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Micronutrient supplementation has limited effects on intestinal infectious disease and mortality in a Zambian population of mixed HIV status: a cluster randomized trial[1-3]

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Abstract

Background: Diarrheal disease remains a major contributor to morbidity and mortality in Africa, but host defense against intestinal infection is poorly understood and may depend on nutritional status.

Objective: To test the hypothesis that defense against intestinal infection depends on micronutrient status, we undertook a randomized controlled trial of multiple micronutrient supplementation in a population where there is borderline micronutrient deficiency.

Design: All consenting adults (≥18 y) living in a carefully defined sector of Misisi, Lusaka, Zambia, were included in a cluster-randomized (by household), double-blind, placebo-controlled trial with a midpoint crossover. There were no exclusion criteria. Participants were given a daily tablet containing 15 micronutrients at just above the recommended nutrient intake or placebo. The primary endpoint was the incidence of diarrhea; secondary endpoints were severe episodes of diarrhea, respiratory infection, nutritional status, CD4 count, and mortality.

Results: Five hundred participants were recruited and followed up for 3.3 y (10 846 person-months). The primary endpoint, incidence of diarrhea (1.4 episodes/y per person), did not differ with treatment allocation. However, severe episodes of diarrhea were reduced in the supplementation group (odds ratio: 0.50; 95% CI: 0.26, 0.92; $P = 0.017$). Mortality was reduced in HIV-positive participants from 12 with placebo to 4 with supplementation ($P = 0.029$ by log-rank test), but this was not due to changes in CD4 count or nutritional status.

Conclusion: Micronutrient supplementation with this formulation resulted in only modest reductions in severe diarrhea and reduced mortality in HIV-positive participants. The trial was registered as ISRCTN31173864.

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The contributions of the authors were as follows—the study was designed by PK, MK, JT, IZ, IRS, and AT; the trial was set up and the data were collected by PK, MK, RB, VY, MF, and FY; the analysis was carried out by PK, MK, EK, IRS, and AT; and the manuscript was written and reviewed by all authors. There were no conflicts of interest and none of the authors had any financial interest in the manufacture or licensing of any micronutrient formulation.

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INTRODUCTION

Defense against infectious disease is a matter of the highest importance for the health and development of tropical populations. Since the observation that mortality in children is higher in children with vitamin A deficiency (1), there has been great interest in determining whether micronutrients interact with immune responses and other aspects of host defense. The idea that micronutrients can enhance defense against infectious disease was considerably boosted by the finding that mortality and diarrheal disease in children could be prevented by high-dose, intermittent retinol supplements (2). There is also evidence that zinc supplementation enhances host defense, including reduced diarrhea in HIV-positive children (3). Zinc has been found to confer benefit in the treatment (4) and prevention (3, 5) of diarrheal disease, but not in all trials (6), and there is divergence between clinical trials that do (4) or do not (7) show significantly reduced mortality. Large studies confirm that multiple micronutrients confer modest benefits in malnourished children (8).

In contrast with the considerable evidence that micronutrients confer benefit in children at risk of malnutrition, fewer data are available relating to adults and children with HIV infection. These were summarized in a systematic review (9) and a Cochrane review (10). Neither review could confirm that there is enough evidence to justify universal supplementation, although HIV-infected adults probably derive some benefit from micro-nutrient supplementation when given at supraphysiologic doses. This benefit included a reduction in some gastrointestinal manifestations (11), and 2 trials showed a reduction in mortality (12, 13). Micronutrients do not appear to reduce mother-to-child transmission (9).

Even less information is available on the impact of physiologic doses of micronutrients in populations that would be expected to have micronutrient deficiencies. Dietary intake of micronutrients in poor persons living in developing countries would be expected to be poor in view of the limited range of foodstuffs available to poor populations (14, 15). In preliminary work, we measured serum retinol and plasma zinc concentrations in samples taken from the community in which the current study was carried out. Of samples from 146 people, 10% had retinol concentrations below the reference range of 1.05 μmol/L, and 17% had plasma zinc below 12.5 μmol/L. Furthermore, among 24 HIV-positive adults, CD4 count and serum retinol were positively correlated (Spearman's rank correlation coefficient $\rho = 0.34$; $P = 0.001$). We set out to test the hypothesis that micro-nutrient supplementation at a level that would be expected to correct deficiencies in the long term [ie, just above the recommended nutrient intake for the United Kingdom (16)] would have a positive impact on morbidity and mortality in HIV-infected and uninfected adults in Lusaka, Zambia, where HIV seroprevalence is 22–30% (17, 18). Cluster randomization by household was used to minimize the risk of contamination of treatment allocation by family members taking the wrong tablets.

