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Assessing the role of inflammation on iron biomarkers in school-age children and adolescents, the BRINDA project

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Abstract

Assessing the role of inflammation on iron biomarkers in school-age children and adolescents, the BRINDA project

By Emily Nieckula

Objectives:

Prevalence estimates for iron deficiency may be under- or overestimated due to inflammation. We examined the relationship between iron biomarkers and inflammation and evaluated the use of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) adjustment method in school-age children (SAC) and adolescents (ADL).

Methods:

Surveys from 15 different countries [8 SAC datasets (n = 26,334; age 5-15 years); 15 ADL datasets (n = 19,595; age 10-20 years)] from the BRINDA project were examined, accounting for complex survey design. The prevalence of inflammation (α-1-acid-glycoprotein [AGP] >1 g/L; C-reactive protein [CRP] > 5 mg/L) and iron deficiency (ferritin < 15 ug/L; soluble transferrin receptor (sTfR) > 8.3 mg/L) and rank correlations between CRP or AGP and ferritin or sTfR were estimated. Ferritin and sTfR were adjusted using the BRINDA inflammation-adjustment method using AGP only, CRP only, and both AGP and CRP. The prevalence of iron deficiency was compared with unadjusted estimates.

Results:

Inflammation prevalence varied by dataset from 1.4% to 33%. Unadjusted iron deficiency prevalence ranged from 0% to 43.4%. Ferritin was positively corelated with AGP in 7 out of 8 datasets (r = 0.06-0.48) and with CRP in 19 out of 22 datasets (r = 0.05-0.48). sTfR was positively correlated with AGP in 6 of 7 datasets (r = 0.11- 0.24). Associations between sTfR and CRP were mixed with 7 positive and 6 null associations (r = 0.02 -0.24). Ferritin adjustment for AGP only had the greatest increase, 4.5 percentage points (pp), compared to 3.7 for AGP and CRP and 3.4 for CRP alone. sTfR adjustment for AGP only had the greatest decrease, 6.4 pp, compared to 5.9 for AGP and CRP and 3 pp for CRP alone.

Conclusions:

Failure to adjust ferritin for inflammation biomarker of AGP may result in iron deficiency underestimation among SAC and ADL. Based on limited data for sTfR, preliminary results suggest inflammation adjustment with AGP may be merited. However, further research is needed given the critical data gaps among these population groups.

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CHAPTER 1: INTRODUCTION

Iron deficiency and iron deficiency anemia (IDA) are significant problems globally, affecting both developed and developing countries. According to the World Health Organization, over two billion people worldwide suffer from anemia, with the highest prevalence found in low- and middle-income countries [1]. The burden of anemia is particularly severe among children and women of reproductive age, affecting an estimated 39.8% in children under five, 29.6% of non-pregnant women of reproductive age, and 36.5% of pregnant women globally [2]. Anemia is caused by dietary iron deficiency; infectious diseases, including malaria, hookworms and schistosomiasis; deficiencies in folate, vitamin B12 and vitamin A; or inherited conditions affecting red blood cells (RBCs), such as thalassemia [1]. It has been estimated that iron deficiency contributes to 30% to 50% of anemia cases worldwide [3], [4]. Iron deficiency and IDA both have significant negative health effects, including impaired cognitive and physical development, weakened immune system, and behavioral problems [5]. Addressing iron deficiency is therefore crucial not only for individual health but also for global public health and development.

However, iron deficiency can be under or overestimated due to inflammation's influence on iron biomarkers like serum ferritin (SF) and soluble transferrin receptors (sTfR) [6]. Thus, an accurate assessment of iron deficiency requires examining the influence of other factors, such as inflammation and infection. Previous studies have addressed the iron biomarkers of SF and sTfR concentrations in women of reproductive age (WRA) and preschool children (PSC), which resulted in guidance on when and how to adjust for iron biomarkers of inflammation in these populations[7]. Currently, there is no guidance for school age children (SAC) and adolescents (ADL); therefore, there is a need to examine the relationship of iron biomarkers and inflammation biomarkers (α-1-acid-glycoprotein (AGP) and C-reactive protein (CRP)) in SAC and ADL.

These child populations are vulnerable to iron deficiency and IDA due to growth at this time of life. During rapid growth periods such as infancy and adolescence, iron demand increases to support the growth of new cells and tissues. Therefore, it's important to ensure that children in these vulnerable age groups consume adequate amounts of iron in their diet. Iron deficiency and IDA in SAC and ADL may result in slowed growth and development and other negative health consequences including behavioral problems, frequent infections, and cardiovascular and respiratory system disorders [8]. An accurate estimation of the prevalence of iron deficiency is needed for planning and implementing effective interventions to reduce iron deficiency and IDA. However, because the biomarkers used to measure iron status, SF and sTfR, are influenced by inflammation, iron deficiency can be under and/or overestimated, which can further result in the improper allocation of prevention and intervention resources.

OBJECTIVE AND AIMS

Investigating the relationship between inflammation and iron biomarkers in SAC and ADL is essential to address a knowledge gap in these understudied population groups. The key objective is to compare different inflammation adjustment methods, AGP only, CRP only, AGP and CRP, for estimating the population prevalence of iron deficiency and to see if inflammation adjustment is warranted in SAC and ADL. The aim of this thesis is to examine the relationship between iron and inflammation biomarkers and compare different inflammation adjustment approaches for estimating the population prevalence of iron deficiency in SAC and ADL. This analysis will help determine whether the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) adjustment method should be used to estimate the population prevalence of iron deficiency in SAC and ADL in settings with inflammation. The results of this analysis will expand BRINDA's existing research and improve our understanding of the relationship between

inflammation adjustment across the lifespan. The findings of this work will be highly relevant to public health researchers, advisory groups, and policymakers who are interested in improving the accuracy of micronutrient assessment in surveillance programs and research by adjusting for inflammation. Within this thesis, a literature review (CHAPTER_2:_COMPREHENSIVE REVIEW OF LITERATURE) a manuscript for publication (CHAPTER 3: MANUSCRIPT), and a conclusion and discussion section (CHAPTER 4: CONCLUSION AND RECOMMENDATIONS) will be presented. The results of this study will enhance the understanding of iron deficiency and its assessment in SAC and ADL for the purpose of public informing and advocating for public health policy and programs.

DEFINITION OF TERMS

α-1-acid glycoprotein (AGP): An acute phase-protein found in blood with a variety of biological functions including the transportation of drugs and activating the immune system. AGP > 1 g/L commonly used cutoff value to indicate the presence of inflammation.

Adolescents (ADL): individuals 10 – 20 years old

C-reactive protein (CRP): An acute phase-protein produced by the liver in response to inflammation in the body. CRP > 5 mg/L commonly used cutoff value to indicate the presences of inflammation.

Inflammation: The presence of AGP > 1 g/L and/or CRP > 5mg/L

Iron Deficiency (ID): A condition that often precedes anemia in which there is a decrease of iron stores, caused by factors such as excessive loss or utilization in the body or persistently low dietary intake or absorption.

Iron Deficiency Anemia (IDA): A type of anemia that occurs when there is not enough iron to make hemoglobin, a protein in red blood cells that carries oxygen throughout the body. Defined as hemoglobin concentration of < 120 g/L and SF <15 ug/L[9].

Preschool-age Children (PSC): individuals 6 months – 5 years old

School-age Children (SAC): individuals 5 – 15 years old

Serum Ferritin (SF): A protein that stores iron in the body. Small amounts of ferritin are secreted into the plasma. The concentration of serum ferritin is positively correlated with the size of total body iron stores. Measuring serum ferritin levels can help diagnose iron deficiency anemia, as well as other conditions related to iron metabolism. Cutoff value of <12 ug/L in children less than 5 and <15 in all other age groups[9] .

Soluble Transferrin Receptor (sTfR): A protein that transports iron into cells. Transferrin is a protein that binds to iron and delivers it to cells that require it. Transferrin receptors, found on cells' surfaces, facilitate the uptake of transferrin-bound iron. Measuring its levels can provide an indirect estimate of the availability of iron for red blood cell formation. sTfR greater than 8.3 mg/L is commonly used as a cutoff value[10].

Women of Reproductive Age (WRA): women 15 – 49 years old

CHAPTER 2: COMPREHENSIVE REVIEW OF LITERATURE

INTRODUCTION

Iron deficiency and iron deficiency anemia (IDA) are significant public health issues globally, affecting both developing and developed countries. According to the World Health Organization (WHO), approximately two billion people worldwide suffer from iron deficiency, and an estimated 50% of anemia cases are due to iron deficiency[2]. Iron deficiency is caused by an insufficient intake of dietary iron, increased iron requirements due to growth or pregnancy, chronic blood loss from menstruation, gastrointestinal bleeding, or other medical conditions that impair iron absorption or utilization, such as celiac disease or inflammatory bowel disease[11]. Iron is essential to produce hemoglobin, which carries oxygen into the blood and for a variety of physiological processes in the body, including energy metabolism, immune function, cognitive function, muscle function, and wound healing[12]. It is involved in the production of adenosine triphosphate (ATP), the proper functioning of immune and muscle cells, and the synthesis of neurotransmitters and collagen. Iron deficiency can impair these processes and lead to health problems. As a result, it should be noted that adequate iron intake can have a significant impact on one's overall health and well-being [13].

Iron deficiency and iron deficiency anemia (IDA) disproportionately affect vulnerable populations, such as women of reproductive age (WRA) and preschool children (PSC)[2]. However, school-age children (SAC) and adolescent (ADL) populations are also at risk of developing iron deficiency and IDA due to their rapid growth and development during these critical periods [14]. Iron deficiency and IDA in SAC and ADL may result in slowed growth and development, behavioral problems, frequent infections, and cardiovascular and respiratory system disorders [8]. Despite the significant health implications of iron deficiency and IDA, there is currently no

guidance on whether and how to adjust iron biomarkers for inflammation in SAC and ADL. Iron biomarkers are tools used to measure iron levels in the body indirectly. The most common biomarkers used to assess iron status in the blood are serum ferritin, soluble transferrin receptors (sTfR), serum iron, total iron-binding capacity (TIBC), and percent transferrin saturation [15]. Among these biomarkers, serum ferritin and sTfR have gained attention for their potential to reflect iron deficiency and inflammation. Therefore, there is a need to examine the role of iron biomarkers, such as serum ferritin (SF) and soluble transferrin receptors (sTfR), and inflammation in SAC and ADL.

This literature review aims to describe the biological plausibility of the association between inflammation and iron biomarkers. It will examine selected previous research on this topic and highlight the need for further research in this area. By examining the relationship between iron biomarkers, inflammation, and anemia in SAC and ADL populations, this review will focus on two key iron biomarkers, SF and sTfR. Additionally, the review will explore the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Adjustment Method, other relevant studies, and the current knowledge gap in this research area. Ultimately, a better understanding of the relationship between inflammation and iron biomarkers in SAC and ADL populations could lead to improved screening and management of iron deficiency and IDA in these vulnerable groups and provide a framework for future research in this field.

IRON BIOMARKERS: SERUM FERRITIN (SF) AND SOLUBLE TRANSFERRIN RECEPTOR (STFR)

Ferritin is an iron storage protein located in the liver, spleen, and bone marrow[16]. Because the concentration of ferritin in the bloodstream is proportional to body iron stores, serum ferritin concentrations can be used to diagnose iron deficiency[17]. During periods of high

inflammation, the body's acute phase response can cause elevated serum ferritin levels, even without true iron overload. The acute phase response is a physiological process that occurs in response to infection, injury, or other types of inflammation. During the acute phase response, the body produces a range of proteins, including ferritin, as part of the immune response[18].

Ferritin is an acute-phase reactant protein, meaning its production increases in response to inflammation. When the body is exposed to an inflammatory stimulus, such as an infection or injury, pro-inflammatory cytokines are released, which stimulate ferritin production. Inflammatory cytokines can stimulate ferritin synthesis by liver cells and macrophages, leading to elevated ferritin levels even in the presence of iron deficiency. Elevated ferritin levels during the acute phase response are thought to be due to increased production by immune cells[17]. In addition, there is decreased clearance of ferritin from the blood. This makes it difficult to distinguish between IDA and anemia of chronic disease or inflammation based on ferritin levels alone. As a result, elevated SF levels during periods of high inflammation may not accurately reflect iron stores in the body[19]. Therefore, when interpreting SF levels in the context of inflammation, it is critical to consider other clinical and laboratory findings.

Soluble transferrin receptors are proteins expressed on the cell membrane that bind to transferrin, a protein that transports iron in the blood. When tissues have an insufficient supply of iron, several cellular mechanisms come into play to maintain iron homeostasis[20]. One such mechanism is the upregulation of transferrin receptors on the cell surface. This upregulation increases iron cellular uptake, ensuring adequate intracellular element levels. The relationship between sTfR and inflammation is conflicting in the scientific literature. On the one hand, some studies suggest that sTfR levels can increase in response to inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[21]. These cytokines upregulate

transferrin receptor expression on cells, leading to an increase in sTfR levels in the bloodstream. Inflammatory states such as infections, autoimmune disorders, and cancer have been associated with elevated sTfR levels, which may reflect an increased demand for iron due to enhanced erythropoiesis or tissue repair [22]. On the other hand, some studies suggest that inflammation can contribute to decreased sTfR levels. Inflammation can interfere with iron metabolism by reducing iron availability to cells [23]. This reduction in iron availability can downregulate the expression of transferrin receptors on cells, leading to a decrease in sTfR levels in the bloodstream. Additionally, inflammation can suppress erythropoiesis, leading to decreased sTfR level. Thus, sTfR concentrations are considered a sensitive and specific biomarker of iron deficiency, particularly without inflammation.

Both ferritin and sTfR have strengths and limitations for assessing iron deficiency. Ferritin is an effective marker when inflammation is not present, but its interpretation can be challenging in inflammation. sTfR may be a more reliable marker in the presence of inflammation, but it may not be as specific. Therefore, it is essential to research both biomarkers to make an accurate diagnosis of iron deficiency.

INFLAMMATION AND IRON BIOMARKERS

To accurately interpret ferritin levels in the context of inflammation, it is important to understand the acute phase response and the proteins used to assess inflammation, such as AGP and CRP. The link between iron and inflammation is supported by evidence in both animal and human studies [24]. Inflammation is the body's natural protective response to injury or infection. During inflammation, there will be an increase in the production of inflammation proteins, AGP and CRP, in response to inflammation [25]. AGP and CRP can be measured to assess inflammation levels. CRP levels, on the other hand, typically rise slightly later than AGP, generally within 6-12 hours

after the onset of inflammation or infection. CRP levels continue to rise for 24-48 hours, and then gradually decline as the acute phase response subsides. AGP levels increase more gradually than CRP and may take a maximum of 2 to 5 days to reach their peak[26]. Therefore, measuring AGP and CRP levels can provide information about the timing and severity of an acute inflammatory response.

Inflammation can affect iron regulation through its impact on the hormone hepcidin, which regulates iron metabolism. Hepcidin, a hormone produced by the liver, plays a central role in iron regulation by regulating iron absorption from the gut, expression of cellular transporters/receptors, and recycling of iron from the spleen. Hepcidin is induced during an acute phase response and leads to a decrease in iron absorption from the gut, sequestration of iron in cells, and a decrease in iron release from cells, including macrophages[27]. These physiological changes can lead to decreased iron availability for erythropoiesis and other essential cellular processes. The decrease in iron release from cells can result in a decrease in serum iron and an increase in ferritin, which is a positive acute-phase protein induced by inflammation. However, the increase in ferritin may not necessarily indicate an increase in iron stores, as its concentration can be elevated in response to inflammation alone. In contrast, sTfR, which reflects cellular iron needs, is not affected by inflammation and can be a more reliable indicator of iron deficiency in the presence of inflammation [10]. The ratio of sTfR to ferritin concentrations can also be used to measure iron deficiency in the presence of inflammation, as it can account for inflammation effects on ferritin levels. However, even the sTfR/ferritin ratio may be affected by inflammation to some extent[18]. A statistical correction for inflammation may be necessary to improve the accuracy of prevalence estimates for iron deficiency at the population level.

Therefore, caution should be taken when using serum ferritin and sTfR concentrations as biomarkers of iron status in individuals with inflammation. Thus, it is essential to consider the relationship between inflammation and iron biomarkers when interpreting iron biomarker measurements. Previous studies have utilized CRP and AGP, to adjust measurements of serum ferritin and sTfR for inflammation in WRA and PSC[7], [19].

