locales in each village on a daily basis. Consumption by pregnant and lactating mothers and children <7 y of age was recorded to the nearest 10 mL. Before 3 mo of age, infants consumed very little supplement; median intakes were about 2 kcal/d for Atole and Fresco children combined. Thereafter, consumption of the supplements in terms of volume was greater for children in the Atole villages until 4 y of age, but greater for children in the Fresco communities between 5 and 7 y of age (Schroeder et al. 1992). With Atole containing three times the energy of Fresco, however, children in the Atole villages consumed greater amounts of energy at all ages.

In addition to the supplementation, all participants were offered free obstetric and medical care, including free medication and vaccination services.

Children were weighed and measured at birth, 15 d of life, and within ±7 days of age 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 60, 72 and 84 mo. Weights (to the nearest 10 g) were obtained using a beam scale with children dressed in a light shift, the weight of which was later subtracted. Supine lengths were measured to the nearest 0.1 cm using a standard measuring table; no standing heights were taken on children. The reliability between measurements was 95% for weight and 99% for length (Habicht et al. 1979).

Twenty-four-hour dietary recalls were conducted every 3 mo between 15 and 36 mo of age and at 42, 48 and 60 mo of age. Unfortunately, few 24-h recalls were done in children <15 mo of age so good information on home diet during the early weaning period is unavailable. An ethnographic study in the four villages found that the foods most frequently consumed by children 3–60 mo of age were: maize (corn) tortilla, black beans, bean soup, bread and coffee; milk products were consumed very rarely (Mejía-Pivaral 1972). Initiation of breastfeeding in these communities was nearly universal with a mean duration of any breastfeeding of ~18 mo (Burger 1992).

Morbidity data were collected during biweekly home visits during which caretakers were asked about their child's illnesses during the previous two weeks. Socioeconomic information such as the physical conditions of the household and parents' schooling was collected during a census conducted between 1974–1975.

Analytic approach. Growth, supplement, illness and home diet data were summarized over ages 3–12 mo and nonoverlapping, yearly intervals thereafter until 84 mo. Very little supplement was consumed before 3 mo of age (Burger 1992) so this period was not included in these analyses. Data for the first interval (3–12 mo) were converted to yearly figures for comparability with the other intervals. Only children who were present in the village for the complete interval were included in the analyses of that interval. Because of the longitudinal nature of the study, children may be represented in more than one interval.

Supplement intake data were analyzed in two forms, first as average kilocalories of supplement ingested per day over the interval. Analyses using this variable may be interpreted as defining the potential impact of a certain amount of supplement on growth regardless of age-related variations of recommended dietary intakes and of growth.

In addition, the percent of the recommended dietary allowances that the supplement represented was calculated. Recommended dietary allowances per kilogram body weight for the interval were based on published values (National Research Council 1989). Because children gained weight at less than the expected rate, RDAs calculated from actual weights may underestimate needs if these children have the potential for growth similar to the reference. RDAs therefore also were calculated using expected weights as indicated by the NCHS/WHO reference (World Health

Organization 1983). The argument against using expected weights is that as the children age, they deviate further from the growth reference and are less and less likely to consume at the expected levels (Burger 1992). Using both approaches gives an envelope within which lies the correct RDA for these children.

Growth data are analyzed in a variety of forms. Raw weight and length increments were calculated by subtracting the value at the beginning of the interval from that at the end. The percent of median of an internal growth reference that this increment represented also was calculated by dividing the child's weight or length velocity by the median value for the entire sample as was done by Lutter et al. (1990). In addition, percent of median of an external growth velocity reference was calculated using actual growth velocities based on the Fels data set as presented by Baumgartner et al. (1986).

Multivariate regression techniques were used to estimate the impact of supplement intake on growth increment after controlling for potential confounding factors. Parameter estimates of the variables of interest were calculated as follows, impact of: 100 kcal (418 kJ) supplement/day or 10% of RDA from supplement on raw growth increment (in millimeters length or grams weight) or percent of median expected growth.

Covariates considered for inclusion in each of the models were: body size, percent of days with diarrheal illness, socioeconomic status and sex. Home diet (energy) was controlled for during all intervals except the first (e.g., 3–12 mo) and last (e.g., 72–84 mo) for which it was not available.

To control for the possibility that larger children would consume supplement or grow differently from smaller children, body size (length or weight) at the measurement period *immediately before* the start of the interval was included as a covariate. For example, for the age interval 12–24 mo, length (or weight) at 9 mo was included in the model. This approach was necessary because body size at the *beginning* of the interval (e.g., 12 mo) was used to create the dependent variable (i.e., the growth increment); the errors between these two variables are thus correlated and the use of both in one model may bias results (Plewis 1985). High correlations between subsequent measurements, >0.90 and >0.80 for most consecutive length and weight measures respectively, further justified this approach.

Though the use of difference scores as dependent variables has been criticized (Bohrnstedt 1969), including a measure of body size before the beginning of the interval avoids the problem of correlated errors, the primary criticism of this approach. An alternate approach that uses the residuals of the later age given the earlier (Bohrnstedt 1969) was used in the previous analysis of these data (Martorell and Klein 1980), but provides less interpretable results (Dalecki and Willits 1991).

Percent of time with diarrheal illness was calculated by dividing the number of days with diarrhea during the interval by the number of days at risk. For the home diet variable, if more than one 24-h recall was available during the interval, these were averaged. Because 24-h recalls were only done between ages 15 and 60 mo, home diet was not controlled for in the regressions for the age intervals 3–12 and 72–84. A summary socioeconomic status (SES) variable was created using principal components analysis and is based on housing quality and possession variables (Rivera et al. 1995); the variable was standardized with mean = 0 and SD = 1. Sex was included as a covariate because growth rates are slightly different for males versus females. Interactions between the above covariates and the independent supplement variables were tested to determine if the impact of supplement varied at different levels of the covariate; interactions were considered significant at $P < 0.10$.

An indicator variable for supplement type (i.e., Atole or Fresco) was *not* included in analyses which pool all villages because of the high correlation between this variable and amount of energy from supplement ingested. In analyses not presented, interactions between supplement type and amount of energy from supplement that might indicate that the impact of energy from Atole differed from that from Fresco were not found to be significant.

All analyses were conducted using the PC-SAS statistical package version 6.04.

RESULTS

Table 1 presents descriptive statistics on children who were included in the regression analyses for a particular age interval, and therefore had information on supplement intake, anthropometric measures at the beginning and end of the interval, a measure at the preceding measuring period, sex, SES, percent of days with diarrhea and, for all but the first and last intervals, energy from home diet. Mean supplement intakes were ~100–170 kcal/d (418–711 kJ) for children consuming Atole. Children in Fresco villages consumed <40 kcal/d (167 kJ) in the first 3 y of life increasing to ~80 kcal/d (335 kJ) at age 7 y.

Differences in energy intake from supplement between the two groups were highly statistically significant at all intervals ($P < 0.001$). Atole intakes represented ~10–16% of the RDAs and Fresco intakes about 1–7% of RDAs if the child's actual weight is used to calculate RDAs (**Figure 1**) and about half this amount if expected weights were used (not shown).

Mean length gains were significantly greater in the Atole villages compared with the Fresco villages for the first three intervals whereas weight gains were greater in the Atole villages only for the first two in-

TABLE 1

***Energy contribution of the supplements and mean annual growth rates in length and weight in Atole and Fresco villages*[1]**

	Supplement intake (kcal/d)[2]				Weight (kg/y)			Length (cm/y)		
	Atole[3]		Fresco[3]							
Age (mo)	n	x̄ ± SD	n	x̄ ± SD	Atole x̄	Fresco x̄	Pooled SD	Atole x̄	Fresco x̄	Pooled SD
3–12	274	90 ± 92 (54)	251	7 ± 9[c] (3)	3.41	3.19	1.01[a]	15.6	14.7	2.7[c]
12–24	180	143 ± 99 (123)	151	14 ± 13[c] (10)	2.17	1.74	0.78[c]	9.2	8.2	2.0[c]
24–36	221	169 ± 114 (145)	198	37 ± 30[c] (27)	2.22	2.09	0.69	8.5	8.1	1.7[b]
36–48	249	158 ± 123 (123)	244	53 ± 37[c] (43)	1.77	1.71	0.65	7.4	7.3	1.6
48–60	274	145 ± 112 (121)	243	69 ± 44[c] (61)	1.57	1.48	0.70	6.5	6.5	1.5
60–72	237	135 ± 108 (107)	204	73 ± 44[c] (68)	1.56	1.53	0.65	5.7	5.7	1.3
72–84	213	128 ± 115 (92)	206	81 ± 49[c] (77)	1.80	1.71	0.73	5.4	5.2	1.4

[1] *t*-test between Atole and Fresco: a = $P < 0.05$; b = $P < 0.01$; c = $P < 0.001$.
[2] To obtain kilojoules, multiply kilocalories by 4.184.
[3] Values in parentheses are medians.

tervals (Table 1). Growth velocities compared with reference data (Baumgartner et al. 1986), however, were depressed greatly until ~3 y of age for both groups but were very similar to the reference data after age 3 (**Figure 2**). Patterns were similar for both males and females.

A sample of the multiple regression models is presented in **Table 2**. In this case, supine length change (millimeters/year) was regressed on energy from supplement (100 kcal/d or 418 kJ) and on covariates for each age interval. Although all covariates were not statistically significant in each of the full models, all covariates were retained in all models (except home diet in the first and last age interval) for ease of comparison. Excluding nonsignificant covariates had little impact on the magnitude and the significance of the parameter estimates of interest. Interactions between supplement and each of the covariates were tested in this series of models as well as those presented below. However, because no interaction was significant for more than one age interval, none were included in the final models.

In the series of models presented in Table 2, an extra 100 kcal/d (418 kJ) of supplement was associated with ~9, 5 and 4 mm in additional annual length gain during the first, second and third year of life, respectively. After age 3, there was no impact of supplement on length gain. Covariates included in the models displayed the expected associations with length gain. The negative association between length at the beginning of the interval and growth during the interval reflects the "regression to the mean" phenomenon docu-

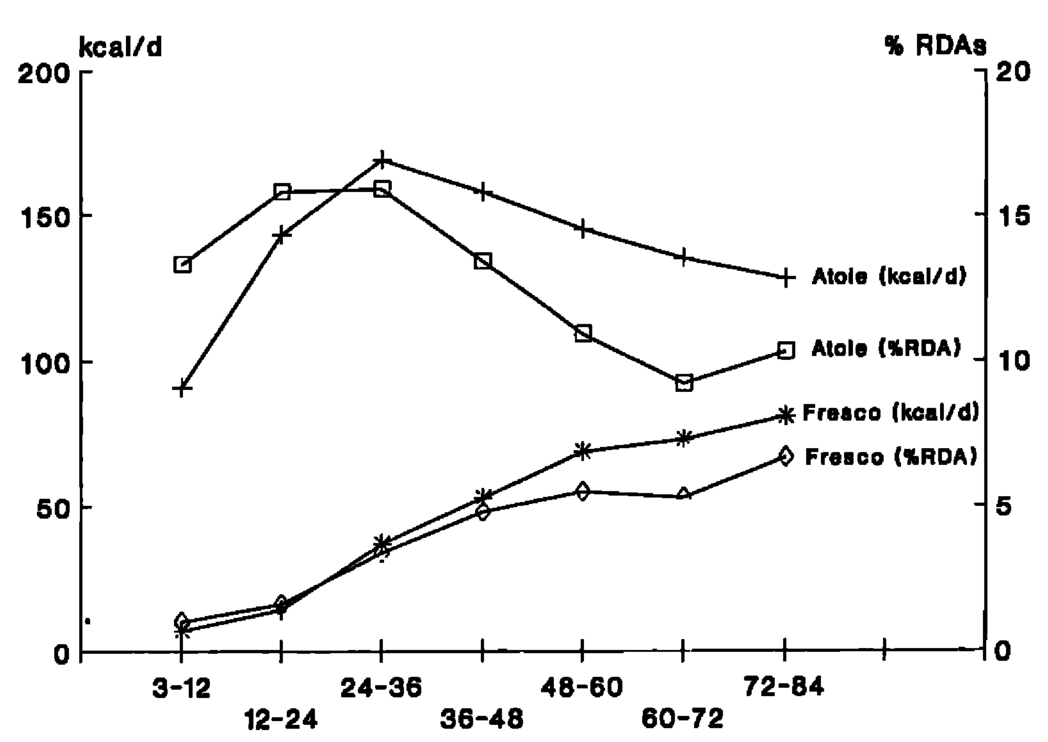

FIGURE 1 Supplement intakes (kcal/d) and percent of RDAs from supplement (calculated using actual weights; see Table 1). To obtain kilojoules, multiply kilocalories by 4.184.

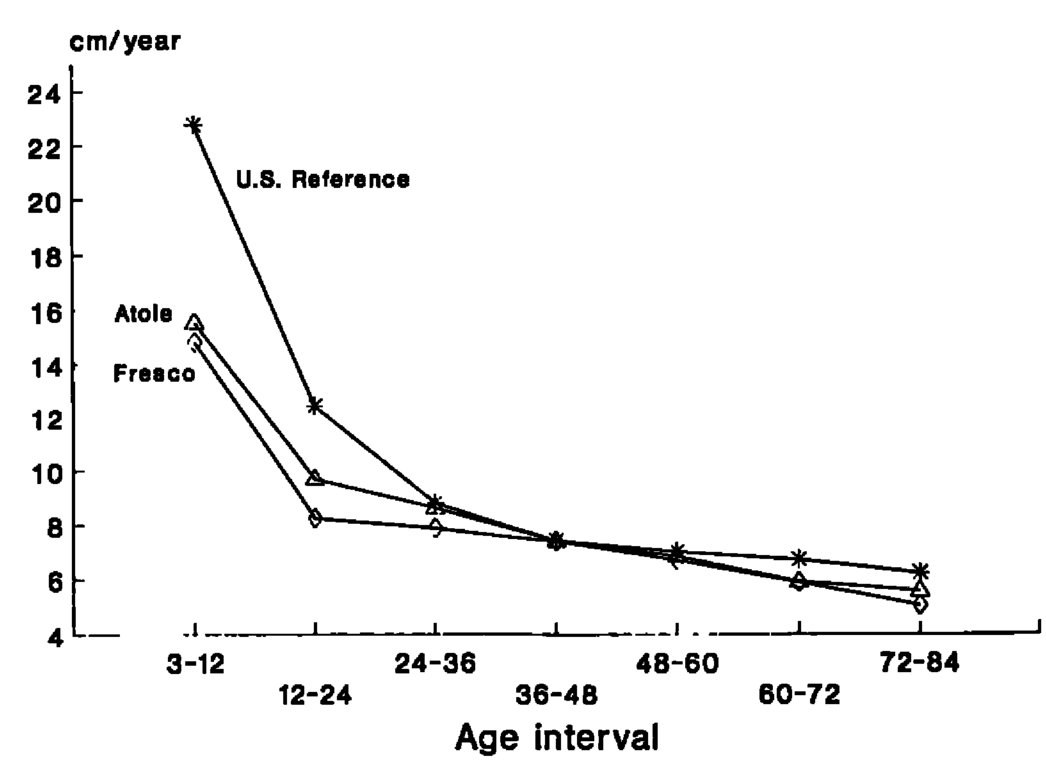

FIGURE 2 Length velocities of Guatemalan females compared with reference data from the Fels Research Institute as published by Baumgartner et al. 1986.

TABLE 2

Multiple variable regression models of supine length change (mm) per year on supplement calories (100 kcal/d or 418 kJ) and covariates

Variable	3–12 (n = 525)	12–24 (n = 331)	24–36 (n = 419)	36–48 (n = 493)	48–60 (n = 517)	60–72 (n = 441)	72–84 (n = 419)
	Age category (mo)						
Intercept	217 ± (25)[1,c]	22 ± (28)	124 ± (19)[c]	92 ± (16)	69 ± (15)	67 ± (15)	87 ± (20)
Supplement (100 kcal/d)	9.2 (1.5)[c]	4.7 (1.1)[c]	3.7 (0.8)[c]	0.8 (0.7)	0.8 (0.7)	1.4 (0.7)	−0.2 (0.8)
Length at T^{-1} (cm)	−1.4 (0.5)[b]	0.8 (0.4)[a]	−0.7 (0.3)[b]	−0.3 (0.2)	−0.1 (0.2)	−0.1 (0.2)	−0.2 (0.2)
Male gender	−0.3 (2.3)	6.2 (2.2)[b]	−0.3 (1.7)	1.1 (1.5)	3.9 (1.4)[b]	2.2 (1.3)	0.04 (1.6)
SES (sd units)	3.2 (1.3)[a]	3.7 (1.2)[b]	0.7 (0.9)	0.3 (0.7)	−1.3 (0.7)	1.0 (0.7)	−0.3 (0.8)
Diarrhea (10% d)	−2.1 (0.9)[a]	−2.5 (1.0)[a]	−2.5 (1.2)[a]	−3.5 (1.7)[a]	−4.3 (1.9)[a]	−2.8 (1.8)	−7.3 (2.8)[a]
Home diet (100 kcal/d)		0.2 (0.4)	0.8 (0.3)[b]	0.4 (0.2)	0.1 (0.2)	−0.2 (0.2)	
R-square	0.10	0.13	0.07	0.02	0.04	0.03	0.02

Separate models by age category. Atole and Fresco children combined.
[1] Parameter estimate (means ± SE. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.

mented elsewhere. Differences in growth rates by gender is inconsistent with significant estimates in favor of the males seen only at the 12- to 24- and 48- to 60-mo age periods. Higher socioeconomic status was associated with greater length gains in the 3- to 12- and 12- to 24-mo age periods, with gains of approximately an extra 3 mm for each standard deviation unit of the factor score. Diarrhea had a consistent negative impact on growth. For the models in Table 2, each additional 10% of days with diarrhea resulted in between 2.1 and 7.3 mm less length gain. Finally, more energy from the home diet was associated with greater length gains in the 24- to 36-mo period. In spite of its questionable validity in the dataset (Habicht et al. 1995), home diet was retained in the models presented in this paper for theoretical reasons and because excluding it had little impact on the parameter estimates of interest.

Table 3 presents the slopes of the regression of supine length and weight change on supplement, stratified by supplement type. Similar to the pooled results, Atole had the most significant impact on length in children <36 mo and on weight in children <24 mo. The impact of Fresco is highly erratic with large standard errors; the only significant impact is that on length at 60–72 mo. Possible explanations for these patterns of effects are discussed in depth elsewhere (Habicht et al. 1995).

The slopes of supplement calories (100 kcal/d or 418 kJ) are compared with those obtained by calculating supplement intakes as 10% of the RDAs (calculated using both actual and expected weights) in **Figure 3**. Though there is some variation depending on how supplement intakes are expressed, the pattern is clear; impact of supplement on length gain is only

TABLE 3

Slopes and standard errors of regression of supine length (mm) and weight (g) change per year[1] on supplement calories (100 kcal/d or 418 kJ)

Age (mo)	Supine length: Atole β^2	SE	Fresco β	SE	Total β	SE	Weight: Atole β	SE	Fresco β	SE	Total β	SE
3–12	9.1	1.6[a]	−22.2	18.9	9.2	1.5[c]	337	58[c]	528	680	338	52[c]
12–24	2.0	1.4	3.8	13.5	4.7	1.1[c]	126	54[a]	404	489	243	42[c]
24–36	3.2	1.0[b]	4.9	4.0	3.7	0.8[c]	21	42	272	170	60	34
36–48	0.3	0.6	1.8	3.5	0.8	0.7	7	32	−152	123	10	29
48–60	0.1	0.7	4.8	2.5	0.8	0.7	17	38	12	100	23	33
60–72	0.6	0.7	5.8	2.5[a]	1.4	0.7	28	37	132	107	28	34
72–84	−0.6	0.8	−0.5	2.2	−0.2	0.8	10	45	194	100	43	39

[1] t-test between Atole and Fresco: [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$.
[2] After controlling for initial body size, diarrheal disease, socioeconomic status, gender and, for all but the first and last intervals, energy from home diet.

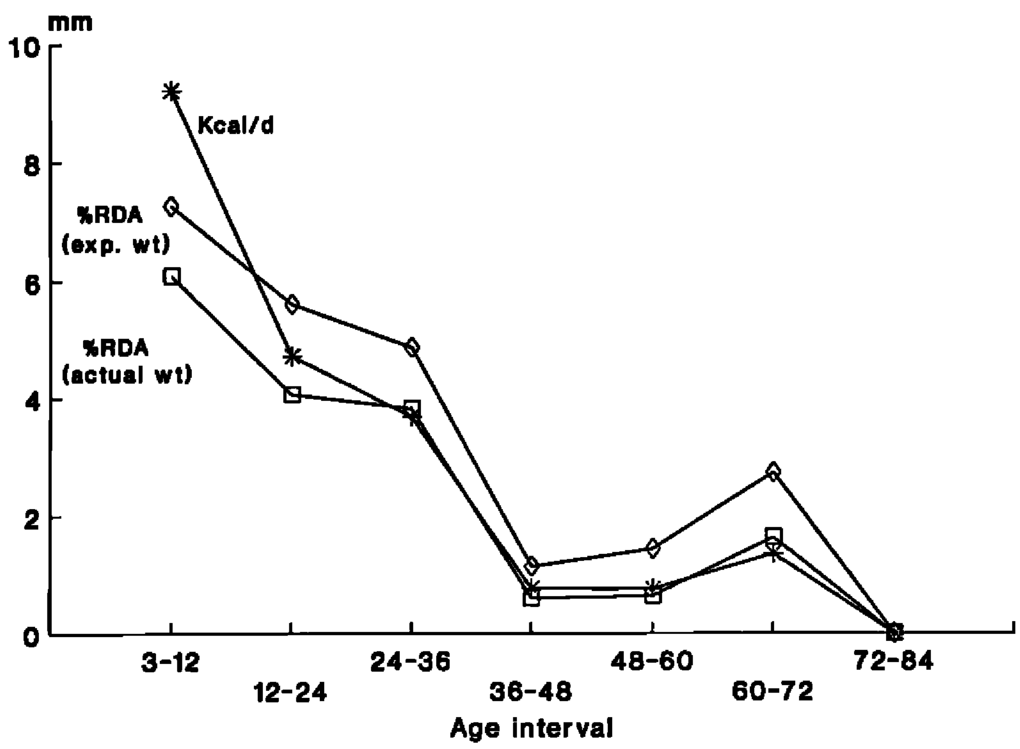

FIGURE 3 Slopes of length gain (millimeters/year) on supplement [100 kcal/d or 10% of RDA/d (calculated using both actual and expected weights)]. Adjusted for initial body size, diarrheal disease, socioeconomic status, gender and, in all but the first and last age intervals, home diet. To obtain kilojoules, multiply kilocalories by 4.184.

seen in the first 3 y of life. A similar pattern is seen with weight gain (**Figure 4**). An extra 100 kcals/d of supplement was associated with an extra 350 and 250 g in annual weight gain during the first and second year of life, respectively, with no significant benefit seen at older ages.

If percent of expected velocity compared with an external or internal reference is used as the dependent variable instead of actual velocity, again the pattern is similar (**Figure 5**). An extra 100 kcal/d of supplement was associated with about a 4–5% increase in expected velocity until the 24- to 36-mo interval using the external reference and slightly higher using an internal reference. Very similar results were obtained if supplement is expressed per 10% of RDAs (not shown).

A summary figure (**Figure 6**) that superimposes the group mean of the percent of time spent with diarrhea, length velocity deficit for the Fresco children compared with an external reference (Baumgartner et al. 1986), and the parameter estimates of supplement intake (100 kcal/d or 418 kJ) on length gain during each of the intervals, demonstrates the close relationship between these factors and the impact of supplement.

DISCUSSION

This paper's analyses, which relate supplement consumption to growth rates in individuals, should be considered jointly with others reviewed by Habicht, Martorell and Rivera (1995). Even though care was taken to control for potentially confounding factors, the present results, by themselves, are open to criticism because they rely on associations involving individual, ad-libitum consumption. Elsewhere, Habicht, Martorell, and Rivera (1995) provide results using the randomized design, with village as the unit of analysis, and show that the nutrition intervention caused the changes in physical growth observed. The present analyses, as well as others reviewed by these authors that involved explorations of "dose-response", complement the randomized design analysis and add strength to a causal inference. Moreover, the present analyses add important information about the age differences in the relationship between supplement intake and growth rates, aspects that could not have been explored with the data available using the randomized design.

Nutritional supplementation of Guatemalan children had the greatest impact on growth in the first 36 mo of life. Consumption of an additional 100 kcals/d of supplement resulted in ~4–9 mm additional gain in length during the first 3 y of life with greatest benefit seen in the first year. Between 3 and 7 y of age, nutritional supplementation had no significant impact on linear growth. Impact on weight gains was greatest during the first 2 y with little benefit thereafter.

If supplement was expressed as a percent of the recommended dietary allowances, the impact of 10% of RDA consumed as supplement during any of the first 3 y of life was approximately the same as consuming 100 kcal of supplement per day. Again, no significant impact on growth was observed after three years of age. Similar patterns were seen if the impact of supplement (either kilocalories/day or as percent of RDAs) was measured as the percent of expected, rather than actual, velocity.

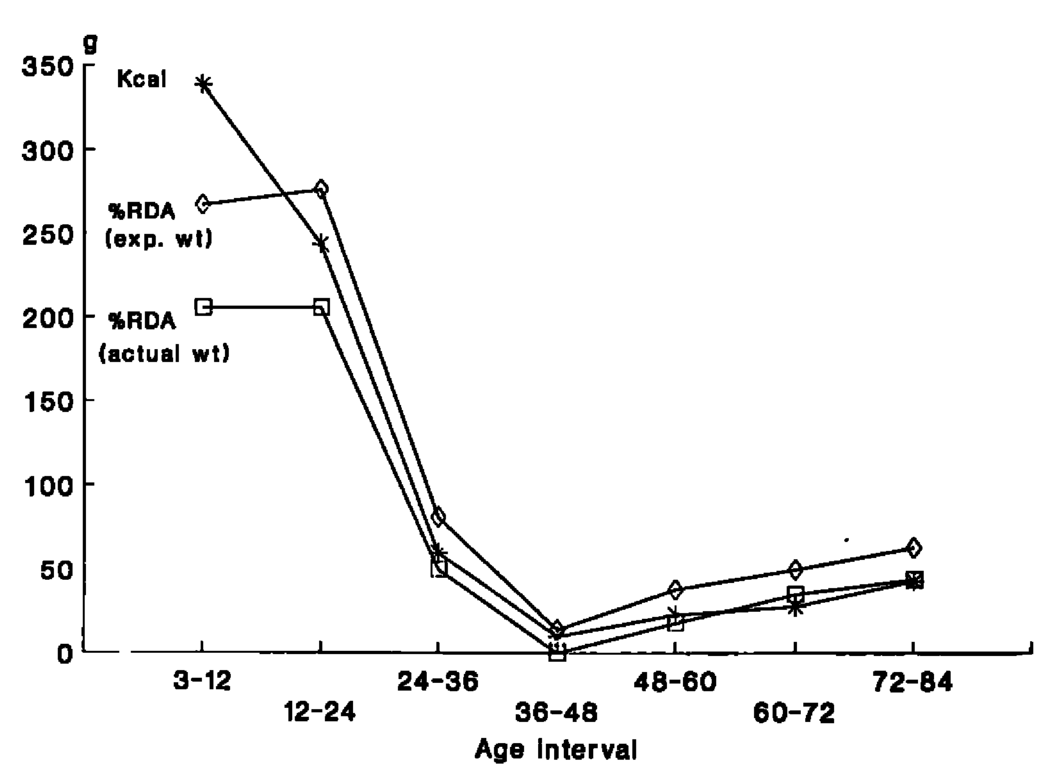

FIGURE 4 Slope of weight gain (grams/year) on supplement [100 kcal/d or 10% of RDA/d (calculated using both actual and expected weights)]. Adjusted for initial body size, diarrheal disease, socioeconomic status, gender and, in all but the first and last age intervals, home diet. To obtain kilojoules, multiply kilocalories by 4.184.

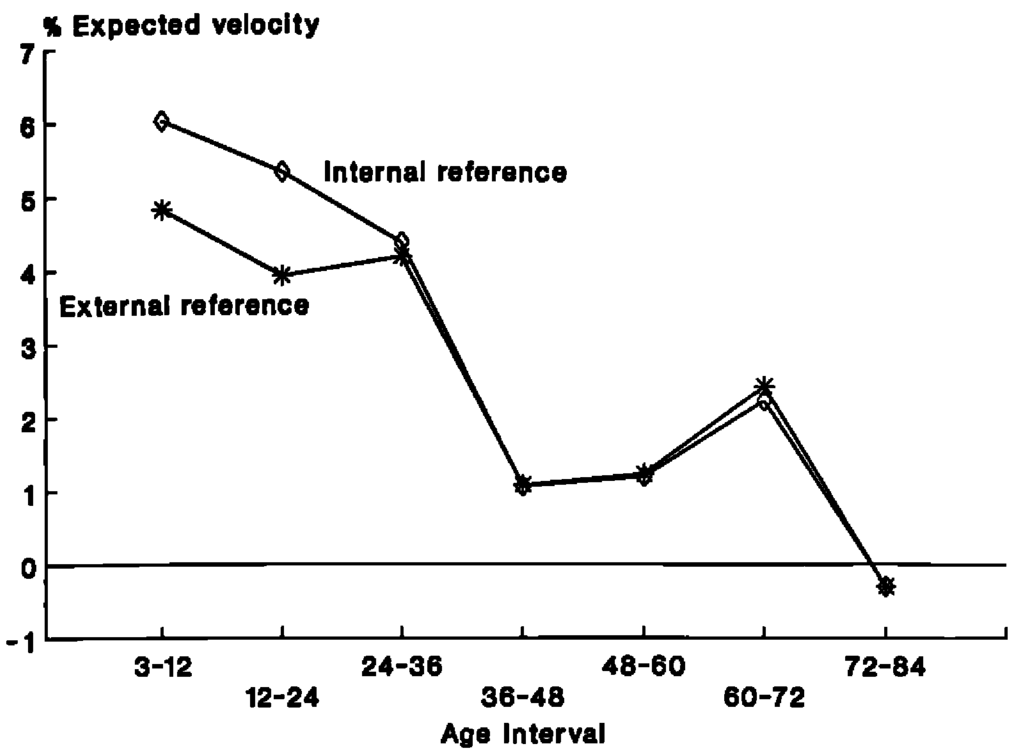

FIGURE 5 Slope of length gain as measured as a percent of expected velocity [calculated using both an external (Baumgartner et al. 1986) and internal reference on supplement (100 kcal/d)]. Adjusted for initial body size, diarrheal disease, socioeconomic status, gender and, in all but the first and last age intervals, home diet.

The results of this study are similar with others that have reported a stratified analysis of the impact of nutritional supplementation by age. Gopalan et al. (1973) found that 1- to 2-y-old Indian children who received ~170 kcal/d of supplement in the form of a sweet cake grew 2.8 cm more during 14 months compared with unsupplemented children. This difference was 1.7 cm for 2- to 3- and 3- to 4-year olds and 1.1 cm for 4- to 5-year olds. Lutter et al. (1990), comparing supplemented and unsupplemented Colombian children and examining generally shorter intervals, found that responsiveness of length and weight to supplementary feeding coincided with the initiation of weaning (3–6 mo of age) and the peak prevalence of diarrhea (9–12 mo). Using the same dataset used in the present analyses, Burger (1992) examined all 3-mo age intervals under two years and found that the 3-to 6-mo age interval was associated with the greatest impact of supplement on length gain.

The most likely explanations for these findings are associated with the relative magnitude of: growth potential as proxied by reference data, deficits in required energy intakes as proxied by the recommended energy intakes and infectious diseases, particularly diarrhea, during these years.

Linear velocity of well-nourished children is greatest during the first year of life at ~20 cm per year (Baumgartner et al. 1986). By the second and third years of life, the rate has declined to ~12 and 9 cm per year, respectively. Between the fourth and seventh years of life, healthy children grow at ~7 cm per year.

Though the lengths of the Guatemalan children in this study were near the 50th percentile at 15 d (Martorell et al. 1995), growth velocities were much less than the reference until age 36 mo. The magnitude of the deficits in length gain per year coincided with rates of greatest expected growth, with the biggest difference during the first year and diminishing until the third year. After age 36 mo, length velocities were approximately as expected. As seen in Figure 2, though consumption of Atole was able to make up some of the growth deficit during the first years of life, the majority of the deficit remained.

The deficits in growth velocity seen during the first 3 y of life are likely due, in part, to inadequate nutrient intakes. Investigations from other developing countries find that the complementary foods given during the weaning period are often insufficiently dense (Ashworth and Draper 1992) or too bulky (Ljungqvist et al. 1981) to provide adequate calories and nutrients to achieve proper growth. In the current study, the fact that supplementation with Atole had an impact on growth is evidence that nutrient deficiencies were limiting growth in this population. The other primary cause of reduced growth velocity seen during these ages is infectious disease.

The negative impact of infectious disease, particularly diarrheal illness, on growth has been well described (Black et al. 1984a, Martorell et al. 1975). In this, as in many populations in the developing world, the highest prevalence of diarrhea coincides with the weaning period when children are first exposed to pathogen containing foods (Black et al. 1982). In this population, children spent on average ~10–12 of every 100 d during their first 2 y with diarrhea, a rate that declined to ~5 d per 100 by the third year of life and about 1 d per 100 by age 7 y (Figure 6).

The effect of these high rates of diarrheal illness on supplement intakes and growth as well as the reverse relationships are complex. Though good nutritional status has been associated with slightly decreased du-

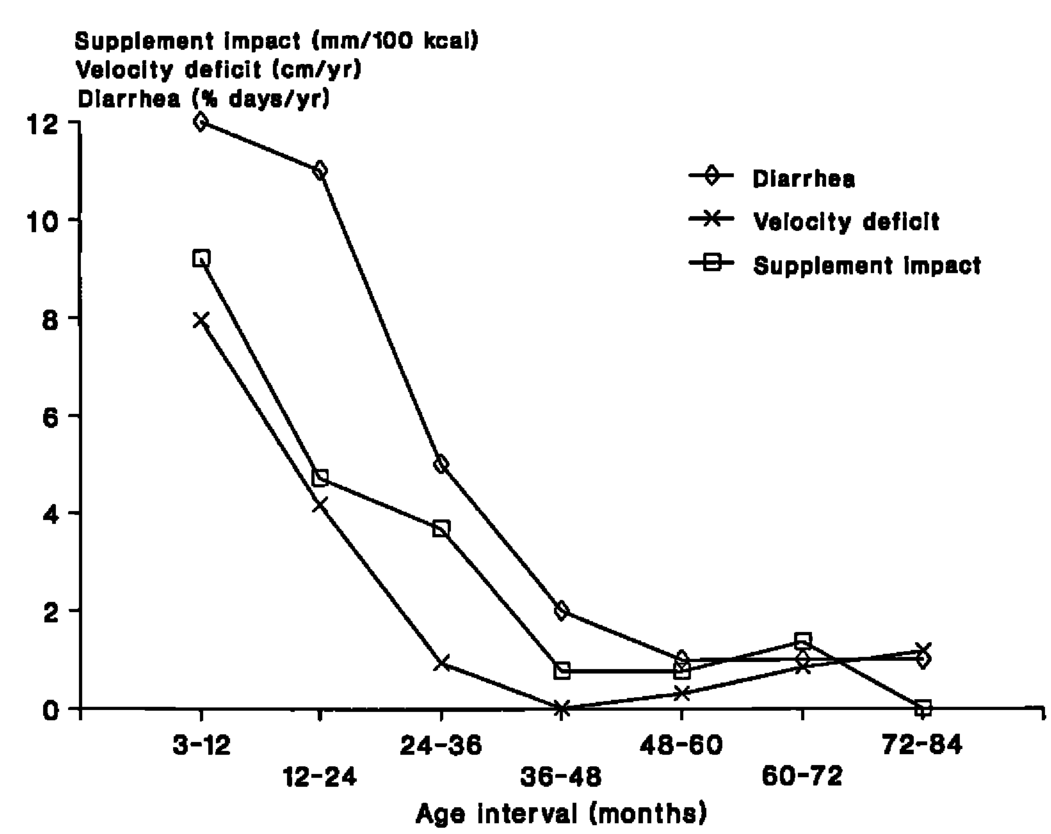

FIGURE 6 Percent of year spent with diarrhea, velocity deficit and adjusted slopes of supplement (100 kcal/d) by age interval.

ration (Black et al. 1984b; Tomkins et al. 1989) of diarrhea, previous analyses of these data did not find that supplementation itself decreased diarrhea prevalence (Rose and Martorell 1992). Nutritional supplementation has, however, been found to partially (Martorell et al. 1990), and nearly completely (Lutter et al. 1989), offset the negative effect of diarrheal illness on linear growth. A similar protective effect of supplementation against growth faltering was found in relation to measles (Gopalan et al. 1973).

The results from the current study, as well as those reported from Colombia (Lutter et al. 1990), suggest that supplementation is most efficacious in very young children. The issue then arises of what the lower age limit for recommending supplementation should be. In an analysis of the same Guatemalan data used here, Burger (1992) found that supplementation *before* 3 mo of age was associated with higher rates of diarrhea whereas children, especially girls, who began receiving supplement at between 3 and 6 mo had better growth rates compared with those who began receiving supplement after 6 mo. Though both this study as well as that from Colombia (Lutter et al. 1990) report benefits for children 3–6 mo of age, it is important to reiterate that these results are based on the use of supplements prepared and served under very hygienic conditions. Large-scale programs that provide supplement under less-controlled conditions may have different results.

The analyses presented here focus on a single component of nutritional supplement (energy) and anthropometric outcomes. Analyses were also carried out that use volume of supplement consumed to explore whether the effects observed could have been due to the nutrients added in equal concentration to the Atole and Fresco (i.e., iron, fluoride, thiamin, riboflavin, niacin, ascorbic acid and vitamin A; see Habicht et al. 1995 for greater details). When volume of supplement consumed was added to the regression models used to generate the combined Atole and Fresco results presented in Table 2, the statistical significance of the variable representing energy from supplement was far greater than that for volume during the first three yearly intervals. Only in the 60- to 72-mo age interval did the variable for volume show a similar level of impact relative to energy. Though these analyses are suggestive that the effects observed are more likely to have been caused by energy as opposed to the other nutrients in the supplements, the issue as to which nutrient or combination of nutrients were responsible remains unsettled. Protein, which the Atole but not the Fresco contained, also could have caused the changes observed. There is little evidence that the Fresco had a nutritional effect (Table 3) which enhances the case for a protein effect. However, the results could be ascribed just as easily to energy; so little energy was contributed by Fresco in the first 3 y of life that an effect would be unlikely, even if energy provided the explanation. Clearly, the fact that energy intakes from both supplements were not similar in children precludes the type of convincing analyses carried out in pregnant women where it was shown that energy and not protein provided a better explanation for the effects on birthweight (Habicht 1995). Additional discussion of the difficulty of differentiating the contribution of energy and other nutrients is found elsewhere in this volume (Habicht et al. 1995). Although there is uncertainty about the nutrient or nutrients responsible for the effects observed, the analyses presented here, together with those given by Habicht, Martorell and Rivera (1995), leave no doubt that ingestion of the Atole caused children to grow better.

Linear growth was of most interest in these analyses because the Guatemalan population is heavily stunted but not wasted. Patterns of wasting and stunting vary drastically throughout the world (Victora 1992); age-specific responsiveness to nutritional supplementation is also likely to vary. In populations where wasting is the main concern, analyses may prioritize examining the age-specific impact of nutritional supplementation on weight gain.

Finally, there are certainly other benefits of nutritional supplementation besides growth (Beaton 1993). Supplementation has been shown to positively affect functional capabilities such as activity level, literacy, and school performance (Pollitt and Gorman 1990)—outcomes that might be most responsive to nutritional supplementation in older rather than younger childhood.

ACKNOWLEDGMENTS

The authors thank Dr. Edward Frongillo of Cornell University for his assistance in developing the analytical strategy used in this paper.

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Patterns of Linear Growth in Rural Guatemalan Adolescents and Children[1,2]

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ABSTRACT Length and weight data from a longitudinal study of rural Guatemalan subjects birth to 7 y of age and height and weight data from a cross-sectional study of the same subjects when they were 11–24.9 y old are compared to reference data for the USA general population and for Mexican-Americans. At birth, the median length of Guatemalan children is at ~ the 16th percentile of the USA reference or ~2 cm shorter. By 6 mo of age, Guatemalan children are shorter, on average, than the 5th percentile of the reference curves and, in absolute terms, are ~5 cm below the median; by 3 y, the difference increases to ~10 cm. As adults, Guatemalans have about the same absolute level of deficit (~13 cm) as they did at age 3 y. If the general USA population is used for comparison, Guatemalans can be said to grow as expected during adolescence, neither recuperating the growth retardation of early childhood nor falling further behind in size. If the Mexican-American sample is selected instead, it would appear that some catch-up in growth occurs in Guatemalan adolescents. Regardless of the choice of reference population, growth is markedly retarded only in early childhood; adolescence is not a period when growth is significantly constrained. J. Nutr. 125: 1060S–1067S, 1995.