SUBJECTS AND METHODS

The trial was a community-based, randomized, placebo-controlled trial of a multiple micronutrient tablet compared with placebo with cluster randomization by household and a crossover midway. The objective was to determine the effect of the intervention at an individual level rather than at a cluster level, because this is the result that might be of greatest utility at a public health level. Ethical approval was obtained from the Research Ethics Committees of the University of Zambia and the London School of Hygiene and Tropical Medicine. The trial was registered as ISRCTN31173864.

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Study population and recruitment

The trial was conducted in Misisi township, Lusaka. All adult residents (defined for this purpose as those ≥18 y of age) of part of section B were eligible for inclusion; there were no exclusion criteria. A household survey of this section in May 2003 showed that there were 733 adults in the defined study area, and 500 volunteered to participate in the trial. The process of recruitment and consent (carried out by PK, RB, VY, and MF) involved 3 stages: a door-to-door invitation to participate, focus group discussions to encourage questions and answers, and then individual counseling sessions before the subjects gave their written consent.

Intervention

The intervention was a multiple micronutrient tablet or matching placebo, both of which were prepared by Dansk Farmaceutic Industri (Ballerup, Denmark). The multiple micronutrient tablet, which included 15 important micronutrients (Table 1), was chosen because it is readily available because it has been used in World Health Organization programs and has a 2-y shelf-life. The placebo tablet was indistinguishable from the multiple micronutrient tablet in appearance and taste.

Randomization and masking of treatment allocation

Households (defined as sharing the same cooking pot; average size 1.86 participating adults) were randomly assigned to one letter of an 8-letter code, and all participating members of the household received the same treatment allocation. The study statistician (JT) generated the code by using a random number sequence and stratified it by household size, which allowed a high degree of matching (Table 2). The code was held by only the study statistician and the manufacturers, both in Europe, during the trial. Tablets were supplied to the study team (RB, VY, and MF) in sealed, light-proof plastic bottles, each of which contained a 28-d supply and were identifiable only by the 8-letter code (4 encoded micronutrient, 4 placebo). After 2 y had elapsed, participants crossed over to the other treatment arm by preparation of a new batch of trial medication with the opposite relation between the 8-letter code and treatment allocation. At the crossover point, any remaining tablets were recalled and destroyed; households retained their original code letter, and new bottles of study medication were issued.

Study procedures, follow-up, and outcomes

We interviewed each participant every 2 wk and asked them directly about any episodes of diarrhea (the primary outcome); the data were then collated for month-by-month analysis. If diarrhea was reported, the incidence, duration, frequency, presence of blood, and time off work or attendance at a health treatment facility for treatment were all recorded. The interview also included a question about cough, as a measure of respiratory infection, and all diagnoses of tuberculosis were recorded. We offered HIV testing (Capillus; Trinity Diagnostics, Dublin, United Kingdom) every year or on demand, and measured CD4 count (FACScount; Becton Dickinson, Franklin Lakes, NJ) if the result was positive. All follow-up assessments were made by 3 nurses trained specifically for this trial. Intestinal infection was assessed in stool samples collected every 4 mo and during every episode of diarrhea. During any episodes of ill health, free care was provided to a standard considerably higher than the prevailing standard of care. For example, treatment of diarrhea usually simply involves provision of oral rehydration therapy, but our study team provided free oral rehydration therapy, investigations (stool microscopy and culture), and specific treatment for pathogens identified. A similar high standard of care was applied to other incident illnesses also.

We measured nutritional status by measuring height, weight, midupper arm circumference, body impedance (Body Stat 1500; Douglas, Isle of Man), and grip strength (by using a Takeida dynamometer; Takeida Corp, Japan). From these measures, body mass index (BMI) was derived, together with fat mass and lean mass from the impedance monitor. Assessments were carried out at 0, 6, 14, 22, and 38 mo. At the same time as nutritional assessment, each participant was given a hygiene score as previously described (19). Briefly, 0, 1, or 2 points were given for each of 5 indicators of household hygiene to make a score between 0 and 10: overall cleanliness, water storage, food storage, access to and cleanliness of latrine, and use of hand washing. Also at each nutritional assessment, participants were asked whether they had difficulty seeing at night.

