Noncommunicable Disease Risk in Global Settings: An Examination of Potential Contributors and Assessment Methods

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[Final note: Chapter 2 (*The relationship between ferritin and body mass index is mediated by inflammation among women in higher-income countries, but not in most lower-income countries nor among young children: a multi-country analysis*) has been accepted for publication as of August 2022 but is not yet published with *Current Development in Nutrition.*]

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ABSTRACT

Noncommunicable diseases, including cardiovascular diseases, diabetes, and others, are the leading cause globally of premature mortality (death before the age of 70), with the majority of deaths occurring in low- and lower-middle-income countries. Two prominent conditions contributing to noncommunicable disease risk include overweight or obesity and hypertension. Worldwide, >40% of adults and almost 6% of children experience overweight and obesity, and >15% of adults have raised blood pressure. Alongside rising prevalence of overweight or obesity and hypertension, micronutrient deficiencies persist, especially in low- and lower-middle-income countries. The United Nations' Sustainable Development Goal (SDG) 2.2 calls for an end to malnutrition in all its forms by 2030, and SDG 3.4 aims to reduce premature deaths from noncommunicable diseases by one-third by 2030 (relative to 2015 levels). However, progress towards these goals is slow, with most countries worldwide off track. Given the potential for conditions of both undernutrition and overnutrition to increase the risk of developing noncommunicable diseases, a greater understanding of the relationships between selected contributors to noncommunicable disease risk, and how they are measured, are needed to reach the SDG targets.

Inflammation is the body's physiological response to injury, illness, infection, or environmental insult, and is characterized by the presence of pro-inflammatory cytokines and acute-phase proteins, such as C-reactive protein (CRP) and α-1-acid glycoprotein (AGP). In populations in LIC and LMIC, inflammation may be more likely associated with illnesses such as diarrhea or helminth infections, while populations in upper-middle and high-income countries may more commonly experience the chronic inflammation associated with obesity. Inflammation confounds the assessment of the micronutrients iron and vitamin A, as the biomarkers commonly used to measure iron and vitamin A status are also acute phase proteins. In the presence of inflammation, serum or plasma concentrations of ferritin transiently decrease, and serum or plasma concentrations of retinol and retinol-binding protein (RBP)

increase. In Chapters 2 and 3, I explored the extent to which adiposity-related inflammation may influence ferritin and retinol or RBP interpretation.

In Chapter 2, I describe relationships between weight status, inflammation, and ferritin among non-pregnant women of reproductive age (15-49 years, WRA) and preschool-age children (6-59 months, PSC) with normal weight to overweight or obesity in differing geographic settings. Cross-sectional data were separately analyzed from n=18 surveys (WRA) and n=25 surveys (PSC) from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project, excluding observations with underweight, wasting, pregnancy, or malaria. Relationships were assessed between BMI (WRA) or BMI-for-age z-score (BAZ, PSC), inflammatory biomarkers CRP and/or AGP, and ferritin by linear regression, and potential mediation by CRP and/or AGP in relationships between BMI or BAZ and ferritin with structural equation modeling. Regression and mediation models accounted for complex survey designs, and results were grouped by World Bank income classifications. In 5 of 6 surveys among WRA from upper-middle and high-income countries, ferritin was significantly positively associated with BMI, and this relationship was partially (or fully in the survey from the United States) mediated by CRP and/or AGP. Mediation was present in 4 of 12 surveys for WRA in low- and lower-middle income countries. Among PSC, ferritin was positively associated with CRP and/or AGP in all surveys, but there were no significant CRP- or AGP-mediated relationships between ferritin and BAZ, except a negative relationship in the Philippines. I concluded that where overweight and obesity are common among WRA, measurement of inflammatory biomarkers and their use in interpreting ferritin may improve iron status assessment. While these relationships were inconsistent among PSC, inflammation was common and should be measured to interpret iron status.

In Chapter 3, I conducted similar analyses to examine relationships between weight status, inflammation, and retinol or RBP among WRA and PSC with normal weight to overweight or obesity. BRINDA data from n=13 surveys (WRA) and n=22 surveys (PSC) were separately analyzed, excluding

observations with underweight, wasting, pregnancy, or malaria. Relationships were assessed between BMI (WRA) or BAZ (PSC), CRP and/or AGP, and retinol or RBP by linear regression, and potential mediation by CRP and/or AGP in relationships between BMI or BAZ and retinol or RBP with structural equation modeling. All regression and mediation models accounted for complex survey designs. Among WRA, greater BMI was positively associated with retinol or RBP in 5 of 13 surveys, BMI was positively associated with CRP and/or AGP in 10 of 13 surveys, but associations between biomarkers of inflammation and retinol or RBP were inconsistent. Among PSC, BMI was not associated with retinol, RBP, CRP, or AGP, but biomarkers of inflammation were consistently negatively associated with retinol or RBP. In 3 of 13 surveys among WRA and 1 of 22 surveys among PSC, inflammation partially mediated the relationship between BMI or BAZ and retinol or RBP, however the direction of association varied. I concluded that in these surveys, inflammation associated with overweight and obesity does not appear to impact vitamin A assessment when measured with retinol or RBP; however, inflammation should continue be measured to interpret vitamin A status among PSC.

Chapter 4 explores salt consumption in Ghana, where salt consumption ranges 6-12 g/d, and salt consumption ≥5 g/d is associated with increased risk of noncommunicable diseases. To develop salt reduction strategies that are relevant to this context, understanding salt usage and consumption patterns is necessary. My objectives for this chapter were to: 1) estimate consumption of salt, including salt from bouillon, among households, women, and children, and compare to global recommendations; 2) estimate the proportion of salt consumed from bouillon; and 3) identify factors, including knowledge, attitudes, and practices (KAP), associated with household salt consumption in 2 districts in Northern Region, Ghana. Employing mixed-methods methodology, households were enrolled from 14 urban and 14 rural clusters from Tolon and Kumbungu districts in a pilot survey and focus group discussions (FGDs, n=20). Using the Fortification Assessment Coverage Toolkit, households (n=369) reported most recent purchases of discretionary salt (DS, 'table salt') and bouillon cubes. From purchase data, median (IQR)

household consumption (g/d) of DS and total salt (TS, DS + salt from bouillon, assumed to be 55% salt) were calculated, including the proportion of salt from bouillon. DS and TS consumption for women (15-49 y) and children (2-5 y) were estimated with the Adult Male Equivalent method and compared to global recommendations. Salt intake from urinary sodium excretion was predicted with the INTERSALT equation (women only). Associations between DS and TS consumption and household and individual (women's) characteristics, including KAP, were tested with mixed effects ANOVA. Minimally-adjusted and multivariable models included district, setting (urban/rural), household size, and participant type (non-lactating or lactating woman) as fixed effects, and the random effect of cluster. Qualitative themes were generated from FGDs using the Framework Method. From reported household purchase data, estimated consumption of DS and TS appeared to exceed global recommendations for many children (TS: 2.9 [1.9, 5.2] g/d) and the majority of women (TS: 6.0 [4.0, 10.2] g/d). Women's mean urinary sodium excretion also suggested high sodium exposure (7.1 g/d). Bouillon contributed <25% to households' daily TS consumption. Household salt consumption was greater among households in 3rd-5th (highest) asset quintiles and those with severe food insecurity. Few other characteristics were associated with household salt consumption. Salient qualitative themes included salt's ubiquity as a seasoning, and how intra-household dynamics, taste preferences, and perceptions about salt and health shaped salt usage and consumption. These results suggest that salt consumption among women and children in this area exceeds recommendations; food prepared outside the home may further contribute to salt consumption. Salt reduction interventions may be warranted in this context.

Together, these studies broaden our understanding of how measuring indicators of iron and vitamin A status relate to noncommunicable disease risk assessment in different global settings, which will aid global nutrition status surveillance efforts. Also, the salt consumption results from Ghana will help inform nutrition and policy discussions related to salt in Ghana, including the development of salt-reduction behavior change communication strategies.

CHAPTER 1. Global Rise of Noncommunicable Diseases: A Review of Selected Implications

Global nutrition trends and the Double Burden of Malnutrition

Noncommunicable diseases (NCDs), including cardiovascular diseases, diabetes, and others, are the leading cause globally of premature mortality (death before the age of 70), with the majority of deaths occurring in low- and lower-middle-income countries (LIC, LMIC) (1). Two prominent conditions contributing to NCD risk include overweight or obesity (OWOB) and hypertension. While these conditions are common in upper-middle and high-income countries (UMIC, HIC), the proportion of the population experiencing OWOB and hypertension in LIC and LMIC has been rising for at least the past three decades (2,3). Alongside rising prevalence of OWOB and hypertension, micronutrient deficiencies persist, especially in LIC and LMIC (4). The United Nations' Sustainable Development Goal (SDG) 2.2 calls for an end to malnutrition in all its forms by 2030 and Goal 3.4 aims to reduce premature deaths from NCDs by one-third by 2030 (relative to 2015 levels); however progress towards these goals is slow, with most countries worldwide off track (5,6). To reach the SDG targets, strategies that address contributors to all forms of malnutrition are needed.

Historically, undernutrition (i.e., stunting, wasting, underweight, low birthweight, anemias, and micronutrient deficiencies) has dominated the health and nutrition landscape of LIC and LMIC, particularly among vulnerable populations, such as women, children, and populations experiencing poverty; conversely, OWOB has concentrated among the wealthy (7). Over the past few decades, changing food systems and food environments due to globalization and urbanization in LIC and LMIC have expanded access to energy-dense and nutrient-poor foods, and made sedentary lifestyles more common (7,8). This shift in dietary patterns and lifestyles, known as the "Nutrition Transition," has contributed to changes in body composition and morbidity, including the rising prevalence OWOB and NCDs in these contexts (9). The concomitant presence of persistent conditions of undernutrition

alongside conditions of overnutrition has been termed the “Double Burden of Malnutrition” (DBM), and can present in individuals, households, and populations, and throughout the life course (10).

