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 (VO_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 VO_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 VO_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

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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

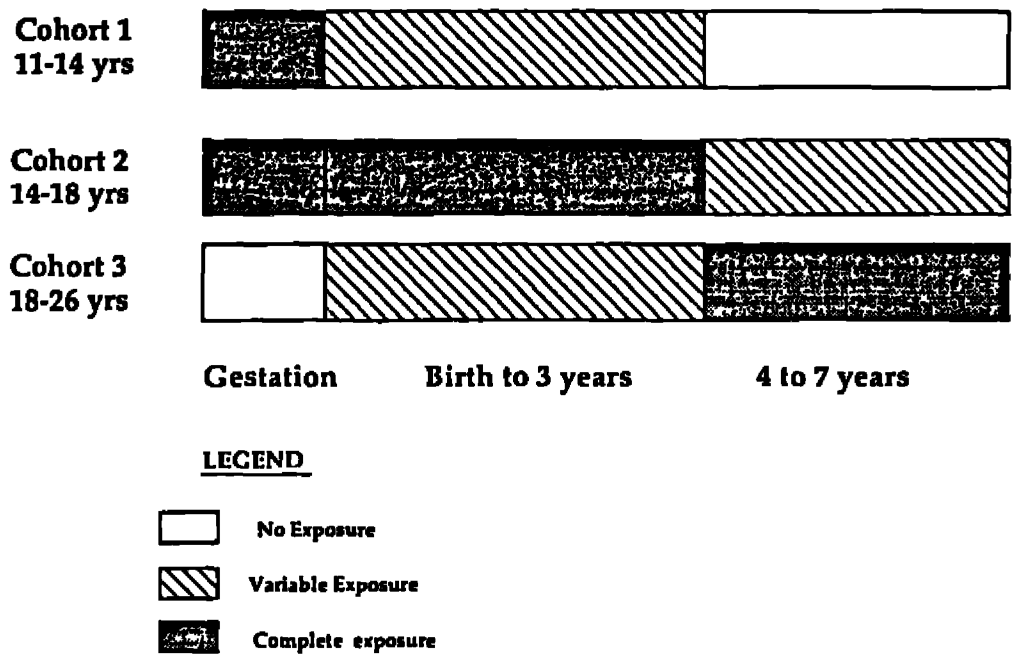

FIGURE 1 Age at exposure to supplement by cohort.

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/m²*			
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/m²*			
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 · 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 · 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 · 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 · 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, age², 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 β	Model 1 P	Model 2 β	Model 2 P	Model 3 β	Model 3 P	Model 4 β	Model 4 P	Model 5 β	Model 5 P	Model 6 β	Model 6 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

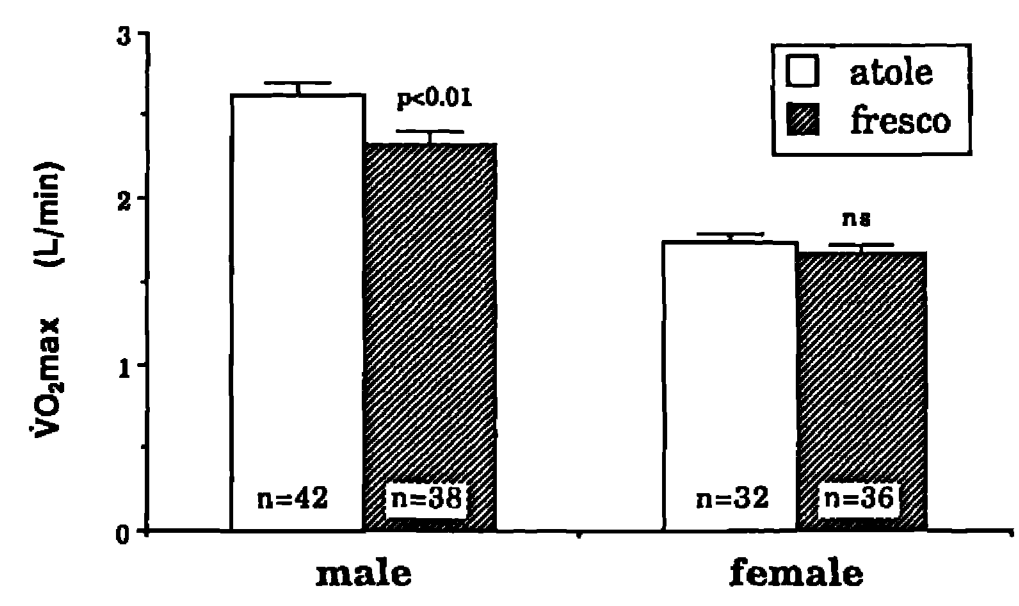

FIGURE 2 Mean $\dot{V}O_2$ max (L/min) of Cohort 2 subjects from Atole and Fresco villages. Statistically significant supplement type effect is seen only in males after controlling for age, SES, village size and level of participation in the supplementation.