INDEXING KEY WORDS:

- *growth* • *anthropometry* • *adolescence*
- *rural Guatemala*

While much is known in developing countries about growth in early childhood and the factors that shape its course, knowledge about growth during later childhood and adolescence is limited. Research to date indicates that growth failure and nutritional stress in poor children from developing countries are greatest in the first 2–3 y of life (Beaton et al. 1990). Growth during later childhood and adolescence in these same societies appears to be considerably less constrained, if at all (Martorell et al. 1994). Some argue that adolescence is a time when the growth retardation produced in early childhood may be recuperated (Delgado et al. 1987). However, the research to support the claim that catch-up growth can occur to a significant degree during adolescence is weak (Martorell et al. 1994). The objective of this paper is to compare patterns of growth of poor Guatemalans during childhood and adolescence to both a racially similar population of Mexican-Americans and a general USA reference. Several key questions guide the analyses: what are the periods in life in rural Guatemala when significant growth failure occurs? Specifically, is adolescence a time of constrained growth or instead, is it a period when some of the growth failure of earlier childhood is overcome by compensatory growth?

MATERIALS AND METHODS

The subjects included in this study were participants of a longitudinal study conducted by the Institute of Nutrition of Central America and Panama (INCAP) in four villages of eastern Guatemala. The study took

[1] Published as a supplement to The Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Data collection and analyses were supported by NIH grant HD22440 and by the International Center for Research on Women (ICRW), Contract # IC-75/03.

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place between 1969 and 1977 in four Ladino (i.e., Spanish speaking, mixed Spanish/Amerindian population) villages. The objectives of the study were to test the effects of improved nutrition achieved through food supplementation on physical growth and mental development. The groups under intensive monitoring were pregnant and lactating women and all children seven years of age and less. Details about the methods of this longitudinal study are found elsewhere (Martorell et al. 1995). Briefly, weight was measured on a beam scale to the nearest 10 g with children wearing a light shift, the weight of which was later subtracted. Supine lengths were measured using a standard measuring table to the nearest 0.1 cm; no standing heights were taken on children <7 y of age.

In 1988–89, a cross-sectional, follow-up study was carried out which included data on height and other measures of linear growth, weight, frame size, circumferences and skinfold thicknesses (Martorell et al. 1995). This article emphasizes the height and weight data. Standard measurement techniques were used (Lohman et al. 1988). Weight was measured to the nearest 100 g using a beam balance scale (Health O Meter). Subjects were given a light shift to wear and shoes were removed. A portable anthropometer in free-standing mode was used to measure height to the nearest 0.1 cm. The body mass index (BMI) was estimated as weight (kilograms) over height squared (meters^2).

The anthropometric data are compared with three reference populations. The first is the United States National Center for Health Statistics/World Health Organization (NCHS/WHO) reference, which is widely used by researchers throughout the world (WHO, 1983). For length data from birth to 36 mo, this reference is based on data collected from children from the Fels Research Institute's (Ohio) longitudinal study of 867 white, middle-class children (Dibley et al. 1987a, Hamill et al. 1979). Height data from 2 to 18 y are from national surveys carried out by NCHS. The appropriateness of these data as a reference (Dibley et al. 1987b) and their comparison with other references are discussed elsewhere (Stephenson et al. 1983).

For adolescents and young adults, a reference based on the first (1971–74) and second (1976–80) National Health and Nutrition Examination Survey (NHANES) published by Frisancho (1990) is used. The NHANES surveys included representative samples of subjects in the United States and as such, reflected the ethnic diversity of the country in the 1970s. For the most part, however, subjects included in these surveys were of European origin; in the combined data set used by Frisancho, 78% of subjects were Caucasian (Frisancho 1990). The reasons for using Frisancho's (1990) reference data for comparison are that percentile values are provided for adults and that additional anthropometric variables (e.g., BMI) are presented. For ages presented in both the Frisancho and NCHS/WHO references, values are very similar. This similarity is not unexpected because both include a common data source, NHANES I; also, average stature did not differ in NHANES I and II. The reason for not using only the NCHS/WHO (WHO 1983) curves is that they do not provide data after age 18 y or for variables other than height and weight. A disadvantage of Frisancho's curves is that the data were not smoothed and this may lead to some irregularity.

A reference population of similar ancestry as the study population is also used for comparison. The reference population selected for this purpose is that of Mexican-Americans measured in the Hispanic HANES of 1982–84 (Najjar and Kuczmarski 1989). Although poverty may have constrained growth, Mexican-Americans are undoubtedly much better off than rural Guatemalans. In fact, the growth patterns of Mexican-Americans are strikingly similar to those of well-to-do samples from the cities of Mexico and Guatemala (Johnston et al. 1973, Johnston et al. 1976, Martorell et al. 1989, Ramos-Galván 1975).

Subjects from all four villages included in the original study are pooled in the analysis because the purpose is to describe general differences in linear growth with respect to the reference populations selected. Differences between Guatemalans and the reference populations are overwhelmingly larger than intervillage differences (see Rivera et al. 1995 for examination of these differences). For the later ages, the study also combines, for the same reason, migrants and nonmigrants (Martorell et al. 1995). The follow-up study was able to collect data on 73% of former participants; coverage was greater for village residents (89%) than for migrants (41%).

For the analyses of child growth, data on 1232 boys and 1160 girls are included in this paper. Because of the longitudinal nature of the earlier study, individual subjects may be represented at more than one age interval.

In 1988–89, the subjects ranged from 11 to 27 y of age (born between 1962 and 1977). Presented in this paper are data for 786 males and 719 females 11.0–24.9 y in age; subjects 25–27 y are excluded to conform with the Frisancho's age groupings (1990), which were yearly to 17 y (e.g., 17.0–17.9), and at longer intervals thereafter (i.e., 18.0–24.9 y, for which we have subjects in the entire age range; 25.0–29.9 y, for which we lack subjects 28–29.9 y).

Mean and median values by age and sex are given in tabular form in this paper only for the rural Guatemalan sample. Medians for Guatemalan and Mexican-American samples are plotted relative to the percentile distribution in the USA reference population. Results of formal statistical tests are not presented because the differences of interest in this study are extremely large and often statistically significant. Rather, readers are encouraged to assess the magnitude of differences relative to the percentile distribution.

TABLE 1

Length or height[1] (cm) of Guatemalan children, adolescents and young adults

	Males				Females			
Age (y)	n	Median	$\bar{x}$	SD	n	Median	$\bar{x}$	SD
0.04[2]	453	50.4	50.2	2.5	409	49.5	49.3	2.3
0.25	507	58.2	58.0	2.5	461	56.7	56.5	2.4
0.5	508	63.5	63.4	2.5	448	62.0	61.8	2.3
0.75	511	67.0	66.7	2.7	450	65.3	65.1	2.5
1.0	504	69.7	69.4	3.0	453	68.0	67.8	2.8
1.5	494	74.4	73.9	3.4	429	72.5	72.4	3.1
2.0	492	78.5	78.2	3.6	427	76.8	76.6	3.4
2.5	466	82.5	82.4	3.7	428	81.0	80.6	4.2
3.0	472	86.4	86.2	3.9	407	85.2	84.7	4.0
3.5	472	89.0	88.9	3.9	391	87.7	87.7	4.1
4.0	466	92.5	92.4	4.1	398	91.2	91.1	4.3
5.0	407	99.0	98.6	4.2	384	97.5	97.6	4.6
6.0	370	104.8	104.4	4.3	357	103.5	103.4	4.5
7.0	339	110.0	110.0	4.2	326	109.0	109.0	4.7
11.0–11.9	61	132.8	132.6	5.6	64	135.0	135.7	7.7
12.0–12.9	72	135.8	137.1	6.9	55	141.3	139.5	5.8
13.0–13.9	67	141.5	142.9	7.9	71	145.6	145.3	5.6
14.0–14.9	62	149.2	147.6	8.1	68	146.6	146.1	5.6
15.0–15.9	58	156.9	155.5	9.4	58	149.8	149.7	5.6
16.0–16.9	82	161.1	159.6	7.6	64	149.3	149.5	4.8
17.0–17.9	64	160.2	160.2	5.3	62	149.4	149.6	5.2
18.0–24.9	281	162.6	162.5	5.6	318	150.4	150.4	5.7

[1] Supine length was measured until 7 y of age; 1 cm was subtracted from length values in children ≥24 mo to approximate height values used in the reference population. Standing height was measured at 11–25 y. Measurement before age 7 y was at exact ages.
[2] Measured at 15 days of life.

RESULTS

Data for length or height, weight and BMI for the Guatemalan sample are given in **Tables 1-3**, respectively. Median lengths or heights by age for the Guatemalan and Mexican American populations are plotted together with the 5th, 50th and 95th percentile values for the USA reference population in **Figures 1** and **2** for children 0–36 mo and subjects 11–25 y old, respectively. At 15 days, the median length of Guatemalan males is at ~ the 16th percentile of the NCHS/WHO reference, declining to less than the 5th percentile by nine months of age and to less than the 3rd percentile by 36 mo. Values remain at the same relative position at ages 3–7 y of age (not shown). Mexican-American boys, on the other hand, are at the 50th percentile at 9 mo (measures at younger ages not available), maintaining this level throughout childhood.

During adolescence, the median height of Guatemalan males remains at below the fifth percentile of the USA reference population. The median height of Mexican-American males is still near the 50th percentile during early adolescence but declines to around the 25th percentile in adulthood. Patterns are similar for females at all ages and for both the Guatemalan and Mexican-American populations.

Absolute differences in length among the populations are shown in **Figure 3** for 0- to 36-mo-old males and females. Differences between Guatemalan and the NCHS reference (noted as Guat-NCHS in the legend) are 2.2 cm at 15 days, ~6.5 and 8 cm by 12 mo and ~10 and 11.5 cm by 36 mo for males and females, respectively. Differences between the Mexican-American population and the NCHS/WHO reference (noted as Mex-NCHS) are negligible throughout young childhood. At 11 y of age, the difference between the Guatemalan and USA samples (Guat-USA) is at ~12.5 and 13.5 cm for males and females, respectively, only slightly greater than it was at 3 y (**Figure 4**). For males, differences increase between ages 13 and 15, but are at ~13 cm again by adulthood for both sexes. Differences in height during adolescence and adulthood between Mexican-American and USA medians (Mex-USA) suggest a different pattern (Figure 4). For both males and females, differences are minimal at 11 to 13 y of age, but increase thereafter so that at adulthood Mexican-Americans are ~6 cm shorter.

Patterns of growth for other anthropometric dimensions also have been examined. In terms of sitting height and bone diameters, patterns generally follow those observed for length and height. However, for bicristal breadth, there is substantial catch-up in late adolescence, particularly in females. On the whole, rural Guatemalans have smaller limb circumferences and reduced skinfold thicknesses than the reference

TABLE 2

Weight (kg) of Guatemalan children, adolescents and young adults

	Males			Females		
Age (y)	Median	x̄	SD	Median	x̄	SD
0.04[1]	3.4	3.4	0.5	3.2	3.3	0.5
0.25	5.7	5.6	0.8	5.2	5.2	0.7
0.5	7.0	7.0	1.0	6.5	6.5	0.9
0.75	7.7	7.6	1.0	7.2	7.1	1.0
1.0	8.1	8.1	1.1	7.5	7.5	1.0
1.5	8.9	9.0	1.1	8.5	8.4	1.0
2.0	10.0	10.0	1.1	9.4	9.4	1.1
2.5	11.1	11.1	1.2	10.5	10.5	1.3
3.0	12.1	12.1	1.3	11.4	11.5	1.4
3.5	13.0	13.1	1.4	12.4	12.5	1.4
4.0	13.9	13.9	1.4	13.1	13.3	1.5
5.0	15.4	15.4	1.6	14.7	14.9	1.6
6.0	16.9	16.9	1.7	16.2	16.2	1.7
7.0	18.7	18.8	1.8	17.9	18.2	2.0
11.0–11.9	29.0	28.9	3.5	30.4	31.0	5.9
12.0–12.9	31.0	31.5	4.6	34.1	33.8	5.3
13.0–13.9	34.4	35.1	5.5	39.6	40.1	6.4
14.0–14.9	39.1	38.6	6.0	41.6	41.1	6.2
15.0–15.9	46.2	45.7	7.1	45.2	46.0	5.6
16.0–16.9	50.0	49.4	6.1	48.0	48.5	6.1
17.0–17.9	52.2	51.7	5.6	48.2	49.5	6.8
18.0–24.9	55.4	56.3	6.5	49.2	50.4	7.9

[1] Measured at 15 days of life.

populations, particularly in males. Median BMI values by age are shown for adolescent and adult Mexican-Americans and Guatemalans relative to USA percentiles in **Figure 5** and **6** for males and females, respectively. The Guatemalan population, particularly in the case of males, is the leanest. Guatemalan males have values that are between the 50th and 5th percentiles. Older Guatemalan female adolescents and young women, on the other hand, have BMI values which are near the 50th percentile of the US population. Mexican-Americans of both sexes have values which are at or slightly above the 50th percentile.

DISCUSSION

A major consideration in any discussion of the results presented above is the choice of reference population. The ideal population to use would be one with similar growth potential as the Guatemalan population but living in an environment that does not constrain growth. Whether the Mexican-American population is the appropriate choice is not entirely clear. Just as Guatemalans, Mexican-Americans are of Spanish-Indian ancestry, though differences may exist in the proportions of admixture. Mexican-Americans are one of the tallest Hispanic populations reported in the literature (Martorell et al. 1989) and are as tall as well-to-do populations from Guatemala (Johnston et al. 1973, Johnston et al. 1976) and Mexico City (Ramos-Galván 1975). Before adolescence, Mexican-Americans are as tall as the general USA population suggesting unconstrained growth but important differences are observed during adolescence (Figure 2). One explanation might be that differences are due to genetic factors that lead to a faster growth tempo in Mexican-Americans and, hence, to a shorter duration of the adolescent growth phase. On the other hand, sexual maturation data for Mexican-Americans from HHANES do not suggest precociousness relative to patterns observed in European longitudinal studies (Villareal et al. 1989). But, data of this type are notoriously difficult to collect reliably and cross-study comparisons may be flawed. Also, the lack of USA reference data for sexual maturation is a serious limitation.

Two of the sets of reference data (i.e., NCHS/WHO, Frisancho) used in this report are derived from cross-sectional data and are subject to biases introduced by cohort effects. Periodic surveys reveal that average heights in the general US population have not changed for some time and hence these biases are unlikely to be important in the US data of Frisancho (1990). In the case of Mexican-Americans, marked changes in stature have occurred over time (Martorell et al. 1989). It is not clear whether future national surveys will reveal differences with respect to HHANES data, now 8–10

TABLE 3

Body mass index (kg/m²) of Guatemalan children, adolescents and young adults

	Males			Females		
Age (y)	Median	x̄	SD	Median	x̄	SD
Birth	13.4	13.4	1.4	13.3	13.3	1.5
0.25	16.6	16.5	1.8	16.2	16.1	1.7
0.5	17.4	17.3	1.8	17.0	17.0	1.6
0.75	17.0	17.1	1.6	16.7	16.8	1.5
1.0	16.7	16.7	1.5	16.4	16.4	1.4
1.5	16.4	16.3	1.3	16.0	16.0	1.3
2.0	16.3	16.4	1.1	16.0	16.0	1.2
2.5	16.3	16.3	1.2	16.0	16.1	1.4
3.0	16.2	16.3	1.2	15.9	16.0	1.2
3.5	16.4	16.5	1.2	16.1	16.2	1.3
4.0	16.2	16.3	1.1	15.8	16.0	1.2
5.0	15.7	15.8	1.1	15.5	15.6	1.2
6.0	15.5	15.5	1.0	15.2	15.3	1.0
7.0	15.6	15.5	1.0	15.2	15.3	1.0
11.0–11.9	16.2	16.4	1.4	16.5	16.7	1.8
12.0–12.9	16.6	16.7	1.3	17.2	17.2	1.8
13.0–13.9	17.2	17.1	1.2	18.8	18.9	2.3
14.0–14.9	17.6	17.6	1.4	19.3	19.2	2.5
15.0–15.9	18.5	18.8	1.5	20.2	20.5	1.7
16.0–16.9	19.3	19.4	1.5	21.6	21.7	2.4
17.0–17.9	20.2	20.1	1.6	21.8	22.1	2.6
18.0–24.9	20.9	21.3	2.1	21.8	22.3	3.2

[1] Measured at 15 days of life.

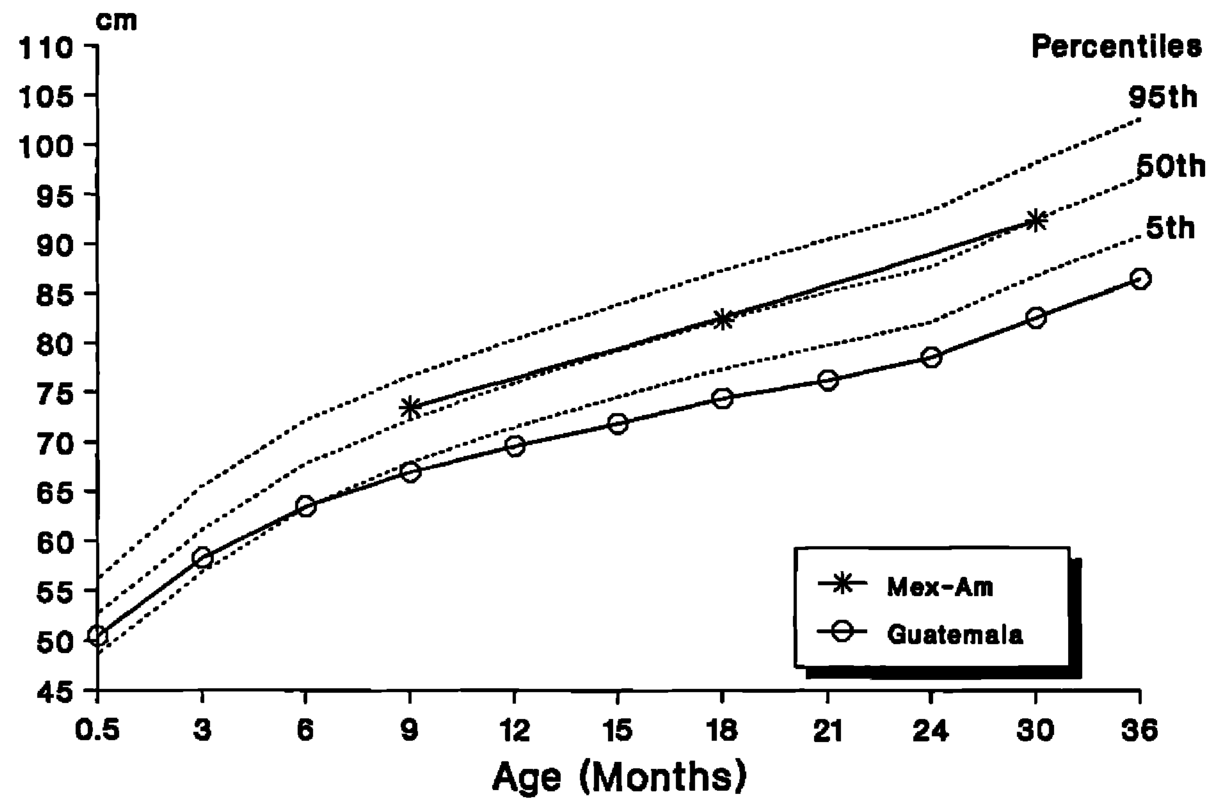

FIGURE 1 Median length in males 0.5–36 mo old: rural Guatemalans and Mexican-Americans compared with reference population [United States National Center for Health Statistics/World Health Organization (NCHS/WHO) 1983].

y old. Cohort effects could invalidate conclusions about growth during adolescence that are derived from cross-sectional studies, as in this case. Suppose that Mexican-American adolescents examined in HHANES grew poorly in early childhood because of poor diets and infection. Also, suppose that conditions changed such that young Mexican-American children measured in HHANES were not constrained in their growth. If these conditions apply, the patterns of adolescent growth that are inferred from the HHANES cross-sectional data would be erroneous. Adolescents would be short, not because of poor growth during adolescence, but because of early childhood retardation and estimates of age at peak height velocity would be biased downward. The same concern applies to the studies of well-to-do populations in Guatemala (Johnston et al. 1973, Johnston et al. 1976) and Mexico City (Ramos-Galván 1975), particularly in view of the fact that the data were collected at least two decades ago. Cohort-like effects also may be produced by migration patterns. The Mexican-American data set includes an unknown proportion of Mexican-born children. If immigration rates were greater for adolescents than for young children, the pattern shown in Figure 4 also could be produced under certain conditions (e.g., if adolescents born in Mexico were subjected to greater nutritional stress and growth retardation than native born adolescents). However, we have no evidence for differential immigration by age.

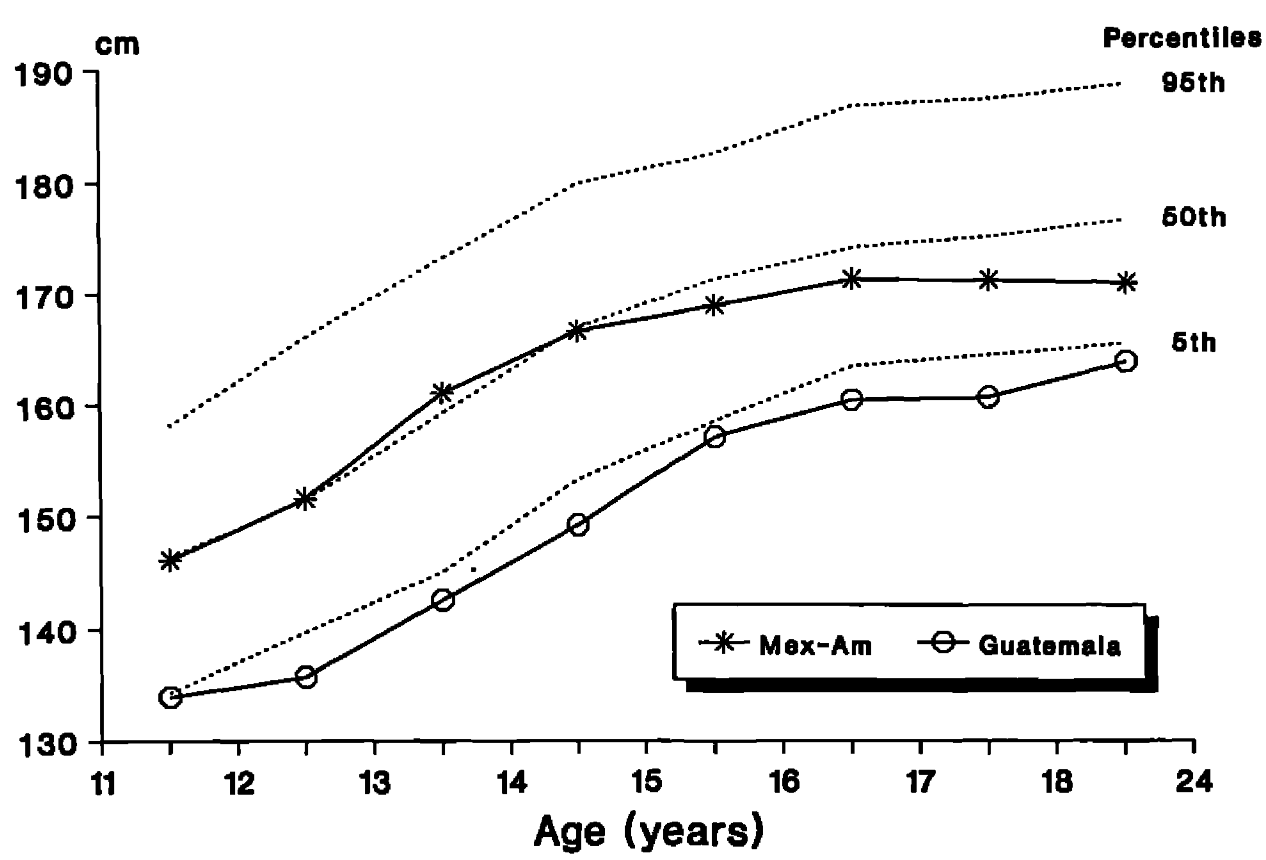

FIGURE 2 Median height in males 11–25 y old: rural Guatemalans and Mexican-American compared with USA reference population (Frisancho 1990).

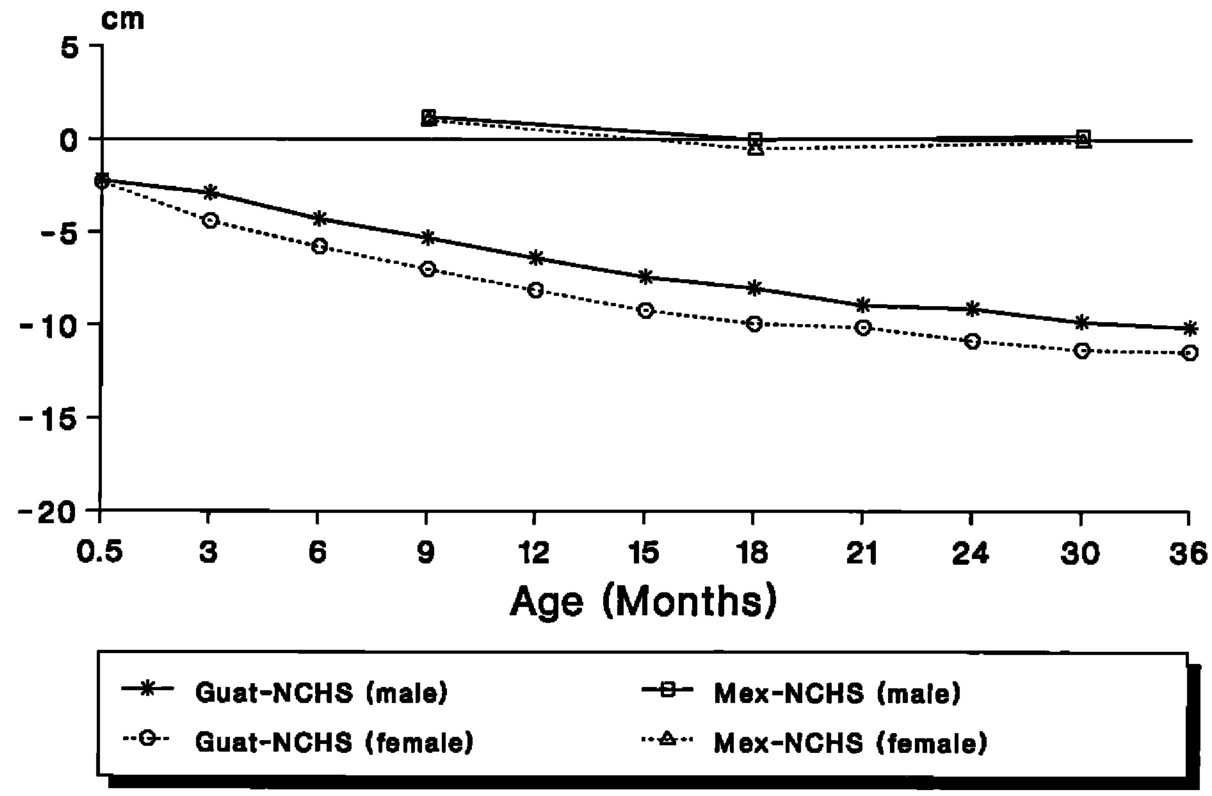

FIGURE 3 Differences in median length by age and sex for 0.5-to 36-mo olds.

At this time, it is not clear which of the two reference populations is most appropriate for assessment of the growth of rural Guatemalans. For assessment of growth during childhood, either reference may be used because patterns exhibited by Mexican-Americans are nearly identical to those of the general US population. Guatemalan children are born retarded in length and begin experiencing additional retardation shortly after birth. Both feeding practices as well as increased frequency of illness are the likely proximate determinants for this further decline. Though breastfeeding is nearly universal in this population, *exclusive* breastfeeding, clearly shown to reduce the risk of infectious illness (Brown et al. 1989), is rarely practiced. In addition, during the second half of infancy, complementary foods are generally inadequate in both quantity and quality to meet requirements.

The infant-feeding practices of the populations upon which the references are based should also be considered. Even under industrialized country conditions, growth patterns differ between breast-fed and bottle-fed infants with bottle-fed infants showing generally greater growth, more so in weight than in length, compared to exclusively breastfed infants (Waterlow 1988). Notably, the Fels children were primarily bottle fed with 85% completely weaned by 3 mo of age (Roche et al. 1989).

In the adolescent period, it is less clear whether Guatemalan growth patterns should be compared with the Mexican-American or USA general reference. If

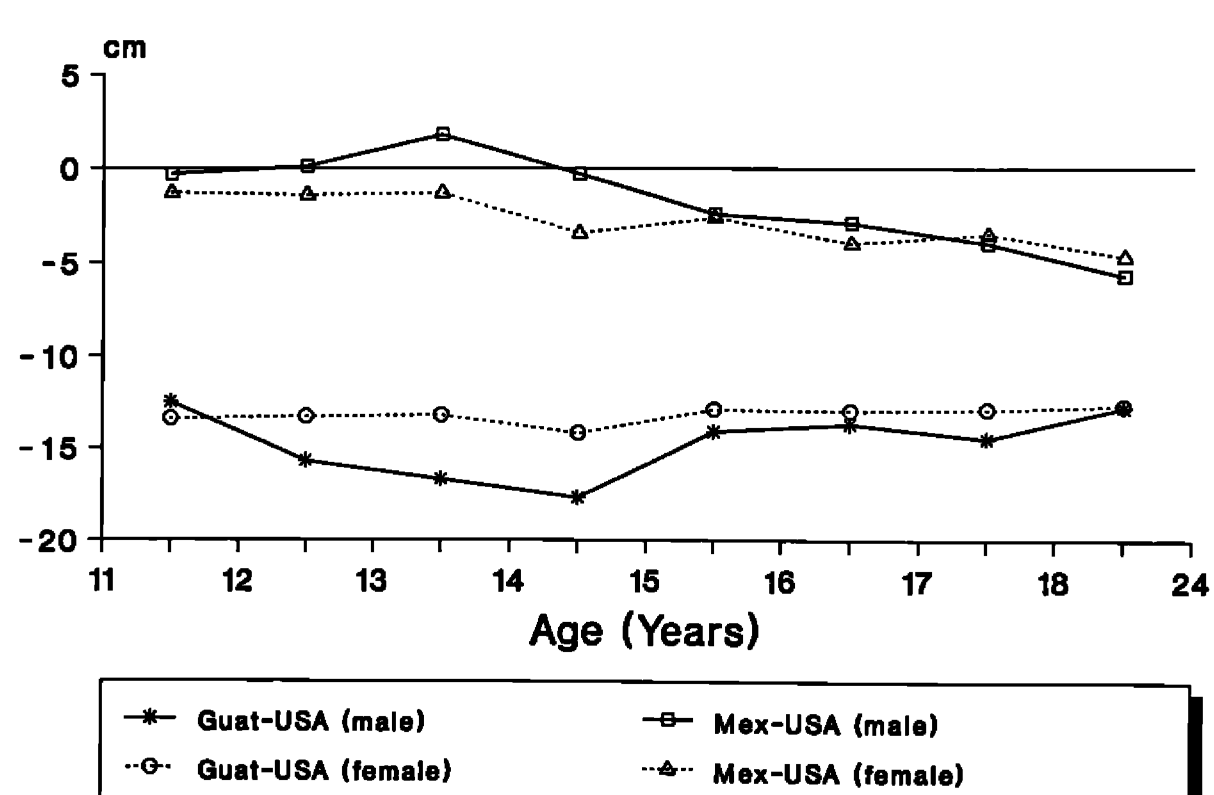

FIGURE 4 Differences in median height by age and sex for 11-to 25-y olds.

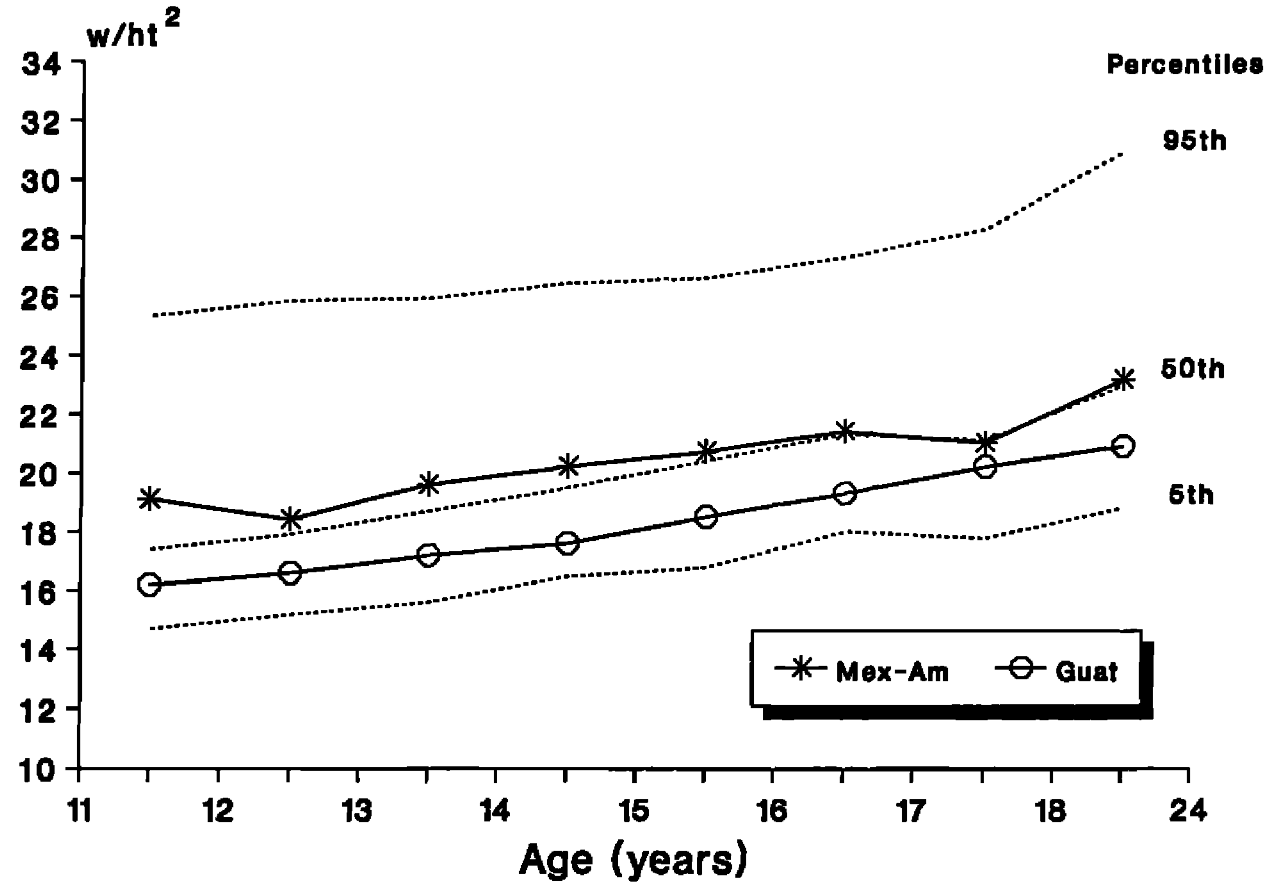

FIGURE 5 Median body mass index in males: rural Guatemalans and Mexican-Americans compared with USA reference population (Frisancho 1990).

the USA data are used as the reference population, one would conclude that absolute linear growth during adolescence is as expected. If Mexican-American data are more appropriate as a reference population, one would be further impressed by the extent to which some of the losses in early childhood are made up during adolescence, perhaps through delayed maturation and longer growth periods (see Martorell et al. 1994 for a discussion of this mechanism). Whether nutrition interventions aimed at adolescents might be helpful in promoting even greater compensatory growth is not clear because these efforts also may accelerate maturation with the unwanted result of reducing growth potential (Proos et al. 1991).

Regardless of the choice of reference population, it is clear that growth is markedly retarded in rural Guatemalans only in early childhood. Adolescence is certainly not a period in life when growth is constrained to a significant degree. In poor Guatemalans, therefore, stature in older children and adults indicates the degree to which growth was constrained in early childhood. Referring to height for age as an indicator of "chronic malnutrition" has brought about much confusion because it may be taken to mean that the process is still

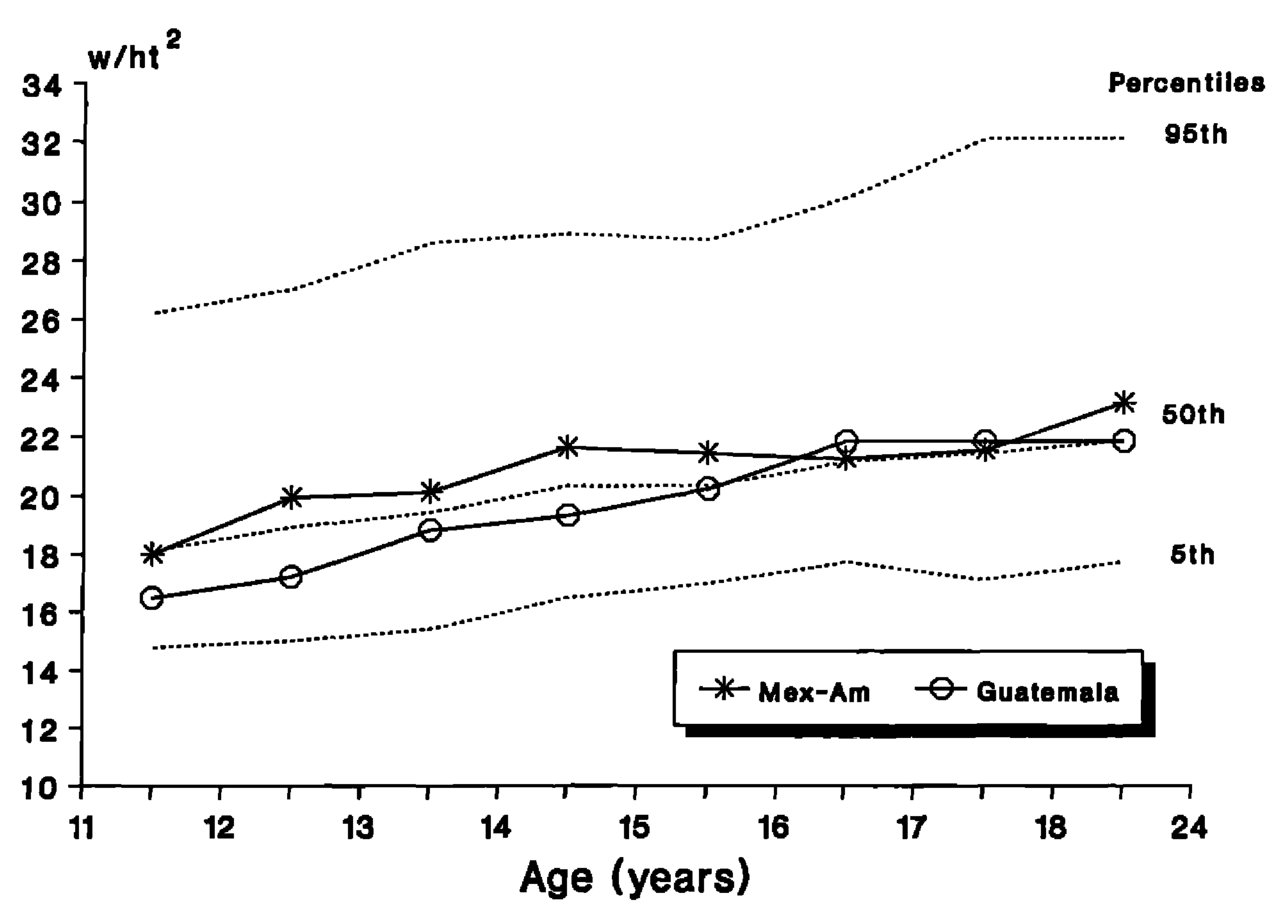

FIGURE 6 Median body mass index in females: rural Guatemalans and Mexican-Americans compared with USA reference population (Frisancho 1990).

continuing. Rather, as noted by Beaton et al. (1990), length for age in the first 2–3 y of life can be said to mark the state of "failing to grow" in early childhood, particularly if repeated measures are taken, whereas in older subjects, height for age marks the state of having "failed to grow". Height for age data at school entry, for example, would be a useful but lagged indicator of nutritional status, in that it reflects conditions at about 4 or more y previously (i.e., when the children were <3 y old).

The patterns of linear growth observed in Guatemala may differ from those common to other areas of the developing world. Skeletal age assessments, made relative to British reference data, indicate a delay of ~1.2 y in boys 11–14 y but none in girls of the same age (Pickett et al. 1995). This may explain the pattern shown in Figure 4; differences in girls with respect to the reference do not vary much with age. In boys however, differences increase temporarily at ages 12–14 y, which suggests earlier ages at peak height velocity in the reference population than in the study sample. Growth in height appears to continue to 19–20 y of age in males (analysis not shown) but little growth takes place in females after 15 y of age.

Menarche occurs at 13.7 y in the study sample, a year or so delayed when compared with US values (Khan et al. 1995). Thus, there is an apparent contradiction between the skeletal age (no delay) and menarche (1-y delay) results in girls but it is important to point out that both assessments of maturation indicate that delays are not severe. In other areas of the world, maturation may be more delayed and a prolonged period of linear growth, for example, might allow for substantial recuperation of the height retardation incurred in early childhood (Martorell et al. 1994).

Our relative ignorance of growth during adolescence in developing countries reflects the long-standing focus of attention on young children, who are at greatest risk of malnutrition. Although the priority remains unchanged, particularly for public health measures, greater attention needs to be given to research on adolescence, if only to understand the full impact of malnutrition in early childhood.

