Current Science
CS 6 (3), 6300-6320 (2026)
Received 04April 2026 | **Accepted 01 May** 2026 | **Online Available 05 May** 2026
https://doi.org/10.52845/CS/2026-6-3-1

Current Science

Current Science

OPEN ACCESS

ISSN(O)2795-8639

Original Article

Nonlinear Association between Serum Folate and Zinc Concentrations in Children with Rickets: A Retrospective Cross-Sectional Study

Chao Ma[1+], Jiawei He[2+]*, Baoxing Huang[1], Bingyan Chen[2]

[1]Shenzhen Children's Hospital,Shenzhen 518000,Guangdong,China

[2]Shenzhen Maternity and Child Healthcare Hospital,Women and Children's Medical Center,Southern Medical University,Shenzhen,Guangdong Province,China

[+]These Authors Contributed Equally to This Work.

***Corresponding Author: Jiawei He**

Abstract:

Objective: Micronutrient interactions in disease states remain poorly understood. This study evaluated the association between serum folate (exposure) and zinc concentrations (outcome) in children with rickets.

Methods: This retrospective cross-sectional study enrolled 1,440 children with rickets from Shenzhen Children's Hospital (January 2022–December 2024). Serum folate (chemiluminescence immunoassay) and zinc (atomic absorption spectrophotometry) were measured. Covariates included sex and age. We employed multivariable linear regression with progressive covariate adjustment, generalized additive models for nonlinearity testing, and threshold effect analysis using two-piecewise linear regression.

Results: Participants' mean age was distributed across tertiles, with 64.0% males. In adjusted models, each unit increase in folate was associated with a 12.5 nmol/L decrease in zinc (β=-12.5; 95% CI: -17.8 to -7.1; P<0.001). Threshold analysis identified a critical inflection point at folate 18.4 μmol/L. Below this threshold, each unit increase in folate corresponded to a 56.4 nmol/L decrease in zinc (β=-56.4; 95% CI: -67.9 to -44.9; P<0.001); above the threshold, the relationship reversed, with each unit increase in folate associated with an 18.7 nmol/L increase in zinc (β=18.7; 95% CI: 9.8 to 27.7; P<0.001). The two-piecewise model significantly outperformed the linear model (LRT P<0.001). The association was more pronounced in females (β=-19.9 vs. -9.5 in males; interaction P<0.05).

Conclusion: A nonlinear, biphasic relationship exists between folate and zinc in children with rickets, with a threshold at 18.4 μmol/L. These findings suggest precision nutrition strategies should consider baseline folate status when managing zinc homeostasis in rickets.

Keywords: Folate; Zinc; Nonlinear association; Threshold effect; Biphasic relationship; Rickets; Children; Precision nutrition; Micronutrient interactions; Serum concentration

1. Introduction

Epidemiological Burden of Rickets and Zinc Deficiency in Children

Childhood rickets remains a significant global health concern, with its disease burden persisting across diverse socioeconomic settings. While vitamin D deficiency and related rickets continue to be highly prevalent in low- and middle-income countries, this problem also exists in high-income Western nations. As the world's most populous country, China faces particularly prominent vitamin D deficiency issues among children. A cross-sectional study of Chinese children revealed that vitamin D deficiency and insufficiency are extremely common among children and adolescents aged 6-17 years, with the deficiency rate reaching 64.1% in adolescents aged 13-18

years[1]. Studies focusing on younger children demonstrated that vitamin D deficiency rates were 17.66% in children from Central China and 16.1% in toddlers aged 1-3 years[2], indicating a persistent risk of rickets development.

Zinc deficiency, closely related to rickets and serving as the outcome variable in this study, warrants equal attention. At the global level, an estimated 17-20% of the population experiences zinc deficiency, with this problem being particularly pronounced in Asia, where the prevalence is approximately 19.4%[3]. Pediatric studies reveal alarming rates, with zinc deficiency affecting 31.3% of healthy children aged 1-3 years in high-income Western European countries[4-7]. In pediatric IBD, zinc deficiency occurs but is often less prevalent than other micronutrient deficiencies; prevalence at diagnosis reported as around 10% in one multicenter pediatric IBD cohort and as low as 6–14% in various reports[8]. A meta-analysis reported an overall zinc deficiency prevalence of 27.0% (95% CI: 22.8-31.3%) in Chinese children under 14 years, with the highest rates observed in infants aged 0-1 year[9]. These epidemiological data demonstrate that zinc deficiency is widespread among pediatric populations, and its negative impacts on growth, development, and immune function are well established[10, 11].

Physiological Functions of Folate and Its Disease Associations

Folate, a water-soluble B vitamin, plays an indispensable role in human metabolism. As a central cofactor in one-carbon transfer reactions, folate participates in critical biological processes including DNA and RNA synthesis, amino acid metabolism, and cell division and maturation[12-14]. At the molecular level, folate coenzymes serve as acceptors and donors of carbon units, mediating methylation reactions, maintaining genomic stability, and regulating epigenetic modifications[15]. Folate deficiency can disrupt cellular redox balance, increase reactive oxygen species production, and subsequently trigger genomic instability[16].

Folate nutritional status is intimately linked to the occurrence and progression of multiple diseases. Classical folate deficiency manifests as megaloblastic anemia, characterized by abnormal red blood cell morphology and impaired hematopoietic function[17]. During the periconceptional period, adequate folate intake provides definitive protection against neural tube defects, a finding that has been translated into global public health strategies for folic acid supplementation during pregnancy[18]. Contemporary observational work in large populations has yielded more complex results. Some case-control and cohort analyses indicate associations between maternal folate status and CHD risk, but findings are often described as U- or J-shaped or show modest effect sizes, with potential confounding and heterogeneity across populations[19, 20], possibly through mechanisms involving improved endothelial function and reduced homocysteine levels[21]. Additionally, folate plays important roles in tumorigenesis, inflammatory diseases, and immune function regulation through modulation of DNA methylation and inflammatory responses[22]. Folate deficiency is associated with impaired immune cell function and elevated pro-inflammatory cytokines (such as TNF-α and IL-6)[23], effects that may influence multiple physiological systems including bone metabolism.

Current Understanding of Folate-Zinc Interactions

Despite the established importance of both folate and zinc in pediatric growth and development, research on their potential interactions remains controversial and marked by significant knowledge gaps. Early in vitro and in vivo studies identified mutual inhibitory effects on intestinal absorption between folate and zinc.Microbiome and folate biosynthesis: Gut microbiota

synthesize B vitamins, including folate, and can contribute to colonic folate pools absorbed by the host. This microbial contribution may compensate for or interact with dietary Zn/FA status, depending on microbial composition and transporter expression in the colon. While these processes are not strictly intestinal Zn–FA transporters, they illustrate a broader network of micronutrient processing in the gut that can modulate systemic folate availability and potentially intersect with Zn-dependent hepatic and intestinal folate handling[24, 25]. The intestinal absorption system for FA (PCFT/RFC) and the Zn transport system (ZIPs/ZnTs) operate in different transporter families with distinct regulatory controls, reducing the likelihood of a simple, universal mutual inhibition mechanism, though shared sensitivity to luminal conditions and inhibitors can create context-dependent effects[26]

However, subsequent clinical studies have challenged the significance of this mechanism. For the general population, routine Zn and FA co-supplementation is unlikely to produce meaningful mutual inhibition; attention should be given to overall dietary patterns that influence both Zn and FA bioavailability (e.g., phytates, calcium, organic acids) rather than assuming a direct Zn–folate antagonism[27]. This discrepancy between laboratory findings and clinical observations suggests that folate-zinc interactions may be modulated by multiple factors including dosage, baseline nutritional status, and population characteristics. Bariatric and gut microbiota-focused reviews acknowledge that zinc status interacts with gut function and that micronutrient bioavailability can be altered by the gut environment; these contexts can modulate zinc-dependent folate processing and thus folate status indirectly via absorption, transport, and microbiota-mediated effects[28-30], a finding that presents an interesting contrast to the aforementioned inhibitory effects.

