Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

_____ _____

Madeleine Zeiler Date: 4/25/2023

Assessing the role of inflammation on serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc in adolescents and school-age children

By

Madeleine Zeiler
MPH

Hubert Department of Global Health

_____
Dr. Melissa Young
Committee Chair

_____
Dr. Hanqi Luo
Committee Member

_____
Dr. Yi-An Ko
Committee Member

_____
Dr. Rochelle Werner
Committee Member

Assessing the role of inflammation on serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc in adolescents and school-age children

By

Madeleine Zeiler
B.A. in Anthropology and Sociology
Western Washington University
2019

Thesis Committee Chair: Dr. Melissa Young, PhD

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2023

Abstract

Assessing the role of inflammation on serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc in adolescents and school-age children

By

Madeleine Zeiler

Objectives: Knowledge gaps remain on the role of inflammation on micronutrient biomarker concentrations among school age children (SAC) and adolescents (ADL). Children and WRA are particularly vulnerable, with at least half of PSC and around two-thirds of non-pregnant WRA experiencing a micronutrient deficiency in some form. Associations between concentrations of inflammation biomarkers C-reactive protein (CRP) and α1-acid glycoprotein (AGP) and serum folate (SFO), red blood cell folate (RBC folate), vitamin B-12, vitamin D, and zinc were examined.

Methods: This study analyzed cross-sectional data from nutrition surveys in multiple countries from 2006-2016 in SAC and ADL. Survey eligibility criteria were as follows: inclusion of either SAC (5-9 years) or ADL (10-19 years), AGP or CRP and ≥ 1 micronutrient of interest, and n > 100. Micronutrient deficiencies were defined as SFO < 10 nmol/L, RBC folate < 340 nmol/L, vitamin B-12 < 150 pmol/L, vitamin D < 25 nmol/L, and zinc < 57 µg/dL – 74 µg/dL depending on specified conditions. Inflammation was defined as AGP > 1 g/L and CRP > 5 mg/L. Prevalence estimates were adjusted for complex survey design, and associations were assessed by rank correlation coefficient.

Results: Correlations between AGP or CRP and SFO, RBC folate, vitamin B-12, vitamin D, and zinc were weak, with no clear pattern of association. Of the 50 observations recorded 11 were statistically significant ($p < 0.05$). 10 of these were negative: 3 between SFO and CRP ($r = -0.08$ to -0.06); 2 between vitamin B-12 and CRP ($r = -0.18$ to -0.09); 1 between vitamin D and CRP (r

= -0.06); 1 between zinc and AGP (r = -0.18); and 3 between zinc and CRP (r = -0.19 to -0.06). 1, vitamin B-12 and CRP (r = 0.10 to 0.13), was statistically significant and positive. Decile plots showed no clear pattern across biomarkers.

Conclusions: Due to weak and inconsistent correlations between the inflammation biomarkers and micronutrient biomarkers, there is no rationale to adjust for inflammation when estimating population prevalence SFO, RBC folate, vitamin B-12, vitamin D or zinc deficiencies in SAC or ADL.

Assessing the role of inflammation on serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc in adolescents and school-age children

By

Madeleine Zeiler
B.A. in Anthropology and Sociology
Western Washington University
2019

Thesis Committee Chair: Dr. Melissa Young, PhD

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Global Health
2023

Acknowledgements

I would like to express my sincere gratitude to my thesis committee members, Dr. Melissa Young, Dr. Hanqi Luo, Dr. Yi-An Ko, and Dr. Rochelle Werner, as well as BRINDA project biostatistician Jiaxi Geng, steering committee member, Dr. Parmi Suchdev, and technical expert Dr. Yaw Addo for their unwavering support, guidance, and invaluable feedback throughout this entire journey. Their expertise, insightful comments, and constructive criticism have been instrumental in shaping this thesis.

I would like to thank my all of my colleagues at the BRINDA project, particularly Chelsea Cole and Emily Nieckula for their generous help, stimulating discussions, and valuable input throughout this project. Their willingness to answer my questions, share their knowledge and expertise, and offer their support made this project both more enjoyable and more productive.

To my partner, Cameron, I extend my heartfelt thanks for their love, encouragement, and understanding during this time. Their steadfast belief in me and constant moral support especially in the most challenging moments were crucial in keeping me motivated and focused throughout this process.

Lastly, I thank my family for their unwavering support, encouragement, and understanding of all my academic and personal endeavors. Their love and belief in me are my foundation. The high bar set on both sides has been an inspiration throughout my life, and I am eternally grateful for their unconditional support.

To all I have mentioned and those not referenced by name, thank you for your time, patience, and encouragement along the way. Thank you all for being an integral part of my journey and for helping me achieve this milestone in my academic career.

Many thanks are given to this projects fundings sources which include the Bill & Melinda Gates Foundation, Centers for Disease Control and Prevention, Eunice Kennedy Shriver National Institute of Child Health and Human Development, HarvestPlus, and the United States Agency for International Development.

Chapter 1: Introduction
Introduction and Rationale

Micronutrient deficiency remains a global public health challenge that affects a substantial proportion of the population, particularly in developing countries.[1] It is estimated that more than half of the world's preschool-aged children (PSC) and approximately two-thirds of non-pregnant women of reproductive age (WRA) experience a micronutrient deficiency in some form.[1][2] However, these data are sparse among adolescents (ADL) and school-age children (SAC). Micronutrient deficiency can be difficult to define given that symptoms can be non-specific, hence the colloquialism to define this phenomenon, "hidden hunger." Deficiencies in micronutrients such as iron, vitamin A, and zinc are widespread, particularly in low- and middle-income countries.[3]

Micronutrients, such as serum folate (SFO), red blood cell (RBC) folate, vitamin B-12, vitamin D, and zinc, are essential for numerous physiological processes and maintaining optimal health.[4–7] Deficiencies in these micronutrients can lead to a variety of negative health outcomes, including impaired growth and development, weakened immune systems, and increased susceptibility to infectious diseases.[8] Moreover, micronutrient deficiency has been linked to long-term cognitive impairment, which can have far-reaching social and economic consequences.[9] There is growing evidence that micronutrient deficiencies are associated with a range of non-communicable diseases (NCDs), including cardiovascular disease, diabetes, and cancer; addressing NCDs early in life can be critical to long term prevention.[10] Addressing this issue requires a comprehensive approach that includes targeted interventions to improve access to nutrient-rich foods, fortification of staple foods, and the use of supplements where necessary.[11]

[1] Abbreviated tables showing prevalence of select micronutrient deficiencies are available in Table 1

Therefore, a thorough understanding of the epidemiology, etiology, and consequences of micronutrient deficiency is necessary for the development of effective public health interventions and policies aimed at mitigating its impact on vulnerable populations.

Problem Statement

Assessment of micronutrient deficiency is critical to identifying and treating patients, evaluating the prevalence of deficiency within a population, and informing the monitoring and evaluation of public health programs. Micronutrient deficiency can have serious and long-term consequences for children's health, growth, and development. Children who do not consume adequate amounts of these micronutrients are at increased risk of developing deficiencies that can impair their immune system, growth, and cognitive development. Assessment of micronutrient deficiencies is complicated for several reasons including the potential for inflammation to suppress plasma concentrations of micronutrients, harmonizing cutoffs across varied analytic methods, sometimes vague deficiency symptoms, and lack of inclusion in population-based surveys.[2] This potentially skews current estimates and explains data gaps worldwide. The past decade has led to tremendous increases in knowledge regarding the role of inflammation on SFO, RBC folate, vitamin B-12, vitamin D, and zinc concentrations, and improved understanding of the necessity to adjust for inflammation to improve the accuracy of prevalence estimates. Past literature demonstrates the need to adjust for inflammation among select biomarkers, particularly those that indicate iron or vitamin A deficiency.[12]

Purpose Statement

This study will assess the prevalence of SFO, RBC folate, vitamin B-12, vitamin D, and zinc deficiency and the impact of adjusting for inflammation by C-reactive protein (CRP) and/or α1-acid glycoprotein (AGP) on these estimates in a population of SAC and ADL. The findings of

this study will be important for informing public health interventions and policies aimed at addressing micronutrient deficiencies among these age groups and improving their long-term health outcomes. Two studies investigating the same effect on iron and vitamin A are currently in process.

Research Question

Should SFO, RBC folate, vitamin B-12, vitamin D, and zinc be adjusted by inflammation biomarkers CRP) and/or AGP when determining population prevalence of related deficiencies?

Significance Statement

Current studies estimate that the impact of malnutrition and related conditions such as overweight or obesity, undernutrition, stunting and wasting, or micronutrient deficiency on the global economy is around $3.5 trillion and $1-2 trillion, respectively.[13] These staggering statistics indicate that combatting nutrition-related adverse health outcomes should be a top priority of public health organizations globally due to their high prevalence and significant impact on public health, particularly in low- and middle-income countries. It must be noted that the prevalence estimates of micronutrient deficiency can be affected by inflammation: populations can have both a high prevalence of micronutrient deficiencies and a high prevalence of inflammation thus potentially confounding prevalence estimates of both. This study will investigate the need for adjusting for inflammation by two different inflammation biomarkers among five micronutrients. Past studies have demonstrated that prevalence estimates may move as many as 25 percentage points after adjustment.[14]

Definition of Terms

- ADL: Adolescents 10–20 years old
- AGP: α1-acid glycoprotein (elevated AGP defined by concentrations > 1 g/L)

- BRINDA: the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia project
- CRP: C-reactive protein (elevated CRP defined by concentrations > 5mg/L)
- IL1: interleukin-1 (a type of cytokine produced primarily by macrophages, monocytes, and dendritic cells that promotes inflammation and recruits immune cells to the site of infection or injury)[15]
- IL6: interleukin-6 (a type of cytokine produced by a variety of cells, including immune cells, endothelial cells, and fibroblasts that is involved in a wide range of physiological processes, including regulating the immune response, promoting the growth and differentiation of certain types of cells, and regulating metabolism)[16]
- Inflammation: elevated α1-acid glycoprotein or C-reactive protein
- PSC: preschool children 6-59 months old
- RBC folate: red blood cell folate (deficiency defined by concentrations < 340 nmol/L)
- RBP: retinol binding protein
- SAC: School-age children 5–15 years old
- SFO: serum folate (deficiency defined by concentrations < 10 nmol/L)
- sTfR: soluble transferrin receptor
- TNF-α: tumor necrosis factor-alpha
- Vitamin B-12: deficiency defined by concentrations < 150 pmol/L
- Vitamin D: deficiency defined by concentrations < 25 nmol/L
- WRA: women of reproductive age 15-49 years old

- Zinc: deficiency determined based on considered age, sex, time of blood draw, and fasting status (population specific cut offs for zinc are presented in supplementary material 2.)
- Zinc: deficiency determined based on considered age, sex, time of blood draw, and fasting status (population specific cut offs for zinc are presented in supplementary material 2.)

Chapter 2: Literature Review

Micronutrient Overview

Folate

Folate was initially discovered in 1931 by Lucy Wills while studying cures for macrocytic anemia in pregnant women.[17] Folate data is limited worldwide due to lack of inclusion of measurement in population-wide surveys and differences in assay approaches, analytes, and antibodies used making estimation of prevalence of deficiency difficult.[18] One study suggests that the prevalence of folate deficiency is >20% in lower income countries and <5% in higher income countries among WRA.[1] In the United States, RBC folate concentrations in participants ≥ 4 years old were higher in females than in males, and highest in non-Hispanic whites compared to non-Hispanic blacks (lowest) and Mexican Americans (in between). The same study showed that serum and RBC folate concentrations were lowest in adolescents and young adults.[20] Another multi-national analysis showed that among PSC, the prevalence of folate deficiency ranged from 1% in Mexico (2012) to 54% in India (2016-2018).[2] Inadequate folate intake in utero may lead to neural tube defects, megaloblastic anemia, poor reasoning and learning skills, and macrocytosis.[21] Folate deficiency must be mitigated early as it can also lead to Alzheimer's disease, and coronary and cardiovascular disease due to hyperhomocysteinemia, and select cancers such as leukemia, lymphoma, and colorectal, breast and prostate cancer as a result of metabolic changes.[17,22] Causes of folate deficiency are numerous and include malabsorptive conditions, metabolic inhibitors, neoplastic diseases, pregnancy, and, particularly in Western cultures, alcoholism.[23] The stages of folate insufficiency include megaloblastic changes in bone marrow, abnormal nuclear changes, and finally, incapacitation of erythroblast replication.[17]

Folate deficiency has been successfully combatted in many populations by supplementation. Interestingly, there is no upper limit for naturally occurring folate, while the upper limit for folic acid, the most biologically active form once converted to L-methylfolate via enzymatic processes and the form most commonly found in supplements, is set at 1000 μg/d.[24,25] It must be noted that as of the 1990s, when folic acid fortification was mandated by the governments of at least 75 countries across the globe in products such as breads, pastas, rice, and cornmeal, the prevalence of populations now regularly experiencing intake above the Tolerable Upper Intake Level (UL) has increased.[17,26] One study showed that individuals exposed to low vitamin B-12 and high folate in utero experienced increased adiposity and insulin resistance by the time they reached six years old age, and high folate has been shown to attenuate improvements in vitamin B-12 status.[26] This is partially explained by overlap vitamin B-12 and folate's metabolic functions particularly in one-carbon metabolism.[27]

Serum Folate

The measurement of serum folate, an indicator of recent folate intake, in ADL and SAC is critical for the identification of folate deficiency and the evaluation of the effectiveness of interventions aimed at increasing folate intake.[28] Because serum folate status is influenced by recent dietary intakes, it cannot be used as an indicator of overall folate status.[25] Several methods are available for the measurement of serum folate, including microbiological assays, immunoassays, protein-binding assays, and chromatographic techniques.[29] Numerous factors can affect the accuracy of serum folate measurement in children and adolescents, including age, gender, ethnicity, BMI, and socioeconomic status.[30] Therefore, these factors should be examined carefully when interpreting serum folate measurements in children and adolescents. Overall, the selection of a reliable and valid measurement strategy, together with appropriate consideration of

confounding factors, is essential for accurate measurement and interpretation of serum folate levels in children and adolescents.

Red Blood Cell Folate

RBC folate is a marker of folate status that reflects long-term folate intake and stores.[28] It is the primary biomarker used when assessing folate status because, unlike serum folate, RBC folate represents the average intake over the lifespan of the red blood cell [2].[31,32] RBC folate is considered a more reliable indicator of tissue folate status than serum folate, as it is less affected by short-term changes in dietary intake or the use of supplements.[33] Folate deficiency can lead to megaloblastic anemia, a condition characterized by the production of large, immature red blood cells that are unable to carry oxygen effectively. The accumulation of tetrahydrofolate (THF) in red blood cells is also associated with the accumulation of homocysteine, an amino acid that is linked to cardiovascular disease and other health problems.[34] The methylation of DNA and other cellular components also requires folate, and folate deficiency can lead to DNA damage and alterations in gene expression.[35,36] These biological mechanisms underscore the importance of maintaining adequate folate intake and status, particularly during periods of rapid growth and development.

Vitamin B-12

Vitamin B-12, which refers to all corrinoids, is most studied in patients with pernicious anemia. It was first studied by Thomas Addison in 1849 as the result of research into the treatment and prevention of this condition. Since then, the scientific community has come to more completely understand not only vitamin B-12's role in anemia but also its biological mechanisms, with contemporary research focusing on determining the most accurate cutoffs and biomarker interpretation.[26] The prevalence of vitamin B-12 deficiency, which manifests as

macrocytic or megaloblastic anemia, varies drastically across countries and population groups. Deficiency prevalence varies by age group and country: in the United States, <1% of children and adolescents are considered deficient, though these estimates are higher for both older and younger groups.[2]37 In Latin America, around 40% of children were deficient in vitamin B-12, and prevalence rose to as high as 80% in African and Asian countries.

Megaloblastic anemia is primary indicator of deficiency and without it, deficiency may go undiagnosed, resulting in permanent neurologic damage. Vitamin B-12 is critical to red blood cell production, and brain and nerve development, and neuronal demyelination in the peripheral and central nervous systems.[26,38] Low vitamin B-12 concentrations can be related to malabsorption due to ileal resection, irritable bowel disease, and bacterial overgrowth, among others; strict adherence to a plant-based diet[3]; and pernicious anemia.[37] Deficiency can lead to megaloblastic anemia with cytopaenias, increased homocysteine in the blood (hyperhomocysteinemia), and neurological abnormalities as well as developmental delays, feeding difficulties, hypotonia, lethargy or hyperirritability, microcephaly, depression (though more research is needed), osteoporosis, and coma in children.[34,39] Hyperhomocysteinemia has been linked to atherosclerosis and venous thrombosis, both placing patients at increased risk for heart attack and/or stroke.[40] Links may exist between low vitamin B-12 and breast cancer in postmenopausal women.[41] Deficiency is typically treated with supplements in the form of intramuscular injections or high oral doses.[26] The stages of vitamin B-12 begins with events in the blood such as increased plasma methylmalonic acid (MMA) concentrations, increased MMA

[2] Prevalence by age follows a U-shaped pattern.
[3] The bioavailability of dietary sources of vitamin B-12 and folate are low compared to synthetic forms[22]

excretions in the urine to demyelination of neurons, and finally brain atrophy, dementia, depression, memory loss, and psychosis.[4]

Vitamin B-12 is absorbed by the body in two unique ways: by active physiological processes and passive diffusion. It is important to note that passive diffusion, which occurs in the gastrointestinal tract, and accounts for only 1-2% of absorption when vitamin B-12 is supplemented orally. Vitamin B-12 is absorbed primarily in the terminal ileum, requiring intrinsic factor which is produced in parietal cells of the gastric body and fundus, and contrary to popular belief, depletion occurs rapidly.[5]42 Malabsorption is determined via dosing with radioactive cobalt.[26] Vitamin B-12 deficiency may result from inadequate dietary intake or insufficient production and activation of intrinsic factor particularly in the stomach lining. The latter may explain why aging populations have a higher prevalence of deficiency, gastritis, and pernicious anemia compared to younger populations.[43] However, older adults may still benefit from dietary interventions with vitamin B12 fortification. One study among older adults in the Netherlands found that vitamin B-12 fortified wheat flour reduced the prevalence of marginal vitamin B12 deficiency.[44] These interventions, however, may be most critical to children and pregnant and lactating women.

Vitamin D

Vitamin D is a fat-soluble vitamin which aids in the function of the immune system, muscles, and brain cell activity and has anti-inflammatory, antioxidant, and neuroprotective properties; inadequate intake may lead to thin, brittle, or misshapen bones.[45] Associations exist between vitamin D deficiency and certain cancers, diabetes, cardiovascular disease, depression,

[4] A full list of the stages of vitamin B-12 insufficiency can be found in Text Box 11 of the Biomarkers of Nutrition for Development's review on vitamin B-12

[5] < 1 year

and autoimmune diseases, and even mild deficiency has been linked to hypocalcemia and hyperparathyroidism.[7] Historically, vitamin D deficiency has been associated with rickets, a condition that leads to poor bone development and thus bone pain and soft, weak bones.[46,47] Rickets has been largely eradicated due to the fortification of milk products in the 1930s.[7,48] Deficiency is most common in individuals with malabsorptive syndromes like inflammatory bowel disease and celiac disease, people with limited access to sunlight and dark-skinned people, and people who suffer from chronic liver disease.[7,49] It is also common in older patients, and those who have obesity, are hospitalized, or live in a nursing home.[50] Because humans require access to sunlight to convert cholecalciferol (vitamin D3) to its active form, 25-hydroxyvitamin D, individuals who cover the majority of their bodies, such as women in the Middle East, or people who live in very hot climates and spend most of their time inside are at increased risk for vitamin D deficiency.[51,52] One study showed that exposure to a commercial portable ultraviolet (UV) indoor tanning lamp in cystic fibrosis and short bowel syndrome patients twice weekly for eight weeks for 5-10 minutes lead to increased serum 25(OH)D levels.[53]

Clinical signs of vitamin D deficiency are notoriously difficult to identify as patients are usually asymptomatic; therefore, identifying deficiencies requires laboratory testing of blood samples, such as those collected in health clinics or through population surveys. Vitamin D deficiency is broken into mild deficiency (25-hydroxyvitamin D < 20 ng/mL), moderate deficiency (25-hydroxyvitamin D < 10 ng/mL), and severe deficiency (25-hydroxyvitamin D < 5 ng/mL).[48] The prevalence of vitamin D deficiency < 25 nmol/L ranges drastically across different populations and surveys, from 4.6% among healthy American adolescents[54] to 71% among adolescents in New Delhi, India.[55] However, these particular estimates do not take skin color into consideration: in the United States, 47% of Black infants experience deficiency

compared to 56% of white infants. These prevalence estimates are low compared to infants in Turkey, Iran, and India, where approximately 90% of infants experience vitamin D deficiency.[56] Current estimates suggest that up to 1 billion people suffer from subclinical vitamin D deficiency and around 50% of the world's population experience insufficiency.[7] Some researchers and clinicians use a cutoff of 30 ng/mL though differences in recommended intakes exist across races as mineral metabolism is unique in each group. Currently, lower intakes are set at 30 ng/mL and upper intakes are around 100 ng/mL, though more research is needed due to the risk for hypercalcemia.[57] Vitamin D deficiency is treated using 6,000 IU daily or 50,000 IU once per week of vitamin D_2 or vitamin D_3 for 6-8 weeks depending on age to attain 25-hydroxyvitamin D levels exceeding 30 ng/mL.[58]

Vitamin D measurement may be confounded by use of medications including clotrimazole, nifedipine, carbamazepine, and phenobarbital.[59] The latter two drugs are both antiepileptics, while the former drugs are used to combat skin infections, and for hypertension and angina, respectively.[60–62] Thyroid disorders and the use of antiepileptics and cholestyramine impact serum 25(OH)D concentrations.[63] 1,25(OH)2 D is also impacted by calcium and phosphate concentrations along with parathyroid hormone, prostaglandins, prolactin, and estradiol and drugs including corticosteroids, antihypertensives, anti-retrovirals, heparin sodium, and biphosphonates.[59] Other confounders include age, gender, nutritional status and habits, mental health, a sedentary lifestyle, smoking, and lung health.[64]

Zinc

The study of zinc began in 1869 when Jules Raulin, a student of Louis Pasteur, identified the role that zinc plays on plant nutrition via microbial systems.[65,66] By 2000, the International Zinc Nutrition Consultative Group (IZiNCG) was founded, and by 2004, a comprehensive report

on zinc assessment had been published.[67] Zinc is essential to immune function, protein and DNA synthesis, wound healing, and cell signaling and division as a structural and catalytic ion.[66] However, zinc deficiency is particularly difficult to identify and therefore treat due to a lack of generalized clinical symptoms.[66] In animals, deficiency manifests in a cycle of anorexia and food ingestion, perhaps extending survival.[68] Zinc is required for the structural integrity of around 8% of the human genome.[66] It serves as an enzyme catalyst, provides enzymatic structure, and regulates gene expression.[69] Zinc deficiency impairs endocrine function, DNA transcription and gene expression, and limits weight gain in animal models.[66] It has significant effects on the immune system, impairing innate immune function and acquired immunity increasing the rate and duration of infection.[70]