Micronutrient assays

Blood samples were collected at approximately annual intervals into EDTA-coated tubes for red blood cell folate estimation, into plain tubes for serum retinol estimation, and into zinc-free lithium heparin tubes for plasma zinc assay. Rubber-free needles and syringes were used throughout, and all samples for retinol assay were protected from light and cooled in a refrigerator as soon as coagulated. Assays were conducted only on a randomly selected subset of 74 samples from participants who had not experienced any illness in the month before sampling. Red blood cell folate was assayed by using a Simultrac folate assay kit (MP Biomedicals UK, London, United Kingdom), retinol was assayed by HPLC as previously described (20), and zinc by atomic absorption spectrometry.

Compliance

At the end of each month, all unused trial medications were retrieved, and new bottles of tablets were distributed. At the crossover, the study statistician (JT) counted all returned bottles and estimated median compliance from the pills returned.

Sample size considerations

Sample size calculations were based on diarrhea incidence from previous studies (1.33 episodes/y in HIV seropositive adults and 0.52/y in seronegative adults). A sample size of 10 272 person-months of observation in each group was needed to show a rate ratio of 0.67 with 80% power and 95% significance. An annual dropout rate of 25% was anticipated (dropouts due to moving house, withdrawal, or death were not replaced), so the initial recruitment was planned for 500 adults. In previous work, the effect of clustering by household and by genetic relatedness on diarrhea incidence was analyzed and estimated to be low (P Kelly, J Todd, unpublished data, 2006); the impact of clustering on sample size was therefore not included in the calculations. To give further weight to these calculations, sample size was also calculated separately for HIV-positive and HIV-negative individuals, and the overall sample size was shown to be adequate for both of these groups and the population overall.

Data analysis

The primary endpoint was incidence of diarrhea, which was analyzed in individuals and not in clusters. Secondary endpoints were incidence of severe episodes of diarrhea and stool frequency, incidence of respiratory infection (cough), changes in CD4 count in HIV-infected participants, changes in nutritional status, and mortality. Diarrhea and respiratory infection incidence were compared in multiple micronutrient and placebo groups, and Poisson and time-series regression models were used to adjust for other potential confounding factors, time trends, and household clustering. The Kruskal-Wallis test was used to compare the duration of diarrhea episodes. Severe episodes of diarrhea were defined as those requiring time off work or the need to visit a health center for treatment. The 5 measures of nutritional

status (BMI, midupper arm circumference, fat and lean body mass, and grip strength) were compared between the multiple micronutrient and placebo groups at each time point, and changes over time were examined by using time series regression analysis. When used in logistic regression as independent variables, nutritional measurements were dichotomized around the median. Life table analysis, Kaplan-Meier curves, and log-rank tests were used to analyze mortality rates. For all analyses, an intention-to-treat basis was used to compare results in the whole group, and subgroup analysis using several potential confounders and effect modifiers was preplanned. These were as follows: sex, age, HIV status, CD4 count < 200 cells/μL, before or after crossover (in case of any carryover effects not eliminated by the washout period of 3 mo after the crossover), and household hygiene score. Because a large program of rollout of antiretroviral therapy began in 2004 (21), use of highly active antiretroviral therapy (HAART) was accounted for by stratification of HIV status: HIV negative, HIV positive with high CD4 count, HIV positive with CD4 count < 200 cells/μL, and HIV positive taking HAART. Serum concentrations of retinol, plasma zinc, and erythrocyte folate were analyzed as continuous variables; for multivariate regression, low zinc was defined as a plasma concentration of ≤12.5 μmol/L and low retinol was defined as ≤0.7 μmol/L in serum. Statistical analysis was carried out by using STATA 8.2 (Stata Corp, College Station, TX).

RESULTS

The trial lasted from August 2003 to December 2006, and a total of 10 846 person-months of observation were evaluated for clinical endpoints. Compliance with study medication was well over 95% before the crossover. The flow of participants through the trial is shown in Figure 1, and the characteristics of trial participants are shown in Table 2. The multiple micronutrient and placebo groups were comparable, although the reported prevalence of poor night vision was higher in the multiple micronutrient group (n = 41; 14%) than in the placebo group (n = 20; 8%; P = 0.01).