While there are common observations related to DBM in LIC and LMIC, such as populations of lower socioeconomic status experiencing more underweight while wealthier populations experience more OWOB, the distribution and manifestation of the multiple forms of malnutrition varies across world regions, countries, and income classifications (11,12). Notably, BMIs are rising in many rural populations across the globe, particularly in UMIC (13,14), which may be predictive of trends in HIC where populations of lower socioeconomic status can experience higher prevalence of OWOB compared to higher socioeconomic populations (15,16).

The name “Double Burden of Malnutrition” implies the coexistence of one condition of overnutrition and one of undernutrition, however in reality, multiple forms of malnutrition often coexist, particularly when viewed over the life course (17). For example, one typology of DBM at the individual-level may include stunting and micronutrient deficiencies in childhood, OWOB with persistent micronutrient deficiencies in adulthood, and then development of heart disease and/or diabetes as adulthood continues. The Developmental Origins of Health and Disease theory offers a biological explanation as to how conditions of undernutrition could lead to the development of NCDs. This theory hypothesizes that fetal undernutrition and poor growth lead to metabolic dysregulation and physical and structural changes to key organs that increase the risk of developing heart disease and diabetes later in life (18). Investigations into the pathways through which these hypothesized outcomes may occur highlighted the micronutrient status of both the mother and fetus as critical in potentially shaping NCD risk (19). Maternal deficiencies in several micronutrients, such as iron, zinc, calcium, folate, and B12, may lead to restricted fetal growth and development, which may in turn impact the development of the kidneys, heart, pancreas, and lungs, as well as altering endocrine capabilities and lipid metabolism and deposition. Development of conditions such as hypertension, OWOB, and insulin resistance may

ensue, increasing the risk of developing NCDs (19,20). Additional influences through fetal epigenetic reprogramming in utero (21,22) and early life consistent exposure to energy-dense, nutrient-poor foods (23) may further predispose a child to poor health outcomes. Importantly, poor fetal growth can occur with both overnourished and undernourished mothers, as maternal OWOB has been associated with fetal growth restriction (21). Thus, prioritizing the nutrition status of women throughout the life course will improve health outcomes for both women and her potential children.

Given the potential for conditions of undernutrition and overnutrition to increase the risk of developing NCDs, a greater understanding of the relationships between selected contributors to NCD risk, including how they are measured, are needed. This review will summarize literature examining the potential influence of inflammation on assessment of selected micronutrients (iron and vitamin A) among populations with OWOB in global settings. It will also summarize how dietary salt is implicated in the rise of NCDs globally, such as through increased risk of developing hypertension, and summarize common methods of dietary salt measurement. This section will particularly focus on salt consumption in Ghana, as that is the setting of the investigation described in Chapter 4.

Micronutrient assessment and associations with overweight or obesity

Global prevalence and definitions of overweight and obesity

The proportion of the global population experiencing OWOB continues to increase. Across the globe, rising body mass is a top contributor to global disease burdens, with LIC and LMIC leading this upward trend for the past 20 years (24). OWOB currently affect approximately 44% of all adults and 20% of all children 5-17 years of age, and overweight affects almost 6% of all children under 5 years of age; the vast majority of these populations reside in LMIC (25,26). In 2016, nearly 40% of adults in Nepal were estimated to be experiencing overweight or obese, nearly 43% of adults in Ghana, and 64% of

adults in Guatemala, a figure that rivals OWOB prevalence among adults in HIC such as Australia (67% in 2017) and the United Kingdom (64% in 2017) (27–31).

OWOB is defined as excessive accumulation of adipose tissue with the risks to health increasing depending on the location and distribution of excess fat accumulation (32). Approximately 80% of adipose tissue is subcutaneous, with the majority of remaining adipose found viscerally, surrounding major abdominal organs. Excessive accumulation of visceral adipose tissue, which is often present in individuals experiencing OWOB, increases the risk of NCDs (33). BMI is often used to screen for OWOB, with overweight in adults is defined as a BMI of ≥25.0-29.9 kg/m^2 and obesity defined as a BMI of ≥30.0 kg/m^2. OWOB are also defined as increased waist circumference (≥ 88 cm for adult women and ≥ 102 cm for adult men) (34). While BMI is limited in its ability to discern body fat location, other direct measures of adiposity, such as waist circumference, waist-to-hip ratio, or skinfold thickness have been found to be highly correlated with BMI when predicting cardiometabolic risk (35).

Children (0-18 years of age) are considered at risk of overweight with a BMI-for-age z-score (BAZ) of ≥ 1 SD, overweight with a BAZ of ≥ 2 SD, and obese at a BAZ of ≥ 3 SD, according to WHO growth standards. According to the United States Centers for Disease Control and Prevention BMI-for-age growth references, children (2-20 years of age) are at risk of overweight at ≥ 85th percentile, and at risk of obesity at ≥ 95th percentile (36–38). Additionally, among Asian adult populations, health risks such as diabetes seem to increase at lower BMIs, with a BMI of 23 kg/m^2 potentially requiring health interventions (39). BMI growth references for children also likely underestimate OWOB among Asian children, however formal recommendations to revise the references have not yet been made (40,41).

Obesity and inflammation

Inflammation is the body's physiological response to injury, illness, infection, or environmental insult, and is characterized by the presence of pro-inflammatory cytokines and acute-phase proteins,

such as C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) (42). Excess adipose tissue, in particular visceral adipose tissue, releases pro-inflammatory cytokines and CRP, which then stimulate the production and release of acute-phase proteins from hepatocytes, macrophages, and others, creating an environment of prolonged, low-grade systemic inflammation that often characterizes obesity (43,44). Abdominal obesity has been found to be strongly related with inflammation among adult women (45,46), and among children and adolescents (47,48). Inflammation associated with obesity affects many bodily systems, and may have life-long effects. A study of adolescents whose mothers experienced obesity during pregnancy had significantly higher levels of CRP than adolescents born to mothers of normal weight, after controlling for BMI (49). As discussed previously, early-life exposure to inflammation may place children and adolescents at risk for developing obesity or metabolic dysregulation later in life (18,50).

Inflammation also influences the assessment of some micronutrients, including iron and vitamin A (51). With obesity specifically, some literature suggests that individuals experiencing obesity may be associated with lower micronutrient status compared to individuals without obesity, however, the direction of association is unclear, and may differ by context (52). Inflammation associated with OWOB may contribute to the variation in micronutrient status evaluation (42).

Iron status assessment

While there are multiple biomarkers available to evaluate a population's iron status (e.g., ferritin, soluble transferrin receptor, serum iron), epidemiological studies of health and nutrition status frequently measure plasma or serum ferritin concentration (53). However, ferritin is a positive acute-phase protein, meaning that in the presence of inflammation ferritin concentrations transiently increase, confounding iron status assessment and potentially resulting in erroneous underestimates of iron deficiency (54). This mechanism occurs because of hepcidin, a hormone released predominantly

from the liver in the presence of inflammation (55). Hepcidin regulates iron homeostasis by binding to and degrading ferroportin, the transmembrane protein used by iron-exporting cells, including enterocytes and macrophages. After degradation of ferroportin, iron is effectively trapped as ferritin within cells (55). The evolutionary purpose of this action is to reduce the availability of circulating iron for use by pathogens, which may be the cause of the inflammation (56). However, persistent inflammation not due to infection, such as in obesity and other chronic diseases, may increase the risk of iron deficiency due to sustained sequestration of cellular iron, leaving the body unable to access iron stores for normal functions such as erythropoiesis (55). Thus, measuring and controlling for inflammation when measuring ferritin concentration is critical for accurate evaluation of iron status (54).

Vitamin A status assessment

Serum or plasma retinol-binding protein (RBP) and retinol are the two principle circulating forms of vitamin A in the body (57). RBP is synthesized in the liver, the primary storage site of vitamin A. After being released from the liver, RBP transports retinol (holo-RBP) through the blood system, and may be bound to transthyretin, which increases the mass of the molecule and delays excretion via the kidneys of the holo-RBP-transthyretin complex (57). Circulating retinol, which comes from dietary or supplemental forms of vitamin A, is primarily bound to RBP or lipoproteins (57). Both RBP and retinol are sensitive to recent intake of vitamin A-rich foods or supplementation, and liver biopsies are the gold standard for measuring vitamin A status (57). However, liver biopsies are not a viable assessment method for evaluations of population vitamin A status, and instead, serum or plasma retinol and RBP concentrations are frequently measured in epidemiological studies (58).

RBP is a negative acute-phase protein. Under inflammatory conditions, the liver will downregulate the release of holo-RBP, and the kidneys will upregulate excretion of both holo-RBP and apo-RBP (57). Thus, in the presence of inflammation, concentrations of RBP and retinol will transiently

decrease, impacting assessments of vitamin A status (59). In evaluations of the extent to which accounting for inflammation is necessary when assessing vitamin A status, researchers found that among preschool-age children (6-59 months) and school-age children (5-15 years), adjusting for inflammation consistently decreased the prevalence of vitamin A deficiency, while among adult women (15-49 years), the inflammation-adjusted results were inconsistent (51,58).

While the highly regulated circulating concentrations of RBP (and by association, retinol) appear to be primarily liver-derived (33–35), RBP (or RBP4) is also expressed in adipose tissue and has been identified as an adipokine (33,36). Because of the hypothesized role as an adipokine, researchers have found differing levels of circulating RBP associated with cardiovascular disease, insulin resistance, obesity, and hypertension in adults (9,12,13,33,36,37), and obesity and metabolic syndrome in children and adolescents (38,39), though the direction of association varies and causal relationships have not been established. Additionally, it is unknown whether and how much of circulating RBP in states of chronic disease are adipocyte-derived (33). Thus, while current understanding of biological pathways supports a negative relationship between inflammation and circulating concentrations of RBP (and retinol), which in some cases may complicate assessment of vitamin A status, additional research is needed to clarify the relationship between chronic disease and RBP and/or retinol, including establishing temporal relationships and measurement of vitamin A stores. Given that global prevalence of overweight and obesity is rising, including among children (40), it will be useful to better understand how measurement and use of retinol and RBP may be influenced by overweight and obesity.