Beyond cellular function and metabolism, zinc is critical to growth, immune and reproductive health, and neurobehavioral development.[66] More specifically, inadequate intake among children and infants may lead to deficits in neuropsychologic functioning, activity, motor development, cognitive performance, diarrhea, alopecia, delayed growth, and frequent infections. Individuals with gastrointestinal or metabolic disorders, and sickle cell anemia or thalassemia; infants, lactating people, and vegetarians are at an increased risk for zinc deficiency.[66] The body is unable to store zinc, making dietary intake essential. In populations where a plant-based diet is dominant, zinc deficiency is typically more prevalent, particularly in children and WRA.[66]

It is estimated that 17% of individuals globally do not get enough zinc through dietary sources, though this estimate may be as many as two times lower than the true prevalence of zinc deficiency.[71] Prevalence of zinc deficiency among children and adolescents ranged from 4% in China to 83% in Cameroon as estimated by national surveys conducted between 2001 and 2014.[72] Another multi-national analysis showed that zinc deficiency ranged from 12% in

Afghanistan (2013) to 67% in Cambodia (2014) among PSC. The World Health Organization (WHO) estimates that 430,000 children die from zinc deficiency annually.[73] These deaths are most often linked to diarrhea, pneumonia, and malaria.[2,74] Deficiency is most common in Southeast Asia and sub-Saharan Africa, with the prevalence of deficiency exceeding 25%.[75] Zinc deficiency and diarrhea are combatted using 20 mg/d for 10-14 days or 10 mg/d for infants < 6 months old.[76] Diarrheal disease treatment uses zinc fortified oral rehydration supplements, particularly among young children in low- and middle-income countries.[77]

Plasma zinc concentrations can fluctuate drastically based on specific conditions; some studies estimate this fluctuation to be as high as 20%.[78] Numerous factors including time of blood draw, pregnancy or use of oral contraceptives, age, sex, altitude, infection, food intake, stress, the position of the patient during blood draw, and smoking status influence serum and plasma zinc levels.[79] Cutoffs for deficiency are available for review in **Table 10.** Zinc function may be altered by the use of antibiotics, penicillamine, and diuretics, and careful notes on the time of last meal, existed of hemolytic conditions, and use of drugs and supplements must be taken and implemented during data cleaning and harmonization.[66] Absorption may also be influenced by pancreatic insufficiency and inflammatory bowel disease. A list of recommendations to limit contamination during zinc sampling is available.[6]

Three indicators are typically used in zinc assessment: dietary assessment, plasma or serum zinc concentration, and stunting. Dietary assessment, which uses food frequency questionnaires, 24-hour food recalls, and food diaries provide information on usual sources and bioavailability of those sources, though they are extremely time consuming and may rely on the use of incomplete food composition tables. Stunting is an excellent indicator of the success of an

[6] These recommendations can be found in Text Box 10 of the Biomarkers of Nutrition for Development's review on zinc.

intervention and is a non-invasive, low-cost technique. However, stunting is a non-specific, long-term indicator that does not respond quickly to changes in zinc status and measurement must be done by skilled anthropometrists. Other potential zinc biomarkers include hair zinc, urinary zinc, and neurobehavioral function.[66]

Deficiency Cutoffs

Folate cutoffs have shifted significantly in the past few decades. Currently accepted cutoffs are <10 nmol/L for serum folate and < 340 nmol/L for RBC folate per the WHO Technical Consultation on folate and vitamin B-12 deficiencies and are derived from NHANES III data. Vitamin B-12 cutoffs are currently set at 148 pmol/L and 221 pmol/L for serum B-12 and methylmalonic acid, respectively. Some argue that a cutoff of >210 nmol/L would be more appropriate.[37] Vitamin D cutoffs are currently set at 30 ng/mL for lower intakes and 100 ng/mL for upper intakes per the Institute of Medicine.[80] Other studies suggest the use of cutoffs for serum 25-hydroxyvitamin D between 20 ng/mL (50 nmol/L) and 30 ng/ml (75 nmol/L).[58] Zinc cutoffs are particularly difficult given numerous confounding variables. Current estimates range from 57 µg/dL to 74 µg/dL depending on age, sex, time of blood draw, and fasting status.[81] AGP cutoffs have been set at 1.0 mg/mL and CRP cutoffs are set at 5 mg/L.[82]

Inflammation Overview

Inflammation is a complex biological process that occurs in response to tissue injury, infection, or other forms of cellular stress.[83] It is defined by the acute phase response (APR) which is activated due to tissue injury, immunologic disorders, microbial invasion, and various forms of stress.[84] It is a crucial component of the immune response, allowing the body to eliminate harmful pathogens and initiate tissue repair.[85] However, when inflammation becomes dysregulated, it can contribute to a variety of chronic diseases including diabetes mellitus,

autoimmune disorders, non-alcoholic fatty liver disease (NAFLD), cancer, stroke, ischemic heart disease, cancer, chronic kidney disease, and neurodegenerative conditions.[86] It is estimated that 50% of all deaths in the entire global population can be attributed to inflammation.[87] Acute inflammation may present with redness, swelling, pain, and heat as well as fever, allergy anaphylaxis, an increase in immune cells, and fibrosis, among others.[88,89] Inflammation, resulting in increased CRP, IL-6, and TNF-α, leads to increased risk for obesity due to increased leptin, adiponectin, and oxidative stress, as well as decreased muscle mass and micronutrient status.[90]

Inflammation may occur in one of several different forms. Acute inflammation typically self-resolves quickly and is a response to infection or tissue injury. Chronic inflammation, however, refers to long-term inflammation and leads to non-communicable diseases such as cancer, obesity, cardiovascular disease, autoimmune diseases, and diabetes. Clinical inflammation relies on the presentation of clear symptoms of inflammation, whereas subclinical inflammation can only be identified biochemically. Subclinical inflammation occurs in four phases: first, the reference period (no evidence of inflammation is observed); second, the "incubation" period (no signs or symptoms of disease have appeared, though trauma or illness has occurred and CRP is elevated), third, the "early convalescence" period (CRP concentrations are elevated though declining and AGP concentrations become elevated); and fourth, the "late convalescence" period (only AGP is elevated and homeostasis approaches restoration).[90,91] At the onset of inflammation, the body releases inflammatory mediators such as bradykinin and histamine which result in small blood vessel dilation for increased blood flow and immune system cells to the injury cite.[88]

α1-Acid glycoprotein (AGP)

α_1-Acid glycoprotein, also known as orosomucoid, is a negatively charged glycosylated serum protein which serves as an excellent indicator of inflammation, liver health, and all-cause mortality.[92] It is synthesized and catalyzed in the liver, and it has a half-life of 2-3 days.[93,94] Healthy adults have concentrations between 0.05 and 0.1 g/dL, though during the acute phase response, serum AGP may rise between two and five times.[95] The levels of AGP in the blood can be measured using various laboratory tests, including immunoassays and chromatographic methods.

AGP is critical to human health as it binds and transports endogenous and exogenous substances. It is highly glycosylated, which allows it to bind and transport a wide range of molecules. This protein primarily inhibits T-cells and the proliferative response of peripheral blood lymphocytes to phytohemagglutinin. AGP also inhibits neutrophils, a common type of white blood cell that is critical to immune response, as well as monocytes and lymphocytes, and is critical to both anti-inflammatory and immunomodulatory responses.[96,97] AGP has been shown to be useful in wound healing, as it stimulates the proliferation of fibroblasts.[97]

C-reactive protein (CRP)

CRP is a highly sensitive marker of inflammation and is widely used in clinical practice to assess the risk of various diseases, including cardiovascular disease, cancer, and autoimmune disorders. The study of C-reactive protein began with its discovery by Tillet and Frances in 1930 via identification in the sera of pneumococcal pneumonia patients. CRP is a biomarker of inflammation that is synthesized by liver hepatocytes in response to various stimuli, such as infection, trauma, or tissue damage.[98-100] The molecular structure of CRP consists of five identical subunits arranged in a pentameric ring. Each subunit contains a calcium ion-binding site, which is essential for its stability and ligand-binding capacity.[101] The ligands of CRP

include various molecules that are involved in the inflammatory process, such as lipopolysaccharides, bacterial cell wall components, and apoptotic cells. CRP binds to these ligands with high affinity, leading to their clearance by phagocytic cells and activation of the complement system.[102] The production of CRP is regulated by several cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha).[103] These cytokines are released by immune cells in response to inflammatory stimuli and stimulate the transcription of the CRP gene in the liver.[98]

The clinical utility of CRP lies in its ability to predict the risk of cardiovascular disease, disease severity and monitor outbreaks, predict mortality, track intervention efficacy, identify individuals at risk of metabolic syndrome, determine response to cancer treatment and vaccinations, and more.[104–109] Elevated levels of CRP have been associated with an increased risk of atherosclerosis, myocardial infarction, stroke, and peripheral artery disease.[110] It is also a useful marker of disease activity in rheumatoid arthritis, systemic lupus erythematosus, and other autoimmune disorders.[111] Recent research has also identified CRP as a potential therapeutic target in various diseases. For example, inhibition of CRP has been shown to reduce the severity of rheumatoid arthritis in animal models and may be a promising treatment option in humans.[112] In addition, CRP has been implicated in the pathogenesis of Alzheimer's disease and may represent a target for disease-modifying therapies.[113]

Laboratory Analysis

Microbiological assays involve the use of microorganisms to measure folate activity and are considered the gold standard for serum folate measurement, though competitive protein binding assays have gained popularity due to ease of use.[114] Microbiological assays, while efficient, are expensive and require specialized expertise and equipment.[115,17] Immunoassays are

rapid and easy to perform, but their accuracy is affected by a lack of standardization, autoantibodies, and anti-reagent antibodies, among others.[116] Chromatographic techniques, such as high-performance liquid chromatography (HPLC), are highly sensitive and specific and can detect both natural and synthetic forms of folate.[117] However, these, techniques are costly, require specialized equipment, and may not be suitable for high-throughput analysis.

Three biomarkers are typically used in the measurement of folate status: serum folate, RBC folate, and homocysteine. Serum folate is an excellent indicator of short-term folate deficiency, takes less time to process, and accurately indicate folic acid exposure. However, it cannot be relied upon to measure the concentration of natural food folates, cutoffs differ by study, and study participants must be fasted before collection. Collection must occur at a laboratory and results are not reliable across studies. Finally, serum folate values are potentially inflated if undergoing hemolysis. RBC folate is a good indicator of long-term folate status, matching liver concentrations, and reflecting folic acid concentrations. However, adjustments must be made for the dietary folate equivalent (the amount of folate actually absorbed by the body),[7] measurement must take place in a laboratory, results cannot be readily compared across laboratories, and multi-country analyses are difficult due to differences in data collection and measurement. Finally, total homocysteine is a sensitive marker for folate status which responds quickly to treatment for folate deficiency. It can also be used to measure vitamin B-12 status. Total homocysteine measurement also must be done in a laboratory by specialists, though results are comparable across laboratories, and plasma homocysteine is a stable analyte.[17] Other

[7] If the research question involves measuring the total folate content of a sample, regardless of its bioavailability, a technique such as microbiological assay may be appropriate. If the research question involves measuring the bioavailable folate content of a sample, a technique such as high-performance liquid chromatography (HPLC) may be more appropriate.

biomarkers used include serum folic acid, urinary folate/folic acid, and urinary and serum para-aminobenzoylglutamate and para-acetamidobenzoylglutamate.[17]

The gold-standard for measuring serum vitamin B-12 concentrations is MMA, which requires the use of mass spectrometry and may be compromised by bacterial overgrowth, using a cutoff of 148–221 pmol/L.[37] Other biomarkers available for vitamin B-12 analysis include serum or plasma B-12 concentration, serum holotranscobalamin concentration, and serum total homocysteine concentration.[26] Each come with their own advantages and disadvantages.

Vitamin D is typically measured using 25(OH)D assay due to a longer half -life of 25(OH)D compared to 1,25(OH)2 D.[118] Possible measurement techniques include competitive protein-binding assays (CPB), tandem mass spectrometry (LC-MS/MS), liquid chromatography-mass spectrometry (LS-MS), radioimmunoassays (RIA), liquid chromatography with UV detection (LC), and chemiluminescence immunoassays (CLIA), though CPB, CLIA, and RIA are the most popular due to efficiency.[119]

Zinc concentrations are typically measured using atomic absorption spectrometry (AAS), though ICP-MS, ICP atomic emission spectrometry, ICP optical emission spectrometry, X-ray spectrometry, proton-induced X-ray emission, instrumental neutron activation analysis, and anodic stripping voltammetry are also popular.[26] Further research is needed to determine the efficacy of measuring bone zinc to determine long-term stores, though no biomarker of changes is currently available. Additionally, zinc assessment relies on the use of a trace-element free laboratory environment, as environmental contamination of biological samples can affect measurements.[120] These contaminants may include preservatives, lubricants, evacuated tubes, water, anti-coagulants, and rubber stoppers.[66]

CRP is commonly measured by enzyme immunoassay, laser nephelometry, and rate immunonephelometry or turbidimetry.[121] The measurement of AGP in the laboratory is typically performed using immunoassays, which detect and quantify the protein based on its specific interaction with antibodies. There are several types of immunoassays that can be used to measure AGP, including enzyme-linked immunosorbent assay (ELISA), nephelometry, and turbidimetry.[122]

The Public Health Implications of Micronutrient Deficiency

A study published this year showed that 372 million PSC and 1.2 billion non-pregnant WRA experience some form of micronutrient deficiency. This is the equivalent of over half of PSC and more than two-thirds of non-pregnant WRA.[2] Deficiencies in micronutrients including iron, vitamin B-12, folate, zinc, copper and vitamin A deficiency can lead to anemia, which can impair cognitive function, weaken the immune system, and increase the risk of infections.[123] Deficiencies in vitamin A and zinc can weaken the immune system and increase the risk of infections, while iodine, iron, and vitamin B-12 deficiency can impair cognitive function via reduced attention span, intelligence, sensory perception, and emotional and behavioral intelligence, and increase the risk of goiter and other thyroid disorders, though links between iron and cognitive warrant further investigation.[124] Zinc and vitamin D deficiencies can have dire consequences for immune and cognitive health. Therefore, ensuring micronutrient adequacy, though not a silver bullet, is critical for supporting children's physical and cognitive development and improving their long-term health outcomes.

Studying micronutrient deficiency from a public health perspective is essential for several reasons: it helps in identifying the populations at risk of micronutrient deficiencies, quantify the burden of micronutrient deficiency, and understand the underlying causes of micronutrient

deficiencies, and in so doing, tailor interventions to prevent and address micronutrient deficiencies effectively and assess their impact on health outcomes. By quantifying the burden of the problem, policymakers and public health practitioners can prioritize interventions and allocate resources to address micronutrient deficiencies effectively. With careful study, public health practitioners can design and implement interventions and allocate resources to prevent and address micronutrient deficiencies. Public health interventions can be categorized into three broad categories: food-based approaches, supplementation, and fortification. Typically, interventions revolve around the use of micronutrient powders, food fortification, and lipid-based nutrient supplements. Food-based approaches include promoting the consumption of nutrient-dense foods and improving access to these foods through agricultural and economic development programs. Supplementation involves providing vitamins and minerals in the form of tablets, capsules, or liquid supplements to populations at risk of micronutrient deficiencies. Fortification involves adding vitamins and minerals to commonly consumed foods such as flour, salt, and cooking oil. Finally, studying micronutrient deficiency from a public health perspective is crucial for monitoring and evaluating the effectiveness of interventions. Monitoring and evaluation involve measuring the impact of interventions on the prevalence and severity of micronutrient deficiencies, health outcomes, and cost-effectiveness. By doing this, public health practitioners can identify successful strategies and make necessary adjustments to ensure the interventions' sustainability and scalability.

Past/related Findings

Several studies have investigated the impact of adjusting for inflammation on the prevalence estimates of micronutrient deficiencies in different populations, including WRA and PSC. A cross-sectional study conducted in PSC and WRA found that the prevalence of vitamin A

deficiency (VAD) was overestimated among PSC; after adjusting retinol binding protein (RBP) and serum retinol for inflammation, the prevalence of VAD decreased by a median of 14 percentage points.[125] The same study showed that inflammation adjustment by serum ferritin increased the estimated prevalence of iron deficiency by a median of 11 percentage points. However, estimates of iron-deficient erythropoiesis for PSC as measured by soluble transferrin receptor (sTfR) decreased by a median of 15 percentage points.[125] These findings were diminished in significance in WRA. The BRINDA project currently recommends adjusting RBP and serum retinol by AGP and CRP in PSC but not WRA, serum ferritin by AGP and CRP for both PSC and WRA, and just by AGP for sTfR, also in both PSC and WRA.[126] Conversely, another study showed that estimated prevalence of depleted iron increased by 7-25 and 2-8 percentage points for PSC and WRA, respectively, when adjusting for inflammation using the CRP plus AGP approach, compared to unadjusted values.[127] A third study demonstrated that when sTfR concentrations were adjusted by CRP or AGP, the estimated prevalence of iron-deficient erythropoiesis decreased by 4.4–14.6 and 0.3–9.5 percentage points in PSC and WRA, respectively, compared with unadjusted values.[128]

The BRINDA project, a multi-agency international collaboration formed to study micronutrient assessment particularly as related to anemia, and have been shown to require adjustment for inflammation.[129] has reviewed the role of inflammation on all five micronutrients investigated here, though in differing populations. The role of inflammation on serum and RBC folate and vitamin B-12 was investigated among PSC and WRA in 13 countries across the globe. Rank correlation coefficients were weak and generally insignificant both for folate and for vitamin B-12, indicating that no adjustment was necessary in either WRA or PSC despite bidirectional associations between inflammation and the three biomarkers.[18] Another BRINDA

project study investigated the need to adjust vitamin D concentrations for inflammation in PSC and WRA. This multi-country analysis, using data from six nationally representative nutrition surveys, also found weak and inconsistent correlations between AGP or CRP and 25(OH)D concentrations in both populations and provided no rationale for adjustment for making vitamin D deficiency prevalence estimates in PSC and WRA.[130] Finally, the BRINDA project investigated the role of inflammation on zinc concentrations in PSC and WRA across 13 nationally representative nutrition surveys, using plasma or serum zinc concentrations. This study found that in general, inflammation was associated with reduced plasma and serum zinc concentrations, and, after regression correction, the prevalence of zinc deficiency decreased by 7.1% on average among PSC suggesting that adjustment is supported among populations of PSC with significant negative associations between plasma or serum zinc and inflammation biomarkers. However, associations were weak and inconsistent for WRA and there is currently no rationale for adjusting for inflammation in this group.[131]

More research is needed to investigate the impact of adjusting for inflammation on the prevalence estimates of micronutrient deficiencies in SAC and ADL, as studies in these age groups are limited. However, two other studies investigating the need to adjust iron and vitamin A in ADL and SAC are currently underway. Nonetheless, the available evidence suggests that adjusting for inflammation is critical for obtaining accurate estimates of micronutrient deficiencies, which can inform effective public health interventions and policies aimed at addressing this important public health issue.

The Case for Adjusting for Inflammation

The accurate estimation of micronutrient deficiencies is critical for informing public health interventions and policies aimed at addressing this issue and requires adjusting for

potential confounding factors, such as inflammation. Some micronutrient biomarkers are also acute-phase proteins (such as AGP and CRP) and may be elevated (or diminished) during infection or inflammation.[132] This work is particularly significant given inflammation and infection's known role in non-nutritional anemias such as anemia from chronic disease, blood loss from hookworm infection, and hemolytic anemia from malaria.[133] Previous studies have primarily focused on investigating the need to adjust for inflammation among WRA and PSC, with limited research available for SAC and ADL. Given that SAC and ADL represent a significant proportion of the population, the lack of research investigating the need to adjust for inflammation among these age groups is a notable gap in the literature. This gap is particularly concerning given that inflammation is prevalent in these populations due to the high burden of infectious diseases, which can influence the concentrations of certain biomarkers of micronutrient status. Therefore, there is a critical need to investigate the use of inflammation-adjustment approaches among SAC and ADL to ensure accurate estimation of micronutrient deficiencies in these populations.

Assessment of micronutrient deficiencies are complicated for several reasons including the potential for inflammation to suppress plasma concentrations of micronutrients, harmonizing cutoffs across varied analytic methods, sometimes vague deficiency symptoms, and lack of inclusion in population-based surveys.[2] This potentially skews current estimates and may explain data gaps worldwide. The past decade has led to tremendous increases in knowledge regarding the role of inflammation on SFO, RBC folate, vitamin B-12, vitamin D, and zinc concentrations, and improved understanding of the necessity to adjust when seeking accurate prevalence estimates. Past literature demonstrates the need to adjust for inflammation among select biomarkers, particularly those that indicate iron or vitamin A deficiency.[12] **Table 12**[134] depicts

when to adjust for inflammation in WRA, PSC, and SAC for RBP and retinol, sTfR, ferritin, zinc, folate, and RBC folate.

Bidirectional Associations of Inflammation and Micronutrients

Inflammation and micronutrients have bidirectional associations, with inflammation affecting the absorption, transport, and utilization of micronutrients, and micronutrient deficiencies contributing to chronic inflammation. Inflammatory cytokines such as interleukin-1 and tumor necrosis factor-alpha can reduce the absorption and availability of nutrients such as iron, zinc, and vitamin B12, leading to deficiencies.[135–137] On the other hand, deficiencies in micronutrients such as vitamin D, vitamin E, and omega-3 fatty acids can contribute to chronic inflammation and increase the risk of chronic diseases such as cardiovascular disease, cancer, and diabetes.[138] Prevalence estimates of micronutrient deficiencies may be artificially inflated (e.g., vitamin A and zinc) or reduced (e.g., ferritin and sTfR) by the presence of inflammation.