Diarrhea

The mean incidence rate overall was 1.41 (95% CI: 1.25, 1.61) episodes/y per person, with 1.10 (95% CI: 0.91, 1.34) in HIV-negative and 1.95 (95% CI: 1.62, 2.35) in HIV-positive participants. There was no significant evidence of benefit on diarrhea incidence while taking micronutrient supplementation compared with placebo in the study cohort as a whole or in HIV seropositive or HIV seronegative groups (Table 3). The intracluster correlation (22) was 0.76, giving a design effect of 2, but no effect of clustering by household was seen in the Poisson regression models of the incidence rate ratio. Diarrhea incidence was higher in women, but there was no interaction between treatment effect and sex either in the Poisson regression models (P = 0.70) or when men and women were analyzed separately. There was no reduction in pathogenic or nonpathogenic parasites or bacteria detected in stool samples (see Supplementary Table 1 in the online journal at www.ajcn.org).

Micronutrient supplementation did not reduce the duration of episodes of diarrhea (mean ± SD): 3.3 ± 2.9 d in the placebo and 3.1 ± 2.7 d in the micronutrient groups (P = 0.38 by Kruskal-Wallis test). Micronutrient supplementation made no difference to the proportion of bloody diarrhea episodes, frequency of diarrhea during episodes, or the number of diarrhea episodes during which participants had taken time off work. However, the number of diarrhea episodes during which participants had reported to a clinical facility of any sort for treatment was reduced from 33 in people taking placebo to 18 in micronutrient recipients (odds ratio: 0.50; 95% CI: 0.26, 0.92; P = 0.017). This effect on severe episodes was confirmed in Poisson regression models.

Respiratory infection

During monthly follow-up visits, participants were also asked about cough and, making the assumption that the great majority of these symptoms would be attributable to respiratory infection, we looked for evidence that micronutrients would reduce respiratory infection. Incidence was 1.24 episodes/y per person in the placebo group and 1.30 in the multiple micronutrient group ($P = 0.36$ by Fisher's exact test). The mean (±SD) duration of each episode was 7.4 ± 11.7 d in the placebo group and 6.8 ± 9.3d in the micronutrient group ($P = 0.36$ by Kruskal-Wallis test). Tuberculosis was diagnosed in 19 participants allocated to placebo and 24 allocated to multiple micronutrient supplementation ($P = 0.54$).

CD4 count in HIV-positive subjects

Excluding 26 patients who received HAART, CD4 counts were taken in 135 HIV-positive persons at baseline, 74 taking active drug and 61 taking placebo. CD4 counts (median, interquartile range) over time are shown in Table 4. The rates of decline in CD4 counts were calculated over time in both the multiple micronutrient and placebo groups. The median change in the group crossing from multiple micronutrient to placebo was −46 (interquartile range: −116, 60) cells $\cdot \mu L^{-1} \cdot y^{-1}$, and 4 (interquartile range: −140, 71) cells $\cdot \mu L^{-1} \cdot y^{-1}$ in the group crossing from placebo to multiple micronutrient, but this was not significant ($P = 0.55$).

Nutritional status

We found no significant differences in measures of nutritional status between the multiple micronutrient and placebo groups at any time point, nor was there any difference in trends over time even after including HIV status or initial nutritional status in time series regression models (Table 5).

Mortality

During 1151 person-years of follow-up, there were 35 deaths, representing an annual mortality rate of 3% (see Supplementary Table 2 in the online journal at www.ajcn.org). There was no benefit of multiple micronutrient supplementation in the whole group, but in known HIV-positive persons, there were 12 deaths while taking placebo and 4 while taking micronutrients ($P = 0.029$ by log-rank test; Figure 2).

Adverse events

There were no adverse events that appeared attributable to study medication. Conversely, pellagra occurred 4 times, 3 of these in participants with high ethanol intakes. All these incident cases of pellagra occurred during allocation to placebo.

Micronutrient assays

Mean (±SD) red blood cell folate was significantly higher in multiple micronutrient (439±346 nmol/L) than in placebo (313 ± 202 nmol/L; $P = 0.007$) recipients, although because of difficulty in obtaining radioisotopes, these assays were carried out only during the months before the crossover. Serum retinol was nonsignificantly higher in multiple micronutrient (0.96 ± 0.51 μmol/L) than in placebo (0.87 ± 0.50 μmol/L; $P = 0.43$) recipients, and no effect of randomization was seen on low serum retinol in the logistic regression. Plasma zinc was also nonsignificantly higher in the multiple micronutrient group (8.34 ± 3.5μmol/L) than in the placebo group (7.1±3.4; $P = 0.09$), but in logistic regression models, multiple micronutrient recipients were less likely to have low plasma zinc ($P = 0.02$).