Variation in sources of inflammation by context

Sources of inflammation differ by context (42). Research examining the relationships between micronutrient status and inflammation due to illness and infection has largely concentrated on data from LIC and LMIC. This is, in part, because of the known direct interactions of micronutrients (e.g., iron)

and pathogens (e.g., malaria, helminths) associated with common illnesses in many of these countries (70–73). In contrast, literature examining adiposity and inflammation and adiposity and micronutrient deficiency has largely been carried out in populations from UMIC and HIC (74–78). Given the body of literature examining inflammation and micronutrient status (42,51,57,79), considering inflammation in nutrition assessments is important for developing interventions and programs that include populations experiencing both undernutrition and overnutrition.

Global health implications of dietary salt consumption

Salt consumption is high worldwide with adults regularly consuming ≥10 grams per day (g/d) (80). While sodium is an essential nutrient necessary for many physiological processes (85), sodium (or salt) consumption and blood pressure are highly correlated, with a linear relationship linking greater risk of hypertension and heart disease to salt consumption >5 g per day (g/d) (86), the recommended daily salt limit of both WHO and the American Heart Association (87,88).

Hypertension affects approximately 1.28 billion adults (aged 30-79 years) globally, and is implicated in 45% of deaths due to cardiovascular disease and 51% of deaths due to stroke, the majority of which occur in LIC and LMIC (81,82). While there are trends of decreasing blood pressure in UMIC and HIC over the past 40 years, mainly due to increased screening and treatment, over the same period, blood pressure and prevalence of hypertension have increased in LIC and LMIC (83). In Ghana specifically, in 2019, prevalence of hypertension (defined as blood pressure ≥140/90 mm Hg [systolic/diastolic] or current use of antihypertensive medication) was 36% for females and 31% for males, aged 30-79 years; approximately 40% of women and 75% of men were unaware of their hypertensive condition (84).

Elevated blood pressure and hypertension definitions

The World Health Organization (WHO) defines hypertension as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg (81) (**Table 1)**. In 2017, the American Heart Association revised their guidelines for detecting hypertension in adults, and recommended lowering the threshold from ≥140/90 mm Hg to ≥130/80 mm Hg (systolic/diastolic). Their recommendation was based on reviews of clinical trial data that showed a two-fold increase in risk of cardiovascular disease when blood pressure exceeded the lower threshold (85). Among children, guidelines for 'at risk' blood pressure are defined by the American Heart Association and the American Academy of Pediatrics, and are based on age, sex, and height according to increased risk of chronic disease (86).

Table 1.1. Blood pressure and hypertension guidelines for adults (18+ years)[1]

Organization	**Normal blood pressure (mm Hg)**	**Elevated blood pressure (mm Hg)**	**Hypertension (mm Hg)**
American Heart Association	SBP <120 and DBP <80	SBP 120-129 and DBP <80	SBP ≥130 or DBP ≥80
International Society of Hypertension	SBP <130 and DBP <85	SBP 130-139 and/or DBP 85-89	SPB ≥140 and/or DBP ≥90
World Health Organization	SBP <120 and DBP <80		SPB ≥140 and/or DBP ≥90

[1] International Society of Hypertension classifies 'Elevated blood pressure' as 'High-normal blood pressure'. References: American Heart Association (85); International Society of Hypertension (87); World Health Organization (81). DBP, diastolic blood pressure; SBP, systolic blood pressure.

Relationships between salt and blood pressure

Salt's relationship with blood pressure is through its physiological role in fluid balance regulation. Salt (sodium chloride) is composed of the main cation (sodium) and anion (chloride) in extracellular fluid (82). Excessive salt intake causes disruptions to the renin-angiotensin-aldosterone system (RAAS), which regulates sodium balance and blood pressure through fluid resorption and excretion (89). The RAAS system increases blood volume and blood pressure by stimulating aldosterone secretion, a hormone secreted from the adrenal cortex. Prolonged excessive salt intake can result in dysregulation to the RAAS system and lead to persistent high blood pressure, which ultimately weakens

the arterial system, contributing to inflammation and arterial plaque accumulation, and putting a person at higher risk for cardiovascular disease and stroke (89).

Populations of African descent, and particularly females of African descent, tend to have higher rates of hypertension than White populations (88,89). This is likely due to a combination of genetic influences, such as greater salt sensitivity (i.e., elevated blood pressure at salt intakes of <5 g per day) and greater arterial wall stiffness (with onset at younger ages in African populations) when compared to White populations. These genetic influences may be enhanced by systemic social-environmental inequities that reduce access to and use of healthcare services, contributing to lower rates of awareness, prevention, and treatment of hypertension (88–91).

Salt consumption in Ghana and dietary sources

Few studies have assessed salt consumption in Ghana. In studies among adults, salt consumption ranged from approximately 6-12 g/d using data from 24-h urinary sodium excretion (92–95), and was 12.5 g/d from analyses of household salt inventories (96). Among children (5-12 y), salt consumption was approximately 6.4 g/d from 24-h urine data (97); take together, these studies suggest that salt consumption in Ghana exceeds global recommendations.

In Ghana, important sources of dietary salt include discretionary salt, which includes salt added at the table or during cooking, and salt from condiments such as bouillon cubes (98–100). Consumption of salt from other sources (i.e., from processed and ultra-processed foods such as snack foods, processed meats, and breads) is becoming more common. The most recent Ghana Demographic and Health Survey (2014) reported that almost 85% of households consumed a salty processed food within the previous twenty-four hours (101).

Salt reduction strategies

Salt reduction strategies are recommended to reduce the risk of hypertension or NCDs (102). The World Health Organization outlines five priority areas of salt reduction: monitoring and surveillance, reformulation of food products, regulations for labeling and marketing, increase consumer knowledge and awareness, and environmental changes to promote healthy eating (103), with multi-component strategies being most effective (104). Many countries around the world have undertaken salt reduction efforts (105). For example, the United Kingdom has successfully lowered average population salt intake and blood pressure through voluntary strategies to reformulate foods to lower-sodium alternatives; other countries are relying on mandated strategies, such as taxation of high-salt foods (Portugal) or regulating front of pack labeling (Chile) (105). In sub-Saharan Africa, South Africa implemented comprehensive legislation in 2016, placing mandatory salt levels on a variety of processed foods; results from two years post-implementation indicate lower levels of urinary sodium excretion compared to urinary sodium excretion levels prior to implementation. However, one concern in South Africa has been that the reduced consumption of iodized salt also reduced iodine consumption, potentially increasing the risk of developing iodine deficiency disorders; preliminary results indicate that urinary iodine excretion did decrease after implementation of the salt reduction legislation (106), and additional monitoring of population iodine intakes was recommended. A proposed intervention to address widespread micronutrient deficiencies is the fortification of salt and salty condiments with multiple-micronutrients in addition to iodine (98,107). Given that salt intakes are high around the world, this intervention will need to address both micronutrient status and reduce salt intake. Thus, measuring and surveilling population salt intake and understanding dietary sources will be essential.

Measurement of dietary salt

Accurate measurement of dietary salt is needed to understand its implication in the development of hypertension or NCDs. Due to salt's ubiquity in many prepared foods (i.e., processed and ultra-processed foods), and as a common addition during cooking and/or at the table (108), many consumers are unaware of the sources of salt in their diet, complicating its assessment (104). In many LIC and LMIC where salt added in cooking is the primary source of dietary salt, variation in food preparation methods and household cooks add additional considerations when assessing salt intakes (104,108). To inform salt reduction strategies and reduce the risk of developing hypertension or other related health outcomes, high quality salt intake estimates are essential.

24-hr urine collection

There are 3 primary methods for assessing dietary salt intake: 24-hr urine collection to assess urinary sodium excretion, spot urine samples to predict 24-hr urinary sodium excretion, and dietary questionnaires to assess dietary salt consumption, such as 24-hr dietary recalls or food frequency questionnaires. As approximately 93% of dietary sodium is excreted in the urine (109), the gold standard salt assessment method is multiple 24-hr urine collections over a series of days in a representative sample of the population (110). Multiple 24-hr urine collections reduce bias due to diurnal variation in urinary sodium excretion compared to single 24-hr urine collections, reduce within- and between-person dietary sodium variability, and the estimates can be used for estimating an individual's usual sodium intake (110). However, collecting urine over 24-hrs places a large burden on participants, particularly among children. Rates of non-compliance and attrition among participants can be high in studies using this method (110,111).

Spot urine samples

Because of these challenges some studies elect to collect spot urine samples once or at multiple time points, and then use a predictive equation (such as the INTERSALT equation (112)) to estimate 24-hr urinary sodium excretion. Due to the variability in urinary sodium excretion, multiple spot urine samples per participant are preferred, though an exact number of samples has not been determined (111). One limitation of spot urine samples is that estimations of salt (or sodium) from spot urine samples have been found to systematically overestimate 24-hr urinary sodium excretion in populations with low salt intake levels, and systematically underestimate 24-hr urinary sodium excretion among populations with high salt intake levels (111). Though the WHO suggests collecting spot urine samples for population salt intake assessment and monitoring when 24-hr urine sample collection is not possible (113), usage of spot urine sample data for predicting salt intake should be restricted to classifying a population above or below a threshold, for example the 5 g/d of salt threshold (111). Spot urine samples should not be used to predict clinical outcomes due to within-person variability in urinary sodium excretion and low precision when using predictive equations (114,115).

Dietary data

Many nutrition surveys collect salt intake data through food frequency questionnaires or 24-hr dietary recalls (116,117). Benefits of these methods include allowing for examination of dietary trends and dietary sources of salt, and estimating the proportion of salt intake from discretionary salt (i.e., table salt) or salt from processed and ultra-processed foods. These data are useful for assessing dietary patterns and potential points of intervention to reduce the risk of diet-related NCDs (118). While these methods are subject to misreporting and recall bias by survey participants, intensive training for those administering dietary recalls and innovations in collection methods, such as via electronic tablet-based sources, can reduce errors in dietary data collection (119,120). A review and meta-analysis comparing

24-hr urine and 24-hr diet recall methods to estimate dietary sodium intake for population monitoring and assessment found that when compared to 24-hr urine collection, 24-hr dietary recalls underestimated mean sodium intake by approximately 600 mg/d (121). However, the review also stated that studies that employed the multipass method of 24-hr dietary recall (i.e., a detailed methodology wherein interviewers iteratively gather dietary data in increasingly more specific 'passes') had smaller differences in mean sodium estimates than studies that did not employ the multipass method (121). Thus, while multiple 24-hr urine collections may be the most precise method to assess dietary salt intake, detailed 24-hr dietary recalls can provide reasonable estimates with consideration of the methodological limitations, such as additional probing for salt added during cooking or at the table to improve participant recall accuracy.