Biological Plausibility for B Vitamins (SFO, RBC folate, and Vitamin B-12)

There is a bidirectional association between B vitamins and inflammation, with B vitamin deficiencies contributing to inflammation and inflammation affecting B vitamin metabolism. B vitamins, such as folate, B6, and B12, play essential roles in one-carbon metabolism, which is involved in DNA synthesis, methylation reactions, and amino acid metabolism.[27] B vitamin deficiencies can lead to elevated levels of homocysteine, which is a pro-inflammatory molecule.[139] On the other hand, inflammation can impair B vitamin absorption and metabolism, leading to decreased levels of circulating B vitamins.[140] Therefore, maintaining adequate B vitamin levels is important for reducing inflammation and promoting overall health. One study showed that serum folate appears to be reduced amidst acute inflammation while red blood cell folate concentrations remained stable, though other studies have demonstrated an inverse

relationship.[132] Inflammation results in increased production of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha).[141] These cytokines can disrupt the normal function of the folate receptor, which is responsible for transporting folate to cells, leading to decreased folate uptake by cells and reducing the amount of folate available for metabolic processes.[142] Interestingly, B vitamins have also been demonstrated to have anti-inflammatory effects in microglia cells.[143] Other studies that use the BRINDA inflammation adjustment method have deemed adjustment for inflammation for vitamin B-12 and folate among PSC and WRA age unnecessary.[144]

Biological Plausibility for Vitamin D

Chronic inflammation has been associated with lower circulating levels of vitamin D.[145] This is thought to be due to the sequestration of vitamin D in adipose tissue and reduced absorption in the gut.[146] Additionally, inflammation can lead to increased expression of 24-hydroxylase, an enzyme which converts vitamin D into less active 25-hydroxyvitamin D (25(OH)D) and 1α,25-dihydroxyvitamin D3 24-hydroxylase, an enzyme responsible for converting 25(OH)D into the biologically inactive metabolite 24,25-dihydroxyvitamin D.[147] Vitamin D also has immunomodulatory properties and can regulate the activity of immune cells, including T cells and macrophages. Inflammatory cytokines can disrupt these effects by altering the expression of vitamin D metabolizing enzymes and the calcitriol receptor, leading to immune dysregulation.[148] One study which investigated the need to adjust biomarkers for iron, vitamin A, vitamin D, vitamin B-12, and folate for inflammation post norovirus exposure (resulting in an inflammatory response) demonstrated that recent infection was correlated with reduced vitamin D concentrations suggesting that adjustment for inflammation may be warranted, though further analysis found that adjustment was only necessary for some iron and vitamin A biomarkers.[149]

Biological Plausibility for Zinc

The relationship between inflammation and zinc concentrations is complex and multifactorial. Zinc deficiency has been demonstrated to compromise epithelial barrier, macrophage, and neutrophil function.[90] Plasma serum zinc concentrations may be reduced in the presence of inflammation, and in settings where infection is common, prevalence of zinc deficiency may be overestimated due to inflammation. Inflammatory cytokines, such as interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α), have been shown to decrease zinc absorption and increase zinc excretion, which can lead to lower zinc concentrations in the body.[150] In addition to the direct effects of inflammatory cytokines on zinc metabolism, inflammation can also lead to changes in the expression of zinc transporters and other proteins involved in zinc homeostasis: the expression of the zinc transporter ZIP14 has been shown to be upregulated in response to inflammation, which can lead to decreased zinc uptake in cells.[151] Previous studies have shown that inflammation leads to exaggerated prevalence of zinc deficiency, particularly in PSC.[131] It is important to note, however, that these findings came from a large multi-country analysis and that associations did not exist across all surveys, therefore, recommendations remain conditional.

Methods of Inflammation Adjustment

Five approaches for adjusting biomarkers for inflammation were identified in Young and Suchdev's seminal work on inflammation adjustment.[152] They include ignoring inflammation, excluding individuals with inflammation, changing nutrient biomarker cutoffs, the Thurnham internal correction factor, and the BRINDA regression correction approach. Each has varying levels of validity, precision, and feasibility and have been designed specifically around micronutrients with a known link to anemia including iron, vitamin A, zinc, folate, vitamin B-12,

and riboflavin. All approaches aim to improve global micronutrient deficiency prevalence estimates for appropriate program design, implementation, and targeting, and effective policy making.[153]

Ignoring inflammation and doing nothing to account for acute phrase proteins (APPs), while precise and feasible, is not valid and is a potentially biased estimate of micronutrient status. This approach is very common across micronutrient surveys, in part due to a lack of inclusion of APPs. Excluding individuals with inflammation by removing individuals with APPs is neither valid nor precise, though it is feasible. Sample size may be lost leading to reduced precision and may introduce bias due to the potential that population subsets differ with and without inflammation. This method also relies on concrete APP cutoffs. This method may be used if neither AGP nor CRP were measured. Changing the nutrient biomarker cutoff, like the exclusion method, is neither valid nor precise, though feasible. It uses higher and lower indicator cutoffs from individuals with inflammation or in settings with high inflammation, respectively. However, this method does not account for varying degrees of inflammation and may introduce bias as cutoffs will differ across groups and context. The four-level categorical inflammation correction factor, also known as Thurnham's internal correction factor, is precise and feasible, but not valid. CRP and AGP are used apply an internal correction factor and to create four categories: no inflammation (CRP < 5 mg/L and AGP < 1 g/L), incubation (CRP > 5 mg/L), early convalescence (CRP > 5 mg/L and AGP > 1 g/L), and late convalescence (AGP > 1 g/L). This method requires that surveys include both AGP and CRP and does not account for confounding and effect modification. Outputs are like those of the exclusion approach.

Finally, the BRINDA regression correction approach is valid, precise, and feasible. It uses linear regression to adjust micronutrient concentrations using AGP and CRP. This method

also requires both AGP and CRP, as well as statistical software to run the correction and knowledge of how to run the correction from a statistical standpoint.[8][153] However, it allows for adjustment for inflammation even in settings where levels are below traditional cutoffs. In the BRINDA approach, the micronutrient biomarker serves as the dependent variable while AGP or CRP serve as the independent variable, using the slope to adjust for inflammation while accounting for the potential for overadjustment. The BRINDA approach has been effectively applied to determine the need for adjustment for inflammation in determining micronutrient deficiency prevalence estimates in RBP and serum retinol, serum ferritin, sTfR, serum zinc, serum and RBC folate, serum B-12, and vitamin, particularly among PSC and WRA. **Table 12**[134] gives recommendations for which micronutrient biomarkers should be adjusted for inflammation. Further instructions for applying the BRINDA approach and answers to frequently asked questions are available.[154]

Potential Confounders

Dose size has a dramatic effect on the amount of vitamin B-12 actually absorbed by the body. Studies show that around 50% of a 1-µg dose, 20% of a 5-µg dose, and 5% of a 25-µg oral dose were absorbed, while only 1% of a 500-µg dose was absorbed.[37,155] As for the absorption of dietary vitamin B-12, absorption ranged from 4.5% to 85% depending on the product.[26]Additionally, differing methods for assessing micronutrient status are often used, all with varying levels of accuracy and different confounders. For example, vitamin B-12 measurement via serum or plasma B-12 is confounded by irregular flow cell cleaning, use of green-top mineral-free tubes, lack of protection of light, and use of antibiotics. Each measurement technique comes with its own specific confounders which must be addressed.

[8] The BRINDA regression correction may be easily applied using the BRINDA inflammation adjustment R package and SAS macro.

Some micronutrients may be compromised by certain medical conditions. Folate status is impacted if a patient is undergoing renal dialysis, has inflammatory bowel disease, tropical sprue, or celiac disease.[17] Chronic alcoholism is also linked to folate deficiency. Various medications can also result in adjusted micronutrient concentrations, that if not controlled, may confound analysis. In folate, status may be compromised as a result of use of anticonvulsant drugs, sulfasalazine, triamterene, and metformin.[17]

Vitamin D concentrations are influenced by the altitude of the primary place of residence.[55] Of the eleven countries studied in this analysis, seven are home to a major city at high altitude. These countries include Ecuador, Malawi, Pakistan, Colombia, Vietnam, the United States, and Mexico. Ultraviolet B (UVB) radiation, which is responsible for the synthesis of vitamin D in the skin, is increased at higher altitudes due to the reduced atmospheric filtering of UVB radiation.[156] This may partially explain differences in vitamin D concentrations across different countries, as well as allow clinicians and designers of interventions to more appropriately target interventions.

A phenomenon called the "folate trap" refers to the idea that a deficiency in vitamin B-12 leads to the inhibition of homocysteine and methyltetrahydrofolate to methione and tetrahydrofolate, leading to megaloblastic anemia and compromised deficiency measurements.[26] Improvements in vitamin B-12 status are attenuated by high folate status, showing that vitamin B-12 metabolism might be impacted by folate status.[157] Oxidative stress has been linked to decreased homocysteine metabolism via increased amino acid catabolism. Cystathionine β-synthase (CBS), the enzyme that initiates pyridoxal-5′-phosphate–dependent homocysteine catabolism and formation of cysteine and glutathione, is inhibited by insulin, leading to decreased homocysteine concentrations in the blood and increased catabolism.[26] Hypothyroidism

and menopause have also been linked to increased homocysteine concentrations in the blood. Other confounders include concomitant depression, ApoE genotype, high total homocysteine, fasting, renal function, and pregnancy. Unmeasured residual confounders can lead to biased or incorrect estimates of the true association between an exposure and an outcome in observational studies, though it must be mentioned that some of these confounders will not be measured in population surveys.

Chapter 3: Manuscript

Assessing the role of inflammation on serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc in adolescents and school-age children

Madeleine Zeiler,[1] Jiaxi Geng, MPH,[1] Dr. Hanqi Luo,[1] Dr. Rochelle Werner,[1] Dr. Yi-An Ko,[1] Dr. Parmi Suchdev,[1,2,3] Dr. Yaw Addo,[1,2] and Dr. Melissa Young[1]

Corresponding Author: Madeleine Zeiler[1]
madeleine.zeiler@emory.edu

[1]Rollins School of Public Health, Emory University, Atlanta, GA, USA
[2]Nutrition Branch, CDC, Atlanta, GA
[3]Emory University School of Medicine, Atlanta, GA, USA

Abstract

Objectives: Knowledge gaps remain on the role of inflammation on micronutrient biomarker concentrations among school age children (SAC) and adolescents (ADL). Children and WRA are particularly vulnerable, with more than half of PSC and approximately two-thirds of non-pregnant WRA experiencing a micronutrient deficiency in some form.[158] Associations between concentrations of inflammation biomarkers C-reactive protein (CRP) and α1-acid glycoprotein (AGP) and serum folate (SFO), red blood cell folate (RBC folate), vitamin B-12, vitamin D, and zinc were examined.

Methods: This study analyzed cross-sectional data from nutrition surveys in multiple countries from 2006-2016 in SAC and ADL. Survey eligibility criteria were as follows: inclusion of either SAC (5-9 years) or ADL (10-19 years), AGP or CRP and ≥ 1 micronutrient of interest, and n > 100. Micronutrient deficiencies were defined as SFO < 10 nmol/L, RBC folate < 340 nmol/L, vitamin B-12 < 150 pmol/L, vitamin D < 25 nmol/L, and zinc < 57 µg/dL – 74 µg/dL depending on specified conditions. Inflammation was defined as AGP > 1 g/L and CRP > 5 mg/L. Prevalence estimates were adjusted for complex survey design, and associations were assessed by rank correlation coefficient.

Results: Correlations between AGP or CRP and SFO, RBC folate, vitamin B-12, vitamin D, and zinc were weak, with no clear pattern of association. Of the 50 observations recorded 11 were statistically significant ($p < 0.05$). 10 of these were negative: 3 between SFO and CRP ($r = -0.08$ to -0.06); 2 between vitamin B-12 and CRP ($r = -0.18$ to -0.09); 1 between vitamin D and CRP ($r = -0.06$); 1 between zinc and AGP ($r = -0.18$); and 3 between zinc and CRP ($r = -0.19$ to -0.06). 1, vitamin B-12 and CRP ($r = 0.10$ to 0.13), was statistically significant and positive. Decile plots showed no clear pattern across biomarkers.

Conclusions: Due to weak and inconsistent correlations between the inflammation biomarkers and micronutrient biomarkers, there is no rationale to adjust for inflammation when estimating population prevalence SFO, RBC folate, vitamin B-12, vitamin D or zinc deficiencies in SAC or ADL.

Introduction

Micronutrient deficiency is a significant public health challenge that affects a substantial proportion of the population, particularly in developing countries. Estimates reflect that around two billion people experience micronutrient deficiency worldwide, though this estimate has been used for the past three decades.[1,2] Children and WRA are particularly vulnerable, with more than half of PSC and approximately two-thirds of non-pregnant WRA experiencing a micronutrient deficiency in some form.[158] ADL (10-20 years old) and SAC (5-15 years old) are also affected, but data on their micronutrient deficiencies are sparse. Deficiencies in micronutrients such as iron, vitamin A, and zinc are widespread, particularly in low- and middle-income countries.[3]

Micronutrients, such as serum folate (SFO), red blood cell folate (RBC folate), vitamin B-12, vitamin D, and zinc, are essential for numerous physiological processes and maintaining optimal health.[4–7] Deficiencies in these micronutrients can lead to a variety of negative health

outcomes, including impaired growth and development, weakened immune systems, and increased susceptibility to infectious diseases.[8] Moreover, micronutrient deficiency has been linked to long-term cognitive impairment, which can have far-reaching social and economic consequences.[9]

Assessment of micronutrient deficiencies is complicated for several reasons, including the potential for inflammation to impact concentrations of micronutrients (which has been demonstrated to both under- and overestimate micronutrient deficiency), harmonizing cutoffs across varied analytic methods, and lack of inclusion in population-based surveys leading to data gaps.[2] This potentially biases current estimates and explains data gaps worldwide. Past literature demonstrates the need to adjust for inflammation among select micronutrients, particularly those that indicate iron or vitamin A deficiency.[12] This study will investigate the need to adjust SFO, RBC folate, vitamin B-12, vitamin D, and zinc for inflammation (AGP or CRP) in SAC and ADL. The findings of this study will be important for informing public health interventions and policies aimed at addressing micronutrient deficiencies among these age groups and improving their long-term health outcomes. Two studies further investigating the same effect on iron and vitamin A are currently in process.

Combatting nutrition-related adverse health outcomes should be among the key priorities of public health organizations across the globe due to their high prevalence and significant impact on public health, particularly in low- and middle-income countries. Current studies estimate that the impact of malnutrition and related conditions such as overweight or obesity, undernutrition, stunting and wasting, or micronutrient deficiency on the global economy is around $3.5 trillion and $1-2 trillion, respectively.[13] The prevalence estimates of micronutrient deficiency is potentially affected by inflammation; past studies have demonstrated that

prevalence estimates may move as many as 25 percentage points after adjustment.[14] This study will investigate the need for adjusting for inflammation by two different inflammation biomarkers, α1-Acid glycoprotein (AGP) and C-reactive protein (CRP) among five micronutrients: SFO, RBC folate, vitamin B-12, vitamin D, and zinc. Overall, a thorough understanding of the epidemiology, etiology, and consequences of micronutrient deficiency and the role that inflammation plays on specific micronutrient concentrations is necessary for the development of effective public health interventions and policies aimed at mitigating its impact on vulnerable populations.

Methods

Study Design and Data Sources

This study was an analysis of cross-sectional surveys from multiple countries acquired by the BRINDA project and later harmonized. A complete list of BRINDA dataset countries, names, and data sources and a report outlining the BRINDA project is available.[159] Data for this analysis came from eleven countries with surveys collected from 2006 to 2016. Eligible studies included data from either SAC (5-15 years old) or ADL (10-20 years old) and a sample size greater than 100. The study sample was restricted to participants with at least one measure for SFO, RBC folate, vitamin B-12, vitamin D, or zinc and with data available for AGP and/or CRP. Twelve deidentified datasets for ADL (n = 14,711) and 7 datasets for SAC (n = 24,523) were included in this analysis. The protocol was determined to be non-human-subjects research by the Institutional Review Board at Emory University. Figure 1: Study Inclusion and Exclusion Criteria depicts inclusion and exclusion criteria for this study. Data sources and availability are presented for review in **Table 9**.

Lab Methods

Lab methods to measure biomarker and micronutrient concentrations varied by survey and biomarker. Methods included Sandwich ELISA, TIA Roche Hitachi 902, nephelometry, particle-enhanced turbidimetric immunoassay (PETIA), atomic absorption, atomic emission spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), chemiluminescence immunoassay (CLIA), microbiological assay, Bio-Rad (BR) Quantaphase II radioassay, and high-performance liquid chromatography (HPLC). Limits of detection were largely unspecified, and quality assurance methods varied across datasets. Complete lab methods for the biomarkers

and age groups of interest are available for review in **Table 11** while laboratory methods for all BRINDA project datasets may be downloaded from the BRINDA website.[159]

Statistical Methods

Medians and IQRs were calculated for AGP, CRP, and all five biomarkers separately for each survey. The prevalence of micronutrient deficiencies was calculated for all biomarkers: SFO < 10 nmol/L and RBC folate < 340 nmol/L[160], vitamin B-12 < 150 pmol/L[161], and vitamin D < 25 nmol/L[130]; zinc considered age, sex, time of blood draw, and fasting status (population specific cut offs for zinc are presented in Table 10.)[131] . Elevated AGP was defined as concentrations > 1 g/L, while elevated CRP was defined as concentrations > 5mg/L. Spearman rank correlation coefficients between SFO, RBC folate, vitamin B-12, vitamin D, and zinc, and AGP or CRP in ADL and SAC were determined using the BRINDA R package, adjusting for complex survey design effects including cluster, strata, and biomarker-specific sampling weights.[162] Unweighted internal deciles for AGP and CRP were calculated and modeled across the pooled geometric means of all five selected biomarkers, using the BRINDA R package to conduct the BRINDA regression adjustment analysis using the internal decile[153]. A flow chart depicting the conditional determinants for zinc adjustment is available.[153]

An additional sensitivity analysis in SAC was conducted to determine the influence of age on the effect of inflammation on micronutrient deficiency prevalence. As age was identified as a potential confounder in this study, we assessed the rank correlation coefficients across biomarkers and AGP or CRP when split by age: ≥ 12 years old and < 12 years old.

Results

Primary Analysis

The final analytic sample contained 12 datasets from 11 different countries. Among ADL, median AGP values ranged from 0.2 (0.2, 0.4) in Colombia to 1.9 (1.9, 2.3) in Ecuador, while the prevalence of elevated AGP ranged from 1.3% in Bangladesh to 23.4% in Cote d'Ivoire (**Table 1**). Among SAC, median AGP values ranged from 0.2 (0.2, 0.7) in Colombia to 1.9 (1.9, 2.2) in Ecuador, and the prevalence of elevated AGP ranged from 4.9% in the UK to 15.7% in Malawi. The prevalence of elevated CRP in ADL ranged from 11.7% in Bangladesh to 24.8% in Cote d'Ivoire. CRP status among SAC was available only in Malawi: 31.5% (27.0, 36.2). Age, AGP, and CRP status data by median, IQR, and the percent of the survey with elevated AGP or CRP are available for review in **Table 3.**

Median SFO concentrations among ADL ranged from 4.2 nmol/L in Cote d'Ivoire to 37.84 nmol/L in Ecuador, while the prevalence of deficiency ranged from 0.1 (0.0, 0.2) to 88.1 (79.1, 94.3) in Mexico and Cote d'Ivoire, respectively. No SFO deficiency was observed among SAC; median SFO concentrations ranged from 34.00 nmol/L in the US to 46.68 nmol/L in Ecuador and the prevalence of deficiency ranged from 0.0 (0.0, 0.1) in Mexico to 0.2 (0.0, 0.4) in the US. Median ADL RBC folate concentrations ranged from 515.00 nmol/L in Malawi to 832.1 nmol/L in Ecuador, and the prevalence of deficiency ranged from 0.7% to 21.5% in Ecuador and Malawi, respectively. Median SAC RBC folate concentrations ranged from 573.00 nmol/L in the US to 912.74 in Ecuador, and the prevalence of deficiency ranged from 0.5% in Ecuador to 2.2% in the US. Median ADL vitamin B-12 concentrations ranged from 130.3 pmol/L in Pakistan to 415.5 pmol/L in the US, and the prevalence of deficiency ranged from 0.7% to 54.8% in the US and Pakistan, respectively. Median vitamin B-12 concentrations for SAC ranged from 296.7

pmol/L in Colombia to 495.2 pmol/L in the US, and the prevalence of deficiency among SAC ranged from 0.1% in the US to 3.8% in Colombia. Median vitamin D concentrations of ranged from 40.2 nmol/L in the UK to 59.9 nmol/L in the US, and the prevalence of deficiency ranged from 17.5% to 30.7% in the US and the UK, respectively. Median SAC concentrations ranged from 46.9 nmol/L in the UK to 64.9 nmol/L in the US, and the prevalence of deficiency ranged from 3.9% in the US to 20.9% in the UK. No zinc deficiency was seen in either ADL or SAC. Six datasets were available for ADL, among whom median zinc concentrations ranged from 62.0 ug/dL in Bangladesh to 93.3 ug/dL in the UK. The prevalence of zinc deficiency ranged from 0.8% in the UK to 57.3% in Malawi. Four datasets were available for analysis of zinc SAC, and median zinc concentration ranged from 61.1 ug/dL in Malawi to 93.1 ug/dL in the UK. The prevalence of deficiency among SAC ranged from 0.4% in the UK to 57.0% in Malawi. Micronutrient status data by median (IQR) and the percent of each survey deficient are available for review in **Table 4**.

There were few significant relationships between micronutrient and inflammation biomarkers. No significant results were observed between SFO and AGP in ADL or SAC, RBC folate and AGP in ADL or SAC, RBC folate and CRP in ADL or SAC, or vitamin B-12 and AGP in ADL or SAC. Serum folate and CRP had significant negative correlations in two of nine ADL datasets (Ecuador, r = -0.08; US, r = -0.07) and one of three SAC datasets (US, r = -0.06) and no significant positive associations in either ADL or SAC. Vitamin B-12 and CRP had significant negative correlations in two of the nine ADL datasets (Malawi, r= –0.18; US, r= -0.09) and no negative correlations among five SAC datasets. However, vitamin B-12 and CRP had significant positive correlations in Mexico for both ADL (r = 0.13) and SAC (r = 0.10). Vitamin D and CRP were significantly negatively associated in one of two SAC datasets (US, r =

–0.06) but not among ADL. No significant positive correlations were observed among SAC or ADL. Zinc and AGP were significantly negatively associated in the one dataset available for SAC in Malawi (r = –0.18). Zinc and CRP were significantly negatively associated in one out of six datasets for ADL in the UK (r = -0.18). No significant positive correlations were observed. Zinc and CRP were significantly negatively associated in two of four associations for SAC in Ecuador (r = -0.06) and Malawi (r = -0.19.) No significant positive correlations were observed in this group. Rank correlation coefficients for each micronutrient by AGP and/or CRP are available for review in **Table 5.**

SFO geometric means varied by CRP decile, and geometric means for ADL females were consistently and visibly below ADL males and both SAC groups across deciles. Values for ADL males and females were consistently below those of SAC for SFO and CRP. When considering geometric means of vitamin B-12 across CRP deciles, SAC were above those of ADL for both sex groups, and SAC females were consistently above the other three groups. For RBC folate and CRP, geometric means for ADL females were lower than the three other groups across deciles and SAC males were consistently higher than the three other groups. ADL values were generally lower than SAC values, though less consistently than in other observations. In considering vitamin D across CRP deciles, geometric means for ADL females were consistently and visibly lower than the three other groups, while values for SAC males were consistently higher than the three other groups. When considering both sex groups, ADL values were not consistently lower than SAC values. Noise was significant across deciles for zinc and AGP. For zinc and CRP, values for SAC and ADL were more clustered across deciles than in other analyses. Values for ADL females were consistently below those of other groups. No other clear patterns were visible. Across all decile plots, geometric means did not clearly differ across

deciles, indicating no clear difference in micronutrient concentration with increase or decrease in inflammation. Decile plots are available for review in **Figure 3** and **Figure 4**.

Sensitivity Analysis

A sensitivity analysis stratified SAC by age: ≥ 12 years old and < 12 years old. Median AGP values among children ≥ 12 years old and children < 12 years old were identical to those of SAC 5-15 years. The prevalence of elevated AGP was 0.9% higher in children ≥ 12 years old than SAC overall. The prevalence of elevated AGP was 3.8% and 1.5% lower among children < 12 years old than SAC. The median CRP level among children ≥ 12 years old was 4.5% higher than SAC. The median CRP level among children < 12 years old in Malawi was 15% lower than SAC. These values are available for review in **Table 6**.