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Nutritional Supplementation during the Preschool Years Influences Body Size and Composition of Guatemalan Adolescents[1,2]

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ABSTRACT Effects of supplementary feeding during early childhood on body size and composition at adolescence are examined in a population with marked growth failure in the first 3 y of life. The data came from a supplementation trial conducted in rural Guatemala from 1969 to 1977 and a 1988–89 follow-up study of the same subjects at adolescence. Two pairs of villages participated in the trial. One village from each pair received a high protein-energy supplement (Atole), which significantly improved dietary intakes, whereas the other village of the pair received a low-energy, no-protein supplement (Fresco), which did not impact appreciably on dietary intakes. Children from Atole villages grew better during the preschool period than children from Fresco villages. At adolescence, subjects from Atole villages were taller, weighed more and had greater fat-free masses than subjects from Fresco villages. Differences in height at adolescence were slightly reduced in magnitude relative to differences at 3 y of age. However, differences in weight were increased in adolescence relative to 3 y of age. J. Nutr. 125: 1068S–1077S, 1995.

INDEXING KEY WORDS:

- *supplementation* • *growth* • *height*
- *adolescence* • *rural Guatemala*

In a review of controlled supplementation trials, clear effects of supplementary feeding on growth were found in populations with evidence of growth retardation when the dietary intakes of young children were truly improved (Habicht and Butz 1979, Rivera 1988). On the other hand, the long-term effects of community-based supplementation programs during early childhood on the growth and body composition at adolescence or adulthood have not been studied.

This article examines effects of supplementary feeding during early childhood on body size and composition at adolescence in a population where effects of supplementation on growth rates were observed during the first 3 y of life but not from 3 to 7 y of age (Martorell et al. 1982, Schroeder et al. 1995). It remains to be shown whether these improvements in growth persist into adolescence.

Tanner (1986) has described human growth as a target-seeking function. In his view, children have their own natural growth trajectories; when deviations occur, restoring forces develop to return children to their original growth curves. Growth after 3 y of age in rural Guatemala is not significantly constrained (Martorell et al. 1995b) and thus, it may be possible for the differences in size in favor of supplemented children observed at 3 y of age to be reduced through faster growth subsequently in nonsupplemented children. In addition, nonsupplemented children may have a greater potential for growth than supplemented children because of delayed maturation. Martorell et al. (1979) found that nonsupplemented children were less mature at 3 y of age than supplemented children in this population. Less mature children, in turn, may have

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-Term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Data analyses were supported by NIH Grant HD22440 and by grant 92–02716–000 from the Pew Charitable Trusts.

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a more prolonged subsequent growth period that could compensate for some of the growth failure incurred in early childhood.

The hypothesis tested in the present study is that effects of supplementation on growth at 3 y of age persist into adolescence and that differences in attained growth at adolescence between supplemented and nonsupplemented groups are of similar magnitude as observed at 3 y of age. The data used in the analysis were collected during a supplementation trial of rural Guatemalan children conducted from 1969 to 1977 and from a follow-up study of the same subjects at adolescence. Results are presented for length and weight at 3 y of age and for height, weight and fat-free mass (FFM) at adolescence and young adulthood.

MATERIALS AND METHODS

Design and sample

Design of the supplementation trial (1969–1977). A controlled supplementation trial was conducted in rural Guatemala between 1969 and 1977 by the Institute of Nutrition of Central America and Panama (INCAP). Detailed descriptions of the sample, methods, and quality control have been published elsewhere (Martorell et al. 1995a). A brief summary of the intervention follows.

Four rural Ladino (i.e., Spanish speaking, mixed Spanish-Indian ancestry) villages located in eastern Guatemala were selected for the study. The villages were selected to be as similar as possible in nutrition, health and demographic characteristics. Two villages were randomly allocated to receive a high-protein (11.5 g per 180 mL or 1 cup serving), high-energy (682 kJ/163 kcal per 180 mL) drink called Atole. The remaining two villages were assigned to receive a low-energy (247 kJ/59 kcal per 180 mL), nonprotein supplement called Fresco. The two drinks contained similar concentrations of vitamins and minerals. A preventive and curative health program was offered in all four villages. The supplements were distributed centrally in supplementary feeding centers and were available daily, on a voluntary basis, to all members of the community. Subjects were free to consume as much as desired and the amounts ingested by children 0–7 y were measured and recorded at each session to the nearest 10 mL.

Design of the follow-up study (1988–89). A follow-up study of the children who participated in the supplementation trial was conducted between 1988 and 1989. At this time, the subjects were between 11 and 27 y of age, of which 2169 were known or believed to be alive. Of these, 1574 (73%) were studied at follow-up (Martorell et al. 1995a).

Sample. The sample for the current analyses consists of 460 children (245 males and 215 females) who were exposed to supplementary feeding in the study villages from birth to 3 y of age (born between March 1969 and February 1974), had anthropometric measurements at 3 y of age (±7 d) and had anthropometric measurements at follow-up, when they were between 14 and 20 y of age (for convenience, this range in age is referred to hereafter as adolescence).

Data utilized

Data from the supplementation trial. Anthropometric measurements at 36 mo, home diet, duration of breastfeeding, diarrhea, maternal height, maternal education and socioeconomic status were used in the present analyses.

Anthropometric measurements at 36 mo. Weight, measured to the nearest 0.01 kg, using a beam balance scale, and recumbent length, measured to the nearest millimeter on a standard measuring table were used in the analyses.

Home diet. Energy intake from the home diet (excluding breastfeeding) was estimated by the 24-h recall method using surveys every 3 mo between 15 and 36 mo of age. The average daily energy intake (kilocalories/day) from the diet between 15 and 36 mo was obtained using all the recalls available during that period. The average was used in the analyses.

Diarrhea. Information about the occurrence and duration of diarrhea, as defined by the mother, was collected every two weeks during home visits. The percentage of time with diarrhea between 0 and 36 mo of age was used in the analyses. This variable was derived by dividing the number of days with diarrhea by the number of days for which information about morbidity was available, multiplied by 100.

Maternal height. Maternal height was measured every 3 mo during pregnancy and lactation starting in 1971. The median value of the repeated measures was used in the analyses.

Socioeconomic status. A socioeconomic score (SES) was generated from factor analysis using information about living conditions of the family in 1975. After initial testing, the model was restricted to one factor. Only variables with factor loadings ≥0.5 were retained. These were house characteristics (type of floor, an overall assessment of the quality of the house construction, type of excreta disposal, the location of the kitchen and facilities for cooking) and possession of household items (radio, TV, record player, bicycle, motorcycle, car, sewing machine and refrigerator). The variance explained by this model was 46%. Standardized factor scores were used in the analyses.

Data from the follow-up study. Anthropometric measurements and maturation were used in the analyses.

Anthropometric measurements. A battery of anthropometric measurements were obtained on the sample (Martorell et al. 1995a). Only height, weight and estimated FFM were used as outcomes in the present analyses. Sex-specific prediction equations for FFM were developed in an urban group specifically selected to match the subjects of the follow-up study on age, anthropometric measurements and ethnic origin. The prediction equation for males included weight, bicristal diameter and arm-fat area as independent variables; the equation for females included weight, height, and waist circumference (Conlisk et al. 1992).

Maturation. The methods of assessing maturation in this study are given by Pickett et al. (1995). Left-hand-wrist X-rays of adolescents up to the age of 18 y, excluding pregnant women, were obtained by field workers who were trained by a radiologist. X-rays in older subjects were not obtained because the probability of finding anyone who had not reached skeletal maturity was very small. All X-rays were read and graded by a single person using the TW-2 (RUS) method (Tanner et al. 1983) in which skeletal maturity is assigned the value of 18.0 y in males and 16.0 y in females. The variable "maturation", which was used in the analyses, was given the hand-wrist X-ray rating value (bone age) if chronological age was <17.9 in males. All males ≥18 y were given a value of 18.0 for maturation. In females, bone age was used if chronological age was <15.9 y. Between chronological ages 16.0 and 17.9, bone age was used if skeletal maturity had not occurred, while 16.0 was assigned when skeletal maturity had occurred. All females ≥18 y were given a value of 16.0 for maturation. Haas et al. (1995) also use this variable as a covariate but refer to it as skeletal age (SA). A nonlinear association between maturation and growth at adolescence was found in males; therefore, a quadratic term was used in the models for males.

Conceptual Model

The variables included in the regression models were based on a conceptual model of the determinants of growth. From evidence in the literature and from previous analyses of the data, the direct determinants of growth during the first 3 y of age are dietary intake (Habicht and Butz 1979) and morbidity, particularly diarrhea (Rivera and Martorell 1988). Two variables representing dietary intake initially were considered: breastfeeding duration and home energy intake. However, breastfeeding duration was dropped from the analyses because of the large number of missing values. Previous analyses in this population showed that maternal height was an important determinant of children's growth. It is probably an indicator of both the genetic potential for growth and of the socioeconomic condition of the family. Maturation was included in all models for adolescents. Socioeconomic status of the family and maternal education were selected for analysis but the latter was dropped because of high rates of missing values. Socioeconomic status operates through dietary intake and morbidity. Because dietary intake and diarrhea, which are direct determinants of growth, were included in the model, incorporation of socioeconomic status may be redundant and should therefore be justified. One important reason for including the three variables in the model is that measures of dietary intake are imprecise (the reliability of the 24-h dietary recall method is low and breast-milk intake was not measured). Also, diarrhea was the only indicator of morbidity used. Therefore, socioeconomic status may capture some of the variability in growth that would be lost because of imperfect measurement of dietary intake and morbidity.

Analysis methodology

Full rather than reduced models (i.e., models in which only those variables found to be statistically significant are retained) were used to decrease biases in the regression coefficients as a result of omitting relevant variables (Johnston 1984). The precision of estimation of the full models was very similar to that observed in the reduced models.

Unadjusted differences in attained growth between supplement groups (Atole and Fresco) were analyzed by t-test. Analysis of variance (ANOVA) and ordinary least squares (OLS) regression analysis were used to control for potential confounding variables and to compute adjusted means. The conceptual model described above guided the choice of variables. The outcome variables analyzed were: length (centimeters) and weight (kilograms) at 3 y of age and height (centimeters), weight (kilograms) and FFM (kilograms) at adolescence. The independent variables included: supplement type (Atole = 1; Fresco = 0), maternal height (centimeters), percent of time with diarrhea between 0 and 3 y of age, SES and home diet (kilocalories). The variable "home diet" was dichotomized using the sex-specific median: diet was considered low (0) if the energy intake from the diet was lower than the median and high (1) if the energy intake was greater than or at the median. The reasons for dichotomizing were that the relationship between energy intake and attained growth is not linear and because of imprecision in the measurement of home diet (Habicht et al. 1995). Maturation was included in all adolescent models. Also included in some adolescent models were anthropometric measurements at 3 y of age and height at adolescence. The former was used to test if differences in attained growth at adolescence were totally explained by differences at 3 y; the latter was used to test if effects on FFM and weight at adolescence were independent of effects on height.

The analytical approach consisted of: testing the effect of supplementation on length, weight and weight adjusting for length, at 3 y of age; testing the long-term effect of supplementation on height, weight and FFM at adolescence and on weight and FFM adjusting for height; and testing the effect of supplementation on weight and height at adolescence, controlling for anthropometric measurements at 3 y of age. These last models tested whether the effect of supplementation on adolescents' outcomes still remained when anthropometric measurements at 3 y of age were included in the model.

Data for males and females were analyzed separately, mainly because of differences in patterns of maturation. In females maturation was related linearly to height at adolescence whereas in males the relationship was quadratic.

Atole versus Fresco differences were considered statistically significant at an alpha level <0.05, using two-tailed tests in descriptive analyses. Statistical power was inadequate to analyze these data according to the intervention design, which would require the unit of analysis to be the village and not the individual (see Habicht et al. 1995 for the application of this approach to size at 3 y of age). However, the important inferences about the long-term effects of supplementation depend more on major changes in the absolute differences in size between Atole and Fresco subjects than on shifts in statistical significance.

All analyses were done using the SAS version 6.04 for microcomputers.

RESULTS

Descriptive statistics for outcome variables (**Table 1**) and independent variables (**Table 2**) are presented by sex and supplement type. Length and weight at 3 y of age were significantly greater in Atole compared with Fresco villages for both genders ($P < 0.05$). Differences in favor of Atole villages were 1.6 and 2.7 cm in length and 0.7 and 1.2 kg in weight for males and females, respectively. Weight, height and FFM at adolescence were also significantly greater in Atole villages in females ($P < 0.05$) but comparisons were not significantly different between Atole and Fresco villages in males. Chronological age as well as maturation were significantly greater in Fresco males (e.g., 16.1 and 16.6 y for Atole and Fresco males, respectively, for chronological age; Table 2); later analyses of size at adolescence adjust for these differences. Table 2 also shows that energy intake from the supplement was significantly greater in Atole villages ($P < 0.05$) for both genders, and that energy intake from home diets, although slightly greater in Fresco villages, was not significantly greater. Maternal height, socioeconomic status and percent time with diarrhea did not differ between supplement types for either gender.

Tables 3–5 present adjusted mean values of the various outcome variables for Atole and Fresco villages, by gender. The regression models (numbered consecutively from 1 to 18) used to compute these adjusted means are presented in **Appendices 1–3**.

At 3 y of age, adjusted lengths and weights for both males and females were significantly greater in Atole compared with Fresco villages (Tables 3 and 4). In length, differences in adjusted means between Atole and Fresco were 2.0 cm in males and 2.9 cm in females, while in weight they were 0.7 kg and 1.3 kg for males and females, respectively. These differences were slightly greater in length compared with those obtained using unadjusted means (unadjusted differences were 1.6 and 2.7 cm, respectively in males and females; from data in Table 1) but nearly similar in the case of weight (unadjusted differences were 0.7 and 1.2 kg, respectively, in males and females; Table 1).

Adolescents from Atole villages were taller (Table 3) and heavier (Table 4) than those of Fresco villages, although the differences were not statistically significant in males. The adjustments, particularly the correction for differences in maturation at adolescence between Atole and Fresco samples, reversed the sign of the anthropometric differences in males to favor Atole (i.e., Fresco males were taller and heavier at adolescence before adjustment but shorter and lighter

TABLE 1

Descriptive statistics for outcome variables by sex and type of supplement

	Males		Females	
Variable	Atole (n = 118)	Fresco (n = 127)	Atole (n = 116)	Fresco (n = 99)
Length at 3 y, *cm*	86.9 ± 3.8	85.3 ± 4.0*	86.3 ± 3.5	83.6 ± 3.6*
Weight at 3 y, *kg*	12.5 ± 1.2	11.8 ± 1.3*	12.1 ± 1.3	10.9 ± 1.1*
Height at adolescence, *cm*	157.5 ± 9.2	158.3 ± 7.6	150.5 ± 5.3	148.8 ± 4.7*
Weight at adolescence, *kg*	48.3 ± 8.1	49.6 ± 7.9	48.3 ± 6.7	46.0 ± 5.7*
Fat-free mass at adolescence, *kg*	41.9 ± 6.7	43.1 ± 6.4	37.3 ± 5.3	35.2 ± 4.6*

Values are means ± SD.

* $P < 0.05$, *t*-test comparing Atole and Fresco. Length at 3 y of age did not differ between Atole and Fresco in 1968, at baseline (Habicht et al. 1995, Ruel et al. 1992). Also note that maternal height (Table 2) did not differ by village type.

TABLE 2

Descriptive statistics for independent variables by sex and village type

	Males				Females			
	Atole		Fresco		Atole		Fresco	
Variable	n[1]		n		n		n	
Supplement energy, *kJ/day*	118	536 ± 377	127	88 ± 75*	116	481 ± 322	99	71 ± 54*
Home diet energy, *kJ/day*[2]	118	2996 ± 912	126	3209 ± 971	113	2795 ± 787	97	2908 ± 1013
Supplement energy, *kcal/day*	118	128 ± 90	127	21 ± 18*	116	115 ± 77	99	17 ± 13*
Home diet energy, *kcal/day*[2]	115	716 ± 218	126	767 ± 232	113	668 ± 188	97	695 ± 242
Maternal height, *cm*	116	148.8 ± 5.1	123	148.8 ± 5.1	114	149.0 ± 5.2	97	149.3 ± 5.6
SES[3]	116	−0.1 ± 0.9	122	−0.1 ± 0.8	114	−0.1 ± 0.9	97	0.0 ± 1.0
Time w/diarrhea, %	117	8.8 ± 8.2	126	7.7 ± 6.7	115	6.4 ± 6.2	97	7.9 ± 7.8
Maturation, *years*	116	16.1 ± 2.0	123	16.8 ± 1.9*	107	16.7 ± 1.8	97	16.7 ± 2.0
Age, *years*	118	16.1 ± 1.4	127	16.6 ± 1.5*	116	16.5 ± 1.4	99	16.4 ± 1.6
Duration of breastfeeding, *months*	113	19.1 ± 6.9	121	19.3 ± 4.5	110	18.0 ± 5.6	94	19.7 ± 5.2*

Values are means ± SD.
* $P < 0.05$, *t*-test comparing Atole and Fresco.
[1] Differences in sample sizes among variables are due to missing values.
[2] The percent with dietary energy intakes equal to the median or greater was 45.2%; (n = 115) and 54% (n = 126) in males and females respectively in Atole villages. The corresponding values for Fresco were 50.4% (n = 113) and 49.5% (n = 97). Differences between Atole and Fresco were not significant for males or females.
[3] Standarized scores from factor analysis are unitless.

after adjustment). Relative to adjusted differences at 3 y of age, adjusted differences at adolescence were smaller for height (1.2 versus 2.0 cm in males and 2.1 versus 2.9 cm in females; Table 3) but larger for weight (1.2 versus 0.7 kg in males and 2.2 versus 1.3 kg in females; Table 4).

Differences between Atole and Fresco villages at adolescence ceased to be significant after adjustment for length or weight at 3 y, indicating that differences observed at adolescence were due to differences already established at 3 y of age (Tables 3 and 4).

When weight at adolescence was adjusted for height at adolescence (Table 4), differences in weight between Atole and Fresco villages were about half the size of the unadjusted values—but still substantial in females—and not statistically significant for either sex.

FFM at adolescence was greater in Atole villages in both males (0.8 kg) and females (2.1 kg). When FFM was adjusted for height, the Atole versus Fresco difference in males was reduced to 0.2 kg and became nonsignificant whereas that in females was reduced to 1.2 kg but remained significant (Table 5).

DISCUSSION

Our results confirm the hypothesis that the positive effects of supplementation on growth at 3 y of age

TABLE 3

Adjusted length/height (cm) of Atole and Fresco subjects

Gender, variable	Atole[1]	Fresco[1]	Difference[2]	P
Males				
Length at 3 y, *cm*	87.3 ± 0.3	85.3 ± 0.3	2.0	0.000
Height at adolescence, *cm*	158.6 ± 0.5	157.4 ± 0.5	1.2	0.111
Height at adolescence adjusted for length at 3 y, *cm*	157.5 ± 0.4	158.4 ± 0.4	−0.9	0.111
Females				
Length at 3 y, *cm*	86.5 ± 0.3	83.6 ± 0.3	2.9	0.000
Height at adolescence, *cm*	150.7 ± 0.4	148.6 ± 0.5	2.1	0.001
Height at adolescence adjusted for length at 3 y, *cm*	149.6 ± 0.4	149.8 ± 0.4	−0.2	0.631

[1] Means ± SE. Means were adjusted for dietary intake, percent of time with diarrhea, socioeconomic status, maternal height and maturation (adolescence only).
[2] Atole minus Fresco.

TABLE 4

Adjusted weight (kg) of Atole and Fresco subjects

Gender, variable	Atole[1]	Fresco[1]	Difference[2]	P
Males				
Weight at 3 y, kg	12.5 ± 0.1	11.8 ± 0.1	0.7	0.000
Weight at adolescence, kg	49.4 ± 0.5	48.2 ± 0.5	1.2	0.084
Weight at adolescence adjusted for weight at 3 y, kg	48.5 ± 0.4	49.1 ± 0.4	−0.6	0.273
Weight at adolescence adjusted for height at adolescence, kg	49.1 ± 0.4	48.6 ± 0.4	0.5	0.347
Females				
Weight at 3 y, kg	12.2 ± 0.1	10.9 ± 0.1	1.3	0.000
Weight at adolescence, kg	48.2 ± 0.6	46.0 ± 0.6	2.2	0.009
Weight at adolescence adjusted for weight at 3 y, kg	46.7 ± 0.5	47.7 ± 0.5	−1.0	0.174
Weight at adolescence adjusted for height at adolescence, kg	47.8 ± 0.5	46.5 ± 0.6	1.3	0.109

[1] Means ± SE. Means were adjusted for dietary intake, percent time with diarrhea, socioeconomic status, maternal height and maturation (in the case of adolescence only).
[2] Atole minus Fresco.

persist at adolescence, although slightly attenuated. Children supplemented with Atole (high-protein/high-energy drink) in their first 3 y of life were taller, heavier and had higher FFMs at adolescence than those supplemented with Fresco (low-energy/no-protein drink). The study also showed that differences between the Atole and Fresco groups at adolescence were largely explained by the effect of supplementation on body size at 3 y of age. This was demonstrated by statistical analyses that showed that Atole and Fresco differences at adolescence disappeared when body size at 3 y of age was included in the models.

Differences between Atole and Fresco were larger in females than in males for all outcomes and at both 3 y of age and adolescence. Differences in favor of females were striking, particularly in FFM, with differences being almost three times larger in females than in males. An intriguing finding was that Atole versus Fresco differences in FFM in females were not totally explained by height at adolescence, suggesting a long-term effect of early supplementation on FFM, independent of the effect on height.

Differences at adolescence relative to those observed at 3 y of age were larger for weight than for height for both genders. Differences in weight were explained mainly by differences in height (i.e., differences in weight disappeared after controlling for height) and for this reason, the remainder of the discussion focuses on height.

The differential response to supplementation between males and females, both at 3 y of age and at adolescence is difficult to explain. It appears that this gender difference at adolescence was mainly due to differences already existing at 3 y of age. Differences between Atole and Fresco in females at 3 y of age were greater by 0.9 cm compared with those found in males. Between 3 y of age and adolescence, both males and females in Fresco villages grew ~0.8 cm more than children in Atole villages (see below). This increased rate of growth among Fresco children resulted in attenuated differences between Atole and Fresco at adolescence in both genders. In males, however, the smaller magnitude of the difference at 3 y of age, combined with larger standard errors at adolescence, resulted in differences between Atole and Fresco at adolescence that were no longer statistically significant. Larger standard errors in adolescent males are thought to be due to variations in maturity. Although a large

TABLE 5

Adjusted fat-free mass (FFM) in Atole and Fresco subjects

Gender, variable	Atole[1]	Fresco[1]	Difference[2]	P
Males				
FFM, not adjusted for height, kg	42.8 ± 0.4	42.0 ± 0.4	0.8	0.162
FFM, adjusted for height, kg	42.5 ± 0.3	42.3 ± 0.3	0.2	0.641
Females				
FFM, not adjusted for height, kg	37.3 ± 0.4	35.2 ± 0.5	2.1	0.002
FFM, adjusted for height, kg	36.9 ± 0.4	35.7 ± 0.4	1.2	0.052

[1] Means ± SE. Means were adjusted for dietary intake, percent time with diarrhea, socioeconomic status, maternal height and for maturation (in the case of adolescence only).
[2] Atole minus Fresco.

proportion of females had reached maturity at the time of the study, this was not the case for males. Although skeletal age was used in the analyses to control for maturation, extreme variations in height associated with differences in maturity could not be completely controlled for.

We have explored possible reasons for the smaller magnitude of difference between Atole and Fresco villages observed in males at 3 y of age. Examination of results within village pairs indicates that differences in favor of Atole in the pair of large villages were statistically significant in males (**Table 6**) and similar to differences seen in females. Also, differences between Atole and Fresco in males in the large villages remained statistically significant at adolescence. In contrast, differences were less and not statistically significant in males in the small villages either at 3 y ($P = 0.28$) or at adolescence ($P = 0.32$). We have no adequate explanation for the lack of a supplement effect in males in the small pair of villages.

Differences in attained height at adolescence in favor of Atole villages disappeared after controlling for length at 3 y of age. This was expected because most of the growth deficit observed in adolescents and adults in this population occurs during the first 3 y of life. Growth after 3 y in this population is generally adequate and may even be greater than that observed in Mexican-Americans (Martorell et al. 1995b). In our study, children in the Fresco villages grew slightly more from 3 y of age to follow-up than children in Atole villages. (72.1 versus 71.3 cm in Fresco and Atole males respectively and 65.0 cm and 64.2 cm, respectively, in females; from data in Table 3). This small difference in growth may be the result of accelerating effects of the Atole on bone maturation in the preschool period (Martorell et al. 1979), which would provide children in the Fresco villages with a greater growth potential after 3 y of age. Another possible explanation for the above is better environmental conditions during school age and adolescence in Fresco villages. Our data suggest that environmental factors may have had an effect on growth from 3 y of age to adolescence (see Appendices 1–3). In females, socioeconomic status was a statistically significant determinant of height at adolescence and the effect continued to be significant after controlling for length at 3 y of age. This suggests a positive effect of socioeconomic factors on growth in height after 3 y of age in females.

A number of variables besides supplementation were statistically significant in the models (see Appendices 1–3). In addition to maturation, maternal height was an important determinant of height and weight at 3 y of age and at adolescence in both sexes. Moreover, maternal height was also a statistically significant predictor of growth at adolescence once size at 3 y of age was controlled for, indicating an effect on growth after 3 y of age. As mentioned earlier, maternal height is thought to be a proxy for both genetic potential and environmental factors. Dietary intake and diarrhea during the first 3 y of life were statistically significant predictors of size at adolescence in some models but were not significant when size at 3 y of age was controlled for. These results suggest that, although the effects of diarrhea and dietary intake on growth take place during the first 3 y of age, the effects persist into adolescence.

In summary, the results show long-lasting effects of supplementation during the first 3 y of life on body size and composition at adolescence. Although differences in height at adolescence were reduced in magnitude relative to those found at 3 y of age, the degree of reduction was similar in both males and females, and the results at adolescence did not change substantially relative to results at 3 y of age. In weight, differences were increased at adolescence relative to 3 y of age.

In this population, the first 3 y of life is the period of maximum growth retardation, whereas growth thereafter may be adequate (Martorell et al. 1995). Our results show that investments in nutrition during early childhood have effects on growth that persist into adulthood. Greater height and FFM in turn may have effects on reproductive performance in females, notably on fetal growth, and consequently on the health and survival of the next generation. Effects on body

TABLE 6

Adjusted length/height (cm) of Atole and Fresco males by village size

	Small villages			Large villages		
Variable	Atole[1]	Fresco[1]	Difference[2]	Atole[1]	Fresco[1]	Difference[2]
Adjusted length at 3 y[1]	86.9 ± 0.48	86.1 ± 0.51	0.8	87.6 ± 0.44	84.7 ± 0.43	2.9*
Adjusted height at adolescence[1]	157.8 ± 0.76	158.9 ± 0.81	−1.1	159.0 ± 0.68	156.2 ± 0.68	2.8*

* $P < 0.05$ (two-tailed).

[1] All means were adjusted for dietary intake, percent time with diarrhea, socioeconomic status, maternal height and maturation (in the case of adolescence only). Values are means ± SE.

[2] Atole minus Fresco.

size in adolescent males, through its association with greater work capacity (Haas et al. 1995), may in turn be associated with increased future earning power.

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APPENDIX 1

Multiple Regression models for length at 3 y and height at adolescence

	Males						Females					
	Length at 3 y (cm)		Height at adolescence (cm)		Height at adolescence controlling for length at 3 y		Length at 3 y (cm)		Height at adolescence (cm)		Height at adolescence controlling for length at 3 y	
Model number	1		2		3		4		5		6	
	β	P	β	P	β	P	β	P	β	P	β	P
Independent variables												
Intercept	48.77	0.000	−76.48	0.000	−109.40	0.000	53.15	0.000	71.17	0.000	30.53	0.001
Supplement[1]	1.97	0.000	1.15	0.111	−0.94	0.111	2.83	0.000	2.03	0.001	−0.27	0.631
Maternal height	0.24	0.000	0.53	0.000	0.29	0.000	0.20	0.000	0.47	0.000	0.31	0.000
SES	0.60	0.025	0.54	0.202	0.01	0.965	0.24	0.320	0.74	0.030	0.55	0.050
Time w/diarrhea, %	−0.07	0.015	−0.04	0.416	0.03	0.454	−0.06	0.063	−0.05	0.280	−0.01	0.893
Home Diet[2]	1.66	0.000	1.76	0.016	0.24	0.677	0.00	0.029	0.51	0.425	−0.27	0.611
Maturation, y	—	—	16.66	0.000	15.16	0.000	—	—	0.44	0.011	0.41	0.004
Maturation sq.	—	—	−0.44	0.000	0.40	0.000	—	—	—	—	—	—
Length 3 y, *cm*	—	—	—	—	0.98	0.000	—	—	—	—	0.77	0.000
Adjusted R sq.	0.25		0.61		0.76		0.27		0.31		0.53	

[1] Supplement type: Atole = 1, Fresco = 0.
[2] Dietary intake: at or above gender specific median = 1, below gender specific median = 0.

APPENDIX 2

Multiple regression models for weight at 3 y of age and at adolescence

	Males								Females							
	Weight at 3 y (kg)		Weight at adolescence (kg)		Weight at adolescence (kg) controlling for weight at 3 y		Weight at adolescence (kg) controlling for height at adolescence		Weight at 3 y (kg)		Weight at adolescence (kg)		Weight at adolescence (kg) controlling for weight at 3 y		Weight at adolescence (kg) controlling for height at adolescence	
Model number	7		8		9		10		11		12		13		14	
	β	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*	*β*	*P*
Independent variables																
Intercept	1.37	0.560	−82.46	0.000	−84.93	0.000	−38.11	0.015	1.86	0.429	−20.75	0.106	−27.22	0.014	−51.78	0.000
Supplement[1]	0.79	0.000	1.17	0.084	−0.66	0.273	0.50	0.347	1.24	0.000	2.16	0.009	−1.08	0.174	1.27	0.109
Maternal height	0.07	0.000	0.28	0.000	0.13	0.031	0.02	0.705	0.06	0.000	0.32	0.000	0.17	0.016	0.11	0.186
SES	0.16	0.084	0.19	0.629	0.52	0.122	0.50	0.106	0.00	0.986	−0.21	0.630	−0.24	0.528	−0.54	0.208
Time w/diarrhea, %	-0.02	0.029	−0.02	0.702	0.03	0.375	0.01	0.875	−0.03	0.031	−0.04	0.508	0.02	0.648	−0.02	0.751
Home Diet[2]	0.43	0.007	1.64	0.016	0.65	0.265	0.61	0.253	0.25	0.125	0.39	0.640	−0.15	0.839	0.17	0.832
Maturation, *y*	—	—	7.71	0.000	7.71	0.000	−1.95	0.283	—	—	1.15	0.000	1.19	0.000	0.96	0.000
Maturation sq.	—	—	−0.14	0.027	0.14	0.008	0.11	0.047	—	—	—	—	—	—	—	—
Weight 3 y, *kg*	—	—	—	—	2.24	0.000	—	—	—	—	—	—	2.56	0.000	—	—
Height at adolescence, *cm*	—	—	—	—	—	—	0.59	0.000	—	—	-	—	—	—	0.44	0.000
Adjusted R sq.	0.21		0.63		0.71		0.77		0.29		0.18		0.40		0.27	

[1] Supplement type: Atole = 1, Fresco = 0.
[2] Dietary intake: at or above gender specific median = 1, below gender specific median = 0.

APPENDIX 3

Multiple regression models for fat-free mass (FFM) at adolescence

	Males				Females			
	FFM (kg) at adolescence		FFM (kg) at adolescence controlling for height at adolescence		FFM (kg) at adolescence		FFM (kg) at adolescence controlling for height at adolescence	
Model number	15		16		17		18	
	β	P	β	P	β	P	β	P
Independent variables								
Intercept	−68.32	0.000	−31.06	0.010	−21.67	0.034	−51.38	0.000
Supplement[1]	0.75	0.162	0.19	0.641	2.04	0.002	1.19	0.052
Maternal height	0.23	0.000	−0.02	0.587	0.28	0.000	0.09	0.192
SES	−0.21	0.509	−0.47	0.050	0.06	0.870	−0.25	0.446
Time w/diarrhea, %	−0.02	0.524	−0.00	0.891	−0.03	0.599	−0.00	0.920
Home diet[2]	1.33	0.014	0.48	0.250	−0.24	0.716	0.03	0.963
Maturation, *y*	6.73	0.000	−1.38	0.323	0.88	0.000	0.70	0.000
Maturation sq.	−0.13	0.012	0.08	0.053	—	—	—	—
Height at adolescence, *cm*	—	—	0.49	0.000	—	—	0.42	0.000
Adjusted R sq.	0.65		0.80		0.19		0.32	

[1] Supplement type: Atole = 1, Fresco = 0.
[2] Dietary intake: at or above gender specific median = 1, below gender specific median = 0.

Nutritional Supplementation during the Preschool Years and Physical Work Capacity in Adolescent and Young Adult Guatemalans[1,2]

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ABSTRACT A follow-up study (1988–89) was carried out in 364 rural Guatemalans, 11–27 y of age, who earlier had participated in a nutritional supplementation experiment. Among its objectives was the assessment of the long-term effects of the nutrition intervention on physical work capacity. Subjects and their mothers from two villages had available a high-energy, high-protein supplement (Atole: 163 kcal/682 kJ and 6.4 g protein per serving or 180 mL), whereas in two other villages a low-energy, no-protein supplement (Fresco: 59 kcal/247 kJ per 180 mL) was provided. Consumption was ad libitum. Maximum oxygen consumption ($\dot{V}O_2max$) at follow-up was significantly greater in Atole compared with Fresco subjects of both sexes. In subjects 14–19 y, exposed to supplementation throughout gestation and the first 3 y of life, Atole males had a significantly higher $\dot{V}O_2max$ (2.62 L/min) than Fresco males (2.24 L/min), the differences remaining significant even after controlling for body weight and fat-free mass; also, there was a significant positive relationship between amount of supplement consumed and $\dot{V}O_2max$. The supplementation effect in females of similar age was not statistically significant. It is concluded that early nutritional improvements can have long-lasting effects on physical performance. J. Nutr. 125: 1078S–1089S, 1995.

INDEXING KEY WORDS:

- *malnutrition* • *growth* • *work capacity*
- *adolescence* • *young adult*

Most evaluations of nutritional interventions in children have focused on outcomes measured during or shortly after the intervention has occurred. Although at least one study has examined the relationship of childhood nutritional status and physical performance during adolescence (Satyanarayana et al. 1979), no studies have reported on the long-term effects of early nutritional interventions on later performance. Achieved growth, as measured by body size and composition, is an indicator of general constitutional development of the individual. It reflects functional aspects of development and performance and thus is a good proxy for overall well being. One important measure of functional performance that has been shown to be related to current nutritional status, as well as to anthropometric indicators of past nutritional status, is physical work capacity (Spurr 1983).

There are very few studies of work capacity in undernourished children. The single prospective study reported in the literature found no relationship between height at 5 y and submaximal work capacity adjusted for body weight at adolescence (Satyanarayana et al. 1979). However, Spurr (1983) noted in these same data a strong negative relationship between height at age 5 and the percentage of maximal work capacity at which the submaximal work load was carried out. This means that the shortest adolescents

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Supported by NIH grant numbers RO1-HD22440 and T32-HD07331.

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would have the least endurance. The most extensive study of anthropometric characteristics and work capacity in undernourished children was conducted by Spurr and colleagues in Cali, Colombia (1982a, 1982b, 1983, 1984). This cross-sectional study of 1013 boys (7–15 y) demonstrated a significantly lower maximal oxygen consumption ($\dot{V}O_2max$) in children with low weight-for-age and low weight-for-height than in anthropometrically normal children. These results, along with those from India (Satyanarayana et al. 1979), Brazil (Desai et al. 1984) and East Africa (Davies 1977), suggest that reduced body weight, probably reflecting less fat-free mass (FFM), accounts for the reduced work capacity seen in adolescents who were undernourished as young children. However, other evidence suggests that concurrent physical activity and anemia affect work capacity independently of variation in muscle mass in Tanzanian youths and young adults (Davies 1974).

None of the studies has examined the possibility that the same factors that cause poor growth also affect maturation during adolescence (Frisancho et al. 1970); thus, the relationship between body size and work capacity during adolescence may be due to retarded maturation because the latter affects work capacity independently (Bouchard et al. 1976, Kemper and Verschuur 1987). No studies have been made of the interrelationships among maturation, body weight, body composition and work capacity in adolescents undernourished as young children. It is not clear how much of the reduced adolescent body weight and its components of fat and lean tissue are a function of growth retardation in height that occurred in early childhood and how much is a reflection of current nutritional problems.

Although the evidence from previous research of the effects of past nutritional status on growth, maturity and physical performance during adolescence have been suggestive of a long-term impact of early nutrition on later development, these studies have been retrospective or used indirect methods to ascribe causality to nutritional effects. In this paper we test the hypothesis that improved nutrition during early life results in improved physical work capacity during adolescence and early adulthood.

MATERIALS AND METHODS

The above hypothesis was tested in a sample of Guatemalan adolescents and young adults who were participants in a nutritional supplementation trial while they were children. From January 1969 to September 1977, the Institute of Nutrition of Central America and Panama (INCAP) carried out a longitudinal study of growth and development in four rural Ladino (i.e., Spanish speaking, mestizo population) communities in eastern Guatemala. The subjects of the study were all village children aged ≤7 y and all pregnant and lactating women. Cohorts of newborns were included for study until February 1977. Data collection for individual children ceased when they reached 7 y of age. All field data collection terminated in September 1977. Martorell et al. (1995) have described the study design, sampling scheme and measurements taken in this study.

The principal hypothesis under study was that improved nutrition results in accelerated mental development and physical growth of preschool-aged children. Two of the villages (one large, one small) consumed a high protein-energy drink (Atole) provided as a supplement to the normal diet. In two other villages (one large, one small) a nonprotein low calorie drink (Fresco) was provided. Atole contained Incaparina (a vegetable protein mixture developed by INCAP), dry skim milk and sugar and had 163 kcal/682 kJ and 11.5 g of protein per cup (180 mL) whereas the Fresco contained no protein and as little sugar and flavoring agents as necessary for palatability. The Fresco provided 59 kcal/247 kJ per cup. Both drinks were distributed in food supplementation centers and were available daily, on a voluntary basis, to all members of the community. A cup containing 180 mL was provided to each individual, but more was given if desired. The unique feature of this study was that individual intake was recorded carefully, on a daily basis, to the nearest 10 mL. A curative-preventive medical care program was also implemented in all four communities.

From 1977 to 1988 no research was conducted in any of these villages. In 1988 INCAP returned to the villages to conduct a follow-up study of the participants in the original intervention trial, by then ranging in age from 11 to 27 y. The design, methods and procedures of this follow-up study are described in detail by Martorell et al. (1995). All participants, who were exposed to the intervention at some time before 7 y of age were candidates for the follow-up study. Because subjects in the original supplementation trial had varying periods of exposure to the intervention depending on their birth cohort, it was necessary to divide the sample into exposure cohorts.

Figure 1 presents the three cohorts chosen on the basis of the ages at which they were exposed to the intervention. Cohort 2, 14–18 years at follow-up, had complete exposure to the intervention throughout gestation and the first 3 y of life and is considered to be the cohort where the effect of the intervention should be most observable. Most of the impact of nutritional supplementation on physical growth in this population was seen before 3 y (Schroeder et al. 1995), therefore, 3 y was considered to be an appropriate cutoff age. Subjects in Cohort 1, the youngest children at follow-up, were born before the intervention stopped but, depending on their birth dates, were exposed for

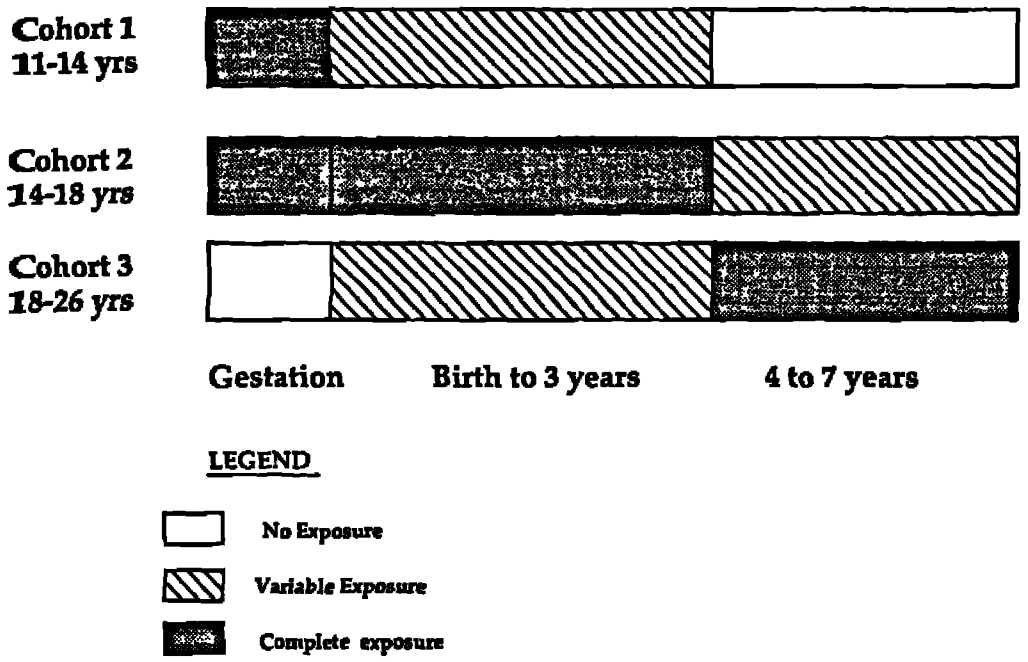

FIGURE 1 Age at exposure to supplement by cohort.

varying lengths of time up to 3 y. Subjects in Cohort 3, the oldest group, were all born before the intervention began in 1969 and had complete exposure from 4 to 7 y of age but variable exposure from birth to 3 y of age. The follow-up sample included approximately 1574 subjects or nearly 73% of all original participants (Martorell et al. 1995).