DISCUSSION

The enormous burden of infectious disease in adult populations in sub-Saharan Africa imparts great significance to the question of the interaction between nutritional status and host defense. There is considerable evidence that micronutrient interventions improve several aspects of host defense (23, 24), but this has never been shown for physiologic doses of multiple nutrients simultaneously. We tested the hypothesis that physiologic replacement-level doses of micronutrients would reduce susceptibility to diarrheal disease in an African population on the edge of micronutrient deficiency and of mixed HIV status. In a randomized controlled trial, we showed that micronutrient supplementation had no effect on incidence of diarrhea, but it did reduce severe episodes of diarrhea (those for which people sought health care). These findings are in some ways similar to the findings of the Ghana Vitamin A supplementation study (2), in which vitamin A supplementation had a more significant impact on severe episodes, rather than on all episodes, of diarrhea.

The design of the trial, including cluster randomization and a crossover, deserves some explanation. Cluster randomization was used to reduce the probability of contamination. The cluster design of the trial had little effect on our estimates of treatment effect, but by reducing the likelihood of contamination within households, it greatly enhances confidence in treatment allocation. The crossover design introduced additional complexity to the analysis of a trial like this, but the absence of effect on the primary endpoint was seen before and after the crossover and there was no suggestion of order effects. A crossover design was used to allay ethical concerns that use of a placebo might deny the advantages of supplementation to potential beneficiaries, even though a situation of equipoise clearly existed surrounding the question of supplementation in HIV-infected adults. There are few data to inform the choice of an appropriate washout period, and the opinions sought suggested that 3 mo would be reasonable in view of the composition of the tablet. In view of the fact that no benefit was obtained on the primary endpoint with this dose level of multiple micronutrients, the crossover design was neither necessary nor does it cloud the interpretation of the results. Our data signify that in future trials a placebo is still required.

Death among adults is still distressingly frequent, despite our best attempts to provide comprehensive medical and nursing care. We observed a 3% overall death rate per year, close to our previous estimate in 1998 (25). Our results are consistent with a previous study of micronutrient supplementation in HIV-infected adults in Thailand that also showed reduced mortality (12). Our study was not powered to examine mortality effects, so this finding should be interpreted with caution, especially because the HIV status of a large proportion of those who died was unknown. However, at least some of these deaths among persons of unknown HIV status were definitely HIV-unrelated deaths from causes that could not be expected to respond to micronutrient supplementation (heart failure, stroke, pulmonary hyper-tension, gastrointestinal bleeding). Also, some of the deaths in the HIV-unknown group occurred early in the study. Consequently, the lack of effect in the group with unknown HIV status is not surprising. The reduction in HIV-related deaths, which could be of great importance, needs to be tested in further large-scale trials powered to analyze mortality. We found no significant effect on rate of change of CD4 count, which was similar to previous measurements we have made in this population and that others have made in Tanzania (26) and South Africa (27), but slower than in Abidjan (28). Two trials from the United States suggest that higher doses of selenium (29) or a multiple micro-nutrient supplement (30) can positively affect CD4 count.

If rises in blood concentrations of zinc and retinol were not detectable, how could there be any benefit on morbidity or mortality? First, we only measured 3 micronutrients, one of which showed a clear difference between multiple micronutrient and placebo groups, and

there are others we did not measure (eg, selenium) that might be just as likely to have an effect on host defense (29, 31). Second, there is a poor relation between serum or plasma micronutrient concentrations and true micronutrient status, especially for vitamin A and zinc (32, 33). Systemic responses to inflammation could probably entirely explain the failure of micronutrients to rise in concentration in blood. Third, there was evidence that the multiple micronutrient group had a higher prevalence of impaired night vision, which might reflect vitamin A deficiency, and this was correlated with incidence of diarrhea. In a previous study, we attributed failure of serum retinol to rise after giving high doses of retinol palmitate orally to severe enteropathy associated with HIV-related diarrhea (20). On balance, our findings suggest that in this population, in which tropical enteropathy is ubiquitous (19), effective micronutrient supplementation may require higher doses than the doses used here.

