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# Micronutrient Supplementation Improves Physical Performance Measures in Asian Indian School-Age Children[1–4]

Mario Vaz,[5,6]* Maria Pauline,[5,6] Uma S. Unni,[5] Panam Parikh,[7] Tinku Thomas,[5] A.V. Bharathi,[5] Sandhya Avadhany,[6] Sumithra Muthayya,[5] Ruchika Mehra,[7] and Anura V. Kurpad[5,6]

[5]St John's Research Institute, St John's National Academy of Health Sciences, Bangalore, India; [6]Department of Physiology, St John's Medical College, Bangalore, India; and [7]GlaxoSmithKline Consumer Healthcare Ltd, Gurgaon, India

## Abstract

Micronutrients are important in physical work capacity and therefore performance. The impact of a multi-micronutrient–fortified nutritional beverage on physical performance measures among clinically healthy school-age children was assessed in a double-blind (for test and placebo groups), placebo-controlled, randomized trial in children aged between 7 and 10.5 y ($n = 300$). The participants with height- and weight-for-age Z-scores between 0 and $\geq -3$ were randomized to 1 of 3 study arms: fortified choco-malt beverage powder (F), matched energy equivalent unfortified placebo (U), and untreated control (C). Participants in the F and C groups were given 40 g fortified (19 key vitamins and minerals) and unfortified choco-malt beverage, respectively, daily for 120 d. Primary efficacy outcomes included endurance and aerobic capacity using a 20-m shuttle test and step test. Other physical performance measures included speed (40-m sprint), visual reaction time, maximal hand grip, and forearm static endurance. Micronutrient status included thiamin, riboflavin, folate, niacin, iron, pyridoxal phosphate, and vitamins B-12 and C. All measurements were made at baseline and the end of the intervention. There was a within-subject increase in aerobic capacity and whole body endurance ($P < 0.05$) accompanied by a significant improvement in the status of iron thiamin, riboflavin, pyridoxal phosphate, folate, and vitamins C and B-12 in the F group compared to the within-subject changes in the other 2 groups ($P < 0.05$). The study suggests that multiple micronutrient supplementation in similar populations may be beneficial in improving micronutrient status and enhancing aerobic capacity and endurance in children. J. Nutr. 141: 2017–2023, 2011.

## Introduction

Childhood undernutrition continues to be a major problem in the developing world and there has been considerable attention given to ways that optimal growth can be achieved (1,2). Although micronutrient deficiencies, particularly those related to anemia and deficiencies of vitamin A, zinc, and iodine have received considerable global attention, less is known about the extent of other micronutrient deficiencies in children and their potential consequences (3,4). The water-soluble B vitamins and several other micronutrients such as iron, magnesium, zinc, and other minerals have been implicated in physical performance (5–7). At a biochemical level, this is explained on the basis of their participation in energy-yielding pathways and indirectly in oxygen carriage through the synthesis of Hb and as regulatory factors in erythropoiesis. Additionally, vitamins A, C, and E have antioxidant properties and may reduce muscle damage associated with high muscle usage (5–7). Experimental evidence for the potential role of micronutrients in physical performance has emerged from micronutrient restriction studies that have indicated that a reduction in the dietary intake of thiamin, riboflavin, and vitamins B-6 and C to about one-third of the RDA is associated with an ~10% reduction in aerobic capacity in young male adults (8). Conversely, there appears to be no strong evidence that supplementation of micronutrients in micronutrient-replete individuals enhances physical performance (9,10).

Relatively few studies have focused on the relationship between nutrition and physical performance in children. Although physical performance measures are less well studied in Indian children, there are well-known fitness batteries such as EUROFIT in Europe and FITNESSGRAM in the USA that have been used in large-scale studies to assess physical performance in children and adolescents (11–13). Given the high prevalence of childhood undernutrition in India and that of multiple micronutrient deficiencies in Indian children, micronutrient supplementation may be beneficial for physical performance in

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[3] This trial was registered at clinicaltrials.gov as NCT00876018.
[4] Supplemental Tables 1 and 2 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
* To whom correspondence should be addressed. E-mail: mariovaz@sjri.res.in.