Sample. The assessment of physical performance is a time-consuming procedure, and not all 1574 subjects could be tested. A representative subsample was identified for the physical performance test. Approximately 25% of the subjects (n = 366) identified as residing in the original study villages at the time of the follow-up were selected at random after stratifying by treatment, sex and cohort. Of this subsample, 206 consented to participate, while 73 individuals (40%) from Atole villages and 87 (48%) from Fresco villages refused to participate, so replacements were selected with a second random selection and eventually with volunteers from the villages. The primary reasons stated for refusal to participate were similar between groups. Fifty-one percent "did not have the time" or "were not interested," 27% were working temporarily or residing permanently in Guatemala City, and a small number were pregnant or had recently delivered (5%), were physically unable to participate (12%) or refused to provide a blood sample (5%). A subsequent round of random sampling yielded a similar response rate (92/163 or 56%) and the remainder (71 subjects) of the subsample was filled by volunteers from the pool of nonsampled subjects. Five subjects were excluded because they did not achieve the criteria for maximal exertion on the exercise test, leaving 364 subjects for analysis.

Compared with the total sample from which it was drawn, the work capacity subsample is not significantly different in height, weight, FFM, percent body fat and body mass index (BMI). The subsample differed slightly but not significantly from the total sample in the distribution of the amount of nutritional supplement ingested. Among Atole subjects of both sexes, the subsample slightly overrepresents the higher consumers of supplement, while the subsample of Fresco males slightly underrepresents the higher consumers. The 71 volunteer subjects did not differ in their anthropometry and supplement ingestion from the 298 subjects drawn at random.

Anthropometry and body composition. All anthropometry was taken by trained personnel using standard procedures (Lohman et al. 1988). All anthropometrists were trained together, which minimized interobserver error. Weight, height and bioelectrical impedance (BIA) were measured at the time of the physical exercise test while all other measurements were taken within the previous 3 wk during an examination conducted in the subjects' home villages. FFM was estimated for each subject from anthropometry and bioelectrical impedance analysis (model BIA-101, RJL Systems, Mt. Clemens, MI) using regression prediction equations specifically developed for this population (Conlisk et al. 1992).

Skeletal maturity. Biological maturity was estimated for all subjects under 18 y of age by assessing skeletal age with the Tanner-Whitehouse-2 (TW2) procedure (Tanner et al. 1983). Maturity is expressed in this study either as skeletal age (SA) or as the difference (SA − CA) between SA and chronological age (CA) where a negative value reflects a delay in maturity relative to expected skeletal development for chronological age. Rivera et al. (1995) also included SA in the analyses but called it maturation. The method for computing SA is reported elsewhere (Pickett et al. 1995); mature girls > 16 y of age were assigned SA equal to their CA.

Physical work capacity. Work capacity was determined as the oxygen consumption at maximum physical exertion ($\dot{V}O_2max$) on a motorized treadmill (model 18–54, Quinton Instruments, Seattle, WA). $\dot{V}O_2max$ was assessed by standard open-circuit spirometry techniques similar to those described by Spurr and Reina (1989). A continuous and progressive test modified from the Balke and Ware (1959) treadmill procedure was administered to all subjects, with oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and cardiac frequency (fH) determined at each work load. Preliminary to the actual test, all subjects were acclimated to the treadmill and face mask and were instructed on the testing procedures. Testing began with a 3-min warm-up on the treadmill at 5% grade and 3.5 mph. Heart rate (fH) during the last 30 s was used to determine the treadmill velocity to be used for the rest of the test (fH < 125, 4.2 mph; fH = 125–140, 3.5 mph; fH > 140, 3.0 mph). Immediately after the warm-up the subject began the continuous test at the specified starting workload. The grade of the treadmill increased by 2.5% every 2 min until maximum effort was achieved. In most tests the final two or three workloads were reduced to 1 min each so that subject fatigue did not result in a premature cessation of the test before $\dot{V}O_2max$ could be observed. Velocity was increased only if the subject had reached the maximum treadmill grade (25%) but had not reached the criteria for $\dot{V}O_2max$. Criteria for maximum effort was the failure to increase $\dot{V}O_2$ by >150 mL between two adjacent work grades on the treadmill while maintaining fH above 190 bpm. Maximum exertion was confirmed in 91% of the subjects who reached this plateau of $\dot{V}O_2$. The remaining 9% of subjects achieved maximum heart rates > −1 SD of the heart rate predicted for their age. Five subjects (1.3% of those tested) did not meet these requirements for maximum exertion and were excluded from the analysis. Pulmonary ventilation ($\dot{V}_E$), $\dot{V}O_2$ and $\dot{V}CO_2$ were determined during the last 30 s of each workload using a Parkinson-Cowen Dry Gas Meter (model CD4, Rayfield Equipment, Waitsfield, VT) and Ametek medical gas analyzers (models S-3A and CD-3A, Thermox Instruments, Pittsburgh, PA). Expiratory gas was sampled through a Respironics (Monroeville, PA) Speakeasy-II face mask-valve and a mixing chamber using a Costill-Wilmore apparatus (R-Pel, Los Altos, CA). Gas analyzers were calibrated after every second subject using room air and factory standardized calibration gases (Fisher Scientific, Springfield, NJ). Heart rate was monitored with a Burdick (Milton, WI) electrocardiograph (model CS-525) with precordial leads at the CM5 position, and backed-up with a Uniq CIC Heartwatch (model 8799, Creative Health Products, Plymouth, MI) remote digital heart rate recorder. Testing was conducted at two laboratory sites because the villages were spread over too large an area to allow for easy transport to one laboratory. Both laboratories were air conditioned to maintain temperatures within the range of 25 to 30°C. The average barometric pressure at the two labs during testing was 748 and 710 mmHg. Twenty-three subjects were retested on a different day within 3 wk to determine test reliability. The technical error of measurement was 0.015 L/min or 8% of the age- and sex-adjusted total variance for $\dot{V}O_2max$.

Statistical analysis. Analyses were conducted in two steps. Analysis of covariance controlling for age was conducted within cohorts to test for differences in work capacity and related measures of body size, composition and maturity between Atole and Fresco subjects. Additional analyses of covariance were carried out to control for possible confounding effects of village size (1 = large, 0 = small), socioeconomic status (SES) and level of individual participation in the supplementation. The SES measure used in these analyses was derived from a factor analysis of characteristics of the home and household possessions (Rivera et al. 1995). Total volume of supplement consumed was used as a proxy for participation. Because participation was dependent on the age when the subjects were born there is a clear effect of child's age and cohort assignment on the amount of supplement ingested during the first 3 y of life. Only Cohort 2 children were exposed to supplementation over the entire age range of birth to 3 y. Therefore, the statistical control for participation was applied only to this cohort. Subjects from Cohort 2 were ranked from lowest to highest volume of supplement consumed during the first 3 y of life. Based on the relative ranking within either Fresco or Atole groups, subjects were given a percentile score. Total supplement consumption ranged from 0 to 386 L over the 3 y. The mean daily intake of Fresco was 43 mL (range = 0–226 mL), while 114 mL (range = 0–350 mL) of Atole was consumed daily. The supplement-specific percentile score (volume ranking) was used as a covariate along with age and SES in regression models that tested for treatment group effects on $\dot{V}O_2max$.

To control for the effects of body size on $\dot{V}O_2max$ and therefore to test for treatment effects on aerobic power, the data are presented in two ways. Tables and figures of mean values for various subgroups (sex × cohort × treatment) express $\dot{V}O_2$ either in L/min or in mL/kg body weight · min^{-1} and mL/kg FFM · min^{-1}. However, the formal testing of treatment effects on $\dot{V}O_2max$ (L/min) includes body weight and FFM as covariates along with potential confounders such as village size, age, SES and volume of supplement consumed in separate regression models. This allows body size to scale itself relative to $\dot{V}O_2max$ and is statistically a preferred means of controlling for these variables because it avoids the restrictive assumptions of a variable computed as a ratio of two variables (Tanner 1949). Differences in $\dot{V}O_2max$ between groups were considered statistically significant if the *P* value was <0.05 on a two-sided test. The *P* value criterion for inclusion of an interactive term in a regression was 0.20; however, a value of ≤0.10 was considered to reflect a strong statistically significant interaction. To maintain consistency across regression models other covariates were retained in all models even if they were not statistically significant.

The second step of analysis tested for the dose-response relationship between amount of supplement ingested and various measures of work capacity. This analysis was limited to Cohort 2 Atole subjects because they were exposed to the intervention during a critical period and consumed a wide enough range of supplement. Multiple regression procedures were used with $\dot{V}O_2max$ (L/min) as the dependent variable and energy consumed from Atole as the independent variable after age and SES were controlled as potential confounders. All statistical analysis was conducted using programs from SAS (SAS Institute, Inc., Cary, NC).

RESULTS

Table 1 presents descriptive information on the 188 male and 176 female subjects according to supple-

TABLE 1

***Mean values for physical characteristics of Atole and Fresco subjects by cohort*[1]**

Variable, group	Cohort 1	Cohort 2	Cohort 3
Males			
Age, *y*			
A	12.8 ± 1.1	16.8 ± 1.3	22.1 ± 1.9
F	12.8 ± 1.0	16.4 ± 1.3	22.6 ± 2.3
Height, *cm*			
A	142.5 ± 9.8*	159.7 ± 9.3	164.2 ± 4.7
F	139.3 ± 8.7	156.4 ± 8.6	162.8 ± 7.5
Weight, *kg*			
A	34.2 ± 6.2	48.1 ± 7.5	57.5 ± 5.4
F	32.5 ± 5.5	45.8 ± 7.7	56.1 ± 7.3
Body mass index, kg/m^2			
A	16.7 ± 1.3	18.8 ± 1.5	21.3 ± 2.0
F	16.6 ± 1.3	18.6 ± 1.8	21.1 ± 2.0
Body fat, %			
A	17.5 ± 4.1	16.9 ± 2.9	19.1 ± 4.7
F	19.0 ± 5.0	18.0 ± 4.2	19.9 ± 4.9
Fat-free mass, *kg*			
A	28.3 ± 5.7*	40.0 ± 6.1	46.4 ± 3.7
F	26.3 ± 4.7	37.5 ± 6.1	44.7 ± 4.5
Skeletal age minus age, *y*			
A	−0.81 ± 2.66	−0.30 ± 2.19	— —
F	−0.97 ± 3.05	−0.45 ± 2.20	— —
Sample size			
A	33	44	24
F	25	42	20
Totals	58	86	44
Females			
Age, *y*			
A	12.7 ± 1.1	16.5 ± 1.4	22.5 ± 1.8
F	13.0 ± 1.0	17.2 ± 1.4	21.6 ± 1.8
Height, *cm*			
A	143.2 ± 7.0	152.5 ± 6.2*	153.3 ± 5.1
F	141.3 ± 8.3	149.3 ± 4.6	151.3 ± 5.9
Weight, *kg*			
A	35.7 ± 6.2	47.6 ± 6.0	49.2 ± 6.2
F	36.3 ± 7.4	47.7 ± 6.2	49.3 ± 5.5
Body mass index, kg/m^2			
A	17.3 ± 2.1	20.5 ± 2.5	20.9 ± 2.2
F	18.0 ± 2.4	21.4 ± 2.5	21.6 ± 2.6
Body fat, %			
A	19.6 ± 3.5	22.6 ± 3.5	23.3 ± 4.0
F	20.6 ± 3.7	23.7 ± 3.4	24.5 ± 4.1
Fat-free mass, *kg*			
A	28.5 ± 4.4	36.7 ± 3.9	37.5 ± 3.4
F	28.6 ± 5.0	36.3 ± 3.9	37.1 ± 3.2
Skeletal age minus age, *y*			
A	0.19 ± 2.60	−0.15 ± 1.01	— —
F	−0.25 ± 2.19	−0.02 ± 0.97	— —
Sample size			
A	26	34	21
F	35	40	20
Totals	61	74	41

[1] Values are means ± SD. Abbreviations used: A = Atole, F = Fresco.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for two-tail *t* test of Atole-Fresco difference in means after adjusting for age.

mentation group and exposure cohort. Mean ages do not differ significantly between treatment groups within cohorts. However, the small differences of up to 0.9 y (Cohort 3 females) were considered sufficient to include age as a covariate in all regression models that follow. Subjects in Atole villages tend to be taller and heavier than those from Fresco villages as reported by Rivera et al. (1995) for the whole sample. Differences are statistically significant for all measures in males and for height in females when all cohorts are combined and age is a covariate (data and analysis not shown). However, they tend to be nonsignificant when exposure cohorts are analyzed separately because of reduced statistical power. FFM is greater in Atole males compared with Fresco males in all cohorts, while differences in females were not seen. BMI is similar between both groups.

Males tend to be delayed in maturation relative to the British children on whom the TW2 method is standardized, but their deviations from CA do not differ according to treatment (Table 1). Girls are less delayed than boys, especially in Cohort 2, where the small age deviations and reduced variances are due to a large number of girls having already achieved skeletal maturity compared with boys. (66% vs. 16%). Because there is no significant difference by supplement type in SA within the two younger cohorts, subsequent analyses do not control for this variable.

Group means for physical performance measures at maximum exertion are presented in **Table 2**. The results of analysis of covariance controlling for age indicate that there are no treatment group differences in maximum heart rate, suggesting that both groups reached similar levels of exertion and that the heart rates along with Respiratory Quotient values are consistent with published values, which suggests these subjects reached maximum exertion (Astrand and Rodahl 1986).

Oxygen consumption at maximum exertion ($\dot{V}O_2max$, L/min) is greater in males than females and increases with age (cohort) in both sexes, except that values are similar in females in Cohorts 2 and 3. $\dot{V}O_2max$ was significantly higher in Atole as compared with Fresco subjects when all cohorts are combined as shown in **Table 3**. This is seen in both sexes and regardless of whether body weight or FFM were controlled in the regression model. Atole vs. Fresco differences also are observed for $\dot{V}O_2max$ (L/min) in each cohort for males (Table 2), but the greatest treatment effect, based on both absolute values and *t* values (effect size), is seen in Cohort 2. Among females (Table 2), the Atole-Fresco differences in $\dot{V}O_2max$ are similar across all cohorts (0.09–0.11 L/min), while only in Cohort 1 is this difference statistically significant at $P < 0.05$.

Although age may be the most important covariate in this analysis, other factors also may confound the relationship between treatment and $\dot{V}O_2max$.

TABLE 2

***Mean values for physiological measurements at maximum exertion in Atole and Fresco subjects by cohort*[1]**

Variable, group	Cohort 1	Cohort 2	Cohort 3
	Males		
Maximum heart rate, *beats/min*			
A	207.1 ± 6.7	205.2 ± 6.3	201.9 ± 7.1
F	208.2 ± 5.2	206.0 ± 5.5	201.5 ± 7.8
$\dot{V}$Emax [BTPS], *L/min*			
A	61.8 ± 11.3	88.7 ± 18.7	103.0 ± 13.4
F	59.4 ± 12.0	83.0 ± 16.9	101.8 ± 21.1
$\dot{V}CO_2$max [STPD], *L/min*			
A	1.86 ± 0.45***	2.89 ± 0.62***	3.29 ± 0.41
F	1.60 ± 0.34	2.43 ± 0.59	3.13 ± 0.45
$\dot{V}O_2$max [STPD], *L/min*			
A	1.70 ± 0.36**	2.62 ± 0.54**	2.98 ± 0.31*
F	1.50 ± 0.30	2.24 ± 0.54	2.77 ± 0.39
$\dot{V}O_2$max [STPD], $mL/kg\ BW \cdot min^{-1}$			
A	49.5 ± 4.4**	54.4 ± 6.4***	52.0 ± 5.1
F	46.1 ± 3.9	48.6 ± 6.5	49.7 ± 7.1
$\dot{V}O_2$max [STPD], $mL/kg\ FFM \cdot min^{-1}$			
A	60.1 ± 6.2	65.5 ± 7.1***	64.3 ± 5.9
F	57.3 ± 6.7	59.2 ± 7.2	61.9 ± 6.5
RQ max			
A	1.10 ± 0.06	1.11 ± 0.06	1.12 ± 0.08
F	1.08 ± 0.05	1.10 ± 0.06	1.15 ± 0.07
O_2 pulse, *mL/beats*			
A	8.2 ± 1.7**	12.8 ± 2.7**	14.8 ± 1.7
F	7.2 ± 1.5	10.9 ± 2.6	13.8 ± 2.2
$\dot{V}E/\dot{V}O_2$ [STPD]			
A	34.3 ± 3.2**	31.8 ± 4.3**	32.4 ± 3.9
F	37.3 ± 4.8	35.2 ± 4.4	34.3 ± 5.5
Sample size			
A	33	44	24
F	25	42	20
Totals	58	86	44
	Females		
Maximum heart rate, *beats/min*			
A	210.4 ± 6.4	208.3 ± 9.4	202.4 ± 8.5
F	208.7 ± 7.3	205.9 ± 6.8	202.1 ± 6.5
$\dot{V}$Emax [BTPS], *L/min*			
A	54.6 ± 9.4	69.4 ± 11.8	68.9 ± 9.4
F	53.3 ± 9.8	69.4 ± 11.3	69.3 ± 9.0
$\dot{V}CO_2$max [STPD], *L/min*			
A	1.56 ± 0.25**	2.02 ± 0.29*	1.98 ± 0.28
F	1.38 ± 0.28	1.85 ± 0.33	1.85 ± 0.37
$\dot{V}O_2$max [STPD], *L/min*			
A	1.40 ± 0.22*	1.74 ± 0.26	1.73 ± 0.21
F	1.29 ± 0.26	1.65 ± 0.31	1.63 ± 0.29
$\dot{V}O_2$max [STPD], $mL/kg\ BW \cdot min^{-1}$			
A	39.5 ± 3.8**	36.6 ± 4.0	35.4 ± 4.3
F	35.9 ± 4.1	34.5 ± 4.5	33.0 ± 4.6
$\dot{V}O_2$max [STPD], $mL/kg\ FFM \cdot min^{-1}$			
A	49.2 ± 4.2**	47.3 ± 4.7	46.1 ± 4.8
F	45.3 ± 5.0	45.3 ± 5.8	43.7 ± 6.1
RQ max			
A	1.12 ± 0.08	1.17 ± 0.07	1.16 ± 0.07
F	1.08 ± 0.08	1.14 ± 0.07	1.15 ± 0.06
O_2 pulse, *mL/beats*			
A	6.7 ± 1.1*	8.4 ± 1.6	8.5 ± 1.0
F	6.2 ± 1.3	8.0 ± 1.6	8.1 ± 1.4
$\dot{V}E/\dot{V}O_2$ [STPD]			
A	36.6 ± 4.8*	37.5 ± 5.1	37.4 ± 4.0
F	38.9 ± 5.4	39.9 ± 6.2	40.4 ± 5.6
Sample size			
A	26	34	21
F	35	40	20
Totals	61	74	41

[1] Values are means ± SD. Abbreviations used: A = Atole, F = Fresco, RQ = respiratory quotient.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for two-tail *t* test of Atole-Fresco difference in means after adjusting for age.

TABLE 3

Mean[1] $\dot{V}O_2$ max for Atole and Fresco subjects: separate sexes, ages 11–26 y

Model no.	Body size variable controlled[1]	Males: Atole (n = 92)[2]	Males: Fresco (n = 77)	t value	Females: Atole (n = 75)	Females: Fresco (n = 84)	t value
		L/min				*L/min*	
I	None	2.43 ± 0.04	2.15 ± 0.04	4.99***	1.63 ± 0.03	1.51 ± 0.03	2.74**
II	Body weight	2.41 ± 0.03	2.18 ± 0.03	5.47***	1.63 ± 0.02	1.52 ± 0.02	3.97***
III	Fat-free mass	2.39 ± 0.03	2.21 ± 0.03	4.60***	1.61 ± 0.02	1.53 ± 0.02	3.03**

[1] Adjusted for age, age^2, socioeconomic status, village size and village size by supplement type interaction plus the body size measure indicated for each regression model. Values are means ± SE.
[2] Sample sizes reduced from Tables 1 and 2 due to missing data for socioeconomic status.
** $P < 0.01$, *** $P < 0.001$, two-tailed t test for difference between Atole and Fresco.

Therefore, the multiple regression analysis was expanded for Cohort 2 to include SES and level of subject participation in the nutritional supplementation program. The effect of supplement type before and after controlling for these covariates is given in **Table 4** where the regression analysis is reported for Cohort 2 males and in **Table 5** for Cohort 2 females. Model 1 in these tables is the same analysis for which the t test results are reported in Table 2; that is, the difference in age-adjusted means for $\dot{V}O_2$max (L/min) between Atole and Fresco groups. Models 2–6 include age, village size, SES and volume of supplement as potential confounders. In all models for females and most models for males, volume of supplement and SES are not significant confounders. However, they are retained in all models for consistency of comparison of coefficients between models and between sexes. Models 3–6 test for the potential mediating effect of body size on the relationship between nutritional supplementation and $\dot{V}O_2$max. Model 3 gives essentially the same results, in terms of the magnitude and level of significance of the nutritional effect, as when $\dot{V}O_2$ is expressed per kg body weight or as maximum aerobic power (mL/kg · min^{-1}) as shown in Table 2.

For males (Table 4) an interaction (P = 0.015) is observed between supplement type and village size but

TABLE 4

Regression models to test the effect of nutritional supplementation on $\dot{V}O_2$ max mediated by various measures of body size: Cohort 2 males[1]

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
	β	P	β	P	β	P	β	P	β	P	β	P
Intercept	−1.33	0.039	−1.10	0.098	−0.52	0.25	−0.85	0.041	−4.81	<0.001	−0.83	0.39
Age, *y*	0.217	<0.001	0.211	<0.001	0.038	0.24	0.048	0.096	0.118	<0.001	0.039	0.22
Village size (1 = large, 0 = small)	—	—	−0.69	<0.001	−0.26	<0.001	−0.22	0.002	−0.30	<0.001	−0.26	<0.001
Socioeconomic status	—	—	0.029	0.67	−0.054	0.21	−0.053	0.18	−0.093	0.075	−0.058	0.20
Volume of supplement ranking, *percentile*	—	—	0.004	0.073	0.0004	0.78	0.001	0.31	0.001	0.46	0.0004	0.79
Supplement type (1 = Atole, 0 = Fresco)	0.311	0.003	0.002	0.99	0.24	<0.001	0.18	0.003	0.23	0.005	0.24	<0.001
Supplement type by village size interaction[2]	—	—	−0.51	0.015	—	—	—	—	—	—	—	—
Weight, *kg*	—	—	—	—	0.053	<0.001	—	—	—	—	0.050	<0.001
Fat-free mass, *kg*	—	—	—	—	—	—	0.067	<0.001	—	—	—	—
Height, *cm*	—	—	—	—	—	—	—	—	0.035	<0.001	0.003	0.71
R^2 (*df*)	0.36	(2, 83)	0.51	(6, 72)	0.77	(6, 72)	0.80	(6, 72)	0.68	(6, 72)	0.77	(7, 71)
RMSE	0.46		0.43		0.28		0.26		0.33		0.28	

[1] $\dot{V}O_2$max was measured in L/min.
[2] Interaction was tested and if P was >0.20, the final model was run without interaction.

TABLE 5

***Regression models to test the effect of nutritional supplementation on $\dot{V}O_2$ max mediated by various measures of body size: Cohort 2 females*[1]**

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6	
	β	P	β	P	β	P	β	P	β	P	β	P
Intercept	2.17	<0.001	2.02	<0.001	0.83	0.036	0.27	0.50	−1.51	0.134	−0.50	0.50
Age, *y*	−0.031	0.22	−0.025	0.37	−0.044	0.023	−0.034	0.061	−0.027	0.29	−0.043	0.023
Village size (1 = large, 0 = small)	—	—	0.02	0.76	−0.09	0.24	−0.09	0.18	−0.013	0.84	−0.09	0.20
Socioeconomic status	—	—	0.016	0.73	−0.011	0.75	−0.003	0.94	−0.022	0.62	−0.024	0.50
Volume of supplement ranking, *percentile*	—	—	−0.0002	0.92	−0.0003	0.78	−0.0003	0.81	0.0001	0.93	−0.0003	0.79
Supplement type (1 = Atole, 0 = Fresco)	0.07	0.32	0.07	0.39	−0.02	0.83	−0.07	0.34	−0.012	0.87	−0.045	0.58
Supplement type by village size interaction[2]	—	—	—	—	0.16	0.15	0.19	0.060	—	—	0.14	0.17
Weight, *kg*	—	—	—	—	0.035	<0.001	—	—	—	—	0.032	<0.001
Fat-free mass, *kg*	—	—	—	—	—	—	0.057	<0.001	—	—	—	—
Height, cm	—	—	—	—	—	—	—	—	0.025	<0.001	0.010	0.042
R^2 (*df*)	0.05	(2, 71)	0.04	(5, 61)	0.59	(7, 59)	0.63	(7, 59)	0.25	(6, 60)	0.61	(8, 58)
RMSE	0.29		0.29		0.20		0.19		0.26		0.19	

[1] $\dot{V}O_2$max measured in L/min.
[2] Interaction was tested and if *P* was >0.20, the final model was run without interaction.

only in Model 2. $\dot{V}O_2$max differences in males are seen exclusively in the large villages where Atole subjects have a 0.509 L/min higher value than Fresco subjects. For the models that include measures of body weight and height (3, 5 and 6), age becomes nonsignificant as expected. However, village size remains a significant contributor to variation in $\dot{V}O_2$max with higher values seen in the large villages. Of importance in these models is that the size of the supplement type effect is fairly constant, the difference between Atole and Fresco ranging from 0.18 to 0.27 L/min.

For females (Table 5) the addition of the four potential confounders in Model 2 does not change the size or significance of the supplement type effect seen in Model 1 and there continue to be no significant differences in $\dot{V}O_2$max associated with supplement type. The interaction between village size and supplement type seen in males (Table 4, Model 2) is not seen in females. When different measures of body mass are included in subsequent models (3, 4 and 6) the supplement type effects approach statistical significance, but only in the context of differences in village size. There is a tendency for girls from Atole villages to have greater $\dot{V}O_2$max per unit of weight than girls from Fresco villages but only in the large villages (Model 3; the *P* value for the interaction is 0.15). When FFM is controlled (Model 4) the interaction is much stronger, with a $P = 0.06$, reflecting a 0.19 L/min higher $\dot{V}O_2$max in the large Atole compared with the large Fresco village. When height is controlled (Model 5) neither the supplement type effect ($P > 0.05$) nor its interaction ($P > 0.20$) with village size are statistically significant. The results of these analyses for main effects of supplement type are presented graphically in **Figures 2** and **3**. The adjusted mean $\dot{V}O_2$max values can be seen to differ significantly between Atole and Fresco males regardless of which covariates are used in the adjustment or whether $\dot{V}O_2$max is expressed as L/min (Fig. 2) or divided by a measure of body size to reflect a measure of aerobic power (Fig. 3). However, the Atole and Fresco $\dot{V}O_2$max means are similar in females.

To assess the plausibility of these treatment effects, measures of physical work capacity were related to

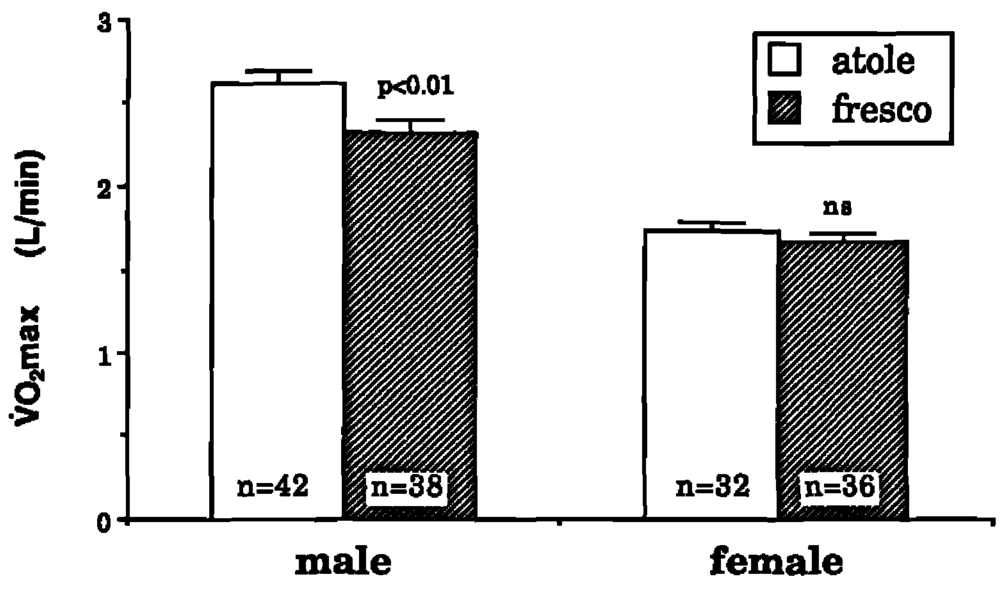

FIGURE 2 Mean $\dot{V}O_2$ max (L/min) of Cohort 2 subjects from Atole and Fresco villages. Statistically significant supplement type effect is seen only in males after controlling for age, SES, village size and level of participation in the supplementation.

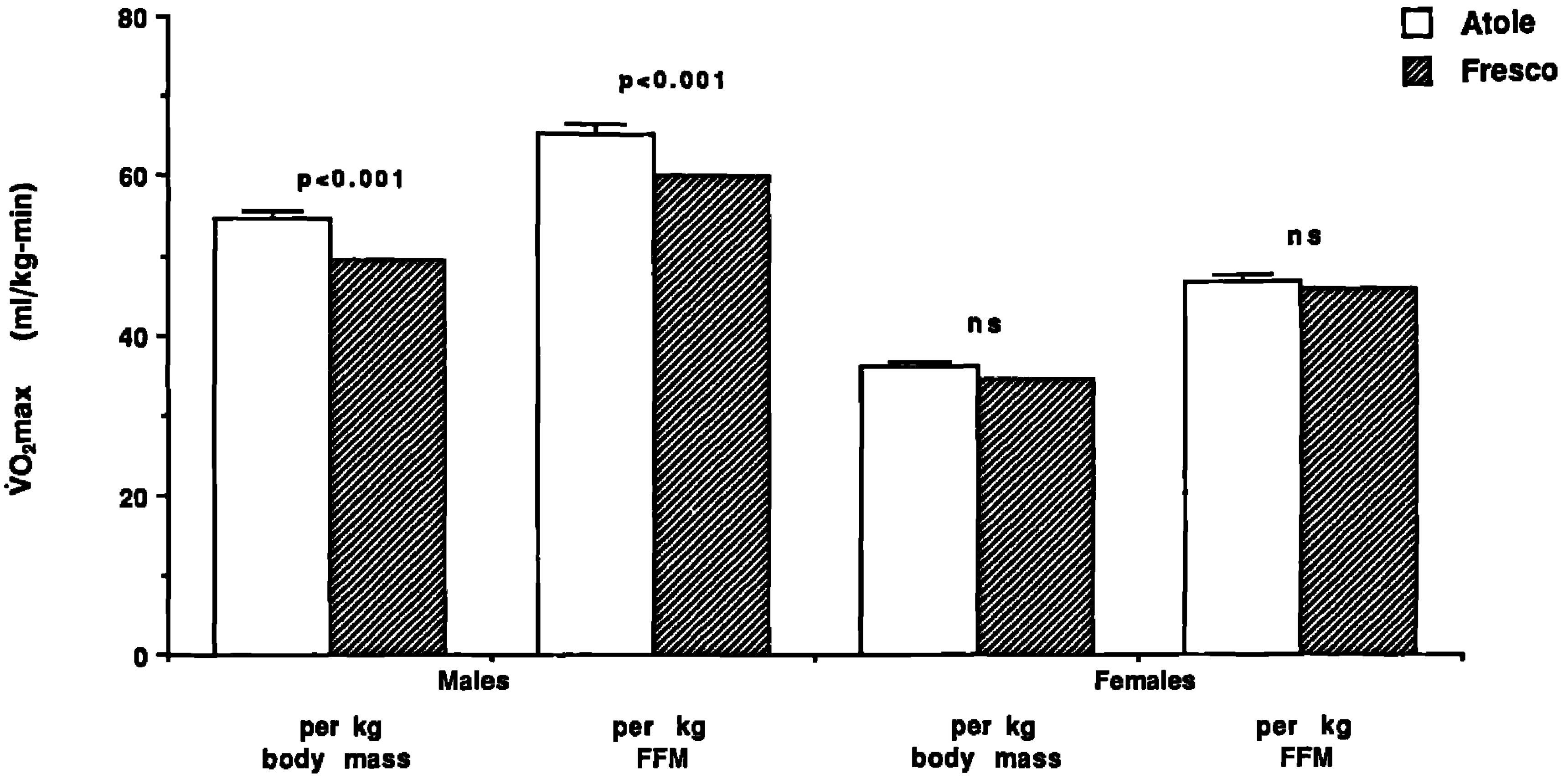

FIGURE 3 Mean maximum aerobic power (oxygen consumption per body mass and per FFM) of Cohort 2 subjects from Atole and Fresco villages. A statistically significant supplement type effect is seen in males after controlling for the same variables reported in Figure 2.

the amount of supplement consumed by individual subjects in Cohort 2 of Atole villages. The analysis, shown in **Table 6**, was conducted using linear regression procedures that modeled $\dot{V}O_2max$ as the dependent variable with age and village size controlled as covariates and kilocalories of supplemental energy consumed during the first 3 y (kcal/d) as the independent variable. SES also was tested as a covariate but was not statistically significant in any model. Also, the interaction between village size and kcal/d was tested to determine if the slope of the relationship between $\dot{V}O_2max$ and kcal/d was different between the large and small Atole villages. No interaction was detected ($P > 0.20$) and the term was excluded from the final models. The statistically significant ($P = 0.044$) coefficient of 0.155 in Model 1 for males indicates that for every 100 kcal/d of Atole consumed over the first 3 y of life, there is a 0.155 L/min increase in $\dot{V}O_2max$ measured at adolescence. When body weight is included in the Model 2, much of the dose effect is reduced and the coefficient drops to a 0.065 L/min increase in $\dot{V}O_2$ for a 100 kcal/d increase in supplement consumption ($P = 0.193$). For females, the dose response is opposite to that seen among males. For every 100 kcal/d increase in Atole consumption, there is a significant ($P = 0.008$) 0.169 L/min decrease in $\dot{V}O_2max$. When body weight is included in this model, the negative effect of supplement is reduced but remains statistically significant ($P = 0.003$).

DISCUSSION

The hypothesized effect of early nutritional supplementation on work capacity at adolescence was observed, particularly in males. Atole males have significantly greater $\dot{V}O_2max$ values (L/min) at all ages or cohorts, while Atole females have significantly greater values only in Cohort 1. The Atole-Fresco differences in Cohort 2 males persist after controlling for SES, village size, age and level of participation in the supplementation trial.

In Cohort 2 Atole males' $\dot{V}O_2max$ was significantly related to the amount of supplement consumed. This positive dose-response relationship appears to be partially, but not totally, mediated by body size, which is also responsive to the amount of supplement consumed. The unexpected negative dose-response seen in girls is difficult to explain. Because Atole-Fresco differences in $\dot{V}O_2max$ are not significant in females in Cohort 2, this may represent a spurious relationship. On the other hand, this may be evidence for self-selection bias related to unmeasured characteristics of the girls who consume higher amounts of Atole.

The subjects who participated in the physical performance testing were meant to be a random subsample of all possible subjects. After two rounds of random sampling, 84 and 77% of the selected subjects in Atole and Fresco villages, respectively, consented to the performance testing. The best response rate was found in the younger subjects (<18 y) who were still in school or working around the home. These included Cohort 1 and most of Cohort 2, the groups most likely to reflect a treatment effect due to their early age at the time of supplementation. Evidence for the subsample being representative of the follow-up sample can be seen in a comparison of anthropometry and amount of supplement ingested. The small biases that occur, although not statistically significant, contribute to greater Atole vs. Fresco differences in height in the

TABLE 6

Relationship of $\dot{V}O_2$ max and amount of supplemental energy consumed in the first 3 y of life in Atole Cohort 2 adolescents, controlling for age, village size and body weight[1]

	Males				Females			
	Model 1		Model 2		Model 1		Model 2	
	β	P	β	P	β	P	β	P
Intercept	−1.25	0.18	−0.79	0.19	2.63	<0.001	1.46	<0.001
Age, y	0.23	<0.001	0.05	0.28	−0.05	0.14	−0.07	0.002
Village size (0 = small, 1 = large)	−0.23	0.13	−0.27	0.009	0.18	0.060	0.14	0.022
Kcal · 100 per d	0.155	0.044	0.065	0.19	−0.169	0.008	−0.122	0.003
Weight, kg	—	—	0.056	<0.001	—	—	0.030	<0.001
R^2	0.34		0.74		0.28		0.73	
RMSE	0.46		0.29		0.24		0.15	
df	3, 39		4, 38		3, 28		4,27	

[1] $\dot{V}O_2$max measured in L/min.

subsample than was observed for the follow-up sample. The greater heights in the subsample of Atole subjects may be due to overrepresentation of adolescents from Atole villages who consumed high quantities of supplement as young children. Thus, the subsample measured may not be a true random subsample of the follow-up sample. Therefore, the internal validity of the supplementation effect can be questioned. Appropriate statistical control for unequal participation can remove some of this disproportionate representation of Atole subjects. Supplement participation and other confounding variables (e.g., age and SES) were controlled through statistical procedures and the results in favor of better performance among Atole subjects remained significant.

Physical work capacity is affected by maturation (Bouchard et al. 1976; Kemper and Verschuur 1987). However, skeletal maturity does not differ between Atole and Fresco villages, thus disqualifying it as a confounder in this analysis. When skeletal age is used as a covariate (in place of CA) to test for Atole-Fresco differences, as done by Rivera et al. (1995) in analyses of body size and composition data, the results are unchanged.

Other individual confounders were not controlled in this analysis, but indirect evidence suggests that they probably did not play an important role in explaining the reported supplementation effects. Most prominent of these is physical activity. Evidence that this is not a serious confounder in this sample was demonstrated by Novak et al. (1990) who studied physical activity patterns by questionnaire and heart rate monitoring in a subsample of 132 subjects drawn from the same subjects reported here. Males were significantly more active than females, but there was no effect of early childhood nutritional supplementation on time spent at levels of physical exertion sufficient to raise heart rates to 75% of maximum.

Another potentially important confounder is iron deficiency anemia. Preliminary analysis of hematological and iron status data for the exercise subsample indicates a very low prevalence (<5%) of anemia severe enough to compromise work capacity, and there are no differences in mean hemoglobin concentration, prevalence of anemia or prevalence of iron deficiency between Atole and Fresco villages.