Female sex was consistently associated with increased risk of diarrhea (Table 3). There are several possible explanations, including exposure to diarrhea pathogens through care of sick children or other relatives or a truly increased susceptibility to intestinal infection. It is also possible that there could be an ascertainment bias if for some reason women are better at recalling or reporting diarrheal illness. Recent studies have suggested that in children the effects of zinc supplementation may be greater in boys (7, 34). We looked for such an interaction between treatment effect and sex and found none, but this intriguing question needs further study.

It is likely that both composition and formulation of a micronutrient supplement make a great deal of difference to efficacy. The formula we chose was determined by the hypothesis we were testing (just above RNI), pharmaceutical compatibility, and shelf-life (considerably increased by using β-carotene as the form of vitamin A). More recent data suggest that high doses, particularly selenium, may be helpful in HIV-infected adults (13, 29, 30, 35), but this was outside the scope of the hypothesis we chose to test, and very high doses of zinc, for example, may be toxic (36). Iron supplementation has now been shown to increase the risk of malaria in children (37-39). If this is also true in adults, the amount of iron may need to be reduced, but in HIV-infected adults there is as yet no direct evidence of accelerated disease progression related to iron supplementation (40). There is also some evidence that vitamin A may have detrimental effects in HIV-infected individuals (11, 41). The complex field of nutrition-infection interactions needs a great deal of further work before significant generalizations are possible, particularly in persons receiving antiretroviral therapy (42), but this study encourages our belief that important benefits are achievable in vulnerable groups once optimal dosages of micronutrients are established.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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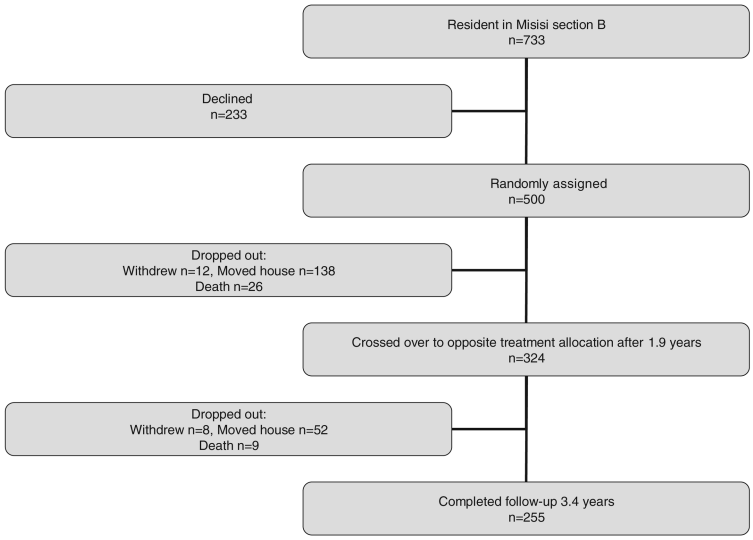

FIGURE 1.
Flow of participants through the trial.

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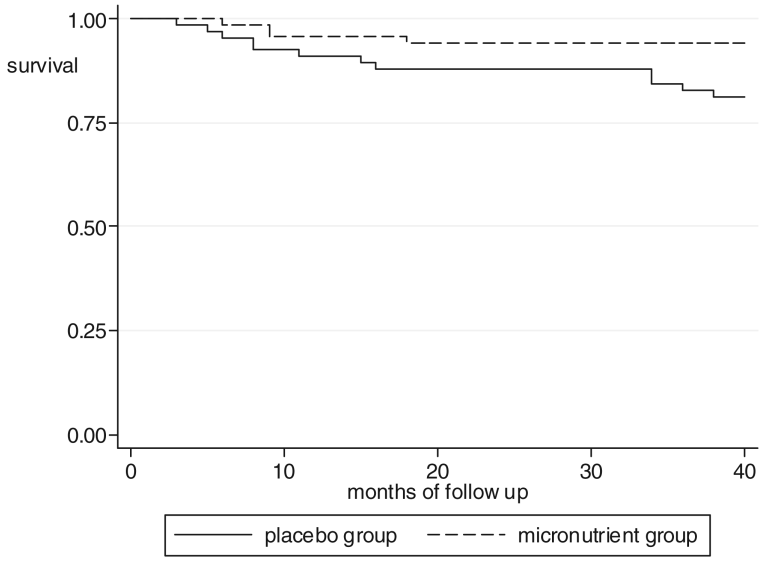

FIGURE 2.
Kaplan-Meier plot of survival in HIV seropositive participants taking micronutrients (dashed line) or placebo (solid line). Differential survival was significantly different by log-rank test ($P = 0.029$).