THE BRINDA ADJUSTMENT METHOD

The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) adjustment method is a statistical tool developed to address the potential confounding effects of inflammation for multiple micronutrient biomarkers. The BRINDA approach is especially important in settings where inflammation is common, as it can impact the measurement of micronutrient biomarkers such as retinol-binding protein (RBP), serum retinol, serum ferritin, soluble transferrin receptor (sTfR), and serum zinc [28]–[30].

The BRINDA Inflammation Adjustment Method is based on the use of inflammation markers AGP and/or CRP to adjust micronutrient biomarkers for inflammation[29]. The adjustment method uses a linear regression model to estimate the effect of inflammation on the micronutrient biomarker. To ensure that the adjustment is appropriate for the population being studied, the BRINDA method recommends using population-specific cutoff values for the inflammation markers and micronutrient biomarkers. These cutoff values should be based on representative samples of the target population to accurately determine the prevalence of deficiency or excess of a specific micronutrient in that population. The adjustment method also uses both internal and external deciles for the inflammation markers and micronutrient biomarkers.

The internal deciles are generated from the user's own data, while the external deciles are based on the BRINDA study reference values; this ensures the accuracy of the adjustment.

However, not all micronutrient biomarkers require adjustment for inflammation, nor do they need to be adjusted by both AGP and CRP[29]. The adjustment method should only be used when there is both biological and statistical evidence of a relationship between micronutrient biomarkers and inflammation markers in the population being studied. It should not be applied indiscriminately to populations with high or low inflammation levels without first establishing such a relationship. Additionally, if only one inflammation biomarker is available for micronutrient biomarkers that need to be adjusted by both AGP and CRP, it is still recommended to use that inflammation marker to adjust for inflammation.

Previous studies have used the BRINDA adjustment method in different populations, including PSC, SAC, and WRA[7], [10], [19]. These studies have shown that the BRINDA adjustment method can improve the accuracy of micronutrient biomarker measurements in the presence of inflammation. The prevalence of elevated sTfR concentrations decreased incrementally as CRP and AGP deciles increased for PSC and WRA; however, the effect was more pronounced for AGP than CRP[10]. Depending on the approach used to adjust for inflammation, the estimated prevalence of iron deficiency by sTfR decreased by 4.4-14.6 and 0.3-9.5 percentage points in PSC and WRA, respectively, compared with unadjusted values. Internal-survey adjustments for SF in children increased the estimated prevalence of depleted iron stores by 11 percentage points and 7 percentage points in women[19]. These studies demonstrate that both AGP and CRP should be used to adjust SF and AGP only should be used to adjust sTfR for both WRA and PSC. The BRINDA adjustment method is not the only method available for adjusting micronutrient biomarkers for inflammation. Other methods include ratio-based adjustments, such

as the soluble transferrin receptor (sTfR)/log ferritin ratio, which has been shown to be a reliable indicator of iron deficiency in the presence of inflammation[31], [32]. For instance, the sTfR ratio may not be an accurate indicator of iron deficiency in the presence of anemia of chronic disease, as SF levels may be falsely elevated due to the presence of inflammatory cytokines. The BRINDA method has several advantages over these methods including its ability to adjust for both AGP and CRP and its use of population-specific cutoffs for inflammation markers. However, it is currently only studied in populations of PSC or WRA, and other populations such as newborns, men, pregnant women, SAC, and ADL need to be researched.

SCHOOL AGE CHILDREN AND ADOLESCENTS

Iron deficiency is a common nutritional deficiency that affects SAC and ADL worldwide. The WHO reports that IDA is one of the most prevalent nutritional disorders, affecting approximately 30% of the world's population[9]. SAC and ADL are particularly susceptible to iron deficiency because of increased nutritional requirements accosted with growth and developed[8]. Research indicates that IDA can have significant short-term and long-term consequences on cognitive and physical development as well as overall health[33].

Several studies have investigated the prevalence of iron deficiency (ID) among SAC and ADL. A cross sectional survey conducted in Hong Kong concluded that 11% of ADL are iron deficient and that ADL girls reported an ID prevalence of 17.1%[34]. Similarly, a study conducted in Bangladesh found that 13.6% of SAC had IDA[35]. Whereas a study southeast Ethiopian study reported 37.3% prevalence among SAC[36]. Although prevalence rates of ID vary among region, these findings highlight that roughly 10 to 25% of SAC and ADL could be suffering from ID. Despite these variations it is crucial to measure the global prevalence of ID among these age groups and account for various factors that may impact ID.

The impact of inflammation on growth and development is particularly important to consider when measuring the prevalence of ID, as inflammation can contribute to the development of ID and impact its severity. Inflammation may lead to impaired nutrient absorption and utilization, which could contribute to impaired growth and development in SAC and ADL[37]–[40]. Several studies have found that children and ADL with higher levels of inflammation had lower levels of height-for-age, weight-for-age, BMI-for-age, and decrease cognitive development and adaptive functioning indicating impaired growth and development[33], [37], [40], [41]. Chronic inflammation can also impact iron availability and utilization, potentially exacerbating iron deficiencies and further impacting growth and development. Furthermore, chronic inflammation may lead to decreased growth hormone secretion, increased insulin resistance, and decreased appetite as other pathways potentially contributing to impaired growth in SAC and ADL.

Although these studies suggest that both inflammation and iron status may be factors influencing growth and development of SAC and ADL, studies that examine the relationship between inflammation and iron biomarkers in SAC and ADL face a major limitation of examining a population group were there are limited studies; most iron prevalence and inflammation research focuses on the population group of WRA or PSC. Addressing the dearth of research in this area is crucial for gaining a comprehensive understanding of the impact of inflammation and iron status on the growth and development of SAC and ADL, especially given the distinct growth patterns observed between boys and girls. Therefore, it is essential to conduct further studies to bridge this knowledge gap and develop effective interventions to promote optimal growth and development in these population groups[38].

Although adjustment and cutoff levels for SF and sTfR during inflammation have not been defined in SAC and ADL, previous studies on micronutrient deficiencies have worked to define the prevalence of iron deficiency in SAC and ADL. In southwestern Ethiopia, the prevalence of IDA was 37.3% in SAC[42]. However, the study failed to account for inflammation when measuring ferritin levels. One study conducted in Senegal defined both the inflammation profile and ID prevalence in SAC and ADL[43]. Of the SAC participants, 5.7% had an elevated CRP and 10.6% had an elevated AGP. The prevalence of ID among both SAC and ADL was 39.1%, while the prevalence of IDA was 10.6%. Additionally, when conducting a review of existing studies, it became difficult to find relevant research as studies have been limited in sample size or have not accounted for the effect of inflammation on SF and sTfR in their analysis. For example, a clinical trial in Burkina Faso analyzed CRP, AGP, SF, and sTfR [44] found a large difference in the estimated prevalence of IDA based on SF and sTfR even after adjustment for confounding by inflammation. The discrepancies in prevalence estimates of ID serve as a call for more research to identify the most effective biomarkers, deficiency cutoffs, and adjustment methods for assessing iron status in SAC and ADL.

Additionally, gender is another significant factor to consider when examining growth and development in SAC and ADL. Studies have shown that boys and girls have different growth patterns, with boys typically experiencing a growth spurt during adolescence that is later but greater in magnitude and duration than that of girls[45], [46]. These gender differences may have implications for the assessment of growth and development in this population. In addition, they may have implications for the development of interventions to promote healthy growth. For example, a study examining the relationship between ID and growth in SAC found that ID affected growth more pronounced in girls than boys. Similarly, a study examining the impact of

inflammation on growth found that inflammation's negative effects were more pronounced in girls than in boys[47], [48]. Therefore, it is imperative to consider gender when examining growth and development in SAC and ADL, as well as when developing interventions to promote healthy growth in this population.

In addition, many studies have been limited by their small sample sizes or cross-sectional design, which prevents us from drawing causal conclusions or assessing changes over time. Furthermore, some studies fail to account for inflammation effects when measuring iron status. This may have led to overestimating ID prevalence. Lastly, while gender differences in growth and development have been identified, there is still a need for more research to explore how these differences impact the relationship between inflammation, iron status, and growth in this population.

In summary, further research is needed to better understand the complex relationships between inflammation, iron status, and growth in SAC and ADL. In addition, it is needed to develop effective interventions to promote healthy growth, prevent anemia, and utilize energy and micronutrients to support cognitive development. However, the interaction between inflammation and iron biomarkers in SAC and ADL remains unclear. Addressing both inflammation and iron status could be critical in improving growth and development outcomes for SAC and ADL.

CONCLUSION AND FUTURE DIRECTIONS

In summary, this literature review explores the various research studies conducted on the relationship between inflammation, iron status, and growth in SAC and ADL. The findings suggest that both inflammation and iron status are significant factors to consider when examining growth and development in this population. This is particularly important given the negative consequences of ID and chronic inflammation on growth and development.

CHAPTER 3: MANUSCRIPT

Adjusting iron biomarkers for inflammation in school-age children and adolescents: A multi-country analysis from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project

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CONTRIBUTION OF STUDENT

The student conducted the analysis, wrote the first draft of the manuscript, participated in the writing process by synthesizing existing literature and revising the final version of the manuscript.

ABSTRACT

BACKGROUND:

Accurate prevalence estimates are needed to monitor and evaluate the reduction of iron deficiency and iron deficiency anemia. Inflammation-adjustment is recommended for assessment of iron in women of reproductive age and preschool children, but guidance is lacking for school-age children (SAC) and adolescents (ADL).

OBJECTIVES:

We examined the relationship between inflammation biomarkers [α-1-acid glycoprotein (AGP) and C-reactive protein (CRP)] and iron biomarkers [serum ferritin and soluble transferrin receptor (sTfR)] and evaluated the use of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) adjustment method on prevalence estimates for iron deficiency in SAC and ADL.

METHODS:

Surveys from 15 different countries [8 SAC datasets (n = 26,334; age 5-15 years); 15 ADL datasets (n = 19,595; age 10-20 years)] from the BRINDA project were examined, accounting for complex survey design. The prevalence of inflammation (AGP >1 g/L; CRP > 5 mg/L) and iron deficiency (serum ferritin < 15 ug/L; sTfR > 8.3 mg/L) and rank correlations between CRP or AGP and ferritin and sTfR were estimated. Ferritin and sTfR were adjusted using the BRINDA inflammation-adjustment method using AGP only, CRP only, and both AGP and CRP. The prevalence of iron deficiency was compared with unadjusted estimates.

RESULTS:

The prevalence of elevated AGP or CRP varied by dataset from 10.6% to 33% and 1.4% to 23.4% respectively. The prevalence of unadjusted iron deficiency ranged from 2.6% to 30.9% in SAC

and 0% to 43.4% in ADL. Correlations between ferritin and AGP were positive in 7 of 8 datasets (SAC: r = 0.24-0.48; ADL: 0.06-0.31) and with CRP in 20 of 23 datasets (SAC: r = 0.09-0.48; ADL: r = 0.05-0.43). sTfR was positively correlated with AGP in the one available SAC dataset (Malawi 2016: r = 0.21) and five of six ADL datasets (r = 0.11- 0.24). Associations between sTfR and CRP were mixed with seven out of 13 datasets reporting significant positive associations and six null associations. (SAC: r = 0.13-0.24; ADL: r = 0.02 – 0.20). Ferritin adjustment for AGP only had the greatest increase, 4.5 percentage points (pp), compared to 3.7 for AGP and CRP and 3.4 for CRP alone. sTfR adjustment for AGP only had the greatest decrease, 6.4 pp, compared to 5.9 for AGP and CRP and 3 pp for CRP alone.

CONCLUSIONS:

Failure to adjust ferritin for inflammation biomarker of AGP may result in iron deficiency underestimation among SAC and ADL. Based on limited data for sTfR, preliminary results suggest inflammation adjustment with AGP may be merited. However, further research is needed given the critical data gaps among these population groups.

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KEYWORDS:

adolescents, anemia, biomarkers, BRINDA, iron deficiency, inflammation, nutrition assessment, school-age children, ferritin, soluble transferrin receptors

INTRODUCTIONS

Iron deficiency is a significant problem globally, affecting both developed and developing countries. According to the World Health Organization, over two billion people worldwide suffer from anemia, with the highest prevalence found in low- and middle-income countries [2], [9]. The burden of anemia is particularly severe among children and women of reproductive age, affecting an estimated 39.8% in children under five, 29.6% of non-pregnant women of reproductive age, and 36.5% of pregnant women globally [2]. Anemia is caused by dietary iron deficiency, infectious diseases, including malaria, hookworms and schistosomiasis, other micronutrition's deficiencies [1]. It can be assumed that iron deficiency contributes to 30% to 50% of anemia cases worldwide [3], [4]. Iron deficiency and iron deficiency anemia (IDA) both have significant negative health effects, including impaired cognitive and physical development, weaken immune system and leads to behavioral problems [3], [8], [33]. Addressing iron deficiency is therefore crucial not only for individual health but also for global public health and development.

Assessments of iron deficiency can be under or overestimated due to inflammation's influence on iron biomarkers like serum ferritin (SF) and soluble transferrin receptors (sTfR). Recommendations for adjusting iron biomarkers for inflammation have been developed for women of reproductive age (WRA) and preschool children (PSC), based in part on previous studies examining the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) linear regression approach[7], [19]. However, no prior research has examined the role of iron biomarkers (SF and sTfR) and inflammation [α-1-acid glycoprotein (AGP) and C-reactive protein (CRP)] in school-age children (SAC) and adolescents (ADL). These child populations are vulnerable to iron deficiency and IDA due to expedited growth at this time in life, which may in turn present with other negative health consequences to child development and behavior, frequent

infections, and cardiovascular and respiratory system disorders[8]. Accurate prevalence estimates for iron deficiency prevalence are needed for planning and implementing effective interventions to reduce iron deficiency and IDA. However, because the biomarkers used to measure iron status are influenced by inflammation, the prevalence of iron deficiency can be underestimated by SF or overestimated by sTfR, which can further result in the improper allocation of prevention and intervention resources.

Investigating the relationship between inflammation and iron biomarkers in SAC and ADL is essential to address knowledge gaps in methods for assessing iron deficiency in these population groups. This analysis builds on previous BRINDA research by 1) examining the relationship between inflammation and iron biomarkers and 2) evaluating the use of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) adjustment method on prevalence estimates for iron deficiency in SAC and ADL. As a result of this analysis, existing knowledge on the association between inflammation and iron biomarkers will be extended to new population groups, providing implications for estimates of iron deficiency prevalence among these populations for programmatic and policy decision-making.

METHODS

Study Design and Data Source

This analysis was conducted using cross-sectional surveys from multiple countries, which were all previously acquired and harmonized by the BRINDA project (http://www.brinda-nutrition.org/) [49], [50]. The BRINDA project was initiated to improve micronutrient assessment and anemia characterization by conducting secondary data analysis on de-identified data. These analyses were determined to be non–human subject research by the Institutional Review Board at Emory University. Datasets from the BRINDA project were included in this analysis based on the

inclusion criteria that the survey must 1) include SAC population group (age 5 to 15 years old) or ADL population group (age 10 to 20 years old), 2) measure at least one biomarker of inflammation (AGP or CRP) and measure at least one iron biomarker of SF or sTfR, and 3) have a sample size greater than 100. Based on this inclusion criteria, datasets from 15 different countries [8 datasets for SAC (n = 26,334) and 15 for ADL (n = 19,595)] were examined individually and combined to investigate the need to adjust for inflammation in iron assessment.

Lab Methods

Venous or capillary blood samples were collected in SAC and ADL. The inflammation biomarkers, AGP and CRP, were measured using the methods Sandwich ELISA, Turbidimetric Agglutination Immunoassay, Nephelometry, or Particle-Enhanced Turbidimetric Immunoassays. Serum ferritin was measured using Sandwich ELISA, Turbidimetric Agglutination Immunoassay, Chemiluminescent Immunoassay, or Chemiluminescent Microparticle Immunoassay. Soluble transferrin receptor was measured Sandwich ELISA, Immunoturbidimetry, or Enzyme Immunoassay.