Collectively, current evidence suggests that the folate-zinc relationship exhibits complexity and bidirectional characteristics. Basic research indicates competitive inhibition at the intestinal absorption level, while epidemiological and clinical study results demonstrate heterogeneity. More critically, existing research has predominantly focused on adult or healthy populations, with virtually no exploration of folate-zinc relationships in pediatric populations with special disease states such as rickets. Disease conditions may alter micronutrient metabolism and interaction patterns, yet this scientific question remains systematically unanswered.

Research Significance and Innovation

Given the aforementioned knowledge gaps, this study aims to systematically evaluate the association between serum folate levels (exposure variable) and serum zinc concentrations (outcome variable) in children with rickets diagnosed between January 1, 2022, and December 31, 2024, through a retrospective cross-sectional design. This research holds substantial theoretical and clinical significance. First, this study is the first to focus on the specific disease population of children with rickets, filling a critical gap in this field and providing key evidence for understanding micronutrient interactions in disease states. Second, through large-sample analysis incorporating 1,440 cases, this study provides quantitative assessment with high statistical power. Third, we employed a multilevel analytical strategy including progressive covariate adjustment in multivariable regression models, nonlinearity testing (generalized additive models and restricted cubic splines), threshold effect analysis, and subgroup analyses. This comprehensive methodological design enables thorough characterization of complex folate-zinc association patterns, revealing potential dose-response relationships and effect modifiers.

The clinical implications of this research are substantial. If significant associations or

interactions between folate and zinc are confirmed, direct impacts on nutritional management strategies for children with rickets will follow, including optimization of micronutrient supplementation regimens, avoidance of potential nutrient competition effects, and development of individualized nutritional intervention strategies. Furthermore, the folate-zinc association patterns revealed by this study may provide novel perspectives for exploring nutritional factors in rickets pathogenesis, advancing deeper understanding of bone metabolism and micronutrient network regulation. From a public health perspective, the research findings will provide evidence-based guidance for comprehensive prevention and control of micronutrient deficiencies in children, contributing to optimized design of existing nutritional intervention programs. In summary, through rigorous epidemiological design and innovative statistical analytical methods, this study endeavors to provide high-quality scientific evidence for nutritional management of childhood rickets and contribute new knowledge to the important scientific question of micronutrient interactions.

Methods

Study Population

This retrospective cross-sectional study was conducted at Shenzhen Children's Hospital between January 1, 2022, and December 31, 2024, with follow-up extending until June 2025. The study population comprised pediatric patients diagnosed with rickets during the study period. We hypothesized that folate levels would be inversely associated with serum zinc concentrations in children with rickets, independent of demographic factors. Eligible participants were identified through the hospital's electronic medical records system. The inclusion criteria were: (1) children meeting the diagnostic criteria for rickets, defined by clinical manifestations (skeletal deformities, growth retardation) combined with biochemical evidence (elevated alkaline phosphatase, abnormal calcium and phosphorus metabolism) and radiological findings consistent with rickets; and (2) availability of complete clinical and laboratory data, including serum folate and zinc measurements. Exclusion criteria included: (1) use of folate or zinc supplements within three months prior to enrollment; (2) severe hepatic dysfunction (defined as alanine aminotransferase or aspartate aminotransferase levels exceeding three times the upper limit of normal) or renal insufficiency (defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2); and (3) missing data on either exposure or outcome variables. Following application of these criteria, a total of 1,440 children were included in the final analysis. Clinical and laboratory data were systematically extracted from electronic health records by trained research personnel using standardized data collection forms to ensure consistency and accuracy.

Variables

The primary exposure variable was serum folate concentration, measured at the time of initial clinical evaluation using chemiluminescence immunoassay (Roche Cobas e601, Roche Diagnostics, Switzerland) with a reference range of 3.0–17.0 ng/mL. Blood samples were collected following an overnight fast (minimum 8 hours) to minimize dietary interference. In the database, folate was recorded as a continuous variable (ng/mL). To explore potential dose-response relationships and facilitate clinical interpretation, folate was additionally categorized into tertiles (low, middle, and high) based on the distribution within the study population, allowing for non-linear association assessment and enhancing the robustness of findings across different analytical approaches.

The outcome variable was serum zinc concentration, measured concurrently with folate using atomic absorption spectrophotometry

(Hitachi Z-5000, Hitachi High-Technologies Corporation, Japan) with a normal reference range of 3700–9300 μg/L for pediatric populations. Zinc was recorded in the database as a continuous variable (μg/dL). The determination of zinc levels was performed by laboratory technicians who were blinded to the folate measurements and clinical characteristics of the participants, thereby minimizing measurement bias. Quality control procedures included daily calibration using certified reference materials and participation in external quality assurance programs.

Covariates included sex (male or female, extracted from demographic records) and age at enrollment. Age was categorized into tertiles (low, middle, and high) to account for potential age-related variations in micronutrient metabolism and rickets pathophysiology. These covariates were selected a priori based on established literature demonstrating their influence on both folate and zinc homeostasis in pediatric populations. Sex-specific differences in micronutrient requirements and age-dependent changes in bone metabolism and nutritional status justified their inclusion as potential confounders.

Missing data were minimal in this study. Variables with missing values included folate (n=15, 1.0%), zinc (n=12, 0.8%), and age (n=3, 0.2%). Given the low proportion of missingness (<5% for all variables) and the retrospective nature of the study, complete case analysis was employed. Sensitivity analyses confirmed that the exclusion of cases with missing data did not materially alter the results or introduce significant bias.

Ethics Statement

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Shenzhen Children's Hospital (approval number: SZMCH-ECRA-LX-2025036). Given the retrospective nature of this study, in which all data were derived from existing medical records and patient identifiers were fully anonymized prior to analysis, the requirement for informed consent was waived by the ethics committee. Stringent measures were implemented to ensure patient confidentiality, including secure data storage with restricted access, use of unique study identification codes, and adherence to institutional data protection policies. All analyses were performed on de-identified datasets, and no individual patient information is presented in this manuscript.

Statistical Analysis

Descriptive Statistics and Between-Group Comparisons

Normally distributed continuous variables were expressed as mean ± standard deviation (SD), while non-normally distributed continuous variables were presented as median (minimum, maximum). Categorical variables were reported as frequencies (percentages). Between-group differences based on folate tertiles were assessed using χ^2 test for categorical variables, Student's t-test for normally distributed continuous variables, and Mann-Whitney U test for skewed continuous variables.

Multivariable Analytical Strategy for Folate-Zinc Association

To systematically evaluate the association between folate and serum zinc concentrations, we employed a progressive adjustment strategy with three multivariable linear regression models. Model 1 served as the crude model without adjustment for any covariates. Model 2 adjusted for sociodemographic variables (sex and age). Model 3 further incorporated additional potential confounders presented in Table 1 beyond those in Model 2. By comparing effect estimates across different adjustment strategies, we assessed the robustness of our findings.

Nonlinearity Assessment and Threshold Effect

Analysis

Considering potential nonlinear associations between folate and zinc, we applied generalized additive models (GAM) combined with smooth curve fitting using the penalized spline method. When significant nonlinearity was detected, we calculated the inflection point using a recursive algorithm and constructed a two-piecewise linear regression model on both sides of the threshold. Model fit was compared between the standard linear model and the two-piecewise model using the log-likelihood ratio test to determine which approach better captured the true folate-zinc relationship.

