Associations between Micronutrient Status and Post-Hospital Discharge Growth among 2–23-Month-Old Children with Acute Illnesses in Africa and South-Asia

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Abstract

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Introduction: The high prevalence of wasting among children under five in low- and middle-income countries remains a significant public health concern, contributing to elevated morbidity and mortality rates. Recent studies have highlighted that even after hospital discharge, these children continue to face an increased incidence of mortality and nutritional factors may contribute to this elevated risk. Malnutrition has been shown to exert detrimental effects on innate and adaptive immune functions, increasing children's susceptibility to infections. At the same time, diseases can deplete vital micro and macronutrients, resulting in impaired growth, which, in turn, is associated with elevated mortality risk. This study aimed to investigate associations between serum micronutrient levels and changes in growth among children aged 2–23 months following hospital discharge for acute illnesses.

Method: This secondary data analysis utilized CHAIN nested case-cohort data. Children aged 2-23 months with acute illnesses who had been admitted to hospitals and discharged as survived across six different countries were included. The associations between micronutrient status at hospital discharge and day 45 follow-up growth outcomes, measured as changes in mid-upper arm circumference Z-score (ΔMUACZ), weight-for-height Z-score (ΔWHZ), length-for-age Z-score (ΔLAZ), and weight-for-age Z-score (ΔWAZ) were examined. Initial univariate regression analyses were conducted to examine the associations between individual micronutrients and early post-discharge growth. Subsequently, multivariate analysis using linear regression was employed to confirm associations between selected micronutrients and growth based on the univariate results and adjusting for potential confounders.

Results: Univariate analysis found significant associations between several micronutrients and growth outcomes. High calcium and copper levels were negatively associated with early post-discharge growth, as reflected by changes in ΔMUACZ, ΔWHZ and ΔWAZ scores. Conversely, serum retinol, magnesium, potassium, calcium, B1, B2, B5, B7 and vitamin E each showed positive associations with at least one measure of growth. However, in multivariate analysis, only vitamin B2 maintained a significant association with increased changes in ΔMUACZ and ΔWAZ scores.

Conclusion: Micronutrients, including copper, calcium, B vitamins, vitamin A, and vitamin E, demonstrated modest associations with early post-discharge growth. However, these associations were attenuated by adjustment for confounders such as preterm birth, suboptimal breastfeeding practices, stunting, diarrhea, pneumonia, and systemic inflammation. Deficiencies in the aforementioned micronutrients may be negatively associated with early post-discharge growth through various physiological mechanisms. However, micronutrient deficiencies alone are not strong independent determinants of early post-discharge growth. While micronutrient supplementation remains an important intervention regardless of its effect on post-discharge growth, promoting early post-discharge growth through micronutrient supplementation alone is unlikely to be effective without addressing underlying factors, such as preterm birth, feeding practices and infectious morbidities.

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[1] The Childhood Acute Illness & Nutrition Network

1. Introduction

Malnutrition continues to be a significant public health challenge worldwide, particularly affecting children in low- and middle-income countries. Wasting, often accompanied by severe medical illnesses, poses a critical threat to child health and development. According to a joint estimation by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), and the World Bank Group in 2021, approximately 45.4 million children worldwide were reported to be affected by wasting, with 13.6 million among them suffering from severe wasting(1). Children suffering from wasting have increased morbidity and mortality risks, primarily due to the vulnerability to severe infections and impaired immune function(2). Furthermore, recent studies have highlighted that even after hospital discharge, these children continue to face an increased mortality risk and nutritional factors contribute to this elevated risk(3–5).

Optimal growth in children depends on a number of nutritional factors, including access to a variety of micronutrients. Micronutrients are necessary for the production of many enzymes, hormones, and biochemical mediators that regulate both growth and immune systems(6–8). Studies have shown that micronutrients, including iron, zinc, iodine, calcium, phosphorus, vitamin D and vitamin A are associated with child growth(9). It is common for children with wasting to suffer from micronutrient deficiencies, which often coexist within the same population or in the same child(8). Similarly, micronutrient deficiencies and infectious diseases frequently co-occur.

The current understanding of the association between micronutrient levels at hospital discharge and childhood growth after treatment of acute illness is limited. The transition phase, which occurs during recovery, is a critical period for optimal child growth as acute illness can increase nutrient requirements and alter metabolism, affecting the utilization and absorption of micronutrients, which may further impede complete recovery and prolong post-illness growth delays in children(10,11). Additionally, the study of micronutrients has predominantly followed a reductionist approach, where individual nutrients are assessed in isolation, limiting the ability to examine complete micronutrient profiles and their interrelatedness comprehensively. This analysis aims to understand the associations between micronutrient profiles with post-discharge growth among hospitalized children. By elucidating the relationship between micronutrient status and growth, this study seeks to contribute to the development of evidence-based interventions that can enhance optimal recovery and long-term health for this vulnerable population.

2. Method

2.1. Participant and setting

This analysis included children aged 2-23 months admitted to the hospital for acute illnesses and participated in the CHAIN (The Childhood Acute Illness & Nutrition Network) Nested Case Cohort (CNCC). The CNCC was a sub-cohort derived from the larger CHAIN cohort study conducted between November 20, 2016, and January 31, 2019, across six different countries. Study sites were selected to serve vulnerable populations and represent a range of environments with varying levels of access to healthcare and different underlying comorbidities. The study sites included:

1. Dhaka Hospital and Matlab Hospital in Bangladesh,
2. Banfora Referral Hospital in Burkina Faso,
3. Kilifi County Hospital, Mbagathi Sub-County Hospital, and Migori County Referral Hospital in Kenya,
4. Queen Elizabeth Hospital in Blantyre, Malawi,
5. Civil Hospital in Karachi, Pakistan, and
6. Mulago Hospital in Kampala, Uganda.

2.2. Study Design

The CHAIN cohort was a prospective cohort study, which enrolled 2-23 months old children admitted to the study hospitals with acute illnesses. The general objective of the primary cohort was to characterize acutely ill children and their outcomes in the hospital and after discharge in order to identify modifiable pathways leading to death. It investigated factors associated with mortality during treatment, mortality after discharge, readmission and poor nutritional recovery among children aged 2 to 23 months.

The enrollment of children into the cohort was stratified by mid-upper-arm circumference (MUAC). Children falling into one of the following three strata were enrolled in a ratio of 2:1:2: those with no wasting (MUAC ≥12.5 cm [age ≥6 months] or MUAC ≥12.0 cm [age <6 months]), moderate wasting (MAM) (MUAC 11.5-12.5 cm [age ≥6 months] or MUAC 11.0-12.0 cm [age <6 months]), and severe wasting (SAM, MUAC <11.5 cm [age ≥6 months] or MUAC <11.0 cm [age <6 months], or kwashiorkor (bilateral pedal edema not explained by other medical causes).

Before the study began, all sites received standardized training on variable definitions, identification of clinical signs, measurement of anthropometry, completion of case report forms (CRFs) and data entry. The staff collected baseline data using a standard CRF, including demographic and social information, clinical examination findings, and vital signs. The study also gathered information such as the number of people living in the homestead, access to clean

water and improved sanitation, occupation, household assets, income and food security using CRF. An anthropometric assessment was performed (head circumference, MUAC, weight, and length).