Median SFO concentrations ranged from 36.20 nmol/L in the US (2006) to 50.08 nmol/L in Ecuador (2012) for SAC >= 12 years old. For SAC < 12 years old, median SFO concentrations ranged from 29.20 nmol/L in the US (2006) to 39.43 nmol/L in Ecuador (2012). Median RBC folate concentrations ranged from 591.20 nmol/L in the US (2006) to 954.89 nmol/L in Ecuador (2012) for SAC >= 12 years old. For SAC < 12 years old, median RBC folate concentrations ranged from 536.80 nmol/L in the US (2006) to 824.37 nmol/L in Ecuador (2012). Median vitamin B-12 concentrations ranged from 301.10 pmol/L in Colombia (2010) to 542.43 pmol/L in the US (2006) for SAC >= 12 years old. For SAC < 12 years old, median vitamin B-12 concentrations ranged from 273.80 pmol/L in Colombia (2010) to 414.76 pmol/L in the US (2006). Median vitamin D concentrations ranged from 49.00 nmol/L in the UK (2014) to 64.90 nmol/L in the US (2006) for SAC >= 12 years old. For SAC < 12 years old, median vitamin D concentrations ranged from 40.10 nmol/L in the UK (2014) to 57.41 nmol/L in the US (2006). Median zinc concentrations ranged from 59.38 µg/dL in Malawi (2016) to 93.14 µg/dL

in the UK (2014) for SAC >= 12 years old. For SAC < 12 years old, median zinc concentrations ranged from 62.50 μg/dL in Malawi (2016) to 91/37 μg/dL in the UK (2014). The prevalence of deficiency was below 0.5% across all observations for SFO, ranged from 0.1% (0.0, 0.3) to 1.2% (0.8, 1.6) in children >= 12 years old and 1.4% (0.7, 2.4) to 4.4% (3.1, 5.9) in children < 12 years old for RBC folate, from 0.0% (0.0, 0.1) to 2.9% (2.3, 3.4) in children >= 12 years old and 0.1% (0.0, 0.3) to 4.6% (3.1, 6.6) in children < 12 years old for vitamin B-12, from 2.2% (1.6, 3.0) to 17.6% (12.7, 23.4) in children >= 12 years old and 7.4% (5.5, 9.8) to 32.5% (23.5, 42.5) in children < 12 years old for vitamin D, and from 0.4% (0.0, 1.7) to 60.0% (51.2, 68.4) in children >= 12 years old and 0.5% (0.0, 2.0) to 58.0% (47.4, 68.2) in children < 12 years old for zinc. All median concentrations and prevalence of deficiency are available for review in **Table 7**.

Like the main analysis, few significant correlations were observed. Three correlations between SFO and CRP were recorded for children ≥ 12 years old: one significant negative correlation was observed in Ecuador (r = -0.09). Neither of the two observations for children < 12 years old were significant. Two correlations between RBC folate and CRP were observed in children ≥ 12 years old and children < 12 years old; neither were significant. Five correlations were observed between vitamin B-12 and CRP: five correlations existed for children ≥ 12 years old and two (both positive) were significant in Mexico (r = 0.10) and Colombia (r = 0.05). None of the four correlations for children < 12 years old were significant. Two correlations existed before vitamin D and CRP for children ≥ 12 years old; one negative correlation was significant in the US (r = -0.07). Two correlations were recorded for children < 12 years old, though neither were significant. One observation was recorded between zinc and AGP for children ≥ 12 years old. This singular observation was significant and negative in Malawi (r = -0.23). The one observation between zinc and AGP for children < 12 years old was not significant. Finally, four

correlations existed between zinc and CRP for children ≥ 12 years old. The two significant correlations were both negative in Ecuador (r = -0.07) and Malawi (r = -0.26). Three observations were recorded for children < 12 years old; none were significant. All rank correlation coefficients are available for review in **Table 8.**

Geometric means were plotted across deciles for children ≥ 12 years old and < 12 years old. AGP deciles were available only for zinc, though no clear pattern was visible. Values for children ≥ 12 years old were higher than those of children < 12 years old across CRP deciles in all five biomarkers. Females ≥ 12 years old saw the highest geometric means across CRP deciles, followed by males ≥ 12 years old, males < 12 years old, and females < 12 years old for SFO and vitamin B-12. Males ≥ 12 years old saw the highest geometric means across CRP deciles, followed by females ≥ 12 years old, males < 12 years old, and females < 12 years old for RBC folate, vitamin D, and zinc. Among children ≥ 12 years old, female values were higher across deciles in SFO and vitamin B-12, while male values were higher for vitamin D and RBC folate. Among children < 12 years old, female values for vitamin B-12 were higher than those of males, while male values were consistently higher in SFO, RBC folate, and vitamin D. Similarly to the previous decile analysis, geometric means did not clearly differ across deciles, indicating no clear difference in micronutrient concentration with increase or decrease in inflammation. The complete deciles plots for the sensitivity analysis are available for review in **Figure 5** and **Figure 6.**

Discussion

The results of this multi-country analysis, which involved 39,234 ADL and SAC, indicate that there are weak and inconsistent correlations between inflammation markers CRP or AGP and micronutrient biomarkers SFO, RBC folate, vitamin B-12, vitamin D, and zinc. The correlations observed were usually either weak ($r < 0.20$) or not statistically significant, with few significant correlations ranging from -0.19 to 0.13 and demonstrating inconsistent directionality. No clear pattern of association was found between inflammation deciles (AGP or CRP) and micronutrient biomarkers. These findings suggest that there is no clear rationale to support adjusting for inflammation when estimating the prevalence of SFO, RBC folate, vitamin B-12, vitamin D, or zinc deficiencies in ADL or SAC.

Prior studies have demonstrated a similar result for several of the micronutrients considered in this analysis, though primarily in WRA and PSC. One showed that there was no rationale to adjust for inflammation when determining the population prevalence of deficiency of vitamin B-12 and folate among WRA or PSC.[144] The BRINDA project has demonstrated rationale to adjust for inflammation by either AGP or CRP in PSC for RBP,[82] serum retinol, serum ferritin,[125] sTfR,[128] and serum zinc.[131] Among WRA, rationale exists to adjust for inflammation for serum ferritin[125] and sTfR.[128] Excluding serum zinc (which only suggested that conditional adjustment was necessary, signifying that further research is necessary), none of the five micronutrients considered in this analysis have demonstrated the need for adjustment among PSC or WRA, showing consistency across numerous studies and population groups.[154]

This study expands the body of literature examining the role of AGP and CRP on SFO, RBC folate, vitamin B-12, vitamin D, and zinc concentrations into population groups for SAC and ADL. It is one of the largest to date, using data from eleven countries across the globe and

potentially increasing the generalizability of our findings to those countries whereas most papers only investigate one country. Likewise, the volume of micronutrient biomarkers analyzed will allow resources to be directed toward expanded investigation of other micronutrient and inflammation biomarkers, age groups, and populations.

This analysis had several limitations. Surveys included in this analysis had a limited number of children with complete measures for CRP, AGP, and one nutritional biomarker, samples were not selected to be nationally- or regionally-representative for SAC or ADL, and data availability was particularly limited for ADL males, AGP in SAC, as well as for RBC folate and vitamin D biomarkers. It is possible that results would differ across biomarkers with increased data availability. Research gaps should be filled by further research using expanded datasets if possible to increase data availability. Finally, laboratory methods varied across the 12 surveys included in this analysis: multiple biomarkers may be used to assess deficiency status in select micronutrients and methods varied by survey. To account for this, data from each survey were analyzed individually. This study did not consider the role that malarial infection can play on concentrations of the selected micronutrients due to limited data on malaria within the surveys used. Limits of detection were largely unavailable for the surveys used. No analysis was done on the impact of existing intervention programs or recent illness as this was outside of the scope of current research.

Overall, this multi-country analysis showed weak and inconsistent correlations between inflammation biomarkers AGP and CRP and SFO, RBC folate, vitamin B-12, vitamin D, or zinc, indicating no rationale to adjust for inflammation when estimating population prevalence of related deficiencies in SAC or ADL. Understanding the role of inflammation on micronutrient biomarker concentrations has important public health implications. Inflammation is a common

occurrence in many chronic diseases and infections, and its presence can impact the interpretation of biomarkers used to assess micronutrient status. Failing to account for inflammation when estimating population-level micronutrient deficiencies could lead to inaccurate estimations of the prevalence of deficiencies and hinder the development of effective interventions. Adjusting for inflammation could also help identify subpopulations with higher risk of micronutrient deficiencies, guide targeted public health interventions, and improve the effectiveness of nutrition programs. Therefore, it is critical to consider the potential impact of inflammation when assessing micronutrient status and to develop standardized approaches for adjusting for inflammation to accurately estimate the prevalence of micronutrient deficiencies in populations.

Chapter 4: Discussion

Overview

This multi-country analysis of 39,234 ADL and SAC showed weak and inconsistent correlations between inflammation (CRP or AGP) and SFO, RBC folate, vitamin B-12, vitamin D, or zinc. All correlations were weak ($r < 0.20$) or non-significant. Statistically significant correlations ranged from -0.19 to 0.13, demonstrating inconsistent directionality. No clear pattern of association was observed between inflammation deciles (AGP and CRP) and micronutrient biomarkers. As a result, this study demonstrates no clear rationale to adjust for inflammation when estimating population prevalence of serum folate, RBC folate, vitamin B-12, vitamin, or zinc deficiencies in SAC or ADL.

Prior studies (summarized in Table 12) have demonstrated a similar result for several of the micronutrients considered in this analysis though in different populations, one showing that there was no rationale to adjust for inflammation when determining the population prevalence of deficiency of vitamin B-12 and folate among WRA or PSC.[144] Currently, the BRINDA project has demonstrated rationale to adjust for inflammation by either AGP or CRP in PSC for RBP,[82] serum retinol, serum ferritin,[125] sTfR,[128] and serum zinc.[131] Among WRA, rationale exists to adjust for inflammation for serum ferritin[125] and sTfR.[128] Excluding serum zinc, none of the five micronutrients considered in this analysis have demonstrated the need for adjustment among PSC or WRA, showing consistency across numerous studies and population groups.[154] It is important to note that conditional recommendations have been made for adjustment by AGP or CRP for serum zinc among PSC as it is recommended to perform both correlation and decile analyses using data from a specific PSC population. The strength and significance of the correlation coefficient between serum zinc and CRP or AGP as well as visual inspection of the

decile plots is needed to corroborate increasing prevalence of zinc deficiency by CRP or AGP decile.[131]

Inflammation and micronutrients are intrinsically linked: malnutrition increases the likelihood and severity of inflammation. Conversely, inflammation impairs nutritional status via reduced intestinal absorption, more nutrient sequestration from the bloodstream, and decreased utilization within tissues.[163] Inflammation is a complex physiological response that plays a crucial role in host defense against infection and injury but can also disrupt micronutrient homeostasis, leading to deficiencies in essential micronutrients. Micronutrient deficiency can compromise the growth and development of children and adolescents. The public health significance of inflammation on this vulnerable population underscores the need for effective strategies to mitigate its adverse effects on micronutrient status and health outcomes. Across all datasets used in this analysis, the median CRP concentration was 0.5 g/L and the median AGP concentration was 0.8 mg/L.

Biological Rationale

Though bidirectional associations exist between SFO, RBC folate, vitamin B-12, vitamin D, and zinc and inflammation biomarkers AGP and CRP exist showing potential biological rationale for adjusting these micronutrient biomarkers for inflammation, studies show varying results. One study demonstrated a link between reduced folate concentrations and inflammation while another demonstrated the inverse.[132] Similar findings exist for vitamin D: while there is an association between inflammation and lower vitamin D concentrations due to the sequestration of vitamin D in adipose tissue and therefore reduced absorption,[145] and increased 24-hydroxylase leading to conversion of 25(OH)D into the biologically inactive metabolite 24,25-dihydroxyvitamin D,[147] past analyses, like this one, have deemed adjustment unnecessary.[149]

Finally, though inflammatory cytokines IL-1 and TNF-α do have the capability to reduce zinc concentrations in the body,[150] inflammation may lead to the upregulated expression of ZIP14,[151] and while previous studies have demonstrated rationale for adjustment in PSC, this study and others have yet to demonstrate the need to adjust in the selected population groups.[131]

Two populations met the conditional determinants for zinc adjustment and showed significant results. Both groups were derived from the Malawi (2016) datasets: SAC showed a rank correlation coefficient of -0.18 and children ≥ 12 years old showed a rank correlation coefficient of -0.23. Therefore, further research may recommend adjusting serum zinc for inflammation by AGP and CRP if both inflammation biomarkers are available. Adjustment by only one biomarker may be sufficient if only one biomarker is available. However, further analysis must be completed prior to expanding recommendations on zinc adjustments for SAC/ADL populations.

Next Steps

Suggested Cutoffs

Debate remains within the nutrition community regarding cutoffs for deficiency. For example, the Institute of Medicine defines deficiency as vitamin D < 30 nmol/L, whereas the Endocrine Society and European Food Safety Authority use vitamin d < 50 nmol/L. Cut offs also range depending on age group. Work is being done to determine appropriate cutoffs levels for hemoglobin to define anemia.[164] One study recommends investing cutoffs for serum vitamin B-12, 25-hydroxyvitamin D, MMA, and RBP. It also recommend continued work on biomarkers of zinc status.[165] Further research must be done to determine ideal deficiency cutoffs across all micronutrient biomarkers.

Clinical Trial Design

Ideally, the impact of the bidirectional associations between inflammation and micronutrient biomarkers would be studied in a clinical trial setting to rule out a lack of precision in survey data collection and analysis. A study is suggested with builds upon Williams et al.'s study which investigated changes in micronutrient and inflammation serum biomarker concentrations after a norovirus human challenge in a hospital setting, though several conditions are adjusted.[149] Free-living participants would be randomized into two groups, a low inflammation group and a high inflammation group. The low inflammation group would receive a standard diet containing adequate amounts of micronutrients, while the high inflammation group will receive a similar diet with additional inflammatory stimuli. This can be achieved by supplementing their diet with a pro-inflammatory agent like lipopolysaccharide or through administering an inflammatory stimulus like the influenza vaccine. For both groups, the primary outcome would be changes in micronutrient biomarkers. Baseline data on demographic, anthropometric, and clinical characteristics would be collected for each participant. Micronutrient biomarkers would be measured at baseline and at the end of the study period. Inflammatory markers would be measured at baseline, during the intervention period, and at the end of the study period. After controlling for confounding factors such as age, sex, BMI, existing inflammatory conditions, existing micronutrient deficiency, diet, altitude, and UVB radiation exposure, the BRINDA inflammation adjustment method would be applied to determine the role of inflammation on micronutrient biomarkers in participants who have undergone the stimulation of an inflammatory response versus those who have not.

Study Strengths

The present analysis is strong in several ways: it is the first of its kind to consider the role of AGP and CRP on SFO, RBC folate, vitamin B-12, vitamin D, and zinc concentrations in SAC and ADL. This study is one of the largest to date, using data from eleven countries across the globe including low-, middle-, and high-income countries potentially increasing the generalizability of our findings to the eleven countries studied in comparison to most papers which only investigate one country. While numerous studies have been published indicating the need to adjust for inflammation in select micronutrients, the five micronutrients investigated here do not appear to require adjustment among ADL and SAC. Additionally, because five micronutrient biomarkers were analyzed, resources can be directed toward expanded investigation of other micronutrient and inflammation biomarkers, age groups, and populations. Accurate estimates are essential to effective program planning and implementation. The public health significance of this is tremendous: the prevalence of deficiency of these micronutrients varies drastically across the globe, indicating that effective interventions are critical to the wellbeing of populations worldwide.

Study Limitations

This study also had significant limitations. The potential for laboratory error in studying the micronutrient biomarkers assessed is vast. Data for this study came from eleven countries, all with varying degrees of medical infrastructure: several micronutrients investigated in this analysis require pristine laboratory conditions for analysis that is both valid and precise. Despite the existence of quality assurance across all surveys included in this analysis, it must be understood that human error may have compromised survey data.

Large data gaps existed in terms of sample size, lack of representation in other regions, and lack of representation of differing groups across surveys. For example, data were limited for male participants. Data availability was limited for RBC folate and vitamin D across both population groups. AGP data was also limited among SAC in particular, restricting analysis to Malawi only. Still, twelve surveys were considered in this analysis, twelve for ADL and seven for SAC. It is possible that results would differ across biomarkers with increased data availability, particularly for CRP in SAC. This study did not consider the role that malarial infection can play on concentrations of the selected micronutrients due to limited data on malaria within the surveys used. Malaria has been linked to increased inflammation and oxidative stress.[166] Limits of detection were largely unavailable for the surveys used, a limitation that should be addressed in future research. No analysis was done on the impact of existing intervention programs or recent illness as this was outside of the scope of current research which focused solely on determining the necessity of adjusting micronutrient biomarkers for inflammation to determine population prevalence estimates. Finally, laboratory methods varied across the twelve surveys included in this analysis: multiple biomarkers may be used to assess deficiency status in select micronutrients and methods varied by survey. To account for this, data from each survey were analyzed individually.

Conclusions

This multi-country analysis of 39,234 ADL and SAC showed weak and inconsistent correlations between inflammation and SFO, RBC folate, vitamin B-12, vitamin D, or zinc, indicating no rationale to adjust for inflammation when estimating population prevalence of related deficiencies in SAC or ADL. This study and previous analyses have deemed adjustment to be unnecessary for the five investigated micronutrients. Although limitations exist, including

data availability and differing laboratory methods across surveys, this study's strength lies in its multi-country analysis and consideration of numerous micronutrients by two different inflammation biomarkers, enhancing the generalizability of findings. This study can serve as a methodological road map for public health researchers in determining the impact of inflammation on micronutrient concentrations in select populations. Public health practitioners will be able to use the accurate prevalence estimates presented in this current analysis to advocate for effective strategies to mitigate the adverse effects of micronutrient deficiency and health outcomes among vulnerable populations.

Supplementary Materials

Table 1: Prevalence of deficiency for each country included in the analysis for PSC and non-pregnant women*

	Folate deficiency	Vitamin B-12 deficiency	Vitamin D deficiency	Zinc deficiency	Any core deficiency (iron, zinc, or vitamin A)
Survey, year					
PSC					
Azerbaijan, 2013	-	-	-	17% (14–20)	40% (36–44)
Bangladesh, 2011	-	-	-	32% (24–41)	52% (41–63)
Cote d'Ivoire, 2007	-	-	-	-	63% (54–73)
Ecuador, 2012	8% (7–10)	-	-	28% (25–31)	43% (40–46)
Malawi, 2015-2016	-	-	-	61% (55–67)	74% (69–78)
Mexico, 2006	-	3% (1–4)	-	27% (24–32)	58% (52–63)
Mexico, 2012	1% (0–1)	0% (0–1)			46% (38–54)
Pakistan, 2011	-	-	13% (12–15)	40% (38–42)	86% (85–87)
UK, 2008-2019	2% (1–5)	0% (0–2)	12% (7–20)		48% (33–63)
Vietnam, 2010	7% (4–13)	-	14% (10–19)	56% (50–61)	66% (61–71)
NPW					
Azerbaijan, 2013	35% (31–39)	20% (16–24)	-	-	76% (66–85)
Bangladesh, 2011	84% (79–87)	20% (16–24)	-	41% (35–48)	76% (66–85)
Cote d'Ivoire, 2007	91% (88–94)	18% (12–26)	-	-	96% (93–99)
Ecuador, 2012	10% (9–11)	1% (1–2)		57% (55–59)	68% (66–69)
Malawi, 2015-2016	23% (18–29)	13% (9–17)	-	58% (52–64)	72% (67–77)
Mexico, 2006	-	-	-	29% (25–34)	61% (54–68)
Mexico, 2012	3% (2–4)	2% (1–2)	-	-	67% (55–79)
Pakistan, 2011	-	52% (50–55)	31% (29–33)	46% (44–48)	78% (72–84)
UK, 2008-2019	19% (16–22)	7% (5–9)	22% (19–25)	10% (8–12)	43% (39–46)
Vietnam, 2010	22% (19–25)	12% (9–15)	9% (7–13)	67% (63–71)	78% (74–81)

* Modified from Stevens et al. Lancet Global Health 2022 Nov; 10(11). PSC, preschool-age children 6-59 months old; NPW, non-pregnant women aged 15-49 years

Table 2: Prevalence of people with deficiencies in one more of three core micronutrients from 2003-2019*

	Prevalence of any deficiency in children age 6-59 months, % (95% UI)	Prevalence of any deficiency in non-pregnant women age 15-49 years, % (95% UI)
World	56% (48-64)	69% (59-78)
East Asia and the Pacific	58% (42-75)	72% (52-88)
Europe and central Asia	45% (27-64)	68% (46-86)
High-income countries	45% (25-68)	48% (26-73)
Latin American and the Caribbean	48% (38-58)	63% (47-79)
Middle East and north Africa	53% (26-78)	68% (34-93)
South Asia	57% (46-68)	74% (61-85)
Sub-Saharan Africa	62% (53-72)	80% (70-89)

* Modified from Stevens et al. Lancet Global Health 2022 Nov; 10(11).