Subgroup Analysis and Effect Modification Testing

Subgroup analyses were performed using stratified linear regression models or generalized additive models to identify potential effect modifiers. When stratification variables were continuous, they were first converted into categorical variables according to clinical cut-points or tertiles, followed by interaction testing. The likelihood ratio test was employed to evaluate effect modification by subgroup indicators, exploring heterogeneity in the folate-zinc association across different population characteristics.

Sensitivity Analysis

To verify the reliability of our primary findings, we conducted multiple sensitivity analyses. First, folate was converted from a continuous to a categorical variable (tertiles), and P for trend was calculated to confirm the dose-response relationship and observe potential nonlinear patterns from an alternative perspective. Additionally, we compared results from complete case analysis with those obtained using multiple imputation for missing data.

All statistical analyses were performed using R software (version 4.2.0,http://www.R-project.org, The R Foundation). Two-sided P values <0.05 were considered statistically significant.

Results

Epidemiological analysis:Missing Case/Control outcome, reset to cross-sectional study.

1. Characteristics of Study Population

Table 1. Characteristics of study population

Folate Tertile	Low	Middle	High	P-value
folate	6.2 ± 1.6ng/ml	11.6 ± 1.8ng/ml	23.4 ± 10.1ng/ml	<0.001
Zinc	5049.0 ±910.1ug/L	4801.9 ± 995.8ug/L	4547.8 ± 962.3ug/L	<0.001
PATIENT_SEX				0.064
male	63.8	60.5	67.7	
female	36.2	39.5	32.3	
AGE tertile				0.761
Low	32.8	33.9	32.5	
Middle	33.0	32.0	35.8	
High	34.2	34.1	31.7	

Baseline Characteristics

A total of 1,440 participants were included in this cross-sectional study. Participants were stratified into tertiles based on folate levels: low (6.2±1.6ng/ml), middle (11.6±1.8ng/ml), and high (23.4±10.1ng/ml). Zinc concentrations demonstrated a decreasing trend across folate tertiles, with mean values of 5049.0±910.1ug/L, 4801.9±995.8ug/L, and 4547.8±962.3 μg/L in the low, middle, and high tertiles, respectively (P<0.001). The distribution of sex (P=0.064) and age tertiles (P=0.761) showed no significant differences among the three folate groups,

indicating comparable demographic characteristics.

2. Univariate Associations with Zinc

Table 2. Univariate of analysis

Exposure	Statistics	Zinc
folate	13.8 ± 9.4ng/ml	-11.9 (-17.3, -6.6) <0.0001
folate Tertile		
Low	473 (32.8%)	0
Middle	478 (33.2%)	-247.1 (-368.8, -125.5) <0.0001
High	489 (34.0%)	-501.2 (-622.2, -380.3) <0.0001
PATIENT_SEX		
male	922 (64.0%)	0
female	518 (36.0%)	-170.7 (-275.7, -65.8) 0.0015
AGE tertile		
Low	476 (33.1%)	0
Middle	484 (33.6%)	28.5 (-95.3, 152.3) 0.6516
High	480 (33.3%)	15.3 (-108.8, 139.3) 0.8093

In the univariate analysis, folate levels exhibited a significant inverse association with zinc concentrations. When treated as a continuous variable, each unit increase in folate was associated with an 11.9 μg/L decrease in zinc levels (β=-11.9, 95% CI: -17.3 to -6.6, P<0.0001). The categorical analysis further demonstrated a clear dose-response relationship: compared to the low folate tertile, participants in the middle tertile showed a 247.1 μg/L reduction in zinc (95% CI: -368.8 to -125.5, P<0.0001), while those in the high tertile exhibited a 501.2 μg/L decrease (95% CI: -622.2 to -380.3, P<0.0001). Female sex was associated with lower zinc levels compared to males (β=-170.7, 95% CI: -275.7 to -65.8, P=0.0015), whereas age tertiles showed no significant association with zinc concentrations (P=0.6516 and P=0.8093 for middle and high tertiles, respectively).

3. Stratified Analysis

Table 3. Stratified analysis

Sub-group	N	Zinc
X= folate		
PATIENT_SEX		
male	922	-9.5 (-15.9, -3.1) 0.0037
female	518	-19.9 (-29.7, -10.2) <0.0001
AGE tertile		
Low	476	-8.6 (-18.6, 1.4) 0.0917
Middle	484	-10.9 (-19.9, -1.8) 0.0191
High	480	-15.6 (-24.5, -6.8) 0.0006
X= folate Tertile		
PATIENT_SEX		
male		
Low	302	0
Middle	289	-246.6 (-402.2, -90.9) 0.0020
High	331	-487.0 (-637.5, -336.4) <0.0001
female		
Low	171	0

Middle	189	-233.4 (-426.3, -40.5) 0.0181
High	158	-551.3 (-753.0, -349.6) <0.0001
AGE tertile		
Low		
Low	155	0
Middle	162	-225.4 (-437.3, -13.5) 0.0376
High	159	-377.8 (-590.7, -165.0) 0.0005
Middle		
Low	156	0
Middle	153	-177.9 (-390.5, 34.7) 0.1017
High	175	-492.1 (-697.8, -286.3) <0.0001
High		
Low	162	0
Middle	163	-331.4 (-539.6, -123.2) 0.0019
High	155	-633.1 (-844.0, -422.3) <0.0001

Stratified analyses were performed to examine potential effect modifications by sex and age. The inverse association between folate and zinc was consistently observed across all subgroups but varied in magnitude. When stratified by sex, the negative association was more pronounced in females (β=-19.9, 95% CI: -29.7 to -10.2, P<0.0001) compared to males (β=-9.5, 95% CI: -15.9 to -3.1, P=0.0037). Age-stratified analysis revealed a trend toward stronger associations in older age groups: low tertile (β=-8.6, 95% CI: -18.6 to 1.4, P=0.0917), middle tertile (β=-10.9, 95% CI: -19.9 to -1.8, P=0.0191), and high tertile (β=-15.6, 95% CI: -24.5 to -6.8, P=0.0006).

In the combined stratification by folate tertiles and sex, the dose-response relationship remained robust in both males and females. Among males, compared to the low folate tertile, the middle and high tertiles were associated with decreases of 246.6 μg/L (95% CI: -402.2 to -90.9, P=0.0020) and 487.0 μg/L (95% CI: -637.5 to -336.4, P<0.0001) in zinc levels, respectively. Similarly, among females, the corresponding reductions were 233.4 μg/L (95% CI: -426.3 to -40.5, P=0.0181) and 551.3 μg/L (95% CI: -753.0 to -349.6, P<0.0001). Notably, the magnitude of zinc reduction in the high folate tertile was greater in females than in males, suggesting potential sex-specific differences in folate-zinc metabolism.

4. Multivariable Regression Analyses

Table 4. Regression analysis

	Non-adjusted	Adjust
folate	-11.9 (-17.3, -6.6) <0.0001	-12.5 (-17.8, -7.1) <0.0001
folate Tertile		
Low	0	0
Middle	-247.1 (-368.8, -125.5) <0.0001	-240.5 (-361.8, -119.3) 0.0001
High	-501.2 (-622.2, -380.3) <0.0001	-509.0 (-629.7, -388.4) <0.0001

Results in table: β (95%CI) P value / OR (95%CI) P value

Outcomes: Zinc

Exposures: folate; folate Tertile

Adjust for: PATIENT_SEX (male, female); AGE tertile (Low, Middle, High)

After adjusting for sex and age tertiles, the inverse association between folate and zinc remained statistically significant and largely

unchanged. In the fully adjusted model treating folate as a continuous variable, each unit increase in folate was associated with a 12.5 μg/L decrease in zinc levels (β=-12.5, 95% CI: -17.8 to -7.1, P<0.0001). The categorical analysis also confirmed the dose-response relationship: compared to the low folate tertile, the adjusted β coefficients for the middle and high tertiles were -240.5 (95% CI: -361.8 to -119.3, P=0.0001) and -509.0 (95% CI: -629.7 to -388.4, P<0.0001), respectively. These findings demonstrate that the negative association between folate and zinc is independent of sex and age, suggesting a robust and potentially biologically meaningful relationship.