At the time of admission, laboratory samples were also obtained, including blood samples (up to 5 mL for research purposes), rectal swabs, and fecal samples. A provider-initiated HIV screening and performed a malaria rapid diagnostic test to all children. Trained healthcare personnel reviewed children admitted to the hospital daily, and specific clinical features indicating disease progression and treatments were documented on a structured daily CRF, which was then entered into the CHAIN database. The same clinical assessment was performed at discharge, including anthropometry and collection of blood and faecal samples. Both water-soluble and fat-soluble vitamins found in serum samples were analyzed via LC−MS/MS methods (liquid chromatography-tandem mass spectrometry (LC−MS/MS) custom assay. For water-soluble vitamins, calibration curves were generated by adding the isotopically labeled internal standard mixture to calibration solutions. Serum samples were prepared by adding the internal standard mixture to serum samples, followed by treatment with trichloroacetic acid solution. LC-MS/MS analysis was performed using an Agilent UHPLC^2 system coupled with an AB Sciex mass spectrometer. For fat-soluble vitamins, precipitation and extraction steps were carried out using methanol, ZnSO4 mixture, and hexane. LC-MS/MS analysis was performed using an Agilent UHPLC system coupled with an AB Sciex mass spectrometer. Data processing was done using Sciex Analyst software.

Children were discharged from the hospitals and followed up at scheduled visits on day 45, day 90 and day 180. At every follow-up visit, the staff performed anthropometry, rectal swabs, faecal specimens, and blood samples again. The CHAIN Nested Case Cohort (CNCC) was also established by drawing the samples from the larger CHAIN Cohort. In the case-cohort sample selection process, a random sample comprising 24% of the original cohort was initially chosen with the additional inclusion of all death outside of the random 24%. Furthermore, an additional 30 community participants from each site (total N=270) were also randomly selected and included in the case cohort.

2.3. Variables

The outcome variables in this analysis were changes in z-scores for mid-upper arm circumference (ΔMUACZ), weight-for-height (ΔWHZ), length-for-age (ΔLAZ), and weight-for-age (ΔWAZ) at visit day 45. These z-scores were calculated based on the World Health Organization (WHO) 2006 growth standards for children under five years. The exposure

variables of interest were serum levels of the following micronutrients measured at hospital discharge; vitamin A, vitamin D, vitamin E (alpha-tocopherol), vitamin E (beta and gamma-tocopherol), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B6 (pyridoxine), vitamin B7 (biotin), vitamin B9 (folic acid), vitamin B12 (cobalamin), vitamin C (ascorbic acid), sodium, magnesium, potassium, phosphorus, calcium, copper, zinc, and selenium.

We also considered additional covariates based on the current literature and maternal and child health conceptual frameworks. These covariates included the study site, child's sex, child's age, preterm birth, feeding practices, health status (diarrhea and pneumonia), maternal age and inflammatory markers were also taken into account. These variables and covariates were considered to explore the associations between micronutrient levels at discharge and post-discharge growth outcomes, while accounting for other relevant factors that may influence child growth and development.

2.4. Data Analysis and Statistical Methods

The statistical analysis was conducted using R-studio version 2022.07.1. All surviving children in the CHAIN Nested Case-Cohort were included in the analysis. Categorical variables were summarized as counts and percentages, while numerical variables were presented as median ± standard deviation. Regarding growth changes at various follow-up time points, the previous studies have observed that substantial increase in WAZ, WHZ and MUAC(cm) occurred during the initial 45-day period, and the growth in the first 45 days post-discharge would be most likely to be influences by micronutrient concentrations at discharge(12–14). Therefore, the primary outcomes in this study were change (Δ) in MUAC, WAZ, WHZ, and LAZ between discharge and day 45 follow-up.

The distribution of both outcome and exposure variables were checked for normality and exposure variables were found be to be right-skewed. Consequently, these variables were log-transformed before regression analysis. The association between micronutrient levels at hospital discharge and post-discharge growth (ΔMUACZ, ΔWHZ, ΔLAZ, and ΔWAZ) at day 45 visits was assessed in two stages. Firstly, univariate analysis using simple linear regression was conducted for each micronutrient to examine its association with changes in growth at day 45 after discharge. In the multivariate analysis, micronutrients that showed significant associations in the univariate analysis, along with other covariates, were adjusted for potential confounders in a linear regression model. The selection of covariates for multivariate analysis was based on a p-value < 0.05 in the univariate analysis or their recognition as risk factors for child growth outcomes in previous literature.

In the multivariate regression models, we included micronutrients that demonstrated a significant association (p-value < 0.05) in the univariate analysis as predictors while designating each outcome variable as the dependent variable. Additionally, variables including child's age, sex, study site, presence of stunting at admission, breastfeeding at admission, birth size, caregiver age group, presence of inflammation (C-Reactive Protein [CRP]) at discharge and the presence of diarrhoea and pneumonia were also included as adjustments. All p-values were adjusted using a false discovery technique to account for multiple testing. Coefficient estimates, 95% confidence intervals (CI), and adjusted p-values were then reported for hypothesis testing. Sensitivity analyses were performed to examine the growth patterns of children, both with and without the exclusion of missing growth data and CRP as a biomarker of systemic inflammation.

2.5. Treatment of missing data and outliers

Outliers in the growth values were defined as values that deviated significantly from the average change between baseline (discharge) and day 45 follow-up visits, with a threshold of +/- 3 standard deviations (SD). Similarly, for the micronutrient values, outliers were identified as values that significantly differed from the mean by more than +/- 3 SD and those that were deemed clinically impossible. These outliers underwent manual review. All implausible values from micronutrients were set to missing and then replaced with imputed values, as outlined below. Forty-six children did not have any growth data at all follow-up and were therefore excluded from the analysis. For those who had missing growth values at discharge or day 45, but had available growth data at other time points, missing values were imputed using a back-interpolation method. Missing values in the micronutrient data were assumed to be missing at random, and were replaced using multiple imputation method. Imputation was based on micronutrient values, age, sex, study sites, presence of breastfeeding, and underlying stunting as predictor variables to impute the missing values.

3. Results

3.1. Baseline Characteristics

Among 1,278 children in the CNCC dataset, 611 children were included in this analysis. Children who died during hospitalization and those who did not have longitudinal growth data were excluded from this analysis (Figure 1).