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micronutrient-deficient participants (14,15). We therefore performed a randomized controlled trial to determine a within-participant change in physical performance measures in response to multiple micronutrient supplementation in nutritionally compromised but clinically healthy Indian children.

## Methods

*Participants and study area.* Apparently healthy boys and girls between the ages of 7 and 10.5 y with a Z-score of 0 to ≥ −3 SD for height-for-age and weight-for-age were recruited from 3 schools in Bangalore, India (eligible $n$ = 1155; screened $n$ = 379; enrolled $n$ = 300) (16). The participants belonged to the 3 middle socio-economic groups (i.e., upper lower, 236; lower middle, 56; and upper middle, 8) based on the modified Kuppuswamy score (17). The participants were randomized to 3 groups: fortified (F)[8], unfortified (U), and control (C). A total of 287 participants (F = 95, U = 95, C = 97) completed the study. All participants randomized were considered for baseline analysis and those who completed the study were considered for the endpoint comparison. Of the 13 participants who did not complete the study, 2 were lost to follow-up and 11 withdrew consent (F = 5, U = 4, C = 2) (**Supplemental Fig. 1**). Participants were included in the study if there was written informed consent from the parents/legal guardians and the participants gave a written assent. Participants were excluded if they suffered from severe anemia (Hb $<80\ g \cdot L^{-1}$), cardiovascular or respiratory disease, physical disability, recent history of serious infections, or surgery or injuries that would impair their ability to perform the study tests. Participants were also excluded if they were already taking nutritional supplements, if they had participated in a nutrition intervention study in the preceding year, or if they were family members of staff employed at the study site or with the sponsor.

*Study design.* This study was designed as a randomized, double-blind (for F and U groups), placebo-controlled, supplementation trial with the children allocated to 1 of 3 study arms. The test group (F) was given a fortified choco-malt beverage powder, one of the control groups (U) was administered an energy equivalent unfortified choco-malt beverage powder, and the second control group received no intervention (C). The primary objective was to determine the within-participant change in physical performance measures in the participants in group F compared to group U and group C over a 4-mo period. The protocol was approved by the Institutional Ethics Review Board of St Johns' Medical College. The participants assented to the study and their parents or legal guardians gave signed informed consent that was translated to the local language.

*Assignment of participants to study groups.* The block randomization technique was employed to generate 20 blocks (10 each of girls and boys to ensure equal gender distribution) each of size 15. The participants in each block were individually randomized to 1 of the 3 treatment groups based on a computer-generated randomization sequence. The computer-generated sequence of randomization with study arm allocation was restricted to a single person (T.T.). The sponsor retained the codes for the product (F and U groups) and a copy was kept with a faculty member not involved with the study at the site in the event of an emergency. These codes were broken once all biochemical assessments (except thiamin and niacin) were completed and after database lock. Statistical analyses were done for the primary outcomes and nutritional biochemistry (except thiamin and niacin) with the codes intact. The biochemist (U.U.) was unaware of the codes until the completion of the thiamin and niacin assays.

*Intervention and administration.* The participants in the F and U groups received 40 g of the nutritional beverage powder in clean water daily for a period of 120 d. This provided ~158 kcal (662 kJ), 3.2 g protein, and 1.4 g fat. The fortified product additionally provided 19 key micronutrients (**Table 1**). The treatments were administered under supervision during school days. During weekends and holidays, in so far as possible, home visits were made to supervise or record compliance to treatment.

*Assessments.* The following assessments, standardized prior to use, were administered by trained personnel at baseline and the end of the intervention.

*Physical performance.* The primary outcome measures were whole body endurance, aerobic capacity, speed, and visual reaction time. Secondary outcome measures were nutritional status, muscle strength, and endurance in the forearm flexor muscle group.