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TABLE 1

Composition of the micronutrient tablet[1]

Micronutrient	Amount	RNI
β-Carotene	4.8 mg	4.2 mg equivalent
Ascorbic acid (vitamin C)	70 mg	40 mg
Cholecalciferol (vitamin D_3)	5 μg	—
Tocopherol (vitamin E)	10 mg	4 mg (uncertain)
Thiamine (vitamin B-1)	1.4 mg	1.0 mg
Riboflavin (vitamin B-2)	1.4 mg	1.3 mg
Niacin	18 mg	17 mg
Vitamin B-6	1.9 mg	1.4 mg
Cyanocobalamin (vitamin B-12)	2.6 μg	1.5 μg
Folic acid	400 μg	200 μg
Iron	30 mg	14.8 mg (women), 8.7 mg (men)
Zinc	15 mg	9.5 mg
Copper	2 mg	1.2 mg
Selenium	65 μg	75 μg
Iodine	150 μg	140 μg

[1]Composition of the trial medication (one tablet was given each day) compared with the UK recommended nutrient intake (RNI; from reference 16). The RNI for vitamin D in healthy adults exposed to sunlight is probably zero if appropriate lipid precursors are present in the diet (16).

TABLE 2

Characteristics of the study group at the time of random assignment and after the crossover date on individual participants and on households (clusters)[1]

	Baseline		After crossover	
	MM (*n* = 249)	**Placebo (*n* = 251)**	**MM (*n* = 156)**	**Placebo (*n* = 141)**
Age (y)	34.2 ± 13[2]	34.2 ± 13	35.8 (13.9)	36.6 (13)
Male (*n*)	79	83	54	49
Female (*n*)	170	168	102	92
Education [*n* (%)]	—	—	—	—
No secondary	185 (74)	193 (77)	119 (76)	107 (76)
Any secondary	64 (26)	58 (23)	37 (24)	34 (24)
Poor housing (ie, domestic hygiene score below median) [*n* (%)]	83 (51) of 164 scored	86 (57) of 151 scored	47 (38) of 124 scored	38 (32) of 119 scored
BMI (kg/m^2)	22.5 ± 4.4	22.5 ± 4.6	24 ± 5.5	23.2 ± 4.5
MUAC (cm)	26.7 ± 3.8	26.9 ± 3.8	28.8 ± 4	27.7 ± 3.7
HIV positive [*n* (%)]	69/169 tested (41)	67/191 (35)	57/134 (43)	54/124 (44)
CD4 count	370 ± 190	365 ± 212	415 ± 242	409 ± 192
Household (cluster) size[3]				
1 participant	49	48	—	—
2 participants	67	67	—	—
3 participants	10	11	—	—
4 participants	4	4	—	—
5 participants	4	4	—	—

[1] Statistical testing of all these factors by either *t* test or chi-square test showed no difference in treatment allocation groups. MM, multiple micronutrient supplementation group.

[2] Mean ± SD (all such values).

[3] Baseline only.

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TABLE 3

Final Poisson regression model of incidence of diarrhea; analysis by individuals[1]

	No. of persons[2]	No. of episodes	Person-months	Unadjusted IRR (95% CI)	*P*	Adjusted IRR (95% CI)	*P*
Treatment allocation							
Placebo	251	619	5135	1.0		1.0	
Multiple micronutrient	249	594	5255	0.94 (0.84, 1.05)	0.26	0.92 (0.79, 1.07)	0.29
Sex							
Male	162	238	2707	1.0		1.0	
Female	338	975	7683	1.51 (1.31, 1.75)	<0.0001	1.44 (1.14, 1.83)	0.002
Night vision							
Good	440	945	6879	1.0		1.0	
Impaired	52	78	304	1.87 (1.46, 2.35)	<0.0001	1.32 (1.00, 1.75)	0.05
Period of study							
Later	315	973	8355	1.0		1.0	
Initial 6 mo	500	240	822	2.50 (2.17, 2.89)	<0.0001	2.08 (1.78, 2.42)	<0.0001
HIV negative	224	521	5136	1.0	—	1.0	—
HIV, CD4 ≥ 200	123	398	2262	1.73 (1.52, 1.98)	<0.0001	1.64 (1.31, 2.05)	<0.001
HIV, CD4 < 200	29	69	283	2.40 (1.84, 3.10)	<0.0001	2.06 (1.36, 3.11)	0.001
HIV, on HAART	26	69	219	3.11 (2.38, 4.00)	<0.0001	2.45 (1.62, 3.69)	<0.001