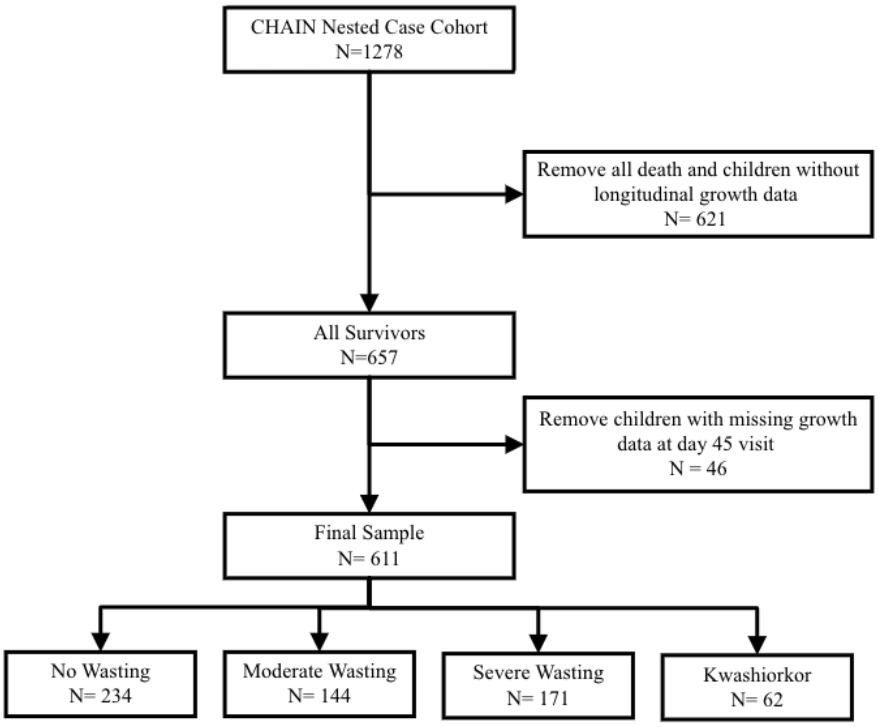

Figure 1.Final Sample Calculation

Table 1 describes the participants who were included in this study. Among these children, 234 (38%) were classified as having normal nutritional status at admission. Moderate wasting (MW) was observed in 144 children (23.5%), while 171 children (27.9%) exhibited severe wasting (SW). Additionally, 62 children (10.1%) presented with kwashiorkor. Across all nutritional status strata, children above 12 months of age were more common other age groups. Irrespective of their nutritional status, approximately 60% of participants were male**.**

The clinical presentation of children in the sample at admission showed that more than half (n=324, 53%) of children were suffering from moderate to severe anemia. Diarrhoea (n=339, 55%) and severe pneumonia (n=133, 22%) were also identified as common clinical conditions among children of all strata. The presence of underlying moderate to severe stunting was very common, affecting more than four out of ten children (n=279, 45%) in all groups. Breastfeeding rates were lowest among children with kwashiorkor (n=18, 29%). The majority of caregivers were identified as mothers, with a substantial portion having completed primary education. Many caregivers reported not having a steady income. Household food insecurity was also prevalent, with more than one-third of households (n=237, 39%) experiencing moderate or severe levels of food insecurity.

Table 1. Characteristics of 611 Study Participants Stratified by WHO Nutritional Status

Characteristics	No wasting N=234	Moderate Wasting N=144	Severe Wasting N=171	Kwashiorkor N=62
Child level factors				
Age Group				
< 6 mo.	64 (27%)	23 (16%)	22 (13%)	5 (8.1%)
> =6 &< 12 mo.	76 (32%)	59 (41%)	62 (36%)	18 (29%)
> =12 mo.	94 (40%)	62 (43%)	87 (51%)	39 (63%)
Sex				
Female	96 (41%)	59 (41%)	71 (42%)	26 (42%)
Male	138 (59%)	85 (59%)	100 (58%)	36 (58%)
Clinical presentation at admission				
Sepsis	36 (15%)	19 (13%)	15 (8.8%)	6 (9.7%)
Severe pneumonia	65 (28%)	33 (23%)	30 (18%)	5 (8.1%)
Diarrhea	107 (46%)	90 (63%)	113 (66%)	29 (47%)
Malaria				
Positive	43 (19%)	26 (18%)	17 (10%)	9 (15%)
Anemia				
Moderate/Severe	121 (55%)	81 (60%)	80 (48%)	42 (69%)
Anthropometry at admission				
MUAC, cm	13 (13, 14)	12 (12, 12)	11 (10, 11)	11 (11, 12)
WLZ	-0.76 (-1.2, 0.05)	-2.2 (-2.6, -1.7)	-3.4 (-4.1, -3.1)	-2.1 (-3.3, -1.0)
WAZ	-1.1 (-1.8, -0.43)	-2.5 (-3.0, -1.8)	-3.9 (-4.6, -3.5)	-2.9 (-3.9, -2.0)
LAZ	-1.2 (-1.9, -0.30)	-1.7 (-2.5, -1.0)	-2.8 (-3.7, -2.0)	-2.9 (-3.7, -1.9)
Underlying chronic conditions				
Stunted				
None	181 (77%)	91 (63%)	42 (25%)	18 (29%)
Moderate	31 (13%)	26 (18%)	51 (30%)	18 (29%)
Severe	22 (9.4%)	27 (19%)	78 (46%)	26 (42%)
Small birth size				
Normal	196 (84%)	113 (80%)	144 (85%)	57 (92%)
Premature/underweight	36 (16%)	28 (20%)	25 (15%)	5 (8.1%)
HIV status				
HIV unexposed, uninfected	220 (94%)	134 (93%)	147 (86%)	53 (85%)
HIV exposed, uninfected	12 (5.1%)	6 (4.2%)	14 (8.2%)	7 (11%)
HIV infected	2 (0.9%)	4 (2.8%)	10 (5.8%)	2 (3.2%)
Nutritional risk exposures				

Recommended adequate diet	128 (55%)	88 (62%)	76 (44%)	10 (16%)
Reported current breastfeeding	192 (82%)	116 (81%)	115 (67%)	18 (29%)
Caregiver characteristics				
Mother is primary care giver	227 (97%)	136 (96%)	158 (94%)	59 (95%)
Caregiver education				
None	42 (18%)	40 (28%)	59 (35%)	15 (24%)
Primary	104 (44%)	54 (38%)	68 (40%)	29 (47%)
Secondary/Tertiary	88 (38%)	50 (35%)	43 (25%)	18 (29%)
Employment				
Employed	24 (10%)	21 (15%)	28 (17%)	14 (23%)
Self-employed	42 (18%)	22 (15%)	24 (14%)	11 (18%)
No income	165 (71%)	100 (70%)	116 (69%)	36 (59%)
Household-level exposures				
Household food insecurity				
Low	147 (63%)	99 (69%)	103 (60%)	25 (40%)
Medium	63 (27%)	28 (19%)	55 (32%)	19 (31%)
High	24 (10%)	17 (12%)	13 (7.6%)	18 (29%)
Toilet				
Improved	184 (79%)	113 (78%)	141 (82%)	40 (65%)
Water source				
Improved	201 (86%)	117 (81%)	154 (90%)	47 (76%)

3.2. Growth Changes at day 45 Follow Up Timepoint

At the day 45 follow up visit, ΔMUACZ showed an increase of 0.47 (SD 0.76), ΔWHZ showed an average increase of 0.56 (SD 0.94), ΔWAZ increased on average by 0.33 (SD 0.65), while ΔLAZ decreased on average by -0.18 (SD 0.41).

3.3. Micronutrient levels at hospital discharge

Table 2 presents the mean values and standard deviations (SD) for different micronutrients that were measured during the hospital discharge of the children participating in the study. The mean values of the micronutrients observed in the sample were within expected ranges based on established reference values(15–20).