The results reported here generally are consistent with those reported by other researchers examining the relationship between anthropometric status and work capacity in adolescents. The values for height and $\dot{V}O_2$max (L/min and mL/kg FFM · min^{-1}) for Atole males and females are similar to those reported by Spurr and Reina (1989) for Colombian subjects who were underweight (<95% of Colombian reference for weight-for-age and weight-for-height). Both the Colombian underweight children and the Atole adolescents of both sexes and all ages are below normal-weight Colombian children in height and $\dot{V}O_2$max (L/min). The Fresco subjects are well below the Colombian underweight children in $\dot{V}O_2$max regardless of age or sex. These two study samples differ when $\dot{V}O_2$max is expressed per kg FFM (**Fig. 4**). Spurr and colleagues (1983, 1989) and others (Desai et al. 1984, Satyanarayana et al. 1979) consistently have reported that the differences in work capacity ($\dot{V}O_2$max in L/min) between subjects with small body size and those with normal size are eliminated when work capacity is expressed per body weight, and often the trend is reversed in favor of the smaller subjects when $\dot{V}O_2$max is expressed per kg FFM. The results from this study indicate that the differences in work capacity, although reduced, remain significant in favor of the Atole subjects after controlling for FFM. Thus, the conclusion of other investigators that the effects of small body size on work capacity are mediated through muscle mass is supported only partially in this sample of Gua-

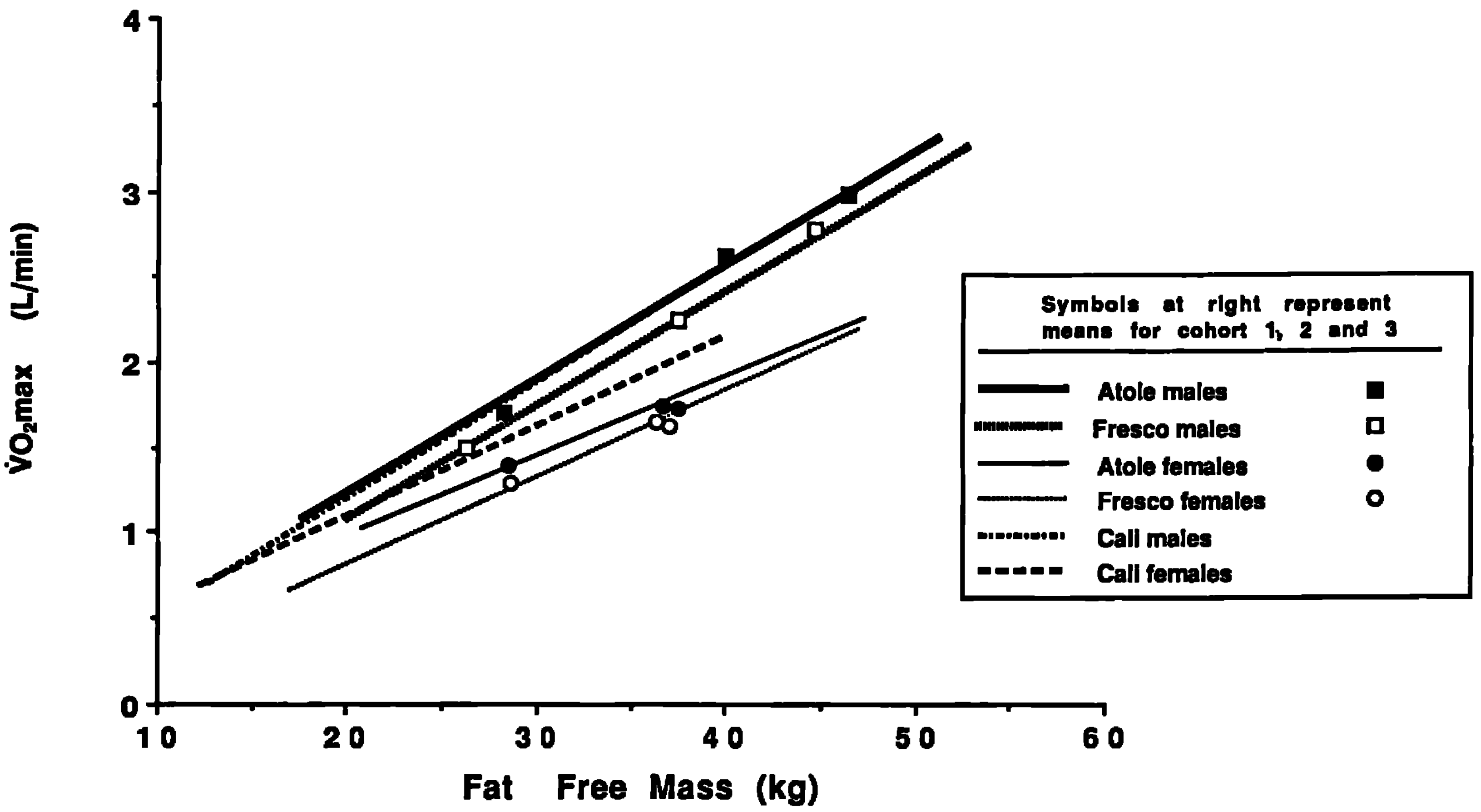

FIGURE 4 Relationship between maximum oxygen consumption ($\dot{V}O_2$ max) and fat-free mass (FFM) for subjects from all cohorts. Thickest and thinnest lines represent regression equations for each sex and nutrition group of Guatemalan subjects and corresponding symbols represent means for each of three cohorts. Lines for Cali males and females represent regression equations reported for Colombian adolescents by Spurr and Reina (1989). Trends for Atole males and Colombian males are nearly identical and greater than Fresco males at any level of FFM. Atole females have lower $\dot{V}O_2$ max for a given FFM than Colombian girls but higher than Fresco females. The statistical analysis for Atole-Fresco differences are reported in Table 3.

temalan adolescents. Several factors could explain these differences.

The relationship between previous nutritional status and work capacity may be nonlinear with the strongest effect seen below a threshold of nutritional status as suggested by a study of Indian male adolescents (Satyanarayana et al. 1979). Relative to the Colombian adolescents studied by Spurr and Reina (1989), the Guatemalan subjects show evidence of a greater degree of growth retardation perhaps as a result of more severe undernutrition in early life.

Another difference between the Colombian and Guatemalan studies is the way subjects were classified. The Colombian subjects, as well as those studied for similar effects in Brazil (Desai et al. 1984) and East Africa (Davies 1977), were classified by current anthropometric indicators of past and/or recent undernutrition. Because current height and weight are only proxies of past nutritional status and are themselves highly correlated to current FFM, the expression of work capacity per kg of weight and FFM would logically lead to a reduction in group differences in uncorrected $\dot{V}O_2$max (L/min). The results reported by Satyanarayana et al. (1979) are more consistent with ours, possibly because they classified nutritional status based on height during the preschool period rather than retrospectively at adolescence.

Yet another difference between the Colombian and Guatemalan studies that might explain the contrasting results is the method used to estimate FFM. By applying prediction equations for FFM developed specifically for this population, most of the bias in the equations used by other authors is reduced. Whatever differences might exist between studies in estimating FFM, they appear to be small. From Figure 4 it is clear that the slopes for the relationship between $\dot{V}O_2$max and FFM are nearly identical among the three groups (Atole, Fresco, Cali) for each sex, suggesting a common effect of FFM on $\dot{V}O_2$max across different populations. However, the group differences in $\dot{V}O_2$ for a given FFM persist.

What explains the fact that differences in work capacity between Atole and Fresco subjects persist after controlling for FFM? Unfortunately, no data were collected to address this question. Future research might consider what role early malnutrition plays in the development of muscle fiber (Bedi et al. 1982, Saltin and Gollnick 1983) and its oxidative capacity in later life.

The reduced $\dot{V}O_2$max of Fresco males compared with Atole males appears not to be related to differences in pulmonary ventilation during maximum exertion. Atole subjects have a significantly lower ventilatory equivalent ($\dot{V}E/\dot{V}O_2$) than Fresco subjects (Table 2), which implies that less ventilation is required for a given amount of oxygen consumed. Also, because maximum heart rates are similar between Atole and Fresco groups, the oxygen pulse is significantly greater in Atole compared with Fresco subjects (Table 2). The exact mechanisms to explain how early chronic undernutrition affects respiratory or circulatory function is not known for human subjects. Future research should focus on the possible long-term or lifelong effects of early malnutrition on development of the lungs, heart and skeletal muscle.

The effects of early nutritional supplementation are much clearer in males than females, both with regard to main effects and to dose-response relationships. This does not seem to be related to differential participation in the supplementation trial or in the physical performance testing. It may be related to different levels of physical activity. Novak et al. (1990) have shown that females in this sample of adolescents are much less active than males and that the sex differences become greater with increasing age, perhaps because of culturally prescribed changes in sex roles associated with biological and social maturation. Spurr and Reina (1989) also observed similar reductions in $\dot{V}O_2max$ among Colombian girls as they matured through adolescence and suggest that age changes in physical activities (Spurr and Reina 1988) might account for this pattern.

Although the results reviewed above indicate that nutrition in early childhood influences adolescent physiological status, little is known about the biological mechanisms through which malnutrition affects oxygen uptake and utilization. Also, not much is known about the practical implications of these results to everyday functioning of individuals living in developing countries.

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Age at Menarche and Nutritional Supplementation[1,2]

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ABSTRACT Retrospective data on age at menarche were collected for 832 Guatemalans 15–30 y in age to test whether exposure to a high energy and high protein supplement (Atole: 163 kcals/682 kJ and 11.5 g protein per cup or 180 mL) during childhood led to earlier menarche than did exposure to a low energy, no protein supplement (Fresco: 59 kcals/247 kJ in 180 mL). Mean age at menarche was similar in Atole (13.75 ± 1.22 y; mean ± SD) and Fresco (13.74 ± 1.36 y) groups. The corresponding value for immigrants (n = 144), subjects not exposed to the supplements, was 13.55 ± 1.20 y. Year of birth as well as socioeconomic status (SES) were associated with age at menarche. Age at menarche declined by 0.69 y over the 15-y period and menarche occurred earlier in higher SES households. Significant positive interactions between supplement type and SES and between supplement type and year of birth were found, but plausible explanations for them could not be advanced. J. Nutr. 125: 1090S–1096S, 1995.

INDEXING KEY WORDS:

- *menarche* • *nutritional supplementation*
- *secular change* • *Guatemala*

Age at menarche occurs earlier than it once did in many parts of the world, especially in Europe and North America (Eveleth 1986, Laslett 1985, Tanner 1973, Wyshak and Frisch 1982). In these regions, age at menarche declined at the rate of ~0.3 y per decade till it stabilized at ~13 y (Eveleth 1986, Poppleton and Brown 1966, Sandler et al. 1984, Tanner 1981). This decline, also seen in some developing countries, has been attributed to improvements in nutrition and health (Bielicki et al. 1986, Eveleth 1986, Hulanicka and Waliszko 1991, Laslett 1985, Low et al. 1982, Malina et al. 1977, Rona 1975, Singh and Malhotra 1988). Similarly, the fact that menarche occurs later in the poorer social classes compared with the well-to-do in developing countries has been ascribed to disparities in nutrition and health (Charzewska et al. 1976, Chowdhury et al. 1977, Eveleth and Tanner 1990, Foster et al. 1986, Gandotra and Das 1982, Rana et al. 1986, Tan-Boom et al. 1983, Uche and Okorafor 1979).

Improvements in the quality of life during early childhood can result in earlier menarche. Indian and Bangladeshi girls adopted between 1 mo and 11 y of age (mean age 3.7 y) by Swedish families experienced earlier pubertal growth and menarche compared with Swedish and affluent Indian girls (Adolfsson and Westphal 1981, Proos et al. 1991a, Proos et al. 1991b). The timing of adoption had a significant but unexpected influence on menarche; girls who were adopted after 3 y of age reached menarche ~9 mo earlier than those adopted between birth and 3 y of age. Catch-up growth occurred in all age cohorts shortly upon arrival but in older girls it may have triggered endocrinological responses and early puberty, whereas in younger girls it did not. However, age at menarche still occurred earlier in girls adopted before 3 y of age than it did in subjects reared in India. The corresponding values were 11.9 and 11.1 y for girls adopted before and after 3 y of age, respectively, compared with 13.7 y for urban and 14.4 y for rural Indian–reared girls.

These findings suggest that broad improvements in nutrition and in living standards are determinants of

[1] Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Supported by National Institute of Health grant HD 22440 and grants from the International Center for Research on Women (LIC-75/03) and the Thrasher Research Fund (#2805–5).

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age at menarche. However, no study has examined the impact of community-based nutrition interventions during childhood on age at menarche. The aim of this study is to test the hypothesis that women who were exposed to a high energy and high protein supplement during childhood reached menarche earlier than those exposed to a low energy, no protein supplement.

MATERIALS AND METHODS

Data source and study sample. Data for this study are derived from a longitudinal nutritional intervention study carried out between 1969 and 1977 in Guatemala and from resurveys of the study subjects starting in 1991 (Martorell et al. 1995). The community-based nutrition intervention study was conducted by the Institute of Nutrition of Central America and Panama (INCAP) and was designed to test whether improved nutrition in early childhood results in better growth and development. Four villages stratified by size (two large and two small) were selected randomly to receive either a high energy, high protein supplement called Atole or a low energy, no protein one called Fresco. Atole contained 163 kcal (682 kJ) and 11.5 g protein per cup or 180 mL, whereas the Fresco had 59 kcal (247 kJ) per 180 mL and no protein. Both supplements contained equal amounts of thiamin (1.1 mg), riboflavin (1.5 mg), niacin (18.5 mg), iron (5.0 mg) and vitamin A (1.2 mg). In addition, the Fresco contained a flavoring agent and Atole contained a small amount of fat (0.7 g), calcium (0.4 g) and phosphorus (0.3 g). More details about the intervention study are given elsewhere (Martorell et al. 1995).

Reproductive histories were collected in 976 women between 15 and 30 y of age in two survey rounds in 1991 and 1992. The target sample for these surveys was all women residents born between January 1962 and August 1977 (i.e., the range in birth dates of the subjects who participated in the longitudinal study). Women who were former participants ($n = 832$) of the longitudinal study are emphasized in this analysis. They represent 72% of the original sample with available supplementation records. Immigrants and subjects who were born after August 1977 ($n = 144$) are excluded from the main analyses but are used as a comparison group in some analyses. Immigrants were those who moved to the study villages because of marriage or the establishment of a new family after 1977 when the intervention ended.

Exposure to nutritional supplementation. Participation in the intervention study was voluntary and open to all members of the community. Intakes of supplement were measured daily for children until 7 y of age, pregnant women and women in the first 24 mo postpartum (Martorell et al. 1995). A limitation of the study is that supplement consumption was not recorded for other subjects. In addition, exposure to supplementation had variable entry ages and durations. For example, if a participant was 5 y old in 1969 when the intervention began, supplement intakes from 5 to 7 y were measured. She then could have received supplement for 7 more years until 1977 when the study ended, but these intakes were not measured.

Age at menarche. Menarche data were gathered during two rounds in 1991 and 1992. During each round, subjects were asked whether they had reached menarche. Those who answered affirmatively were then asked to recall the year and month of occurrence. All interviews were conducted at home.

Most of the subjects reported having reached menarche (i.e., 826/839). A total of 13 subjects had reached menarche but could not remember the date of the event in either round. This left 813 cases who had reached menarche and who reported some data in at least one of the rounds. Comparison of the reported age at menarche for subjects giving both year and month in both rounds ($n = 420$) revealed much inconsistency in response, with the dates matching in only 176 subjects. When differences were between 1 and 24 mo, values were averaged and included in the analyses ($n = 237$); cases with differences of >24 months were excluded ($n = 7$). If age at menarche was missing or the month was not recalled in one of the two rounds, the nonmissing value was selected or a value was assigned as described below ($n = 393$). When a month was not reported in either round but the reported year was the same, menarche was assumed to have occurred in midyear and an additional 6 mo of age was added to the reported year ($n = 21$). For cases where years differed by 1 or 2 but months were not given, the 1991 record for year was selected and an additional 6 mo was added to the reported year to estimate age at menarche ($n = 90$). The final sample consisted of 806 subjects who had reached menarche and 13 who had not.

Statistical analysis. Statistical analyses included Student's two-tailed t test of unequal sample size for comparison of mean ages at menarche in Atole and Fresco groups. Linear regression was used to evaluate the effects of possible covariates on age at menarche. The 13 cases that had not reached menarche, said to be "right censored," were left out of the above analyses ($n = 806$). Survival analysis techniques, primarily the SAS procedure PROC LIFEREG (SAS Institute, 1987), were used to include these cases ($n = 819$).

SES, village size and year of birth were included as predictors of age at menarche in the regression model. The SES score was derived from a principal components analysis and is based on household characteristics and an inventory of possessions in 1975; as such, the score measures accumulated wealth within the household (Rivera et al. 1995). The SES score was

TABLE 1

Exposure to supplementation (1969–1977) by year of birth for cases that had reached menarche by 1992[1]

Birth year	Age at exposure	Potential years of exposure	Age at interview	Sample size Atole	Sample size Fresco
	y		y		
1962	7–16	9	29–30	17	23
1963	6–15	9	28–29	17	25
1964	5–14	9	27–28	13	21
1965	4–13	9	26–27	21	18
1966	3–12	9	25–26	26	26
1967	2–11	9	24–25	20	21
1968	1–10	9	23–24	19	21
1969	0–9	9	22–23	30	24
1970	0–8	8	21–22	27	22
1971	0–7	7	20–21	26	34
1972	0–6	6	19–20	33	27
1973	0–5	5	18–19	32	32
1974	0–4	4	17–18	35	34
1975	0–3	3	16–17	28	38
1976	0–2	2	15–16	33	22
1977	0–1	1	14–15	19	22
Total				396	410

[1] Thirteen cases had not reached menarche by 1992; $n = 806$.

TABLE 2

Mean age at menarche by supplement type[1]

Birth year	Supplement type Atole	Supplement type Fresco	Difference[2]
	y		
1962	13.74 ± 1.22	13.55 ± 1.31	+0.19
1963	13.88 ± 1.32	14.01 ± 1.44	−0.13
1964	13.68 ± 1.62	13.77 ± 1.40	−0.09
1965	13.84 ± 1.21	14.50 ± 1.32	−0.66
1966	13.98 ± 1.01	14.37 ± 1.39	−0.39
1967	14.01 ± 1.43	13.93 ± 1.14	+0.08
1968	14.03 ± 1.09	14.11 ± 2.03	−0.08
1969	14.02 ± 1.24	13.75 ± 1.24	+0.27
1970	13.91 ± 1.31	14.29 ± 1.40	−0.38
1971	13.61 ± 1.42	14.01 ± 1.17	−0.40
1972	13.73 ± 1.51	13.89 ± 1.37	−0.16
1973	13.93 ± 1.40	13.35 ± 1.22	+0.58
1974	13.51 ± 1.44	13.57 ± 1.16	−0.06
1975	13.53 ± 0.73	13.29 ± 1.12	+0.24
1976	13.49 ± 1.04	13.18 ± 1.02	+0.31
1977	13.16 ± 0.93	12.80 ± 0.95	+0.36
Mean	13.75 ± 1.22	13.74 ± 1.36	+0.01
Overall	13.71 ± 1.29		

[1] Thirteen cases had not reached menarche and are not included; $n = 799$.

[2] Difference = Atole − Fresco. T tests comparing differences at each birth year were all nonsignificant at $P < 0.05$.

normalized within village with a mean of 0 and an SD of 1.

Differences in mean age at menarche were considered statistically significant at $P < 0.05$. Main effects and interactions were considered significant at $P < 0.05$ and $P < 0.10$, respectively. All analyses were done using SAS-PC version 6.04.

RESULTS

Supplementation and age at menarche. Ages at exposure, potential years of exposure to supplements, age at interview and sample sizes of those who had reached menarche by 1992 are presented in **Table 1**. The mean age at menarche for the entire sample is 13.71 ± 1.29 y (mean ± SD, and does not differ by supplement type; the values are 13.75 ± 1.22 y for Atole and 13.74 ± 1.36 y for Fresco villages (**Table 2**). Age at menarche does, however, vary by year of birth (**Fig. 1**). There is a decline in mean age at menarche over time for both groups; the overall rate of decline is ~0.69 y over the 15-y period as calculated by a regression of age at menarche on year of birth.

Determinants of age at menarche. **Table 3** shows the results of two separate regression models: with age at menarche as the dependent variable and supplement type, year of birth, a second degree polynomial of year of birth, SES score and village size as covariates in both models. Model 2 includes these variables and interactive terms. In Model 1, supplement type is not a significant predictor of menarche but all other covariates are. Older subjects reached menarche later and higher SES was associated with earlier menarche. Village size (large = 1, small = 0) was associated positively with age at menarche.

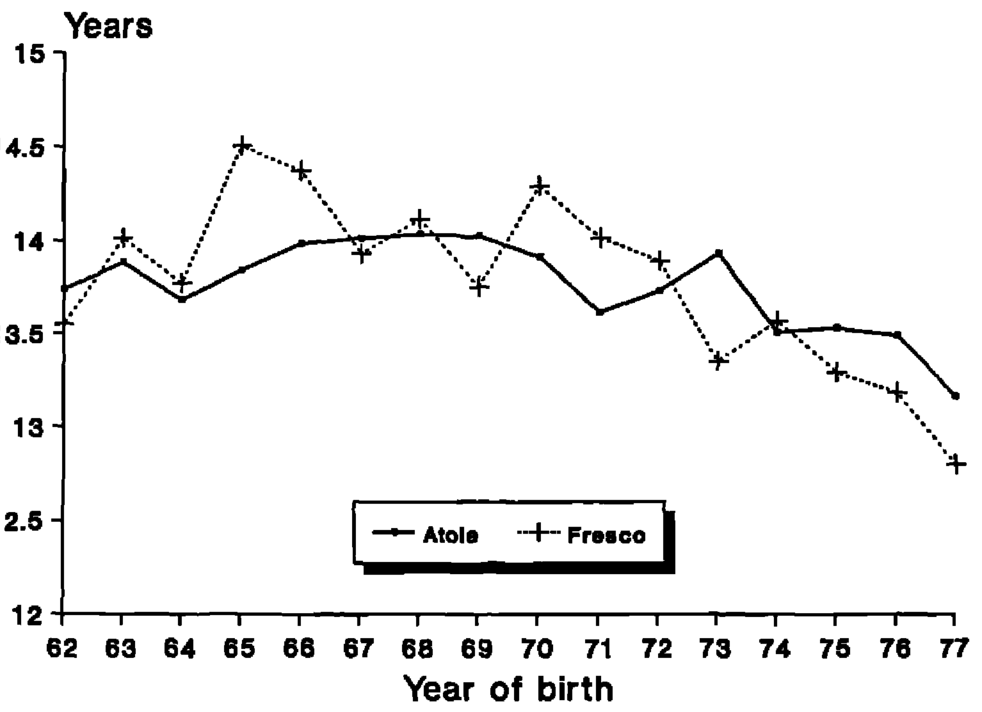

FIGURE 1 Mean age at menarche by supplement type and year of birth (n = 799).

TABLE 3

Determinants of age at menarche: multiple linear regression analyses

Independent variable	Model 1 b	Model 1 P	Model 2 b	Model 2 P
Supplement type[1]	0.069 ± 0.094	0.464	−0.337 ± 0.215	0.117
Year of birth[2]	0.130 ± 0.047	0.006	0.125 ± 0.047	0.009
Square of YRBIRTH[3]	−0.011 ± 0.003	0.001	−0.012 ± 0.003	0.001
SES[4]	−0.145 ± 0.050	0.004	−0.279 ± 0.073	0.001
Village size[5]	0.256 ± 0.097	0.008	0.317 ± 0.099	0.001
YRBIRTH*SUPPL[6]			0.046 ± 0.021	0.032
SES*SUPPL[7]			0.255 ± 0.102	0.012
CONSTANT	13.499 ± 0.193	0.000	13.549 ± 0.211	0.000
	R^2 = 0.07		R^2 = 0.09	
	df = 688[8]		*df* = 688	

[1] 1 for Atole and 0 for Fresco supplements, and abbreviated as SUPPL.
[2] Coded as 1962 = 1, 1963 = 2, etc., and abbreviated as YRBIRTH.
[3] Second degree polynomial term for year of birth.
[4] Composite score based on characteristics of home and household possessions in 1975. Scores are a standardized variable with a mean of zero. SES = socioeconomic status.
[5] 1 for large and 0 for small villages.
[6] Interaction of year of birth and supplement type.
[7] Interaction of SES and supplement type.
[8] The degrees of freedom are fewer than the original number of subjects because of missing data for some of the independent variables.

In Model 2, significant two-way interactions between year of birth and supplement type and between SES score and supplement type are included. Other two-way interactions between year of birth and SES score and between village size and supplement type also were tested but were not found to be statistically significant. The interaction between year of birth and supplement type in Model 2 reflects the fact that for cases who were exposed to the supplements at older ages, menarche occurred earlier in Atole villages compared with Fresco villages. In cases exposed at younger ages, menarche was later in Atole compared with Fresco villages (see Fig. 1 and Table 2).

The positive interaction between SES score and supplement type suggests that, at high levels of SES, exposure to Atole was associated with later menarche; at lower levels of SES, on the other hand, exposure to Atole was associated with earlier menarche.

Results using survival analysis to incorporate cases who had not reached menarche (n = 13) were nearly identical to those in Table 3 and therefore are not presented.

DISCUSSION

The mean age at menarche in subjects exposed to Atole, a high energy and high protein supplement was 13.74 y, nearly identical to the value of 13.75 y found in subjects exposed to Fresco, a low energy, no protein supplement. The mean age at menarche for immigrants (n = 144), subjects not exposed to either supplement, was 13.55 ± 1.20 y. Most of these immigrants married village men and are likely to be of similar SES and education as the sample of women exposed to the supplements. The fact that immigrants, in effect a control group, reached menarche at about the same age as Atole and Fresco women provides additional evidence that supplementation did not influence age at menarche.

Some reports of age at menarche in Guatemala are available, Sabharwal et al. (1966) reported a mean age at menarche of 12.8 y for 230 upper socioeconomic urban girls and 14.5 y for 218 lower socioeconomic rural Mayan Indian girls. Notably, a later recalculation using probit analysis estimated those values to be 13.3 and 15.1 y, respectively (Eveleth and Tanner, 1976). Delgado et al. (1984) reported that the median age of menarche of girls living under poor conditions on 12 coffee plantations located in Suchitepequez, Quezaltenango and Alta Verapaz was 14.81 y. The average values in the present study are only slightly later than found in the well-to-do urban sample (i.e., 13.71 vs. 13.3 y). In our study, women with higher SES (1.0 SD above the mean) reached menarche ~0.30 y earlier than those of lower SES (1.0 SD below the mean). Thus, women of higher SES in our sample had nearly identical mean age at menarche as the urban sample, if the recomputed value of 13.3 y is used.

Age at menarche showed a tendency to decline over time in Atole and Fresco groups as well as in immigrants. The observed rate of decline is slightly greater (0.50 y per decade) than reported in other studies. It may be that this value is an overestimation because 13 cases had not reached menarche by the end of data collection, artificially depressing the mean age at menarche. To test this theory, we assumed conservatively that all 13 subjects were "late" maturers and assigned them a value of 17.58 y, the overall mean plus 3 SD. Inclusion of these 13 subjects using assigned values did not alter the basic observation that menarche declined over time; however, the rate of decline was reduced to 0.39 y per decade.

Comparable data on changes in age at menarche over time in other Latin American populations are limited. A study from Chile (Rona 1975) found that the rate of decline was, on average, 0.29 y per decade between 1940 and 1970. Using recall data of women age 20–59 y, Malina et al. (1983) found no decline in age at menarche for girls in Oaxaca, Mexico.

A differential rate of decline in age at menarche according to SES has been reported (Low et al. 1982, Padro 1984, Rona 1975, Singh and Malhotra 1988). In Chile, Rona (1975) found that the rate of decline from 1940 to 1970 was greater in the middle classes than in the upper classes. In Madrid and a nearby suburb, Padro (1984) reported that menarche declined from 1935 and 1965 by 0.34 y per decade in the upper classes compared with 0.43 y in the lower classes. Similarly, among Malaysian Chinese, Indian and Malay females, ages at menarche fell on average 0.27 y per decade for women born between 1926 and 1961, with a greater decline observed in those raised in poorer households; about one half of the decline was accounted for by improved SES over the period studied (Tan-Boom et al. 1983). In summary, the secular trend in age at menarche is often greater in lower compared with higher SES groups.

In the present study, an interaction between year of birth and SES was not statistically significant in either Model 1 or 2, suggesting that the rate of decline in age at menarche was not influenced by SES. It may be that the variance in SES in our study is too small to pick up an interaction; in other studies (e.g., Padro 1984, Rona 1975), the socioeconomic differences were more extreme.

In the present study, statistically significant interactive effects of supplementation with SES and year of birth on menarche were found. At high SES (1.0 SD above the mean), subjects exposed to Atole reached menarche 0.90 mo later than those exposed to Fresco. At low SES, (1.0 SD below the mean) subjects exposed to Atole reached menarche 0.72 mo earlier than those exposed to Fresco. The strength of the interaction with year of birth can be ascertained from Figure 1. Reasonable and plausible explanations for these interactions have not been found despite considerable effort. In view of the moderate to small magnitude of the interactive effects and, more importantly, due to the lack of plausible explanations, these results are disregarded in drawing up the main conclusion of the analyses.

Intake of Atole was associated positively with childhood maturity as measured by the number of ossification centers present in subjects < 3 y of age (Martorell et al. 1979). Limiting the analysis of menarche to subjects exposed to supplement from birth to 3 y of age, the mean age at menarche (years) is exactly the same for both groups: 13.78 ± 1.26 for Atole ($n = 178$) and 13.78 ± 1.27 for Fresco ($n = 167$), respectively. This suggests that the previously demonstrated impact of supplementation on maturity during early childhood did not continue into early adulthood.

The relationship between supplementation and skeletal age in adolescence in our study population has been investigated by Pickett et al. (1995) using the Radius-Ulna-Short bones (RUS) option of the Tanner-Whitehouse-2 (TW2) method (Tanner et al. 1983). This method yields skeletal ages and relative maturation can be assessed by comparisons to chronological ages, with British data used as the reference population. Skeletal maturation was significantly delayed ($P < 0.05$) in the combined sample only in boys 11–14 y of age (by 1.2 y); in girls 11–14 years, values were slightly accelerated (+0.23 y) with respect to the reference. Many girls 14–18 y had reached maturity and a reliable estimate of maturational delay could not be made; in boys 14–18 y, there was a delay of 0.16 y, but this was not statistically significant. There were no significant differences ($P < 0.05$) in skeletal ages between Atole and Fresco subjects except in females 11–14 y of age, with those exposed to Atole being 0.4 y more advanced; however, these differences ceased to be statistically significant after controlling for SES. These findings corroborate those on menarche; there is no evidence of marked delays in maturation in the population and exposure to Atole has little or no impact.

There is the possibility that supplement intake in children older than 7 y of age may have attenuated Atole and Fresco differences in maturity. Very little Fresco was consumed in the first 3 y of life; however, by 5–7 y, consumption of Fresco was greater than that of Atole in terms of volume, such that high levels of energy and micronutrients were consumed at these ages in both Atole and Fresco villages. It is possible that these high levels of consumption persisted later into adolescence. This is unlikely, however, because former field workers recall that it was mostly women and the target children under 7 y of age who frequented the supplement centers. Nonetheless, the possibility should be entertained. Assuming that intakes of both Fresco and Atole remained high after 7 y of age and that these influenced age at menarche, one would have expected lower ages at menarche in the study sample compared with immigrants, but such was not the case.

The association between energy intakes and age at menarche has been examined previously, mostly in industrialized countries (Kissinger and Sánchez 1987, Maclure et al. 1991, Meyer et al. 1990, Moisan et al. 1990). In these studies, dietary intakes in the 1–3 y before menarche were examined; none looked at intakes during childhood as we have done. Results from these studies suggest that energy intakes in the years immediately before menarche have little or no relation to age at menarche, consistent with our own findings.

A limitation with this study is that the data on age at menarche were collected retrospectively. There is no reason to believe, however, that responses were biased either toward younger or older ages or that subjects of one supplement type responded differently from the other. Limiting the analyses to subjects who recalled their age at menarche within ±3 mo in the two surveys, the values were 13.78 ± 1.32 y (n = 111) and 13.56 ± 1.25 (n = 105) y for Atole and Fresco villages, respectively.

To our knowledge, no previous investigation of the effects of nutritional supplementation on menarche has been reported. Our study found that mean age at menarche was similar for subjects who received either a high energy and high protein supplement (Atole) or a low energy, no protein supplement (Fresco). No differences between supplement type were found when analyses were limited to those who accurately recalled their age at menarche or to those subjects who were exposed to supplements in the first 3 y of age. In addition, immigrants of similar SES background who were not exposed to any supplement had a similar mean age at menarche. Therefore, we conclude that nutritional supplementation did not have an affect on age at menarche in the present study. Nonetheless, the fact that both SES and year of birth were found to be associated with age at menarche indicates that menarche is subject to general environmental, and possibly nutritional, influences.

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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

[1] Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

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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] $n = 187$.

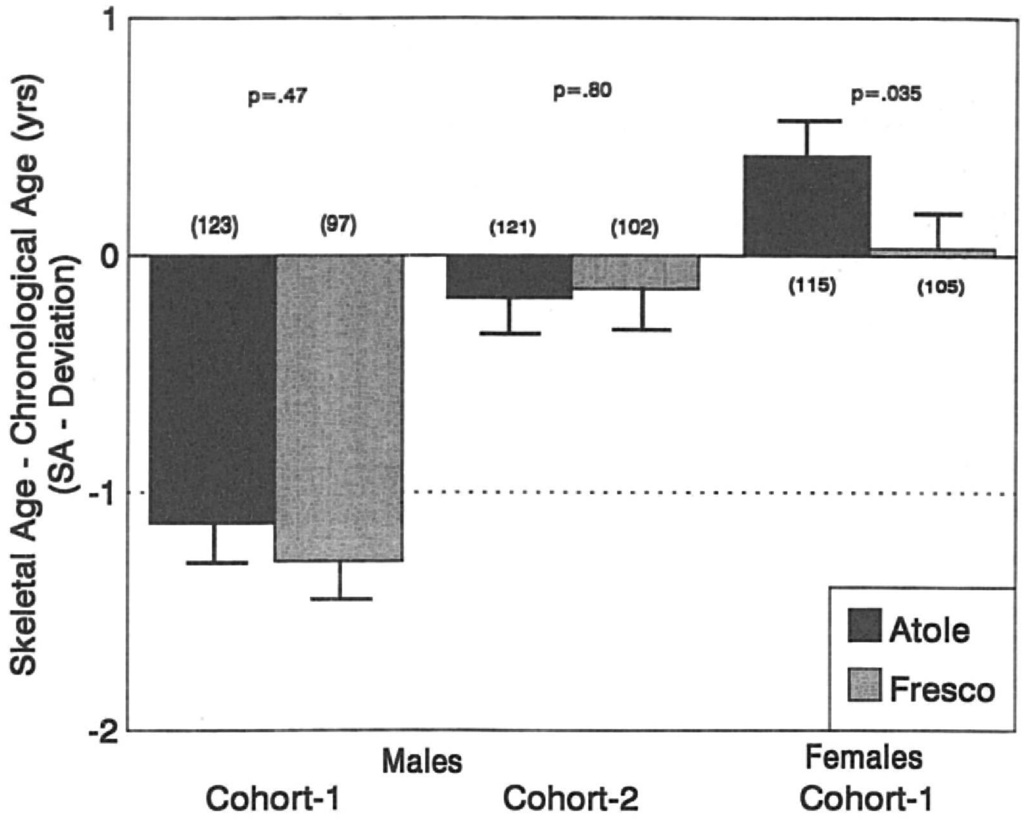

FIGURE 1 Atole and Fresco differences in the deviation of SA from CA among male and female Guatemalan adolescents. Height of the bars represent means after adjusting for age and village size (see Table 2). Brackets represent SE. Sample size for each sample in parenthesis. *P* values are for test of Atole-Fresco 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, 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 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.
* $P \le 0.05$ on two-sided test.

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

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

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.

ACKNOWLEDGMENTS

We thank Hilda Castro, Elizabeth Conlisk, Edward Frongillo, Jr., John Himes and Kimberly Suriano.

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Nutritional Supplementation During Early Childhood and Bone Mineralization During Adolescence[1,2]

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ABSTRACT To assess the long-term impact of nutritional supplementation on bone mineralization during adolescence, we studied 356 Guatemalan adolescents who participated from birth to 7 y of age in a controlled supplementation trial. Bone mineralization of the distal radius was assessed using single photon absorptiometry. Children who consumed more cumulative energy from the supplement during childhood had greater bone mineral content, bone width and bone mineral density during adolescence than those who consumed less energy. The associations remained after controlling for each subject's age and gender, and for the type of supplement consumed, but became statistically nonsignificant after adjusting for weight and stature. Because intake of supplement also was associated positively with weight and stature during adolescence, it is concluded that supplementing malnourished children can have a demonstrable long-term impact on bone mineralization, but that the effects are probably not beyond those due to improvements in overall somatic growth associated with supplementation. J. Nutr. 125: 1104S–1110S, 1995.

INDEXING KEY WORDS:

• *bone mineralization* • *nutritional supplementation* • *adolescence* • *malnutrition*

Adult levels of bone mineralization are largely achieved during the first two decades of life (Garn and Wagner 1969, Newton-John and Morgan 1971). Consequently, there is considerable interest in identifying determinants of this process in childhood and adolescence. Malnutrition is associated with low levels of bone density, diminished cortical bone and delays in skeletal maturation (Himes 1978, Himes et al. 1975, Martorell et al. 1979). Dietary interventions can enhance bone mineralization and skeletal maturation in infants and children who are undernourished in the short-term (Guzmán et al. 1965, Himes et al. 1990, Martorell et al. 1979), but the long-term permanency of such improvements is unknown.

From 1969 to 1977, the Institute of Nutrition of Central America and Panama (INCAP) conducted a longitudinal study of child growth and development in four rural communities in Guatemala (Martorell et al. 1995a). As part of this study, free food supplements were provided on demand to all inhabitants in the four communities. Findings from this study showed that early nutritional intervention can improve significantly the physical growth and development, bone growth and skeletal maturation of mild to moderately malnourished infants and children (Himes et al. 1990, Martorell et al. 1979, Schroeder et al. 1995).

In 1988–89, the former participants of the INCAP longitudinal study, by then adolescents and young adults, were the subjects of a follow-up study. This has provided a unique opportunity to assess the long-term impact of an intervention designed to improve the growth and development of malnourished children.

Specifically, the objective of the analyses presented here was to determine whether there is an effect of early nutritional supplementation on bone mineralization during adolescence. Further, because some evidence indicates that enhancement in bone mineralization may be independent of other more general so-

[1] Presented in part at the Annual Meeting of the American Public Health Association, October 1990, New York, NY. Caulfield, L. E. & Himes, J. H. (1990) Nutritional supplementation during early childhood and bone mineralization during adolescence. Published as a supplement to The Journal of Nutrition. Guest editors for this supplemental publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA, and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Supported partially by grants HD-24684 and HD-22440 from the National Institutes of Health, Bethesda, MD, USA.

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matic and maturational responses to supplementation (Himes 1978, Himes et al. 1990), a secondary objective was to determine if there is evidence of a long-term, bone-specific response to supplementation, beyond what may be expected from concurrent somatic responses in body size.

MATERIALS AND METHODS

The design and methods of the original INCAP longitudinal study have been described in detail elsewhere (Martorell et al. 1995a); thus, only details of the study pertinent to the analyses presented here are described. Two supplements were provided on demand in four rural communities in Guatemala; two of the villages received Atole, a high-calorie, high-protein drink, whereas the other two villages received Fresco, a low-calorie, no-protein drink. Also, Atole contained calcium and phosphorus whereas Fresco did not contain these minerals. Both supplements contained identical concentrations of iron, fluoride and some vitamins. Villagers were allowed to consume as much or as little supplement as they desired, and consumption was measured to the nearest 10 mL on a daily basis for all pregnant and/or lactating mothers and children 0–7 y of age. The growth and development of children during the first 7 y of age were studied in all subjects who were ≤7 y of age in 1969, when the study began, and in all children born into the study from 1969 to 1977, when the study ended.

The follow-up study, which began in 1988, was a cross-sectional follow-up of participants from the four study villages (Martorell et al. 1995a). Bone mineralization was measured on a subsample of 372 healthy adolescents and young adults stratified by age, gender and village, who had been exposed to nutritional supplementation during early childhood and who agreed to participate in the assessments of physical performance (for details, see Haas et al. 1995). At the time of examination, the subjects were 11—27 y of age. Measurements of bone mineral concentration (BMC), radial bone width (BW) and bone density (BD = BMC/BW) were made at the one-third distal site using a Norland Model 2780 Digital Bone Densitometer (Fort Atkinson, WI). The bone measures were obtained using single-photon absorptiometry from a ^{125}I source (Sorenson and Camerson 1967). Such measures are highly correlated with body calcium and with skeletal weight and are an appropriate method for assessing bone mineralization in the appendicular skeleton (Mazess 1983). Anthropometric dimensions, including stature and weight, were taken by trained observers using recommended methods (Lohman et al. 1988). The exact age of each subject was known from the birth records of the longitudinal study.

Of those 372 subjects examined, the files of 11 subjects were found to have missing data for either height, weight or bone mineralization measures and the files of 5 subjects could not be linked with those from the original INCAP longitudinal study and, therefore, no information was available on supplement intakes during early childhood. The analyses presented here relate to those 356 subjects for whom complete data on all variables of interest were available.

The effects of early nutritional supplementation on adolescent bone mineralization, weight and stature were analyzed relative to the total cumulative amount of energy consumed from the supplements by the subjects during the first 7 y of their lives. Cumulative intakes were determined in part by age; for example, those born in 1962 could only have been supplemented in their seventh year of life whereas those born in 1969 could have received supplement from birth to 7 y. Cumulative intakes were also determined by individual patterns of attendance and consumption (Schroeder et al. 1992). Because of the upwardly skewed nature of the distribution of values of cumulative energy intake of supplement, a square root transformation was used for all analyses. Preliminary analyses demonstrated that such transformation reduced skewness and kurtosis to acceptable levels. The five outcome measures were transformed to gender specific z-scores (standard deviation scores) to allow for comparisons of the magnitude of the response to energy supplementation among outcomes. The results are presented in standard deviation units for each outcome measure per 100 square root of kcal, (SD/100$\sqrt{\text{kcal}}$) which can be thought of as the effects on status during adolescence of consuming ~10,000 kcal/41,840 kJ of supplemental energy during childhood.

The effects of early supplemental energy intake on adolescent bone mineralization, weight and stature were examined using the multiple linear regression techniques of Statistical Analysis Systems (SAS Institute 1992a) to control for covariates and potentially confounding variables. Random effects models (SAS Institute 1992b) were developed to estimate the unadjusted effects of energy supplementation on each of the three bone measures, weight and stature and the effects after adjusting for age, gender (males = 1, females = 0) and supplement type (Atole = 1, Fresco = 0). Use of these models allowed us to treat supplement type as a random variable. Finally, weight and/or stature were included in the bone mineralization models to address the secondary objective of testing for the presence of a bone-specific response to supplementation. Effects were considered statistically significant at $P < 0.05$.