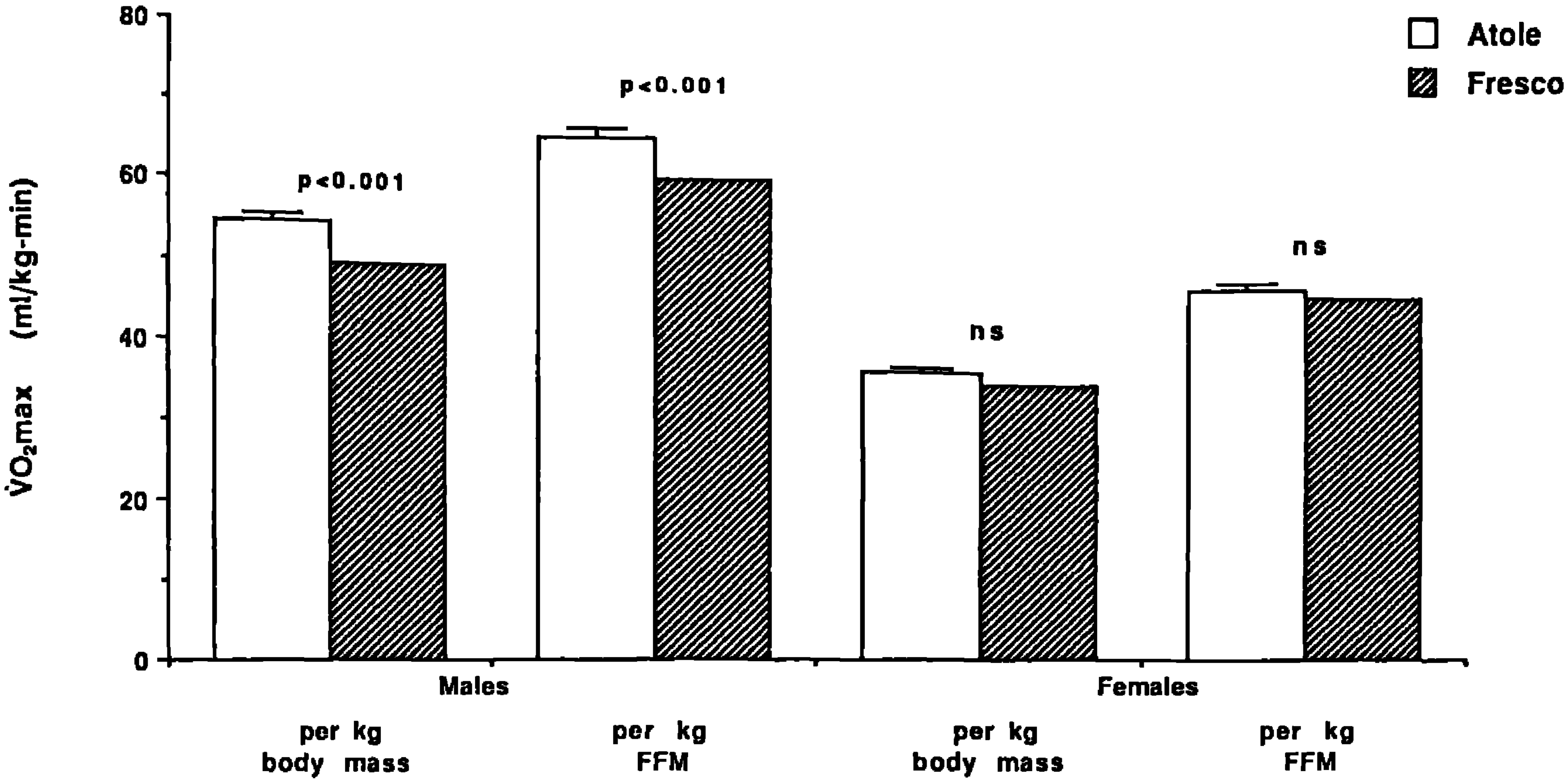

FIGURE 3 Mean maximum aerobic power (oxygen consumption per body mass and per FFM) of Cohort 2 subjects from Atole and Fresco villages. A statistically significant supplement type effect is seen in males after controlling for the same variables reported in Figure 2.