[1] Unadjusted incidence rate ratio (IRR) for risk factors for diarrhea and the adjusted IRR of the same factors after inclusion in a Poisson regression model. Only factors included in the final regression model are shown, and treatment allocation was retained even though not significant because this was the primary hypothesis being examined. HAART, highly active antiretroviral therapy.

[2] The number of participants in different HIV groups changed over time as CD4 counts declined (or climbed) and participants were started on HAART; figures shown are numbers ever included in that category.

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TABLE 4

CD4 counts over time according to treatment allocation

Group	Year 1	Year 2	Year 3	Year 4 (4 mo only)
Whole group	296 (201–410) [135]	412 (249–578) [83]	392 (263–591) [78]	420 (231–486) [26]
MM at close of period	286 (186–379) [74]	386 (255–527) [40]	394 (257–591) [42]	438 (265–622) [12]
Placebo at close of period	318 (236–450) [61]	429 (244–529) [43]	384 (276–608) [36]	400 (217–480) [14]
P[2]	0.42	0.44	0.95	0.33

[1] CD4 counts are given as median (interquartile range) of all counts taken during the year stated (these were undertaken annually). *n* in brackets. The crossover was at the end of year 2. MM, multiple micronutrient.

[2] Kruskal-Wallis test of difference between MM and placebo.

TABLE 5

Time series regression models of changes in nutritional status[1]

Dependent variable	Independent (explanatory) variable	Regression coefficient	95% CI	*P*
BMI (kg/m^2)	MM versus placebo	−0.082	−0.285, 0.122	0.43
	Female versus male	3.031	2.049, 4.013	<0.001
	Age <40 y	−2.661	−3.634, −1.688	<0.001
	HIV	−1.172	−1.921, −0.422	0.002
	Hygiene score <5	0.135	0.051, 0.218	0.002
	Initial BMI <18.5	−4.592	−6.011, −3.173	<0.001
Midupper arm circumference (cm)	MM versus placebo	0.0354	−0.287, 0.358	0.83
	Female versus male	1.479	0.667, 2.290	<0.001
	Age <40 y	0.070	0.041, 0.098	<0.001
	HIV	−0.953	−1.685, −0.222	0.01
	Hygiene score <5	0.218	0.099, 0.337	<0.001
	Initial BMI <18.5	−4.395	−5.562, −3.227	<0.001
Fat body mass (kg)	MM versus placebo	0.113	−0.490, 0.716	0.71
	Female versus male	8.147	6.383, 9.911	<0.001
	Age <40 y	0.335	0.273, 0.398	<0.001
	HIV stage	−0.969	−1.795, −0.143	0.02
	Initial BMI <18.5	−5.227	−7.703, −2.750	<0.001
Lean body mass (kg)	MM versus placebo	0.117	−0.584, 0.819	0.74
	Female versus male	−7.989	−9.538, −6.6441	<0.001
	Age <40 y	−0.116	−0.170, −0.063	<0.001
	Hygiene score <5	0.594	0.343, 0.845	<0.001
	Initial BMI <18.5	−5.104	−7.305, −2.903	<0.001
Grip strength (kg)	MM versus placebo	−0.026	−0.751, 0.698	0.943
	Female versus male	−7.020	−8.204, −5.834	<0.001
	Age <40 y	−0.127	−0.169, −0.086	<0.001
	HIV stage	−1.508	−2.280, −0.734	<0.001
	Hygiene score < 5	0.370	0.143, 0.596	<0.001

[1]Time series regression models were constructed to analyze the contributions of various factors (including treatment allocation) to time trends in the dependent variables shown. Final regression models are shown for each dependent variable, and treatment allocation is included even when not significant because this was the primary hypothesis being examined. MM, multiple micronutrient.