Detailed dietary data is lacking in many countries throughout the world, often due to its complicated data collection and analysis methodology, such as with the multipass 24-hr recall. In the absence of dietary or biomarker data, household-level data from Household Income and Expenditure Surveys and a variety of other household surveys that are completed periodically in the majority of LIC and LMIC may be used to generate estimates of food and nutrient intake, such as salt or sodium (122). These surveys generally provide data on household purchases of specific staple foods, including but not limited to how much of a food item was purchased, the cost of that quantity of purchased food, and the duration of time that quantity of food typically lasts (123). This information yields a household's estimated consumption of a specific food or nutrient, and while the data can be 'noisy' (e.g., household members may misreport costs, quantities, and duration), it is helpful for estimating a population's food consumption patterns when detailed dietary data are not available.

Additionally, for nutrition policymaking and program development that prioritizes specific population subgroups, for example children, adolescents, or adult women, estimating individual consumption behaviors within households is useful. One method of disaggregating household-level food

and nutrient consumption data to individual 'apparent intake' data is through the Adult Male Equivalent (AME) method. The AME method assigns an adjustment factor (AME unit) to each member of a household based on sex- and age-specific energy expenditure and energy requirements, where adult males have an AME value of 1.0 and are the reference category (i.e., AME=1 for adult males aged 19-30 years, AME=0.7 for adult females aged 19-30 years, and AME=0.5 for children) (124). This method assumes that food is shared within a household based on individual energy needs, and the calculation yields apparent intake of a food or nutrient by demographic group. However, food is not always shared equitably among household members with male household members often receiving more and/or higher quality food than their female counterparts (125), and the AME method has also been shown to systematically underestimate individual consumption (126). Regardless, the method is useful for using household-level data to generate baseline (or "proxy" (122)) estimations of food and nutrient consumption among population subgroups. For example, it may be used to generate broad estimates of salt consumption above or below the 5 g/d threshold.

In summary, there are multiple methods of salt measurement available, and the method selection should be determined by the study objectives and priorities for data usage. Use of spot urine samples or household-level data estimations may be useful in population assessments and monitoring of salt consumption, but are limited in their precision and ability to predict clinical outcomes.

Summary and Research Gaps

Conditions of both undernutrition and overnutrition contribute to NCD risk globally. High prevalence of OWOB is common worldwide, and associated conditions such as inflammation and micronutrient deficiencies may require additional consideration when evaluating OWOB prevalence and intervention or programmatic impacts. Globally, higher than recommended salt intakes likely contribute to the high prevalence of hypertension and to increased cardiovascular disease morbidity and mortality.

Strategies that simultaneously address all forms of malnutrition ('Double-Duty Actions') are needed to address these public health priorities, and to meet the Sustainable Development Goals, including accurate assessment and evaluation of conditions contributing to NCD morbidity (127,128).

Previous analyses have highlighted the influence of inflammation on iron and vitamin A status in different populations (58,129), as well as the prevalence and independence of intra-individual OWOB and micronutrient deficiencies among children and adult women (130,131). However, these analyses focused on inflammation generally and did not explore weight status as a source of inflammation. In Chapters 2 and 3, I build upon these prior analyses to examine whether the relationship between BMI and micronutrient status indicators may be influenced by inflammation. This research will broaden our understanding of the extent to which adiposity-related inflammation may influence interpretation of micronutrient assessment in contexts with varying etiologies of inflammation.

In Ghana, salt iodization programs successfully reduced iodine-deficiency disorders (132), and fortification of other condiments such as bouillon with multiple micronutrients is being considered as an additional intervention to address micronutrient deficiencies (133). Modeling results indicate that multiple-micronutrient fortified bouillon has the potential to be cost-effective and adequately reach the majority of the population in Ghana; however, salt reduction efforts likely need to be undertaken simultaneously (107). In Chapter 4, I estimate salt consumption among households, women, and children in two districts in the Northern Region, Ghana, including the proportion of salt consumed from bouillon, to inform a planned randomized controlled trial testing multiple-micronutrient fortified bouillon cubes. With mixed-methods methodology using data from a pilot survey and focus group discussions, I identify factors, including knowledge, attitudes, and practices, associated with household salt consumption. These data will help inform nutrition and policy discussions related to salt in Ghana, including the development of salt-reduction behavior change communication strategies.

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CHAPTER 2: The relationship between ferritin and body mass index is mediated by inflammation among women in higher-income countries, but not in most lower-income countries nor among young children: a multi-country analysis[1-4]

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Abstract

Background: In the presence of inflammation, serum or plasma ferritin concentration ('ferritin') transiently increases, confounding its interpretation as an iron status marker. The extent to which adiposity-related inflammation may influence ferritin interpretation is uncertain.

Objective: To describe relationships between weight status, inflammation, and ferritin among non-pregnant women of reproductive age (15-49 years, WRA) and preschool-age children (6-59 months, PSC) with normal weight to overweight or obesity (OWOB) in differing geographic settings.

Methods: Cross-sectional data were separately analyzed from n=18 surveys (WRA) and n=25 surveys (PSC) from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project, excluding observations with underweight, wasting, pregnancy, or malaria. Relationships were assessed between BMI (WRA) or BMI-for-age z-score (BAZ, PSC), inflammatory biomarkers C-reactive protein (CRP) and/or α-1-acid glycoprotein (AGP), and ferritin by linear regression, and potential mediation by CRP and/or AGP in relationships between BMI or BAZ and ferritin with structural equation modeling. Regression and mediation models accounted for complex survey designs. Results were grouped by World Bank income classifications. Included Kenya trial data registered at http://clinicaltrials.gov, identifier NCT01088958.

Results: In 5 of 6 surveys among WRA from upper-middle and high-income countries, ferritin was significantly positively associated with BMI, and this relationship was partially (or fully in the United States) mediated by CRP and/or AGP. Mediation was present in 4 of 12 surveys for WRA in low- and lower-middle income countries. Among PSC, ferritin was positively associated with CRP and/or AGP in all surveys, but there were no significant CRP- or AGP-mediated relationships between ferritin and BAZ, except a negative relationship in the Philippines.

Conclusions: Where OWOB are common among WRA, measurement of inflammatory biomarkers and their use in interpreting ferritin may improve iron status assessment. While these relationships were inconsistent among PSC, inflammation was common and should be measured to interpret iron status.

Introduction

Epidemiological studies of health and nutrition status frequently measure plasma or serum ferritin concentration (hereafter referred to as 'ferritin') to evaluate a population's iron status (1). However, the presence of inflammation confounds iron status assessment by transiently increasing ferritin concentrations, potentially resulting in erroneous underestimates of iron deficiency (2). Thus, to accurately assess and interpret the ferritin results of a population assessment, researchers should measure inflammation biomarkers, identify potential sources of inflammation, and consider their influence on iron status assessment.

Sources of inflammation often differ by setting. For example, inflammation due to malarial illness or diarrheal disease may be more common in low-income and lower-middle income countries (LIC and LMIC) (3). In contrast, inflammation associated with overweight and obesity (OWOB) may be more common in upper-middle income and high-income countries (UMIC and HIC) (4), though OWOB are increasingly prevalent across global contexts (5,6). Inflammation is the body's physiological response to injury, illness, infection, or environmental insult, and is characterized by the presence of pro-inflammatory cytokines and acute-phase proteins (APP), such as C-reactive protein (CRP) and α-1-acid glycoprotein (AGP) (4). In obesity, excess adipose tissue, in particular visceral adipose tissue, releases pro-inflammatory cytokines and CRP, which then stimulate the production and release of APPs from hepatocytes, macrophages, and others, creating an environment of prolonged, low-grade systemic inflammation (7,8). Persistent inflammation may increase the risk of iron deficiency due to sustained disruptions to intestinal iron absorption and systemic iron distribution (9).

Research examining the relationship between iron status and inflammation due to illness and infection has largely concentrated on data from LIC and LMIC. This is, in part, because of the direct interaction of iron and pathogens (e.g., malaria, helminths) associated with common illnesses in many of

these countries (10–13). In contrast, literature examining adiposity and inflammation and adiposity and iron deficiency has largely been carried out in populations from UMIC and HIC (14–18). Much of the research on adiposity and iron status has yielded mixed results in the magnitude or direction of association of this relationship due to variation in evaluation methods (19,20), population assessed (21,22), and the degree of iron deficiency at the time of assessment (18,23). As obesity prevalence increases globally, research exploring the combined influence of inflammation and adiposity on iron status is needed in countries with and without a high infectious disease burden.

The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project previously presented analyses of national survey data from multiple countries highlighting the influence of inflammation in iron status assessment in different populations (24). The BRINDA project also established a statistical method to adjust for inflammation when measuring iron status (2,25–27), an approach adopted by the World Health Organization in its most recent guidelines on the use of ferritin to assess iron status of populations (1). Additionally, the BRINDA project recently reported the prevalence and independence of intra-individual OWOB and iron deficiency among adult women and young children (28,29), and determined that in both groups, OWOB was not associated with iron deficiency based on inflammation-adjusted ferritin. However, this analysis estimated iron deficiency with inflammation-adjusted ferritin, and did not examine whether the relationship between BMI and iron status indicators may be influenced by inflammation.

Therefore, we analyzed data from the BRINDA project to explore the following objectives: 1) to determine the relationships between weight status (BMI), inflammation (CRP and/or AGP), and ferritin among adult women and young children with normal weight to OWOB in differing geographic settings; and 2) to examine whether inflammation mediates the relationship between BMI (women) or BMI-for-age z-score (BAZ, children) and ferritin in these same settings.