Table 2.Micronutrients (Mean, Standard Deviation) at hospital discharge

Micronutreints	Mean ± SD (Unit)	Range
Sodium	137.60 ± 9.56 (mmol/L)	[108.3 - 167.25]
Magnesium	0.82 ± 0.11 (mmol/L)	[0.48 - 1.17]
Phosphorous	1.15 ± 0.24 (mmol/L)	[0.56 - 1.91]
Potassium	4.66 ± 1.01 (mmol/L)	[2.32 - 12.3]
Calcium	2.15 ± 0.26 (mmol/L)	[1.31 - 2.89]
Copper	21.85 ± 7.63 (µmol/L)	[5.96 - 48.37]
Zinc	12.7 ± 3.97 (µmol/L)	[4.62 - 27.25]
Selenium	1.29 ± 0.5 (µmol/L)	[0.35 - 2.82]
B1	0.30 ± 0.28 (µmol/L)	[0.00 - 1.51]
B2	0.07 ± 0.06 (µmol/L)	[0.01 - 0.32]
B3.amide	0.43 ± 0.23 (µmol/L)	[0.06 - 1.51]
B5	0.59 ± 0.42 (µmol/L)	[0.05 - 2.06]
B6	0.02 ± 0.02 (µmol/L)	[0.01 - 0.34]
B7	0.01 ± 0.01 (µmol/L)	[0.00 - 0.04]
B9	0.02 ± 0.10 (µmol/L)	[0.00 - 0.79]
C	1.87 ± 2.36 (µmol/L)	[0.01 - 16.8]
Retinol	0.79 ± 0.58 (µmol/L)	[0.01 - 2.92]
25.Hydroxy.Vitamin.D3	0.10 ± 0.05 (µmol/L)	[0.01 - 0.31]
alpha Tocopherol	8.89 ± 13.58 (µmol/L)	[0.03 - 99]
beta and gamma Tocopherol	1.34 ± 1.87 (µmol/L)	[0.00 - 13.5]

3.4. Univariate Analysis

The univariate regression analysis revealed significant associations between ΔMUACZ at day 45 and multiple individual micronutrients. Specifically, calcium, copper, vitamin B1, vitamin B2, vitamin B5, vitamin B7, vitamin B9, alpha-tocopherol, and beta and gamma-tocopherol displayed significant associations with ΔMUACZ, as indicated by p-values below 0.05. Similarly, for ΔWHZ at day 45, calcium, copper, vitamin B1, vitamin B2, vitamin B5, retinol, alpha-tocopherol, and beta and gamma-tocopherol exhibited significant associations. Likewise, in the case of ΔWAZ at day 45, copper, vitamin B1, vitamin B2, vitamin B5, retinol, alpha-tocopherol, and beta and gamma-tocopherol demonstrated significant associations. Moreover, the analysis of ΔLAZ at day 45 revealed significant associations with magnesium, potassium, calcium, vitamin B1, vitamin B2, and beta and gamma-tocopherol. No other

micronutrients displayed statistically significant associations with the growth indicators (Table 3).

Table 3. Simple Linear Regression: Coefficient Table

	ΔMUACZ	**ΔWHZ**	**ΔWAZ**	**ΔLAZ**
	Estimate [95%CI], p-value			
Sodium	0.21[-0.67, 1.09] 0.642	-0.26 [-1.34, 0.83] 0.641	-0.18 [-0.93, 0.58] 0.643	0.25 [-0.22, 0.73] 0.300
Magnesium	-0.32 [-0.76, 0.11] 0.146	-0.13 [-0.66, 0.41] 0.639	0.05 [-0.32, 0.42] 0.798	**0.27 [0.03, 0.50] 0.025**
Phosphorous	-0.17 [-0.45, 0.11] 0.233	-0.17 [-0.51, 0.17] 0.337	-0.06 [-0.30, 0.18] 0.643	0.13 [-0.02, 0.28] 0.082
Potassium	0.12 [-0.17, 0.41] 0.411	-0.02 [-0.37, 0.33] 0.907	0.13 [-0.11, 0.38] 0.293	**0.20 [0.05, 0.36] 0.010**
Calcium	**-0.60 [-1.10, -0.10] 0.019**	**-0.74 [-1.35, -0.13] 0.018**	-0.32 [-0.75, 0.11] 0.146	**0.28 [0.01, 0.55] 0.040**
Copper	**-0.42 [-0.59, -0.25] 0.000**	**-0.42 [-0.63, -0.21] 0.000**	**-0.32 [-0.46, -0.17] 0.000**	0.01 [-0.09, 0.10] 0.903
Zinc	-0.06 [-0.26, 0.14] 0.537	-0.14 [-0.39, 0.11] 0.266	-0.03 [-0.20, 0.14] 0.731	0.09 [-0.02, 0.19] 0.120
Selenium	-0.01 [-0.17, 0.14] 0.863	0.05 [-0.14, 0.24] 0.610	0.05 [-0.09, 0.18] 0.498	0.06 [-0.03, 0.14] 0.175
B1	**0.13 [0.07, 0.18] 0.000**	**0.10 [0.03, 0.17] 0.006**	**0.09 [0.04, 0.14] 0.000**	**0.04 [0.01, 0.07] 0.016**
B2	**0.22 [0.15, 0.29] 0.000**	**0.21 [0.12, 0.29] 0.000**	**0.16 [0.10, 0.22] 0.000**	**0.05 [0.01, 0.09] 0.007**
B3.amide	0.08 [-0.04, 0.20] 0.190	0.08 [-0.07, 0.23] 0.277	0.07 [-0.04, 0.17] 0.199	0.02 [-0.05, 0.08] 0.632
B5	**0.22 [0.13, 0.31] 0.000**	**0.27 [0.16, 0.37] 0.000**	**0.21 [0.14, 0.28] 0.000**	0.03 [-0.02, 0.07] 0.258
B6	0.12 [-0.05, 0.29] 0.152	0.08 [-0.13, 0.28] 0.456	0.09 [-0.05, 0.23] 0.215	0.03 [-0.06, 0.12] 0.494
B7	**0.26 [0.13, 0.39] 0.000**	0.14 [-0.03, 0.30] 0.099	0.10 [-0.01, 0.22] 0.072	0.04 [-0.03, 0.11] 0.270
B9	**0.04 [0.01, 0.08] 0.022**	0.04 [-0.00, 0.09] 0.073	0.02 [-0.01, 0.06] 0.165	-0.01 [-0.03, 0.01] 0.176
C	0.00 [-0.04, 0.05] 0.872	-0.01 [-0.07, 0.05] 0.798	0.01 [-0.03, 0.05] 0.701	0.02 [-0.00, 0.05] 0.094
Retinol	0.07 [-0.00, 0.15] 0.067	**0.15 [0.06, 0.24] 0.001**	**0.10 [0.03, 0.16] 0.003**	0.00 [-0.04, 0.04] 0.886
25-hydroxy vitamin D3	0.00 [-0.11, 0.12] 0.978	0.07 [-0.07, 0.22] 0.310	0.04 [-0.06, 0.14] 0.409	0.00 [-0.06, 0.07] 0.930
alpha Tocopherol	**0.09 [0.05, 0.13] 0.000**	**0.10 [0.05, 0.16] 0.000**	**0.08 [0.04, 0.12] 0.000**	0.02 [-0.00, 0.05] 0.059
beta and gamma Tocopherol	**0.08 [0.04, 0.12] 0.000**	**0.09 [0.04, 0.15] 0.000**	**0.09 [0.05, 0.12] 0.000**	**0.04 [0.01, 0.06] 0.001**

3.5. Multivariate Analysis

In the multivariate analysis, after adjusting for micronutrients that demonstrated significant association in the univariate analysis and additional covariates listed in the methods, only vitamin B2 maintained a significant association with ΔMUACZ, with a coefficient of 0.13 (95% CI: 0.04, 0.21, p-value = 0.043). Similarly, vitamin B2 was the only micronutrient significantly associated with ΔWAZ (coefficient: 0.10, 95% CI: 0.03, 0.17, p-value = 0.044) in the adjusted analysis (Table 4). None of the micronutrients showed significant associations with ΔWHZ and ΔLAZ in the multivariate analysis.