Statistical Analysis

The cutoffs applied to iron and inflammation biomarkers in this analysis were consistent with variable definitions used in previous BRINDA publications and WHO recommendations. Elevated AGP was defined by concentrations of > 1 g/L, while elevated CRP was defined by concentrations of > 5mg/L. Iron deficiency was defined by SF concentrations of < 15 ug/L and sTfR concentrations of > 8.3 mg/L[9], [10]. For each survey, median (IQR) was calculated for age, inflammation status (AGP, CRP), and iron status (SF, sTfR), and the prevalence of iron deficiency was calculated for the two iron biomarkers. Rank correlation coefficients between SF or sTfR, and AGP or CRP were calculated to determine the relationship between inflammation biomarkers and

iron. For each survey, we calculated unweighted internal deciles for inflammation biomarkers and pooled geometric means of SF and sTfR. Ferritin and sTfR were adjusted by linear regression using the BRINDA R package [51], using AGP only, CRP only, and both AGP and CRP when inflammation indices exceeded the first internal decile. The prevalence of inflammation-adjusted estimates of iron deficiency was calculated and compared with unadjusted estimates. Sensitivity analyses were conducted to investigate the role of sex and age to explore the possible influence of menarche and potential confounding effect of age. We determined the rank correlation coefficients when iron and inflammation biomarkers were stratified by sex as well as for SAC younger or older than 12 years. All analyses were conducted using R version 4.2.1 and adjusted for complex survey design effects including cluster, strata, and biomarker-specific sampling weights as applicable. All analyses were conducted independently by two analysts to ensure reproducibility.

RESULTS

The analysis included 8 SAC datasets (n = 26,334) and 15 ADL (n = 19,631) datasets after applying inclusion criteria, with a total of 23 datasets from 14 different countries. **Table 1** illustrates count, median, IQR for age, AGP, CRP and the percent of elevated AGP and CRP by dataset. In SAC, two datasets had values for AGP while all eight datasets had values for CRP. Among SAC, the median AGP values ranged from 0.7 g/L in Bangladesh to 0.8 g/L in Malawi and CRP values ranged from 0.3 mg/L in Bangladesh and the United States to 1.9 mg/L in Ecuador. The prevalence of elevated AGP in SAC ranged from 15.4% in Bangladesh to 31.8% in Malawi and elevated CRP ranged from 4.2% in Bangladesh to 15.9% in Malawi. In ADL, seven datasets had values for AGP while all 15 datasets had values for CRP. Among ADL, the median AGP values ranged from 0.7 g/L in Bangladesh and Malawi to 0.9 g/L in Papua New Guinea and CRP values ranged from 0.1 mg/L in Laos and 1.9 mg/L in Ecuador. The prevalence of elevated AGP

in ADL ranged from 10.6% in Laos to 26.5% in Cote D'Ivoire and elevated CRP ranged from 1.4% in Laos and 23.4% in Cote D'Ivoire. In SAC, the prevalence of unadjusted iron deficiency based on SF ranged from 2.6% in Ecuador to 17.3% in Mexico (2006) and based on sTfR ranged from 0% in Georgia to 30.9% in Malawi. In ADL, the prevalence of unadjusted iron deficiency based on SF ranged from 0% in Georgia to 29.3% in Laos and based on sTfR ranged from 6.9% in the United States to 41.7% in Liberia.

Table 2 presents micronutrient status data by median and IQR for each dataset. In SAC, all eight datasets had values for SF while three datasets had values for sTfR. Among SAC, the median SF values ranged from 28 ug/L in the United Kingdom to 56.1 ug/L in Malawi and sTfR values ranged from 5.9 mg/L in the United States to 7.0 mg/L in Malawi. In ADL, 14 datasets had values for SF while nine datasets had values for sTfR. Among ADL, the median serum ferritin values ranged from 28 ug/L in the United Kingdom to 103.9 ug/L in Georgia and sTfR values ranged from 3.8 mg/L in Laos and 7.8 mg/Lin Liberia.

Rank correlation coefficients for each micronutrient by AGP and/or CRP are available for review in **Table 3**. All survey specific rank correlation coefficients between inflammation biomarkers (AGP, CRP) and iron biomarkers (SF, sTfR) in SAC were statistically significant with coefficients ranged from 0.09 to 0.48. For SF, positive correlations with AGP ranged from 0.24 in Bangladesh to 0.48 in Malawi and with CRP ranged from 0.09 in Colombia to 0.48 in Malawi in SAC datasets. For sTfR, a positive correlation with AGP was 0.21 in Malawi, the only dataset available with sTfR and AGP, and with CRP coefficients ranged from 0.13 in Mexico (2006) to 0.24 in the United Kingdom. In ADL results varied and were less consistent in the degree of significance. For SF, positive correlations were present with AGP in five of six ADL datasets with coefficients ranged from 0.06 in Laos to 0.31 in Malawi and with CRP in 11 of 14 ADL datasets

with coefficients ranging from 0.05 in Vietnam to 0.43 in Mexico. For sTfR, positive correlations were present with AGP in five of six ALD datasets with coefficients ranging from 0.11 in Azerbaijan to 0.24 in Liberia and with CRP in three of nine ADL datasets with coefficients ranging from 0.02 in Azerbaijan to 0.2 in Malawi. Associations between sTfR and CRP were null in more than half, six out of none, of the ADL datasets.

Decile plots of geometric mean of iron biomarker by inflammation biomarker are available in **Figure 1**. In both SAC and ADL, the geometric means of iron biomarkers increased with increasing CRP and AGP deciles; larger increases in iron deficiency are seen beyond the 7th decile of CRP and AGP in SAC, and beyond the 5th for ADL however this trend is a less clear.

Figures 2 and 3 illustrate the estimated prevalence of iron deficiency using different BRINDA adjustment approaches. Comparison of inflammation-adjustment methods for iron deficiency were limited to two SAC and six ADL datasets for SF and one SAC and six ADL datasets for sTfR. Using SF, all inflammation adjustments increased the prevalence of iron deficiency (0.2 percentage points (pp) to 6.2 pp increase) compared to unadjusted models. In SAC, ferritin adjustment for both AGP and CRP increased the estimated prevalence of iron deficiency by a median of 1.18 pp (range 0.4 – 2 pp) compared to 1.3 pp for AGP alone (range 0.7 – 1.9 pp) and 0.3 pp for CRP alone (range 0.2 – 0.4 pp). In ADL adjustment for both AGP and CRP increased by median of 7.3 pp (range 0.6 – 14 pp) compared to 5 pp (range 2.7 – 9.7 pp) for AGP alone and 7.1 pp for CRP alone (range 0.5 – 10 pp). When combining both population groups, adjustment for AGP only had the greatest increase, 4.5 pp, compared to 3.7 for AGP and CRP and 3.4 for CRP alone. Using sTfR to estimate the prevalence of iron deficiency, results decreased the prevalence of iron deficiency in all but one country, Azerbaijan, and ranged from a 14.7 pp decrease to a 3.5 pp increase compared to unadjusted models. When adjusting sTfR in SAC, Malawi was the only

dataset available to compare adjustment methods. For Malawi all adjustment methods decreased the prevalence of iron deficiency, in which AGP and CRP and AGP only had the greatest decrease of 7.7 pp each compared to a 4 pp decrease for CRP only. In ADL, sTfR adjustment for AGP only decreased by median of 6.2 pp (range -14.7 – -0.1 pp) compared to 4.2 pp for both AGP and CRP (range -14 – 1.7 pp) and 2.8 pp for CRP alone (range -10.7 – 3.5 pp). When combining both population groups, adjustment for AGP only had the greatest decrease, 6.4 pp, compared to 5.9 for AGP and CRP and 3 pp for CRP alone.

Sensitivity Analysis

For each country, a sensitivity analysis stratified results by females only and SAC females by age: < 12 years old and ≥ 12 years old. Results from these analyses highlight the role age and sex impacted inflammation adjustment, however there wasno significant impact on the interpretation of the original analysis (Supplementary Material).

Table OSM1 illustrates count, median, IQR for age, AGP, CRP and the percent of elevated AGP and CRP by dataset in SAC females by age group. The female SAC by age group analysis included 8 datasets with 9,939 surveyed < 12 years old and 4,447 surveyed ≥ 12 years old. For female SAC, the median AGP values ranged from 0.7 g/L to 0.8 g/L in < 12 years old compared to 0.6 g/L to 0.7 g/L in ≥ 12 years old. The prevalence of elevated AGP ranged from 17.7% to 35.7% in < 12 years old compared 12.7% to 15.9% in ≥ 12 years old. For female SAC, the median CRP values ranged from 0.2 g/L to 1.9 g/L in < 12 years old and ≥ 12 years old. The prevalence of elevated AGP ranged from 4.9% to 14.8% in < 12 years old compared 1.5% to 11.5% in ≥ 12 years old.

Table OSM2 presents micronutrient status data by median and IQR for SAC females by age group. In female SAC, all eight datasets had values for SF while four datasets had values for

sTfR. Among female SAC < 12 years old, the median SF values ranged from 28 µg/L in the United Kingdom to 57.6 µg/L in Malawi and sTfR values ranged from 5.3 mg/L in the United Kingdom to 6.9 mg/L in Malawi. Among female SAC ≥ 12 years old, the median SF values ranged from 26 µg/L in the United Kingdom to 46.1 µg/L in Bangladesh, and sTfR values ranged from 4.3 mg/L in the United Kingdom to 6.6 mg/L in Malawi.

Rank correlation coefficients for each micronutrient by AGP and/or CRP in SAC females by age group are available for review in **Table OSM3**. Differing from main analysis results, when SAC females were stratified by age, 21 out of 30 survey specific rank correlation coefficients between inflammation biomarkers (AGP, CRP) and iron biomarkers (SF, sTfR) were statistically significant with a positive association. For SAC females < 12 years old, nine out of 10 of the datasets had a positive correlation between an inflammation biomarker and SF (ranged 0.09 – .53), and three out of five for inflammation biomarkers and sTfR (range 0.1 – 0.2). For SAC females ≥ 12 years old, six out of 10 of the datasets had a positive correlation with inflammation biomarkers and SF (ranged 0.2 – 0.55), and three out of five for inflammation biomarkers and sTfR (range 0.14 – 0.33).

Decile plots of geometric means for iron biomarkers by inflammation biomarkers among females only are available in **Figure OSM1**. In both female only SAC and female only ADL datasets, the estimated prevalence of iron deficiency increased with increasing CRP and AGP deciles (**Figure OSM1**).

Figures OSM2 and OSM3 illustrate the estimated prevalence of iron deficiency using different BRINDA adjustment approaches. Similar to the main analysis, for females only in SAC and ADL using SF to estimate the prevalence of iron deficiency, all inflammation adjustments increased the prevalence (0.4 pp to 6.3 pp) compared to unadjusted models. Differing from main

analysis results, using sTfR to estimate the prevalence of iron deficiency in females only had consistent effects as all adjustment methods decreased the prevalence of iron deficiency, coefficients ranged from a 6.4 pp to a 2.5 decrease compared to unadjusted models.

Figures OSM4, OSM5, and OSM6 display decile plots of geometric means of iron biomarkers by inflammation biomarkers for females only by country. Like the main analysis, geometric means of the iron biomarkers for females only in SAC and ADL datasets increased with increasing CRP and AGP deciles; similar results were seen by country as well.

Figures OSM7 and OSM8 illustrate the estimated prevalence of iron deficiency using different BRINDA adjustment approaches. When estimating the prevalence of iron deficiency based on SF for females only in SAC by age group, all inflammation adjustments increased the prevalence compared to unadjusted models. Differing from main analysis results, using sTfR to estimate the prevalence of iron deficiency in SAC by age group had consistent effects as all adjustment methods decreased the prevalence of iron deficiency compared to unadjusted models.

DISCUSSION

Using geographically diverse data from ~ 26,000 SAC and ~20,000 ADL, we found statistically significant positive associations between inflammation (AGP and CRP) and indicators of iron status (SF and sTfR) were consistent, except for sTfR and CRP in ADL, indicating the need for further investigation of sTfR as an inflammation-adjusted iron status biomarker. Associations with CRP in ADL were mixed, indicating that additional research is needed to determine the optimal approach for adjusting sTfR for inflammation in these populations.

Our analysis found significant correlations between inflammation and iron biomarkers as well as rational for adjustment, with adjustments for SF and sTfR resulting in different effects. In contrast to previous studies on PSC and WRA, which showed an increase of 11 pp when adjusting

for SF, our analysis increased estimates by 0.2 pp to 6.2 pp compared to unadjusted models in all SAC and ADL datasets. Several population group factors could potentially account for the smaller increase in estimates observed in our analysis compared to previous studies on WRA and PSC, the most notable one is that WRA and PSC had a significantly higher prevalence of iron deficiency and inflammation. While adjusting sTfR for inflammation resulted in a decrease in all but one survey. Consistent with prior studies, adjustment of inflammation resulted in a reduced prevalence of iron deficiency based on sTfR levels. For example, one study observed a decrease in iron deficiency prevalence by a median of 15 pp, while another reported reduction of 4.4–14.6 and 0.3–9.5 pp in PSC and WRA, respectively [10], [19]. Our results fall within the range of these previous findings, demonstrating that adjusting for inflammation can lead to a reduction in the estimated prevalence of iron deficiency based on sTfR levels. However, comparing the adjustment method using both CRP and AGP had limited data available as only a few datasets had measurements for both biomarkers.

Iron deficiency and inflammation are commonly observed together in many populations, creating a vicious cycle in which inflammation exacerbates iron deficiency and iron deficiency increases inflammation. This is especially concerning in vulnerable populations where malnutrition can lead to iron deficiency, which increases the likelihood and severity of inflammation[21], [52]. On the other hand, inflammation impairs iron metabolism by reducing intestinal absorption, increasing iron sequestration within cells, and decreasing iron utilization by tissues. This disruption of iron homeostasis can lead to deficiencies, which in turn compromises children's growth and development[24]. Thus, there is rationale for adjusting for inflammation, as accurate prevalence estimates are needed for effective program planning and policies[52].

This study extends the previous work conducted by the BRINDA project, which focused on the assessment of inflammation's impact on iron biomarkers in various populations. Unlike previous studies, this multi-country analysis examines the necessity of adjusting iron biomarkers for inflammation in SAC and ADL, a population group not previously explored in the literature[7], [10]. Moreover, the study utilized a diverse set of data from various income countries. This enhanced the generalizability of the results to a broader population of SAC and ADL than a single-country analysis. This analysis highlights the importance of adjusting for inflammation when assessing iron status in SAC and ADL populations. Failure to do so may result in incorrect estimates of iron deficiency prevalence. These findings have significant implications for the design and interpretation of studies aimed at assessing iron status in these populations. In addition, they have implications for public health interventions aimed at addressing iron deficiency. Moreover, this study provides significant information that can be used to inform the development and implementation of micronutrient programs targeting SAC and ADL populations. However, it is critical to note that additional research is needed to fully understand the impact of inflammation on iron biomarkers in this population.

The study has several limitations that should be considered when interpreting the findings. The conclusions from this analysis were based on eight SAC and 15 ADL datasets, which may not represent all SAC and ADL populations. Data availability was limited to only three datasets for AGP among SAC. All surveys were cross-sectional studies thus this analysis is unable to establish causality between inflammation and iron deficiency. Future research should consider using longitudinal data to investigate the relationship between in these variables over time, especially in populations of growth like SAC and ADL. Additionally, this analysis did not examine the effects of other potential confounders such as dietary intake, infection (e.g. malaria), or coverage of iron

supplementation programs. Additional datasets would allow for a more robust analysis. Finally, laboratory methods differed across the 23 surveys included in this analysis which could lead systematic difference in results.

CONCLUSION

In summary, associations between SF or sTfR and CRP or AGP are consistently positive in SAC and ADL. Failing to adjust SF levels for inflammation may lead to an underestimation of iron deficiency in SAC and ADL. Though the available data for sTfR is limited, preliminary findings indicate that adjusting for inflammation using AGP may be necessary. Nevertheless, given the significant gaps in data for these population groups, further research is necessary to confirm these findings. Our study suggests that it is imperative to consider measuring inflammation biomarkers when assessing iron status. Failing to account for inflammation could lead to incorrect iron deficiency estimation. These errors in assessment could result in inadequate resource distribution and imprecise targeting of micronutrient programs.