Methods

Data source and inclusion criteria

Using secondary de-identified data from nationally- or regionally-representative surveys from the BRINDA project, we analyzed 18 datasets from 17 countries with data on non-pregnant women of reproductive age (WRA, 15-49 years) and 25 datasets from 22 countries with data on preschool-aged children (PSC, 6-59 month). Criteria for survey inclusion in the BRINDA project, dataset harmonization, and methodology of anthropometric calculations and biochemical collection have been previously documented (28–31). For all surveys, all participants provided informed consent, referrals were made for severe anemia and/or severe acute malnutrition, participants did not directly benefit from their participation in the survey, and participants were not informed of the results of the current study. The Kenya data included in BRINDA were part of a clinical trial registered at http://clinicaltrials.gov, identifier NCT01088958.

Our inclusion criteria matched that of two previous analyses examining the intra-individual double burden of malnutrition among PSC(28) and WRA (29). Additional inclusion criteria specific to the present analyses were surveys that included a marker of inflammation (CRP and/or AGP) and measured serum or plasma ferritin. As the present analyses examined relationships between BMI, ferritin, and inflammation among populations with normal weight to OWOB, observations with BMI <18.5 kg/m^2 (WRA), or BAZ or weight-for-height z-score (WHZ) <-2 SD (PSC) were excluded due to concerns that individuals with underweight or wasting were likely to have inflammation from other sources, such as infectious diseases. Other excluded observations were those with zero values for ferritin, WRA who were pregnant or whose height or weight was outside the ranges of 101.6-219.9 cm and 22.7-222.2 kg, respectively (29), PSC with BAZ less than -5 SD or greater than 5 SD (28), and observations with a positive malaria result (to minimize the influence of inflammation from infectious disease; n=4 [WRA]

and n=8 [PSC] surveys measured malaria). Observations with other morbidity symptoms (i.e., reported fever or diarrhea) were not excluded because the variables were not reported uniformly across surveys, and prior BRINDA analyses showed that reported morbidity was not consistently associated with inflammation (32). We did not apply a sample size cutoff for excluding surveys after application of exclusion criteria; however, we did ensure the analytical sample sizes met criteria for mediation analysis (33). BAZ and WHZ were recalculated for the BRINDA database using the WHO growth standards (30). The proportion of observations excluded overall and by individual exclusion criterion are presented in **Supplemental Tables 2.1 and 2.2**.

Variable definitions

The outcome variables were unadjusted ferritin (μg/L), CRP (mg/L), and AGP (g/L); the predictor variables were BMI (kg/m^2, WRA) or BAZ (PSC), CRP (mg/L), and AGP (g/L). For consistency with prior BRINDA analyses (28), we applied BAZ to all age groups, though WHZ is recommended for use for children <24 months (34). Ferritin was measured in all surveys. CRP was measured in all WRA and n=21 PSC surveys, AGP in n=11 WRA and n=18 PSC surveys, and both CRP and AGP were measured in n=11 WRA and n=15 PSC surveys. For the mediation analysis, the outcome variable was ferritin, the predictor variable was BMI or BAZ, and the mediating variables were CRP and/or AGP.

Covariates were defined for each survey as reported by the survey representative, unless otherwise indicated (30): age (years [WRA] or months [PSC]); sex (PSC only); urban residence (compared to rural residence); high household socio-economic status (SES, the ordinal 3-category SES variable from the harmonized BRINDA dataset (30) was dichotomized into a binary variable of low SES versus high SES, where high SES included both medium and high categories); access to an improved water source (compared to no access or access only to an unimproved water source); access to an improved toilet (compared to no access or access only to an unimproved toilet); and high education level (compared to

no education or primary school only) measured as respondent (WRA) or maternal (PSC) education level except in surveys that reported household head education level (Burkina Faso [PSC, WRA], Colombia [PSC], Mexico 2006 [PSC, WRA], and the United States [PSC]). Covariates with more than two categories (SES, water, sanitation, and education level) were dichotomized for ease of interpretation.

The following variables described the nutrition and health status of the survey populations: BMI (WRA) categorized as normal (18.5-24.9 kg/m^2), overweight (25.0-29.9 kg/m^2), and obesity (≥30.0 kg/m^2); BAZ (PSC) categorized as normal (-2 to 2 SD), overweight (>2 SD ≤3 SD), and obesity (>3 SD); any inflammation defined as CRP >5 mg/L and/or AGP >1 g/L; iron deficiency defined as inflammation-adjusted ferritin <15 µg/L (WRA) or <12 µg/L (PSC).

Statistical analyses

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, North Carolina, USA) and STATA version 16 (StataCorp, College Station, Texas, USA). All analyses accounted for complex survey designs (cluster and strata) by calculating variance estimates and applying survey weights, except Mongolia which used simple random sampling. Analyses were conducted separately by survey and by population using continuous variables available in each dataset. P-values were considered statistically significant at $p<0.05$. Covariates available for analysis are listed by survey in **Supplemental Table 2.3**.

Means (95% confidence interval [CI]) are presented for continuous variables, except CRP, AGP, and ferritin are presented as geometric means (95% CI) due to their non-normal distributions, and age is presented as the median (interquartile range); percent (95% CI) are presented for categorical variables. For descriptive analyses, ferritin and iron deficiency were adjusted for inflammation following the BRINDA regression correction approach (2,30). Previous BRINDA work has examined the impact of this adjustment on estimates of iron deficiency prevalence, and found the prevalence increased after adjustment (2,25,26).

Bivariate and multivariable analyses

In surveys for which >30% of the analytical sample values had a single low value (representing the lower limit of detection in the lab analysis), we generated and applied a random number between 0 and the lowest value (Afghanistan [PSC], Colombia [WRA and PSC], Georgia [PSC], and Zambia [PSC]). For example, more than half of the CRP values in the surveys from Colombia were reported as 0.2 (WRA n=4838, 58% of analytical sample; PSC n=2476, 66%); for these observations we generated and applied a random value between 0 and 0.2 for CRP (35,36), and confirmed that the direction and strength of association did not differ with randomly generated values by re-running all regression models with multiple random seeds. For all surveys, ferritin, CRP, and AGP were natural log (*ln*) transformed to achieve normal distributions and the residuals visually examined. For any surveys where the distribution of residuals appeared abnormal after transformation of the outcome variables, a sensitivity analysis compared the Spearman's rank correlation coefficient with the bivariate linear regression estimate, and the results examined to determine the appropriateness of continuing with linear regression analyses. All regression analyses were conducted with the unadjusted ferritin variable.

We assessed the following bivariate linear regression models in each survey:

1. $Y(ln\text{Ferritin}) = B_0 + B_1(\text{BMI})$*
2. $Y(ln\text{CRP}) = B_0 + B_1(\text{BMI})$*
3. $Y(ln\text{AGP}) = B_0 + B_1(\text{BMI})$*
4. $Y(ln\text{Ferritin}) = B_0 + B_1(ln\text{CRP})$
5. $Y(ln\text{Ferritin}) = B_0 + B_1(ln\text{AGP})$

*PSC models used BAZ in place of BMI.

Relationships between the previously defined covariates and outcome variables were assessed in separate bivariate linear regression analyses, with marginally significant covariates (at least $p<0.10$) included in multivariable adjusted models. Models were assessed for collinearity with variance inflation factors (>5) and tolerance (>0.1). CRP and AGP variables were analyzed in separate models, rather than combined into a single variable, in order to examine the individual associations between CRP or AGP with iron and inflammation status. As pre-specified in our analysis plan, all bivariate WRA analyses were also completed in stratified analyses by age (15-29 years and 30-49 years) as inflammation due to chronic disease has been associated with age (37). For consistency with prior BRINDA analyses, PSC surveys were stratified separately by age (6-23 months, 24-59 months) and by sex (28).

Results are presented grouped by World Bank country income-level classifications (LIC, LMIC, UMIC, HIC) according to their ranking at the time the survey was conducted (38). While the country groupings do not imply that the results may be generalized by income classification, the groupings have historically represented differences in potential sources of inflammation among populations with regard to non-communicable and communicable disease burdens (39,40). We also examined heterogeneity among surveys to decide whether to proceed with pooling by income classification. Within each income-level, we tested for heterogeneity by constructing pooled regression models using the individual-level data from each survey, and evaluating the interaction between the survey and predictor variables.

Mediation analysis

We used mediation analysis to test the hypothesis that inflammation mediates part or all of the relationship between BMI or BAZ and ferritin among WRA or PSC with normal to elevated BMI or BAZ. Mediation analyses were conducted using Structural Equation Modeling (SEM) procedures in STATA according to the path diagram, with the path through CRP or AGP eliminated in surveys without the variable (**Figure 2.1**). Mediation was considered present if both the total effect (the effect of BMI or BAZ

on ferritin) and the indirect effect (the effect of BMI or BAZ on ferritin as mediated by the effect of CRP and/or AGP) were significant at $p<0.05$ (41,42). Linearized standard errors and 95% CI were generated and adjusted for the complex survey design by calculating variance estimates and applying survey weights. Final mediation estimates were exponentiated, and adjusted mediation results are presented as percent change in ferritin concentration for every 1-unit change in BMI or BAZ. Survey-specific SEM models were adjusted for the same marginally significant covariates found in bivariate models. All mediation analyses met the minimum sample size requirement of at least 10 observations per linear relation (i.e., n=40 observations for models with CRP or AGP only; n=50 observations for models with both CRP and AGP) (33).

Sensitivity analyses

We conducted sensitivity analyses by including values for BMI <18.5 kg/m^2 (WRA) or BAZ/WHZ <-2 SD (PSC) for surveys where greater than 50% of the observations were excluded due to our pre-specified exclusion criteria (n=5 surveys WRA; n=8 surveys PSC). Sensitivity mediation analyses were also conducted in datasets with available malaria data (n=4 surveys WRA; n=8 surveys PSC) to examine the inflammation-mediated relationship between BMI or BAZ and ferritin among all individuals versus those without a positive malaria result. An additional sensitivity mediation analysis was conducted in a subset of surveys to assess the effect of excluding observations with reported morbidity symptoms (fever or diarrhea in the past 24 hours [Kenya 2010, PSC] or past 2 weeks [Liberia, PSC; Côte d'Ivoire and Malawi, WRA]; results were compared qualitatively.