Table 4. Multiple Linear Regression: Coefficient Table

	ΔMUACZ	**ΔWHZ**	**ΔWAZ**	**ΔLAZ**
	Estimate [95%CI], adjusted p-value			
Magnesium	--	--	--	0.19[-0.08, 0.45] 0.264
Potassium	--	--	--	0.04[-0.13, 0.22] 0.695
Calcium	-0.21[-0.74,0.31] 0.634	-0.45[-1.10, 0.20] 0.342	--	0.25[-0.05, 0.56] 0.180
Copper	-0.24[-0.46, -0.02] 0.137	-0.23[-0.50, 0.05] 0.309	-0.16[-0.33, 0.02] 0.186	--
B1	0.04[-0.03, 0.11] 0.403	-0.01[-0.09, 0.08] 0.887	0.01[-0.05, 0.07] 0.789	0.03[-0.01, 0.07] 0.261
B2	**0.13[0.04, 0.21] 0.043**	0.12[0.01, 0.22] 0.127	**0.10[0.03, 0.17] 0.044**	0.05[0,0.09] 0.120
B5	0.03[-0.08, 0.14] 0.763	0.15[0.02,0.29] 0.127	0.07[-0.03, 0.16] 0.262	-0.06[-0.12,0] 0.123
B7	0.05[-0.09, 0.20] 0.634	--	--	--
B9	0.01[-0.03, 0.05] 0.763	--	--	--
Retinol	--	0.04[-0.07,0.14] 0.704	-0.01[-0.08, 0.06] 0.846	-0.05[-0.1, -0.01] 0.085
Alpha-Tocopherol	0.02[-0.04, 0.07] 0.652	0.04[-0.03,0.10] 0.500	0.01[-0.03, 0.06] 0.756	--
beta and gamma-Tocopherol	0.01[-0.04, 0.07] 0.763	0.02[-0.05, 0.09] 0.705	0.04[-0.01,0.09] 0.245	0.03[0,0.06] 0.085

The plots provided below serve as a visual representation, allowing us to examine the dynamic changes in the relationship between micronutrients and growth outcomes as a result of introducing adjustment variables. By including both adjusted and unadjusted data, we comprehensively understand the effect sizes and detect the underlying pattern. It becomes apparent from these plots that the introduction of adjustment variables suggests confounding

effects, leading to changes in the strength of the relationships. The direction of associations between the micronutrients and growth outcomes remain consistent, regardless of the adjustment. However, the effect sizes depicted in the plots clearly demonstrate that the associations between micronutrients and growth outcomes are attenuated when adjustment variables are considered.