TABLES

Table 1: Age and inflammation status in School-age Children and Adolescents

	Age		AGP, g/L			CRP, mg/L		
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP %, (95% CI)**	**n**	**Median (IQR)**	**Elevated CRP %, (95% CI)**
School-age Children								
Bangladesh(2012)	1275	9(7, 11)	1274	0.7(0.6, 0.9)	15.4(12.3, 18.8)	1274	0.3(0.3, 0.7)	4.2(2.3, 6.9)
Colombia(2010)	8604	10(8, 12)	–	–	–	8604	0.2(0.2, 0.6)	13.3(12.2, 14.4)
Ecuador(2012)	6062	10(8, 13)	–	–	–	6062	1.9(1.9, 2.2)	7.2(5.8, 8.8)
Malawi(2016)	758	9(7, 12)	758	0.8(0.6, 1.1)	31.8(27.2, 36.5)	758	0.8(0.3, 2.2)	15.9(12.2, 20.2)
Mexico(2006)	3660	9(6, 12)	–	–	–	3660	0.5(0.2, 1.6)	9.4(7.8, 11.1)
Mexico(2012)	4328	9(7, 10)	–	–	–	4328	0.5(0.2, 1.5)	8.5(7.3, 9.9)
United Kingdom(2014)	590	9(7, 12)	–	–	–	590	1.4(0.8, 2.0)	4.9(2.8, 7.8)
United States(2006)	1057	13(6, 14)	–	–	–	1057	0.3(0.1, 1.0)	7.2(5.1, 9.7)
Adolescents								
Azerbaijan(2013)	363	17(16, 19)	363	0.8(0.7, 0.9)	20.5(16.0, 25.6)	363	0.4(0.2, 1.1)	5.1(2.9, 8.1)
Bangladesh(2012)	798	12(11, 14)	797	0.7(0.6, 0.8)	10.9(8.0, 14.3)	797	0.3(0.3, 0.7)	2.2(1.1, 3.7)
Colombia(2010)	7015	14(12, 16)	–	–	–	7015	0.2(0.2, 0.6)	12.6(11.4, 13.8)
Cote D'Ivoire(2007)	110	17(16, 18)	110	0.8(0.7, 1.0)	26.5(19.6, 34.1)	110	1.7(0.5, 4.9)	23.4(15.9, 32.2)
Ecuador(2012)	4152	14(12, 15)	–	–	–	4152	1.9(1.9, 2.3)	7.7(6.5, 9.0)
Georgia(2009)	178	17(16, 18)	–	–	–	178	0.5(0.5, 2.1)	12.0(6.9, 18.8)
Laos(2006)	170	16(15, 18)	170	0.8(0.6, 0.8)	10.6(6.8, 15.4)	170	0.1(0.0, 0.6)	1.4(0.4, 3.4)
Liberia(2011)	378	18(17, 19)	378	0.8(0.6, 0.9)	11.9(8.4, 16.2)	378	0.7(0.4, 2.2)	12.9(9.4, 17.2)
Malawi(2016)	514	14(12, 16)	514	0.7(0.5, 0.9)	17.4(13.8, 21.5)	514	0.6(0.2, 1.9)	10.4(7.5, 13.9)
Mexico(2006)	1897	14(12, 17)	–	–	–	1897	0.6(0.2, 1.6)	8.2(6.5, 10.2)
Mexico(2012)	1269	11(10, 12)	–	–	–	1269	0.5(0.2, 1.7)	9.6(6.9, 12.9)
Papua New Guinea(2005)	133	17(16, 18)	133	0.9(0.7, 1.1)	33.0(24.0, 42.9)	133	0.6(0.1, 1.5)	8.8(3.6, 17.1)
United Kingdom(2014)	545	14(12, 16)	–	–	–	545	1.5(1.0, 2.1)	6.1(3.7, 9.3)
United States(2006)	1881	16(14, 18)	–	–	–	1881	0.5(0.1, 1.6)	10.5(8.8, 12.3)
Vietnam(2010)	192	17(16, 18)	–	–	–	192	0.3(0.2, 0.7)	4.7(2.3, 8.3)

*AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range; Elevated AGP defined as AGP > 1 g/L; Elevated CRP defined as CRP > 5 mg/L

Table 2: Serum ferritin and sTfR in School-age Children and Adolescents

	Serum ferritin, ug/L		sTfR, mg/L	
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**
School-age Children				
Bangladesh(2012)	1275	51.9(37.3, 73.2)	_	_
Colombia(2010)	8604	33.5(23.1, 48.3)	_	_
Ecuador(2012)	6062	43.0(30.0, 60.0)	_	_
Malawi(2016)	758	56.1(38.4, 83.7)	758	7.0(6.1, 8.9)
Mexico(2006)	3650	31.6(19.0, 48.5)	3635	6.6(5.6, 7.9)
Mexico(2012)	4328	30.6(21.7, 43.0)	_	_
United Kingdom(2014)	586	28.0(20.0, 39.0)	-	-
United States(2006)	1057	33.0(22.0, 47.0)	1039	5.9(5.0, 6.9)
Adolescents				
Azerbaijan(2013)	363	29.1(13.6, 49.0)	363	5.6(4.6, 7.1)
Bangladesh(2012)	798	47.1(34.2, 67.8)	-	-
Colombia(2010)	7015	30.8(19.6, 47.0)	-	-
Cote D'Ivoire(2007)	110	52.2(29.6, 79.6)	110	7.5(6.3, 8.7)
Ecuador(2012)	4152	41.0(27.0, 58.0)	-	-
Georgia(2009)	178	103.9(76.8, 172.8)	-	-
Laos(2006)	170	29.2(12.5, 63.4)	170	3.8(3.0, 5.2)
Liberia(2011)	378	25.7(14.3, 42.8)	378	7.8(6.2, 9.5)
Malawi(2016)	514	50.7(37.3, 75.4)	514	6.9(5.9, 8.5)
Mexico(2006)	1890	31.3(17.9, 48.0)	1889	6.0(4.8, 7.5)
Mexico(2012)	1269	32.6(21.4, 45.8)	-	-
Papua New Guinea(2005)	-	-	133	5.2(4.0, 8.7)
United Kingdom(2014)	542	28.0(18.0, 41.0)	430	4.7(3.9, 5.8)
United States(2006)	1880	33.0(19.0, 49.0)	1860	5.4(4.8, 6.4)
Vietnam(2010)	192	44.4(25.2, 71.4)	-	-

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range

Table 3: Rank correlation coefficients between Serum ferritin, sTfR, AGP, and CRP concentrations in School-age Children and Adolescents

	Serum ferritin				sTfR			
	AGP*Serum ferritin		CRP*Serum ferritin		AGP*sTfR		CRP*sTfR	
Dataset(year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
School-age Children								
Bangladesh(2012)	1274	0.24*	1274	0.27*	-	-	-	-
Colombia(2010)	-	-	8604	0.09*	-	-	-	-
Ecuador(2012)	-	-	6062	0.27*	-	-	-	-
Malawi(2016)	758	0.48*	758	0.48*	758	0.21*	758	0.14*
Mexico(2006)	-	-	3650	0.29*	-	-	3645	0.13*
Mexico(2012)	-	-	4328	0.36*	-	-	-	-
United Kingdom(2014)	-	-	586	0.13*	-	-	385	0.24*
United States(2006)	-	-	1057	0.25*	-	-	1039	0.17*
Adolescents								
Azerbaijan(2013)	363	0.21*	363	0.16*	363	0.11	363	0.02
Bangladesh(2012)	797	0.15*	797	0.22*	-	-	-	-
Colombia(2010)	-	-	7015	0.08*	-	-	-	-
Cote D'Ivoire(2007)	110	0.24*	110	0.26*	110	0.20*	110	0.11
Ecuador(2012)	-	-	4152	0.20*	-	-	-	-
Georgia(2009)	-	-	178	0.10	-	-	-	-
Laos(2006)	170	0.06	170	0.14	170	0.22*	170	0.04
Liberia(2011)	378	0.24*	378	0.22*	378	0.24*	378	0.12
Malawi(2016)	514	0.31*	514	0.30*	514	0.22*	514	0.20*
Mexico(2006)	-	-	1890	0.18*	-	-	1889	0.11*
Mexico(2012)	-	-	1269	0.43*	-	-	-	-
Papua New Guinea(2005)	-	-	-	-	133	0.21*	133	0.12
United Kingdom(2014)	-	-	542	0.16*	-	-	430	0.08
United States(2006)	-	-	1880	0.15*	-	-	1860	0.12*
Vietnam(2010)	-	-	192	0.05	-	-	-	-

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Figure 1: Geometric means of serum ferritin by (A) AGP deciles and (B) CRP deciles in School-age Children and Adolescents; Geometric means of sTfR by (C) AGP deciles and (D) CRP deciles in School-age Children and Adolescents

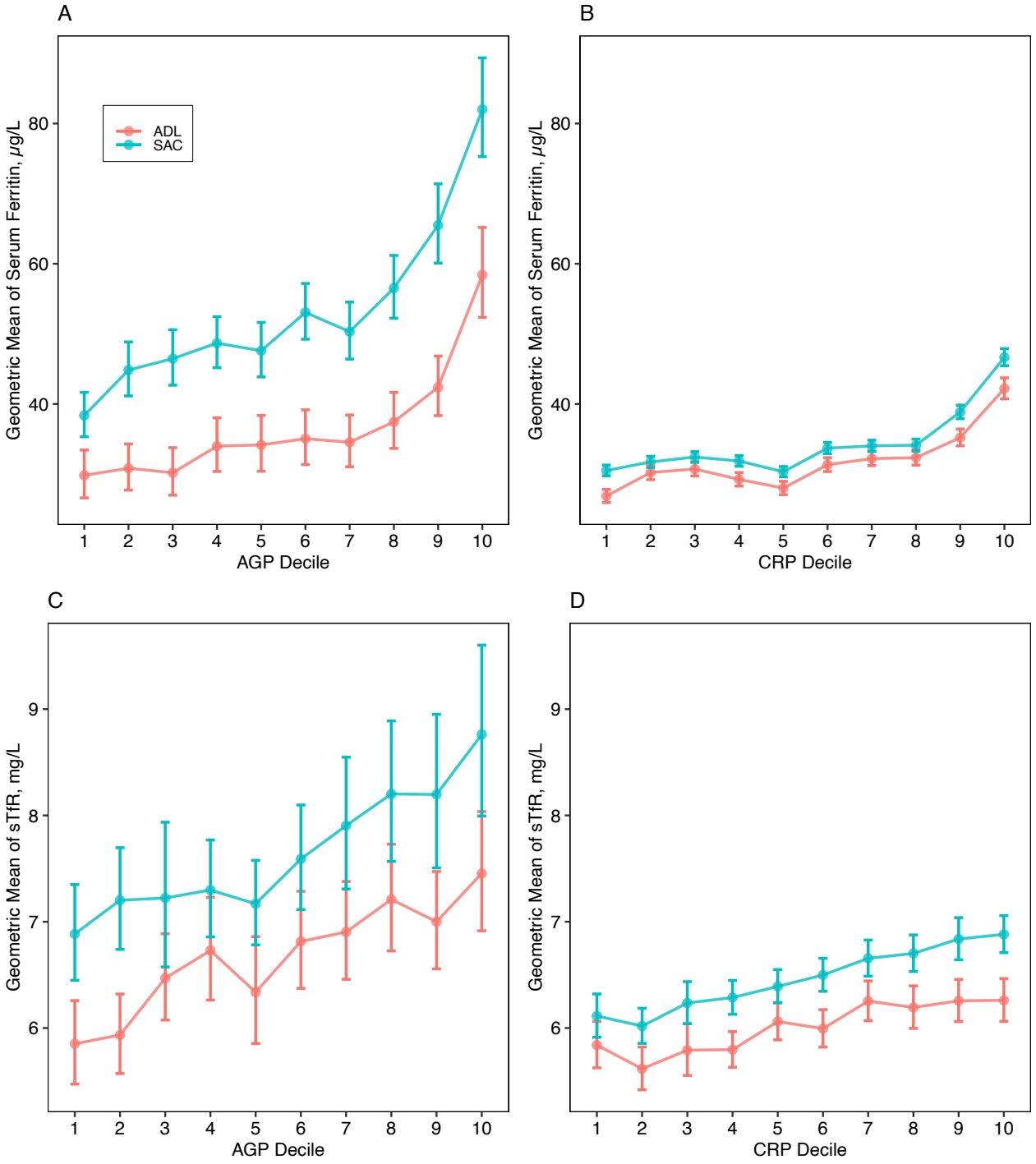


Figure 2: Estimated prevalence of Iron deficiency using serum ferritin < 15ug/L in (A) School-age Children and (B) Adolescents with the use of different BRINDA adjustment approaches