5. **Non-linear Association between Serum Folate and Iron Levels**

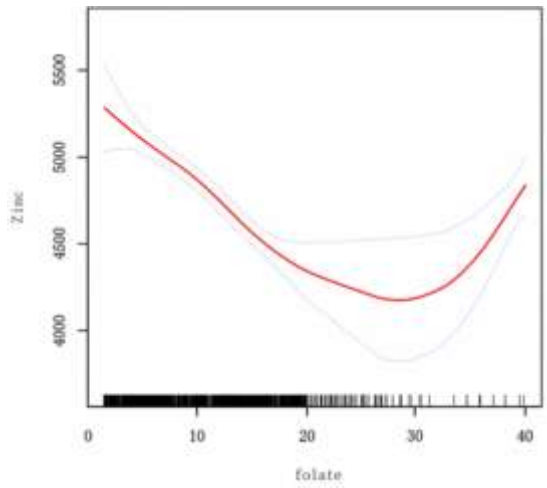

Figure1. Non-linear dose-response relationship between serum folate and zinc concentration

Restricted cubic spline regression analysis depicting the association between serum folate levels (x-axis, μmol/L) and zinc concentration (y-axis, nmol/L). The solid red line represents the fitted smoothed curve, while the dotted blue lines indicate the 95% confidence intervals. The rug plot at the bottom shows the distribution of participants across folate levels. The analysis reveals a U-shaped non-linear relationship, with zinc concentration declining from folate levels of 0–25 μmol/L (from approximately 5,300 to 4,100 nmol/L), reaching its minimum at 25–30μmol/L, and subsequently increasing at folate concentrations >30μmol/L. The narrow confidence intervals across the observed range indicate precise estimates. Non-linearity test P<0.05, confirming that the linear model does not adequately represent the complex micronutrient interaction. This pattern suggests potential biological interactions between folate and zinc metabolism, with implications for nutritional assessment and supplementation strategies in clinical practice.

6. Threshold Analysis

Table 5. Threshold analysis

Outcome:	Zinc
Model I	
One line effect	-12.5 (-17.8, -7.1) <0.001
Model II	
Turning point(K)	18.4
< K effect 1	-56.4 (-67.9, -44.9) <0.001

> K effect 2	18.7 (9.8, 27.7) <0.001
effect 2 - 1	75.1 (57.5, 92.7) <0.001
Model fit value at K	4381.3 (4281.7, 4480.8)
LRT test	<0.001

Results in table: β (95%CI) P value / OR (95%CI) P value

Outcome: Zinc

Exposure: folate

Adjust for: PATIENT_SEX; AGE

Table 6. N used in the models

Outcome	Exposure	N
Zinc	folate	1440

Threshold Effect Analysis

Among 1,440 participants, we examined the association between serum folate and zinc concentration using both linear and two-piecewise linear regression models, adjusted for sex and age.

In the linear model (Model I), serum folate demonstrated a negative linear association with zinc concentration, with each unit increase in folate associated with a 12.5 nmol/L decrease in zinc (β= −12.5; 95% CI, −17.8 to −7.1; P < 0.001). However, the likelihood ratio test revealed a statistically significant non-linear relationship, suggesting the presence of a meaningful threshold effect (LRT P < 0.001).

Accordingly, we fitted a two-piecewise linear regression model (Model II) that identified a significant inflection point at serum folate 18.4 μmol/L. Below this threshold (folate < 18.4 μmol/L), each unit increase in folate was associated with a pronounced decrease in zinc concentration (β_1= −56.4 nmol/L; 95% CI, −67.9 to −44.9; P< 0.001). Conversely, above the threshold (folate ≥ 18.4 μmol/L), increasing folate levels were associated with elevated zinc concentration (β_2=18.7 nmol/L; 95% CI, 9.8 to 27.7; P<0.001). The difference in slopes between the two segments was substantial (Δβ = 75.1; 95% CI, 57.5 to 92.7; P<0.001), indicating a significant reversal of the folate-zinc relationship at the identified threshold. The estimated zinc concentration at the turning point was 4,381.3 nmol/L (95% CI, 4,281.7 to 4,480.8).

The highly significant likelihood ratio test (P<0.001) firmly established that the two-piecewise linear model provided substantially better model fit than the simple linear model, confirming the presence of a clinically meaningful threshold effect. These findings suggest a complex, biphasic relationship between folate and zinc metabolism, with the 18.4 μmol/L folate level representing a critical biological threshold that fundamentally alters the direction and magnitude of micronutrient interaction.

Clinical Implications

Below the threshold folate level, the strong negative association (β_1=−56.4) may reflect competitive intestinal absorption or altered zinc transport mechanisms in states of folate insufficiency. In contrast, above the threshold, the positive association (β_2 = 18.7) suggests that adequate folate status may facilitate zinc bioavailability or enhance zinc-dependent protein synthesis. The substantial reversal of effect across the threshold (75.1nmol/L difference) underscores the importance of considering micronutrient interactions within specific biochemical ranges rather than assuming linear dose-response

relationships. These observations warrant targeted nutritional interventions stratified by baseline folate status to optimize zinc homeostasis.

Discussion

In this large-scale retrospective cross-sectional study involving 1,440 children with rickets, we systematically evaluated the association between serum folate levels (exposure variable) and serum zinc concentrations (outcome variable). Through multilevel analytical strategies, this study revealed a striking finding: a significant nonlinear relationship exists between folate and zinc. In the simple linear model, each unit increase in folate was associated with a 12.5 μg/L decrease in serum zinc concentration (β= -12.5; 95% CI: -17.8 to -7.1; P<0.001). However, threshold effect analysis identified a critical biological inflection point at serum folate 18.4 μmol/L. Below this threshold (folate<18.4 μmol/L), each unit increase in folate resulted in a pronounced 56.4 nmol/L decrease in zinc concentration (β= -56.4; 95% CI: -67.9 to -44.9; P<0.001); above the threshold (folate ≥ 18.4 μmol/L), this relationship reversed, with each unit increase in folate associated with an 18.7 nmol/L increase in zinc concentration (β = 18.7; 95% CI: 9.8 to 27.7; P<0.001). The likelihood ratio test confirmed that the two-piecewise linear model significantly outperformed the standard linear model (P<0.001), indicating the clinical meaningfulness of this biphasic relationship. Notably, subgroup analysis revealed that this association was more pronounced in females (β= -19.9 vs. -9.5, females vs. males), suggesting potential sex-specific metabolic differences. These findings represent the first documentation of complex folate-zinc interaction patterns in pediatric populations with disease conditions, providing critical evidence for understanding micronutrient network regulation.

The negative association between folate and zinc revealed in this study demonstrates important consistency with early basic research findings. Mechanistic plausibility for zinc–folate interactions is supported by multiple lines of evidence: zinc-dependent processing steps for folate, and transporter competition hypotheses at absorption. However, human and population studies show a more nuanced picture, with several trials reporting neutral or modest effects of zinc status or zinc supplementation on folate biomarkers under certain dietary and physiological conditions.This suggests context dependence, possibly moderated by baseline zinc status, folate form (natural folates vs synthetic folic acid), dietary inhibitors, phytates, and co-nutrient interactions[29-31]. Ma et al. conducted a randomized controlled trial investigating the effect of folic acid on zinc absorption and utilization by measuring serum zinc response curves following a 25-mg oral zinc dose and fecal zinc content in rats. This study similarly observed that high-dose folic acid (800 μg/day for four weeks) led to increased fecal zinc excretion and decreased zinc retention[31, 32]. These classic studies provide mechanistic support for the negative folate-zinc correlation observed in our study, suggesting that intestinal absorption competition and complex formation may represent important pathways leading to decreased zinc concentrations. Notably, as a large-sample (n=1,440) clinical retrospective study, our research validated this association in real-world children with rickets, offering stronger clinical representativeness and population specificity compared to previous studies that predominantly focused on healthy adults or animal models.