Figure 1: Study Inclusion and Exclusion Criteria

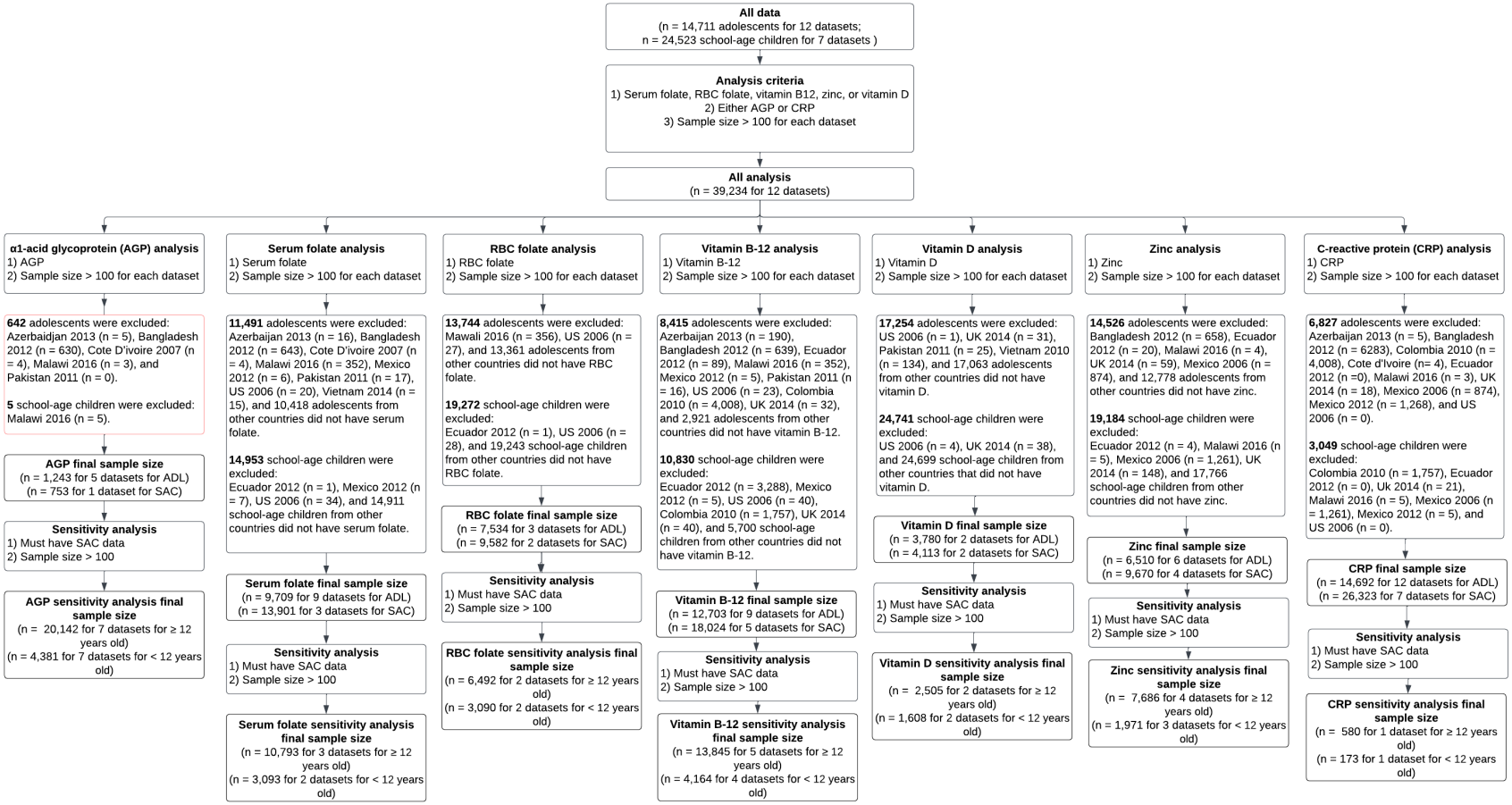

Table 3: Age, AGP, and CRP status in adolescents and school-age children

	Age		CRP, g/L			AGP, mg/L		
Survey (year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP, % (95% CI)**	**n**	**Median (IQR)**	**Elevated CRP, % (95% CI)**
Adolescents								
Azerbaijan(2013)	358	17(16, 19)	358	0.4(0.2, 1.1)	4.5(2.4, 7.4)	358	0.8(0.7, 0.9)	20.4(15.9, 25.5)
Bangladesh(2012)	176	16(16, 18)	176	0.3(0.3, 1.3)	1.3(0.5, 2.7)	176	0.7(0.6, 0.9)	11.7(3.7, 25.6)
Cote D'ivoire(2007)	106	17(16, 18)	106	1.7(0.5, 4.9)	23.4(15.7, 32.5)	106	0.8(0.7, 1.0)	24.8(17.8, 32.8)
Colombia(2010)	3007	12(11, 12)	3007	0.2(0.2, 0.4)	12.5(10.7, 14.4)	_	_	_
Ecuador(2012)	4153	14(12, 15)	4153	1.9(1.9, 2.3)	7.7(6.5, 9.0)	_	_	_
UK(2014)	544	14(12, 16)	544	1.5(1.0, 2.1)	6.2(3.8, 9.4)	_	_	_
Malawi(2016)	511	14(12, 16)	511	0.6(0.2, 1.9)	10.5(7.6, 13.9)	511	0.7(0.5, 0.9)	17.4(13.7, 21.5)
Mexico(2006)	1023	17(16, 19)	1023	0.7(0.3, 1.6)	8.4(6.4, 10.8)	_	_	_
Mexico(2012)	1268	11(10, 12)	1268	0.5(0.2, 1.7)	9.6(6.9, 12.9)	_	_	_
Pakistan(2011)	123	18(18, 19)	104	0.8(0.4, 2.1)	11.0(5.2, 19.6)	92	0.8(0.6, 1.0)	19.1(11.7, 28.3)
US(2006)	3250	14(12, 16)	3250	0.4(0.1, 1.5)	9.0(7.7, 10.5)	_	_	_
Vietnam(2010)	192	17(16, 18)	192	0.3(0.2, 0.7)	4.7(2.3, 8.3)	_	_	_
School-age children								
Colombia(2010)	6847	9(7, 11)	6847	0.2(0.2, 0.7)	13.6(12.3, 14.9)	_	_	_
Ecuador(2012)	6063	10(8, 13)	6063	1.9(1.9, 2.2)	7.2(5.8, 8.8)	_	_	_
UK(2014)	586	9(7, 12)	586	1.4(0.8, 2.0)	4.9(2.8, 7.8)	_	_	_
Malawi(2016)	753	9(7, 12)	753	0.8(0.3, 2.2)	15.7(12.0, 19.9)	753	0.8(0.6, 1.1)	31.5(27.0, 36.2)
Mexico(2006)	2399	7(6, 10)	2399	0.5(0.2, 1.7)	10.0(7.9, 12.3)	_	_	_
Mexico(2012)	4327	9(7, 10)	4327	0.5(0.2, 1.5)	8.5(7.3, 9.9)	_	_	_
US(2006)	3548	10(8, 13)	3548	0.3(0.1, 1.1)	6.6(5.4, 8.0)	_	_	_

* AGP, Alpha(1)-acid glycoprotein; CRP, C-reactive protein; IQR, Interquartile Range. Elevated AGP and CRP defined as an AGP > 1 g/L and CRP > 5 mg/L, respectively

Table 4: Serum folate, RBC folate, vitamin B-12, vitamin D, and zinc status in adolescents and school-age children

	Serum folate, nmol/L			RBC folate, nmol/L			Vitamin B12, pmol/L			Vitamin D, nmol/L			Zinc, µg/dL		
Survey (year)	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**
Adolescents															
Azerbaijan(2013)	347	10.89(9.10, 12.90)	37.6(30.9, 44.6)	-	-	-	173	260.37(178.03, 344.61)	21.9(14.3, 30.9)	-	-	-	-	-	-
Bangladesh(2012)	164	10.96(8.40, 14.23)	43.4(26.4, 61.4)	-	-	-	168	351.14(274.17, 481.77)	2.6(0.5, 7.4)	-	-	-	149	62.00(56.00, 70.00)	30.4(17.0, 46.5)
Cote D'ivoire(2007)	106	4.20(2.50, 5.90)	88.1(79.1, 94.3)	-	-	-	-	-	-	-	-	-	-	-	-
Ecuador(2012)	4153	37.84(26.74, 53.70)	0.7(0.4, 1.2)	4153	832.08(641.28, 1062.75)	0.9(0.6, 1.3)	4064	394.00(296.00, 538.00)	1.3(0.6, 2.2)	-	-	-	4133	71.00(63.00, 80.00)	14.0(11.8, 16.4)
Malawi(2016)	162	18.90(13.00, 35.30)	16.4(8.9, 26.2)	158	515.00(352.00, 747.00)	23.5(12.9, 37.1)	162	259.04(184.50, 382.28)	11.5(6.1, 19.0)	-	-	-	510	62.50(50.00, 71.88)	58.8(51.9, 65.4)
Mexico(2012)	1267	34.88(30.35, 38.28)	0.1(0.0, 0.2)	-	-	-	1268	398.00(309.00, 519.00)	1.1(0.4, 2.3)	-	-	-	-	-	-
Pakistan(2011)	103	7.14(2.19, 23.34)	59.4(49.0, 69.4)	-	-	-	104	130.26(46.81, 262.14)	56.4(46.1, 66.4)	-	-	-	-	-	-
US(2006)	3230	28.80(22.00, 37.40)	0.7(0.4, 1.2)	3223	539.10(455.30, 634.20)	5.2(4.3, 6.3)	3227	415.49(315.13, 533.57)	0.6(0.3, 0.9)	3249	59.90(47.42, 72.38)	8.3(6.3, 10.7)	-	-	-
Vietnam(2010)	177	16.20(11.40, 22.90)	13.0(8.3, 18.9)	-	-	-	-	-	-	-	-	-	192	62.40(54.40, 72.00)	38.5(30.9, 46.6)
Colombia(2010)	-	-	-	-	-	-	3007	285.61(221.40, 364.57)	4.2(3.3, 5.2)	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	530	292.00(231.00, 351.00)	2.8(1.5, 4.6)	531	40.20(26.10, 56.40)	29.8(23.9, 36.2)	503	93.27(83.99, 103.07)	0.6(0.1, 1.5)
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	-	-	1023	82.61(64.09, 116.38)	18.4(14.1, 23.2)
School-age children															
Ecuador(2012)	6062	46.68(33.76, 63.22)	0.1(0.0, 0.2)	6062	912.74(716.96, 1154.98)	0.5(0.3, 0.8)	2775	401.00(298.00, 538.00)	1.2(0.6, 2.2)	-	-	-	6059	73.00(65.00, 81.00)	12.4(10.7, 14.3)
Mexico(2012)	4325	35.79(31.94, 38.51)	0.0(0.0, 0.1)	-	-	-	4327	457.00(345.00, 598.00)	0.6(0.3, 1.1)	-	-	-	-	-	-
US(2006)	3514	34.00(26.50, 42.40)	0.2(0.0, 0.4)	3520	573.00(496.00, 665.90)	2.2(1.7, 2.8)	3508	495.20(385.97, 638.37)	0.1(0.0, 0.1)	3544	64.90(52.42, 74.88)	3.9(2.9, 5.0)	-	-	-
Colombia(2010)	-	-	-	-	-	-	6847	296.68(231.73, 381.55)	3.1(2.6, 3.7)	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	567	346.00(274.00, 444.00)	0.3(0.1, 0.9)	569	46.90(32.80, 62.20)	20.9(16.5, 25.8)	459	93.14(84.12, 101.50)	0.4(0.1, 1.3)
Malawi(2016)	-	-	-	-	-	-	-	-	-	-	-	-	753	61.08(50.00, 71.88)	59.6(51.8, 67.0)
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	-	-	2399	83.23(63.10, 111.02)	19.5(16.4, 22.8)

* IQR, Interquartile Rangel; RBC folate, red blood cell folate. Serum folate deficiency is defined as < 10 nmol/L. Serum folate deficiency is defined as < 340 nmol/L. Vitamin B12 deficiency is defined as < 150 pmol/L. Vitamin D deficiency is defined as < 30 nmol/L. Definition of zinc deficiency depends on unique combinations in age, sex, blood draw time, and fasting status.

Table 5: Rank correlation coefficient between serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc and AGP or CRP in adolescents and school-age children

	SFO & AGP		SFO & CRP		RBC folate & AGP		RBC folate & CRP		Vit B12 & AGP		Vit B12 & CRP		Vit D & CRP		Zinc & AGP		Zinc & CRP	
Survey (year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
Adolescents																		
Azerbaijan(2013)	347	-0.06	347	-0.01	-	-	-	-	173	0.12	173	0.05	-	-	-	-	-	-
Bangladesh(2012)	164	-0.06	164	-0.23	-	-	-	-	168	0.14	168	0.20	-	-	149	0.03	149	-0.16
Cote D'ivoire(2007)	106	-0.04	106	-0.04	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ecuador(2012)	-	-	4153	-0.08*	-	-	4153	-0.01	-	-	4064	-0.05	-	-	-	-	4133	-0.09
Malawi(2016)	162	-0.13	162	-0.06	158	-0.12	158	-0.13	162	-0.07	162	-0.18*	-	-	510	-0.03	510	-0.04
Mexico(2012)	-	-	1267	0.08	-	-	-	-	-	-	1268	0.13*	-	-	-	-	-	-
Pakistan(2011)	-	-	103	-0.01	-	-	-	-	-	-	104	-0.16	-	-	-	-	-	-
US(2006)	-	-	3230	-0.07*	-	-	3223	0.02	-	-	3227	-0.09*	3249	-0.03	-	-	-	-
Vietnam(2010)	-	-	177	0.08	-	-	-	-	-	-	-	-	-	-	-	-	192	0.00
Colombia(2010)	-	-	-	-	-	-	-	-	-	-	3007	0.02	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	-	-	-	-	530	-0.05	531	0.07	-	-	503	-0.18*
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1023	0.05
School-age children																		
Ecuador(2012)	-	-	6062	-0.06	-	-	6062	-0.04	-	-	2775	0.03	-	-	-	-	6059	-0.06*
Mexico(2012)	-	-	4325	0.04	-	-	-	-	-	-	4327	0.10*	-	-	-	-	-	-
US(2006)	-	-	3514	-0.06*	-	-	3520	0.03	-	-	3508	0.01	3544	-0.06*	-	-	-	-
Colombia(2010)	-	-	-	-	-	-	-	-	-	-	6847	0.03	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	-	-	-	-	567	0.03	569	0.00	-	-	459	-0.07
Malawi(2016)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	753	-0.18*	753	-0.19*
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2399	0.01

* AGP, Alpha(1)-acid glycoprotein; CRP, C-reactive protein; RBC folate, red blood cell folate; SFO, serum folate; vit B12, vitamin B1; vit D, vitamin D. r represents spearman rank correlation coefficients. * indicates p-values for spearman rank correlation coefficients < 0.05.

Figure 3: Estimated geometric means of (A) serum folate by AGP, (B) serum folate by CRP, (C) vitamin B12 by AGP, (D) vitamin B12 by CRP, (E) RBC folate by AGP and (F) RBC folate by CRP in school-age children by sex and age group

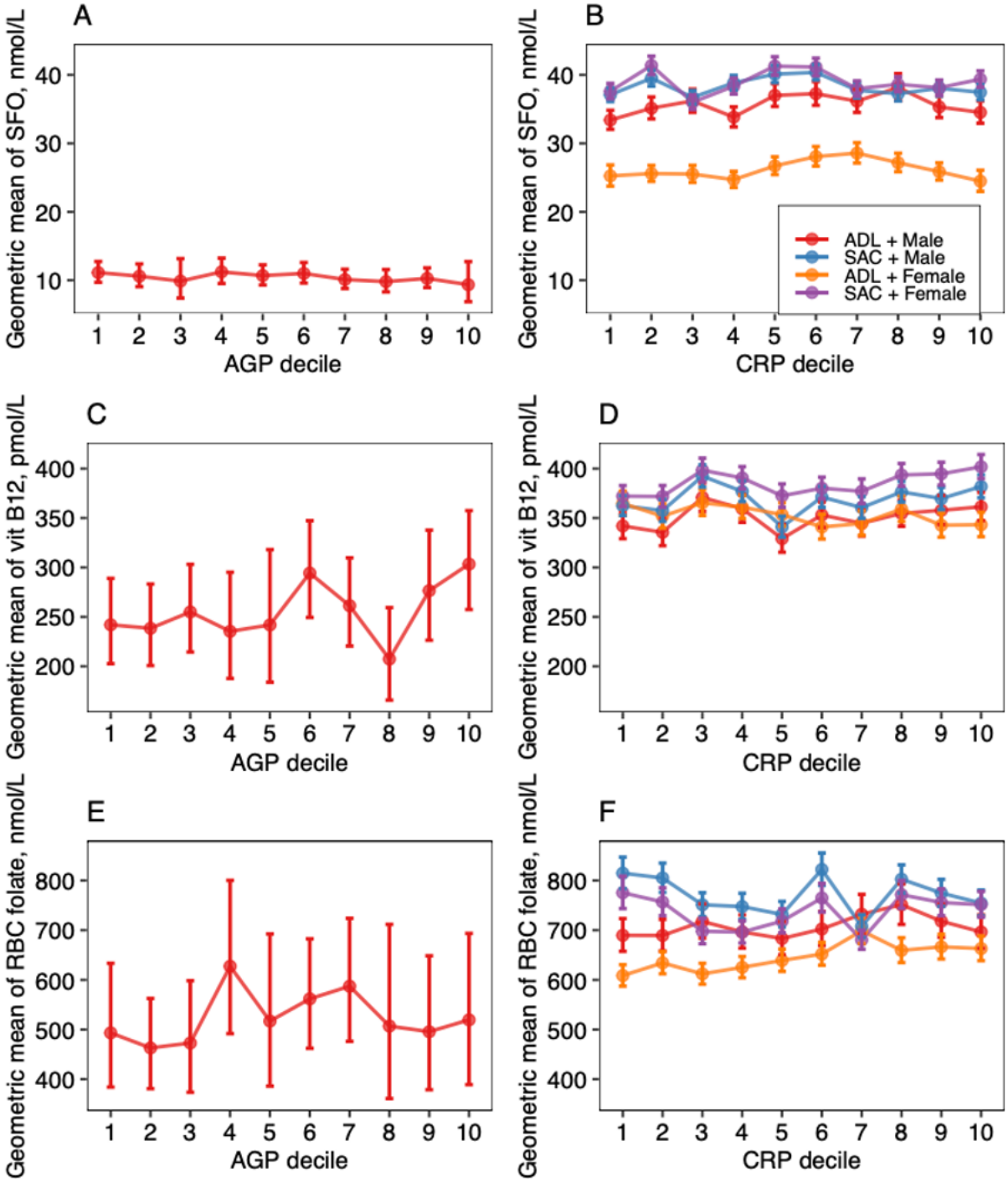

Figure 4: Estimated geometric means of (A) serum folate by AGP, (B) serum folate by CRP, (C) vitamin B12 by AGP, (D) vitamin B12 by CRP, (E) RBC folate by AGP and (F) RBC folate by CRP in school-age children by sex and age group. AGP, α1-Acid glycoprotein; CRP, C-reactive protein.

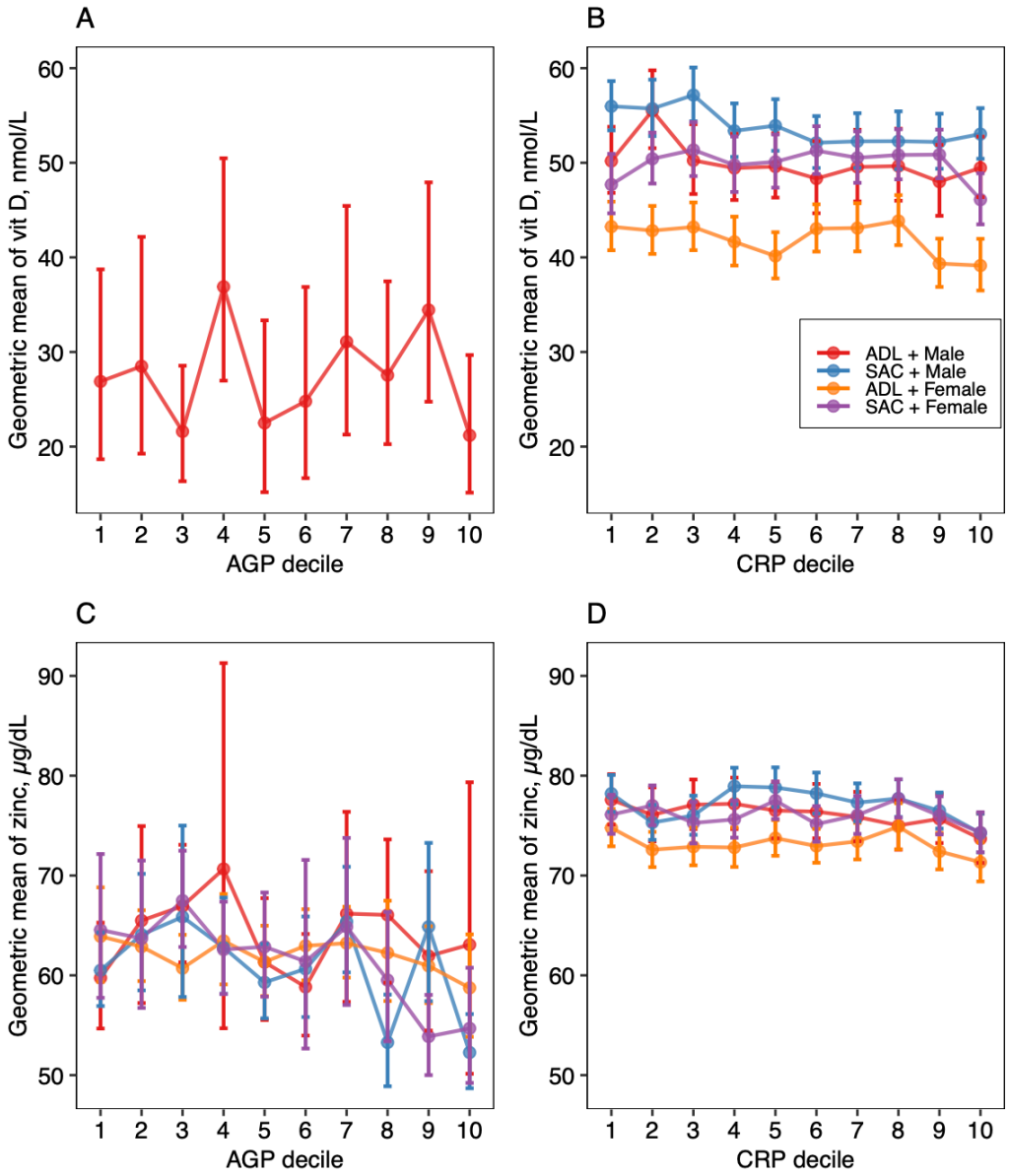

Table 6: Age, AGP, and CRP status in school-age children by age

	Age		CRP, mg/L			AGP, g/L		
Survey (year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP, % (95% CI)**	**n**	**Median (IQR)**	**Elevated CRP, % (95% CI)**
Age >= 12 years								
Colombia(2010)	5931	9(7, 10)	5931	0.2(0.2, 0.7)	13.5(12.2, 14.7)	_	_	_
Ecuador(2012)	4413	9(7, 10)	4413	1.9(1.9, 2.2)	7.6(6.0, 9.5)	_	_	_
UK(2014)	423	9(6, 10)	423	1.4(0.8, 2.1)	5.8(3.1, 9.4)	_	_	_
Malawi(2016)	580	8(7, 10)	580	0.9(0.3, 2.8)	16.6(12.6, 21.2)	580	0.8(0.6, 1.2)	36.0(30.9, 41.3)
Mexico(2006)	2386	7(6, 10)	2386	0.5(0.2, 1.7)	10.0(8.0, 12.4)	_	_	_
Mexico(2012)	4312	9(7, 10)	4312	0.5(0.2, 1.5)	8.6(7.3, 9.9)	_	_	_
US(2006)	2097	9(7, 10)	2097	0.3(0.1, 1.1)	6.0(4.7, 7.6)	_	_	_
Age < 12 years								
Colombia(2010)	916	13(12, 13)	916	0.2(0.2, 0.8)	14.2(10.6, 18.4)	_	_	_
Ecuador(2012)	1650	13(13, 14)	1650	1.9(1.9, 2.1)	6.4(4.9, 8.1)	_	_	_
UK(2014)	163	14(13, 14)	163	1.6(0.8, 1.9)	2.0(0.4, 5.4)	_	_	_
Malawi(2016)	173	14(13, 14)	173	0.6(0.3, 1.5)	12.6(7.0, 20.1)	173	0.7(0.5, 0.9)	16.5(10.4, 24.2)
Mexico(2006)	13	12(12, 12)	13	0.2(0.1, 0.6)	2.3(NA, 13.7)	_	_	_
Mexico(2012)	15	12(12, 13)	15	0.8(0.1, 1.3)	1.1(NA, 67.6)	_	_	_
US(2006)	1451	14(13, 14)	1451	0.3(0.1, 1.1)	7.8(5.8, 10.1)	_	_	_