Whole body endurance and aerobic capacity were measured using a 20-m shuttle test and a step test. The 20-m shuttle test was performed in groups of between 3 to 5 participants to improve motivation and maximize performance. The test started with an initial speed of 4 $km \cdot h^{-1}$ ($1.11\ m \cdot s^{-1}$) and increased by 0.5 $km \cdot h^{-1}$ ($0.14\ m \cdot s^{-1}$) every minute until exhaustion or when 2 consecutive markers were not reached within the stipulated time. Estimated $VO_2$ peak (peak oxygen uptake) from the 20-m shuttle was calculated using multiple prediction equations (11,18–20). There are no published data to our knowledge on the validation of 20-m shuttle or step-derived $VO_2$ max (maximal oxygen uptake) in Asian Indian children. An externally paced single-step test (30.48 cm step at 22 steps $\cdot\ min^{-1}$) for 3 min was performed. Heart rate/pulse rate was measured manually within 15 s of completing the step test and $VO_2$ max was estimated (11).

Speed was measured by a 40-m sprint in groups of 3–5 participants with the time to completion of the race recorded manually using a digital stop watch (Racer). Visual reaction time (based on the best of 3 tests) was assessed using a customized computer-based program with target images randomly interspersed between nontarget images. Children were asked to place their hands on either side of the computer and strike the space bar as fast as possible when a target image was seen. Maximal handgrip (as the best of 3 values using a Jamar hydraulic hand dynamometer, SI Instruments) and static endurance of forearm flexors were measured on the nondominant forearm rested at right angles on a table to prevent weight bearing. The grip width was determined for each participant based on individual comfort and kept constant pre- and postintervention. As a field test, static endurance of the forearm flexors (also using the

**TABLE 1** Micronutrient composition of the nutritional beverage[1]

| Nutrient | *unit/40 g* |
|---|---|
| Vitamin A, *μg* | 250 |
| Thiamin, *mg* | 1.1 |
| Riboflavin, *mg* | 1.1 |
| Vitamin B-6, *mg* | 1.3 |
| Vitamin B-12, *μg* | 2.3 |
| Niacin, *mg* | 12 |
| Folate, *μg* | 225 |
| Biotin, *μg* | 10 |
| Pantothenic acid, *mg* | 2 |
| Vitamin C, *mg* | 75.2 |
| Vitamin D, *μg* | 1.3 |
| Calcium, *mg* | 231 |
| Copper, *μg* | 350 |
| Iodine, *μg* | 79.2 |
| Iron, *mg* | 17.8 |
| Magnesium, *mg* | 33.0 |
| Zinc, *mg* | 1.8 |

[1] Content as provided by the manufacturer: GlaxoSmithKline Consumer Healthcare. Other information is proprietary.

[8] Abbreviations used: C, untreated control; F, fortified or test group; Hb, hemoglobin; MET, metabolic equivalent of task; sTfR, soluble transferrin receptor; U, unfortified or placebo group; $VO_2$ max, maximal oxygen uptake; $VO_2$ peak, peak oxygen uptake.

**TABLE 2** Characteristics of the participants at baseline in the C, F, and U groups[1]

| | C | F | U |
|---|---|---|---|
| Age, *y* | 8.29 ± 1.04 | 8.26 ± 1.02 | 8.18 ± 1.01 |
| Socioeconomic score | 8.98 ± 2.43 | 8.78 ± 2.86 | 8.78 ± 2.74 |
| Height, *cm* | 122.3 ± 6.1 | 123.1 ± 7.2 | 122.3 ± 6.1 |
| Weight, *kg* | 21.5 ± 3 | 21.9 ± 3.4 | 21.2 ± 3 |
| Body fat, *%* | 13.1 ± 3.6 | 13.3 ± 3.5 | 12.9 ± 3.5 |
| Energy intake,[2] $kJ \cdot d^{-1}$ | 5150 ± 1660 | 5000 ± 1680 | 4770 ± 1650 |
| Protein intake,[2] $g \cdot d^{-1}$ | 33 ± 10 | 27 ± 9 | 31 ± 11 |
| Fat intake,[2] $g \cdot d^{-1}$ | 32 ± 16 | 23 ± 13 | 28 ± 14 |
| Carbohydrate intake,[2] $g \cdot d^{-1}$ | 203 ± 65 | 172 ± 52 | 190 ± 69 |
| Moderate to vigorous physical activity, $MET \cdot min \cdot wk^{-1}$ | 1970 ± 1100 | 1980 ± 1290 | 1860 ± 1590 |
| Sedentary activity, $MET \cdot min \cdot wk^{-1}$ | 1680 ± 680 | 1590 ± 586 | 1680 ± 745 |

[1] Values are mean ± SD, $n = 100$. C, control group; F, fortified group; U, unfortified group.
[2] $n = 98$ in F.