Ethical approval and human subject research protocol

The BRINDA protocol was reviewed by the institutional review boards of the National Institute of Health and was deemed to be non-human-subjects research.

Results

Participant characteristics

Among WRA, the analytical sample size ranged from n=61 (Burkina Faso) and n=147 (India) to n=8300 (Colombia), with a total sample size of 33,429. The median age was approximately 30 years. The proportion of the analytical sample residing in rural areas ranged from 20.8% (Mexico 2012) to 89.7% (Malawi); the Indian and Nigerian surveys were conducted in rural areas only. In surveys with SES data (n=14), more than 60% of the population was classified as high SES, except in Mexico (2006) where the high SES proportion was 53% (**Supplemental Table 2.4**).

Among PSC, the analytical sample size ranged from n=63 (Burkina Faso) to n=5824 (Pakistan), with a total sample size of 28,727. Most surveys (n=13) included children 6-59 months of age, while other surveys included the following age ranges: Bangladesh (2010), 6-24 months; Kenya (2007, 2010), Liberia, and Mongolia, 6-35 months; Vietnam, 10-59 months; Cameroon, Georgia, and Mexico (2006, 2012), 12-59 months; and Burkina Faso and the Philippines, 24-59 months. In the analytical sample, more than 80% of participants resided in rural areas in Cambodia, Laos, Malawi, and the Philippines; the surveys from Kenya (2007 and 2010) and Nigeria were conducted in rural areas only. Among surveys that measured SES (n=18), the proportion of the analytical sample classified as low SES ranged from 5.1% (Afghanistan) to 84.0% (Philippines) (**Supplemental Table 2.5**).

Prevalence of OWOB, iron deficiency, and inflammation among WRA

Overall, the prevalence of OWOB among WRA was generally greater in UMIC and HIC than LMIC and LIC, with the greatest prevalence in Mexico 2012 (UMIC) (72% [CI: 69.0, 75.1]). The prevalence of inflammation-adjusted iron deficiency among WRA ranged from 20.8% to 44.3% in UMIC and HIC, and from 2.0% to 63.9% in LIC and LMIC. The prevalence of inflammation (elevated CRP and/or AGP) ranged

from 16.0% to 34.9% among surveys from UMIC and HIC, and from 7.1% to 74.5% among surveys from LIC and LMIC **(Table 2.1).**

Prevalence of OWOB, iron deficiency, and inflammation among PSC

Among PSC, prevalence of OWOB was ≤11.6% across all surveys from LIC and LMIC, except in Georgia (19.6 [16.7, 22.6]). Among UMIC and HIC, Azerbaijan had the greatest prevalence of OWOB at 15.6% (12.7, 18.5). Across income classifications and world regions, the prevalence of inflammation-adjusted iron deficiency among PSC varied, ranging from 0.6% [0.2, 1.0] in Georgia to >60% in Kenya (2007 and 2010), Nicaragua, and Pakistan. The prevalence of any inflammation ranged from 10.7% to 92.4% among surveys from LIC and LMIC, and from 6.1% to 30.8% among UMIC and HIC. **(Table 2.2).**

Associations between BMI, ferritin, and inflammation among WRA

Results from adjusted linear models that examined the relationship between BMI and ferritin varied among income classifications (**Supplemental Table 2.6**). Greater BMI was consistently and significantly associated with greater ferritin concentration in all surveys from UMIC and HIC (n=6, Azerbaijan, Colombia, Mexico 2006, Mexico 2012, United Kingdom, United States), and in 4 of 12 surveys from LIC and LMIC (Cambodia, Laos, Georgia, and Pakistan). Among UMIC and HIC, the percent increase in ferritin concentration for every 1-unit increase in BMI ranged from 1.0% (95% CI: 0.3%, 1.7%) (USA) to 3.5% (2.5%, 4.4%) (Azerbaijan), and ranged from 0.8% (0.1%, 1.5%) (Pakistan) to 5.5% (2.4%, 8.7%) (Laos) among the 4 LIC and LMIC surveys. BMI was also significantly positively associated with inflammation (CRP and/or AGP) across all surveys except Afghanistan, Burkina Faso, and Nigeria (Supplemental Table 6). Ferritin was significantly positively associated with inflammation across all surveys except in Malawi where the relationship was positive but non-significant (CRP only, Supplemental Table 2.6). Stratified results by age are presented in **Supplemental Table 2.7**, however no obvious trends emerged.

Associations between BAZ, ferritin, and inflammation among PSC

Among PSC, the relationship between BAZ and ferritin varied across surveys and income classifications in the adjusted models (**Supplemental Table 2.8**). BAZ was significantly negatively associated with ferritin in 5 of 19 surveys from LIC and LMIC (Bangladesh (2010), Nicaragua, Pakistan, the Philippines, and Vietnam), and 2 of 5 surveys from UMIC and HIC (Mexico 2012 and the US). Among these surveys, the percent change in ferritin concentration for every 1-unit increase in BMI ranged from -3.5% (-6.2%, -0.8) (Pakistan) to -11.8% (-17.9, -5.3%) (Vietnam). BAZ was significantly positively associated with ferritin in Colombia. In the majority of other surveys (n=11), the relationship between BAZ and ferritin was negative but non-significant. BAZ was significantly positively associated with CRP in the surveys from Malawi (LIC) and the USA (HIC). In all other surveys (n=23), the relationship between BAZ and inflammation (CRP and/or AGP) was non-significant and the direction of association varied. Across all surveys, greater ferritin was significantly associated with greater inflammation, except in Georgia; in Afghanistan, only the relationship with CRP was significant, and in Burkina Faso only the relationship with AGP was significant (Supplemental Table 2.8). Stratified results by age and sex are presented in **Supplemental Tables 2.9 and 2.10**. There was variation by age in relationships between inflammation and BAZ and between ferritin and inflammation in some surveys. In the surveys from Cambodia, Côte d'Ivoire, Nicaragua, and Nigeria, the relationship between inflammation and BAZ changed from negative to positive as age increased.

Mediation analysis between ferritin, BMI, and inflammation

In 9 of 18 surveys included in the WRA analyses, inflammation partially and positively mediated the relationship between ferritin and BMI in adjusted models (**Figure 2.2, Table 2.3**). Among LIC and LMIC surveys with mediation by inflammation, the percentage of the total effect mediated by inflammation was on average 27%. Among UMIC and HIC, the surveys in Azerbaijan and Mexico (2006

and 2012) had similar mediated effects of 60-70%, while Colombia's mediated effect was 19%. In the US, 100% of the total effect was mediated by inflammation. Among four surveys which measured both CRP and AGP, CRP accounted for >50% of the mediated effect in Laos and Azerbaijan, but in Cambodia and Pakistan, AGP accounted for >50% of the mediated effect. No mediation was present in the survey from Vietnam after adjusting for age.

No significant mediated relationships emerged among PSC, except in the Philippines where the mediated relationship was negative, suggesting inconsistent mediation (-2% [95% CI: -3.7, -0.2] (**Table 2.4**). Unadjusted mediation results for both WRA and PSC are presented in **Supplemental Table 2.11.**

Sensitivity analyses

Overall, pooled analyses and generation of pooled estimates were not possible due to extensive heterogeneity in all WRA and PSC surveys, with the exception of one model (CRP=BMI) for the WRA LIC pooled grouping (pooled estimate β=-0.02, p=0.2; data not presented). Among surveys that measured malaria status (n=4 WRA, n=8 PSC), results from the sensitivity mediation analysis that included all observations regardless of malaria test result were similar to the main mediation results (**Supplemental Table 2.12**). Results were also similar to the main results in sensitivity analyses that included all values for BMI<18.5 kg/m^2 (WRA) or BAZ/WHZ<-2 SD (PSC) in surveys where greater than 50% of the observations were excluded due to our pre-specified exclusion criteria, and among surveys with the additional excluded morbidity data of reported fever or diarrhea (data not presented).

Discussion

We explored relationships between BMI or BAZ, inflammation, and ferritin concentrations in 18 datasets from 17 countries with data on WRA, and 25 datasets from 22 countries with data on PSC, with data drawn from both high- and low-income contexts according to World Bank classifications. We found

that for WRA with BMI >18.5 kg/m^2 residing in UMIC and HIC, greater ferritin was associated with greater BMI, and this relationship could be partially (or fully in the case of the US survey) explained by the inflammatory markers CRP or AGP. This pattern was present in fewer surveys for WRA in LIC and LMIC, and was often not mediated by the presence of inflammation. Among PSC, greater ferritin was significantly associated with greater inflammation in all but two surveys; however, significant associations between ferritin and BAZ and inflammation and BAZ were not observed. Additionally, neither CRP nor AGP mediated the relationship between ferritin and BAZ among children, except in the Philippines, where the relationship was negative.

Our findings suggest that in settings where OWOB are common among WRA, measurement of inflammatory biomarkers and their use in interpreting ferritin concentrations may improve iron status assessment, even where infections are less common or less severe. These findings may also be helpful in clinical settings as ferritin concentration is the most commonly used measurement of iron status (43–46). While some clinical guidelines suggest including a measure of inflammation to interpret iron status when inflammatory conditions are present, often OWOB is not highlighted as contributing to inflammation (43–46). Further research is needed for individual patient care in clinical settings as cases of iron deficiency may be missed among WRA with OWOB if inflammation is not measured or accounted for in interpretation of ferritin concentrations. Additional consideration of the impacts of inflammation on iron status assessment and potential iron deficiency may be necessary in individuals with anemia of chronic disease (37,47).