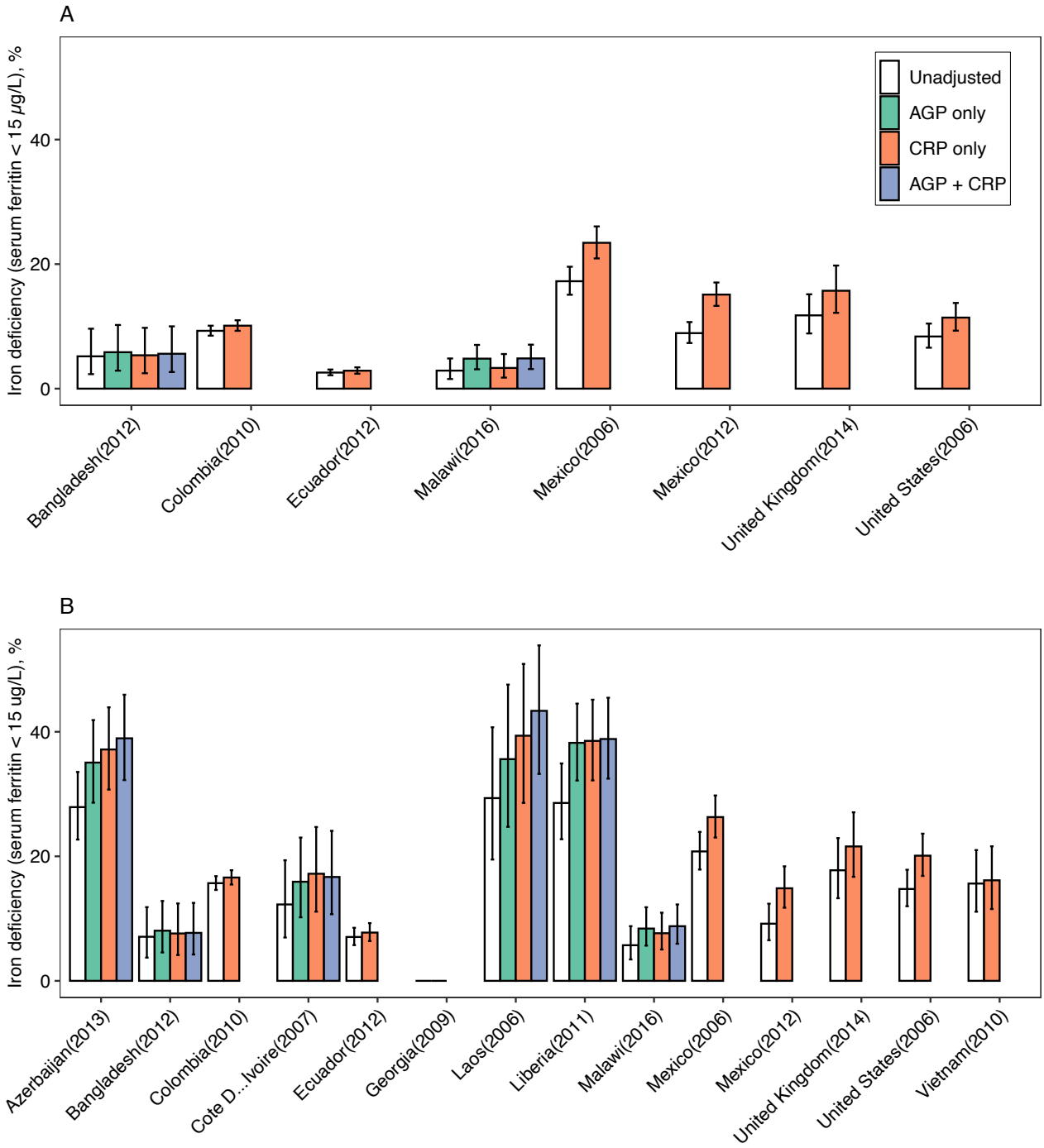
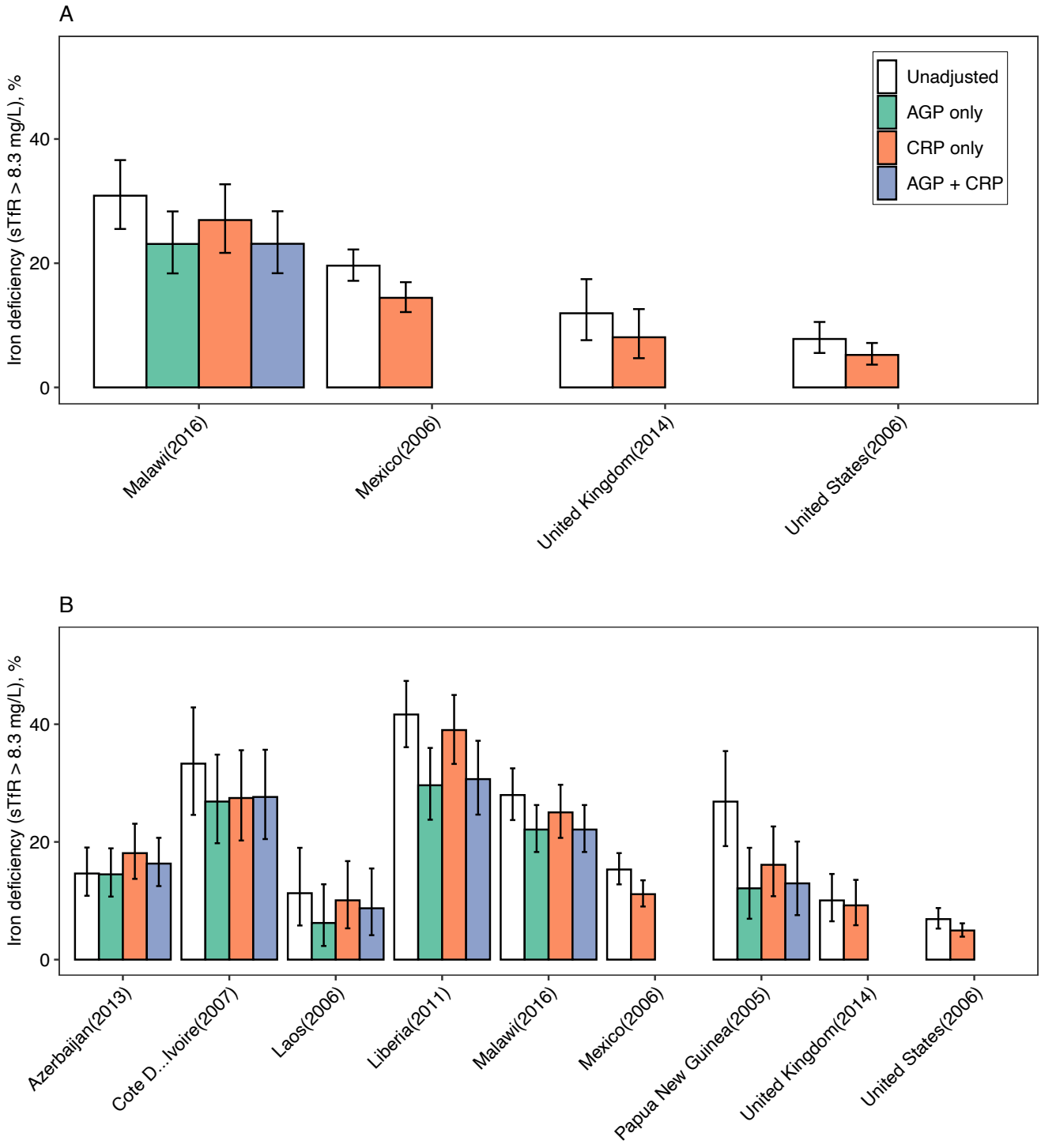


Figure 3: Estimated prevalence of Iron deficiency using sTfR >8.3 mg/L in (A) School-age Children and (B) Adolescents with the use of different BRINDA adjustment approaches



SUPPLEMENTARY MATERIAL

Table OSM1: Age and inflammation status in School-age Children by age group

	Age		AGP, g/L			CRP, mg/L		
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**	**Elevated AGP %, (95% CI)**	**n**	**Median (IQR)**	**Elevated CRP %, (95% CI)**
Age < 12								
Bangladesh(2012)	492	8(7, 10)	492	0.7(0.6, 0.9)	17.7(12.4, 23.9)	492	0.3(0.3, 0.9)	6.2(3.5, 9.9)
Colombia(2010)	3051	9(7, 10)	–	–	–	3051	0.2(0.2, 0.7)	13.3(11.7, 15.0)
Ecuador(2012)	2156	9(7, 10)	–	–	–	2156	1.9(1.9, 2.4)	8.8(6.1, 12.0)
United Kingdom(2014)	166	8(6, 9)	–	–	–	166	1.4(1.1, 2.1)	4.9(1.6, 10.8)
Malawi(2016)	282	8(6, 10)	282	0.8(0.6, 1.2)	35.7(29.2, 42.7)	282	0.8(0.2, 2.1)	14.8(10.4, 20.1)
Mexico(2006)	1538	7(6, 10)	–	–	–	1538	0.6(0.2, 1.7)	9.8(7.8, 12.1)
Mexico(2012)	2098	9(7, 10)	–	–	–	2098	0.6(0.3, 1.7)	8.5(6.8, 10.4)
United States(2006)	156	6(5, 6)	–	–	–	156	0.3(0.1, 1.1)	8.4(3.7, 15.7)
Age > 12								
Bangladesh(2012)	162	13(12, 13)	161	0.7(0.5, 0.8)	15.9(8.6, 25.7)	161	0.3(0.3, 0.4)	1.5(0.3, 4.4)
Colombia(2010)	1924	14(13, 14)	–	–	–	1924	0.2(0.2, 0.2)	11.3(9.2, 13.8)
Ecuador(2012)	840	14(13, 14)	–	–	–	840	1.9(1.9, 2.0)	4.7(3.0, 6.9)
United Kingdom(2014)	112	13(12, 14)	–	–	–	112	1.4(0.8, 2.0)	4.0(1.1, 9.5)
Malawi(2016)	90	14(13, 14)	90	0.6(0.5, 0.9)	12.7(5.3, 23.9)	90	0.5(0.2, 1.4)	11.5(4.0, 23.9)
Mexico(2006)	577	13(13, 14)	–	–	–	577	0.5(0.2, 1.4)	7.8(5.1, 11.3)
Mexico(2012)	9	12(12, 12)	–	–	–	9	0.9(0.6, 1.3)	2.2(NA, 14.4)
United States(2006)	733	14(13, 14)	–	–	–	733	0.3(0.1, 0.9)	6.9(4.6, 9.6)

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range; Elevated AGP defined as AGP >1 g/L; Elevated CRP defined as CRP >5 mg/L.

Table OSM2: Serum ferritin and sTfR in School-age Children by age group

	Serum ferritin, ug/L		sTfR, mg/L	
Dataset(year)	**n**	**Median (IQR)**	**n**	**Median (IQR)**
Age < 12				
Bangladesh(2012)	492	54.4(39.8, 75.3)	-	-
Colombia(2010)	3051	35.0(24.6, 49.8)	-	-
Ecuador(2012)	2156	44.0(32.0, 62.0)	-	-
Malawi(2016)	282	57.6(37.8, 85.0)	282	6.9(6.2, 8.4)
Mexico(2006)	1536	31.1(19.8, 48.6)	1533	6.7(5.7, 7.9)
Mexico(2012)	2098	30.2(21.4, 43.4)	-	-
United Kingdom(2014)	164	28.0(19.0, 41.0)	101	5.3(4.4, 6.1)
United States(2006)	156	31.0(20.0, 43.0)	150	6.4(5.8, 7.0)
Age > 12				
Bangladesh(2012)	162	46.1(36.1, 67.1)	-	-
Colombia(2010)	1924	28.5(18.0, 43.8)	-	-
Ecuador(2012)	840	39.0(25.0, 55.0)	-	-
Malawi(2016)	90	45.2(36.4, 76.7)	90	6.6(6.0, 8.3)
Mexico(2006)	574	30.4(18.9, 47.6)	573	5.6(4.6, 6.8)
Mexico(2012)	9	36.8(13.4, 84.6)	-	-
United Kingdom(2014)	111	26.0(19.0, 37.0)	87	4.3(3.5, 5.4)
United States(2006)	733	34.0(22.0, 48.0)	727	5.6(4.8, 6.4)

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; IQR, Interquartile Range

Table OSM3: Rank correlation coefficients between Serum ferritin, sTfR, AGP, and CRP concentrations in Female School-age Children by age group

	AGP * ferritin		CRP * ferritin		AGP * sTfR		CRP * sTfR	
Dataset(year)	**n**	**r**	**n**	**r**	**n**	**r**	**n**	**r**
Age < 12								
Bangladesh(2012)	492	0.24*	492	0.30*	-	-	-	-
Colombia(2010)	-	-	3051	0.09*	-	-	-	-
Ecuador(2012)	-	-	2156	0.23*	-	-	-	-
Malawi(2016)	282	0.45*	282	0.53*	282	0.17*	282	0.16*
Mexico(2006)	-	-	1536	0.26*	-	-	1533	0.10*
Mexico(2012)	-	-	2098	0.32*	-	-	-	-
United Kingdom(2014)	-	-	164	0.08	-	-	101	0.20
United States(2006)	-	-	156	0.41*	-	-	150	0.13
Age > 12								
Bangladesh(2012)	161	0.12	161	0.34*	-	-	-	-
Colombia(2010)	-	-	1924	-0.01	-	-	-	-
Ecuador(2012)	-	-	840	0.32*	-	-	-	-
Malawi(2016)	90	0.45*	90	0.55*	90	0.28	90	0.20
Mexico(2006)	-	-	574	0.24*	-	-	573	0.14*
Mexico(2012)	-	-	9	0.54	-	-	-	-
United Kingdom(2014)	-	-	111	0.13	-	-	87	0.33*
United States(2006)	-	-	733	0.20*	-	-	727	0.19*

* AGP, Alpha(1)-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Figure OSM1: Geometric means of serum ferritin by (A) AGP deciles and (B) CRP deciles in female School-age Children and female Adolescents; Geometric means of sTfR by (C) AGP deciles and (D) CRP deciles in female School-age Children and female Adolescents

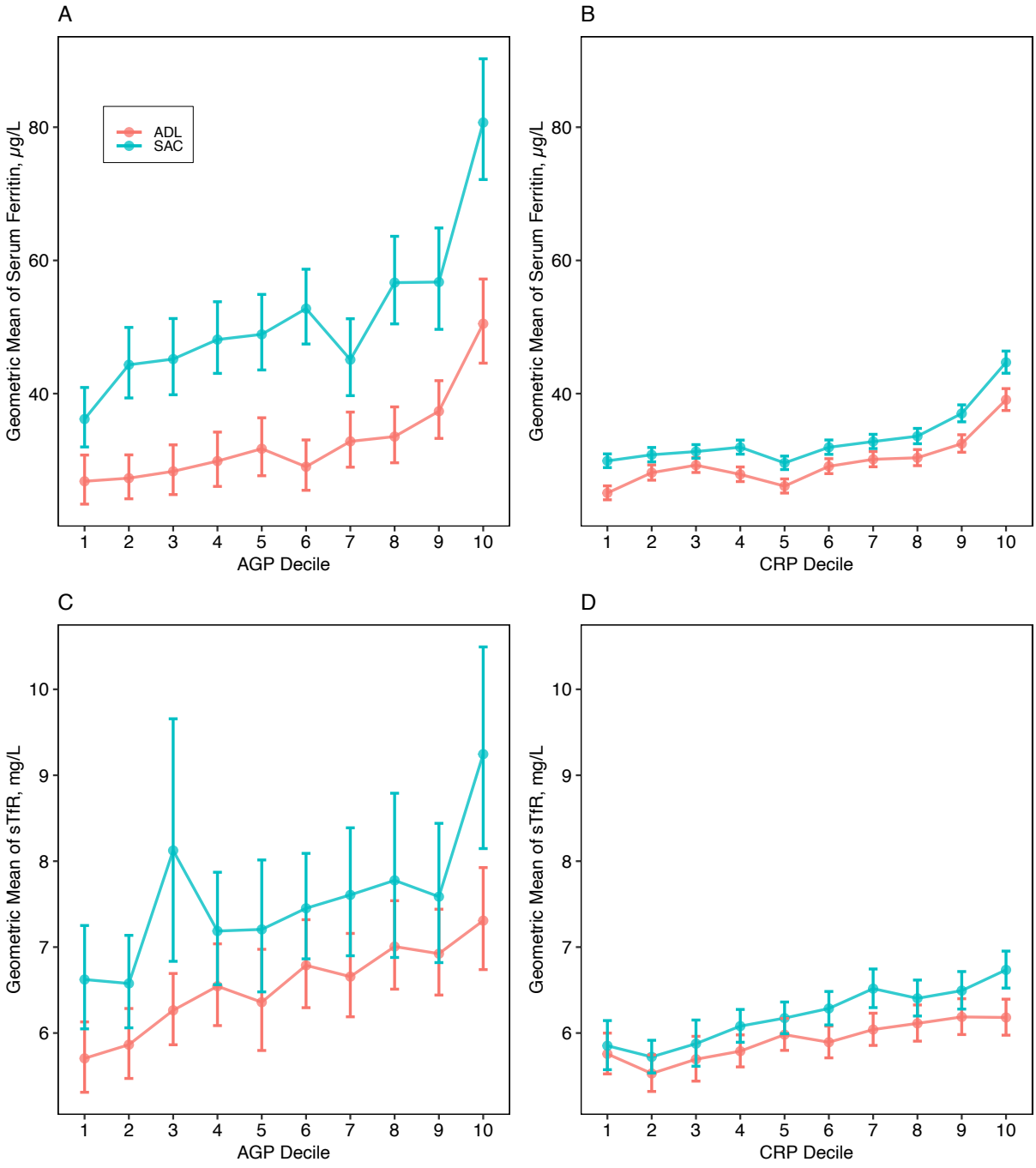


Figure OSM2: Estimated prevalence of Iron deficiency using serum ferritin < 15ug/L in (A) School-age Children and (B) Adolescents with the use of different BRINDA adjustment approaches