Jamar handgrip dynamometer) was measured as the time taken for the handgrip strength to fall to 50% of its starting value as determined visually by the investigators. The time was assessed manually using a digital stop watch.

*Anthropometry.* Height was recorded to the nearest 0.1 cm using a calibrated, locally made stadiometer and weight to the nearest 100 g in standard school clothing without footwear (Salter Digital Weighing Scale). This was typically a cotton shirt and shorts for the boys and a cotton blouse and skirt for the girls. Mid-upper arm circumference and calf circumference were recorded to the nearest 0.1 cm and triceps and calf skinfolds to the nearest 0.2 cm (Holtain) were measured. Percent body fat was computed using a gender-specific prediction equation using the triceps and calf skinfolds (21).

*Biochemical assessment.* Nonfasting blood samples were collected by venipuncture from the participants and plasma and serum stored and analyzed for micronutrient markers at St John's Research Institute. The samples collected at both time points were analyzed in the same batch except for Hb, which had an intra and inter-assay CV% <2. Hb was analyzed by spectrophotometric measurement of cyanmethemoglobin on the ABX Pentra 60C+ (Horiba Medical). Plasma ferritin was measured by electro-chemiluminescence method using the principle of sandwich on Elecsys 2010 (Roche Diagnostics Mannheim). Plasma sTfR and serum CRP were measured by particle-enhanced immunoturbidimetric method on the Roche/Hitachi 902 (Roche Diagnostics Mannheim). Plasma sTfR values were corrected to obtain data comparable to the Ramco sTfR assay (22). The intra-assay CV% for the trilevel level controls for ferritin and bilevel controls for sTfR and CRP were 4.0, 2.9, 3.7; 4.2, 4.1; 4.4, 3.0 and inter-assay CV% were 4.9, 5.1, 2.9; 7.1, 5.0; 4.4, 3.1; respectively.

RBC riboflavin was measured by the modified erythrocyte glutathione reductase coefficient method (23–25). Plasma pyridoxal phosphate, the main cofactor form of vitamin B-6, was separated by reverse-phase HPLC using the principle of ion exchange and detected by fluorescence detector (Shimadzu). Total serum vitamin C was assayed using a modification of the Roe and Kuetherl method (26). RBC riboflavin, plasma pyridoxal phosphate, and serum vitamin C had an intra-assay CV of 3.0, 9.5, and 7.3 and inter-assay CV of 3.6, 13.4, and 10.4, respectively. RBC folate and plasma vitamin B-12 were measured by the electro-chemiluminescence method using the principle of competition on Elecsys 2010 (Roche Diagnostics Mannheim). The intra-assay CV% for the trilevel controls for RBC folate and vitamin B-12 were 2.6, 2.4, and 1.7; and 4.6, 4.2, and 2.8, respectively, and the inter-assay CV% were 6.4, 5.1, and 5.8; and 6.8, 5.3, and 3.2, respectively. RBC thiamin diphosphate, which forms 90% of thiamin, was extracted from the erythrocytes and oxidized to thiochromes. This was separated on a reverse-phase HPLC column using a step gradient of methanol and phosphate buffer and detected by a fluorescence detector (Shimadzu) (27). The niacin number was calculated as the ratio of NAD:NADP in whole blood samples × 100. NAD and NADP were extracted from the sample and measured using the method of Jacobson and Jacobson (28). RBC thiamin and niacin had an intra-assay CV of 3.2 and 9.6 and inter-assay CV of 3.2 and 8.9, respectively.

*Diet and physical activity.* Diet and physical activity information was recorded using standardized questionnaires at baseline and at the end of the intervention period. In addition, data were collected at 2 mo on available participants to monitor any change in the pattern and quality of the diet or physical activity during the intervention period, but these 2-mo data were not used in the final analyses. Dietary intake was obtained primarily from a 24-h recall questionnaire using standard food measures during the interview at the baseline to facilitate the recall of portion size. Nutrient composition of the diet was estimated using food composition tables as described in previous papers (29,30). In addition, a 30-item FFQ was also administered at baseline and the end of the intervention to capture the frequency of consumption of a variety of food items (31).