Various functional forms of all variables were considered. For example, we compared the fit of gender-specific simple linear and curvilinear models relating each subject's age and the five outcome measures. Although in general the curvilinear relationships provided better fits to the data, the addition of either weight or height to the bone mineralization models removed the effects of the higher-order age terms and interactions, suggesting that the higher-ordered terms for age were colinear with height and weight and thus, their inclusion would "over-control" for the effects of age on bone mineralization status in the models. Therefore, gender-specific linear terms for age (ie., age + gender + gender*age) were fit in the models for bone mineralization, and the higher order terms, age^2 and age^2*gender were added to the models to describe age-related variations in weight and stature. Interactions between amount and type of supplement consumed also were evaluated for inclusion in the models. Because we were interested in evaluating the potential importance of these interactions, they were considered statistically significant at $P < 0.10$.

RESULTS

Presented in **Table 1** are descriptive characteristics of the study sample stratified by supplement type. In general, there are few significant differences in the variables associated with supplement type. The means for most outcome measures appear greater among adolescents who consumed Atole during childhood, but the differences reach statistical significance ($P < 0.05$) for stature and radial bone width only among girls.

There are few adolescent samples available that provide mean radial density measures that may be used as comparisons. In **Figure 1**, the means for bone mineral content (a,b) and bone density (c,d) are plotted against age for boys (a,c) and for girls (b,d) with data for German adolescents (Runge et al. 1980). Guatemalan boys and girls have less bone mineral content and bone density compared with German adolescents but the overall pattern of age-associated changes appears similar. The Guatemalan adolescents approximate the fifth percentile of the NCHS reference data for stature and weight (Martorell et al. 1995b).

Effects of energy supplementation on bone mineralization, weight and stature are presented in **Table 2** with the genders pooled. Presented in the first column, are estimates of the unadjusted effect of supplementation on each outcome measure. Again, the effects on each variable are measured in standard deviation units per 100 square root of kcal, ($SD/100\sqrt{kcal}$) which translated back, means the consumption of ~10,000 kcal/41,840 kJ of supplement during childhood. As shown, early energy supplementation significantly increased each of the outcome measures to similar degrees.

In the next column of Table 2, are estimates of the effects of supplement consumed on each outcome variable after adjusting for each subject's gender and age. As stated earlier, the specification of the age variables in the models depended on the outcome of interest (see Materials and Methods for specification). The effects of supplementation are diminished by this adjustment, but significant effects of supplementation on bone mineralization can still be demonstrated. The effects of supplementation on weight and stature are reduced to a greater extent by this adjustment, and remain statistically significant at the 0.05 level only for stature. The P level for the coefficient in the weight model is 0.08, however, and is still suggestive of a supplementation effect.

Finally, in the third column of Table 2, are estimates of the effect of supplement consumed on bone mineralization, weight and stature after adjusting for the type of supplement consumed during early childhood. Differences in bone mineralization associated with village of residence (as opposed to supplement type, per se) were observed; however, after adjustment, statistically significant effects of the amount of calories from supplement consumed during childhood on bone mineralization during adolescence are still observed, irrespective of supplement type. The magnitude of these effects is similar

TABLE 1

Descriptive characteristics of the sample by gender and type of supplement[1]

	Boys		Girls	
Characteristics	Atole	Fresco	Atole	Fresco
n	100	88	79	89
Age *y*	16.7 ± 0.4	16.7 ± 0.4	16.8 ± 0.4	16.6 ± 0.4
Weight *kg*	44.9 ± 1.1	43.3 ± 1.2	41.8 ± 0.9	42.0 ± 1.0
Stature *cm*	153.6 ± 1.2	151.4 ± 1.3	148.6 ± 0.8*	145.7 ± 0.8
BMC *g/cm*	0.78 ± 0.02	0.77 ± 0.02	0.66 ± 0.01	0.64 ± 0.01
BW *cm*	1.22 ± 0.02	1.18 ± 0.02	1.06 ± 0.01*	1.03 ± 0.01
BD *g/cm²*	0.63 ± 0.01	0.64 ± 0.01	0.61 ± 0.01	0.62 ± 0.01
Supplement *kcal*	161,531 ± 17,063*	42,346 ± 4,514	161,493 ± 20,911*	50,979 ± 5,670

[1] Values are means ± SE. * $P < 0.05$ (Atole versus Fresco). Abbreviations used: BMC, bone mineral content; BW, bone width; BD, bone density.

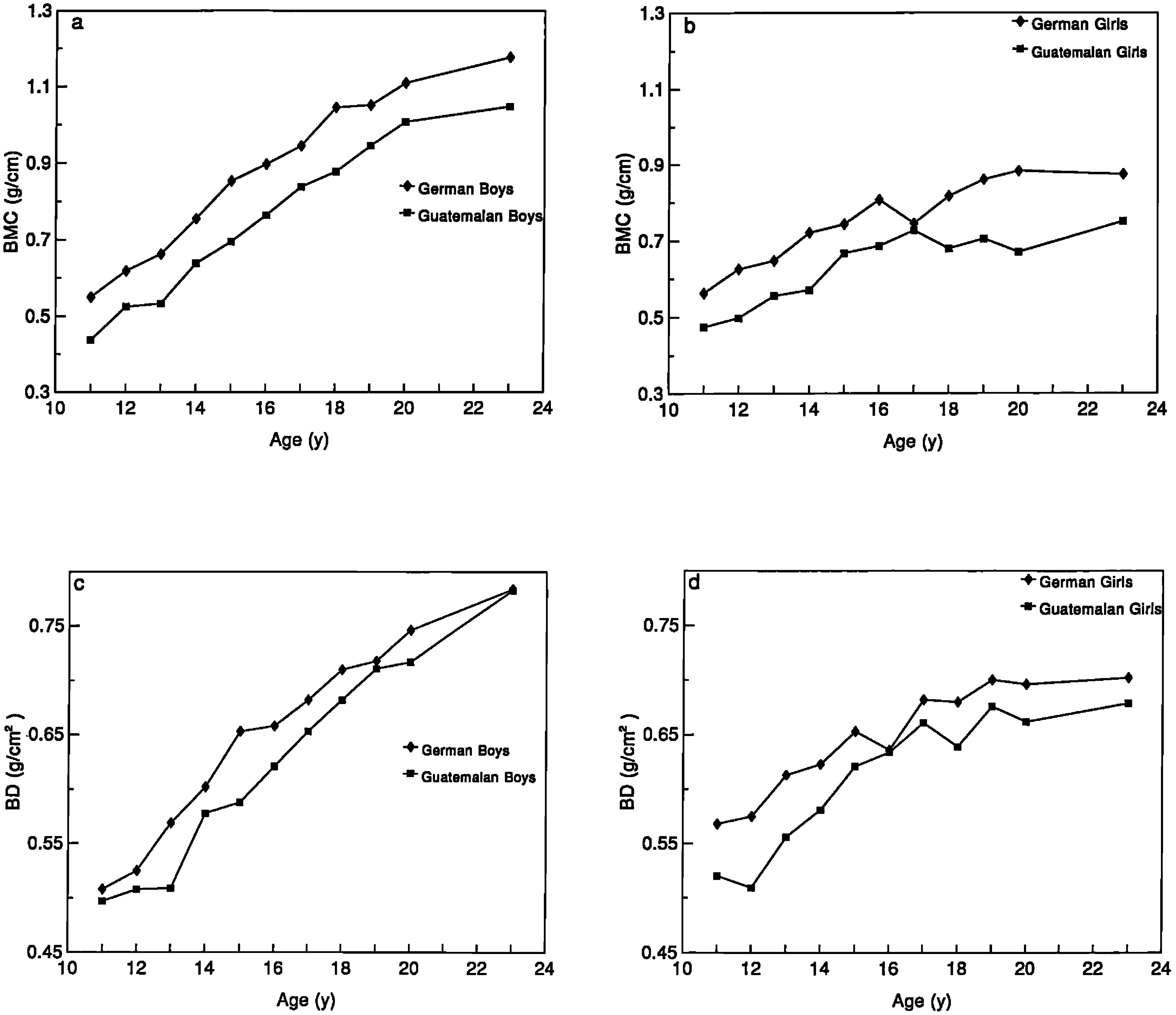

FIGURE 1 Bone mineral content (BMC) and bone density (BD) in Guatemalan and German boys and girls (Runge et al. 1980). Values are the means of BMC (a,b) and BD (c,d) over 1-y age intervals plotted at each interval midpoint for boys (a,c) and girls (b,d) separately.

across the three bone measures (~0.06–0.09 SD/100 $\sqrt{\text{kcal}}$). As shown, the effects of supplementation on weight and stature become statistically nonsignificant for both weight and stature after adjusting for type of supplement consumed.

To test for bone-specific effects of the supplementation, weight and stature, were added, separately and in combination, to the regression models (**Table 3**). As shown here, the effects of supplement on bone mineralization are no longer statistically significant.

Interaction terms between supplement type and cumulative supplemental energy intake were not statistically significant in any models, again supporting the conclusion of energy effects per se, excluding effects due to protein, calcium or phosphorus.

DISCUSSION

Our results suggest that the amount of energy consumed from supplement during early childhood had a significant and positive effect on the bone mineralization of these Guatemalan adolescents and young adults. Although studies have related short-term improvements in diet and nutritional status to enhanced bone growth, mineralization, and skeletal maturation

TABLE 2

***Effects of supplementation during early childhood on bone mineralization, weight and stature during adolescence*[1]**

Outcome measures (SD units)	Supplementation effects (100γ/kcal)		
	Unadjusted[2]	Adjusted[3]	Adjusted[4]
BMC	0.198 ± 0.026*	0.072 ± 0.019*	0.076 ± 0.020*
BW	0.182 ± 0.026*	0.097 ± 0.024*	0.094 ± 0.024*
BD	0.152 ± 0.022*	0.040 ± 0.015*	0.058 ± 0.016*
Weight	0.212 ± 0.025*	0.035 ± 0.020	0.033 ± 0.021
Stature	0.220 ± 0.025*	0.054 ± 0.023*	0.028 ± 0.025

[1] Values are the beta coefficients ± SE for the supplementation terms estimated using linear regression. All are expressed in SD/100γ/kcal units. * $P < 0.05$. Abbreviations used: BMC, bone mineral content; BW, bone width; BD, bone density; SD, standard deviation.

[2] Unadjusted effect of supplementation.

[3] Effect adjusted for each subject's gender, age (see text).

[4] Effect adjusted for gender, age and for type of supplement consumed.

of malnourished children (Guzmán et al. 1965, Himes et al. 1990, Martorell et al. 1979), we are unaware of other studies reporting positive effects of nutritional supplementation during early childhood on bone mineralization during adolescence and young adulthood.

The magnitude of the supplementation effects diminished, but remained significant after controlling for the three most important confounding factors: age, gender and supplement type. This is an important point, because these three factors together account for 33–65% of the variation in bone mineralization in these data. Further, because the correlations between age and body size are high (0.6–0.8) in this age group, the presence of age in the model also controls partially for variation in bone mineralization associated with body size. The persistence of the effects of energy supplementation after controlling for supplement type is important in light of the fact that only Atole contained protein, calcium and phosphorus—nutrients often cited for their important roles in bone development. Available evidence indicates no major deficiencies in calcium or phosphorus in this population (Flores 1971, INCAP 1969, Lechtig et al. 1972).

The effects of amount of supplemental energy consumed on bone mineralization became statistically nonsignificant after controlling for weight and stature at adolescence. The amount of supplemental energy consumed was associated positively with weight and with stature in these adolescents and young adults, after controlling for age and gender, but not supplement type. These results suggest that the effects of supplemental energy on bone mineralization were operating through the increases in weight and stature associated with supplementation.

The interpretation of these results also did not change in further analyses ($n = 352$) considering maturational age (deviation in RUS age from chronological age) as well as chronological age in the models. Further, analyses by Pickett et al. (1995) and Khan et al. (1995) suggest no overall effects of supplementation on skeletal maturation or menarcheal status respectively. Thus, although maturation and menarcheal status influence bone mineralization during adolescence apart from chronological age (Himes and Huang 1993), these results suggest that the observed effects of supplementation on bone mineralization are not likely to be operating through changes in maturation related to supplementation. From this, we conclude that the observed effects of energy supplementation on bone mineralization were probably no greater than those associated with the overall somatic growth response in body size associated with supplementation and were probably mediated by this general somatic response to supplementation.

Two methodological limitations of the study, however, should be mentioned. These relate to the lack of randomization at the level of the individual in the design of the longitudinal study and the selection of subjects for the bone mineralization substudy.

In the longitudinal study, the subjects were not randomly assigned to differing levels of supplementation; that is, the children chose how much supplement they would consume (Schroeder et al. 1992). Thus, the positive relationships between amount of supplement consumed during early childhood and bone mineralization during adolescence could result from selection bias if better-off children (i.e., larger children with bigger, more dense bones) chose to consume more supplement. Results from analyses of the longitudinal study have provided arguments against this possibility by demonstrating that rates of growth in weight and length were associated positively with level of sup-

TABLE 3

***Effects of supplementation during early childhood on bone mineralization during adolescence: effects adjusted for current weight and stature*[1]**

Bone status measures (SD units)	Supplementation effects (100γ/kcal)		
	Adjusted[2]	Adjusted[3]	Adjusted[4]
BMC	0.013 ± 0.016	0.015 ± 0.017	0.024 ± 0.018
BW	0.020 ± 0.023	0.017 ± 0.022	0.005 ± 0.022
BD	0.019 ± 0.014	0.024 ± 0.015	0.015 ± 0.014

[1] Values are the beta coefficients (±SE) for the supplementation terms estimated using linear regression. All are expressed in SD/100γ/kcal units. * $P < 0.05$. Abbreviations used: BMC, bone mineral content; BW, bone width; BD, bone density; SD, standard deviation.

[2] Adjusted for each subject's gender, age, the type of supplement consumed and weight.

[3] Adjusted for each subject's gender, age, the type of supplement consumed and stature.

[4] Adjusted for each subject's gender, age, the type of supplement consumed and weight and stature.

plementation after controlling for important confounding factors such as initial body size, socioeconomic status, home diet and diarrheal diseases (Schroeder et al. 1995). The limitations of this type of analysis are discussed in greater detail by Habicht et al. (1995).

Although the preceding arguments lend credence to the interpretation of the results as effects of energy supplementation on bone mineralization, it is important to note, that this selection bias issue could have been addressed directly if we had controlled for variation in bone mineralization during early childhood. This was not possible because such bone measures were not collected during the longitudinal study. Measures of the dimensions of the second metacarpal, however, were available for the majority of the subjects (n = 314; median age of measure = 3 mo), and from these measures, estimates of the bone cortical area can be made (Garn 1970). This area estimate has been shown to be closely related to bone mineral content (Horsman and Kirby 1972). When the regression models were fit again, controlling for this indicator of early childhood bone mineral content, the basic conclusions drawn from the analyses remained the same, although, in these analyses, there was some evidence of bone-specific effects of supplementation on both BMC and BD (P levels of 0.05–0.10).

During the follow-up study, bone mineralization measures were taken on only a subsample of the subjects. The subjects were selected based on their age, gender, village and willingness to participate in another substudy on physical work capacity (Haas et al. 1995). Given that this sample of adolescents and young adults may be different from the rest of the follow-up sample, sample-selection bias could exist. Therefore, analyses were performed comparing the weight, stature, and intake of supplement of subjects in the follow-up study who did, or did not, have bone measurements taken. Subjects who participated in the bone mineralization substudy were younger, lighter and shorter, but had consumed the same amount of supplemental energy during early childhood as compared with the rest of the subjects in the follow-up study. Further analyses demonstrated that the groups were not different in weight, but were shorter after controlling for age, gender and supplement type. The positive relationship between the amount of supplemental energy consumed during childhood and weight and stature during adolescence was not different between the groups for weight, but was for stature, with the bone group having a significantly more positive effect of supplementation. In summary, as suggested previously by Rivera et al. (1992), these analyses indicate that the subjects chosen for the subsample were not representative of all subjects participating in the follow-up study, and were, perhaps, those who responded more to supplementation. Thus, the magnitude of the supplementation effects on bone mineralization presented here may be somewhat overstated.

Despite these limitations, the results provide important evidence that participation in nutritional supplementation programs can have demonstrable long-term nutritional benefits on bone mineralization for mild to moderately malnourished children. Supplementation trials of mild to moderately malnourished children using different designs and methodologies have been conducted in other populations in developing countries (Gopalan et al. 1973, Mora et al. 1981). Following up these participants could provide further evidence of the long-term impact of food supplementation programs and provide further support for the results presented here.

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Nutrition in Early Life and the Fulfillment of Intellectual Potential[1,2]

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ABSTRACT The effects of early supplementary feeding on cognition are investigated using data collected during two periods in four Guatemalan villages. The first was the Institute of Nutrition of Central America and Panama (INCAP) longitudinal study from 1969 to 1977 and the second was a cross-sectional follow-up of former participants carried out in 1988–1989. The principal objective of these studies was to assess the differential effect of two dietary supplements, Atole containing 163 kcal/682 kJ and 11.5 g protein per cup or 180 mL and Fresco containing 59 kcal/247 kJ and 0 g protein per cup, that were given to mothers, infants and young children. Performance was assessed on a battery of psychoeducational and information processing tests that were administered during adolescence. Consistent differences between groups were observed on psychoeducational tests. Subjects receiving Atole scored significantly higher on tests of knowledge, numeracy, reading and vocabulary than those given Fresco. Atole ingestion also was associated with faster reaction time in information processing tasks. In addition, there were significant interactions between type of dietary supplement and socioeconomic status (SES) of subjects. In Atole villages, there were no differences in performance between subjects in the lowest and highest SES categories. On the other hand, performance in Fresco villages was best in the highest compared with the lowest SES group. After close scrutiny of alternative hypotheses, it is concluded that dietary changes produced by supplementation provide the strongest explanation for the test performance differences observed in the follow-up between subjects exposed to Atole and those exposed to Fresco supplementation. J. Nutr. 125: 1111S–1118S, 1995.

INDEXING KEY WORDS:

- *cognitive development* • *intellectual achievement*
- *school achievement* • *nutritional supplementation*

In addition to this one, there are at least six other studies on the effects of prenatal and early postnatal supplementary feeding on behavioral development. All test the hypothesis that protein-energy malnutrition in early life has adverse developmental consequences, but research designs and methods differ widely among them. One study conducted by Rush et al. (1980) restricted supplementation to pregnancy whereas other investigations involved prenatal as well as postnatal supplementation (Chávez and Martínez 1982, Waber et al. 1981). Work by Grantham-McGregor et al. (1990) and Husaini et al. (1991), on the other hand, focused exclusively on postnatal supplementation. Most reported results refer to the first 2 y of life. In general, nutritional interventions accounted for a small (~0.20 SD) but significant proportion of variability in performance among infants and toddlers on mental and motor developmental scales.

Some information about long-term effects of nutritional interventions on behavioral outcomes of older children are available from two follow-up studies (Hsueh and Meyer 1981, Super et al. 1991) but, unfortunately, the reports were limited to abstracts in conference proceedings. In the study by Super et al.

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] Data collection and analyses were supported by NIH grant HD22440 and by Pew Charitable Trusts grant 90-00210-000.

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(1991) carried out in children (mean age = 6.74 y) from Bogotá, achievement was measured using a test that included reading readiness, arithmetic and basic knowledge. Statistically significant effects of treatment, accounting for ~8 mo of cognitive development, were observed in reading readiness, particularly among subjects whose mothers had strong psychological and social resources. In an earlier study in Taiwan, Hsueh and Meyer (1981) found that a nutrition supplement administered to Taiwanese mothers during pregnancy and lactation had no effect on an intelligence test administered to the offspring at 5 y of age.

In the Guatemalan study reported here, an assessment is made of the differential impact of the nutritional supplements on functional outcomes designed to be relevant to the local context. The outcomes were measured more than 10 years after the administration of the supplements was discontinued. The study design called for two villages to receive a high energy, high protein supplement called Atole and two to receive a low calorie supplement devoid of protein called Fresco. Data for this paper were collected as part of the Institute of Nutrition of Central America and Panama (INCAP) longitudinal study from 1969 to 1977 and the follow-up study carried out in 1988 and 1989 (Martorell et al. 1995).

Detailed results about the effects of early supplementary feeding on performance are provided in a separate publication[4] for a wide range of psychoeducational tests measured in adolescents and young adults (Pollitt et al. 1993). A summary of these results is given here to provide a more complete presentation of the follow-up results, with emphasis on selected outcome measures that reflect distinct cognitive domains. The analyses focus on performance differences between treatment groups (i.e., Atole and Fresco) and on the interactive effects of type of supplement with social and economic characteristics.

MATERIALS AND METHODS

Design of the study

The study villages. Four villages were included in the study; at baseline, two had populations of ~900 each and two were smaller, ~500 people each. Within each pair, assignment to Atole or Fresco supplementation was random (Martorell et al. 1995). The villages are located in the Department of El Progreso, a dry mountainous area northeast of Guatemala City. The temperature ranges from 14 to 38°C, with the rainy season occurring from June to October.

[4] Available from the Nestle Foundation, P.O. Box 581, 4 Place de le Gare, 1001 Lausanne, Switzerland.

Throughout the course of the studies, from 1969 to 1989, the primary source of income for most villagers was agriculture. The majority of the children's fathers made their living as wage laborers, tenant farmers or small land owners. No one in any of the villages reported being a large landholder. Few adult males reported being skilled tradesmen or merchants. Women infrequently reported having an occupation outside the home. Adult literacy rates continue to be low but have improved over time. Literacy among mothers increased from 30 to 60%, whereas, among fathers, rates increased from 46 to 67% from 1969 to 1989.

Subjects. At the time of the initial study in 1969 potential subjects were all children ≤ 7 y old living in the villages (i.e., all those born since 1962). In addition, all children who moved into the villages during the course of the project and all children born in the villages from January 1, 1969 through February 28, 1977 were also potential study participants.

Subjects born between 1962 and 1965 were excluded from the behavioral assessments to lower costs and to focus on the children exposed to supplement at younger ages. The analysis reported here focuses on those subjects exposed to the nutrition treatment during the prenatal period and the first 2 y of life. This is a period of accelerated brain growth and is perhaps the time when development of the brain is most sensitive to the effects of nutrition. The critical period of exposure to supplementation is expanded to 3 y of age in analyses of physical growth data (Haas et al. 1995, Rivera et al. 1995) because of evidence of continued but declining effects of supplement intake on growth rates between 24 and 36 mo of age (Schroeder et al. 1995). All subjects selected in this paper were born between 1970 and 1974 and ranged in age from 13 to 19 y at the time of the follow-up study. The sample was comprised of 636 subjects, divided almost equally among Atole and Fresco villages.

Experimental intervention

The Atole supplement was a warm, thick, brown, sweet drink, similar to corn gruels given to children in rural Guatemala. It contained 11.5 g of protein and 163 kcal/682 kJ of energy per cup (180 mL). The Fresco supplement was a cool, clear, sweet drink like KoolAid®, also similar to common village drinks. It contained no protein but it had 59 kcal/247 kJ per cup, approximately one third the energy of the Atole. Both supplements were fortified with vitamins and minerals. At the time the longitudinal study began, it was assumed that the energy concentration of Fresco was insufficient to have a developmental effect. The supplements were available at a central feeding station 7 d/wk to every resident of the village twice daily (1000 and 1400 h). Ingestion was recorded only for target subjects, that is, pregnant and lactating women and children up to age seven. Because consumption was ad libitum, attendance at the feeding centers, after controlling for consumption, was used in the analyses to control for factors associated with supplement participation. Additional details are given in Martorell et al. (1995).

Socioeconomic indicators. Three socioeconomic variables are included in the analyses as potential confounders: house quality, maternal education and father's occupation. Data for each of these three variables were derived from census data obtained in 1987.

Because of the difficulty of obtaining direct measures of income in developing countries, a measure of house quality often is used as a proxy for social-environmental variables that affect cognitive growth and educational development (Johnston et al. 1987). Nine variables describing house quality were assessed: an overall rating of the type of house (1–4 scale), ownership of house (no = 0, yes = 1), number of rooms, type of floor (1–5), type of walls (1–7), type of roof (1–4), location of the kitchen (1–3), type of toilet (1–4) and number of household possessions (1–6). In all instances, higher scale scores reflected the higher quality of the dwelling. The measure used in these analyses was generated using factor analysis on the within-village standardized variables.

Maternal education consistently has been shown to be related positively to the cognitive development of the offspring (LeVine et al. 1991). In this case, informants reported both literacy (coded 0 = none, 1 = some) and the number of years of schooling completed successfully. The mean number of years of schooling for mothers was 2.1 y.

Occupational status is a carrier variable that may be associated with income, status in the community, availability of resources and family socialization practices. The indirect effects of parental occupation on the cognitive development of children are thought to occur through the earning capacity of the parent and the consequent resources for stimulation that it permits. Both mothers' and fathers' occupations were assessed; however, because only ~20% of women at follow-up reported having an occupation, mother's occupation was excluded from further analysis.

Data on 19 occupational categories were obtained and then subsequently collapsed into six categories for comparison with earlier census data from 1967; the original and recorded scales are highly correlated ($r = 0.88$). In preliminary analyses, the recorded scale demonstrated adequate linear properties and was used in all subsequent statistical calculations as an ordinal variable. A detailed description of the specific procedures used in the construction of these three variables and the related reliability and validity data have been reported elsewhere (Pollitt et al. 1993). The three variables were standardized within village and a composite score was constructed using the sum of the three scores.

Schooling variables. At the time of testing, all subjects had reached school age and most had received some schooling. Given the well-established relation between schooling and cognition (Ceci 1991), performance on psychoeducational tests was adjusted for schooling experience. This was particularly important for the assessment of treatment effects given that differences between Atole and Fresco villages on important schooling indicators (e.g., maternal and paternal education) favored Fresco villages before the intervention (Engle et al. 1992). Two schooling variables in the statistical analysis were the age the child started school and the maximum grade attained in primary school.

The psychological test battery

Two psychological test batteries were used in the follow-up study. One includes psychoeducational tests and the other includes information processing tasks. The psychoeducational test battery included tests of literacy, numeracy, general knowledge, two standardized educational achievement tests and the Raven's Progressive Matrices. The achievement tests were part of the Interamerican Series used extensively in Guatemala by faculty from the Universidad del Valle in Guatemala City (for a detailed description of the tests see Pollitt et al. 1993). The purpose of this battery was to acquire a measure of general abilities, aptitudes and achievements that are influenced heavily by experience, education and cultural upbringing.

Information processing. Tests of simple, choice and memory reaction time (RT) (Sternberg 1966) comprised the computerized battery of tests to assess information processing. In addition, a paired associate test was administered as part of this battery. The intent of the battery was to assess the efficiency with which an individual processes information by focusing on speed of response in elementary cognitive tasks. In addition to measures of RT from three tests, two of the RT tests (i.e., choice and memory) also yielded a performance score (i.e., number of errors). In general, between-subject variability in RT tests is not accounted for by schooling and cultural background, yet test performance still maintains a low level correlation (−0.10 to −0.30) with g, a general ability factor. Theoreticians presently claim that RT is a sensitive indicator of differences in brain function (Eysenck 1986, Jensen 1991, Vernon 1987).

Procedure. Each of the four villages was visited twice by a research team, once during the dry season and once during the rainy season. The teams were rotated and each team visited each village during one round of testing. The presence of the team in the village varied from 3 to 9 wk depending on village size.

TABLE 1

***Results of hierarchical regression analyses for vocabulary*[1]**

Step	Variables	R^2	F(eq.)	F-to-enter	β	Direction of effects favors
1	Sex	0.03	3.16*	0.01	−0.122	—
	Age			8.01**	3.290	Older subjects
	Attendance			1.48	−0.571	—
2	Socioeconomic status	0.11	10.66***	32.26***	1.168	Higher SES
3	Age at entry	0.21	14.25***	18.98***	−1.064	Younger subjects
	Maximum grade			19.17***	2.327	Higher grade
4	Treatment	0.26	16.20***	22.35***	3.930	Atole
5	Treatment by grade	0.30	15.13***	6.13**		
	Treatment by socioeconomic status			11.28***		

[1] Adapted from Pollitt et al. (1993).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

In each community, two staff members recruited subjects and made appointments for testing. All testing was done in community houses rented by the project and adapted accordingly. In addition to psychological data, subjects were given several examinations, including medical and anthropometric assessments. They also were interviewed extensively about sociodemographic characteristics.

Analytical strategy. The data were analyzed using a hierarchical regression model. This approach permits the estimation of variance accounted for by treatment alone as well as the identification of differential effects of treatment that may be related to particular characteristics of the population. By including interactive terms in the model, it is possible to identify the potential indirect pathways through which supplement could also have affected the outcomes of concern.

All independent variables were standardized. Individual characteristics (sex, age at testing and attendance at the feeding center with consumption partialled out) were entered first, followed by the socioeconomic status (SES) composite (sum of mother's education, father's occupation and house quality factor score), and then the two school indicators (age at school entry, maximum grade attained) and finally the treatment variable (entered as a categorical (1/−1). In this way the percent of variance accounted for by the different predictors was estimated. In a subsequent step, two interaction terms were entered: treatment by SES and treatment by maximum grade. The results presented include the percent of variance accounted for at each step (R^2), and F values and regression coefficients for each variable in the step in which it was entered, controlling for all other variables entered before this step.

A three-way interaction term (treatment by SES by grade attained) also was entered into the model but the results of these analyses were nonsignificant and will not be presented. Similarly, treatment by gender terms were entered in preliminary analyses but also were nonsignificant and dropped from the final model.

RESULTS

Psychoeducational tests

Vocabulary. **Table 1** presents the results of the hierarchical regression analyses for the vocabulary test. In general, the results are in the predicted direction with older adolescents, subjects from higher SES families and those who entered school earlier and stayed in longer performing better on the measure of vocabulary. After controlling for all of these variables, the treatment contributed an additional 5% of the variance ($F = 22.35, P < 0.001$) in performance with Atole subjects performing significantly better ($b = 3.93$) than Fresco subjects.

When entered into the model, both interactive terms were significant and accounted for an additional 4% of the variance. In the case of the SES-by-treatment interaction, the slope for Atole subjects was nonsignificant whereas that for Fresco subjects was positive and significant ($b = 1.373, P < 0.001$). Although there was no relation between SES and performance in Atole villages, performance improved in Fresco villages with increasing SES level (**Fig. 1**). At lower ends of the SES distribution, subjects who received Atole supplements performed significantly better than those who received Fresco supplements; whereas at higher SES levels there were no differences between them.

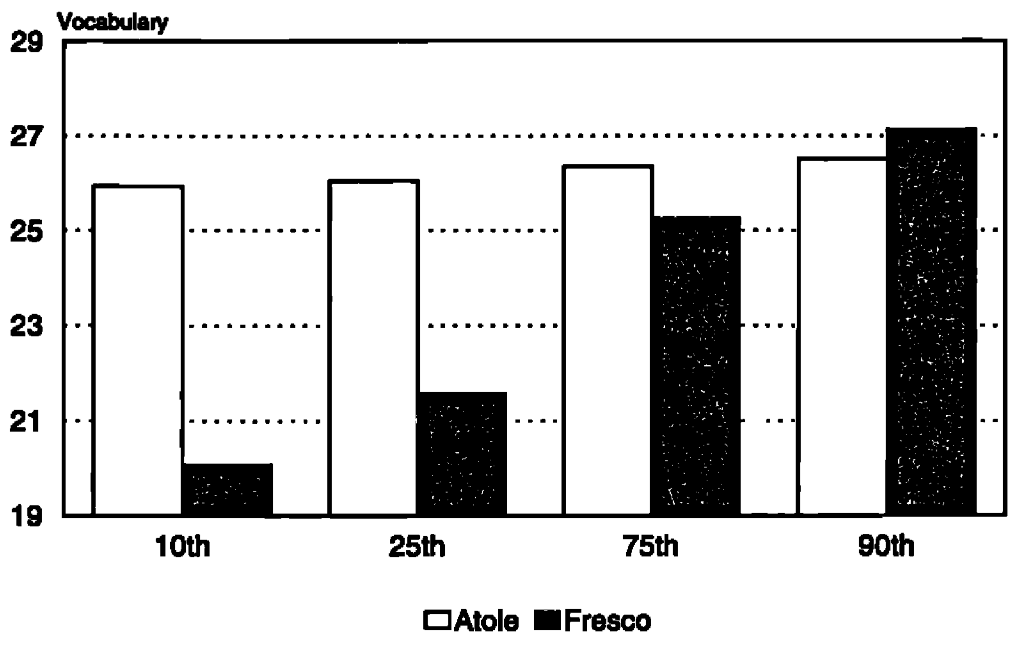

FIGURE 1 SES-by-treatment interactions for vocabulary.

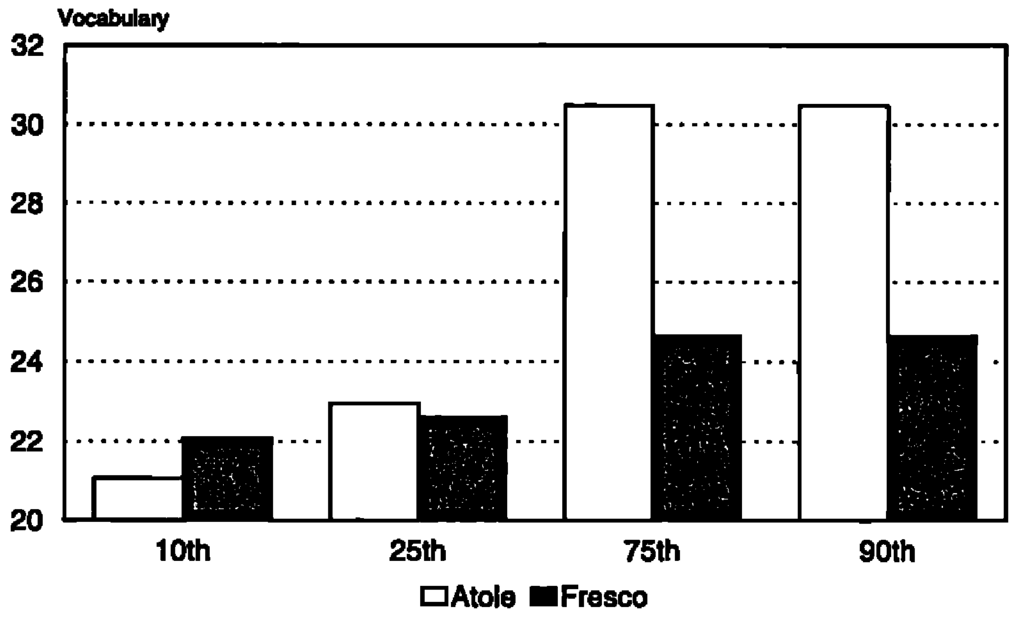

FIGURE 2 Maximum grade-by-treatment interaction for vocabulary.

The grade-by-treatment interaction showed a different pattern. The slope was positive and significant for Atole ($b = 3.861$, $P < 0.001$) but not for Fresco subjects. Differences between treatment groups increased with grade attained, such that children from Atole villages scored significantly higher than Fresco children at the upper ends of the grade distribution (**Fig. 2**). For those at the lower end of grade attainment, there were no differences between Atole and Fresco subjects.

Results of the hierarchical analyses for other outcome variables were similar (**Table 2**). After controlling for potentially confounding variables, there were significant effects of Atole on performance on tests of numeracy, knowledge, vocabulary and reading achievement. The percent of variance accounted for by inclusion of the treatment variable was generally small, yet statistically significant, ranging between 1 and 5%. Examination of significant interactive terms permits the identification of subgroups in whom effects were greatest. In almost all instances, effects of Atole were evident in children from families at the lowest levels of SES. In several cases (e.g., reading, vocabulary and reading achievement), effects were observed in children with the highest levels of education.

Information processing

Results of the regression analyses on reaction time of the memory task are presented in **Table 3**. In contrast to the results on the psychoeducational tests, none of the predictor variables were associated with performance, with the exception of grade attainment. After controlling for all potential confounders, treatment was associated significantly with performance, with Atole subjects having significantly faster reaction times ($b = -0.321$, $P < 0.01$) than Fresco subjects. Neither of the interactive terms was significant.

Results of hierarchical analyses for other information processing outcome variables were similar (**Table 4**). In general, the percent of variance accounted for by the models was small (between 3 and 10%), with males, higher SES, earlier school entry and higher grade attainment associated with enhanced performance. In seven analyses, there were three significant main effects of treatment. Atole subjects responded faster and more efficiently than Fresco subjects on the memory task and reached criterion faster on the paired associates task. None of the interactive terms was significant.

DISCUSSION

The results of the INCAP follow-up study show that, after controlling for socioeconomic factors and

TABLE 2

Summary of results of hierarchical regression analyses for psychoeducational tests[1]

	R^2	*F* values		
Dependent variable	Full model	Treatment†	SES by treatment	Treatment by grade
Literacy	56	0.44	0.14	0.54
Numberacy	48	7.75**	10.06***	0.01
Knowledge	27	8.57**	6.74**	0.79
Raven	15	0.22	8.39**	2.18
Reading	30	0.03	2.49	5.36*
Vocabulary	30	22.35***	11.28***	6.13**
Reading Achievement	30	20.05***	14.91***	13.14***

[1] Adapted from Pollitt et al. (1993).

† After controlling for age at testing, gender, attendance, SES, age at school entry and maximum grade attained. SES = socioeconomic status.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

formal educational experience, subjects exposed to Atole during the pre- and early postnatal period obtained significantly higher scores on measures of general intellectual abilities than subjects exposed to Fresco. Furthermore, better information processing abilities were observed in subjects exposed to Atole. There was great consistency to the results when examined by specific test; Atole subjects performed significantly better than Fresco subjects on tests of numeracy, general knowledge, reading and vocabulary achievement (Pollitt et al. 1993). Furthermore, significant interactions with SES were found for tests of numeracy, knowledge, reading achievement, vocabulary and the Raven's Progressive Matrices. Finally, significant interactions with grade attainment were reported on the three reading-related tests.

Elsewhere (Pollitt et al. 1993) we reported that mother's education, father's occupation and house quality, the three indicators of SES, correlated positively with scores from all the psychoeducational tests (including vocabulary) administered. As expected, subjects at the lowest end of the SES distribution were at the highest risk of poor cognitive test performance. Because of it, the finding that the interaction between treatment and SES accounted for significant portions of the vocabulary test scores and of other tests of the battery administered is particularly important for developmental theory and public health. The direction of the interaction shows that the strongest beneficial effects of the Atole on cognition were observed among those at the lowest end of the SES distribution. This selective effect was sufficient to cancel the expected test score differences between those at the lowest and highest end of the SES distribution in the group that received Atole. Conversely, the distribution of cognition test scores in the Fresco group follows what is generally a truism in developmental psychology: cognitive competence varies as a positive function of SES status. Accordingly, in this study, we view the effect of the Atole as a social equalizer.

TABLE 4

Summary of results of hierarchical regression analyses for information processing tests[1]

Dependent variable	R^2 Full model	F value Treatment†
Simple RT	03	0.03
Choice RT	02	1.01
Trials to criterion	06	3.65*
Memory RT	04	9.25**
Memory efficiency	10	8.40**
Memory impulsivity	04	2.06
Memory percent correct	07	1.02

[1] Adapted from Pollitt et al. (1993).
† After controlling for age at testing, gender, attendance, socioeconomic status, age at school entry and maximum grade attained.
* $P < 0.05$.
** $P < 0.01$.

Particular characteristics of the subjects also modified the effects of the treatment, as suggested by the significant interaction of treatment and maximum grade attained. Whereas maximum grade attained was independent of test performance among those that received Fresco, this was not the case among those subjects that received Atole. In this last group, the scores in the vocabulary test varied as a positive function of maximum grade. In our view, this effect indicates that Atole provided an impetus to take advantage of the formal educational experiences to which they were exposed.

Are the results due to the supplementation program? Answering this question requires critical examination of the internal validity of the data (Cook and Campbell 1979). Among the potential threats to internal validity are differences between Atole and Fresco in several

TABLE 3

Results of hierarchical regression analyses for memory reaction time[1]

Step	Variables	R^2	F(eq.)	F-to-enter	β	Direction of effect favors
1	Sex	0.002	0.24	0.03	−0.018	—
	Age			0.00	−0.051	—
	Attendance[a]			0.69	0.046	—
2	Socioeconomic status	0.004	0.43	1.01	−0.024	—
3	Age at entry	0.02	1.51	3.03	0.037	—
	Maximum grade			4.25*	−0.117	Higher grade
4	Treatment	0.04	2.64**	9.25**	−0.321	Atole

[1] Adapted from Pollitt et al. (1993).
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

areas: 1) pretreatment differences in social and economic characteristics; 2) differences in the provision of Atole and Fresco treatments; 3) differences in attrition and recruitment rates to the follow-up study; and 4) differential patterns of community development since the end of the longitudinal study in 1977 to the 1988 follow-up study. The possibility that differences in these areas provide an explanation for differences in test performance between Atole and Fresco subjects has been considered carefully elsewhere and rejected (Pollitt et al. 1993). Rather, the conclusion reached was that the nutritional differences between the Atole and Fresco supplements is the best explanation for the behavioral differences observed in the INCAP follow-up study.

A related issue regarding treatment effects and their programmatic implications is the nutritional status of the target population. The prevalence of growth retardation in the study population indicates high levels of malnutrition among infants and children. About 26% of the sample had severe stunting (≥3 SD below the reference median) and 43% had moderate stunting (2.9–2.0 SD below the median) at 3 y of age (Martorell et al. 1992).

Nutritional status is likely to interact with supplementary feeding to determine outcome. Thus, the external validity of the Guatemalan findings must be assessed in context and generalizations should be restricted to populations with nutritional status similar to that in the study population. However, it must be noted that there is no theoretical or empirical reason to suspect that the benefits of early supplementary feeding are observed exclusively below a certain level of nutritional risk.