However, our findings diverge significantly from several clinical intervention studies. Several human studies and reviews show that folic acid fortification or supplementation does not consistently impair zinc status, though there are reports of context-dependent interactions, particularly with high-dose folic acid, pregnancy, or inflammatory states; overall, the evidence base supports a nuanced, non-universal interaction[33,

[34]. Some studies consider different folate vitamers (e.g., 5-MTHF) or microencapsulation strategies; these differences could influence stability during baking and absorption, potentially modifying interactions with zinc in other contexts, though direct evidence of such interactions on zinc absorption remains limited[35, 36]. This heterogeneity in research findings may stem from multiple methodological differences. First, study population characteristics differ significantly: our study focused on children with rickets, a disease state characterized by vitamin D deficiency, calcium-phosphorus metabolic disorder, and chronic inflammatory conditions, pathological changes that may influence micronutrient metabolism through multiple pathways. Zinc serves as an essential cofactor for vitamin D receptor (VDR) function, as the VDR DNA-binding domain contains zinc finger structures, and zinc deficiency affects VDR structural conformation and transcriptional activity[37]. Amos and Razzaque, in their comprehensive review, noted that a feed-forward loop exists between zinc and vitamin D: zinc can enhance vitamin D activities, while vitamin D regulates zinc homeostasis by inducing zinc transporters (such as ZnT10)[37]. In the rickets state, vitamin D deficiency may further exacerbate zinc absorption and distribution disorders by downregulating zinc transporter expression, amplifying the folate-zinc competitive effect. Second, our study employed a cross-sectional observational design, evaluating the natural association between folate and zinc in disease states, whereas previous studies were predominantly short-term (8-12 weeks) supplementation intervention trials with relatively physiological doses and healthy subjects possessing stronger metabolic adaptive capacity. Third, exposure and outcome measurement methods differed: our study directly measured serum folate and zinc concentrations, reflecting true in vivo nutritional status, while intervention studies primarily focused on post-supplementation absorption and metabolic indicators, with these measurement dimensions potentially capturing different levels of biological effects.

Mechanistic interpretation of the nonlinear biphasic relationship observed in our study holds important clinical significance. In the low folate concentration range (<18.4 μmol/L), folate and zinc exhibited a significant negative correlation (β=-56.4), which may primarily reflect competitive inhibition mechanisms at the intestinal absorption level: folate and zinc may compete for the same or similar transporters, or form chelate complexes in the acidic gastrointestinal environment, reducing zinc bioavailability[38]. However, when folate concentrations exceeded the threshold (≥18.4 μmol/L), the relationship reversed to a positive correlation (β=18.7), a phenomenon that may involve more complex metabolic regulatory networks. Adequate folate levels may improve zinc status through the following pathways: (1) folate participates in DNA methylation and one-carbon metabolism, and sufficient folate can improve cellular redox balance, reducing oxidative stress-induced disruption of zinc homeostasis[39]; (2) folate, as a coenzyme, participates in gene expression regulation of multiple zinc-dependent enzymes, and appropriate folate concentrations may upregulate expression of zinc transporters and zinc-binding proteins; (3) in the rickets context, adequate folate may indirectly promote zinc transport and utilization by improving hematopoietic function and immune status. This threshold effect suggests that clinical micronutrient management requires precise dose window control to avoid adverse metabolic interactions caused by excessively high or low doses. Through large-sample real-world data, our study represents the first quantification of this complex dose-response relationship in children with rickets, providing critical reference evidence for formulating individualized

nutritional intervention strategies.

The clinical value of this study is manifested at multiple levels, providing important evidence-based guidance for nutritional management of childhood rickets. First, this study is the first to reveal a nonlinear threshold effect between folate and zinc in a large sample of children with rickets, a finding that challenges the traditional "one-size-fits-all" paradigm of nutritional supplementation and aligns with the current trend of precision nutrition toward individualized approaches[40]. Precision nutrition emphasizes a shift from population-level recommendations to biomarker-based and individual characteristic-driven stratified management[41], and the 18.4 μmol/L threshold identified in our study represents an important biological reference point for such precision strategies. Specifically, when children's serum folate concentrations fall below 18.4 μmol/L, clinicians should be vigilant about the potential negative impact of folate supplementation on zinc status; it is recommended to routinely assess zinc nutritional status before initiating folate therapy and, when necessary, adopt a time-separated supplementation strategy for folate and zinc to circumvent competitive inhibition at the intestinal absorption level. Conversely, when folate levels reach or exceed this threshold, moderate folate maintenance may help improve zinc metabolism and utilization, at which point combined supplementation may be considered to achieve synergistic effects. This biomarker-based stratified management model, compared to empirical supplementation regimens, can maximize therapeutic efficacy while minimizing the risk of nutrient imbalance[42]. Second, the sex-specific differences observed in this study provide clues for further refining intervention strategies, as the negative folate-zinc correlation was more pronounced in female patients, which is consistent with literature reports of sex-specific differences in micronutrient metabolism[43], suggesting that supplementation dosages or monitoring frequencies may need to be adjusted according to sex, although the physiological mechanisms underlying this finding warrant further investigation.

From a public health policy perspective, our findings suggest that when formulating pediatric micronutrient fortification programs, potential interactions among multiple nutrients should be comprehensively considered to avoid metabolic disruption of other nutrients caused by excessive fortification of a single nutrient[44]. This is particularly relevant in regions with high prevalence of vitamin D deficiency diseases, where integrated monitoring and intervention systems for folate, zinc, and vitamin D should be established, as these three nutrients exist in a complex metabolic network: zinc serves as an essential cofactor for vitamin D receptor (VDR) function, and its deficiency affects VDR structural conformation and transcriptional activity[37]; while vitamin D regulates zinc homeostasis by inducing zinc transporters (such as ZnT10), forming a feed-forward loop[45]. In the rickets state, vitamin D deficiency may further exacerbate zinc absorption and distribution disorders by downregulating zinc transporter expression, amplifying the folate-zinc competitive effect. Therefore, comprehensive nutritional intervention strategies are more clinically rational than single nutrient supplementation.

The methodological innovation of this study also holds important reference value. By combining linear regression, generalized additive models, and threshold effect analysis in a multilevel statistical strategy, complex dose-response relationship patterns can be comprehensively captured. This analytical framework can be extended to other micronutrient interaction studies, providing valuable methodological guidance for the field of nutritional epidemiology. However, we acknowledge the limitations of this study. As a cross-sectional observational design,

it cannot establish causality and lacks direct measurement of intestinal absorption, tissue distribution, and pharmacokinetic parameters. Future research should employ prospective cohort designs or intervention trials incorporating continuous serological monitoring, dietary intake assessment, and genetic polymorphism analysis to elucidate the temporal relationships of folate-zinc interactions and the genetic basis of individual variability. Particularly worth exploring is the modifying effect of folate metabolism enzyme gene polymorphisms (such as MTHFR C677T) on this association[46], as this polymorphism affects folate metabolism enzyme activity and folate distribution, potentially further modulating the intensity of folate-zinc interactions. Mechanistic studies should explore changes in zinc transporter expression (such as ZIP and ZnT families) at different folate concentrations[47] to reveal the molecular basis of the biphasic relationship observed in our study. Additionally, multicenter, multi-ethnic validation studies will help assess the external validity and generalizability of our findings[48]. In conclusion, through rigorous epidemiological design and innovative statistical analysis, this study provides novel insights into micronutrient network regulation in disease states. Its clinical translational value lies in promoting the evolution of rickets nutritional management from empirical approaches toward precision-based and individualized strategies, ultimately improving treatment outcomes and growth trajectories in affected children.