The physical activity questionnaire aimed to capture reported duration and type of activity during the day at school or at home, such as transport used to travel to school, activities during physical education periods, recess time, household chores, and weekend activities, etc. Other activities such as tutoring at home, and television viewing were also recorded. This study used a modified version of the physical activity questionnaire previously used in an Indian school study (31). MET were assigned for all activities that were reported (32,33). Because physical performance measures in children are best correlated with moderate to vigorous activity, this component was calculated by extracting all moderate to vigorous activities reported using a MET cutoff ≥3. Activities below a MET of 3 were used for sedentary and light activity.

*Statistics.* A minimum sample size of 94 for each group was calculated to allow for a mean difference of 10% in estimated aerobic capacity between groups while allowing for all relevant between-group comparisons at an $\alpha$ level of 1% with a power of 80%. Descriptive statistics (mean ± SD) are presented for baseline, end of intervention, and change from baseline for normally distributed data. Between-group comparisons at baseline for normally distributed data were done using ANOVA. For data that were not normally distributed, a Kruskal-Wallis test was done and data reported as median (25th, 75th percentiles).

All participants randomized into the study and having completed 2 measurements (baseline and end of study) were included for analysis of time point comparison. Efficacy of the intervention was tested using 2 independent sample *t* tests to compare the change (end of intervention − baseline) between the F group and U or C group; the U and C groups were not compared to one another. Data are presented as means and SD for normally distributed parameters and median (1st quartile, 3rd quartile) for non-normal data. Delta values that were not normally distributed were compared between groups using the Mann-Whitney U test. For variables with differences at baseline, ANCOVA was per-

**TABLE 3** Physical performance measures at baseline and change with intervention in the C, F, and U groups[1]

| | C | F | U |
|---|---|---|---|
| 20-m shuttle (laps) | | | |
| Baseline | 50 (44, 58)[b] | 48 (41, 55)[a] | 52 (44, 60)[b] |
| Change[2] | 4 (−5, 13.5)* | 12 (0, 21) | 4 (−6, 12)* |
| 20-m shuttle [predicted $VO_2$ peak (11)], $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 32.4 (29.1, 35.6) | 32.2 (29.1, 34.6) | 33.2 (29.7, 35.9) |
| Change | 5.8 (3.9, 8.3)* | 7.3 (5, 10) | 3.4 (3.7, 7.8)* |
| 20-m shuttle [predicted $VO_2$ peak (18)], $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 43.7 (40.3, 46.1)[b] | 42.3 (38.7, 44.9)[a] | 43.8 (40.5, 46.4)[b] |
| Change | 7.9 (5.3, 11.2)* | 10.2 (6.8, 13.9) | 8.2 (5.2, 10.8)* |
| 20-m shuttle [predicted $VO_2$ peak (prediction equation a) (19)], $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 46.7 (42.8, 50.3) | 45.7 (42.3, 49.5) | 46.5 (43.2, 50.5) |
| Change | 5.5 (3.8, 7.9)* | 7.2 (4.8, 9.4) | 5.7 (3.5, 7.4)* |
| 20-m shuttle [predicted $VO_2$ peak (prediction equation b) (19)], $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 43.7 (40.9, 47.3) | 43.2 (39.9, 45.7) | 43.8 (41.2, 47.5) |
| Change | 5.9 (3.9, 8.4)* | 7.5 (5.1, 10.2) | 6.2 (3.7, 7.9)* |
| 20-m shuttle [predicted $VO_2$ peak (20)], $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 44.1 (41.2, 46.9)[b] | 43.2 (40.8, 45.9)[a] | 44 (41.8, 47.6)[b] |
| Change | 7 (4.9, 10.1)* | 9.3 (5.8, 11.7) | 7.2 (4.2, 9.5)* |
| Step test (predicted $VO_2$ max), $mL \cdot kg^{-1} \cdot min^{-1}$ | | | |
| Baseline | 35.9 (33.3, 40.4) | 35.9 (33.3, 39.5) | 37.2 (33.3, 41.9) |
| Change | 1.18 (−4.1, 5.1) | 2.3 (−2.1, 5.8) | 0 (−4.4, 3.4)* |
| 40-m sprint, *s* | | | |
| Baseline | 8.8 (8.3, 9.5) | 8.9 (8, 9.6) | 9.1 (8.2, 9.6) |
| Change | −0.2 (−1.0, 0.3) | −0.4 (−0.7, 0.2) | −0.2 (−0.7, 0.5) |
| Visual reaction time, *ms* | | | |
| Baseline | 946 (797, 1104) | 938 (832, 1094) | 922 (801, 1016) |
| Change | −63 (−249, 71) | −78 (−218, 17) | −47 (−141, 32) |
| Handgrip – nondominant, *kg force* | | | |
| Baseline | 8.0 (6, 10) | 8 (6, 11) | 8 (6, 10) |
| Change | 1 (0, 2) | 1 (−1, 3) | 0 (−1, 2) |
| Static endurance (time to 50% of maximal force), *s* | | | |
| Baseline | 7.1 (6.5, 14) | 10.6 (6.9, 15.4) | 10.2 (6.7, 16.4) |
| Change | 2.7 (−1, 7.3) | 2.4 (−1.5, 7.3) | 2.1 (−2.6, 8.8) |