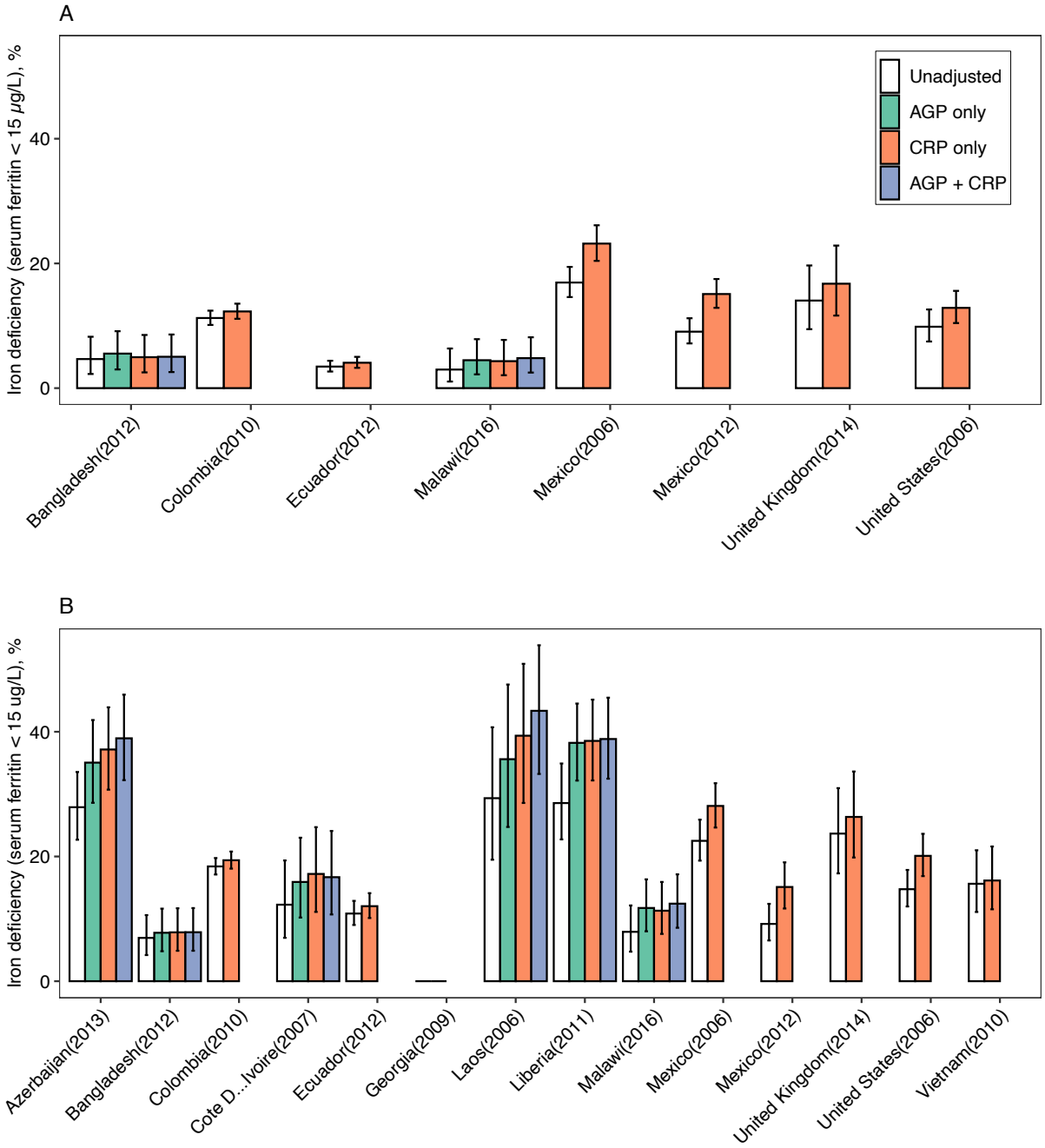


Figure OSM3: Estimated prevalence of Iron deficiency using sTfR >8.3 mg/L in (A) female School-age Children and (B) female Adolescents with the use of different BRINDA adjustment approaches

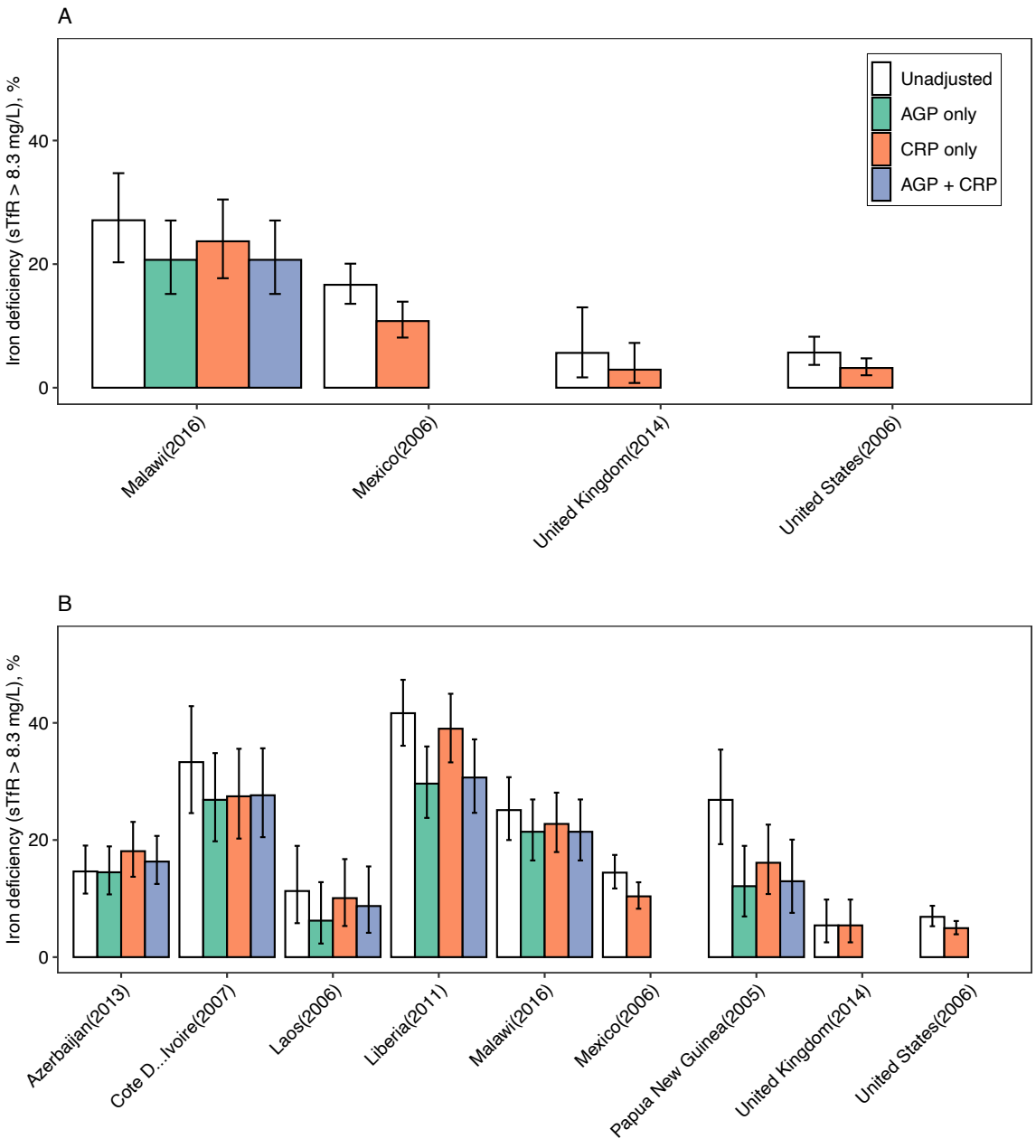


Figure OSM4: Geometric means of serum ferritin by (A) AGP deciles and (B) CRP deciles in School-age Children and Adolescents by country

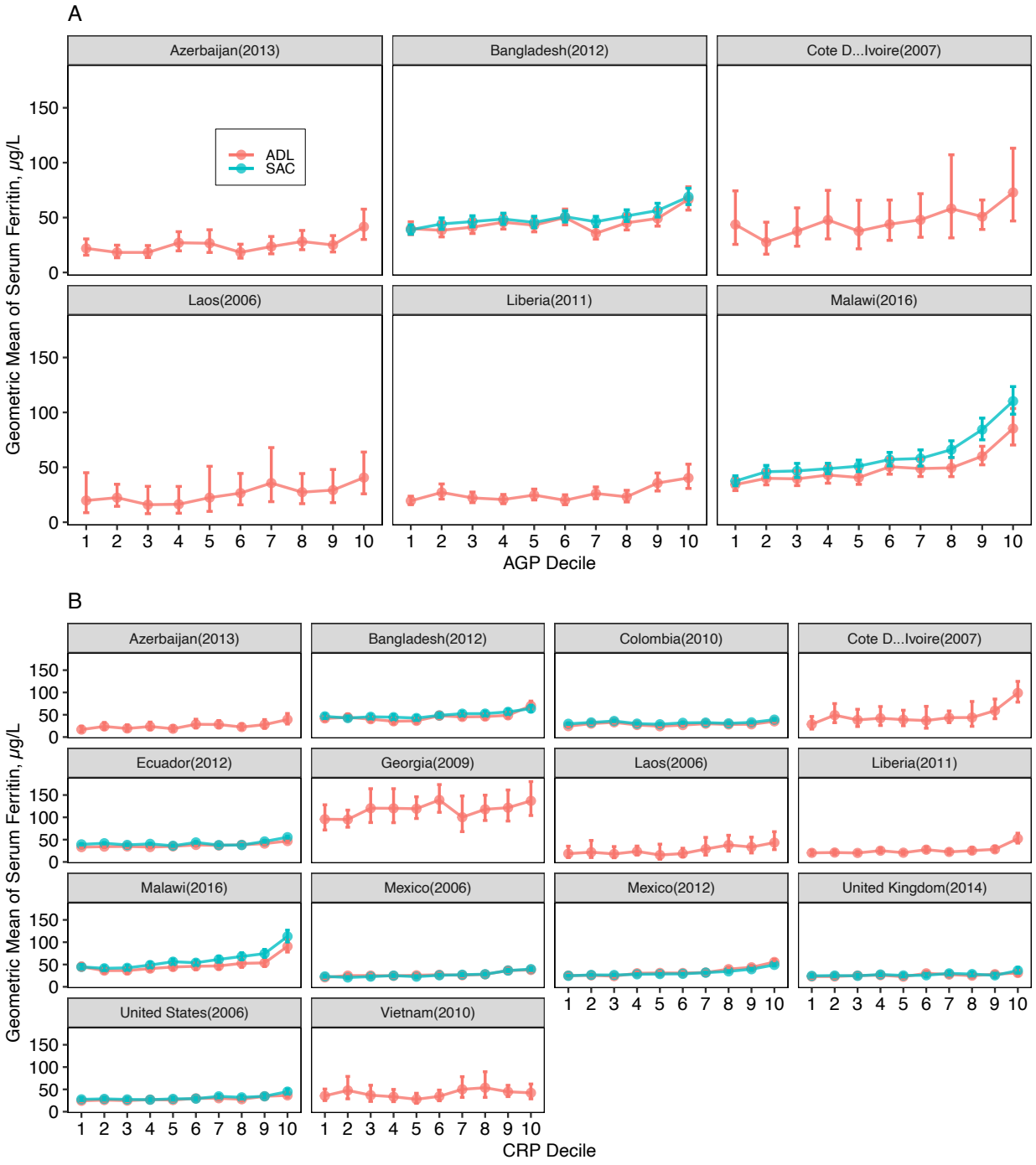


Figure OSM5: Geometric means of sTfR by (A) AGP deciles and (B) CRP deciles in School-age Children and Adolescents by country

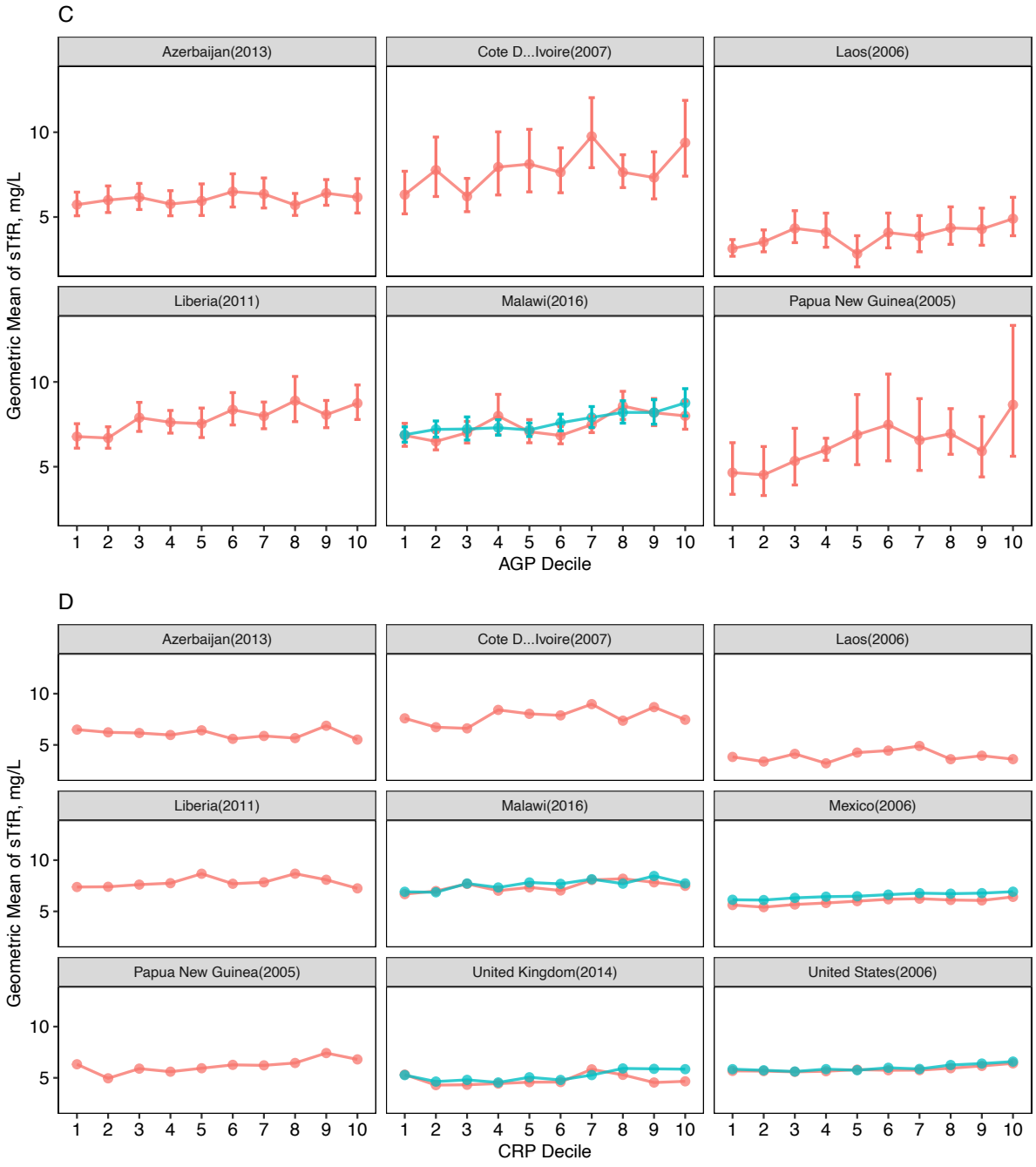


Figure OSM6: Geometric means of serum ferritin by (A) AGP deciles and (B) CRP deciles in female School-age Children; Geometric means of sTfR by (C) AGP deciles and (D) CRP deciles in female School-age Children by age group

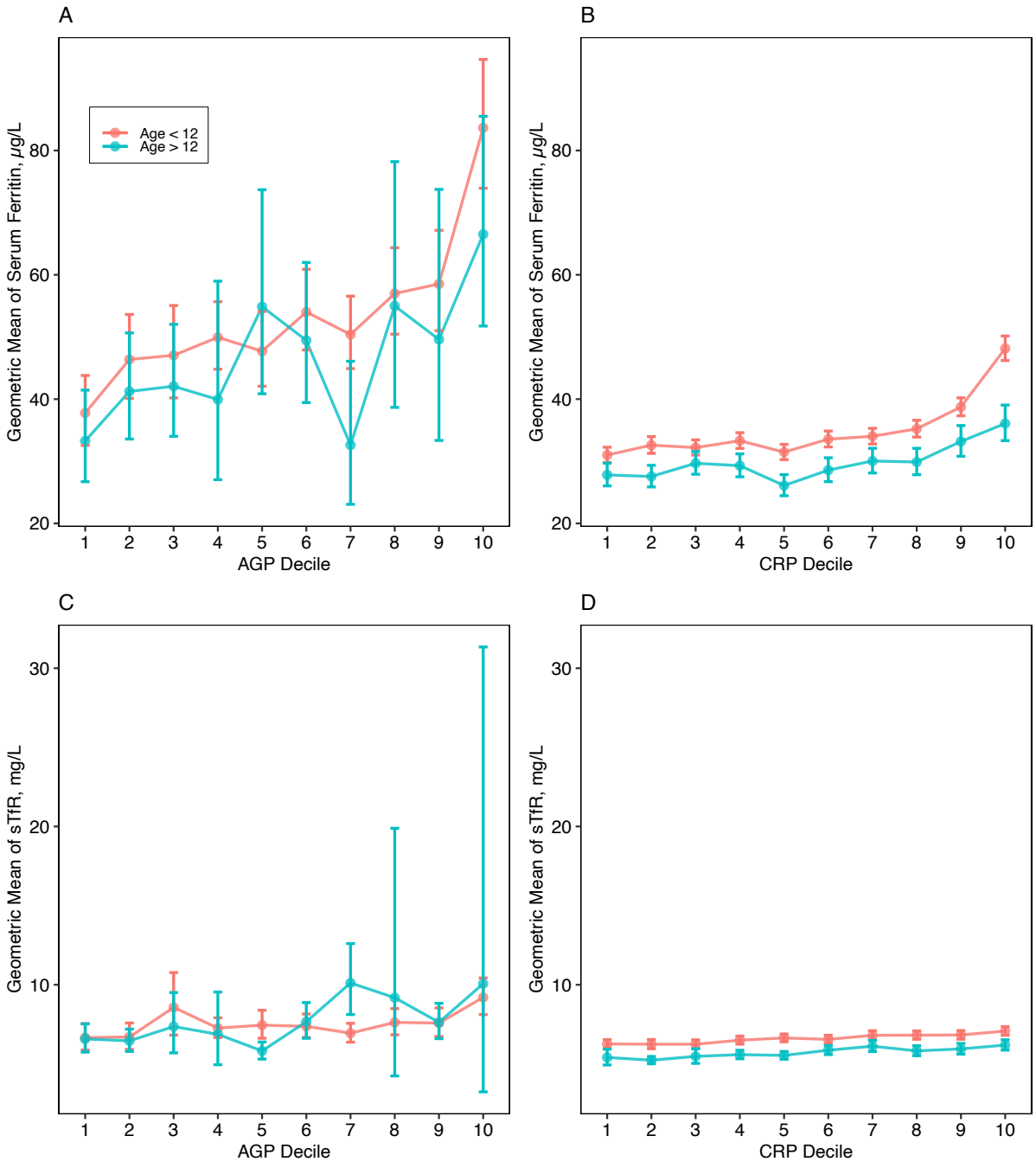


Figure OSM7: Estimated prevalence of Iron deficiency using sTfR >8.3 mg/L in School-age Children by age group (A) Age < 12 (B) Age >12 with the use of different BRINDA adjustment approaches