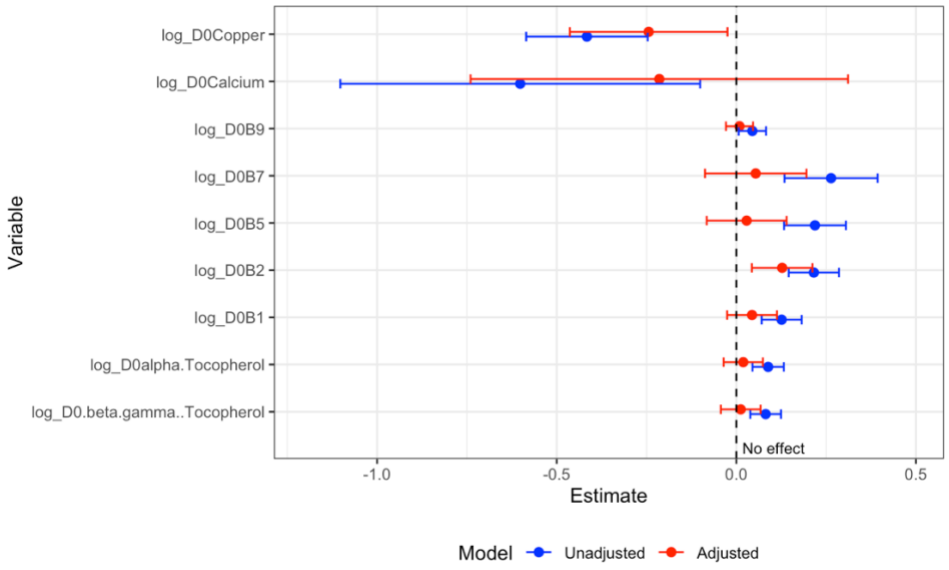

Figure 2.Coefficient and 95%CI comparison for ΔMUACZ

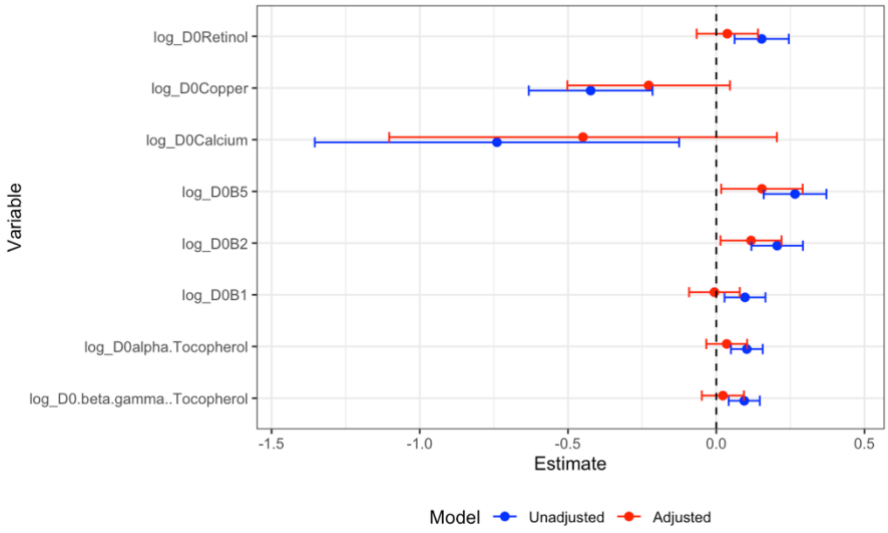

Figure 3.Coefficient and 95%CI comparison for ΔWHZ

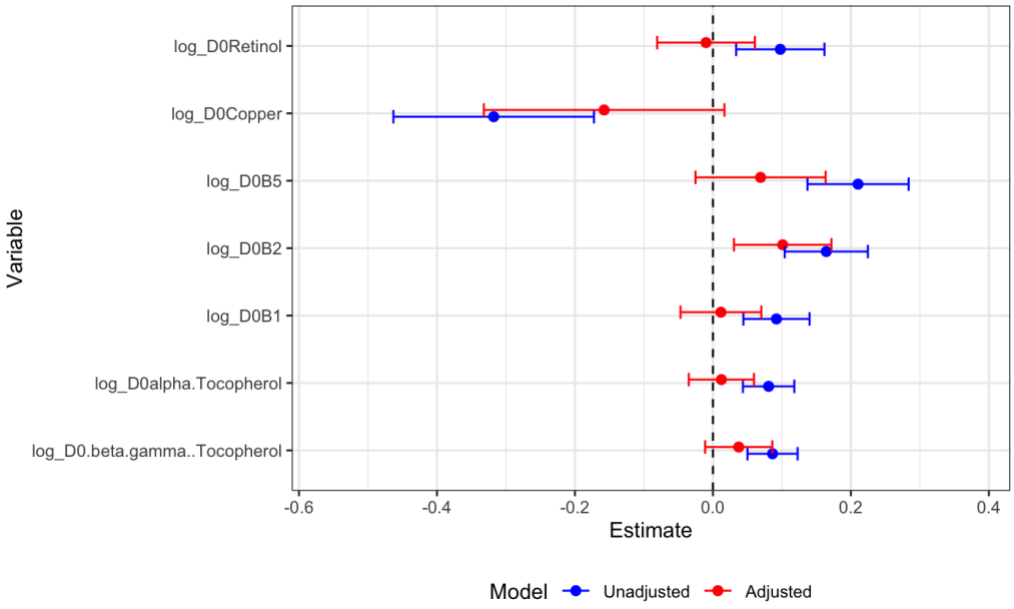

Figure 4.Coefficient and 95%CI comparison for ΔWAZ

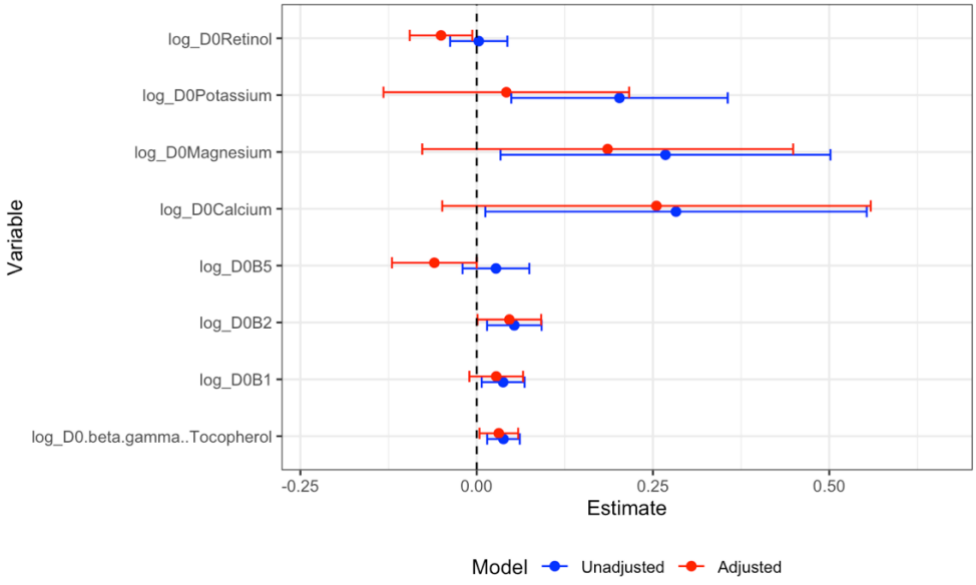

Figure 5.Coefficient and 95%CI comparison for ΔLAZ

4. Sensitivity Analysis

We performed sensitivity analyses to ensure the robustness of our findings. Firstly, we assessed the impact of missing values by comparing analyses with and without imputation. We utilized multiple imputation and back interpolation to handle missing data, aiming to obtain more reliable estimates and account for potential biases. Additionally, we examined the role of CRP as a covariate, considering its association with both micronutrient status and growth outcomes. While the presence of CRP did not significantly alter the majority of associations between micronutrients and growth outcomes, there was a non-significant change in the association involving retinol(Table.5). These findings suggest that CRP may not be a major confounding factor, except for a potential influence on the association with retinol as it is supported by the observed change in the direction of the association between ΔWAZ and retinol when comparing the unadjusted model to the adjusted model. These sensitivity analyses strengthen the validity and generalizability of our results, providing additional insights into the stability of our findings.

5. Discussion

We aimed to investigate the association between serum micronutrients and growth outcome changes at day 45 post-hospital discharge in a case cohort of 2–23-month-old children with acute illnesses. The univariate analysis revealed significant associations between several micronutrients and growth outcomes. Higher calcium and copper were associated with lower ΔMUACZ, ΔWHZ, and ΔWAZ at day 45 post-discharge. On the other hand, retinol, magnesium, potassium, calcium, B1, B2, B5, and vitamin E were all positively associated with at least one measure of growth at day 45 post-discharge. However, in multivariate analysis, only vitamin B2 maintained a significant association with increased ΔMUACZ and ΔWAZ. After adjusting for potential confounding factors, most associations between the other micronutrients and growth outcomes lost their significance.

The inclusion of covariates such as CRP, underlying stunting, common infections and suboptimal breastfeeding led to the attenuation or elimination of the associations observed in the univariate analysis. Systemic inflammation, commonly observed in acute illnesses, can disrupt normal nutrient metabolism and utilization in the body(12,21,22). It can decrease the uptake of essential micronutrients by affecting nutrient transporters in cells, leading to reduced absorption from the gastrointestinal tract. Additionally, inflammation can increase the utilization of micronutrients by immune cells, induce changes in carrier molecules responsible for nutrient transport in the bloodstream, and cause sequestration of micronutrients within cells, potentially leading to deficiencies in other tissues (23–25). Our results suggest that micronutrients do not have a substantial impact on early post-discharge growth when factors including systemic inflammation, birth size, breastfeeding status, and underlying stunting status are considered.

Micronutrients are essential in many physiological processes, and correcting micronutrient deficiencies does not need to be justified by improvements in growth. Copper, calcium, B vitamins, vitamin A, and vitamin E play essential roles in many physiological processes, including neurocognitive development, protein synthesis, energy metabolism and immune functions, which are crucial for growth and development (26–29). They are essential in neuronal signaling, synaptic plasticity, and neurotransmitter synthesis. Insufficient levels of these micronutrients can impair these processes, leading to neurocognitive delays and impairments in learning, memory, and overall cognitive function(30). Secondly, micronutrients are involved in protein synthesis, essential for growth and development. Copper, for example, is a cofactor for enzymes involved in collagen and connective tissue formation(31). Calcium is necessary for proper bone development and muscle function. B vitamins are critical for the synthesis of DNA, RNA, and proteins and are essential for the metabolism of carbohydrates, fats, and proteins, converting them into energy (32–34). Insufficient levels of these micronutrients can disrupt protein synthesis, leading to impaired tissue repair. Lastly, micronutrients are vital for proper immune function. Vitamin A, for instance, is involved in developing and maintaining immune cells, while vitamin E acts as an antioxidant to protect immune cells from oxidative stress. Therefore, the justification for micronutrient supplementation can be based on multiple physiological pathways, including neurocognitive development, protein synthesis, compromised energy metabolism, and weakened immune function.