[1] Values are median (Q1, Q3), $n$ = 100 (baseline), or 95–97 (change). *Different from F, $P < 0.05$. Labeled baseline medians with superscripts without a common letter differ, $P < 0.05$. C, control group; F, fortified group; U, unfortified group.
[2] Change: final (postintervention) − baseline.

formed for end-of-intervention values, keeping treatment as a fixed effect and the baseline value as the covariate. Percentage differences between groups were examined using a chi-square test. An $\alpha$ level of <0.025 was considered significant for the delta change analysis to allow for multiple comparisons and 0.05 level for all other analyses. All analyses were performed using SPSS version 13.0.

## Results

*Baseline characteristics.* The 3 arms were balanced for socioeconomic strata, age, gender, anthropometric status, dietary intakes, and physical activity patterns (**Table 2**). The physical performance measures were comparable across groups, with the exception of the number of shuttles in the 20-m shuttle test and the corresponding $VO_2$ peak. The number of shuttles run at baseline was ~4 fewer in the F group compared to the C or U group ($P < 0.05$) (**Table 3**). The 3 groups had comparable micronutrient status with 2 exceptions: ferritin in the F group was significantly greater than in the U group and riboflavin in the C group was greater than in the U group (**Table 4**). Overall, micronutrient deficiency was 88 and 17% for riboflavin and vitamin B-12, respectively. The percentage of ferritin deficiency was 21, 17, and 32 for the C, F, and U groups, respectively, and deficiency was greater in the U group compared to the F group ($P = 0.02$) (Supplemental Table 1).

*Effect of the intervention.* At the end of the study, 93 and 92% of the treatments were administered to the F and U groups, respectively, under supervision. Following the intervention, there was an increase from baseline to endpoint in the number of shuttles run in all study arms. However, the increase in the number of shuttles in the F group was greater than in the U ($P = 0.001$) or C ($P = 0.005$) groups (Table 3). This increase remained significant after adjusting for baseline values ($P < 0.02$). Regardless of the prediction equation used, the $VO_2$ peak/max derived from the 20-m shuttle test was also significantly greater in the F group compared to both the U and C groups. The increase from baseline to endpoint in $VO_2$ max derived from the step test differed between the F and U groups ($P = 0.004$) but not between the F and C groups ($P = 0.15$). The change in other