On the basis of the results presented here and elsewhere (Pollitt et al. 1993), the internal validity of the effects of supplementation is high. However, although there is strong evidence for a nutritional effect, the specific nutrient or nutrients responsible for the changes observed cannot be identified from this study. Rather, the findings are informative of the potential of efforts to improve diets more generally. Thus, programs that are effective in improving diets in deficient areas, whether educational, food-based or other in nature, will achieve the same results.

The effects of public health programs, particularly as they refer to behavior, need to be evaluated in the context of a society's explicit and implicit social policy. In the context of rural Guatemala, the benefits and costs of nutrition programs to enhance development must be contrasted with those related to efforts to address other existing conditions that limit development. The school system, for example, is vastly inefficient and does not meet the basic educational needs of the population. Only about one half of the children enrolled in first grade ever finish primary school and a large percentage (>20%) remain functionally illiterate.

Programmatic actions that focus on unmet nutritional needs and have beneficial effects on human cognitive development are potentially a step forward in social policy. However, in our view, such actions are deceptive if framed in the context of a social policy that disregards other basic human needs and does not attend to the overall quality of life. Unmet nutritional needs generally coexist with, among others, unmet needs in education, housing, sanitation and health care. Only by meeting all these needs in conjunction with nutritional needs will we truly have moved forward toward a fair humane society that sustains the rights of children and fosters cognitive, social and emotional development.

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Malnutrition and Human Function: A Comparison of Conclusions from the INCAP and Nutrition CRSP Studies[1,2]

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ABSTRACT The overall objective of both the Institute of Nutrition of Central America and Panama (INCAP) studies and the Nutrition Collaborative Research Support Program (CRSP) was to determine if marginal malnutrition affects human function. The conclusions from the two studies were consistent, notably that growth stunting occurs early in life and is accompanied by functional impairments. These consequences of early malnutrition persist later in life. The comparison of INCAP and Nutrition CRSP results also illustrates that the Guatemalan children were more malnourished and stunted than those in Kenya, Mexico or Egypt and that this greater degree of stunting occurs before 18 months of age. Even the Atole did not bring the average size of supplemented children in Guatemala up to those of the unsupplemented children in the CRSP populations. It is also likely that their functional potential was not realized fully by supplementation. The CRSP studies provide evidence of multiple micronutrient deficiencies associated with poor growth and function and it is probable that these associations exist in Guatemala as well. The overall conclusion is that our attention should be directed to determining the adequacy of micronutrient status in the perinatal period and to the development of approaches that prevent early growth failure. J. Nutr. 125: 1119S–1126S, 1995.

INDEXING KEY WORDS:

- *malnutrition* • *function* • *energy* • *growth*
- *micronutrients* • *human*

The Institute of Nutrition of Central America and Panama (INCAP) studies described in the preceding articles and the Nutrition Collaborative Research Support Program (CRSP) are two of the largest field investigations of marginal malnutrition ever conducted. Such studies are inherently difficult to design, conduct and control so that the interpretation of their results is always open to question. Because the INCAP and CRSP research had similar goals and measured many of the same outcomes and possible confounding variables, a comparison of the results can extend the generalizability of the conclusions and add new insights into their interpretation. There is no doubt that the general conclusions from the two studies are in agreement and are useful for developing appropriate nutrition policy. However, some important unanswered questions also emerge from this comparison.

Comparison of hypotheses tested

Both projects had the main goal of testing whether marginal malnutrition affects human function. The major hypothesis addressed by the original design of the INCAP longitudinal study (1969–77) was that improved nutrition results in accelerated physical growth and mental development of preschool-aged children; in the follow-up study the hypothesis was extended to apply to the same children at adolescence or adulthood (Martorell et al. 1995a). The major CRSP hypothesis was that marginal malnutrition affects function, namely growth, cognitive performance/behavior, morbidity, reproduction and social competence (Calloway et al. 1988).

Comparison of study designs

There are marked similarities and differences between the studies. The INCAP project was located in

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-Term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication were Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

[2] The Nutrition CRSP was supported by USAID grants no. DAN-1309-G-SS-1070-00 and DAN-1309-A-00-9090-00.

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rural Guatemala and the Nutrition CRSP was located in rural Kenya and Mexico and a periurban area of Egypt. The CRSP was conducted in three locations to test the generalizability of the results across different staple diets, environments and cultures. Nevertheless, the CRSP investigators worked together closely to ensure that the types of data collected and the research methods were as similar as possible across the three projects. In general, based on anthropometry and dietary information, the nutritional status of the populations was poorest in Kenya, where famines occurred during the project, intermediate in Mexico and best in Egypt. The CRSP results discussed in this article are described in detail elsewhere (Allen et al. 1992a, Allen 1993, Calloway et al. 1992, Kirksey et al. 1992, Neumann et al. 1992).

The original INCAP study was a controlled intervention design, providing energy and protein supplements for pregnant women and children under seven years of age (Martorell et al. 1995a). When this project was designed it was the current belief that protein would be the critical growth-limiting nutrient. INCAP analyses have tended to focus on comparisons of Atole and Fresco groups and to analyses of the relationship between supplement intake, often expressed as energy, and outcomes (Habicht et al. 1995). The Nutrition CRSP was a nonintervention observational study in which the intent was to describe relationships between intake and function of several household members consuming their usual diet. When the CRSP was designed, in the late 1970s and early 1980s, it was believed that inadequate energy (food) intake was the dietary problem most likely to limit growth and other human functions (National Research Council, 1977). Thus, energy intake was the main independent variable. However, because energy intake was assessed by frequent measurements of food intake, the consumption of other nutrients and food intake patterns were also used as independent variables during subsequent data analyses.

Pregnant and lactating women, and children were subjects in both studies. The INCAP project was longitudinal, originally studying children for up to 7 y of age and, in the follow-up, adolescents and adults. The CRSP only followed each type of "target" subject for 1 y, or slightly longer for most pregnant or lactating women. CRSP subjects also included schoolers, adult males and nonpregnant nonlactating women.

Many similar functional outcomes of nutritional status were measured, including maternal and child anthropometry and cognitive development and behavior. Morbidity and socioeconomic and demographic data were used as potential confounding variables. The INCAP follow-up study included work capacity measures (Haas et al. 1995) whereas the CRSP has information on the observed activity patterns of some preschoolers and adults. In general, the CRSP focused more on food intake data, measuring 2 days of intake per month during the year of study, on each individual as well as at the household level. Dietary variables constructed during data analysis included micronutrient intakes, estimates of mineral bioavailability and food patterns. Hematological and biochemical measures, included to examine potential confounding effects of vitamin and mineral (primarily iron) deficiency in functional tests, provided an indication of the status of some micronutrients.

Patterns of growth stunting

The INCAP longitudinal study (1969–77), similar to many previous and subsequent studies in developing countries, showed that growth stunting occurred very early in life. The follow-up study was relatively unique in that it followed the same individuals in late adolescence and adulthood, so proving that this early growth failure persisted (Martorell et al. 1995b). The CRSP does not have equivalent longitudinal data across childhood and adolescence because each individual was followed for only 1 year. However, because infants, preschoolers and schoolers were studied from the same sample of households, it is reasonable to compare growth at these ages and make some inference about the longitudinal development of the CRSP children.

One of the most important conclusions from the INCAP follow-up is that size (length) at 3 y of age is a strong predictor of adolescent size (Martorell et al. 1992; Rivera et al. 1995). The situation seems to be

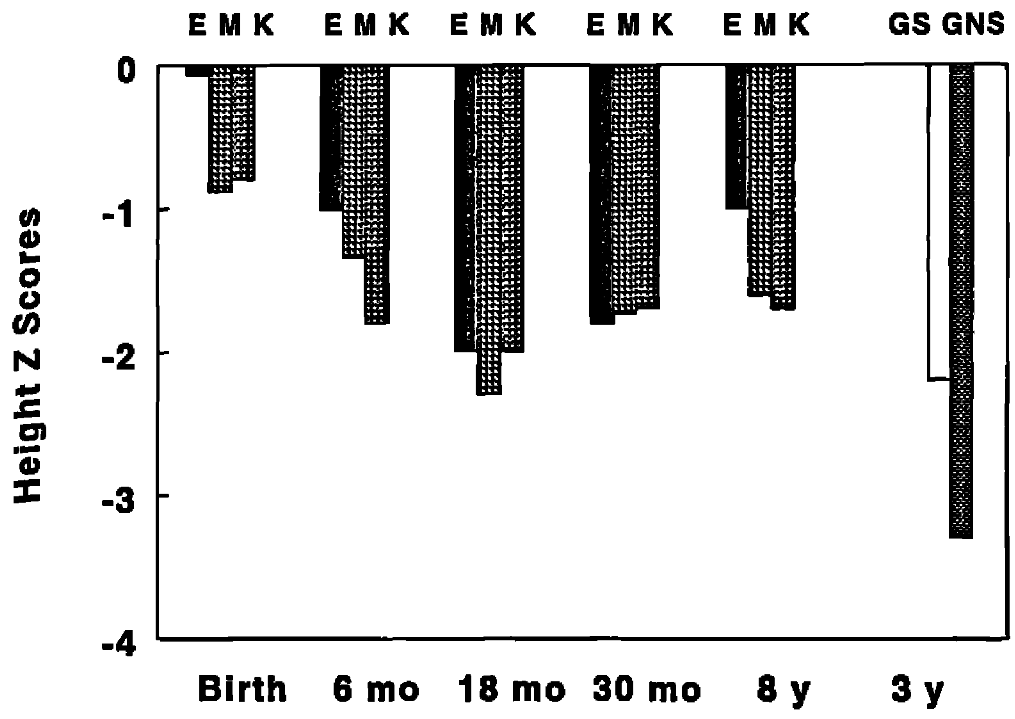

FIGURE 1 Height Z-scores of target children in the Nutrition CRSP compared with the Guatemalan children. E = Egypt; M = Mexico; K = Kenya; GS = Guatemalans supplemented with Atole; GNS = Guatemalans supplemented with Fresco. CRSP children were 30 mo old and Guatemalans were 36 mo old. A Z-score of zero represents the NCHS reference value at each age.

similar in the Nutrition CRSP. **Figure 1** shows the length/height Z-scores of the target children in each country, in each target age group. At birth, infants were slightly shorter than reference values (Hamill et al. 1979) in all three countries. The most rapid period of growth failure started at ~3–4 mo after birth. Growth stunting relative to the reference values essentially was complete before 18 mo, the age at which observations were started on preschoolers; length Z-scores actually improved slightly between 18 and 30 mo of age. In Egypt, height Z-scores improved slightly for schoolers, but comparison with cross-sectional data on adolescents in the same households and with the adult target subjects suggests that in none of the three CRSP locations will there be a significant change in height status during adolescence. The INCAP follow-up supports this assumption, because growth in adolescence occurred at a rate similar to that of adolescents in the United States (Martorell et al. 1995b). The pattern for weight Z-scores in the CRSP was similar to that for height, although these were higher than those for height at all ages.

Because both height and weight were stunted, weight-for-height was relatively normal at all ages. Weight-for-height was also relatively normal in Guatemala, in both initial (Martorell et al. 1982) and follow-up studies (Rivera et al. 1995).

Growth-stunting predicts impaired function

INCAP studies indicate that short size was associated with adverse cognitive and behavioral outcomes in infancy and childhood (Lasky et al. 1981) and that this association persisted to adulthood (Martorell et al. 1992). It is somewhat difficult to compare specific outcomes across the studies because there were differences among tests and many of the scores were grouped or scaled differently during analysis. However, it is clear that short stature also predicted poorer outcomes in the Nutrition CRSP. Most of the associations between height and these functional outcomes in both the INCAP and CRSP subjects were identified in multivariate analyses that controlled for variables such as socioeconomic status, years of schooling and parental and household factors.

CRSP preschoolers who were taller, in Kenya and Mexico but not Egypt, had better cognitive and behavioral performance. In Mexico, the taller preschoolers performed better on the Bayley Mental examination (Allen et al. 1992a). They played, verbalized and interacted more frequently and were crying or doing nothing (apathetic) less often (Allen et al. 1992a, Scanlon 1989). In Kenya, less-stunted preschoolers had higher scores on the Bayley Mental and Motor tests and played and verbalized more (Sigman et al. 1989a). In general, similar associations were seen with weight but not with weight-for-height.

In schoolers, cognitive scores and verbal comprehension, behavior and school performance tended to be even more strongly correlated with height and weight (Allen et al. 1992a, Oh 1990, Sigman et al. 1989b). Although schoolers' size was probably predominantly determined by the second year of life, we assume that longer exposure to an inadequate diet and performance deficits already obvious in the preschool period created cumulative deficits in the children's performance.

The nutritional causes of growth stunting

Both the CRSP and INCAP data provide very strong evidence that early growth stunting predicts both current and later functional performance of individuals. Certainly, it can no longer be argued that small size is an adaptation to malnutrition, reducing the lifelong need for food but unaccompanied by ill health or poor function (Seckler 1980).

This moves us to the need for a new research agenda, which is to understand the reasons *why* growth stunting occurs in developing countries and the extent to which poor dietary patterns and specific nutrient deficiencies are responsible. A comparison of the INCAP and CRSP results provides some insight into this question, although not nearly enough.

Comparing the height Z-scores of the CRSP and INCAP preschoolers illustrates the relative degree of early malnutrition across projects (Fig. 1). The closest ages for which published data are available for comparison are 30 mo in the CRSP populations and 36 mo in the Guatemalan children. This comparison illustrates the important point that the Guatemalan children were substantially shorter than in the other three countries, even after supplementation with Atole. Thus, it is likely that the Guatemalan children were by far the most malnourished group.

These differences in nutritional status should be attributable to differences in dietary patterns or nutrient intake. The dietary patterns across the three Nutrition CRSP countries are described in **Table 1** (Calloway et al. 1992). Mexico and, to a substantial extent, Kenya is similar to Guatemala in that maize and beans supply a large proportion of the dietary energy. Egypt uses more wheat and rice. Animal product and fat consumption is low in all three locations, especially in Kenya and Mexico, where intakes of these dietary constituents are most similar to those in Guatemala.

The energy intakes of the children in the four locations are shown in **Table 2**. For the CRSP projects the values represent average energy intakes between 18 and 30 mo of age (Beaton et al. 1992). For the Guatemalan children the data are an average of intakes between 15 and 36 mo, from the home diets of the Fresco group and excluding the small amount of energy provided by this supplement (Martorell et al. 1982).

Clearly, there is a gradation of energy intake across the projects. The CRSP analyses showed that energy intake was adequate for the Egyptian and Mexican preschoolers. In Kenya, where food shortages occurred during the project, energy intakes averaged less than recommended levels. The Guatemalan children consumed even less energy from their home diets than the Kenyan children.

Protein intakes followed a similar pattern because the correlation between protein and energy intake is very high in these populations. In the CRSP, the calculated probability of digestible protein and essential amino acid intakes being inadequate was essentially zero (Beaton et al. 1992). In Guatemala, protein intake is likely to be adequate for those children consuming adequate amounts of energy, but not otherwise (Torún and Viteri 1981).

Because energy intake was inadequate for some Kenyan and Guatemalan children, an energy supplement, such as the Atole used as one of the INCAP supplements, would be expected to benefit the growth of the Guatemalan children and, to a lesser extent, the Kenyan children. Intervention with dietary energy would not be expected to improve the linear growth of the Egyptian or Mexican groups. This is supported by the fact that in both Kenya (Neumann et al. 1992) and Guatemala (Martorell et al. 1982) the children's energy intake during the preceding 12 mo was a predictor of their height at 30 mo (Kenya) and 36 mo (Guatemala) and of functional outcomes. In Egypt (Kirksey et al. 1992) and Mexico (Allen et al. 1992b), where food shortage was not seen, dietary energy intake was not associated with growth or other functions.

Micronutrient deficiencies

Several lines of evidence suggest that micronutrient (mineral and/or vitamin) deficiencies may explain at least some of the early growth failure. As summarized in **Table 3**, there was a high predicted prevalence of inadequate vitamin and mineral intakes in all three CRSP countries (Calloway et al. 1992, Murphy et al. 1992). The estimates of mineral adequacy were based on algorithms that took into consideration the high intake of phytate (especially in Mexico) and the low consumption of animal products and ascorbic acid. The Guatemalan diet is also high in fiber and phytate and low in ascorbic acid and animal products.

There was a very high prevalence of anemia and iron deficiency (low ferritin) in all three CRSP studies. For Egypt, Mexico and Kenya, respectively, low hemoglobin was present in 73, 62 and 74%, and iron deficiency (low ferritin) was present in 38, 45 and 40% of preschoolers (Murphy et al. 1992). There is a strong possibility that the Guatemalan children were also iron deficient based on the similarity of their diet to that of the Mexican children, the poor absorption of iron from maize (Martinez-Torres et al. 1987) and the high prevalence of iron-deficiency anemia in Central America (DeMaeyer and Adiels-Tegman 1985). Habicht et al. (1973) reported that anemia was not found

TABLE 2

Energy and protein intakes of preschoolers in the four countries[1]

	Egypt	Mexico	Kenya	Guatemala
Energy, *kJ/d*	5,003 ± 1237	4,640 ± 1095	3,540 ± 748	3,322 ± 895
Protein, *g/d*	36 ± 10	33 ± 8	23 ± 5	21 ± 6

[1] Values are means ± SD. Data for CRSP locations are from Beaton et al. (1992). Values for Guatemala are calculated from Martorell et al. (1982).

TABLE 1

Foods consumed by Egyptian, Mexican and Kenyan children[1]

	Preschoolers			Schoolers		
	Egypt (*n* = 96)	Mexico (n = 59)	Kenya (n = 100)	Egypt (n = 63)	Mexico (*n* = 84)	Kenya (*n* = 138)
		kcal/d[2]			*kcal/d*	
Maize	169	551	369	291	1185	877
Wheat	292	75	24	358	97	26
Rice	126	26	41	187	28	29
Legumes	43	80	83	62	201	121
Dairy	59	66	59	72	40	35
Meat, egg	85	60	6	111	76	11
Fats	162	98	29	221	126	41

[1] From analyses by Calloway et al. (1992).
[2] To obtain kilojoules, multiply kilocalories by 4.184.

TABLE 3

***Predicted prevalence of children with inadequate nutrient intakes*[1]**

	Preschoolers			Schoolers		
	Kenya (n = 96)	Egypt (n = 59)	Mexico (n = 100)	Kenya (n = 63)	Egypt (n = 84)	Mexico (n = 138)
		%			%	
Thiamin	1.4	5.9	0.0	0.0	7.4	0.0
Riboflavin	20.4	51.7	1.9	16.3	83.4	1.6
Vitamin B-12	3.2	8.0	44.2	23.6	38.3	86.9
Vitamin C	3.1	62.7	1.2	1.6	34.6	0.0
Vitamin A	2.2	19.5	0.4	9.2	24.4	0.6
Vitamin E	21.5	91.8	85.1	4.5	86.0	43.5
Iron	65.3	88.5	36.2	70.4	87.3	31.4
Zinc	9.8	25.2	57.0	3.5	9.2	29.5

[1] From analyses by Calloway et al. (1992) and Murphy et al. (1992). Intakes of niacin, folate, vitamin B-6 and copper were adequate in all three locations. For vitamin A, iron, zinc and copper comparisons are against FAO/WHO basal requirements, i.e., that needed to prevent clinically detectable signs of functional impairment. The prevalence of low intakes would have been substantially higher if based on normative requirements.

in 1971 in unsupplemented 2-y olds; however, information about iron deficiency without anemia was not provided. Finally, iron supplements have improved the cognitive (mental) performance of rural Guatemalan children in other studies (Lozoff et al. 1985).

Preschoolers' growth in Mexico and Kenya was related positively to the amount of animal products consumed in their usual diet (Allen et al. 1992b, Neumann et al. 1992); animal products supply more nutrients, such as available iron and zinc, and vitamin A and are markers for a diet that is generally of higher quality and lower in fiber and phytate (Allen et al. 1992a). Animal product consumption, as well as other indicators of a better quality diet, also predicted a number of cognitive and behavioral outcomes in children in all three countries.

Micronutrient content of the INCAP supplements

The comparative data presented in Figure 1 also illustrates the important point that *even the Guatemalan children supplemented with Atole* did not attain the average size of those in any of the CRSP countries and that the supplement failed to prevent all but a very small amount of their growth faltering. Because even the smaller degree of growth stunting of CRSP children was associated with functional deficits, it is reasonable to assume that even those Guatemalan individuals who benefited from the Atole supplement still suffered from a substantial amount of functional impairment.

INCAP's Atole supplement was designed to provide additional energy and protein. It consisted of (g/180 mL) Incaparina, a vegetable protein mixture (13.5), dry skim milk (21.6) and sugar (9.0) (Martorell et al. 1995a). The Fresco, which contained only sugar, provided only one third as much energy and no protein. The Atole contained calcium and phosphorus whereas the Fresco did not. Because the Atole ingredients also contained micronutrients, the *concentrations* of several vitamins and minerals were matched in the two supplements with the aim of preventing differences in these from confounding the interpretation of the results. However, between birth and 2 y of age, the children unexpectedly drank about four times as much Atole as Fresco and twice as much between birth and 3 y (Schroeder et al. 1992). This means, as presented for the 0–2-y age period in **Table 4**, that there was a difference in the actual micronutrient intake between the two groups. Estimated intakes of four micronutrients (zinc, vitamin B-6, folacin and vitamin B-12) not originally considered in the formulation have been calculated here from the dry skim milk in the Atole. Data are not available for the micronutrient composition of the Incaparina. The 1989 RDA values are used (NRC, 1989).

The difference between the micronutrients consumed in the two supplements raises two concerns. First, it is impossible to be certain that the greater benefits of the Atole supplement on postpartum growth and development are attributable to its energy content alone. [We note that this was not true for the relationship between energy supplementation of the pregnant woman and birth weight, where this association remained significant when the amount of supplement was controlled for (Habicht et al. 1995).] Second, the supplements failed to improve the intake of some micronutrients substantially. Notably, neither

TABLE 4

Nutrients in INCAP supplements consumed by children 1–2 y

	Percent RDA per cup		Percent RDA supplemented	
	Atole	Fresco	Atole	Fresco
Energy	13	6	12	6
Protein	72	0	66	0
Calcium	50	0	46	0
Phosphorus	40	0	37	0
Iron	54	50	50	21
Ascorbic acid	10	0	9	0
Thiamin	157	157	143	71
Riboflavin	188	188	175	133
Niacin	150	150	189	86
Vitamin A	300	300	275	125
Zinc[1]	10	0	9	0
Vitamin B-6[1]	7	0	7	0
Folacin[1]	21	0	19	0
Vitamin B-12[1]	124	0	114	0

[1] Calculated contribution from the skim milk in Atole.

supplement contributed much zinc, which may be growth limiting in this population, or ascorbic acid, which might have improved iron absorption and subsequently growth, or vitamin B-6, which might have improved infant cognitive development. Although this is speculation, there is no doubt that even the Atole did not supply enough of some nutrient(s) to prevent growth stunting during the first 2 y after birth.

The nutritional implications of early growth stunting

Figure 2 compares children's average length growth rates between 18 and 30 mo of age for the three CRSP projects, the INCAP study (Martorell and Klein 1980) and the National Center for Health Statistics (NCHS) reference population (Hamill et al. 1979). The rate of increase in the four poorer countries was less than that of the reference children so that some growth failure was still occurring during this period. However, given that the Guatemalan children were so much shorter than CRSP children at 30–36 mo (Fig. 1), it is remarkable that even the Guatemalans supplemented with Fresco were growing at least as fast as children in Egypt and Kenya, and those supplemented with *Atole* grew as rapidly as children in all three CRSP locations. The pattern for weight was similar, the Atole-supplemented Guatemalans growing faster than the Mexicans or Kenyans during this period (**Fig. 3**). This means that the greater degree of growth failure of the Guatemalan children occurred *before* the age of 18 mo (see Martorell et al. 1995b). Infants in all four countries were breast-fed, the majority receiving almost all of their nutrients from breast milk through at least the first 8 mo of life. Guatemalan infants consumed an average of ~10% of their energy requirements from Atole between 3 and 12 mo of age (Schroeder et al. 1995), most of this presumably toward the end of the first year. Some children still received small amounts of breast milk beyond 18 mo. Considering that the most rapid growth failure occurred within a few months of birth in all the population samples, the conclusion must be that either maternal milk or weaning foods failed to supply the nutrients needed by the children.

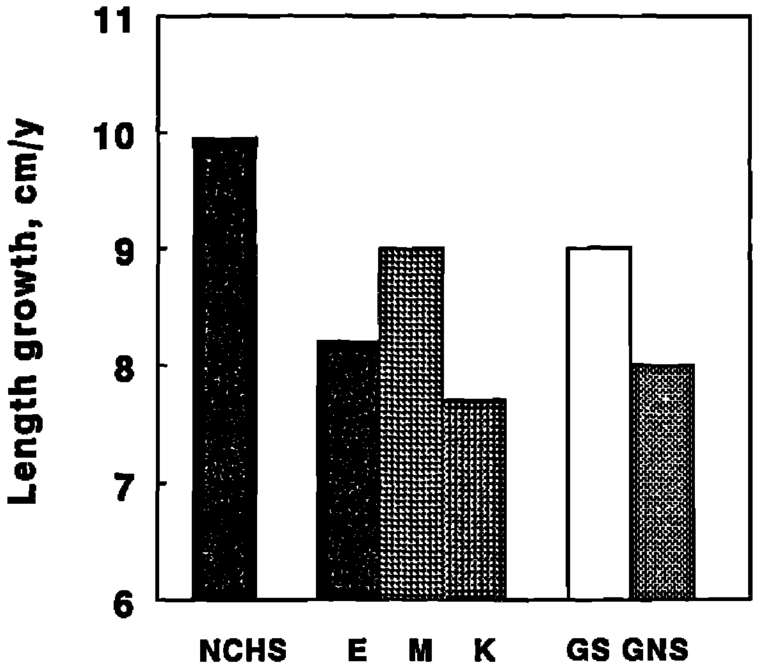

FIGURE 2 Rate of length growth of NCHS reference, Nutrition CRSP and Guatemalan children. E = Egypt; M = Mexico; K = Kenya; GS = Guatemalans supplemented with Atole; GNS = Guatemalans supplemented with Fresco. Values are for the 18–30-mo age period for all groups.

The CRSP found some evidence that the poor micronutrient status of the mothers had an adverse effect on breast milk composition. The concentrations of

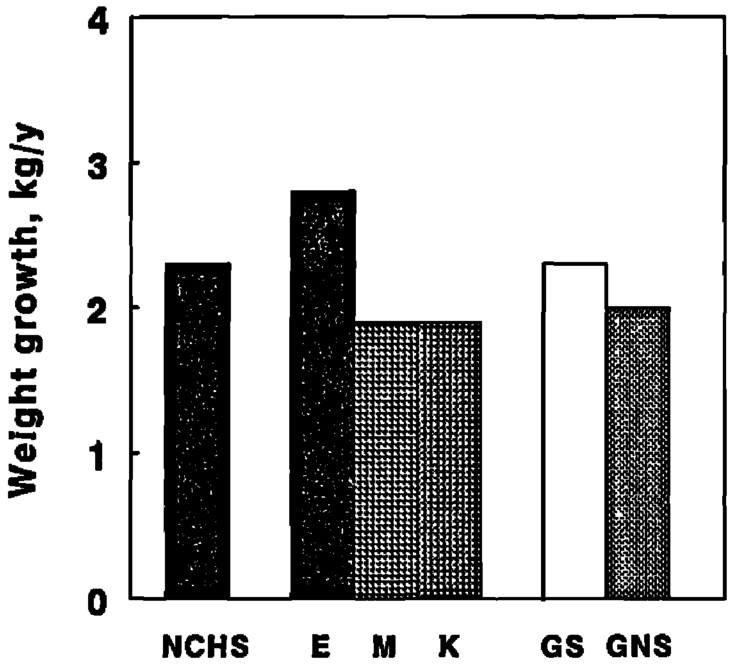

FIGURE 3 Rate of weight growth of NCHS reference, Nutrition CRSP and Guatemalan children. E = Egypt; M = Mexico; K = Kenya; GS = Guatemalans supplemented with Atole; GNS = Guatemalans supplemented with Fresco. Values are for the 18–30-mo age period for all groups.

water soluble vitamins in breast milk are especially responsive to maternal dietary intake. In Egypt, breast milk concentrations of vitamin B-6 were only about one half those in milk of nonsupplemented American women and predicted the behavior of the infants at 6 mo of age (McCullough et al. 1990). The milk content of this vitamin was not analyzed in Kenya or Mexico. The only other vitamin measured in breast milk was vitamin B-12 in Kenya and Mexico. The content was deficient in the milk of all the Kenyan mothers (Neumann et al. 1992) and in 62% of the milk samples in Mexico (Black et al. 1994). More data are needed concerning whether the micronutrient content of maternal milk is adequate to support infant growth and development in regions where dietary quality is poor.

The INCAP and Nutrition CRSP studies show that growth failure occurs very early in life in marginally malnourished populations. They leave little doubt that early malnutrition usually causes permanent growth stunting and associated functional deficits. However, we still do not know why it occurs or which nutrients are growth limiting. In Kenya and Guatemala, where there was a shortage of food, inadequate energy intake probably explains some, but not all, of the growth failure. However, the energy provided by the INCAP Atole supplement was not very effective at preventing growth stunting and children given this supplement were still smaller than the average CRSP child at ~30 mo of age.

It is highly probable that, as in the Nutrition CRSP, micronutrient deficiencies played a role in the failure of Guatemalan children to grow adequately. In preschoolers and schoolers this can be attributed in part to their low intake of animal products and the poor bioavailability of minerals from dietary constituents. But the growth failure occurs during the first few months after birth when breast milk provides all or by far the majority of infant's nourishment. It is also evident from these comparisons that the greater severity of growth stunting in the Guatemalan children can be attributed to their nutritional status before the age of 18 mo; between 18 and 30 mo they grew as well or faster than the CRSP children.

For many compelling reasons nutritionists support breast milk as the sole food for infants during the first 4–6 mo of life. Certainly, however, breast milk was not adequate to support optimal growth of either the CRSP or the INCAP infants. Although the introduction of supplementary foods and infant morbidity (especially subclinical) cannot be ruled out definitively as causes of the inadequate growth, in none of the four locations have these explained more than a small proportion of the growth faltering. Further attention should be paid to the possibility that maternal micronutrient deficiencies result in infants having stores at birth and/or intakes from breast milk that are inadequate to support optimal growth and development in such environments. Efforts should now be focused on understanding when to intervene, how to intervene and what nutrients to provide to prevent early growth stunting and its associated persistent functional deficits.

ACKNOWLEDGMENTS

The author is indebted to her coprincipal investigators in the Mexico Nutrition CRSP, Gretel H. Pelto of the University of Connecticut and Adolfo Chávez who headed the research team at the Instituto Nacional de la Nutrición in Mexico City. Thanks are also due to the principal investigators of the Egypt and Kenya projects, the team at Berkeley and the INCAP investigators, who produced the data discussed here and made helpful comments on this paper.

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Results and Implications of the INCAP Follow-up Study[1,2]

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ABSTRACT This article is a critical synthesis of 12 papers included in this supplement. The set deals with the short- and long-term effects of improving nutrition in Guatemalan villages characterized by deficient diets, high rates of infection and pronounced growth retardation in the first 3 y of life. The data reviewed come from two studies carried out over two decades: the Institute of Nutrition of Central America and Panama (INCAP) longitudinal study (1966–1977) and its follow-up (1988–1989). The longitudinal study included a nutrition intervention that improved the energy and nutrient intakes of women and preschool children. Its effects included improved birthweights, reduced infant mortality rates and improved growth rates in children < 3 y of age. Growth rates from 3 to 7 y of age, similar to those of well-nourished children, were not affected by the intervention. The follow-up study was conducted when the subjects were 11–27 y old. Among the long-term effects found were greater stature and fat-free mass, particularly in females, improved work capacity in males and enhanced intellectual performance in both genders. The nutrition intervention did not, on the other hand, accelerate maturation during adolescence, as measured by skeletal age or age at menarche. It is concluded that improved nutrition in early childhood has important long-term effects in the adolescent and adult. J. Nutr. 125: 1127S–1138S, 1995.

INDEXING KEY WORDS:

- *nutritional supplementation* • *adolescence*
- *malnutrition* • *growth and development*

This article is an overview of papers included in this volume, with emphasis on the main findings and policy implications of the Institute of Nutrition of Central America and Panama (INCAP) follow-up study (1988–89). A previous set in the Food and Nutrition Bulletin (Vol. 14, number 3, 1992) concentrated on the study which preceded the follow-up, namely the INCAP longitudinal study (1969–77).

To provide readers with the context necessary to interpret the findings of the follow-up study, the first three papers of this volume (Habicht et al. 1995, Martorell et al. 1995a, Schroeder et al. 1995) present essential information from the earlier INCAP longitudinal study, the next seven papers deal with the follow-up study and the last one is a comparison of our findings to those of the Nutrition Collaborative Research Support Program (CRSP) studies (Allen 1995).

This effort is an attempt to provide a critical summary of the findings and is guided by the following questions: What has been learned from the INCAP studies that is important? Are these results believable and internally consistent? What lessons for policies and programs can be drawn from these results? The first section is a discussion of the original INCAP longitudinal study and its results and is followed by sections dealing with design and analytic issues and with the key findings of the follow-up study. The article ends by addressing the program and policy implications of the results of the INCAP and follow-up studies.

The INCAP Longitudinal Study (1969–77)

The villages. The studies took place in four small villages of eastern Guatemala. When chosen in 1968, two had ~900 inhabitants each and two had ~500 each but had grown to 1,200 and 800 by 1975, respectively. Mean household incomes per year in these villages, where the primary occupation was subsistence agriculture, ranged from US $291 to $463 in 1975. Maternal literacy ranged from 25 to 40% in 1967, and 10 years later, this had risen from 35 to 50%. Although all villages had schools when the study began, it was not until around 1980 that instruction

[1] Presented in the symposium on Nutrition in Early Childhood and its Long-term Functional Significance, FASEB, April 6, 1992, Anaheim, CA. Published as a supplement to The Journal of Nutrition. Guest editors for this supplement publication are Reynaldo Martorell, The Rollins School of Public Health of Emory University, Atlanta, GA and Nevin Scrimshaw, The United Nations University, Boston, MA.

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up to 6 y of elementary schooling was offered. Electricity became available in the midst of the study in the two small villages and, in the 1980s, in the larger ones. Most families obtained water from wells or rivers and few had latrines. Typical houses were small, one to two rooms, and made of adobe. Driving into these villages was difficult, particularly during the rainy season, but one village close to the highway was more accessible. The dietary staples, as in most of rural Guatemala, were corn and beans, complemented occasionally by small amounts of meat and by fruits and vegetables when in season. Additional details are provided by Bergeron (1992), Engle et al. (1992) and Martorell et al. (1995a) but enough information has been given to illustrate the setting of poverty and rural isolation in which the study took place.

The supplementation program. The longitudinal study was designed to test the hypothesis, in vogue in the 1960s, that malnutrition retards mental development. Researchers operationalized the study as an assessment of the impact of significantly improving protein intakes in mothers during pregnancy and lactation and in children during the first 7 y of life. The treatment was a drink called Atole, rich in high quality protein, the nutrient then thought to be the main constraint to nutrition in developing countries. However, the Atole contributed much more than protein because it is impossible to provide a food-based supplement that is rich in it without simultaneously providing energy, as well as important amounts of some vitamins and minerals. There were additional considerations about the treatment. The researchers feared that the setting in which the Atole was made available, an attractively painted large room with blue tables and chairs set about and with uniformed attendants serving the Atole in brightly colored cups, would be conducive to considerable social interactions among consumers that, in turn, might affect psychological development in the target sample, independent of the nutritional effects of the Atole. Consequently, a protein-free beverage called Fresco was provided under the same set of conditions as the Atole in two villages to serve as control to the two receiving Atole. The careful almost fastidious recording of attendance and amount of supplement provided (subjects were free to drink as many cups as desired, each containing 180 mL) and of the measurement of leftovers was a feature in both Atole and Fresco villages. Attendance and consumption records were kept for pregnant and lactating women and for children ≤7 y; although all villagers were free to attend, the participation of men, older women and children > 7 y old was unrecorded.

Four villages were selected in 1969 for the study. The pair of large villages was randomized to receive Atole or Fresco, as was the pair of small villages. Although great care was taken to choose pairs of villages that were similar to each other, important differences existed then or surfaced later in nutrition and health as well as in social, educational and demographic conditions, but these differences do not favor any village systematically; rather, there is considerable variability in the comparative rankings by village depending upon the aspect considered (Bergeron 1992, Engle et al. 1992, Martorell et al. 1995a).

The Fresco was devoid of protein and because empty calories were thought to be potentially harmful, it contained only enough sugar and flavoring to make a palatable refreshing drink. A number of vitamins (thiamin, riboflavin, niacin, vitamin A, ascorbic acid) and minerals (iron, fluoride) were added to the Fresco early in the study to make its composition similar to that of the Atole except with regard to protein; the obvious differences in terms of energy, which favored the Atole, were not thought to be nutritionally significant at the time. The Atole, but not the Fresco, also contained calcium and phosphorus, as well as other nutrients whose presence has gone unrecognized in publications about the study, such as zinc, vitamin B-6, folacin and B-12 (Allen 1995). The content per cup of the Atole was 11.5 g of protein and 163 kcal/682 kJ per cup (180 mL) and of the Fresco was 0 g protein and 59 kcal/247 kJ per cup. Both the Atole and Fresco drinks were similar to local preparations and both were liked, but patterns of intake differed. In mothers, attendance was similar but the Fresco was consumed, on a volume basis, at about twice the rate of the Atole (Delgado et al. 1982). This is understandable in that the Fresco was light and thirst quenching and the Atole was thick and served hot. Patterns in children were much different (Schroeder et al. 1992). Attendance below 3 y of age was greater in Atole villages as was the volume consumed; after 3 y, attendance was similar but the volume consumed was greater in Fresco villages (**Fig. 1, Fig. 2**).

The nature of the intervention. What was the nature of the intervention? This can be answered in

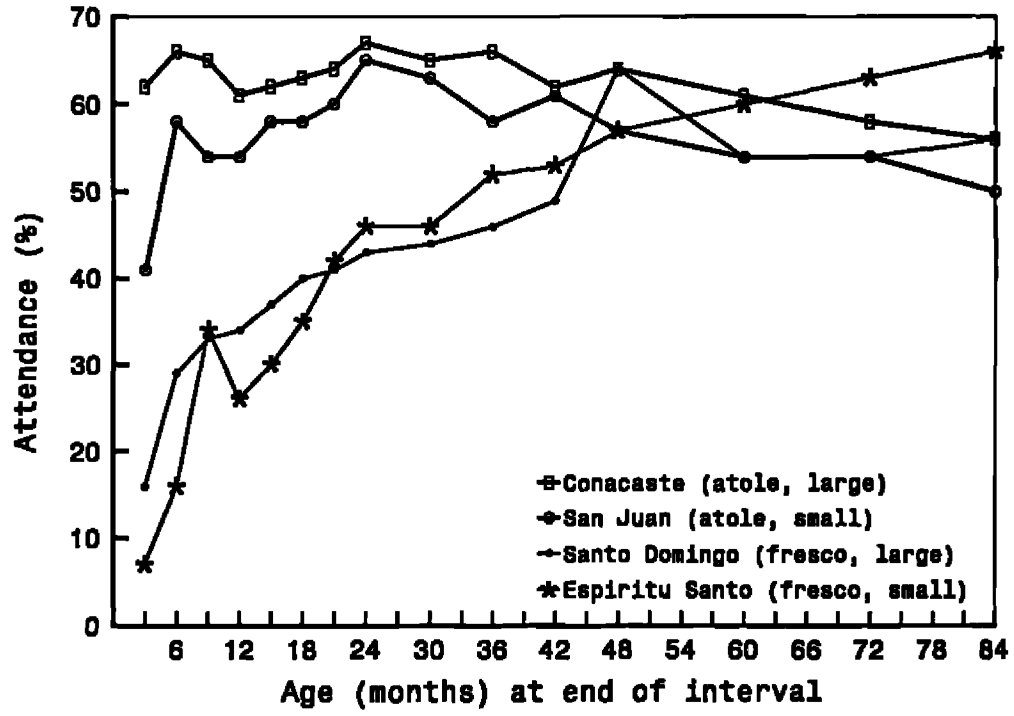

FIGURE 1 Attendance of participants at the feeding center (percentage of days in interval) by age and village. From Schroeder et al. 1992.

the narrow sense in terms of improvements in nutrient intakes in mothers and children. In mothers, average intakes of energy from the supplements during the entire pregnancy were 107 kcal/448 kJ per day in Atole villages and 81 kcal/339 kJ per day in Fresco villages (Delgado et al. 1982). Thus, the greater consumption of the Fresco nearly made up for its lower energy concentration. Average daily protein intakes from the Atole were ~7.5 g; the Fresco had no protein. Intake of a number of vitamins and minerals common to both drinks was about twice greater in Fresco villages, because Fresco was consumed that much more and the concentration was similar in Atole and Fresco villages. The rate of replacement of home diet is not known with precision but is estimated to have been 22% for energy (Habicht et al. 1995).

In children 15–36 mo, average total energy intakes per day (i.e., home diet and supplement combined) were greater in Atole compared with Fresco villages by ~101 kcal/423 kJ in boys and 89 kcal/372 kJ in girls; this is equivalent to ~11% of the total intake in Fresco villages. Comparisons of total intake of protein favored Atole boys by 8.6 g and Atole girls by 8.8 g, equivalent to ~40% of the intake in Fresco villages (**Table 1**). Children in Atole villages, because they ingested significantly greater volumes of supplement from 15 to 36 mo of age than children in Fresco villages did (Fig. 2), also had significantly greater intakes of many vitamins and minerals.