The effect sizes estimated in this analysis, as indicated by the magnitude of regression coefficients and the coefficients of determination (R2) values, were small. These findings suggest that micronutrients may have some associations with improved post-discharge growth, but these associations are not substantial. Additionally, confounding factors weaken the association between micronutrients and post-discharge growth. Further research, including randomized controlled trials and systematic reviews, is needed to provide more robust evidence on the effectiveness, potential adverse effects, cost-effectiveness, and long-term outcomes of micronutrient supplementation interventions in promoting post-discharge growth.

Our research demonstrates several strengths. Firstly, we conducted a large study with good statistical power and generalizability. Secondly, we employed rigorous data collection methods, ensuring reliable and accurate information. Additionally, we conducted thorough and standardized anthropometric measurements, providing precise data on growth outcomes. Lastly, our study examined a broad panel of micronutrients, allowing for a comprehensive understanding of their collective impact and complex relationship. These strengths enhance the reliability and significance of our findings. However, the study also had several limitations. The observational nature of the data limits our ability to establish causality or infer long-term effects. Active follow-ups and intensive care offered by the program could have led to the

overidentification of severe illnesses and deficiencies, leading to over-reporting compared to children in the community. Children who participated in the study received standard treatment as recommended by the WHO, although there might be minor differences in this management between sites. Many included children may have also been receiving micronutrient supplements through therapeutic foods, which may have obscured the natural association between micronutrient deficiency and post-discharge growth. There were also possible measurement errors and performance biases in the anthropometry assessments.

Multiple complex factors such as psychosocial, environmental and socioeconomic differences influence optimal growth in children. This study was conducted among complicated acutely malnourished children who required hospitalization. The findings are representative of similar groups but may differ for acutely malnourished children in the community or those with subclinical micronutrient deficiencies.

6. Conclusion

Our study highlights the association between serum micronutrients and post-discharge growth outcomes in children with acute illnesses. The findings indicate that micronutrient status at the time of hospital discharge is modestly associated with early post-discharge growth. These associations, however, were attenuated by the presence of confounding factors such as preterm birth, suboptimal breastfeeding practices, stunting, diarrhea, pneumonia, and systemic inflammation. Micronutrient deficiencies do not appear to be strong independent determinants of early post-discharge growth. While micronutrient supplementation remains important, regardless of the direct effect on post-discharge growth, promoting early post-discharge growth through micronutrient supplementation alone will not be effective without adequately addressing multiple other underlying factors driving poor growth recovery, such as preterm birth, breastfeeding support, and preventing and managing recurrent infections.

7. Supplementary Materials

Table 5.Comparison of Estimates Between Models with and without CRP

	Coefficients		Adjusted p-values		95%CI	
Variables	**CRP**	**No CRP**	**CRP**	**No CRP**	**CRP**	**No CRP**
Δ MUACZ						
Calcium	-0.21	-0.48	0.634	0.188	[-0.74, 0.31]	[-0.99, 0.04]
Copper	-0.24	-0.19	0.137	0.178	[-0.46, -0.02]	[-0.39, 0.00]
B1	0.04	0.03	0.403	0.474	[-0.03, 0.11]	[-0.04, 0.09]
B2	0.13	0.14	**0.043**	**0.009**	[0.04, 0.21]	[0.06, 0.22]
B5	0.03	0.05	0.763	0.474	[-0.08, 0.14]	[-0.05, 0.16]
B7	0.05	0.07	0.634	0.474	[-0.09, 0.20]	[-0.07, 0.21]
B9	0.01	0.02	0.763	0.474	[-0.03, 0.05]	[-0.02, 0.05]
alpha Tocopherol	0.02	0.04	0.652	0.353	[-0.04, 0.07]	[-0.01, 0.09]
beta and gamma Tocopherol	0.01	0.01	0.763	0.638	[-0.04, 0.07]	[-0.04, 0.06]
Δ WHZ						
Calcium	-0.45	-0.41	0.342	0.409	[-1.10, 0.20]	[-1.06, 0.24]
Copper	-0.23	-0.26	0.309	0.195	[-0.50, 0.05]	[-0.53, 0.01]
B1	-0.01	-0.01	0.887	0.930	[-0.09, 0.08]	[-0.09, 0.08]
B2	0.12	0.12	0.127	0.137	[0.01, 0.22]	[0.01, 0.22]
B5	0.15	0.16	0.127	0.137	[0.02, 0.29]	[0.02, 0.29]
Retinol	0.04	0.05	0.704	0.505	[-0.07, 0.14]	[-0.05, 0.15]
alpha Tocopherol	0.04	0.04	0.500	0.432	[-0.03, 0.10]	[-0.03, 0.11]
beta gamma Tocopherol	0.02	0.02	0.704	0.85	[-0.05, 0.09]	[-0.05, 0.09]
Δ WAZ						
Copper	-0.16	-0.19	0.186	0.096	[-0.33, 0.02]	[-0.36, -0.01]
B1	0.01	0.01	0.789	0.767	[-0.05, 0.07]	[-0.05, 0.07]
B2	0.1	0.1	**0.044**	**0.045**	[0.03, 0.17]	[0.03, 0.17]
B5	0.07	0.07	0.262	0.286	[-0.03, 0.16]	[-0.02, 0.17]
Retinol	**-0.01**	**0.01**	0.846	0.950	[-0.08, 0.06]	[-0.06, 0.08]
alpha Tocopherol	0.01	0.02	0.756	0.627	[-0.03, 0.06]	[-0.03, 0.06]

beta gamma Tocopherol	0.04	0.03	0.245	0.390	[-0.01, 0.09]	[-0.02, 0.08]
Δ LAZ						
Magnesium	0.19	0.2	0.264	0.238	[-0.08, 0.45]	[-0.06, 0.46]
Potassium	0.04	0.04	0.695	0.727	[-0.13, 0.22]	[-0.13, 0.22]
Calcium	0.25	0.26	0.180	0.209	[-0.05, 0.56]	[-0.05, 0.56]
B1	0.03	0.03	0.261	0.238	[-0.01, 0.07]	[-0.01, 0.07]
B2	0.05	0.05	0.120	0.149	[0.00, 0.09]	[0.00, 0.09]
B5	-0.06	-0.06	0.123	0.183	[-0.12, 0.00]	[-0.12, 0.00]
Retinol	-0.05	-0.04	0.085	0.209	[-0.1, -0.01]	[-0.08, 0.01]
beta and gamma Tocopherol	0.03	0.03	0.085	0.149	[0.00, 0.06]	[0.00, 0.06]