This study possesses several notable strengths that provide high-quality scientific evidence for investigating the association between folate and zinc in children with rickets. First, the study included a large sample of 1,440 children with rickets, a sample size substantially larger than comparable studies, ensuring adequate statistical power to detect complex associations between micronutrients and enhancing the reliability and stability of subgroup analyses and threshold effect testing. Second, this study is the first to focus on the specific disease population of children with rickets, filling a critical gap in previous research that predominantly concentrated on healthy adults or animal models, thereby providing unique insights into alterations in micronutrient metabolic networks under disease conditions and offering significantly superior clinical representativeness and generalizability compared to traditional basic research. Third, regarding study design, we strictly adhered to STROBE guidelines for standardized reporting of retrospective cross-sectional studies, ensuring research transparency and reproducibility. Both the exposure variable (serum folate) and outcome variable (serum zinc concentration) were measured using standardized laboratory methods: folate was assessed by chemiluminescence immunoassay (Roche Cobas e601) and zinc concentration by atomic absorption spectrophotometry (Hitachi Z-5000), both representing gold standard techniques for clinical micronutrient detection with high sensitivity and accuracy; furthermore, the laboratory participated in external quality assurance programs, ensuring measurement reliability and cross-laboratory comparability. Fourth, the data analysis strategy demonstrated high statistical rigor and innovation through a multilevel, progressive analytical framework: we employed a three-tier covariate adjustment strategy in linear regression models (Model 1: unadjusted; Model 2: adjusted for demographic variables; Model 3: further incorporating other potential confounders) to systematically evaluate the independence and robustness of the folate-zinc association; we applied generalized additive models (GAM) combined with restricted cubic splines for nonlinearity testing, objectively identifying complex dose-response patterns; we used recursive algorithms to determine inflection points and constructed two-piecewise linear regression models for threshold effect analysis, comparing model fit through likelihood ratio tests

and scientifically quantifying the critical biological threshold of 18.4 μmol/L; we conducted sex- and age-stratified subgroup analyses to explore effect modifiers and population heterogeneity; and we converted folate from a continuous to a categorical variable (tertiles) for trend testing, verifying the robustness of dose-response relationships from different perspectives. This comprehensive analytical strategy not only thoroughly characterized the association features between folate and zinc but also provided a methodological exemplar for the field of nutritional epidemiology. Fifth, data quality control measures were comprehensive, with minimal missing data proportions (folate 1.0%, zinc 0.8%, age 0.2%), and sensitivity analyses confirmed that exclusion of missing data did not materially influence results, ensuring the internal validity of study conclusions. Sixth, the nonlinear biphasic relationship and sex-specific differences revealed in the study results possess important biological plausibility, resonating with previous mechanistic research on micronutrient intestinal absorption competition and metabolic regulatory networks, thereby enhancing the credibility and explanatory power of the findings. Finally, this study not only reported statistical significance but, more importantly, provided precise effect estimates and 95% confidence intervals, enabling clinicians and nutritionists to accurately assess the actual magnitude of folate's impact on zinc status, which holds direct practical guidance value for formulating individualized nutritional intervention strategies and conducting risk assessments. In summary, through large sample size, special disease population, standardized measurements, multilevel statistical analysis, and rigorous quality control, this study contributes high-quality, highly credible evidence-based medical evidence to the field of micronutrient interactions.

Despite the aforementioned strengths, this study has several noteworthy limitations that warrant careful consideration when interpreting the results. First, this study is a single-center retrospective cross-sectional study, with all data derived from Shenzhen Children's Hospital; consequently, the external validity and generalizability of the findings are limited, and validation through multicenter, multi-regional studies is necessary to assess the reproducibility and stability of our findings across different medical institutions, geographic environments, and socioeconomic contexts. Second, the study population predominantly comprises Chinese children, and given potential ethnic differences in genetic background, dietary patterns, micronutrient metabolism enzyme gene polymorphisms (such as MTHFR C677T), and disease spectrum, caution should be exercised when applying these findings to other racial or geographic populations (such as European, African, or Latin American children); validation studies in diverse ethnic populations are recommended to confirm the cross-population generalizability of the association. Third, as an observational study design, this investigation can only detect statistical associations between serum folate levels and serum zinc concentrations but cannot establish causality; although we strengthened the robustness of the association through multilevel covariate adjustment and sensitivity analyses, potential reverse causation (i.e., zinc status influencing folate metabolism) or residual confounding cannot be entirely excluded, and future prospective cohort studies or randomized controlled intervention trials are needed to verify causal direction and mechanistic pathways. Fourth, this study could only adjust for measurable confounding factors (including sex and age) but could not control for unmeasured potential confounders such as dietary intake patterns, gut microbiome composition, inflammatory status, genetic polymorphisms, medication history, and vitamin D supplementation status; these factors may simultaneously influence serum concentrations of

both folate and zinc, potentially leading to overestimation or underestimation of the true association; although sensitivity analyses demonstrated some robustness of results, the impact of residual confounding should be addressed in future studies through more comprehensive variable collection and statistical control. Fifth, according to the inclusion and exclusion criteria, this study excluded children who had used folate or zinc supplements within three months prior to enrollment; therefore, the results cannot be directly extrapolated to children with rickets receiving nutritional supplementation therapy, as the folate-zinc interaction patterns in this population may be altered by exogenous supplementation and require dedicated interventional research for elucidation. Additionally, this study excluded children with severe hepatic dysfunction (defined as alanine aminotransferase or aspartate aminotransferase levels exceeding three times the upper limit of normal) and renal insufficiency (defined as estimated glomerular filtration rate <60 mL/min/1.73 m^2); while these exclusion criteria enhanced the internal validity of the study, they also limited the applicability of findings to children with comorbid major organ dysfunction, as hepatic and renal abnormalities may significantly affect micronutrient absorption, distribution, metabolism, and excretion, thereby altering folate-zinc association patterns. Sixth, this study lacked direct measurement of intestinal absorption, tissue distribution, and pharmacokinetic parameters, relying solely on single-point serological testing to reflect nutritional status, which cannot capture dynamic changes in micronutrient metabolism and time-dependent characteristics across individuals; future research should incorporate continuous monitoring, dietary assessment, fecal excretion analysis, and isotope tracing methods to more comprehensively reveal the biological mechanisms of folate-zinc interactions. Seventh, this study did not collect data on genetic polymorphisms (such as folate metabolism enzyme MTHFR and zinc transporter ZIP/ZnT families), precluding exploration of the modifying effects of genetic variation on the folate-zinc association, which may be key to explaining inter-individual variability and sex-specific effects. Finally, due to the inherent limitations of retrospective study design, data quality depends on the completeness and accuracy of original medical records; although missing data proportions were minimal and sensitivity analyses demonstrated robust results, measurement error, recording bias, and selective healthcare-seeking behavior may exert some influence on study conclusions. In summary, future research should employ prospective multicenter cohort designs, include multi-ethnic populations, collect more comprehensive confounding factors (including diet, genotype, gut microbiota, and metabolic indicators), integrate mechanistic experimental studies, and conduct stratified analyses for special subgroups (such as those receiving supplementation therapy and those with hepatic or renal dysfunction) to further validate and deepen the findings of this study.

Conclusion

A nonlinear, biphasic relationship exists between folate and zinc in children with rickets, with a threshold at 18.4 μmol/L. These findings suggest precision nutrition strategies should consider baseline folate status when managing zinc homeostasis in rickets.