The preceding makes it clear that the intent in 1969 of creating a contrast between village types in terms of protein was realized but along with other effects. Supplement intakes during pregnancy in Atole women were improved substantially in terms of protein, but both Atole and Fresco women received nearly similar amounts of energy from the supplements and the Fresco group ingested greater amounts of a number of vitamins and minerals. In children < 3 y of age, intakes of protein were increased in Atole villages but so were those of energy, vitamins and minerals. In children 3–7 y of age, protein intakes were increased in Atole villages, but despite the greater volume of Fresco consumed as children got older, the energy consumed from the Fresco was still less than that from the Atole, but the gap narrowed considerably as children neared 7 y of age (**Fig. 3**). Finally, the contribution from the supplements of those vitamins and minerals found in equal concentrations in both drinks was greater from Fresco than from Atole after ~4 y of age when more of the Fresco began to be consumed.

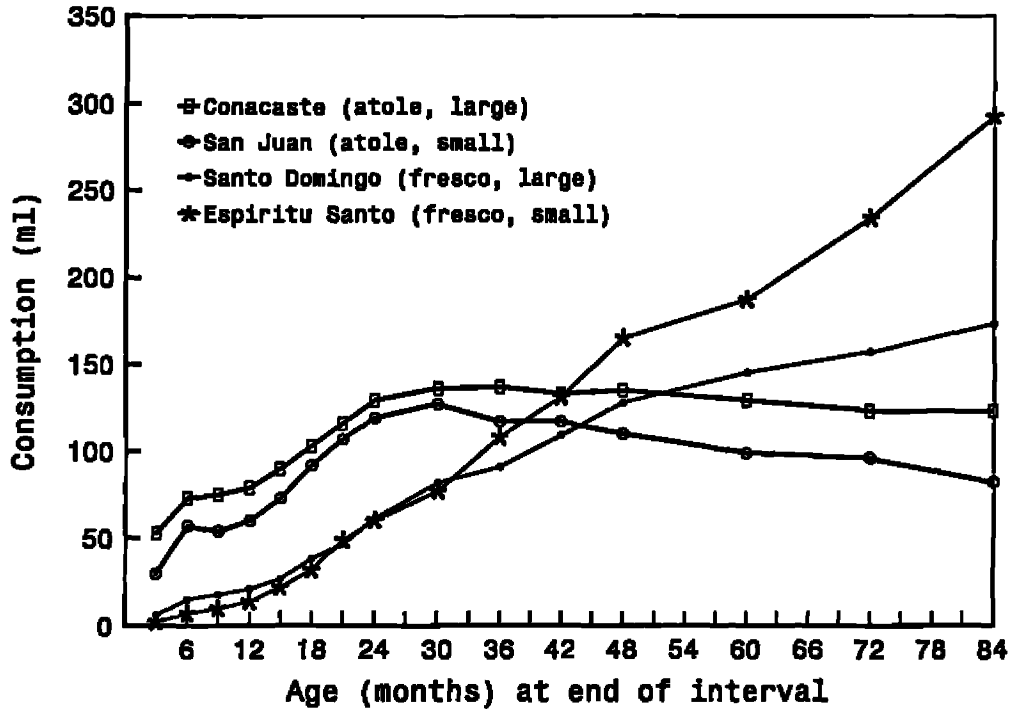

FIGURE 2 Mean volume of supplement consumed per day by age and village (nonparticipants included). From Schroeder et al. 1992.

TABLE 1

Contribution of the supplements to total energy and protein intakes in children 15–36 mo of age[1]

	Males		Females		
	Atole (n = 128)	Fresco (n = 135)	Atole (n = 118)	Fresco (n = 104)	Pooled SD
Energy, *kcal/d*					
Home diet	785	814	718	756	213
Supplement	156	26[a]	150	23[a]	79
Total Intake	941	840[a]	868	779[b]	226
Energy, *kJ/d*					
Home diet	3,284	3,406	3,004	3,163	891
Supplement	653	109[a]	628	96[a]	331
Total Intake	3,937	3,515[a]	3,632	3,259[b]	946
Protein, *g/d*					
Home diet	20.1	22.5	19.3	21.0	5.9
Supplement	11.0	0.0[a]	10.5	0.0[a]	5.4
Total Intake	31.1	22.5[a]	29.8	21.0[a]	7.5

[1] Home diet values are averages of as many as eight recall surveys conducted at 15 mo and every 3 mo thereafter until 36 mo (adapted from Martorell, 1982).

[a] $P < 0.001$.

[b] $P < 0.01$.

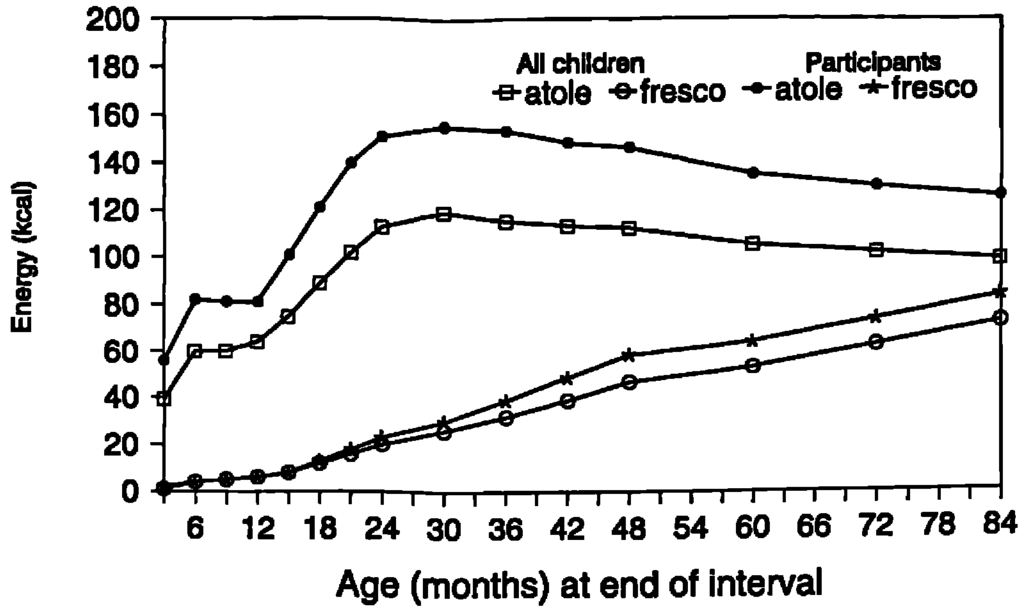

FIGURE 3 Mean energy intake per day from supplement by age and supplement type (with and without nonparticipants). From Schroeder et al. 1992.

Was the Fresco, as originally intended, an adequate control for the socialization effects that might result from the social interaction at the feeding centers? The answer appears to be a qualified yes in that patterns of attendance were similar in Atole and Fresco villages in the case of pregnant women and older children but not in young children. Fortunately, attendance and consumption of supplement were measured, permitting researchers to control for participation in contrasting Atole and Fresco communities, particularly important in the analyses of behavioral outcomes (Pollitt et al. 1995). Living closer to the feeding center and being from a larger family predicted participation in children in both village types; however, children of poorer families had greater participation in the supplementation program only in Atole villages (Schroeder et al. 1992). Failure to control for socioeconomic status in analyses of the relationship between Atole intake and outcomes might therefore bias the results against the hypothesis that consumption leads to improved status.

In addition to the supplementation program, INCAP established a medical care program in all four communities (Martorell et al. 1995a). These services were provided by auxiliary nurses under the careful supervision of a physician. A range of curative services, with appropriate referral in case of complications, was given. Also, periodic examinations during the prenatal period and during early childhood were carried out. Vaccines were administered in these visits. These efforts, although important, fell short of the ideal in that community nutrition and health education were not provided outside the context of medical care visits. Also, there were no programs implemented in environmental sanitation, formal education or economic development.

Finally, another component of the intervention, the very presence of INCAP investigators asking questions and examining women and children, must have had some unspecified effects, but these, because of careful rotation among personnel and similar research activities in all four villages, cannot bias the comparison of Atole and Fresco villages (Habicht et al. 1995).

In summary, the nature of the intervention is a contrast between villages receiving different types of supplements but identical medical services and similar but unintended effects of having a research team present for about 8 years. In children *15–36 mo of age*, exposure to different supplements increased total energy intakes by 11% and total protein intakes by 40% in Atole villages relative to Fresco villages. Also, total intakes of many vitamin and minerals also were substantially greater in Atole compared with Fresco villages. Because of the uncertainty about the energy requirements of children, it is impossible to say how far the extra 100 kcal/418 kJ per day went toward meeting needs, but it is unlikely that adequate levels were reached. Average energy intakes of Atole and Fresco children, combining home diet and supplement, were ~93 and 83 kcal/kg (389 and 347 kJ/kg) at 24 mo of age, respectively, compared with estimated requirements of ~110 kcal/kg (460 kJ/kg) given by FAO/WHO/UNU (1985). This would mean that children in Atole and Fresco villages had intakes equivalent to 85% and 75%, respectively, of the requirement. Allowances for catch-up growth, usually taken into account by using ideal rather than actual body weights, would of course increase the apparent energy gap substantially. For protein, on the other hand, average intakes exceeded allowances even before considering the contribution of the Atole; average total protein intakes were 3.1 and 2.2 g/kg in Atole and Fresco villages compared with the safe level of 1.8 g/kg at 24 mo of age (adjusted conservatively for an amino acid score of 60 and a digestibility score of 85).

Approaches to analyses. A meta-analysis of nutrition interventions during pregnancy and birthweight excluded the findings of the INCAP longitudinal study from all consideration (Kramer, 1993). The reason given was that the analyses were not carried out according to the randomized design.

The randomized design calls for village to be the unit of analysis. Because the comparison of village means involves only two Fresco and two Atole villages, statistical power is extremely low (Habicht et al. 1995). It is doubtful that those who designed the study intended for it to be analyzed in this manner. If the randomized design is used to test for birthweight differences, no effect is found; mean birthweights in Atole are slightly greater than those in Fresco villages, but this small difference is not statistically significant (3077 ± 334 and 3027 ± 461 g, respectively; Delgado et al. 1982). As reviewed earlier, the mean intake of energy from the supplements during pregnancy also was slightly greater in Atole than in Fresco villages. It turns out that energy intakes from the supplements, irrespective of whether accompanied by protein, were related to birthweight (Habicht et al. 1995). The similarity in the dose-response relationship in Atole and Fresco samples, when expressed in terms of energy, implies that vitamins and minerals present in both drinks in equal concentrations could not account for the findings. If this were the case, regression coefficients would be more than twice larger for Fresco, when expressed per units of energy, and similar to those for Atole, when given per units of volume. Thus, because energy turned out to be limiting for birthweight and because both the Atole and Fresco contributed nearly equal amounts of energy during pregnancy, the randomized design became inappropriate.

The reasons for abandoning the randomized design in the analyses of birthweight are justified but the credibility of the results, as exemplified by Kramer's decision to exclude the study, suffers. However, others have given considerable importance to the individual level analyses of supplement intake and birthweight, giving due weight to their internal consistency and the careful efforts to control for confounding. Thus, the finding that 20,000 kcal/83,684 kJ provided during pregnancy will reduce the prevalence of low birthweight by half (Lechtig et al. 1975) has helped to justify maternal nutrition programs worldwide.

The randomized design is used by Habicht et al. (1995) to analyze the effects of exposure to Atole on attained lengths at 3 y of age. Baseline values of length at 3 y of age in 1968 (before the study) are used to estimate, for each of the four villages, the differences in length with respect to values for children born after the study began in 1969. Using village means, Habicht et al. (1995) estimated that the Atole sample grew ~2.5 cm more than children in Fresco villages ($P < 0.005$, $df = 2$). This may be a conservative estimate of the impact of Atole because children in Fresco villages grew ~0.5 cm more compared with baseline values, perhaps because of the small amounts of energy and other nutrients received from the Fresco and/or because of the medical care program, among other possible reasons. It should be noted that the small degree of change in Fresco villages implies that the medical care program and energy supplementation during pregnancy appear to have had little impact on *postnatal growth*.

An extension of the randomized design also has been used by Martorell (1992) in examining time trends in severe stunting (i.e., lengths that are ≥3 SD below the reference mean) in Fresco and Atole villages. The prevalence of severe stunting was ~45% in both Atole and Fresco villages at the beginning of the study in 1969 and declined at the end of the study, in 1976–77, to <20% in Atole villages but remained at about the same level in Fresco villages. A logistic regression of trends by village with treatment, sex and year as independent variables and village as the unit of analysis showed a statistically significant decline only in Atole villages ($P < 0.01$).

Also, a village level analysis has been used in assessing changes in infant mortality, using data collected through retrospective women's life histories (Rose et al. 1992). Relative to baseline levels in 1949–68, infant mortality rates declined by 66% in Atole villages and by 24% in Fresco villages, but the rates of decline were not significantly different from each other ($P > 0.05$). Data collected in three of the villages considered for selection but not included in the longitudinal study showed a rate of decline of 19% over the same period, presumably reflecting general trends in rural Guatemala. The rates of decline in Atole villages, but not those in Fresco villages, were significantly different ($P < 0.05$) from those observed in these three undisturbed villages.

Thus, despite low statistical power, analyses using the randomized design have demonstrated a significant impact of Atole ingestion on physical growth at 3 y of age and on infant mortality. In both these situations we had baseline data available that permitted a before/after design, thereby increasing precision of the estimate of change in each village. We lack baseline data for birthweight (never collected) and for all the psychological variables in the infant and preschool batteries (some data apparently were collected but these cannot be found despite careful searches).

Instead of the randomized design, analyses of the psychological data, as indeed many of those dealing with physical growth, have used the individual as the unit of analysis, which vastly increases statistical power. These analyses are of two types. One compares means in Atole villages to those in Fresco villages, in analyses of covariance that adjust for potentially confounding factors such as socioeconomic status, with the degrees of freedom determined by the number of individuals and the model used. A second type of analysis examines the dose-response relationship between supplement intake and outcomes, controlling for potentially confounding factors.

These analyses do not have the rigor of the randomized design, which incorporates the potential effects of confounding factors in the design (Habicht et al. 1995). Instead, the individual level analyses deal with confounding through the analyses. Although previous analyses of our data almost always have included adjustment for potentially confounding factors, this was necessarily limited to those aspects measured in the study and incorporated in the analysis.

Follow-up study (1988–89)

Design and analyses issues. The follow-up study was a cross-sectional evaluation of former participants of the INCAP longitudinal study of 1969–77 (Martorell et al. 1995a). The main hypothesis was that the nutritional improvements in the critical period of gestation and the first 3 y of life ultimately produce adolescents with a greater potential for leading healthy productive lives. Anyone ≤7 y when the study began or who was born into the study was included in the target sample. Migrants were measured as well but, because of costs, only those moving to certain urban centers were included. The main areas of data collection were anthropometry, medical examinations, hand-wrist X-rays, blood samples (for iron measures), psychological tests, retrospective life histories and work capacity. About 73% (1574/2169) of subjects in the four study villages were included in the follow-up. Coverage was 41% (296/727) in migrants and 89% (1278/1442) in nonmigrants.

The follow-up was an ambitious attempt, the first of its kind, to look at the long-term effects of a nutrition intervention in a developing country. The range of outcomes measured was broad and was meant to provide indicators of human function across a range of domains. The follow-up adds new knowledge because it extends the usual horizon for evaluating nutrition interventions to tap functions and abilities in the adolescent and young adult that are not present or not yet developed in their entirety in young children. At the same time, the follow-up has important design limitations that limit the range of possible analyses and their credibility. These limitations are discussed below.

At the time of the follow-up, the subjects ranged from ~11 to 27 y of age. Subjects were exposed to supplementation over a wide range of ages and for varying durations, from those who were 7 in 1969 when the study began (exposed for 9 y from 7 to 16 y of age) to those born in 1977 when the study ended (exposed only during infancy). Ages at exposure and years of exposure by year of birth are given by Khan et al. (1995). Our ability to study the differential impact of supplementation at various ages is limited by the fact that recording of ingestion of supplement and measurement of other outcomes in the INCAP longitudinal study were carried out only until 7 y of age.

The main hypothesis of the follow-up study states that nutritional improvements during pregnancy and the first 3 y of life should result in improved outcomes in adolescence. Most of the papers test this hypothesis by comparing outcomes in Atole and Fresco subjects born on or after March 1, 1969 to February 28, 1974 (designated as Cohort 2 in Martorell et al. 1995a). This is the group exposed during what we have called the critical period (pregnancy and the first 3 y of life). The expectation is that examination of effects in this group is more likely to uncover differences between Atole and Fresco. This approach, to be complete, should include comparison of results for this cohort to those observed in cohorts exposed to supplementation at other periods, but this has been done only in some areas (Haas et al. 1995; Khan et al. 1995). Because of the lack of baseline data (i.e., pre-1969 data) on adolescent outcomes, the randomized design has not been used in analyzing the results of the follow-up.

The basis for designating pregnancy and the first 3 y of life as the critical period comes from the analyses on birthweight reviewed above, from results presented by Schroeder et al. (1995) and Martorell et al. (1995b) as well as from other considerations. Adolescents and adults in our sample are short in height compared with reference values but growth rates are retarded only in the first 2–3 y of age. This is a time when nutritional needs (per kg of body weight) are greater, when growth in length is still rapid, although decelerating, and when diarrheal diseases are more common. The above are some of the reasons that might explain why a striking relationship between supplement intake and growth is found in the first 3 y of life, but not from 3 to 7 y of age. Pollitt et al. (1995) assessed effects on behavioral outcomes and reduced the upper bound of the critical period from 3 to 2 y of age, on the basis that pregnancy and the first 2 y are periods of marked brain development and perhaps the times most sensitive to the effects of nutrition. The group considered by Pollitt includes all Cohort 2 subjects as well as some Cohort 1 subjects, specifically those fully exposed to the nutritional intervention during pregnancy and the first 2 y of life but with variable exposure from 24 to 36 mo of age; the birthdates for the cohorts are given by Martorell et al. (1995a).

The analyses that contrast Cohort 2 subjects in Atole and Fresco villages control for potentially confounding factors but the models used in the various papers rarely are identical. This reflects in part the diverse nature of the outcomes analyzed (e.g., maximal oxygen consumption and reading achievement), which requires a different set of covariates, and the equally varied disciplinary approaches of the various members of the research team.

A major difficulty in the analyses was the need to control for maturation and/or age because many of the subjects were adolescents. This increased the complexity of the models and complicated the interpretation of the results, as exemplified by the analyses of body size by Rivera et al. (1995). Adolescent males in Fresco villages were taller than their counterparts in Atole villages but they were also older by 0.5 y; controlling for this difference reversed the trend and made Atole males taller, as predicted. At the same time, the fact that adolescents were included in the follow-up study permits analyses of effects on maturation per se (Pickett et al. 1995) and on growth during adolescence (Martorell et al. 1995b); these are significant contributions to the literature on adolescence, a group largely ignored in previous studies in developing countries.

A related problem is that effects of the nutrition intervention on productivity, measured in terms of goods produced, income or their proxies, could not be studied adequately in a young sample. Rather, the young age of the sample limits one to study potential in human capital through variables such as body size and composition, work capacity and intellectual performance. The issue of the links with productivity is discussed in more detail later.

As reviewed earlier, coverage in the follow-up was ~72%, introducing a source of potential bias in the analyses, an issue addressed generally by Rivera et al. (1992). The availability of data from the preschool period allows examination of characteristics of measured and nonmeasured subjects. Participants in both Atole and Fresco villages tended to be better off in terms of birthweight, physical growth, days ill with diarrhea, home diet and supplement intake. In two economic analyses (Alba 1992, Chung 1992), adjustments were made directly for sample selectivity as recommended by Heckman (1979), but these complex procedures have not been applied in the papers in this series.

Another design limitation of the follow-up study is that it picks up the sample after a hiatus of 11 y (i.e., from 1977, when the longitudinal study ended, to 1988, when the follow-up study began). In some areas the gap has been filled through review of records and through recall interviews or surveys. In this manner we have reconstructed the social and economic development of the communities (Bergeron, 1992), collected information on menarche (Khan et al. 1995) and reconstructed schooling histories (Pollitt et al. 1995). For variables such as diet, morbidity and growth, however, we have no direct measures for these 11 years. In other words, the analyses compare outcomes measured at adolescence or adulthood in Atole and Fresco subjects, often adjusting for covariates in early childhood but not for conditions after the supplementation program ended. This clearly is a limitation. However, socioeconomic status, a variable often included in the models, might be viewed as a proxy for health and diet during the unmeasured period. Also, the period from 7 y of age to adulthood does not appear to be a time of significant stress from the point of view of health and nutrition and omission of information for this period may not be critical in our case. For example, growth in height appears to be similar to that observed in a U.S. reference population beyond the first 3 y of life (Martorell et al. 1995b).

Findings from the follow-up study. What are the key findings from the follow-up study? Are these results internally consistent? Are they of an important magnitude? What potential significance do they have for programs and policies about nutrition? These are some of the questions explored below.

Patterns of growth in height. The follow-up sample was compared with a U.S. reference population and to Mexican-Americans (Martorell et al. 1995b). As young children, Guatemalans grow very poorly relative to both of these U.S. samples. Slightly different characterizations of growth during adolescence are obtained depending upon which is used as the reference, the Mexican-American sample, which is similar in racial ancestry but may not yet show unconstrained growth, or the general U.S. population, largely of European ancestry but probably exhibiting patterns reflective of unconstrained growth potential (i.e., not affected significantly by such factors as infections and dietary deficiencies). Differences in height between Mexican-Americans and the U.S. population are not large but are important. Whereas similar heights are observed up to ~12 y of age, differences begin to appear thereafter; as young adults Mexican Americans are 6 cm shorter, on average, which places them about the 25th percentile of the U.S. reference. Whether these differences reflect genetic or environmental causes is unclear.

Relative to the U.S. reference population, Guatemalans are below the 5th percentile as adults, just as they were as children. Absolute differences in height are slightly greater in adulthood than at 3 y of age (i.e., 13 cm as adults compared with 10–11 cm as children). Thus, growth failure would appear to be confined largely to early childhood. On the other hand, relative to Mexican-Americans, the growth failure of early childhood would appear to be reduced by about one half as a result of gains made up during adolescence. However, regardless of the choice of reference, adolescence, unlike early childhood, does not appear to be a period of constrained growth.

Body size and composition. Adolescents and adults in Atole villages (Cohort 2) were taller, weighed more and had greater fat-free masses compared with subjects in Fresco villages (Rivera et al. 1995). The differences seen at follow-up were shown to have been explained by differences already present at 3 y of age, which in turn have been shown to have been caused by the supplementation program (Habicht et al. 1995). There was some attenuation at adolescence of the differences in length observed at 3 y of age because subjects in Fresco villages grew slightly more from 3 y of age to the time they were measured in the follow-up study compared with subjects from Atole villages. Male subjects grew by 72.1 and 71.3 cm after 3 y of age in Fresco and Atole villages, respectively (difference = 0.8 cm) and by 65.0 and 64.2 cm in Fresco and Atole females, respectively (diff = 0.8 cm). Differences between Atole and Fresco subjects were greater in females than in males both at 3 y of age and at adolescence. At adolescence, the differences in height favoring Atole were 1.2 cm in males and 2.0 cm in females (compared with 2.0 and 2.8 cm, respectively, at 3 y of age) whereas differences in weight were 1.2 kg in males and 2.2 kg in females (compared with 0.8 and 1.2 kg, respectively, at 3 y of age). Atole subjects, particularly females, also had greater fat-free masses; the differences with respect to Fresco subjects being 0.8 and 2.0 kg in males and females, respectively. Thus, we can state that most of the gains achieved by the food supplementation program in early childhood were maintained at adolescence and adulthood.

Maturation. A review of the literature indicates that maturational delays will prolong the period of growth and lead to compensatory growth (Martorell et al. 1994). Thus, the slightly greater growth in height after 3 y of age (i.e., 0.8 cm) in Fresco villages may be due to minor differences in maturation with respect to Atole subjects. As children, subjects in Atole villages were more advanced than those in Fresco villages, as indicated by the greater number of centers of ossification recorded (Martorell et al. 1979) Differences in skeletal maturation at follow-up have been assessed by Pickett et al. (1995), using the Tanner and Whitehouse-2 method in subjects < 18 y (virtually all reached maturity after this age). In Cohort 1, the youngest age group (11–14 y of age), Atole girls were found to be 0.4 y more advanced than those of Fresco; the analyses for girls 14–18 y, corresponding roughly to Cohort 2, were disregarded because many in the sample were approaching skeletal maturity or already had reached it. No differences were found in skeletal maturation between boys in Atole and Fresco villages at either younger (Cohort 1) or older ages (Cohort 2).

Menarche data were collected through recall interviews in 1991 and 1992 in all former participants of the longitudinal study. In analyses that combined all cohorts, mean ages at menarche were 13.75 ± 1.22 y in Atole villages and 13.74 ± 1.36 y in Fresco (Khan et al. 1995). Restricting the analyses to Cohort 2 females showed nearly the same results (13.78 ± 1.28 y for both groups). Using data given in Khan et al. (1995) mean age at menarche for Cohort 1 girls [comparable to the 11–14-y group studied by Pickett et al. (1995)] is 13.45 y in Atole and 13.26 y in Fresco, values not significantly different from each other. The fact that values for Cohort 1 are lower than for Cohort 2 reflects a declining trend in menarche over time.

The skeletal age and menarche results indicate that the food supplementation program had little or no effect on maturation. The results in girls are contradictory (i.e., in Cohort 1, skeletal ages indicate Fresco girls are 0.4 y more retarded than those in Atole but mean ages at menarche are nearly identical in both groups) but the disparity is minor. It is also the case that the population as a whole (i.e., Atole and Fresco combined) does not seem to be markedly delayed. Menarche is perhaps a year or so delayed compared with data from developed countries (Eveleth and Tanner, 1990) and, relative to British children, there is a significant delay in skeletal age of 1.2 y in boys 11–14 y. In boys 14–18 y, skeletal ages differ by only 0.2 y, but this is not statistically significant. Finally, in girls 11–14 y, skeletal age was advanced by 0.2 y, but again, this minor difference was not statistically significant. As noted earlier, maturational delays could not be ascertained reliably in older girls (i.e., 14–18 y).

Thus, unlike some populations in Asia and Africa where the mean age at menarche may be as late as 15 or 16 y, our sample does not appear to be delayed significantly in maturation. Although reference data from a more appropriate group (i.e., upper class subjects from Guatemala City) may clarify the minor discrepancies observed between skeletal age and menarche data, it is unlikely that our conclusions that maturation is delayed at the most by ~1 y and that the food supplementation program had little or no effect on maturation are unlikely to change.

Work capacity. Work capacity in our study was assessed in a subsample as maximal oxygen consumption (VO_2 max). In subjects 14–19 y at follow-up (the age range corresponding to Cohort 2), VO_2 max was significantly greater in males in Atole villages (2.62 and 2.24 L/min for Atole and Fresco samples, respectively). Significant differences also were found, but to a lesser extent, in other cohorts (Cohort 1: 1.70 vs. 1.50; Cohort 3: 2.98 vs. 2.77 L/min; for Atole and Fresco subjects, respectively). Interestingly, the differences remained significant after controlling for body weight and fat-free mass. A dose-response relationship also was found in males in Cohort 2; greater Atole intake was associated with greater VO_2 max. Although Atole females had a higher VO_2 max values in all cohorts, differences with respect to Fresco subjects were less than observed in males and were statistically significant only in Cohort 1 (Cohort 1: 1.40 vs. 1.29; Cohort 2: 1.74 vs. 1.65; Cohort 3: 1.73 vs. 1.63 L/min in Atole and Fresco subjects, respectively).

Given the findings in Rivera et al. (1995) that fat-free mass differences between Atole and Fresco were greater in females than in males, one might have expected that VO_2 max differences between Atole and Fresco would also be greater in females. The reverse was actually found by Haas et al. (1995); the effect found in males was strong and statistically significant whereas that in females was weak for Cohorts 1 and 2 and statistically significant only for the former. One possibility to explain this difference is that the potential for a greater VO_2 max may be unrealized in females because of patterns of physical activity that are much less active than in males, this gender difference increasing with age (Novak et al. 1990). Another potential explanation is that the subsample measured in the work capacity study is not representative of the total sample; this issue was addressed by Haas et al. (1995) who reported no statistically significant differences between samples in supplement ingestion and anthropometric characteristics. However, the direction of the differences in weight and fat-free mass are interesting. Rivera et al. (1995) presented results for the full sample that showed that Atole females have significantly greater fat-free masses than females from Fresco. However, in the subsample, Haas et al. (1995) found that females in Fresco villages have similar weights and fat-free masses compared with subjects in Atole villages, despite being shorter. The reverse was the case in males; differences in weight and fat-free masses between Atole and Fresco subjects actually were greater in the subsample than in the full sample. These observations might appear to provide an explanation, but further analyses suggest otherwise. After controlling for fat-free mass, VO_2 max differences between Atole and Fresco villages continued to be significantly different for males but not females, suggesting that the gender difference in the effect of supplementation is independent of fat-free mass differences between village types. To date, we have no adequate explanation for these differences or for those discussed earlier between the sexes in the response to supplementation in height and fat-free mass.

Bone density. Caulfield et al. (1995) studied the relationship between the supplementation program and bone mineral content, bone width and bone mineral density in adolescence, aspects measured using single-photon absorptiometry at the distal radius. The study sample had less bone mineral content and bone density than German adolescents. Comparison between Atole and Fresco samples showed similar mean values in both sexes, except in females in the case of bone width, where the means were significantly greater for the Atole sample. These village type comparisons, however, did not control for potentially confounding factors. Also, the comparison included all cohorts and may have obscured stronger effects in subjects exposed to the nutrition intervention at particular ages during childhood. In other analyses that used regressions but that also combined all study cohorts, the authors showed that energy intakes from the supplement from birth to 7 y were related positively to the bone mineral outcomes. The relationships were still evident after controlling for age and gender as well as for type of supplement, but socioeconomic status was not included among the potentially confounding variables. When weight and height were included in the analyses, the relationship between energy intakes from supplement and bone density was attenuated and became nonsignificant. Thus, one view of the findings is that the relationship between supplement intake and bone mineral measures was mediated through increased body size. However, the lack of a demonstration of main effects (i.e., differences between Atole and Fresco subjects in mean measures of bone density) and the omission of socioeconomic status in the analyses suggests caution with this interpretation.

Intellectual performance. The effects of the supplementation program on behavioral outcomes were much more evident and consistent in adolescence than in the preschool period. A reanalysis of the 1969–77 data shows that subjects exposed to the Atole during gestation and the first 24 mo of life, when compared with those exposed to Fresco, had improved motor development scores at 2 y and higher scores as well at 4 and 5 y of age in a factor representing perceptual-organizational tests but not in another representing verbal ability tests (Pollitt et al. 1993). At the follow-up, when subjects were 13–19 y of age, Atole exposure was significantly related to tests of knowledge, numeracy, reading and vocabulary and, to a lesser extent, to information processing (Pollitt et al. 1993; Pollitt et al. 1995). No relationship was found on the other hand, in terms of intelligence, assessed with scales A, B and C of the Raven's Progressive Matrices. In older subjects exposed to supplement after 24 mo of age, the pattern of relationships was similar but the number and magnitude of significant associations was greatly reduced, suggesting that exposure before 24 mo was more beneficial. Interactions involving treatment were observed with socioeconomic status and maximum grade attained. Atole exposure, according to Pollitt et al. (1995), acted as a social equalizer, by erasing the relationship between socioeconomic status and performance that was observed in children exposed to Fresco. In terms of the interaction with maximum grade attained, Atole can be characterized as an enhancer of the educational returns to schooling. Atole exposure markedly improved the performance of those with more schooling but had little effect in those with only low levels of schooling.

The findings presented by Pollitt et al. (1995) controlled for sex, age, socioeconomic status, schooling (age at entry, maximum grade attained) and attendance (residual after regressing attendance on consumption). The inclusion of schooling variables in particular was quite important because a number of unadjusted comparisons favored Fresco subjects. Controlling for schooling is justified because of its importance as a determinant of performance and to control for the greater degree of schooling in Fresco villages, a characteristic that predates the beginning of the study in 1969 (Engle et al. 1992).

How can the relatively weak findings observed in the preschool period be reconciled with the more consistent and stronger effects in the follow-up? There is no easy explanation. In a sense, the outcome measures used during the longitudinal study and the follow-up study are not comparable. Unlike variables such as height, there is little assurance in the case of the psychological variables that one is measuring the same underlying aspect or function in young children and in adolescents. In our case, there were no concerted attempts to tap the same constructs at the two periods in life. The longitudinal study battery used adaptations of widely known tests of cognitive development (e.g., embedded figures, verbal inferences) as well as Piagetian concepts (e.g., conservation) whereas the follow-up assessment emphasized psychoeducational tests designed to identify potential to contribute to social and economic development. This led to the inclusion of tailor-made tests of general knowledge, reading and numeracy, all using locally relevant material. The Raven's Test of Intelligence, as noted earlier, was also used in the follow-up.

Pollitt et al. (1993) propose the hypothesis that the nutritional effects of the Atole are mediated through effects on body size, motor maturation and physical activity. For example, smaller children may be treated as if they were younger and low physical activity may limit interaction with the environment. Under this model, effects on intellectual performance are produced slowly through time, as children interact with their family, school and community. Greater effects would be expected in adolescence than in early childhood as, by then, full expression of the psychoeducational growth of subjects would be measurable.

A recent reviewer of these findings is somewhat skeptical of the results because he doubts it is possible to control adequately through confounding for the complexities of the human environment (Dobbing 1994). Astonishingly, he recommends animal experiments because they allow one "to control environmental factors and impose interventions in a structured manner" (p. 602). No doubt the context in which we grow and develop is complex, but animal experimentation, however enlightening it might be, can never be a substitute for human research precisely for this very reason.

Summary and significance of the key findings

The INCAP studies reviewed here provide information about the short- and long-term effects of a nutrition intervention carried out in early childhood. Much of the first part of this paper dealt with the nature of this intervention. Evidence was provided that showed that the supplementation program impacted significantly on energy and protein intakes, raising them by 10 and 40%, respectively, in children 15–36 mo of age, as well as on some other nutrients. Effects on growth were demonstrated, confirming that this was a biologically effective intervention; for example, the prevalence of severe stunting (i.e., >3 SD below the reference median) was reduced from ~45 to 20% in Atole villages but remained about the level of 45% in Fresco villages throughout the study.

These and other effects described (e.g., birthweight, infant mortality) are important from a public health point of view. At the same time, the limited nature of both the intervention and its effects must be recognized. The nutrition intervention was unable to improve the diets to a point where children met their nutritional needs and hence the effects observed can never be taken as a measure of the full potential of nutrition interventions. Although the Atole contributed significant amounts of many nutrients (e.g., protein, thiamin, riboflavin, niacin, vitamin A, etc.), it contributed very little of some other nutrients as Allen (1995) points out: "Notably, neither supplement contributed much zinc, which may be growth limiting in the population, or ascorbic acid, which might have improved iron absorption and subsequently growth, or vitamin B-6, which might have improved cognitive development." Energy needs, as suggested earlier, may not have been met fully in children consuming Atole.

Another important aspect is that the infectious load was not diminished except through vaccinations. Although a medical care program was provided, programs in environmental and personal hygiene were not carried out. Diarrheal diseases, therefore, remained common in Atole and Fresco villages, most likely limiting the utilization of the dietary improvements and reducing the magnitude of the effects observed.

It is no surprise, therefore, that despite important improvements in physical growth, the children born to mothers exposed to Atole and themselves exposed to Atole for the first 3 y of life, still exhibited a substantial degree of growth retardation. Children in Atole villages grew 2.5 cm more than children in Fresco villages and the latter, whether because of the small nutritional contributions of the Fresco and/or because of the medical care program, grew 0.5 cm more relative to baseline values. Thus, the total effect of Atole on growth may have been ~3 cm of length at 3 y of age. However, children measured during the baseline were ~12 cm shorter than reference children, meaning that the intervention erased only 25% of the original growth deficit observed at 3 y of age.

Other than unintended effects of the INCAP presence, there were no elements designed to foster psychological development other than through the nutrition intervention, as made explicit in the principal hypothesis. For this reason, many researchers have not been surprised by the small magnitude of the effects on mental development observed in the preschool children.

Although it did not meet the full nutritional needs of the children, the INCAP intervention was conducted carefully and its design permits one to document that important effects occurred on infant mortality, birthweight and postnatal physical growth. Programs that include more than just supplementation, such as actions to bring about better feeding and child care practices together with improvements in environmental sanitation, personal hygiene and infectious diseases, among others, if properly implemented and effective, should achieve more. In retrospect, the INCAP longitudinal study demonstrates the power of nutrition interventions in that so much was achieved by so little.

The INCAP longitudinal study provides strong justification for focusing nutrition interventions on mothers and young children. Growth retardation in Guatemala, as is the case in other areas as well (Allen, 1995), is largely confined to the first 3 or even just the first 2 y of life. Why then, spend resources monitoring the growth of children 3–5 y of age, as many programs do, when the real concern should be with younger children? The results about the differential effects of the supplementation by age are clear; supplementation had a demonstrable impact on growth in the first 2 or 3 y of life but not from 3–7 y. This also may be taken as an argument for targeting scarce resources to very young children.

The follow-up study showed that the effects on height remained at adolescence and adulthood, although attenuated. The differences observed between Atole and Fresco subjects in the follow-up were small, 1.2 cm in males and 2.0 cm in females. Still, the prevalence of short maternal stature was reduced significantly in Atole compared with Fresco villages. Using the commonly accepted cut-off point of <149 cm as a measure of obstetric risk, we find that 34% of women were very short in Atole villages compared with 49% in Fresco women (Martorell, 1993). Few women in the United States would be this short; for example, the 5th percentile in young women in the United States is 152 cm according to Frisancho (1990). The effects on fat-free mass were also greater for women than for men, 2.0 and 0.8 kg, respectively. These differences between women in Atole and Fresco villages are important, equivalent to ~0.5 SD units. Such improvements in body size and composition are likely to lead to improved reproductive outcomes, a subject under investigation in the villages.

Maturation during adolescence, whether measured in terms of skeletal maturation or age at menarche, was not significantly affected by the intervention. On the other hand, age at menarche was found to be associated with socioeconomic status and a downward trend through time was observed. These findings suggest that maturation is under environmental influence, possibly of a nutritional nature. However, we were unable to confirm the latter. Planners and policy makers who might be concerned by possible effects of nutrition interventions in early childhood on the timing of puberty will be reassured by our findings. Ongoing research is assessing possible effects of the intervention on fertility milestones (e.g., age at marriage or union, age at first birth) and should address more fully the demographic implications of the nutrition intervention.

Another important effect, this time more apparent in men than in women, was on work capacity. The effect on maximal oxygen consumption, viewed in terms of standard deviation units, was large, equivalent to 0.7 SD. Differences between Atole and Fresco males remained significant even after controlling for fat-free mass, suggesting qualitative differences in tissue composition and function. One would expect from the literature that improvements in work capacity would result in increased productivity and incomes. However, Chung (1992) failed to demonstrate that work capacity resulted in higher wages. Several design and methodological problems may account for this. Also, few of the subjects were wage earners and instead most contributed labor to family agricultural activities; lack of adequate data on the labor inputs made by individuals and poor measures of household production made inclusion of nonwage earners difficult and resulted in small sample sizes. One problem was the young age of the subjects, many of them unmarried adolescents and young men when the data were collected in 1988–89 (the range in age was 11–26 y). Thus, better data on economic productivity collected when the sample settles into more permanent occupations and jobs a few years from now are needed to test the hypothesis that improvements in work capacity led to increased incomes.

The findings on intellectual development are surprising as noted earlier because of the larger magnitude observed in adolescence than in childhood. Using a composite variable (i.e., a factor score combining literacy, numeracy, general knowledge, Raven's Progressive Matrices, reading and vocabulary), the Atole-Fresco differences can be estimated as ~0.6 SD (compared with <0.2 SD in the preschool period). However, it is not the main effects but the interactions that should be emphasized; to repeat, these indicate that the nutrition intervention attenuated the negative relationship between socioeconomic status and performance and that it increased the educational returns to schooling. Schooling and intellectual capital, according to the literature, results in improved wages. Alba (1992), facing the same methodological difficulties described above, was nonetheless able to confirm that this was also the case for the study sample of wage earners. The additional rate of return to an additional year in school was found to be ~6%; weaker relationships were found when skills, such as vocabulary and numeracy, were used as measures of educational capital instead of years of school. Again, this and other questions concerning productivity and incomes are best addressed through better data collection.

In summary, the follow-up study has shown that nutrition interventions in pregnancy and early childhood culminate in the adolescent and adult in improvements in body size and composition and in intellectual performance. We were unable to link these improvements convincingly to economic productivity largely because of methodological reasons, but expect that these may be discerned later with better data. This means that advocates of nutrition programs aimed at mothers and children have an additional argument for them, namely that they improve the physical and intellectual endowment of adults. They also may refer to the reasonable but yet unproven hypothesis that these improvements in human capital will result in enhanced economic productivity. No doubt future extensions of our studies will provide a more comprehensive assessment of the value of nutrition programs for mothers and young children. Enough is known, however, to give these actions the highest priority, no matter how poor or rich the country may be.

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