* AGP, Alpha(1)-acid glycoprotein; CRP, C-reactive protein; IQR, Interquartile Range. Elevated AGP and CRP defined as an AGP > 1 g/L and CRP > 5 mg/L, respectively

Table 7: Serum folate, RBC folate, vitamin B-12, vitamin D, and zinc status in school-age children by age group

	Serum folate, nmol/L			RBC folate, nmol/L			Vitamin B12, pmol/L			Vitamin D, nmol/L			Zinc, µg/dL		
Survey (year)	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**	**n**	**Median (IQR)**	**Deficiency (95% CI)**
Age >= 12															
Ecuador(2012)	4412	50.08(36.71, 66.39)	0	4412	954.89(774.29, 1190.56)	0.1(0.0, 0.3)	1126	423.00(323.00, 564.00)	0.6(0.1, 1.8)	-	-	-	4412	74.00(66.00, 82.00)	11.1(9.5, 12.7)
Mexico(2012)	4310	35.79(31.94, 38.51)	0.0(0.0, 0.1)	-	-	-	4312	457.00(345.00, 598.00)	0.6(0.3, 1.1)	-	-	-	-	-	-
US(2006)	2071	36.20(29.20, 44.20)	0.0(0.0, 0.1)	2080	591.20(514.20, 679.50)	1.2(0.8, 1.6)	2069	542.43(427.30, 693.72)	0.0(0.0, 0.1)	2094	64.90(54.91, 77.38)	2.2(1.6, 3.0)	-	-	-
Colombia(2010)	-	-	-	-	-	-	5931	301.10(234.68, 388.19)	2.9(2.3, 3.4)	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	407	376.00(292.00, 474.00)	0.0(NA, 0.1)	411	49.00(34.20, 64.30)	17.6(12.7, 23.4)	308	93.14(84.25, 100.85)	0.4(0.0, 1.7)
Malawi(2016)	-	-	-	-	-	-	-	-	-	-	-	-	580	59.38(50.00, 71.88)	60.0(51.2, 68.4)
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	-	-	2386	83.26(63.10, 111.11)	19.5(16.4, 22.9)
Age < 12															
Ecuador(2012)	1650	39.43(27.87, 55.52)	0.2(0.1, 0.5)	1650	824.37(628.82, 1059.58)	1.4(0.7, 2.4)	1649	379.00(286.00, 517.00)	1.6(0.8, 2.8)	-	-	-	1647	71.00(63.00, 80.00)	15.3(11.8, 19.2)
US(2006)	1443	29.20(22.70, 37.60)	0.4(0.1, 1.1)	1440	536.80(457.50, 629.70)	4.4(3.1, 5.9)	1439	414.76(318.82, 520.29)	0.1(0.0, 0.3)	1450	57.41(44.93, 69.89)	7.4(5.5, 9.8)	-	-	-
Colombia(2010)	-	-	-	-	-	-	916	273.80(216.97, 340.96)	4.6(3.1, 6.6)	-	-	-	-	-	-
UK(2014)	-	-	-	-	-	-	160	286.00(225.00, 335.00)	1.3(0.2, 4.0)	158	40.10(26.00, 52.90)	32.5(23.5, 42.5)	151	91.37(83.73, 105.16)	0.5(0.0, 2.0)
Malawi(2016)	-	-	-	-	-	-	-	-	-	-	-	-	173	62.50(54.65, 71.88)	58.0(47.4, 68.2)

* IQR, Interquartile Rangel; RBC folate, red blood cell folate. Serum folate deficiency is defined as < 10 nmol/L. Serum folate deficiency is defined as < 340 nmol/L. Vitamin B12 deficiency is defined as < 150 pmol/L. Vitamin D deficiency is defined as < 30 nmol/L. Definition of zinc deficiency depends on unique combinations in age, sex, blood draw time, and fasting status.

Table 8: Rank correlation coefficient between serum folate, red blood cell folate, vitamin B-12, vitamin D, and zinc and AGP or CRP in school-age children by age group.

	SFO & CRP		RBC folate & CRP		Vit B12 & CRP		Vit D & CRP		Zinc & AGP		Zinc & CRP	
Survey (year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
Age >= 12												
Ecuador(2012)	4412	-0.09*	4412	-0.05	1126	0.13	-	-	-	-	4412	-0.07*
Mexico(2012)	4310	0.03	-	-	4312	0.10*	-	-	-	-	-	-
US(2006)	2071	-0.07	2080	0.02	2069	0.02	2094	-0.07*	-	-	-	-
Colombia(2010)	-	-	-	-	5931	0.05*	-	-	-	-	-	-
UK(2014)	-	-	-	-	407	0.03	411	-0.02	-	-	308	-0.05
Malawi(2016)	-	-	-	-	-	-	-	-	580	-0.23*	580	-0.26*
Mexico(2006)	-	-	-	-	-	-	-	-	-	-	2386	0.01
Age < 12												
Ecuador(2012)	1650	0.00	1650	-0.02	1649	-0.04	-	-	-	-	1647	-0.04
US(2006)	1443	-0.04	1440	0.06	1439	0.02	1450	-0.04	-	-	-	-
Colombia(2010)	-	-	-	-	916	-0.08	-	-	-	-	-	-
UK(2014)	-	-	-	-	160	0.12	158	0.13	-	-	151	-0.18
Malawi(2016)	-	-	-	-	-	-	-	-	173	0.11	173	0.12

* AGP, Alpha(1)-acid glycoprotein; CRP, C-reactive protein; RBC folate, red blood cell folate; SFO, serum folate; vit B12, vitamin B1; vit D, vitamin D. r represents spearman rank correlation coefficients. * indicates p-values for spearman rank correlation coefficients < 0.05.

Figure 5: Estimated geometric means of (A) serum folate by AGP, (B) serum folate by CRP, (C) vitamin B12 by AGP, (D) vitamin B12 by CRP, (E) RBC folate by AGP and (F) RBC folate by CRP in school-age children by sex and age group. AGP, α1-Acid glycoprotein; CRP, C-reactive protein.

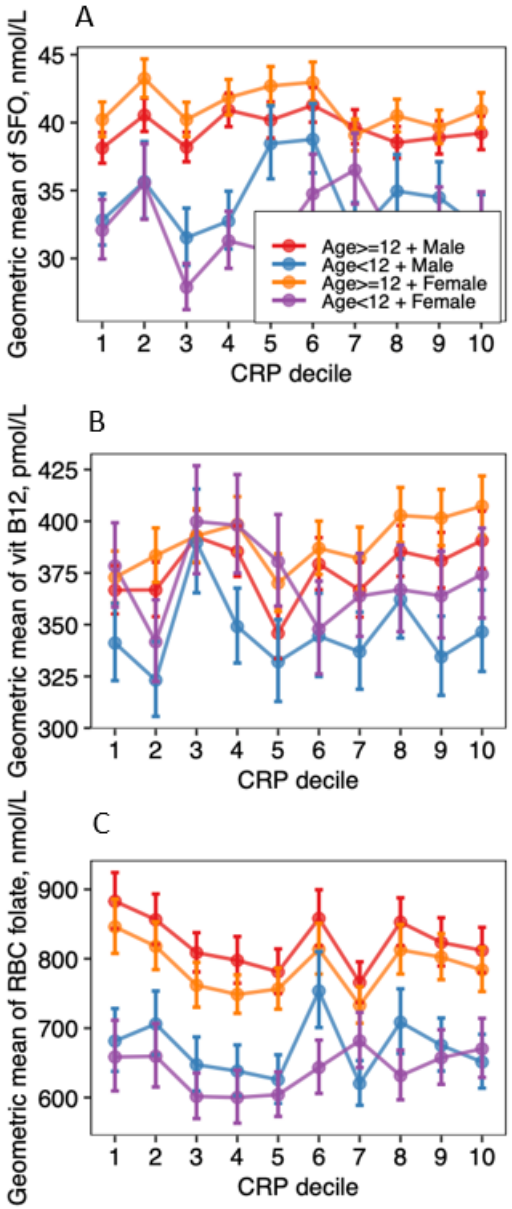

Figure 6: Estimated geometric means of (A) vitamin D by AGP, (B) vitamin D by CRP, (C) zinc by AGP, (D) zinc by CRP in school-age children by age group and sex. AGP, α1-Acid glycoprotein; CRP, C-reactive protein.

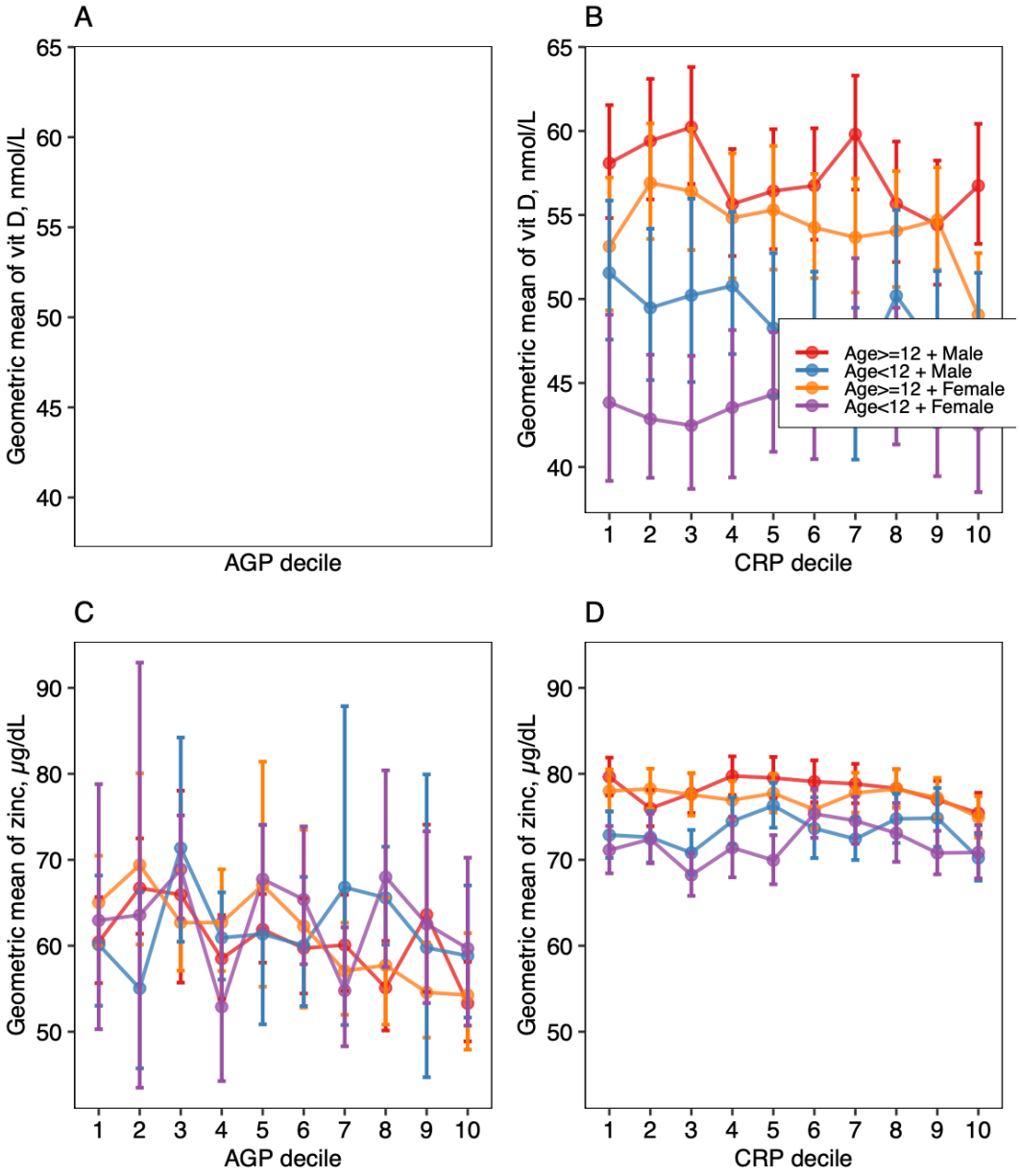

Table 9: Data Sources and Availability[167]

Country (year)	Survey Name	SFO	RBC Folate	Vitamin B-12	Vitamin D	Zinc	Data Source
Azerbaijan (2013)	Azerbaijan Nutrition Survey (AzNS)	ADL	-	ADL	-	-	UNICEF; Ministry of Health of the Republic of Azerbaijan. Azerbaijan Nutrition Survey (AzNS); 2013.
Bangladesh (2012)	National Micronutrient Survey 2011-2012	ADL	-	ADL	-	ADL	Institute of Public Health Nutrition (Bangladesh); UNICEF; iicddr,b; GAIN. National Micronutrient Survey 2011-12, Final Report. Dhaka, Bangladesh; 2012.
Colombia (2010)	Columbia National Survey of the Nutrition Situation	-	-	ADL, SAC	-	-	Columbian Family Welfare Institute; Ministry of Social Protection (Columbia); National Institute of Health (Columbia); Profamilia (Columbia). Colombia National Survey of the Nutritional Situation (ENSIN); Bogotá, D.C., 2010.
Cote D'Ivoire (2007)	Cote D'Ivoire multiple indicator cluster survey 2006	ADL	-	-	-	-	Rohner, F.; Tschannen, A. B.; Northrop-Clewes, C.; Kouassi-Gohou, V.; Bosso, P. E.; Mascie-Taylor, N. Comparison of a Possession Score and Poverty Index in Predicting Anaemia and Undernutrition in Pre-School Children and Women of Reproductive Age in Rural and Urban Cote d'Ivoire. Public Health Nutrition 2012, 15 (9), 1620–1629.
Ecuador (2012)	Ecuador's National Health and Nutrition Survey	ADL, SAC	ADL, SAC	ADL, SAC	-	ADL, SAC	Freire, W. B.; Belmont, P.; Lopez-Cevallos, D. F.; Waters, W. F. Ecuador's National Health and Nutrition Survey: Objectives, Design, and Methods. Annals of Epidemiology 2015, 25 (11), 877–878.

Malawi (2016)	Malawi Demographic and Health Survey 2015-2016	ADL	ADL	ADL	-	ADL, SAC	National Statistics Office (NSO); ICF. Malawi Demographic and Health Survey 2015-2016; 2016.
Mexico (2006)	Encuesta Nacional de Salud y Nutrición 2006	-	-	-	-	ADL, SAC	Abúndez, C. O.; Cázares, G. N.; Cordero, C. J.; Zetina, D. A.; Angona, S. R.; de Voghel Gutiérrez, S.; Rivera-Dommarco, J. Encuesta Nacional de Salud y Nutrición 2006 [National Health and Nutrition Survey 2006].; 2006.
Mexico (2012)	Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales	ADL, SAC	-	ADL, SAC	-	-	Gutiérrez, J. P.; Rivera-Dommarco, J.; Shamah-Levy, T.; Villalpando-Hernández, S.; Franco, A.; Cuevas-Nasu, L.; Romero-Martínez, M.; Hernández-Ávila, M. Encuesta Nacional de Salud y Nutrición 2012. Resultados Nacionales [National Health and Nutrition Survey 2012. National Results]; 2012.
Pakistan (2011)	Pakistan National Nutrition Survey 2011	ADL	-	ADL	-	-	Bhutta, Z. A.; Soofi, S. B.; Zaidi, S. S.; Habib, A. Pakistan National Nutrition Survey 2011; 2011.
United Kingdom (2014)	National Diet and Nutrition Survey Results	-	-	ADL, SAC	ADL, SAC	ADL, SAC	Public Health England; Food Standards Agency. National Diet and Nutrition Survey Results from Years 5 and 6 (Combined) of the Rolling Programme (2012/2013 - 2013/2014); 2016.
USA (2006)	National Health and Nutrition Examination Survey 2003-2006	ADL, SAC	ADL, SAC	ADL, SAC	ADL, SAC	-	CDC. Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population; 2012.

Vietnam (2010)	2010 micronutrient status survey	ADL	-	-	-	ADL	Laillou, A.; Van Pham, T.; Tran, N. T.; Le, H. T.; Wieringa, F.; Rohner, F.; Fortin, S.; Bach Le, M.; Tran, D. T.; Moench-Pfanner, R.; Berger, J. Micronutrient Deficits Are Still Public Health Issues among Women and Young Children in Vietnam. PLoS ONE 2012, 7 (4).

Table 10: Suggested lower* cutoffs for the assessment of serum zinc**[81]

Time of measurement	Serum zinc (µg/dL [µmol/L])[1]		
	< 10 years old	≥ 10 years old	
		Females	Males
Morning fasting[2]	NA	70 (10.07)	74 (11.3)
Morning non-fasting	65 (9.9)	66 (10.1)	70 (10.7)
Afternoon	57 (8.7)	59 (9.0)	61 (9.3)

* 2.5% percentiles
** For use in population studies, derived from NHANES II
NA, not available
[1] Conversion factor: µmol/L = g/dL = 6.54.
[2] Based on data from subjects aged ≥ 20 years.

Table 11: Laboratory methods by biomarker and age group

	Adolescent methods	School-age children methods
AGP	Sandwich ELISA, TIA (Roche Hitachi 902)	Sandwich ELISA
CRP	Sandwich ELISA, TIA (Roche Hitachi 902), PETIA, nephelometry	Sandwich ELISA, TIA (Roche Hitachi 902), PETIA, nephelometry
Serum folate	BR Quantaphase II radioassay, CLIA, CMIA, ECLIA, microbiological assay	CLIA, CMIA, BR Quantaphase II radioassay
RBC Folate	BR Quantaphase II radioassay, CLIA	CLIA, BR Quantaphase II radioassay
Vitamin B-12	CLIA, CMIA, ECLIA, immunoassay, microbiological assay	CLIA, CMIA, immunoassay
Zinc	AAFS, AAS, atomic emission spectrometry, ICP-MS, ICP-OES	AAS, atomic emission spectrometry, ICP-MS, ICP-OES

Table 12: When to apply the BRINDA inflammation adjustment method and by AGP and/or CRP[134]

	WRA	PSC	SAC
Retinol binding protein and retinol	X	CRP and AGP	X
sTfR	AGP	AGP	X
Ferritin	CRP and AGP	CRP and AGP	X
Zinc	X	CRP and AGP	*
Folate	X	X	*
RBC Folate	X	X	*
Vitamin B-12	X	X	*

* Indicates that more research is needed to determine the need for adjustment; "X" indicates that adjustment is not recommended

References

(1) Müller, O.; Krawinkel, M. *Malnutrition and health in developing countries - PMC*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1180662/ (accessed 2023-04-05).

(2) Stevens, G. A.; Beal, T.; Mbuya, M. N. N.; Luo, H.; Neufeld, L. M.; Global Micronutrient Deficiencies Research Group. Micronutrient Deficiencies among Preschool-Aged Children and Women of Reproductive Age Worldwide: A Pooled Analysis of Individual-Level Data from Population-Representative Surveys. *Lancet Glob. Health* **2022**, *10* (11), e1590–e1599. https://doi.org/10.1016/S2214-109X(22)00367-9.

(3) Olson, R.; Gavin-Smith, B.; Ferraboschi, C.; Kraemer, K. Food Fortification: The Advantages, Disadvantages and Lessons from Sight and Life Programs. *Nutrients* **2021**, *13* (4), 1118. https://doi.org/10.3390/nu13041118.

(4) Maxfield, L.; Shukla, S.; Crane, J. S. Zinc Deficiency. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2022.

(5) Khan, K. M.; Jialal, I. Folic Acid Deficiency. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(6) Ankar, A.; Kumar, A. Vitamin B12 Deficiency. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(7) Sizar, O.; Khare, S.; Goyal, A.; Givler, A. Vitamin D Deficiency. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.

(8) Dao, M. C.; Meydani, S. N. Micronutrient Status, Immune Response and Infectious Disease in Elderly of Less Developed Countries. *Sight Life Mag.* **2009**, *3*, 6–15.

(9) Mustafa Khalid, N.; Haron, H.; Shahar, S.; Fenech, M. Current Evidence on the Association of Micronutrient Malnutrition with Mild Cognitive Impairment, Frailty, and Cognitive Frailty among Older Adults: A Scoping Review. *Int. J. Environ. Res. Public. Health* **2022**, *19* (23), 15722. https://doi.org/10.3390/ijerph192315722.

(10) Balbus, J. M.; Barouki, R.; Birnbaum, L. S.; Etzel, R. A.; Gluckman, S. P. D.; Grandjean, P.; Hancock, C.; Hanson, M. A.; Heindel, J. J.; Hoffman, K.; Jensen, G. K.; Keeling, A.; Neira, M.; Rabadán-Diehl, C.; Ralston, J.; Tang, K.-C. Early-Life Prevention of Non-Communicable Diseases. *Lancet* **2013**, *381* (9860). https://doi.org/10.1016/S0140-6736(12)61609-2.

(11) Howson, C. P.; Kennedy, E. T.; Horwitz, A. *Key Elements in the Design and Implementation of Micronutrient Interventions*; National Academies Press (US), 1998.

(12) Kanu, F.; Sharma, A.; Addo, O. Y.; Suchdev, P. Association Between Hemoglobin and Elevation Among School-Aged Children: A Verification of Proposed Adjustments. *Curr. Dev. Nutr.* **2020**, *4* (Supplement_2), 1717–1717. https://doi.org/10.1093/cdn/nzaa064_007.

(13) Global Panel on Agriculture and Food Systems for Nutrition. The Cost of Malnutrition: Why Policy Action Is Urgent, 2016. https://glopan.org/sites/default/files/pictures/CostOfMalnutrition.pdf (accessed 2023-03-31).

(14) Larson, L. M.; Namaste, S. M. L.; Williams, A. M.; Engle-Stone, R.; Addo, O. Y.; Suchdev, P. S.; Wirth, J. P.; Temple, V.; Serdula, M.; Northrop-Clewes, C. Adjusting Retinol-Binding Protein Concentrations for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2017**, *106* (Suppl 1), 390S-401S. https://doi.org/10.3945/ajcn.116.142166.

(15) Kaneko, N.; Kurata, M.; Yamamoto, T.; Morikawa, S.; Masumoto, J. The Role of Interleukin-1 in General Pathology. *Inflamm. Regen.* **2019**, *39* (1), 12. https://doi.org/10.1186/s41232-019-0101-5.

(16) Tanaka, T.; Narazaki, M.; Kishimoto, T. IL-6 in Inflammation, Immunity, and Disease. *Cold Spring Harb. Perspect. Biol.* **2014**, *6* (10), a016295. https://doi.org/10.1101/cshperspect.a016295.

(17) Bailey, L. B.; Stover, P. J.; McNulty, H.; Fenech, M. F.; Gregory, J. F.; Mills, J. L.; Pfeiffer, C. M.; Fazili, Z.; Zhang, M.; Ueland, P. M.; Molloy, A. M.; Caudill, M. A.; Shane, B.; Berry, R. J.; Bailey, R. L.; Hausman, D. B.; Raghavan, R.; Raiten, D. J. Biomarkers of Nutrition for Development—Folate Review. *J. Nutr.* **2015**, *145* (7), 1636S-1680S. https://doi.org/10.3945/jn.114.206599.