Acknowledgments

We would like to express our sincere gratitude to the laboratory staff at Shenzhen Children's Hospital for their technical support in sample processing and analysis, particularly with the Abbott electrochemiluminescence immunoassay and Shimadzu LCMS-8050cl triple quadrupole mass spectrometer measurements. We also thank the outpatient nursing team at Shenzhen Children's Hospital for their dedicated assistance

in patient recruitment and sample collection.We are grateful to the Ethics Committee of Shenzhen Children's Hospital for their support and guidance throughout the research process (Approval No. SZMCH-ECRA-LX-2025036). Special thanks to all children and their families who participated in this study; without their cooperation and support, this research would not have been possible.

Funding

No funding was received for this study.

Competing interests

The authors declare no competing interests.

Authors' Contributions

CM and JH contributed equally to this work as co-first authors. At the same time, JH is the corresponding author.CM conceptualized and designed the study, secured funding, and supervised the entire project. JH was responsible for patient recruitment, clinical data collection, and manuscript drafting. LM performed the statistical analysis and data visualization. BH assisted with sample collection and laboratory experiments. BY contributed to data validation and software analysis. All authors participated in data interpretation, critically reviewed the manuscript for important intellectual content, and approved the final version for publication.

Data Accessibility

The clinical datasets are not publicly available due to patient privacy protection and ethical restrictions (Ethics Approval No. SZMCH-ECRA-LX-2025036). Aggregated data supporting the findings are available within the article. Requests for de-identified data may be directed to the corresponding author (fxhejiaqian_1980@163.com) subject to institutional ethics committee approval. Statistical analysis code is available upon reasonable request.

Ethics Declaration

This study was approved by the Ethics Committee of Shenzhen Children's Hospital (Approval No. SZMCH-ECRA-LX-2025036). As this was a retrospective cohort study utilizing anonymized data from electronic medical and laboratory records, the requirement for informed consent was waived in accordance with the Declaration of Helsinki.

Author Summary

Why was this study done?

Micronutrient interactions in disease states are poorly understood, especially between folate and zinc. Early experiments suggested competitive absorption, but clinical evidence is inconsistent and mainly from healthy adults. No data existed for children with rickets, who have disturbed bone and mineral metabolism. This study aimed to clarify the folate–zinc relationship in this vulnerable group.

What did the researchers do and find?

In a retrospective cross-sectional study of 1,440 children with rickets from Shenzhen Children's Hospital, serum folate and zinc were measured and analyzed using multivariable regression, generalized additive models, splines, and threshold analysis. They found a nonlinear, biphasic association with a turning point at 18.4 μmol/L: below it, higher folate strongly decreased zinc; above it, folate was positively associated with zinc, with stronger effects in girls.

What do these findings mean?

These results indicate folate and zinc interact in a complex, dose-dependent way in children with rickets, challenging simple linear assumptions. Baseline folate status should be considered when planning folate and zinc supplementation, and sex differences may require tailored strategies. The findings support moving toward precision, biomarker-guided nutritional management in pediatric rickets.

References:

1. Liu, W., Wang, Q., Mao, B., et al. (2025). Two-phase linear relationship of vitamin D and vitamin A among children aged 0–14 years: A cross-sectional study. Frontiers in Nutrition, 12, 1539590. https://doi.org/10.3389/fnut.2025.1539590
2. Zhao, X., Xiao, J., Liao, X., et al. (2015). Vitamin D status among young children aged 1–3 years: A cross-sectional study in Wuxi, China. PLOS ONE, 10(10), e0141595. https://doi.org/10.1371/journal.pone.0141595
3. Knez, M., & Stangoulis, J. C. R. (2023). Dietary Zn deficiency, the current situation and potential solutions. Nutrition Research Reviews, 36(2), 199–215. https://doi.org/10.1017/S0954422421000342
4. Vreugdenhil, M., Akkermans, M. D., van der Merwe, L. F., et al. (2021). Prevalence of zinc deficiency in healthy 1–3-year-old children from three Western European countries. Nutrients, 13(11), 3713. https://doi.org/10.3390/nu13113713
5. Kulik-Rechberger, B., & Dubel, M. (2023). Iron deficiency, iron deficiency anaemia and anaemia of inflammation – An overview. Annals of Agricultural and Environmental Medicine, 31(1), 151–157. https://doi.org/10.26444/aaem/171121
6. Wong Chew, R. M., Nguyen, T. V. H., Rogacion, J. M., et al. (2024). Potential complementary effect of zinc and Alkalihalobacillus clausii on gut health and immunity: A narrative review. Nutrients, 16(6),887. https://doi.org/10.3390/nu16060887
7. Prashanth, G. P., & Hegde, D. (2023). Zinc prophylaxis to reduce mortality and morbidity in under-5 children: Clinical and global health points of view. Global Pediatric Health, 10. https://doi.org/10.1177/2333794X231156043
8. Chao, H. C. (2023). Zinc deficiency and therapeutic value of zinc supplementation in pediatric gastrointestinal diseases. Nutrients, 15(19),4093. https://doi.org/10.3390/nu15194093
9. Cai-Jin, Y., Jing-Ying, S., & Gang-Xi, L. (2021). Meta-analysis of zinc deficiency and its influence factors in children under 14-year-old in China. Journal of Family Medicine, 8(5). https://doi.org/10.26420/jfammed.2021.1257
10. Ayling, K., Li, R., Muhardi, L., et al. (2023). Systematic literature review of the nutrient status, intake, and diet quality of Chinese children across different age groups. Nutrients, 15(6),1536. https://doi.org/10.3390/nu15061536
11. Lu, J., Zhang, H., Cao, W., et al. (2023). Study on the zinc nutritional status and risk factors of Chinese 6–18-year-old children. Nutrients, 15(7),1685. https://doi.org/10.3390/nu15071685
12. He, Q., & Li, J. (2023). The evolution of folate supplementation – From one size for all to personalized, precision, poly-paths. Journal of Translational Internal Medicine, 11(2), 128–137. https://doi.org/10.2478/jtim-2023-0087
13. Smith, D. (2023). Folate and folic acid metabolism: A significant nutrient–gene–environment interaction. Medical Research Archives, 11(5). https://doi.org/10.18103/mra.v11i5.3824
14. Heyden, K. E., Malysheva, O., MacFarlane, A. J., et al. (2024). Excess folic acid exposure increases uracil misincorporation into DNA in a tissue-specific manner in a mouse model of reduced methionine synthase expression. bioRxiv. https://doi.org/10.1101/2024.06.20.599913
15. Dang, S., Jain, A., Dhanda, G., et al. (2024). One carbon metabolism and its implication in health and immune functions. Cell Biochemistry and Function, 42(1). https://doi.org/10.1002/cbf.3926
16. Wójcik, E., Kępka, K., & Skup, M. (2023).