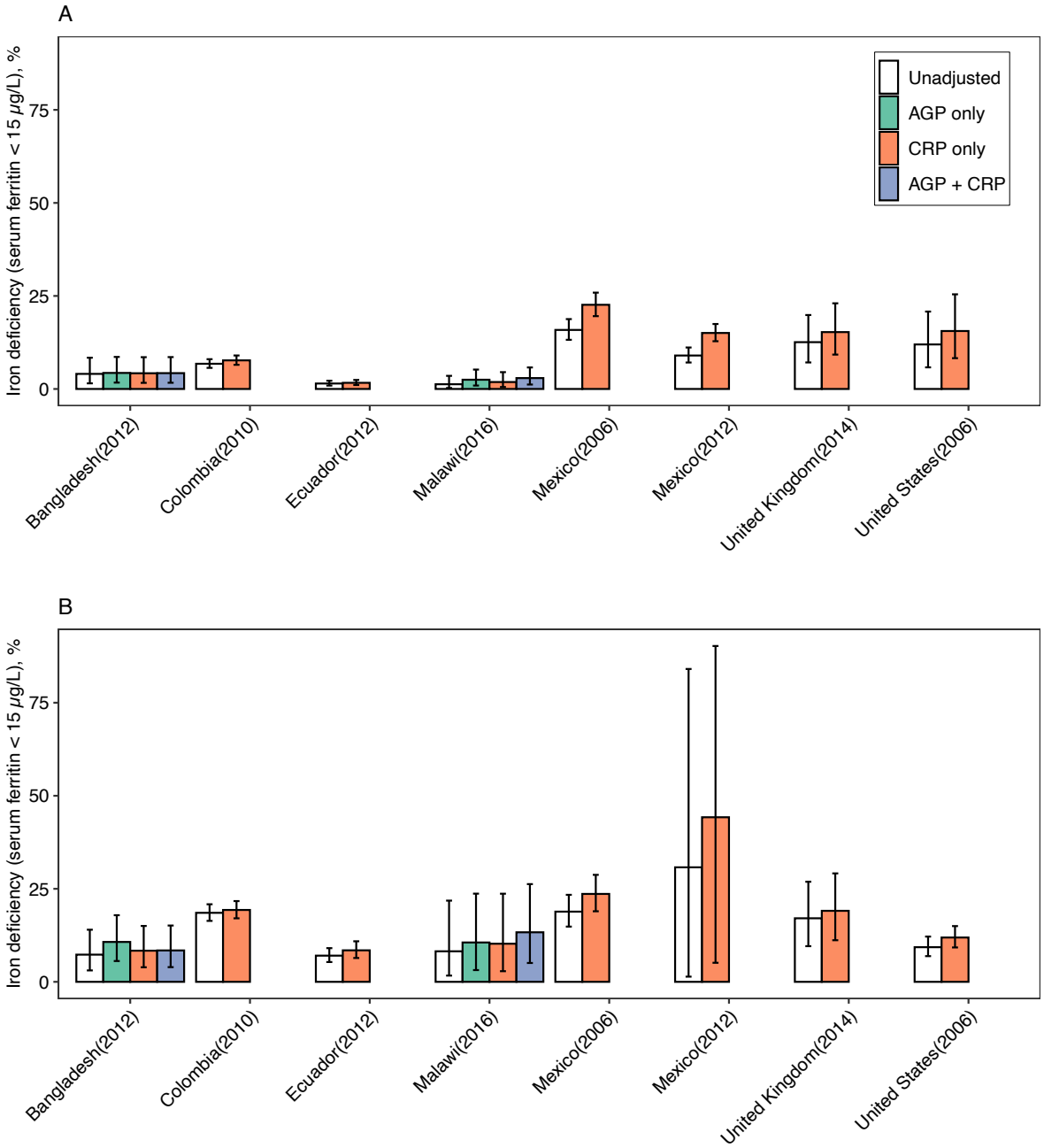
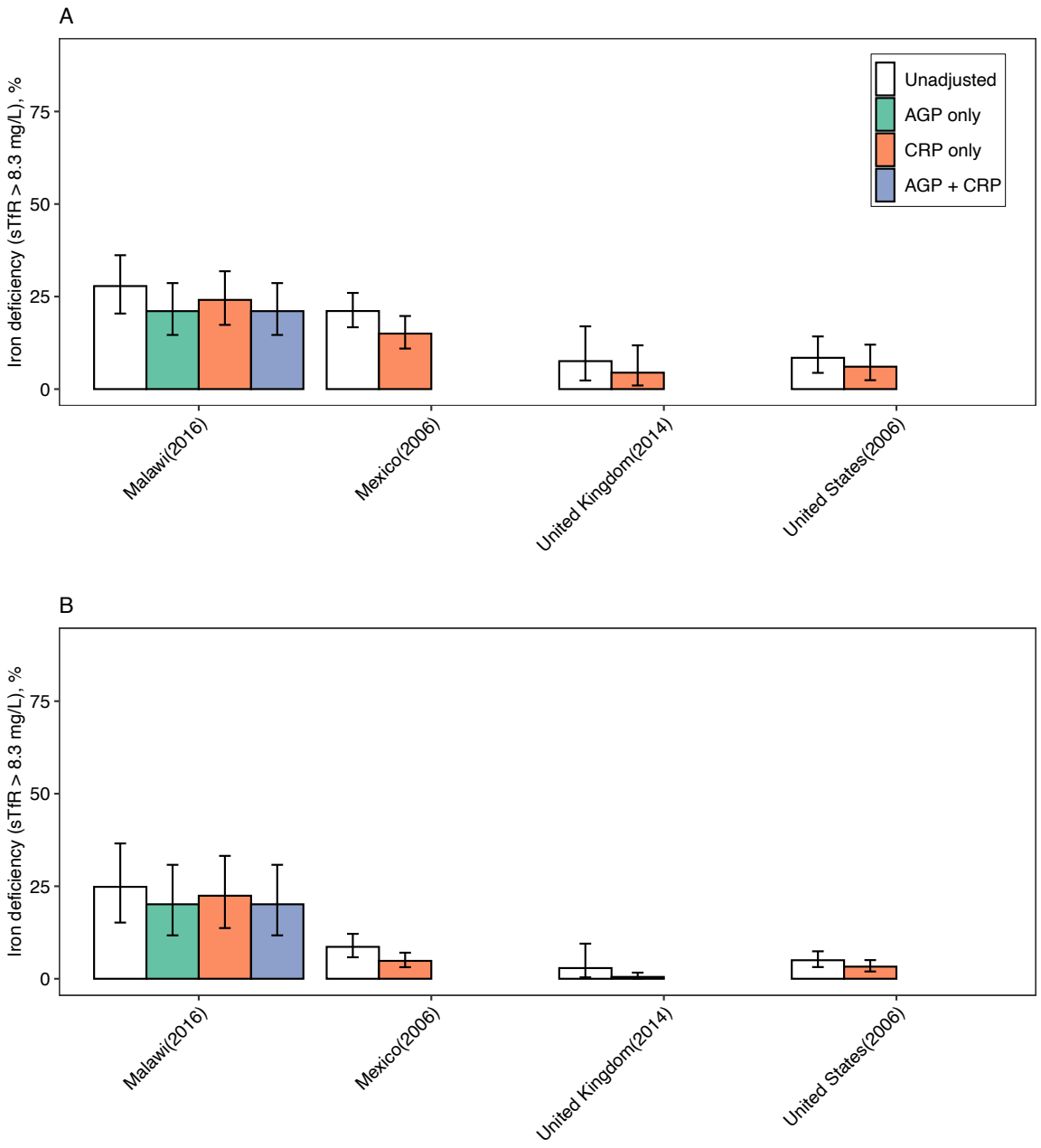


Figure OSM8: Estimated prevalence of Iron deficiency using sTfR >8.3 mg/L in School-age Children by age group (A) Age < 12 (B) Age >12 with the use of different BRINDA adjustment approaches



CHAPTER 4: CONCLUSION AND RECOMMENDATIONS

STUDY OVERVIEW

In summary, iron deficiency and iron deficiency anemia (IDA) are significant problems globally, affecting vulnerable populations such as children and women of reproductive age[2]. An accurate assessment of iron deficiency is essential for individual health and global public health and development. However, inflammation can influence iron biomarkers, such as serum ferritin (SF) and soluble transferrin receptors (sTfR), leading to over- or underestimation of iron deficiency, respectively. This thesis aimed to examine the relationship between inflammation and iron biomarkers in SAC and ADL to address the knowledge gap in these understudied populations. The study analyzed data from over 26,000 SAC and ~20,000 ADL and found a consistent positive association between inflammation biomarkers (α-1-glycoprotein (AGP) and C-reactive protein (CRP) and indicators of iron status, except for sTfR and CRP in ADL. Adjusting for inflammation had different effects on SF and sTfR, with adjusting for SF increasing estimates of iron deficiency prevalence while adjusting for sTfR resulted in a decreased prevalence. Failure to adjust for inflammation could lead to incorrect estimates of iron deficiency prevalence, with significant implications for program planning and policy. This could result in inadequate resource distribution and imprecise targeting of micronutrient interventions. Therefore, it is imperative to consider measuring inflammation biomarkers when assessing iron status. This will improve the accuracy of micronutrient assessments in surveillance programs and research. Further research is necessary to confirm these findings due to the significant gaps in data for these population groups. Gaps including but not limited to the lack of collection of both inflammation biomarkers and a need for more diverse datasets. Many studies on SAC and ADL inflammation and health outcomes tend to focus on female populations.

Strengths

This study's multi-country analysis of SAC and ADL populations is a significant strength because it examines a diverse set of data from 15 different countries with various income levels and geographical regions. This approach enhances the generalizability of the study's findings and provides a more comprehensive understanding of the impact of inflammation on iron biomarkers in these populations. Additionally, the study expands on BRINDA's previous work, exploring a population group not previously studied. It highlights the importance of adjusting for inflammation when assessing iron status in SAC and ADL populations. Furthermore, it provides valuable information for micronutrient research, program development, and implementation.

Limitations

The study had several limitations, including the limited number of datasets and cross-sectional study design. Data availability for iron biomarkers was limited for some groups, such as sTfR in SAC. AGP data was limited among SAC, restricting the analysis of adjustment methods to Bangladesh and Malawi. It is possible that results would differ with increased data availability, particularly AGP for SAC. Additionally, it is important to note that this analysis did not explore the impact of other factors that may influence iron status, such as dietary intake, infection, or coverage of iron deficiency programs. These factors could potentially confound the relationship between inflammation and iron deficiency prevalence. For example, inadequate dietary intake of iron-rich foods or poor absorption of dietary iron due to other micronutrient deficiencies could contribute to iron deficiency, regardless of the presence of inflammation. Similarly, infections, such as malaria, can also impact iron status as it causes inflammation and alter iron metabolism. Additionally, the effectiveness of iron supplementation programs can impact iron status, as the coverage and quality of such programs may vary across different countries and populations.

Finally, it is important to acknowledge that there may be potential for laboratory error in the assessment of biomarkers, given that data for this study came from multiple countries with varying degrees of laboratory infrastructure. Furthermore, laboratory methods differed across the surveys included in this analysis, which could lead to systematic differences in results. While quality assurance measures were in place, there is always the possibility of human error compromising survey data. Therefore, caution should be exercised when interpreting the findings of this study and future research should aim to address these limitations to provide a more comprehensive understanding of the relationship between inflammation and iron deficiency.

IMPLICATIONS FOR POLICY AND PRACTICE

Policymakers and health professionals should recognize that inflammation can significantly impact iron biomarkers and lead to incorrect estimates of iron deficiency prevalence. Public health interventions should address both inflammation and iron deficiency, particularly in vulnerable populations, such as children and women of reproductive age. Neglecting to consider inflammation may result in an inaccurate estimation of iron deficiency. This could lead to improper allocation of resources and imprecise targeting of micronutrient initiatives, affecting policy implementation. Since inflammation can contribute to levels that do not reflect an individual's true iron status, it is important to account for inflammation when assessing iron deficiency. Without doing so, there is a risk of over or underestimating the prevalence of iron deficiency and allocating resources to the wrong population groups. This can lead to ineffective or harmful policy implementation. Although it is essential that inflammation is accounted for in intervention strategies, more research is needed to understand the mechanisms underlying the relationship between inflammation and iron status in understudied populations, such as SAC and ADL, to better address these issues in public health policy and practice. Additionally, policies that focus solely

on specific nutrient deficiencies may not be effective in improving overall health outcomes. Policies are more effective if they take a holistic approach, by taking account for other factors such as socioeconomic factors, cultural and dietary practices, health behaviors, environment, to addressing nutrient deficiencies and improving overall health outcomes.

FUTURE RECOMMENDATIONS

The findings of this study have highlighted the need for further research on the relationship between iron status and inflammation in SAC and ADL. One potential area for future research is the need for longitudinal studies to examine the long-term implications of iron deficiency and inflammation on health outcomes. Longitudinal studies would provide insight into how changes in iron status and inflammation over the growth period experienced by SAC and ADL populations. Additional research is needed in different populations and settings to assess the generalizability of the findings. Within expanded research, it is also important to consider other variables that may confound the relationship between inflammation and iron status, such as diet, infection, genetics, and socioeconomic status.

Future studies could explore several potential research questions related to iron deficiency and inflammation among SAC and ADL populations. One area of focus could be clarifying the age analysis of this child population group. Based on recommendations by the CDC, the assessment of nutritional biomarkers for SAC and ADL should be combined into one population group to adhere to previously defined age groups by the WHO. This analysis should be replicated with this population group known as children and adolescents, 5-19 years[53]. The CDC recommends this analysis as SAC and ADL population groups have age overlap and sensitivity at, below, or above specific ages can be conducted. This study will minimize confusion of the age overlap and help provide clearer understanding among children and adolescents.

Another important research question is to determine the true prevalence of iron deficiency among SAC and ADL, and how it varies by geography and other demographic factors such as sex, ethnicity, and socioeconomic status. This could be achieved through large-scale longitudinal studies that assess multiple biomarkers of iron status and inflammation, as well as dietary intake and other potential confounding factors. Although this research would be costly, it is important to account for the confounding factors to better understand the relationship between biomarkers and one's environment.