(18) Young, M. F.; Guo, J.; Williams, A.; Whitfield, K. C.; Nasrin, S.; Kancherla, V.; Suchdev, P. S.; Crider, K. S.; Pfeiffer, C. M.; Serdula, M. Interpretation of Vitamin B-12 and Folate Concentrations in Population-Based Surveys Does Not Require Adjustment for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2020**, *111* (4), 919–926. https://doi.org/10.1093/ajcn/nqz303.

(19) Rogers, L. M.; Cordero, A. M.; Pfeiffer, C. M.; Hausman, D. B.; Tsang, B. L.; De-Regil, L. M.; Rosenthal, J.; Razzaghi, H.; Wong, E. C.; Weakland, A. P.; Bailey, L. B. Global Folate Status in Women of Reproductive Age: A Systematic Review with Emphasis on Methodological Issues. *Ann. N. Y. Acad. Sci.* **2018**, *1431* (1), 35–57. https://doi.org/10.1111/nyas.13963.

(20) *National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 2012 | CDC*. https://www.cdc.gov/nutritionreport/index.html (accessed 2023-04-11).

(21) *Folate - Health Encyclopedia - University of Rochester Medical Center*. https://www.urmc.rochester.edu/encyclopedia/content.aspx?contenttypeid=167&contentid=folate (accessed 2022-10-07).

(22) Steur, H.; Gellynck, X.; Storozhenko, S.; Liqun, G.; Lambert, W.; Van Der Straeten, D.; Viaene, J. Health Impact in China of Folate-Biofortified Rice. *Nat. Biotechnol.* **2010**, *28*, 554–556. https://doi.org/10.1038/nbt0610-554.

(23) Cotoraci, C.; Ciceu, A.; Sasu, A.; Hermenean, A. Natural Antioxidants in Anemia Treatment. *Int. J. Mol. Sci.* **2021**, *22* (4), 1883. https://doi.org/10.3390/ijms22041883.

(24) Greenberg, J. A.; Bell, S. J. Multivitamin Supplementation During Pregnancy: Emphasis on Folic Acid and l-Methylfolate. *Rev. Obstet. Gynecol.* **2011**, *4* (3–4), 126–127.

(25) *Office of Dietary Supplements - Folate*. https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/ (accessed 2023-04-05).

(26) Allen, L. H.; Miller, J. W.; de Groot, L.; Rosenberg, I. H.; Smith, A. D.; Refsum, H.; Raiten, D. J. Biomarkers of Nutrition for Development (BOND): Vitamin B-12 Review. *J. Nutr.* **2018**, *148*, 1995S-2027S. https://doi.org/10.1093/jn/nxy201.

(27) Lyon, P.; Strippoli, V.; Fang, B.; Cimmino, L. B Vitamins and One-Carbon Metabolism: Implications in Human Health and Disease. *Nutrients* **2020**, *12* (9), 2867. https://doi.org/10.3390/nu12092867.

(28) Berti, C.; Fekete, K.; Dullemeijer, C.; Trovato, M.; Souverein, O. W.; Cavelaars, A.; Dhonukshe-Rutten, R.; Massari, M.; Decsi, T.; van't Veer, P.; Cetin, I. Folate Intake and Markers of Folate Status in Women of Reproductive Age, Pregnant and Lactating Women: A Meta-Analysis. *J. Nutr. Metab.* **2012**, *2012*, 470656. https://doi.org/10.1155/2012/470656.

(29) Pfeiffer, C. M.; Zhang, M.; Jabbar, S. FRAMEWORK FOR LABORATORY HARMONIZATION OF FOLATE MEASUREMENTS IN LOW-AND-MIDDLE-INCOME COUNTRIES AND REGIONS. *Ann. N. Y. Acad. Sci.* **2018**, *1414* (1), 96–108. https://doi.org/10.1111/nyas.13532.

(30) Kreusler, P.; Vogel, M.; Willenberg, A.; Baber, R.; Dietz, Y.; Körner, A.; Ceglarek, U.; Kiess, W. Folate and Cobalamin Serum Levels in Healthy Children and Adolescents and Their Association with Age, Sex, BMI and Socioeconomic Status. *Nutrients* **2021**, *13* (2), 546. https://doi.org/10.3390/nu13020546.

(31) Folate, I. of M. (US) S. C. on the S. E. of D. R. I. and its P. on; Vitamins, O. B.; Choline, A. *Folate*; National Academies Press (US), 1998.

(32) Franco, R. S. Measurement of Red Cell Lifespan and Aging. *Transfus. Med. Hemotherapy* **2012**, *39* (5), 302–307. https://doi.org/10.1159/000342232.

(33) *Folate - an overview | ScienceDirect Topics*. https://www.sciencedirect.com/topics/neuroscience/folate (accessed 2023-04-05).
(34) Tjong, E.; Dimri, M.; Mohiuddin, S. S. Biochemistry, Tetrahydrofolate. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.
(35) Crider, K. S.; Yang, T. P.; Berry, R. J.; Bailey, L. B. Folate and DNA Methylation: A Review of Molecular Mechanisms and the Evidence for Folate's Role2. *Adv. Nutr.* **2012**, *3* (1), 21–38. https://doi.org/10.3945/an.111.000992.
(36) Unnikrishnan, A.; Prychitko, T. M.; Patel, H. V.; Chowdhury, M. E.; Pilling, A. B.; Ventrella-Lucente, L. F.; Papakonstantinou, E. V.; Cabelof, D. C.; Heydari, A. R. FOLATE DEFICIENCY REGULATES EXPRESSION OF DNA POLYMERASE β IN RESPONSE TO OXIDATIVE STRESS. *Free Radic. Biol. Med.* **2011**, *50* (2), 270–280. https://doi.org/10.1016/j.freeradbiomed.2010.11.003.
(37) Allen, L. H. How Common Is Vitamin B-12 Deficiency? *Am. J. Clin. Nutr.* **2009**, *89* (2), 693S-696S. https://doi.org/10.3945/ajcn.2008.26947A.
(38) Boston, 677 Huntington Avenue; Ma 02115 +1495-1000. *Vitamin B12*. The Nutrition Source. https://www.hsph.harvard.edu/nutritionsource/vitamin-b12/ (accessed 2022-10-06).
(39) *Immigrant Health Service : Vitamin B12*. https://www.rch.org.au/immigranthealth/clinical/Vitamin_B12/ (accessed 2023-02-13).
(40) Varga, E. A.; Sturm, A. C.; Misita, C. P.; Moll, S. Homocysteine and MTHFR Mutations. *Circulation* **2005**, *111* (19), e289–e293. https://doi.org/10.1161/01.CIR.0000165142.37711.E7.
(41) *Vitamin B12 deficiency: A new risk factor for breast cancer? - ProQuest*. https://www.proquest.com/openview/4b3526149b5797c4754c98742914c299/1?pq-origsite=gscholar&cbl=42187 (accessed 2023-04-11).
(42) Al-Awami, H. M.; Raja, A.; Soos, M. P. Physiology, Gastric Intrinsic Factor. In *StatPearls*; StatPearls Publishing: Treasure Island (FL), 2023.
(43) *Fundic Atrophic Gastritis in an Elderly Population: Effect on Hemoglobin and Several Serum Nutritional Indicators - Krasinski - 1986 - Journal of the American Geriatrics Society - Wiley Online Library*. https://agsjournals.onlinelibrary.wiley.com/doi/abs/10.1111/j.1532-5415.1986.tb03985.x (accessed 2023-04-11).
(44) Winkels, R. M.; Brouwer, I. A.; Clarke, R.; Katan, M. B.; Verhoef, P. Bread Cofortified with Folic Acid and Vitamin B-12 Improves the Folate and Vitamin B-12 Status of Healthy Older People: A Randomized Controlled Trial. *Am. J. Clin. Nutr.* **2008**, *88* (2), 348–355. https://doi.org/10.1093/ajcn/88.2.348.
(45) CDC. *Vitamin D - Infant and Toddler Nutrition*. Centers for Disease Control and Prevention. https://www.cdc.gov/nutrition/infantandtoddlernutrition/images/FB-banner.jpg (accessed 2022-09-12).
(46) *Rickets and osteomalacia*. nhs.uk. https://www.nhs.uk/conditions/rickets-and-osteomalacia/ (accessed 2023-04-11).
(47) Engelsen, O. The Relationship between Ultraviolet Radiation Exposure and Vitamin D Status. *Nutrients* **2010**, *2* (5), 482–495. https://doi.org/10.3390/nu2050482.
(48) Gani, L. U.; How, C. H. PILL Series. Vitamin D Deficiency. *Singapore Med. J.* **2015**, *56* (8), 433–436; quiz 437. https://doi.org/10.11622/smedj.2015119.
(49) Czernichow, S.; Fan, T.; Nocea, G.; Sen, S. S. Calcium and Vitamin D Intake by Postmenopausal Women with Osteoporosis in France. *Curr. Med. Res. Opin.* **2010**, *26* (7), 1667–1674. https://doi.org/10.1185/03007995.2010.483658.
(50) Kweder, H.; Eidi, H. Vitamin D Deficiency in Elderly: Risk Factors and Drugs Impact on Vitamin D Status. *Avicenna J. Med.* **2018**, *8* (4), 139–146. https://doi.org/10.4103/ajm.AJM_20_18.
(51) Iftikhar, R.; Kamran, S. M.; Qadir, A.; Haider, E.; Bin Usman, H. Vitamin D Deficiency in Patients with Tuberculosis. *J. Coll. Physicians Surg.--Pak. JCPSP* **2013**, *23* (10), 780–783.

(52) AlFaris, N. A.; AlKehayez, N. M.; AlMushawah, F. I.; AlNaeem, A. N.; AlAmri, N. D.; AlMudawah, E. S. Vitamin D Deficiency and Associated Risk Factors in Women from Riyadh, Saudi Arabia. *Sci. Rep.* **2019**, *9*, 20371. https://doi.org/10.1038/s41598-019-56830-z.
(53) *Treatment of vitamin D deficiency with UV light in patients with malabsorption syndromes: a case series - PMC*. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2846322/ (accessed 2023-04-11).
(54) Gordon, C. M.; DePeter, K. C.; Feldman, H. A.; Grace, E.; Emans, S. J. Prevalence of Vitamin D Deficiency among Healthy Adolescents. *Arch. Pediatr. Adolesc. Med.* **2004**, *158* (6), 531–537. https://doi.org/10.1001/archpedi.158.6.531.
(55) Garg, M. K.; Tandon, N.; Marwaha, R. K.; Menon, A. S.; Mahalle, N. The Relationship between Serum 25-Hydroxy Vitamin D, Parathormone and Bone Mineral Density in Indian Population. *Clin. Endocrinol. (Oxf.)* **2014**, *80* (1), 41–46. https://doi.org/10.1111/cen.12248.
(56) Palacios, C.; Gonzalez, L. Is Vitamin D Deficiency a Major Global Public Health Problem? *J. Steroid Biochem. Mol. Biol.* **2014**, *144PA*, 138–145. https://doi.org/10.1016/j.jsbmb.2013.11.003.
(57) Amrein, K.; Scherkl, M.; Hoffmann, M.; Neuwersch-Sommeregger, S.; Köstenberger, M.; Tmava Berisha, A.; Martucci, G.; Pilz, S.; Malle, O. Vitamin D Deficiency 2.0: An Update on the Current Status Worldwide. *Eur. J. Clin. Nutr.* **2020**, *74* (11), 1498–1513. https://doi.org/10.1038/s41430-020-0558-y.
(58) *Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline | The Journal of Clinical Endocrinology & Metabolism | Oxford Academic*. https://academic.oup.com/jcem/article/96/7/1911/2833671 (accessed 2023-04-11).
(59) Gröber, U.; Kisters, K. Influence of Drugs on Vitamin D and Calcium Metabolism. *Dermatoendocrinol.* **2012**, *4* (2), 158–166. https://doi.org/10.4161/derm.20731.
(60) *Summary of Antiepileptic Drugs*. Epilepsy Foundation. https://www.epilepsy.com/stories/summary-antiepileptic-drugs (accessed 2023-04-18).
(61) *Nifedipine (Oral Route) Description and Brand Names - Mayo Clinic*. https://www.mayoclinic.org/drugs-supplements/nifedipine-oral-route/description/drg-20071680 (accessed 2023-04-18).
(62) *Clotrimazole Topical: Uses, Side Effects, Interactions, Pictures, Warnings & Dosing - WebMD*. https://www.webmd.com/drugs/2/drug-4316/clotrimazole-topical/details (accessed 2023-04-18).
(63) Wakeman, M. A Literature Review of the Potential Impact of Medication on Vitamin D Status. *Risk Manag. Healthc. Policy* **2021**, *14*, 3357–3381. https://doi.org/10.2147/RMHP.S316897.
(64) DATTA, S.; PAL, M.; DE, A. The Dependency of Vitamin D Status on Anthropometric Data. *Malays. J. Med. Sci. MJMS* **2014**, *21* (3), 54–61.
(65) Maret, W. *Zinc: the galvanizing chameleon*. De Gruyter Conversations. https://blog.degruyter.com/zinc-the-galvanizing-chameleon/ (accessed 2023-04-11).
(66) King, J. C.; Brown, K. H.; Gibson, R. S.; Krebs, N. F.; Lowe, N. M.; Siekmann, J. H.; Raiten, D. J. Biomarkers of Nutrition for Development (BOND)-Zinc Review. *J. Nutr.* **2015**, *146* (4), 858S-885S. https://doi.org/10.3945/jn.115.220079.
(67) International Zinc Nutrition Consultative Group (IZiNCG); Brown, K. H.; Rivera, J. A.; Bhutta, Z.; Gibson, R. S.; King, J. C.; Lönnerdal, B.; Ruel, M. T.; Sandtröm, B.; Wasantwisut, E.; Hotz, C. International Zinc Nutrition Consultative Group (IZiNCG) Technical Document #1. Assessment of the Risk of Zinc Deficiency in Populations and Options for Its Control. *Food Nutr. Bull.* **2004**, *25* (1 Suppl 2), S99-203.
(68) Williams, R. B.; Mills, C. F. The Experimental Production of Zinc Deficiency in the Rat. *Br. J. Nutr.* **1970**, *24* (4), 989–1003. https://doi.org/10.1079/BJN19700102.
(69) Cousins, R. J.; Liuzzi, J. P.; Lichten, L. A. Mammalian Zinc Transport, Trafficking, and Signals *. *J. Biol. Chem.* **2006**, *281* (34), 24085–24089. https://doi.org/10.1074/jbc.R600011200.

(70) Fraker, P. J.; King, L. E. Reprogramming of the Immune System During Zinc Deficiency. *Annu. Rev. Nutr.* **2004**, *24* (1), 277–298. https://doi.org/10.1146/annurev.nutr.24.012003.132454.
(71) *Nutrition and Health in a Developing World*; de Pee, S., Taren, D., Bloem, M. W., Eds.; Springer International Publishing: Cham, 2017. https://doi.org/10.1007/978-3-319-43739-2.
(72) Hess, S. Y. National Risk of Zinc Deficiency as Estimated by National Surveys. *Food Nutr. Bull.* **2017**, *38* (1), 3–17. https://doi.org/10.1177/0379572116689000.
(73) *New online nutrition initiative can help protect lives and health of millions of children*. https://www.who.int/news/item/21-10-2011-new-online-nutrition-initiative-can-help-protect-lives-and-health-of-millions-of-children (accessed 2023-03-28).
(74) Fischer Walker, C. L.; Ezzati, M.; Black, R. E. Global and Regional Child Mortality and Burden of Disease Attributable to Zinc Deficiency. *Eur. J. Clin. Nutr.* **2009**, *63* (5), 591–597. https://doi.org/10.1038/ejcn.2008.9.
(75) *Estimating the Global Prevalence of Zinc Deficiency: Results Based on Zinc Availability in National Food Supplies and the Prevalence of Stunting | PLOS ONE*. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0050568 (accessed 2023-04-11).
(76) Larson, C. P.; Roy, S. K.; Khan, A. I.; Rahman, A. S.; Qadri, F. Zinc Treatment to Under-Five Children: Applications to Improve Child Survival and Reduce Burden of Disease. *J. Health Popul. Nutr.* **2008**, *26* (3), 356–365.
(77) Lazzerini, M.; Wanzira, H. Oral Zinc for Treating Diarrhoea in Children. *Cochrane Database Syst. Rev.* **2016**, *12* (12), CD005436. https://doi.org/10.1002/14651858.CD005436.pub5.
(78) *Daily Variation in Plasma Zinc Concentrations in Women Fed Meals at Six-Hour Intervals | The Journal of Nutrition | Oxford Academic*. https://academic.oup.com/jn/article-abstract/124/4/508/4724504 (accessed 2023-04-11).
(79) Greffeuille, V.; Fortin, S.; Gibson, R.; Rohner, F.; Williams, A.; Young, M. F.; Houghton, L.; Ou, J.; Dijkhuizen, M. A.; Wirth, J. P.; Lander, R. L.; McDonald, C. M.; Suchdev, P. S.; Berger, J.; Wieringa, F. T. Associations between Zinc and Hemoglobin Concentrations in Preschool Children and Women of Reproductive Age: An Analysis of Representative Survey Data from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *J. Nutr.* **2021**, *151* (5), 1277–1285. https://doi.org/10.1093/jn/nxaa444.
(80) Medicine, I. of; Board, F. and N.; Calcium, C. to R. D. R. I. for V. D. and. *Dietary Reference Intakes for Calcium and Vitamin D*; National Academies Press, 2011.
(81) Hotz, C.; Peerson, J.; Brown, K. Suggested Lower Cutoffs of Serum Zinc Concentrations for Assessing Zinc Status: Reanalysis of the Second National Health and Nutrition Examination Survey Data (1976-1980)1-3. *Am. J. Clin. Nutr.* **2003**, *78*, 756–764. https://doi.org/10.1093/ajcn/78.4.756.
(82) Larson, L.; Guo, J.; Williams, A.; Young, M.; Ismaily, S.; Addo, O.; Thurnham, D.; Tanumihardjo, S.; Suchdev, P.; Northrop-Clewes, C. Approaches to Assess Vitamin A Status in Settings of Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Nutrients* **2018**, *10* (8), 1100. https://doi.org/10.3390/nu10081100.
(83) Chen, L.; Deng, H.; Cui, H.; Fang, J.; Zuo, Z.; Deng, J.; Li, Y.; Wang, X.; Zhao, L. Inflammatory Responses and Inflammation-Associated Diseases in Organs. *Oncotarget* **2017**, *9* (6), 7204–7218. https://doi.org/10.18632/oncotarget.23208.
(84) Suchdev, P. S.; Boivin, M. J.; Forsyth, B. W.; Georgieff, M. K.; Guerrant, R. L.; Nelson, C. A. Assessment of Neurodevelopment, Nutrition, and Inflammation From Fetal Life to Adolescence in Low-Resource Settings. *Pediatrics* **2017**, *139* (Supplement_1), S23–S37. https://doi.org/10.1542/peds.2016-2828E.

(85) Medzhitov, R. Inflammation 2010: New Adventures of an Old Flame. *Cell* **2010**, *140* (6), 771–776. https://doi.org/10.1016/j.cell.2010.03.006.
(86) Furman, D.; Campisi, J.; Verdin, E.; Carrera-Bastos, P.; Targ, S.; Franceschi, C.; Ferrucci, L.; Gilroy, D. W.; Fasano, A.; Miller, G. W.; Miller, A. H.; Mantovani, A.; Weyand, C. M.; Barzilai, N.; Goronzy, J. J.; Rando, T. A.; Effros, R. B.; Lucia, A.; Kleinstreuer, N.; Slavich, G. M. Chronic Inflammation in the Etiology of Disease across the Life Span. *Nat. Med.* **2019**, *25* (12), 1822–1832. https://doi.org/10.1038/s41591-019-0675-0.
(87) *Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017 - The Lancet*. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(18)32203-7/fulltext (accessed 2023-04-11).
(88) *What Is an Inflammation?*; Institute for Quality and Efficiency in Health Care (IQWiG), 2018.
(89) *Inflammatory Response Pathway - US*. https://www.thermofisher.com/us/en/home/life-science/cell-analysis/signaling-pathways/inflammatory-response-pathway.html (accessed 2023-04-11).
(90) Raiten, D. J.; Ashour, F. A. S.; Ross, A. C.; Meydani, S. N.; Dawson, H. D.; Stephensen, C. B.; Brabin, B. J.; Suchdev, P. S.; van Ommen, B.; the INSPIRE Consultative Group. Inflammation and Nutritional Science for Programs/Policies and Interpretation of Research Evidence (INSPIRE). *J. Nutr.* **2015**, *145* (5), 1039S-1108S. https://doi.org/10.3945/jn.114.194571.
(91) Thurnham, D. I.; Northrop-Clewes, C. A.; Knowles, J. The Use of Adjustment Factors to Address the Impact of Inflammation on Vitamin A and Iron Status in Humans123. *J. Nutr.* **2015**, *145* (5), 1137S-1143S. https://doi.org/10.3945/jn.114.194712.
(92) Bteich, M. An Overview of Albumin and Alpha-1-Acid Glycoprotein Main Characteristics: Highlighting the Roles of Amino Acids in Binding Kinetics and Molecular Interactions. *Heliyon* **2019**, *5* (11), e02879. https://doi.org/10.1016/j.heliyon.2019.e02879.
(93) Sarcione, E. J. Synthesis of A1-Acid Glycoprotein by the Isolated Perfused Rat Liver. *Arch. Biochem. Biophys.* **1963**, *100* (3), 516–519. https://doi.org/10.1016/0003-9861(63)90120-6.
(94) Brée, F.; Houin, G.; Barré, J.; Moretti, J.-L.; Wirquin, V.; Tillement, J.-P. Pharmacokinetics of Intravenously Administered 125I-Labelled Human A1-Acid Glycoprotein. *Clin. Pharmacokinet.* **1986**, *11* (4), 336–342. https://doi.org/10.2165/00003088-198611040-00006.
(95) Huang, Z.; Ung, T. Effect of Alpha-1-Acid Glycoprotein Binding on Pharmacokinetics and Pharmacodynamics. *Curr. Drug Metab.* **2013**, *14* (2), 226–238. https://doi.org/10.2174/138920013804870709.
(96) Nathan, C. Neutrophils and Immunity: Challenges and Opportunities. *Nat. Rev. Immunol.* **2006**, *6* (3), 173–182. https://doi.org/10.1038/nri1785.
(97) Hochepied, T.; Berger, F. G.; Baumann, H.; Libert, C. A1-Acid Glycoprotein: An Acute Phase Protein with Inflammatory and Immunomodulating Properties. *Cytokine Growth Factor Rev.* **2003**, *14* (1), 25–34. https://doi.org/10.1016/S1359-6101(02)00054-0.
(98) Sproston, N. R.; Ashworth, J. J. Role of C-Reactive Protein at Sites of Inflammation and Infection. *Front. Immunol.* **2018**, *9*, 754. https://doi.org/10.3389/fimmu.2018.00754.
(99) Gewurz, H.; Mold, C.; Siegel, J.; Fiedel, B. C-Reactive Protein and the Acute Phase Response. *Adv. Intern. Med.* **1982**, *27*, 345–372.
(100) Du Clos, T. W. Pentraxins: Structure, Function, and Role in Inflammation. *ISRN Inflamm.* **2013**, *2013*, 379040. https://doi.org/10.1155/2013/379040.
(101) Mullenix, M. C.; Mortensen, R. F. Calcium Ion Binding Regions in C-Reactive Protein: Location and Regulation of Conformational Changes. *Mol. Immunol.* **1994**, *31* (8), 615–622. https://doi.org/10.1016/0161-5890(94)90169-4.