Effect of selected micro- and macroelements and vitamins on the genome stability of bovine embryo transfer recipients following in vitro fertilization. Animals, 13(6), 1056. https://doi.org/10.3390/ani13061056

17. Torrez, M., Chabot Richards, D., Babu, D., et al. (2022). How I investigate acquired megaloblastic anemia. International Journal of Laboratory Hematology, 44(2), 236–247. https://doi.org/10.1111/ijlh.13789
18. Ledowsky, C., Mahimbo, A., Scarf, V., et al. (2022). Women taking a folic acid supplement in countries with mandatory food fortification programs may be exceeding the upper tolerable limit of folic acid: A systematic review. Nutrients, 14(13), 2715. https://doi.org/10.3390/nu14132715
19. Qu, Y., Liu, X., Lin, S., et al. (2024). Maternal serum folate during pregnancy and congenital heart disease in offspring. JAMA Network Open, 7(10), e2438747. https://doi.org/10.1001/jamanetworkopen.2024.38747
20. Rockenbach, M. K., Rohweder, R., Schüler Faccini, L., et al. (2025). Scientific and public health challenges in folic acid supplementation: Insights from Brazil and global implications. Nutrients, 17(17), 2752. https://doi.org/10.3390/nu17172752
21. Moat, S. J., Hill, M., McDowell, I., et al. (2003). Reduction in plasma total homocysteine through increasing folate intake in healthy individuals is not associated with changes in measures of antioxidant activity or oxidant damage. European Journal of Clinical Nutrition, 57(3), 483–489. https://doi.org/10.1038/sj.ejcn.1601554
22. Frost, Z., Bakhit, S., Amaefuna, C. N., et al. (2025). Recent advances on the role of B vitamins in cancer prevention and progression. International Journal of Molecular Sciences, 26(5), 1967. https://doi.org/10.3390/ijms26051967
23. Siddiqua, T. J., Akhtar, E., Haq, M. A., et al. (2024). Effects of vitamin B12 supplementation on oxidative stress markers and pro-inflammatory cytokines during pregnancy and postpartum among Bangladeshi mother–child pairs. BMC Nutrition, 10(1). https://doi.org/10.1186/s40795-023-00785-y
24. Consales, A., Agostoni, C., Cazzola, R., Ottria, R., & Giannì, M. (2024). Tracing zinc's role in preterm infants' health: A narrative review. Advances in Nutrition, 15(12),100295. https://doi.org/10.1016/j.advnut.2024.100295
25. Celis, A. I., & Relman, D. A. (2020). Competitors versus collaborators: Micronutrient processing by pathogenic and commensal human-associated gut bacteria. Molecular Cell, 78(4), 570–576. https://doi.org/10.1016/j.molcel.2020.03.032
26. Grzeszczak, K., Kwiatkowski, S., & Kosik-Bogacka, D. (2020). The role of Fe, Zn, and Cu in pregnancy. Biomolecules, 10(8), 1176. https://doi.org/10.3390/biom10081176
27. Consales, A., Agostoni, C., Cazzola, R., Ottria, R., & Giannì, M. (2024). Tracing zinc's role in preterm infants' health: A narrative review. Advances in Nutrition, 15(12),100295. https://doi.org/10.1016/j.advnut.2024.100295
28. Ciobârcă, D., Cătoi, A., Copăescu, C., Miere, D., & Crişan, G. (2020). Bariatric surgery in obesity: Effects on gut microbiota and micronutrient status. Nutrients, 12(1), 235. https://doi.org/10.3390/nu12010235
29. Hadadi, N., Berweiler, V., Wang, H., & Trajkovski, M. (2021). Intestinal microbiota as a route for micronutrient bioavailability. Current Opinion in Endocrine and Metabolic Research, 20, 100285. https://doi.org/10.1016/j.coemr.2021.100285
30. Reytor-González, C., Frías-Toral, E., Núñez-Vásquez, C., et al. (2025). Preventing and managing pre- and postoperative micronutrient deficiencies: A vital component of long-term success in bariatric surgery.

Nutrients, 17(5), 741. https://doi.org/10.3390/nu17050741
31. Kharve, K., Engley, A. S., Paine, M. F., et al. (2024). Impact of drug-mediated inhibition of intestinal transporters on nutrient and endogenous substrate disposition…an afterthought? Pharmaceutics, 16(4), 447. https://doi.org/10.3390/pharmaceutics16040447
32. Ma, H., Liu, H., Yang, Y., et al. (2024). The effect of folate deficiency and different doses of folic acid supplementation on liver diseases. British Journal of Nutrition, 133(1), 37–47. https://doi.org/10.1017/S000711452400285X
33. Durrani, A., & Parveen, H. (2021). Zinc deficiency and its consequences during pregnancy (pp. 69–82). https://doi.org/10.1007/978-3-030-76609-2_3
34. Alonge, S., Melandri, M., Leoci, R., Lacalandra, G., Caira, M., & Aiudi, G. (2019). The effect of dietary supplementation of vitamin E, selenium, zinc, folic acid, and n-3 polyunsaturated fatty acids on sperm motility and membrane properties in dogs. Animals, 9(2), 34. https://doi.org/10.3390/ani9020034
35. Liu, F., Edelmann, M., Piironen, V., & Kariluoto, S. (2022). 5-methyltetrahydrofolate is a crucial factor in determining the bioaccessibility of folate in bread. Journal of Agricultural and Food Chemistry, 70(41), 13379–13390. https://doi.org/10.1021/acs.Jafc.2c03861
36. Zheng, J., Wu, F., Wang, F., Cheng, J., Zou, H., Li, Y., … & Kan, J. (2023). Biomarkers of micronutrients and phytonutrients and their application in epidemiological studies. Nutrients, 15(4),970. https://doi.org/10.3390/nu15040970
37. Amos, A., & Razzaque, M. (2022). Zinc and its role in vitamin D function. Current Research in Physiology, 5, 203–207. https://doi.org/10.1016/j.crphys.2022.04.001
38. Barchielli, G., Capperucci, A., & Tanini, D. (2024). Glutamate carboxypeptidase II (pp. 305–319). https://doi.org/10.1016/B978-0-12-823974-2.00002-4
39. Revelli, A. (2025). Effects of homocysteine circulating levels on human spontaneous fertility and in vitro fertilization outcomes: A literature review. Nutrients, 17(20), 3211. https://doi.org/10.3390/nu17203211
40. Murrin, E., Saad, A., Sullivan, S., Millo, Y., & Miodovnik, M. (2024). Innovations in diabetes management for pregnant women: Artificial intelligence and the Internet of Medical Things. American Journal of Perinatology, 42(12), 1540–1549. https://doi.org/10.1055/a-2489-4462
41. Mogos, R., Gheorghe, L., Cărăuleanu, A., Vasilache, I., Munteanu, I., Mogos, S., … & Preda, C. (2024). Predicting unfavorable pregnancy outcomes in polycystic ovary syndrome (PCOS) patients using machine learning algorithms. Medicina, 60(8), 1298. https://doi.org/10.3390/medicina60081298
42. Voulgaridou, G., Papadopoulou, S., Detopoulou, P., Tsoumana, D., Giaginis, C., Kondyli, F., … & Pritsa, A. (2023). Vitamin D and calcium in osteoporosis, and the role of bone turnover markers: A narrative review of recent data from RCTs. Diseases, 11(1), 29. https://doi.org/10.3390/diseases11010029
43. Tinsley, G. (2026). Sex differences in nutrient gaps among active adults. Journal of Nutritional Science, 15. https://doi.org/10.1017/jns.2025.10070
44. Bell, V., Rodrigues, A., Ferrão, J., Varzakas, T., & Fernandes, T. (2024). The policy of compulsory large-scale food fortification in sub-Saharan Africa. Foods, 13(15), 2438. https://doi.org/10.3390/foods13152438
45. Kippler, M., & Oskarsson, A. (2024). Manganese – a scoping review for Nordic Nutrition Recommendations 2023. Food & Nutrition Research, 68. https://doi.org/10.29219/fnr.v68.10367
46. Golja, M., Šmid, A., Kuželički, N., Trontelj, J., Geršak, K., & Mlinarič-Raščan, I. (2020).

Folate insufficiency due to MTHFR deficiency is bypassed by 5-methyltetrahydrofolate. Journal of Clinical Medicine, 9(9),2836. https://doi.org/ 10.33 90/jcm9092836

47. Franco, C., & Canzoniero, L. (2024). Zinc homeostasis and redox alterations in obesity. Frontiers in Endocrinology, 14. https:// doi.org/10.3389/fendo.2023.1273177
48. 48. Dinardo, G., Indolfi, C., Klain, A., Grella, C., Tosca, M., Ruocco, E., … & Ciprandi, G. (2025). The role of zinc in pediatric asthma and allergic rhinitis: Mechanisms and clinical implications. Nutrients, 17(16), 2660. https:// doi. org/10.3390/nu17162660.