In addition to research questions, it is worthwhile to consider potential methodological issues in future studies related to iron deficiency and inflammation. One significant consideration is the standardization of measurement protocols for biomarkers of iron status and inflammation. This can include the use of standardized laboratory procedures, such as quality control and assurance. This will ensure data accuracy and comparability across settings. Future studies should consider the availability of limits of detection for biomarkers used in assessing iron status and inflammation. The current study did not have access to limits of detection for the surveys used, which could have impacted the accuracy and precision of the biomarker measurements. Future studies should aim to include limits of detection for all biomarkers to ensure accurate and reliable measurements. This will help to minimize measurement error risk and ensure that the results are robust and reliable. Overall, addressing these methodological considerations will improve the accuracy and reliability of research findings related to iron deficiency and inflammation among SAC and ADL populations.

CONCLUSION

In conclusion, this thesis highlights the critical importance of accurately assessing iron deficiency in vulnerable populations such as SAC and ADL to inform effective program planning

and policy development. The study findings demonstrate the significant impact of inflammation on iron biomarkers and the need for adjustment to obtain precise estimates of iron deficiency prevalence. Failure to do so can lead to inadequate resource distribution and imprecise micronutrient targeting, potentially compromising individual health and global public health and development. Therefore, measuring inflammation biomarkers is essential when assessing iron status in these populations. While this study contributes to addressing the knowledge gap in SAC and ADL populations, further research is necessary to confirm the findings due to the limited research in these population groups.

ADDITIONAL PAGES

ACRONYMS

ADL	Adolescents
BRINDA	Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia
CDC	Center for Disease Control
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
PSC	Preschool-age Children
SAC	School-age Children
SF	Serum Ferritin
sTfR	Soluble Transferrin Receptor
TIBC	Total Iron-Binding Capacity
WHO	World Health Organization
WRA	Women of Reproductive Age

REFERENCES

[1] "Assessing the iron status of populations: including literature reviews," Aug. 18, 2007. https://www.who.int/publications-detail-redirect/9789241596107 (accessed Apr. 11, 2023).

[2] "Anaemia in women and children," 2021. https://www.who.int/data/gho/data/themes/topics/anaemia_in_women_and_children (accessed Dec. 09, 2022).

[3] "Iron Deficiency: Global Prevalence and Consequences." https://journals.sagepub.com/doi/epdf/10.1177/15648265030244S206 (accessed Apr. 11, 2023).

[4] J. L. Miller, "Iron Deficiency Anemia: A Common and Curable Disease," *Cold Spring Harb. Perspect. Med.*, vol. 3, no. 7, p. a011866, Jul. 2013, doi: 10.1101/cshperspect.a011866.

[5] World Health Organization, "Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity," World Health Organization, WHO/NMH/NHD/MNM/11.1, 2011. Accessed: Apr. 18, 2023. [Online]. Available: https://apps.who.int/iris/handle/10665/85839

[6] P. S. Suchdev *et al.*, "Assessment of iron status in settings of inflammation: challenges and potential approaches," *Am. J. Clin. Nutr.*, vol. 106, no. Suppl 6, pp. 1626S-1633S, Dec. 2017, doi: 10.3945/ajcn.117.155937.

[7] S. M. L. Namaste, J. Ou, A. M. Williams, M. F. Young, E. X. Yu, and P. S. Suchdev, "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.*, vol. 112, no. Supplement_1, pp. 458S-467S, Aug. 2020, doi: 10.1093/ajcn/nqaa141.

[8] H. Saloojee and J. M. Pettifor, "Iron deficiency and impaired child development," *BMJ*, vol. 323, no. 7326, pp. 1377–1378, Dec. 2001.

[9] "WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations." 2021. Accessed: Apr. 18, 2023. [Online]. Available: https://www.who.int/publications-detail-redirect/9789240000124

[10] F. Rohner *et al.*, "Adjusting soluble transferrin receptor concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project," *Am. J. Clin. Nutr.*, vol. 106, no. suppl_1, pp. 372S-382S, Jul. 2017, doi: 10.3945/ajcn.116.142232.

[11] M. J. Warner and M. T. Kamran, "Iron Deficiency Anemia," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2023. Accessed: Apr. 18, 2023. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK448065/

[12] N. Abbaspour, R. Hurrell, and R. Kelishadi, "Review on iron and its importance for human health," *J. Res. Med. Sci. Off. J. Isfahan Univ. Med. Sci.*, vol. 19, no. 2, pp. 164–174, Feb. 2014.

[13] S. Lynch *et al.*, "Biomarkers of Nutrition for Development (BOND)—Iron Review," *J. Nutr.*, vol. 148, pp. 1001S-1067S, Jun. 2018, doi: 10.1093/jn/nxx036.

[14] M. Jain, "Correlation between haematological and cognitive profile of anaemic and non anaemic school age girls," *Curr. Pediatr. Res.*, vol. 16, pp. 145–149, Jan. 2012.

[15] C. M. Pfeiffer and A. C. Looker, "Laboratory methodologies for indicators of iron status: strengths, limitations, and analytical challenges," *Am. J. Clin. Nutr.*, vol. 106, no. suppl_6, pp. 1606S-1614S, Dec. 2017, doi: 10.3945/ajcn.117.155887.

[16] H. SAITO, “METABOLISM OF IRON STORES,” *Nagoya J. Med. Sci.*, vol. 76, no. 3–4, pp. 235–254, Aug. 2014.
[17] W. Wang, M. A. Knovich, L. G. Coffman, F. M. Torti, and S. V. Torti, “Serum Ferritin: Past, Present and Future,” *Biochim. Biophys. Acta*, vol. 1800, no. 8, pp. 760–769, Aug. 2010, doi: 10.1016/j.bbagen.2010.03.011.
[18] A. Dignass, K. Farrag, and J. Stein, “Limitations of Serum Ferritin in Diagnosing Iron Deficiency in Inflammatory Conditions,” *Int. J. Chronic Dis.*, vol. 2018, p. 9394060, Mar. 2018, doi: 10.1155/2018/9394060.
[19] S. M. Namaste *et al.*, “Adjusting ferritin concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project,” *Am. J. Clin. Nutr.*, vol. 106, no. Suppl 1, pp. 359S-371S, Jul. 2017, doi: 10.3945/ajcn.116.141762.
[20] D. F. Wallace, “The Regulation of Iron Absorption and Homeostasis,” *Clin. Biochem. Rev.*, vol. 37, no. 2, pp. 51–62, May 2016.
[21] G. Weiss, T. Ganz, and L. T. Goodnough, “Anemia of inflammation,” *Blood*, vol. 133, no. 1, pp. 40–50, Jan. 2019, doi: 10.1182/blood-2018-06-856500.
[22] O. Yokus, B. Yilmaz, M. Albayrak, O. S. Balcik, M. R. Helvaci, and E. Sennaroglu, “The Significance of Serum Transferrin Receptor Levels in the Diagnosis of the Coexistence of Anemia of Chronic Disease and Iron Deficiency Anemia,” *Eurasian J. Med.*, vol. 43, no. 1, pp. 9–12, Apr. 2011, doi: 10.5152/eajm.2011.02.
[23] M. Restrepo-Gallego, L. E. Díaz, and P. H. C. Rondó, “Classic and emergent indicators for the assessment of human iron status,” *Crit. Rev. Food Sci. Nutr.*, vol. 61, no. 17, pp. 2827–2840, Sep. 2021, doi: 10.1080/10408398.2020.1787326.
[24] M. Wessling-Resnick, “Iron Homeostasis and the Inflammatory Response,” *Annu. Rev. Nutr.*, vol. 30, pp. 105–122, Aug. 2010, doi: 10.1146/annurev.nutr.012809.104804.
[25] W. Ansar and S. Ghosh, “Inflammation and Inflammatory Diseases, Markers, and Mediators: Role of CRP in Some Inflammatory Diseases,” *Biol. C React. Protein Health Dis.*, pp. 67–107, Mar. 2016, doi: 10.1007/978-81-322-2680-2_4.
[26] D. I. Thurnham, C. A. Northrop-Clewes, and J. Knowles, “The Use of Adjustment Factors to Address the Impact of Inflammation on Vitamin A and Iron Status in Humans123,” *J. Nutr.*, vol. 145, no. 5, pp. 1137S-1143S, May 2015, doi: 10.3945/jn.114.194712.
[27] T. Ganz and E. Nemeth, “Hepcidin and iron homeostasis,” *Biochim. Biophys. Acta BBA - Mol. Cell Res.*, vol. 1823, no. 9, pp. 1434–1443, Sep. 2012, doi: 10.1016/j.bbamcr.2012.01.014.
[28] P. S. Suchdev, S. M. Namaste, G. J. Aaron, D. J. Raiten, K. H. Brown, and R. Flores-Ayala, “Overview of the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) Project1234,” *Adv. Nutr.*, vol. 7, no. 2, pp. 349–356, Mar. 2016, doi: 10.3945/an.115.010215.
[29] H. Luo *et al.*, “A Practical Guide to Adjust Micronutrient Biomarkers for Inflammation Using the BRINDA Method,” *J. Nutr.*, Feb. 2023, doi: 10.1016/j.tjnut.2023.02.016.
[30] K. Begum *et al.*, “Prevalence of and factors associated with antenatal care seeking and adherence to recommended iron-folic acid supplementation among pregnant women in Zinder, Niger,” *Matern. Child. Nutr.*, vol. 14 Suppl 1, Feb. 2018, doi: 10.1111/mcn.12466.
[31] R. Castel *et al.*, “The transferrin/log(ferritin) ratio: a new tool for the diagnosis of iron deficiency anemia,” *Clin. Chem. Lab. Med.*, vol. 50, no. 8, pp. 1343–1349, Feb. 2012, doi: 10.1515/cclm-2011-0594.

[32] I. Infusino, F. Braga, A. Dolci, and M. Panteghini, “Soluble Transferrin Receptor (sTfR) and sTfR/log Ferritin Index for the Diagnosis of Iron-Deficiency Anemia A Meta-Analysis,” *Am. J. Clin. Pathol.*, vol. 138, no. 5, pp. 642–649, Nov. 2012, doi: 10.1309/AJCP16NTXZLZFAIB.
[33] I. Jáuregui-Lobera, “Iron deficiency and cognitive functions,” *Neuropsychiatr. Dis. Treat.*, vol. 10, pp. 2087–2095, Nov. 2014, doi: 10.2147/NDT.S72491.
[34] Y. T. Cheung *et al.*, “Iron Deficiency among School-Aged Adolescents in Hong Kong: Prevalence, Predictors, and Effects on Health-Related Quality of Life,” *Int. J. Environ. Res. Public. Health*, vol. 20, no. 3, Art. no. 3, Jan. 2023, doi: 10.3390/ijerph20032578.
[35] S. Kundu *et al.*, “Prevalence of Anemia among Children and Adolescents of Bangladesh: A Systematic Review and Meta-Analysis,” *Int. J. Environ. Res. Public. Health*, vol. 20, no. 3, p. 1786, Jan. 2023, doi: 10.3390/ijerph20031786.
[36] E. Z. Tariku *et al.*, “Anemia and its associated factors among school-age children living in different climatic zones of Arba Minch Zuria District, Southern Ethiopia,” *BMC Hematol.*, vol. 19, p. 6, Apr. 2019, doi: 10.1186/s12878-019-0137-4.
[37] B. Sederquist, P. Fernandez-Vojvodich, F. Zaman, and L. Sävendahl, “RECENT RESEARCH ON THE GROWTH PLATE: Impact of inflammatory cytokines on longitudinal bone growth,” *J. Mol. Endocrinol.*, vol. 53, no. 1, pp. T35–T44, Aug. 2014, doi: 10.1530/JME-14-0006.
[38] “What Is Inflammation? And Why Does it Matter for Child Development?,” *Center on the Developing Child at Harvard University*. https://developingchild.harvard.edu/resources/what-is-inflammation-and-why-does-it-matter-for-child-development/ (accessed Dec. 09, 2022).
[39] C. C. John, M. M. Black, and C. A. Nelson, “Neurodevelopment: The Impact of Nutrition and Inflammation During Early to Middle Childhood in Low-Resource Settings,” *Pediatrics*, vol. 139, no. Suppl 1, pp. S59–S71, Apr. 2017, doi: 10.1542/peds.2016-2828H.
[40] M. Adelantado-Renau, M. R. Beltran-Valls, and D. Moliner-Urdiales, “Inflammation and Cognition in Children and Adolescents: A Call for Action,” *Front. Pediatr.*, vol. 8, 2020, Accessed: Apr. 13, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fped.2020.00583
[41] F. Amaro and F. Chiarelli, “Growth and Puberty in Children with Inflammatory Bowel Diseases,” *Biomedicines*, vol. 8, no. 11, Art. no. 11, Nov. 2020, doi: 10.3390/biomedicines8110458.
[42] A. Gebrie and A. Alebel, “A systematic review and meta-analysis of the prevalence and predictors of anemia among children in Ethiopia,” *Afr. Health Sci.*, vol. 20, no. 4, pp. 2007–2021, Dec. 2020, doi: 10.4314/ahs.v20i4.59.
[43] M. Fiorentino *et al.*, “Anthropometric and micronutrient status of school-children in an urban West Africa setting: a cross-sectional study in Dakar (Senegal),” *PloS One*, vol. 8, no. 12, p. e84328, 2013, doi: 10.1371/journal.pone.0084328.
[44] B. Cichon *et al.*, “Assessment of Regression Models for Adjustment of Iron Status Biomarkers for Inflammation in Children with Moderate Acute Malnutrition in Burkina Faso,” *J. Nutr.*, vol. 147, no. 1, pp. 125–132, Jan. 2017, doi: 10.3945/jn.116.240028.
[45] A. Soliman, V. De Sanctis, R. Elalaily, and S. Bedair, “Advances in pubertal growth and factors influencing it: Can we increase pubertal growth?,” *Indian J. Endocrinol. Metab.*, vol. 18, no. Suppl 1, pp. S53–S62, Nov. 2014, doi: 10.4103/2230-8210.145075.

[46] R. M. Viner, N. B. Allen, and G. C. Patton, “Puberty, Developmental Processes, and Health Interventions,” in *Child and Adolescent Health and Development*, D. A. P. Bundy, N. de Silva, S. Horton, D. T. Jamison, and G. C. Patton, Eds., 3rd ed.Washington (DC): The International Bank for Reconstruction and Development / The World Bank, 2017. Accessed: Apr. 18, 2023. [Online]. Available: http://www.ncbi.nlm.nih.gov/books/NBK525269/

[47] C. P. Fagundes, J. M. Bennett, H. M. Derry, and J. K. Kiecolt-Glaser, “Relationships and Inflammation across the Lifespan: Social Developmental Pathways to Disease,” *Soc. Personal. Psychol. Compass*, vol. 5, no. 11, pp. 891–903, Nov. 2011, doi: 10.1111/j.1751-9004.2011.00392.x.

[48] I. Martínez de Toda, M. González-Sánchez, E. Díaz-Del Cerro, G. Valera, J. Carracedo, and N. Guerra-Pérez, “Sex differences in markers of oxidation and inflammation. Implications for ageing,” *Mech. Ageing Dev.*, vol. 211, p. 111797, Apr. 2023, doi: 10.1016/j.mad.2023.111797.

[49] “Nutrition | The Brinda Project,” *BRINDA*, 2023. https://www.brinda-nutrition.org (accessed Apr. 12, 2023).

[50] S. M. Namaste, G. J. Aaron, R. Varadhan, J. M. Peerson, and P. S. Suchdev, “Methodologic approach for the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project,” *Am. J. Clin. Nutr.*, vol. 106, no. Suppl 1, pp. 333S-347S, Jul. 2017, doi: 10.3945/ajcn.116.142273.

[51] H. Luo [cre, aut, O. Y. Addo, and J. Geng, “BRINDA: Computation of BRINDA Adjusted Micronutrient Biomarkers for Inflammation.” Oct. 16, 2022. Accessed: Apr. 12, 2023. [Online]. Available: https://CRAN.R-project.org/package=BRINDA

[52] M. Nairz, I. Theurl, D. Wolf, and G. Weiss, “Iron deficiency or anemia of inflammation?,” *Wien. Med. Wochenschr. 1946*, vol. 166, no. 13, pp. 411–423, 2016, doi: 10.1007/s10354-016-0505-7.

[53] M. de Onis, A. W. Onyango, E. Borghi, A. Siyam, C. Nishida, and J. Siekmann, “Development of a WHO growth reference for school-aged children and adolescents,” *Bull. World Health Organ.*, vol. 85, no. 9, pp. 660–667, Sep. 2007, doi: 10.2471/BLT.07.043497.