Among WRA, the prevalence of OWOB was greater than 20% in the majority of surveys where inflammation mediated the relationship between ferritin and BMI, suggesting that inflammation associated with adiposity may influence iron status assessment. This is consistent with literature suggesting that adiposity and inflammation — primarily measured by CRP — are strongly correlated in WRA (48–50), which may explain our result from the US survey where inflammation (measured only

with CRP in this survey) explained 100% of the inflammatory effect. In our analyses, where mediation was present and both CRP and AGP were measured (WRA only: Azerbaijan, Cambodia, Laos, and Pakistan), AGP explained 3-21% of the inflammatory effect in each case. Previous literature suggests that CRP and AGP reflect different phases of the inflammatory acute phase response, with CRP levels rising and falling quickly after the initial insult, and AGP levels rising later, and staying elevated longer, potentially indicating longer-term or chronic inflammation (51). This framework does not translate easily to OWOB or other chronic conditions where inflammation is not the response to a single infection, and is not consistent with observations of associations between OWOB and CRP. Moreover, a study comparing the kinetics of CRP and AGP found similar inflammatory response patterns between the two proteins in relation to ferritin (52). Further research that includes AGP and other APPs and adipocytokines may help to understand which ones are most useful for iron assessment, as inflammatory biomarkers such as interleukin-6 and α-1-antitrypsin have been shown to be important in characterizing inflammation associated with obesity (4,7,49). Measurement of hepcidin may also provide insight into the corresponding effects on iron metabolism.

Among PSC, little evidence of mediation was present, and we did not see much relationship between BAZ and inflammation, likely due to the overall low prevalence of OWOB across PSC surveys. Although our results indicate that OWOB was not strongly associated with inflammation among PSC, we observed a strong relationship between inflammation and ferritin, which likely indicates a burden of inflammation from causes other than OWOB, and underscores the need to measure inflammation when assessing iron status among PSC. An additional consideration is many countries globally are not on track to meet the World Health Assembly nutrition target for 2025 to prevent increases in child OWOB (5,53), which compels increased monitoring of indicators of body composition and inflammation in this group. Literature examining iron deficiency, inflammation, and adiposity among WRA and PSC suggests

adiposity could be a risk factor for iron deficiency, and thus, iron deficiency should be monitored, particularly in countries with rapidly increasing OWOB (14,54).

We grouped our findings by country income classifications to aid interpretation of findings by likely sources of inflammation (i.e., greater BMI and lower prevalence of infections in UMIC/HIC), under the assumption that inflammation due to common infectious illnesses such as malarial or helminth infections would be more common in LIC and LMIC, and inflammation due to OWOB would be more common in UMIC and HIC (4). We recognize that sources of inflammation and patterns of malnutrition are not uniform across or within these groupings. However, the groupings did allow for a pattern to emerge among WRA: inflammation mediated the relationship between BMI and ferritin in all four UMIC surveys (Azerbaijan, Colombia, and Mexico 2006 and 2012). One interpretation of these findings is that these countries have a high prevalence of OWOB alongside persistent but decreasing prevalence of infectious diseases (e.g., malaria and diarrhea) that lead to inflammation (39). Similarly, CRP entirely explained the relationship between ferritin and BMI among WRA in the USA, where infectious illnesses prevalent in LIC and LMIC are less common, but the OWOB prevalence is high. Thus, interpretation of iron status in the context of inflammation is necessary in settings of high BMI, such as HIC, as is continued surveillance of iron deficiency, as chronic inflammation may increase the risk of iron deficiency over time (37). This recommendation is in line with literature examining the likelihood of overlapping iron deficiency and OWOB across global settings (55–59). We did not find that inflammation mediated the relationship between ferritin and BMI in the UK survey, the only other HIC, though the survey prevalence of OWOB was 50% and iron deficiency was 29%. We found no obvious explanation for this observation, which warrants further investigation.

A limitation of our analyses was the use of BMI alone as an indicator of adiposity, though other direct measures, such as waist circumference, waist-to-hip ratio, or skinfold thickness have been found to be highly correlated with BMI when predicting cardiometabolic risk (60). Of greater consideration is

the relation of inflammation to the distribution of adipose tissue, as abdominal obesity has been found to be strongly related with inflammation among adult women (61,62), and among children and adolescents (16,63), as well as having significant influence on iron status.(62) Additionally, we were limited in our choice of inflammatory markers to CRP and AGP. For example, in the Colombia survey specifically, the low levels of inflammation made it difficult to assess the relationship between CRP and other factors. Though CRP and AGP are often measured in health surveys (64,65), different biomarkers, such as the APP interleukin-6 or the hepatic hormone hepcidin, have also been reported as elevated among individuals with obesity (57,62), and their inclusion may help further characterize the relationships between iron status markers and inflammation.

Another limitation is the loss of sample size in some surveys due to our exclusion criteria. Though our exclusion criteria were selected *a priori* to align with our research objective, in some surveys, such as India (WRA only), Burkina Faso (WRA, PSC), and Afghanistan (WRA, PSC), the exclusion of participants with underweight or wasting and those that tested positive for malaria resulted in a >80% loss of sample size. For these surveys, we interpreted the results with caution. Additionally, the reduced sample may have biased some results towards the null hypothesis if a large proportion of the analytical sample was excluded. To examine the potential effects the loss of sample size had on our outcomes, we conducted sensitivity mediation analyses that included all malaria observations, with results similar to our main results. We also conducted sensitivity analyses including all observations categorized as underweight or wasted in surveys where >50% of the observations were excluded; these observations were originally excluded to avoid U-shaped distributions (and violation of regression assumptions) as conditions of underweight are also inflammatory. Sensitivity analyses that included these observations did not change the analysis linearity, and in retrospect, their exclusion may not have been necessary. However, their exclusion allowed us to focus our analyses on our population of interest:

those with normal weight to OWOB. Finally, the cross-sectional nature of the data prevented temporal interpretations.

Conclusion

It has become standard to include measures of inflammation to interpret iron status in large nutrition and health surveys in LIC and LMIC, mainly due to the role of infections in systemic inflammation, but the role of adiposity and its associated inflammation in iron status assessment has been less clear. Our findings suggest that adiposity may affect iron status assessment, particularly among WRA in countries where prevalence of OWOB is high, an observation that has implications in both public health and clinical settings. These results suggest that inflammation should be measured and considered alongside ferritin in assessment of iron status in contexts where OWOB is common, even if inflammation related to infections is expected to be low. While the results were not consistent with adiposity-related inflammation influencing iron status assessment among children, inflammation was nevertheless common among children and should continue to be measured to interpret iron status. With the nutrition transition persisting in LIC and LMIC, and the prevalence of OWOB increasing across the globe (5,40), the contribution of adiposity-related inflammation may become more important, and other potential sources of inflammation beyond infections likewise merit exploration.

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Authors contributions to the manuscript

A.W., M.Y., P.S. and R.E.S. were responsible for designing the research; J.N.D. performed statistical analyses and wrote the paper; R.E.S. had primary responsibility for the final content; and all authors: contributed to interpretation of the results, revision of the manuscript, and read and approved the final manuscript.

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Tables

Table 2.1. Biological and nutritional characteristics for women of reproductive age (15-49 years) with normal weight to overweight /obesity by survey: BRINDA project[1]

CIC[2]	Country, survey year	n	BMI, kg/m^2 mean (95% CI)	Overweight/ obesity[3] % (95% CI)	CRP, mg/L mean (95% CI)	AGP, g/L mean (95% CI)	Any inflammation[4] % (95% CI)	Ferritin[5], µg/L mean (95% CI)	Iron Deficiency[6] % (95% CI)
Low	Afghanistan, 2013	571	25.1 (24.6, 25.6)	42.0 (35.9, 48.1)	0.7 (0.5, 0.8)	0.7 (0.7, 0.7)	18.1 (13.9, 22.4)	24.1 (20.4, 27.8)	30.2 (23.3, 37.1)
	Burkina Faso, 2010	61	20.8 (20.0, 21.7)	3.1 (0.0, 7.8)	1.8 (1.3, 2.2)	1.2 (1.1, 1.3)	74.5 (63.5, 85.5)	31.9 (25.6, 38.3)	14.0 (2.9, 25.1)
	Cambodia, 2014	609	22.9 (22.5, 23.3)	22.5 (17.1, 27.9)	0.8 (0.7, 0.9)	0.7 (0.7, 0.8)	35.9 (25.9, 45.8)	57.8 (54.5, 61.1)	3.5 (1.8, 5.2)
	Côte d'Ivoire, 2007	706	23.5 (23.1, 23.8)	26.0 (22.3, 29.8)	1.5 (1.3, 1.7)	0.8 (0.8, 0.8)	31.9 (27.9, 35.9)	26.7 (24.4, 29.0)	23.4 (19.6, 27.3)
	Laos, 2006	690	22.4 (22.0, 22.8)	17.1 (12.5, 21.7)	0.4 (0.3, 0.5)	0.7 (0.7, 0.7)	14.1 (10.8, 17.4)	28.9 (23.3, 34.5)	28.7 (22.0, 35.5)
	Malawi, 2016	594	22.5 (22.1, 23.0)	17.2 (13.3, 22.0)	0.7 (0.6, 0.8)	0.6 (0.6, 0.6)	11.7 (8.4, 15.1)	33.0 (30.3, 35.7)	14.1 (10.3, 17.9)
Low-middle	Cameroon, 2009	594	24.7 (24.3, 25.1)	39.0 (34.4, 43.6)	0.9 (0.8, 1.1)	0.7 (0.7, 0.8)	16.3 (13.2, 19.4)	25.7 (24.3, 27.0)	19.2 (15.6, 22.7)
	Georgia, 2009	1605	26.0 (25.6, 26.3)	46.3 (43.2, 49.5)	2.2 (2.0, 2.4)	--	30.6 (27.5, 33.7)	99.2 (93.9, 104.4)	2.0 (1.1, 3.0)
	India, 2011	147	21.5 (20.9, 22.0)	12.9 (6.2, 19.7)	0.5 (0.3, 0.6)	0.8 (0.8, 0.8)	21.1 (15.4, 26.8)	10.8 (9.3, 12.3)	63.9 (57.8, 70.1)
	Nigeria, 2012	506	24.2 (23.6, 24.7)	35.2 (29.5, 40.9)	1.6 (1.4, 1.8)	0.7 (0.7, 0.8)	25.7 (21.6, 29.8)	31.3 (28.3, 34.3)	17.0 (12.8, 21.2)
	Pakistan, 2011	5004	24.4 (24.2, 24.5)	36.5 (34.6, 38.3)	1.0 (0.9, 1.0)	0.8 (0.8, 0.8)	32.5 (30.8, 34.3)	18.4 (17.8, 19.0)	42.1 (40.3, 44.0)
	Vietnam, 2010	1178	21.7 (21.6, 21.9)	10.0 (8.2, 11.8)	0.9 (0.8, 0.9)	--	7.1 (5.9, 8.4)	37.6 (34.6, 40.7)	17.9 (15.1, 20.7)
Upper-middle	Azerbaijan, 2013	2528	26.8 (26.5, 27.1)	57.3 (54.7, 59.8)	1.1 (1.1, 1.2)	0.9 (0.9, 0.9)	34.9 (32.5, 37.4)	16.2 (15.3, 17.1)	44.3 (41.7, 46.8)
	Colombia, 2010	8300	25.2 (25.1, 25.3)	44.4 (43.0, 45.8)	0.4 (0.4, 0.4)	--	22.1 (20.9, 23.4)	26.2 (25.5, 26.9)	24.4 (23.1, 25.6)
	Mexico, 2006	2910	27.7 (27.4, 28.1)	65.3 (62.4, 68.3)	1.9 (1.8, 2.0)	--	24.8 (22.2, 27.3)	18.0 (16.7, 19.3)	35.8 (32.7, 38.9)
	Mexico, 2012	3540	28.7 (28.3, 29.1)	72.0 (69.0, 75.1)	1.8 (1.7, 2.0)	--	21.2 (18.6, 23.9)	15.3 (14.3, 16.3)	42.7 (39.3, 46.1)