8. References

1. World Health Organization, Fund (UNICEF) UNC, Bank W. Levels and trends in child malnutrition: UNICEF [Internet]. World Health Organization; 2021 [cited 2023 January 8]. 31 p. Available from: https://apps.who.int/iris/handle/10665/341135
2. Bhutta ZA, Berkley JA, Bandsma RHJ, Kerac M, Trehan I, Briend A. Severe childhood malnutrition. Nat Rev Dis Primer. 2017 September 21;3:17067.
3. Diallo AH, Shahid ASMSB, Khan AF, Saleem AF, Singa BO, Gnoumou BS, et al. Childhood mortality during and after acute illness in Africa and south Asia: a prospective cohort study. Lancet Glob Health. 2022 May 1;10(5):e673–84.
4. Walson JL, Berkley JA. The impact of malnutrition on childhood infections. Curr Opin Infect Dis. 2018 Jun;31(3):231–6.
5. Chisti MJ, Graham SM, Duke T, Ahmed T, Faruque ASG, Ashraf H, et al. Post-Discharge Mortality in Children with Severe Malnutrition and Pneumonia in Bangladesh. PLOS ONE. 2014 Sep 16;9(9):e107663.
6. Bourke CD, Berkley JA, Prendergast AJ. Immune Dysfunction as a Cause and Consequence of Malnutrition. Trends Immunol. 2016 Jun;37(6):386–98.
7. Pecora F, Persico F, Argentiero A, Neglia C, Esposito S. The Role of Micronutrients in Support of the Immune Response against Viral Infections. Nutrients. 2020 Oct 20;12(10):3198.
8. Jamison DT, Breman JG, Measham AR, Alleyne G, Claeson M, Evans DB, et al., editors. Chapter 28. Stunting,Wasting, and Micronutrient Deficiency Disorders. In: Disease Control Priorities in Developing Countries (2nd Edition) [Internet]. World Bank Publications; 2006 [cited 2023 May 23]. p. 551–68. Available from: http://www.dcp2.org/pubs/DCP/28/FullText
9. Bailey RL, West KP, Black RE. The epidemiology of global micronutrient deficiencies. Ann Nutr Metab. 2015;66 Suppl 2:22–33.
10. Versloot CJ, Voskuijl W, van Vliet SJ, van den Heuvel M, Carter JC, Phiri A, et al. Effectiveness of three commonly used transition phase diets in the inpatient management of children with severe acute malnutrition: a pilot randomized controlled trial in Malawi. BMC Pediatr. 2017 Apr 26;17(1):112.
11. Bwakura-Dangarembizi M, Dumbura C, Amadi B, Chasekwa B, Ngosa D, Majo FD, et al. Recovery of children following hospitalisation for complicated severe acute malnutrition. Matern Child Nutr. 2022;18(2):e13302.
12. Njunge JM, Gonzales GB, Ngari MM, Thitiri J, Bandsma RHJ, Berkley JA. Systemic inflammation is negatively associated with early post discharge growth following acute illness among severely malnourished children - a pilot study. Wellcome Open Res. 2021 March 16;5:248.

13. Ngari MM, Iversen PO, Thitiri J, Mwalekwa L, Timbwa M, Fegan GW, et al. Linear growth following complicated severe malnutrition: 1-year follow-up cohort of Kenyan children. Arch Dis Child. 2019 Mar 1;104(3):229–35.
14. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet. 2013 Jul 20;382(9888):209–22.
15. Trapani S, Rubino C, Indolfi G, Lionetti P. A Narrative Review on Pediatric Scurvy: The Last Twenty Years. Nutrients. 2022 Jan;14(3):684.
16. Verive MJ, Irazuzta J, Steinhart CM, Orlowski JP, Jaimovich DG. Evaluating the frequency rate of hypomagnesemia in critically ill pediatric patients by using multiple regression analysis and a computer-based neural network. Crit Care Med. 2000 Oct;28(10):3534.
17. Cordano A. Clinical manifestations of nutritional copper deficiency in infants and children. Am J Clin Nutr. 1998 May 1;67(5):1012S-1016S.
18. Feld LG, Neuspiel DR, Foster BA, Leu MG, Garber MD, Austin K, et al. Clinical Practice Guideline: Maintenance Intravenous Fluids in Children. Pediatrics. 2018 Dec 1;142(6):e20183083.
19. Rempel J, Grover K, El-Matary W. Micronutrient Deficiencies and Anemia in Children with Inflammatory Bowel Disease. Nutrients. 2021 Jan;13(1):236.
20. Whitfield KC, Bourassa MW, Adamolekun B, Bergeron G, Bettendorff L, Brown KH, et al. Thiamine deficiency disorders: diagnosis, prevalence, and a roadmap for global control programs. Ann N Y Acad Sci. 2018 Oct;1430(1):3–43.
21. Wen B, Njunge JM, Bourdon C, Gonzales GB, Gichuki BM, Lee D, et al. Systemic inflammation and metabolic disturbances underlie inpatient mortality among ill children with severe malnutrition. Sci Adv. 8(7):eabj6779.
22. DeBoer MD, Scharf RJ, Leite AM, Férrer A, Havt A, Pinkerton R, et al. Systemic inflammation, growth factors, and linear growth in the setting of infection and malnutrition. Nutr Burbank Los Angel Cty Calif. 2017 Jan;33:248–53.
23. Tickell KD, Walson JL. Nutritional Enteric Failure: Neglected Tropical Diseases and Childhood Stunting. PLoS Negl Trop Dis. 2016 Apr 28;10(4):e0004523.
24. Campbell DI, McPhail G, Lunn PG, Elia M, Jeffries DJ. Intestinal Inflammation Measured by Fecal Neopterin in Gambian Children With Enteropathy: Association With Growth Failure, Giardia lamblia, and Intestinal Permeability. J Pediatr Gastroenterol Nutr. 2004 Aug;39(2):153.

25. Pham VT, Dold S, Rehman A, Bird JK, Steinert RE. Vitamins, the gut microbiome and gastrointestinal health in humans. Nutr Res. 2021 Nov 1;95:35–53.
26. Castillo-Duran C, Uauy R. Copper deficiency impairs growth of infants recovering from malnutrition. Am J Clin Nutr. 1988 May 1;47:710–4.
27. Farag M. Dietary Vitamin B Complex: Orchestration in Human Nutrition throughout Life with Sex Differences. Nutrients. 2022 September 22;14.
28. Suárez-Ortegón MF, Jiménez P, Mosquera M, Pradilla AG, Gracia AB, Aguilar de Plata C. Inverse Correlation Between Serum Calcium and Copper Levels in Male Urban Colombian Preschool Children: Relationships with Anthropometry and Age. Biol Trace Elem Res. 2011 Dec 1;144(1):445–53.
29. van Stuijvenberg ME, Nel J, Schoeman SE, Lombard CJ, du Plessis LM, Dhansay MA. Low intake of calcium and vitamin D, but not zinc, iron or vitamin A, is associated with stunting in 2- to 5-year-old children. Nutrition. 2015 Jun 1;31(6):841–6.
30. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. Am J Clin Nutr. 2007 Feb 1;85(2):614S-620S.
31. Harris ED, Rayton JK, Balthrop JE, DiSilvestro RA, Garcia-de-Quevedo M. Copper and the synthesis of elastin and collagen. Ciba Found Symp. 1980;79:163–82.
32. Powers HJ. Riboflavin (vitamin B-2) and health. Am J Clin Nutr. 2003 Jun 1;77(6):1352–60.
33. Garcia BA, Luka Z, Loukachevitch LV, Bhanu NV, Wagner C. Folate deficiency affects histone methylation. Med Hypotheses. 2016 March 1;88:63–7.
34. Fenech M. The role of folic acid and Vitamin B12 in genomic stability of human cells. Mutat Res Mol Mech Mutagen. 2001 Apr 18;475(1):57–67.