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 $\dot{V}O_2$max 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 $\dot{V}O_2$max 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 $\dot{V}O_2$max 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 $\dot{V}O_2$max. 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 $\dot{V}O_2$max 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' $\dot{V}O_2$max 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 $\dot{V}O_2$max 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-

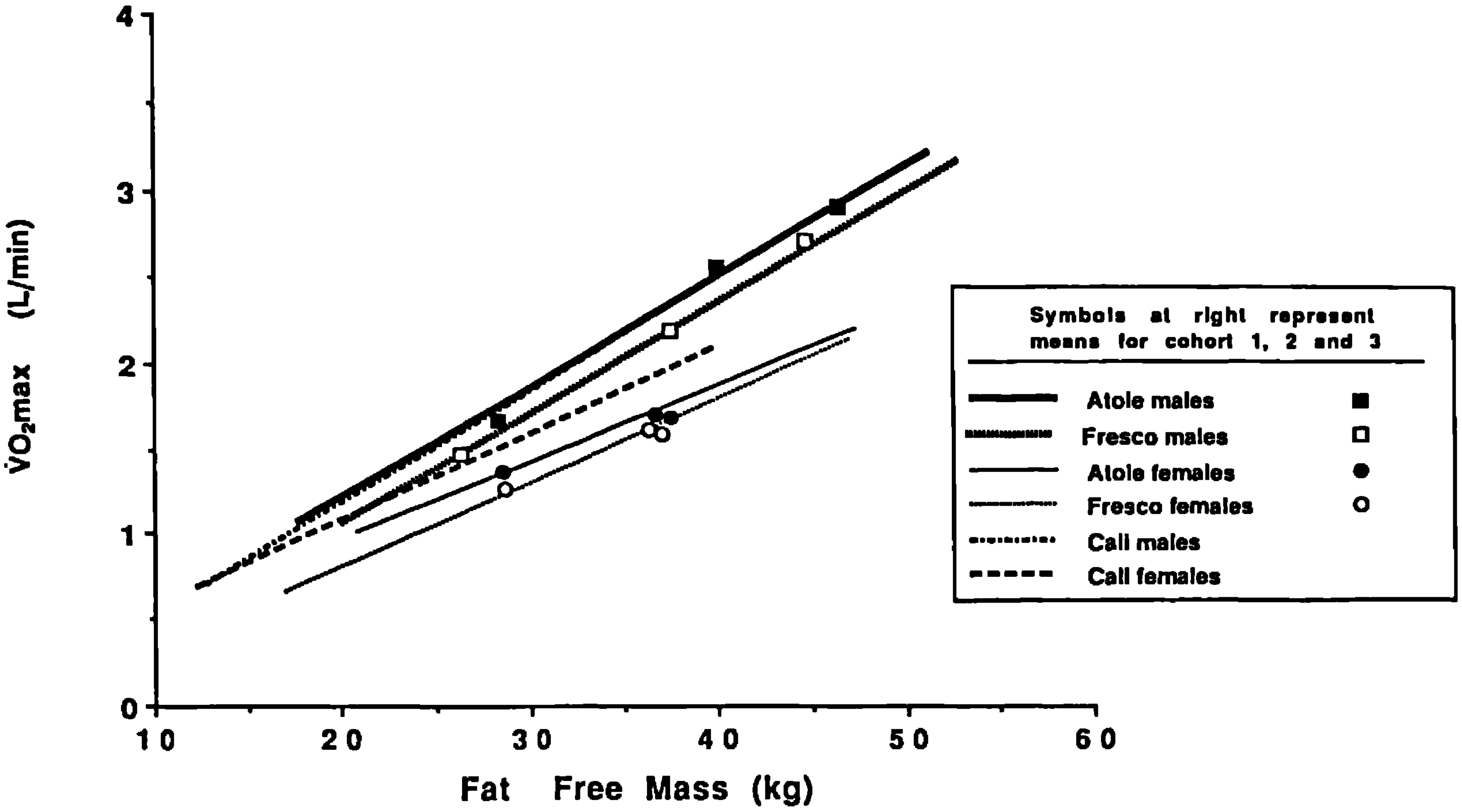

FIGURE 4 Relationship between maximum oxygen consumption ($\dot{V}O_2$ max) and fat-free mass (FFM) for subjects from all cohorts. Thickest and thinnest lines represent regression equations for each sex and nutrition group of Guatemalan subjects and corresponding symbols represent means for each of three 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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