**TABLE 4** Baseline and postintervention biochemical status in the C, F, and U groups[1]

| Variables (*unit*) | C | F | U |
|---|---|---|---|
| RBC riboflavin, (*coefficient*) | | | |
| Baseline | 1.57 (1.48, 1.70)[b] | 1.56 (1.47, 1.64)[b] | 1.52 (1.43, 1.61)[a] |
| Change[2] | 0 (−0.08, 0.07)* | −0.32 (−0.41, -0.26)* | −0.04 (−0.10, 0)* |
| Plasma pyridoxal phosphate,[1] $nmol \cdot L^{-1}$ | | | |
| Baseline | 39.3 (21.8, 52.0) | 33.8 (20.7, 50.3) | 34.3 (22.4, 50.7) |
| Change | 2.5 (−11.0, 19.6)* | 24.0 (6.7, 45.0) | 4.7 (−5.4, 16.1)* |
| Plasma vitamin B-12, $pmol \cdot L^{-1}$ | | | |
| Baseline | 224 (164, 270) | 210 (167, 286) | 213 (163, 297) |
| Change | −10 (−39, 15)* | 211(165, 266) | 5(−26, 29)* |
| RBC folate, $nmol \cdot L^{-1}$ | | | |
| Baseline | 1345 (1158, 1546) | 1323 (1207, 1517) | 1326 (1235, 1459) |
| Change | −22 (−128, 110)* | 900 (650, 1126) | −30 (−112, 91)* |
| RBC thiamin,[1] $nmol \cdot L^{-1}$ | | | |
| Baseline | 88.0 (68.0, 106.6) | 81.6 (64.7, 105.2) | 85.8 (61.6, 105.7) |
| Change | 0.9 (−17.7, 13.0)* | 11.4 (−4.2, 27.8) | 1.4 (−11.5, 16.1)* |
| Niacin,[1] *n* | | | |
| Baseline | 82.6 (64.5, 98.6) | 81.6 (66.3, 103.4) | 86.0 (68.6, 101.1) |
| Change | −2.5 (−13.7, 7.6)* | 2.6 (−4.0, 14.2) | −0.1 (−11.5, 11.4) |
| Serum vitamin C,[1] $\mu mol \cdot L^{-1}$ | | | |
| Baseline | 41.1 (26.8, 70.7) | 39.1(24.2, 58.8) | 35.2 (23.3, 56.1) |
| Change | 5.0 (−25.8, 25.3)* | 47.1(27.3, 61.9) | 7.2 (−18.9, 25.6)* |
| Plasma ferritin,[3,4] $pmol \cdot L^{-1}$ | | | |
| Baseline | 55.0 (37.9, 89.4)[ab] | 62.0 (38.9, 102.1)[b] | 45.3 (29.5, 65.1)[a] |
| Change | 0.4 (−12.2, 14.9)* | 21.0 (5.4, 39.6) | 4.4 (−2.6, 20.0)* |
| Serum CRP, $nmol \cdot L^{-1}$ | | | |
| Baseline | 3.81 (0, 7.62) | 1.90 (0, 6.67) | 2.86 (0, 7.43) |
| Change | 0 (−3.81, 2.86) | 0 (−1.90, 3.81) | 0 (−4.76, 2.86) |
| Plasma sTfR, $mg \cdot L^{-1}$ | | | |
| Baseline | 5.98 (5.29, 6.94) | 5.91 (5.27, 7.16) | 6.20 (5.56, 7.34) |
| Change | −0.02 (−0.39, 0.43)* | −0.33 (−0.95, 0.20) | −0.24 (−0.69, 0.24)* |

[1] Values are median (Q1, Q3), $n = 100$, except where specified. *Different from F, $P < 0.05$. Labeled baseline medians with superscripts without a common letter differ, $P < 0.05$. C, control group; F, fortified group; sTfR, soluble transferrin receptor; U, unfortified group.
[2] Change: final (postintervention) − baseline.
[3] $n = 93$–99.
[4] Analysis only for individuals with CRP < 47.62, $nmol \cdot L^{-1}$.

physical performance measures (visual reaction time, 40-m sprint, handgrip strength, and static forearm endurance) did not differ between study groups following intervention. Height and weight of the participants increased from baseline to endpoint in all study arms ($P < 0.01$). However, the increase did not differ among the groups. Similarly, the unsupplemented energy and macronutrient intakes decreased from baseline to endpoint ($P < 0.05$) except for fat in the U group. The changes did not differ among the groups (Supplemental Table 2). Moderate to vigorous physical activity was significantly reduced between the 2 assessments in the C and F groups ($P < 0.05$). However, there were no differences in the observed changes in diet during the intervention period between the study arms among the 3 groups (Supplemental Table 2).