(102) Marnell, L.; Mold, C.; Du Clos, T. W. C-Reactive Protein: Ligands, Receptors and Role in Inflammation. *Clin. Immunol.* **2005**, *117* (2), 104–111. https://doi.org/10.1016/j.clim.2005.08.004.
(103) Giovannini, S.; Onder, G.; Liperoti, R.; Russo, A.; Carter, C.; Capoluongo, E.; Pahor, M.; Bernabei, R.; Landi, F. Interleukin-6, C-Reactive Protein, Tumor Necrosis Factor-Alpha as Predictors of Mortality in Frail, Community-Living Elderly Individuals. *J. Am. Geriatr. Soc.* **2011**, *59* (9), 1679–1685. https://doi.org/10.1111/j.1532-5415.2011.03570.x.
(104) Potempa, L. A.; Rajab, I. M.; Hart, P. C.; Bordon, J.; Fernandez-Botran, R. Insights into the Use of C-Reactive Protein as a Diagnostic Index of Disease Severity in COVID-19 Infections. *Am. J. Trop. Med. Hyg.* **2020**, *103* (2), 561–563. https://doi.org/10.4269/ajtmh.20-0473.
(105) Shah, S. H.; Newby, L. K. C-Reactive Protein: A Novel Marker of Cardiovascular Risk. *Cardiol. Rev.* **2003**, *11* (4), 169–179. https://doi.org/10.1097/01.CRD.0000077906.74217.6E.
(106) Qu, R.; Hu, L.; Ling, Y.; Hou, Y.; Fang, H.; Zhang, H.; Liang, S.; He, Z.; Fang, M.; Li, J.; Li, X.; Chen, C. C-Reactive Protein Concentration as a Risk Predictor of Mortality in Intensive Care Unit: A Multicenter, Prospective, Observational Study. *BMC Anesthesiol.* **2020**, *20* (1), 292. https://doi.org/10.1186/s12871-020-01207-3.
(107) Sharma, A.; Gokkulakrishnan, S.; Shahi, A. K.; Kumar, V. Efficacy of Serum CRP Levels as Monitoring Tools for Patients with Fascial Space Infections of Odontogenic Origin: A Clinicobiochemical Study. *Natl. J. Maxillofac. Surg.* **2012**, *3* (2), 148–151. https://doi.org/10.4103/0975-5950.111369.
(108) den Engelsen, C.; Koekkoek, P. S.; Gorter, K. J.; van den Donk, M.; Salomé, P. L.; Rutten, G. E. High-Sensitivity C-Reactive Protein to Detect Metabolic Syndrome in a Centrally Obese Population: A Cross-Sectional Analysis. *Cardiovasc. Diabetol.* **2012**, *11* (1), 25. https://doi.org/10.1186/1475-2840-11-25.
(109) Cooper, J.; Pastorello, Y.; Slevin, M. A Meta-Analysis Investigating the Relationship between Inflammation in Autoimmune Disease, Elevated CRP, and the Risk of Dementia. *Front. Immunol.* **2023**, *14*, 1087571. https://doi.org/10.3389/fimmu.2023.1087571.
(110) Polyakova, E. A.; Mikhaylov, E. N. The Prognostic Role of High-Sensitivity C-Reactive Protein in Patients with Acute Myocardial Infarction. *J. Geriatr. Cardiol. JGC* **2020**, *17* (7), 379–383. https://doi.org/10.11909/j.issn.1671-5411.2020.07.007.
(111) Karlsson, J.; Wetterö, J.; Weiner, M.; Rönnelid, J.; Fernandez-Botran, R.; Sjöwall, C. Associations of C-Reactive Protein Isoforms with Systemic Lupus Erythematosus Phenotypes and Disease Activity. *Arthritis Res. Ther.* **2022**, *24* (1), 139. https://doi.org/10.1186/s13075-022-02831-9.
(112) Liang, C.; Li, J.; Lu, C.; Xie, D.; Liu, J.; Zhong, C.; Wu, X.; Dai, R.; Zhang, H.; Guan, D.; Guo, B.; He, B.; Li, F.; He, X.; Zhang, W.; Zhang, B.-T.; Zhang, G.; Lu, A. HIF1α Inhibition Facilitates Leflunomide-AHR-CRP Signaling to Attenuate Bone Erosion in CRP-Aberrant Rheumatoid Arthritis. *Nat. Commun.* **2019**, *10*, 4579. https://doi.org/10.1038/s41467-019-12163-z.
(113) Tao, Q.; Alvin Ang, T. F.; Akhter-Khan, S. C.; Itchapurapu, I. S.; Killiany, R.; Zhang, X.; Budson, A. E.; Turk, K. W.; Goldstein, L.; Mez, J.; Alosco, M. L.; Qiu, W. Q. Impact of C-Reactive Protein on Cognition and Alzheimer Disease Biomarkers in Homozygous APOE Ɛ4 Carriers. *Neurology* **2021**, *97* (12), e1243–e1252. https://doi.org/10.1212/WNL.0000000000012512.
(114) Shane, B. Folate Status Assessment History: Implications for Measurement of Biomarkers in NHANES12345. *Am. J. Clin. Nutr.* **2011**, *94* (1), 337S-342S. https://doi.org/10.3945/ajcn.111.013367.
(115) Franco-Duarte, R.; Černáková, L.; Kadam, S.; S. Kaushik, K.; Salehi, B.; Bevilacqua, A.; Corbo, M. R.; Antolak, H.; Dybka-Stępień, K.; Leszczewicz, M.; Relison Tintino, S.; Alexandrino de Souza, V. C.; Sharifi-Rad, J.; Melo Coutinho, H. D.; Martins, N.; Rodrigues, C. F. Advances in Chemical and Biological Methods to Identify Microorganisms—From Past to Present. *Microorganisms* **2019**, *7* (5), 130. https://doi.org/10.3390/microorganisms7050130.

(116) Hoofnagle, A. N.; Wener, M. H. The Fundamental Flaws of Immunoassays and Potential Solutions Using Tandem Mass Spectrometry. *J. Immunol. Methods* **2009**, *347* (1–2), 3–11. https://doi.org/10.1016/j.jim.2009.06.003.
(117) Zayed, A.; Bustami, R.; Alabsi, W.; El-Elimat, T. Development and Validation of a Rapid High-Performance Liquid Chromatography–Tandem Mass Spectrometric Method for Determination of Folic Acid in Human Plasma. *Pharmaceuticals* **2018**, *11* (2), 52. https://doi.org/10.3390/ph11020052.
(118) Lips, P. Relative Value of 25(OH)D and 1,25(OH)2D Measurements. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* **2007**, *22* (11), 1668–1671. https://doi.org/10.1359/jbmr.070716.
(119) Stokes, C. S.; Lammert, F.; Volmer, D. A. Analytical Methods for Quantification of Vitamin D and Implications for Research and Clinical Practice. *Anticancer Res.* **2018**, *38* (2), 1137–1144.
(120) Li, L.; Xu, G.; Shao, H.; Zhang, Z.-H.; Pan, X.-F.; Li, J.-Y. Analysis of Blood Concentrations of Zinc, Germanium, and Lead and Relevant Environmental Factors in a Population Sample from Shandong Province, China. *Int. J. Environ. Res. Public. Health* **2017**, *14* (3), 227. https://doi.org/10.3390/ijerph14030227.
(121) Clyne, B.; Olshaker, J. S. The C-Reactive Protein11Clinical Laboratory in Emergency Medicine Is Coordinated by Jonathan S. Olshaker, MD, of the University of Maryland Medical Center, Baltimore, Maryland. *J. Emerg. Med.* **1999**, *17* (6), 1019–1025. https://doi.org/10.1016/S0736-4679(99)00135-3.
(122) Gannon, B. M.; Glesby, M. J.; Finkelstein, J. L.; Raj, T.; Erickson, D.; Mehta, S. A Point-of-Care Assay for Alpha-1-Acid Glycoprotein as a Diagnostic Tool for Rapid, Mobile-Based Determination of Inflammation. *Curr. Res. Biotechnol.* **2019**, *1*, 41–48. https://doi.org/10.1016/j.crbiot.2019.09.002.
(123) Petry, N.; Jallow, B.; Sawo, Y.; Darboe, M. K.; Barrow, S.; Sarr, A.; Ceesay, P. O.; Fofana, M. N.; Prentice, A. M.; Wegmüller, R.; Rohner, F.; Phall, M. C.; Wirth, J. P. Micronutrient Deficiencies, Nutritional Status and the Determinants of Anemia in Children 0–59 Months of Age and Non-Pregnant Women of Reproductive Age in The Gambia. *Nutrients* **2019**, *11* (10), 2275. https://doi.org/10.3390/nu11102275.
(124) Jáuregui-Lobera, I. Iron Deficiency and Cognitive Functions. *Neuropsychiatr. Dis. Treat.* **2014**, *10*, 2087–2095. https://doi.org/10.2147/NDT.S72491.
(125) Namaste, S. M. L.; Ou, J.; Williams, A. M.; Young, M. F.; Yu, E. X.; Suchdev, P. S. Adjusting Iron and Vitamin A Status in Settings of Inflammation: A Sensitivity Analysis of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Approach. *Am. J. Clin. Nutr.* **2020**, *112* (Supplement_1), 458S-467S. https://doi.org/10.1093/ajcn/nqaa141.
(126) *Publications*. BRINDA. https://www.brinda-nutrition.org/publications (accessed 2023-04-11).
(127) Namaste, S. M. L.; Rohner, F.; Huang, J.; Bhushan, N. L.; Flores-Ayala, R.; Kupka, R.; Mei, Z.; Rawat, R.; Williams, A. M.; Raiten, D. J.; Northrop-Clewes, C.; Suchdev, P. S. Adjusting Ferritin Concentrations for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2017**, *106* (Suppl 1), 359S-371S. https://doi.org/10.3945/ajcn.116.141762.
(128) Rohner, F.; Namaste, S. M. L.; Larson, L. M.; Addo, O. Y.; Mei, Z.; Suchdev, P. S.; Williams, A. M.; Sakr Ashour, F. A.; Rawat, R.; Raiten, D. J.; Northrop-Clewes, C. Adjusting Soluble Transferrin Receptor Concentrations for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2017**, *106* (Suppl 1), 372S-382S. https://doi.org/10.3945/ajcn.116.142232.
(129) *Nutrition | The Brinda Project*. BRINDA. https://www.brinda-nutrition.org (accessed 2023-02-20).
(130) Young, M. F.; Ou, J.; Duong, C.; Luo, H.; Beyh, Y. S.; Meng, J.; Gernand, A. D.; Roth, D. E.; Suchdev, P. S. Assessment of Vitamin D Status and Association with Inflammation: Biomarkers Reflecting

Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2023**, *117* (1), 175–181. https://doi.org/10.1016/j.ajcnut.2022.10.018.
(131) McDonald, C. M.; Suchdev, P. S.; Krebs, N. F.; Hess, S. Y.; Wessells, K. R.; Ismaily, S.; Rahman, S.; Wieringa, F. T.; Williams, A. M.; Brown, K. H.; King, J. C. Adjusting Plasma or Serum Zinc Concentrations for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2020**, *111* (4), 927–937. https://doi.org/10.1093/ajcn/nqz304.
(132) Tomkins, A. Assessing Micronutrient Status in the Presence of Inflammation. *J. Nutr.* **2003**, *133* (5), 1649S-1655S. https://doi.org/10.1093/jn/133.5.1649S.
(133) Chaparro, C. M.; Suchdev, P. S. Anemia Epidemiology, Pathophysiology, and Etiology in Low- and Middle-Income Countries. *Ann. N. Y. Acad. Sci.* **2019**, *1450* (1), 15–31. https://doi.org/10.1111/nyas.14092.
(134) *Effects of Inflammation on Micronutrient Biomarkers Associated with Anemia | SpringerLink*. https://link.springer.com/chapter/10.1007/978-3-031-14521-6_5 (accessed 2023-04-05).
(135) Kilby, K.; Mathias, H.; Boisvenue, L.; Heisler, C.; Jones, J. L. Micronutrient Absorption and Related Outcomes in People with Inflammatory Bowel Disease: A Review. *Nutrients* **2019**, *11* (6), 1388. https://doi.org/10.3390/nu11061388.
(136) Oz, H. S. Nutrients, Infectious and Inflammatory Diseases. *Nutrients* **2017**, *9* (10), 1085. https://doi.org/10.3390/nu9101085.
(137) Ott, L. W.; Resing, K. A.; Sizemore, A. W.; Heyen, J. W.; Cocklin, R. R.; Pedrick, N. M.; Woods, H. C.; Chen, J. Y.; Goebl, M. G.; Witzmann, F. A.; Harrington, M. A. Tumor Necrosis Factor-α -and Interleukin-1-Induced Cellular Responses: Coupling Proteomic and Genomic Information. *J. Proteome Res.* **2007**, *6* (6), 2176–2185. https://doi.org/10.1021/pr060665l.
(138) Eggersdorfer, M.; Berger, M. M.; Calder, P. C.; Gombart, A. F.; Ho, E.; Laviano, A.; Meydani, S. N. Perspective: Role of Micronutrients and Omega-3 Long-Chain Polyunsaturated Fatty Acids for Immune Outcomes of Relevance to Infections in Older Adults—A Narrative Review and Call for Action. *Adv. Nutr.* **2022**, *13* (5), 1415–1430. https://doi.org/10.1093/advances/nmac058.
(139) Fratoni, V.; Brandi, M. L. B Vitamins, Homocysteine and Bone Health. *Nutrients* **2015**, *7* (4), 2176–2192. https://doi.org/10.3390/nu7042176.
(140) Kennedy, D. O. B Vitamins and the Brain: Mechanisms, Dose and Efficacy—A Review. *Nutrients* **2016**, *8* (2), 68. https://doi.org/10.3390/nu8020068.
(141) Medzhitov, R. Origin and Physiological Roles of Inflammation. *Nature* **2008**, *454* (7203), 428–435. https://doi.org/10.1038/nature07201.
(142) Kolb, A. F.; Petrie, L. Folate Deficiency Enhances the Inflammatory Response of Macrophages. *Mol. Immunol.* **2013**, *54* (2), 164–172. https://doi.org/10.1016/j.molimm.2012.11.012.
(143) Cianciulli, A.; Salvatore, R.; Porro, C.; Trotta, T.; Panaro, M. A. Folic Acid Is Able to Polarize the Inflammatory Response in LPS Activated Microglia by Regulating Multiple Signaling Pathways. *Mediators Inflamm.* **2016**, *2016*, 5240127. https://doi.org/10.1155/2016/5240127.
(144) Young, M. F.; Guo, J.; Williams, A.; Whitfield, K. C.; Nasrin, S.; Kancherla, V.; Suchdev, P. S.; Crider, K. S.; Pfeiffer, C. M.; Serdula, M. Interpretation of Vitamin B-12 and Folate Concentrations in Population-Based Surveys Does Not Require Adjustment for Inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project. *Am. J. Clin. Nutr.* **2020**, *111* (4), 919–926. https://doi.org/10.1093/ajcn/nqz303.
(145) Zhou, A.; Hyppönen, E. Vitamin D Deficiency and C-Reactive Protein: A Bidirectional Mendelian Randomization Study. *Int. J. Epidemiol.* **2023**, *52* (1), 260–271. https://doi.org/10.1093/ije/dyac087.

(146) Migliaccio, S.; Di Nisio, A.; Mele, C.; Scappaticcio, L.; Savastano, S.; Colao, A. Obesity and Hypovitaminosis D: Causality or Casualty? *Int. J. Obes. Suppl.* **2019**, *9* (1), 20–31. https://doi.org/10.1038/s41367-019-0010-8.
(147) Jones, G.; Prosser, D. E.; Kaufmann, M. 25-Hydroxyvitamin D-24-Hydroxylase (CYP24A1): Its Important Role in the Degradation of Vitamin D. *Arch. Biochem. Biophys.* **2012**, *523* (1), 9–18. https://doi.org/10.1016/j.abb.2011.11.003.
(148) Athanassiou, L.; Mavragani, C. P.; Koutsilieris, M. The Immunomodulatory Properties of Vitamin D. *Mediterr. J. Rheumatol.* **2022**, *33* (1), 7–13. https://doi.org/10.31138/mjr.33.1.7.
(149) Williams, A. M.; Ladva, C. N.; Leon, J. S.; Lopman, B. A.; Tangpricha, V.; Whitehead, R. D.; Armitage, A. E.; Wray, K.; Morovat, A.; Pasricha, S.-R.; Thurnham, D.; Tanumihardjo, S. A.; Shahab-Ferdows, S.; Allen, L.; Flores-Ayala, R. C.; Suchdev, P. S. Changes in Micronutrient and Inflammation Serum Biomarker Concentrations after a Norovirus Human Challenge. *Am. J. Clin. Nutr.* **2019**, *110* (6), 1456–1464. https://doi.org/10.1093/ajcn/nqz201.
(150) Foster, M.; Samman, S. Zinc and Regulation of Inflammatory Cytokines: Implications for Cardiometabolic Disease. *Nutrients* **2012**, *4* (7), 676–694. https://doi.org/10.3390/nu4070676.
(151) Liuzzi, J. P.; Lichten, L. A.; Rivera, S.; Blanchard, R. K.; Aydemir, T. B.; Knutson, M. D.; Ganz, T.; Cousins, R. J. Interleukin-6 Regulates the Zinc Transporter Zip14 in Liver and Contributes to the Hypozincemia of the Acute-Phase Response. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (19), 6843–6848. https://doi.org/10.1073/pnas.0502257102.
(152) Young, M. F.; Suchdev, P. S. Effects of Inflammation on Micronutrient Biomarkers Associated with Anemia. In *Nutritional Anemia*; Karakochuk, C. D., Zimmermann, M. B., Moretti, D., Kraemer, K., Eds.; Nutrition and Health; Springer International Publishing: Cham, 2022; pp 63–73. https://doi.org/10.1007/978-3-031-14521-6_5.
(153) Luo, H.; Geng, J.; Zeiler, M.; Nieckula, E.; Sandalinas, F.; Williams, A.; Young, M. F.; Suchdev, P. S. A Practical Guide to Adjust Micronutrient Biomarkers for Inflammation Using the BRINDA Method. *J. Nutr.* **2023**. https://doi.org/10.1016/j.tjnut.2023.02.016.
(154) *Adjustment Theory and Methods*. BRINDA. https://www.brinda-nutrition.org/adjustmenttheoryandmethods (accessed 2023-03-21).
(155) Berlin, H.; Berlin, R.; Brante, G. Oral Treatment of Pernicious Anemia with High Doses of Vitamin B12 Without Intrinsic Factor. *Acta Med. Scand.* **1968**, *184* (1–6), 247–258. https://doi.org/10.1111/j.0954-6820.1968.tb02452.x.
(156) Wacker, M.; Holick, M. F. Sunlight and Vitamin D. *Dermatoendocrinol.* **2013**, *5* (1), 51–108. https://doi.org/10.4161/derm.24494.
(157) Brito, A.; Verdugo, R.; Hertrampf, E.; Miller, J. W.; Green, R.; Fedosov, S. N.; Shahab-Ferdows, S.; Sanchez, H.; Albala, C.; Castillo, J. L.; Matamala, J. M.; Uauy, R.; Allen, L. H. Vitamin B-12 Treatment of Asymptomatic, Deficient, Elderly Chileans Improves Conductivity in Myelinated Peripheral Nerves, but High Serum Folate Impairs Vitamin B-12 Status Response Assessed by the Combined Indicator of Vitamin B-12 Status1,2. *Am. J. Clin. Nutr.* **2016**, *103* (1), 250–257. https://doi.org/10.3945/ajcn.115.116509.
(158) Stevens, G. A.; Paciorek, C. J.; Flores-Urrutia, M. C.; Borghi, E.; Namaste, S.; Wirth, J. P.; Suchdev, P. S.; Ezzati, M.; Rohner, F.; Flaxman, S. R.; Rogers, L. M. National, Regional, and Global Estimates of Anaemia by Severity in Women and Children for 2000–19: A Pooled Analysis of Population-Representative Data. *Lancet Glob. Health* **2022**, *10* (5), e627–e639. https://doi.org/10.1016/S2214-109X(22)00084-5.
(159) BRINDA data management team. BRINDA Data Source, 2022. brinda-nutrition.org/_files/ugd/c11534_f4692f7dbc42463bb9fdc92fa2c57e6f.pdf (accessed 2023-03-31).

(160) *Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population | The American Journal of Clinical Nutrition | Oxford Academic*. https://academic.oup.com/ajcn/article/104/6/1607/4564692 (accessed 2023-03-15).
(161) Ko, Y.-A.; Williams, A. M.; Peerson, J. M.; Luo, H.; Flores-Ayala, R.; Wirth, J. P.; Engle-Stone, R.; Young, M. F.; Suchdev, P. S. Approaches to Quantify the Contribution of Multiple Anemia Risk Factors in Children and Women from Cross-Sectional National Surveys. *PLOS Glob. Public Health* **2022**, *2* (10), e0001071. https://doi.org/10.1371/journal.pgph.0001071.
(162) Luo, H.; Addo, O. Y. BRINDA: Computation of BRINDA Adjusted Micronutrient Biomarkers for Inflammation. *10/16/2022*.
(163) Gombart, A. F.; Pierre, A.; Maggini, S. A Review of Micronutrients and the Immune System–Working in Harmony to Reduce the Risk of Infection. *Nutrients* **2020**, *12* (1), 236. https://doi.org/10.3390/nu12010236.
(164) *Evaluation of Hemoglobin Cutoff Levels to Define Anemia Among Healthy Individuals | Global Health | JAMA Network Open | JAMA Network*. https://jamanetwork.com/journals/jamanetworkopen/fullarticle/2782758 (accessed 2023-04-12).
(165) Raghavan, R.; Ashour, F. S.; Bailey, R. A Review of Cutoffs for Nutritional Biomarkers12. *Adv. Nutr.* **2016**, *7* (1), 112–120. https://doi.org/10.3945/an.115.009951.
(166) Ty, M. C.; Zuniga, M.; Götz, A.; Kayal, S.; Sahu, P. K.; Mohanty, A.; Mohanty, S.; Wassmer, S. C.; Rodriguez, A. Malaria Inflammation by Xanthine Oxidase-Produced Reactive Oxygen Species. *EMBO Mol. Med.* **2019**, *11* (8), e9903. https://doi.org/10.15252/emmm.201809903.
(167) *BRINDA Countries*. BRINDA. https://www.brinda-nutrition.org/brinda-countries (accessed 2023-02-20).