The increase in iron status and the status of several measured vitamins (thiamin, riboflavin, pyridoxal phosphate, vitamins B-12 and C, folate, and niacin) from baseline to endpoint in the F group was significantly greater than in the C or U group (Table 4). The postintervention difference in micronutrient status between groups remained significant even after adjusting for baseline values. Consequently, there was a significantly lower percentage of participants with biochemical deficiency at the time of assessment (using standard cutoffs) of these micronutrients in the F group, compared to the 2 other groups (Supplemental Table 1).

## Discussion

The data demonstrate that a 4-mo intervention with multiple micronutrients improves whole body endurance and predicted aerobic capacity. The children were nutritionally compromised as seen by the prevalence of multiple micronutrient deficiencies at baseline. The increase in the number of shuttles in the F group was ~25% over the baseline value. Groups U and C also demonstrated an increase of ~8% in the number of shuttles over the duration of the intervention period likely linked to growth. Given that the growth of children during the intervention period was similar in the F, U, and C groups, a 17% increase in shuttles over baseline can be specifically attributed to the micronutrient supplement. The findings were particularly apparent with the 20-m shuttle test but less so with the step test. This may have been due to the fact that pulse rates were recorded manually for the step test, increasing the random error in measurement and thereby limiting the chances of detecting significant differences. In addition, in other ethnic groups, the step test has shown lower validity and reliability compared to the 20-m shuttle; this may

have made it more difficult to demonstrate differences with the step test (34). Nonetheless, the increases in the estimated maximal aerobic capacity in the F group tended to be higher than those in the C group ($P = 0.14$) and supportive of the findings from the more objective and robust shuttle test. These findings were not confounded by selective changes in anthropometry, dietary intake (excluding the supplement), and physical activity patterns in the F group, because increases in anthropometry and changes in physical activity and dietary intakes were similar across all the study groups during the intervention period. Micronutrient supplementation did not result in a specific increase in muscle strength or speed or visual reaction times. The significant effect of the micronutrient supplement was on the aerobic tests of physical performance and not the muscle strength tests (which are anaerobic in nature). The findings of the present study are internally consistent, because the available literature suggests that micronutrient supplementation largely affects aerobic performance (5–7). Static forearm endurance also did not change, though the method followed was less robust than obtaining a continuous record of the handgrip with appropriate curve-fitting to determine time to fatigue, as we have done earlier (35).

It has been suggested that micronutrient supplementation is most likely to have beneficial effects on physical performance when there is a preexisting deficiency (36,37). Data from the late 1970s indicated high levels of biochemical riboflavin and vitamin B-6 deficiency in rural school boys (38). More recent data from a middle-income residential school setting indicated that apparently healthy school children have subclinical deficiencies, with ~45–65% children deficient in riboflavin and vitamin B-12 (15). Other studies report the prevalence of iron and vitamin B-6 deficiencies in this age group, which are at much higher levels (42–65%) than in the present study (15,39–41). These differences may reflect differences in the study populations and the dietary patterns across India. Despite the considerable heterogeneity, these data suggest that micronutrient deficiencies in Indian school children are common wherever they have been tested. Multiple micronutrient supplementation in such populations may, therefore, be beneficial in improving micronutrient status and enhancing aerobic capacity and endurance.

There is a broader, long-term context for the need to improve physical fitness and aerobic capacity in children who are nutritionally compromised. Physical fitness begins to track early in childhood (42) and into adulthood (43). In addition, cardiovascular fitness relates more closely than physical activity to cardiovascular disease risk factors in healthy children and adolescents (44). Given that South Asians who undergo a nutritional and epidemiological transition experience a first myocardial infarction at a younger age compared to other countries (45), it would be important to optimize physical fitness as early in life as possible. For individuals who do not experience a nutritional transition, continued undernutrition in adulthood may be associated with functional disadvantages, including decreased work capacity (46).


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