High	United Kingdom, 2014	862	26.5 (25.9, 27.1)	49.6 (44.5, 54.8)	2.2 (2.1, 2.4)	--	16.0 (12.8, 19.2)	23.5 (21.6, 25.4)	28.9 (24.4, 33.3)
	United States, 2006	3024	27.9 (27.4, 28.5)	56.3 (52.7, 59.9)	1.8 (1.7, 1.9)	--	26.3 (24.1, 28.5)	28.6 (27.5, 29.8)	20.8 (18.7, 22.8)

[1]CRP, AGP, and ferritin values presented as geometric mean (95% CI) due to non-normal distributions. All estimates account for survey design variables (cluster, strata, weight). '--' indicates the variable was unavailable in that survey. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. AGP, α-1-acid glycoprotein; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CI, confidence interval; CIC, country income classification; CRP, C-reactive protein.

[2]Country income classification (CIC) defined according to the World Bank definition for the year in which the survey was conducted (38).

[3]Overweight/obesity defined as a BMI ≥25.0 kg/m^2.

[4]Any inflammation defined as CRP >5 mg/L or AGP >1 g/L.

[5]Ferritin measured in plasma or serum, as reported in the survey.

[6]Iron deficiency defined as serum or plasma ferritin concentration <15 µg/L, adjusted for inflammation using the BRINDA regression correction approach (2).

Table 2.2. Biological and nutritional characteristics for preschool-aged children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

CIC[2]	Country, survey year	n	BAZ mean (95% CI)	Overweight/ Obesity[3] % (95% CI)	CRP, mg/L mean (95% CI)	AGP, g/L mean (95% CI)	Any inflammation[4] % (95% CI)	Ferritin, µg/L[5] mean (95% CI)	Iron Deficiency[6] % (95% CI)
Low	Afghanistan, 2013	595	0.2 (0.1, 0.3)	7.1 (3.8, 10.3)	0.3 (0.2, 0.3)	0.8 (0.8, 0.8)	10.7 (7.1, 14.3)	21.7 (18.9, 24.4)	30.3 (25.8, 34.9)
	Bangladesh, 2010	1179	-0.6 (-0.7, -0.5)	2.1 (1.3, 2.9)	0.8 (0.6, 0.9)	0.9 (0.9, 0.9)	34.4 (30.8, 38.1)	23.8 (22.2, 25.4)	25.1 (21.7, 28.6)
	Bangladesh, 2012	368	-0.4 (-0.6. -0.2)	4.3 (1.1, 7.6)	0.7 (0.6, 0.9)	0.8 (0.8, 0.9)	27.8 (20.0, 35.5)	25.0 (21.7, 28.3)	18.8 (11.2, 26.4)
	Burkina Faso, 2010	63	-0.1 (-0.5, 0.3)	1.5 (0, 5.2)	4.9 (2.9, 6.9)	1.6 (1.5, 1.7)	92.4 (87.0, 97.7)	32.2 (25.1, 39.3)	16.3 (0.9, 31.8)
	Cambodia, 2014	599	-0.4 (-0.5, -0.4)	0.8 (0, 1.7)	0.6 (0.5, 0.7)	0.8 (0.7, 0.9)	38.5 (30.5, 46.5)	43.8 (41.5, 46.1)	8.0 (5.8, 10.1)
	Côte d'Ivoire, 2007	435	0.1 (0.0, 0.2)	6.2 (3.7, 8.6)	2.0 (1.6, 2.4)	1.1 (1.1, 1.1)	59.3 (54.3, 64.3)	15.4 (14.0, 16.8)	48.6 (44.0, 53.3)
	Kenya, 2007	665	0.2 (0.2, 0.3)	4.2 (2.5, 5.9)	1.0 (0.8, 1.2)	1.1 (1.1, 1.1)	59.4 (54.6, 64.2)	7.0 (6.5, 7.6)	80.0 (76.7, 83.3)
	Kenya, 2010	551	0.3 (0.2, 0.3)	4.5 (3.0, 6.1)	0.9 (0.7, 1.1)	1.0 (0.9, 1.0)	47.9 (42.0, 53.8)	10.7 (9.8, 11.7)	64.4 (59.9, 69.0)
	Laos, 2006	443	-0.2 (-0.3, -0.1)	0.7 (0, 1.7)	0.5 (0.4, 0.7)	0.9 (0.9, 1.0)	43.2 (35.7, 50.8)	21.2 (18.7, 23.8)	32.1 (27.5, 36.6)
	Liberia, 2011	956	-0.1 (-0.2, 0)	2.5 (1.4, 3.7)	1.4 (1.2, 1.6)	1.0 (0.9, 1.0)	50.4 (45.9, 54.8)	12.3 (11.4, 13.2)	58.4 (54.1, 62.7)
	Malawi, 2016	748	0.1 (-0.1, 0.2)	5.3 (2.9, 7.8)	1.0 (0.8, 1.2)	1.0 (1.0, 1.1)	49.0 (42.5, 55.6)	24.2 (21.2, 27.2)	25.7 (18.4, 33.1)
	Mongolia, 2006	239	0.8 (0.7, 0.9)	10.5 (6.9, 15.1)	--	0.8 (0.8, 0.8)	24.7 (19.4, 30.7)	11.9 (10.4, 13.4)	55.7 (49.1, 62.1)
	Nicaragua, 2005	946	-0.9 (-1.0, -0.7)	5.1 (3.5, 6.8)	--	0.8 (0.8, 0.9)	26.9 (21.3, 32.6)	13.4 (12.3, 14.5)	60.1 (54.3, 65.9)
	Zambia, 2009	330	0.4 (0.3, 0.6)	6.1 (2.8, 9.3)	1.5 (0.8, 2.1)	1.0 (0.9, 1.0)	70.3 (66.5, 74.1)	27.8 (25.0, 30.6)	24.2 (16.0, 32.5)
Low-middle	Cameroon, 2009	556	0.4 (0.3, 0.5)	4.2 (2.0, 6.3)	1.3 (1.1, 1.5)	0.9 (0.9, 0.9)	37.7 (32.7, 42.7)	17.3 (16.0, 18.5)	37.6 (33.5, 41.7)
	Georgia, 2009	2064	1.1 (1.0, 1.1)	19.6 (16.7, 22.6)	0.9 (0.8, 1.1)	--	24.6 (21.7, 27.5)	124.3 (118.9, 129.8)	0.6 (0.2, 1.0)
	Nigeria, 2012	303	0.4 (0.2, 0.7)	11.6 (5.9, 17.2)	2.0 (1.5, 2.4)	1.0 (0.9, 1.0)	56.1 (49.1, 63.1)	22.6 (20.5, 24.7)	25.4 (19.0, 31.9)
	Pakistan, 2011	5824	-0.1 (-0.1, 0)	5.5 (4.8, 6.2)	--	0.9 (0.9, 0.9)	35.6 (34.0, 37.2)	11.7 (11.3, 12.2)	59.7 (58.1, 61.4)
	Philippines, 2011	1656	-0.1 (-0.2, 0)	2.1 (1.0, 3.3)	0.7 (0.6, 0.9)	0.8 (0.8, 0.8)	25.9 (22.3, 29.4)	14.8 (13.8, 15.7)	45.0 (41.4, 48.6)
	Vietnam, 2010	344	-0.1 (-0.3, 0)	4.1 (2.0, 6.2)	0.7 (0.6, 0.8)	--	11.9 (9.2, 14.6)	24.9 (22.6, 27.1)	24.4 (19.5, 29.3)

Upper-middle	Azerbaijan, 2013	987	0.9 (0.8, 1.0)	15.6 (12.7, 18.5)	0.3 (0.3, 0.4)	0.8 (0.8, 0.9)	30.8 (27.0, 34.6)	20.4 (19.1, 21.7)	29.1 (25.0, 33.2)
	Colombia, 2010	7753	0.4 (0.4, 0.4)	3.9 (3.1, 4.7)	0.6 (0.6, 0.7)	--	18.8 (17.1, 20.5)	23.5 (22.7, 24.2)	21.5 (19.8, 23.3)
	Mexico, 2006	1562	0.6 (0.5, 0.6)	7.6 (5.9, 9.2)	0.6 (0.6, 0.7)	--	11.1 (8.9, 13.4)	14.3 (13.3, 15.3)	45.8 (42.0, 49.6)
	Mexico, 2012	2454	0.6 (0.5, 0.6)	8.4 (6.6, 10.2)	0.5 (0.4, 0.6)	--	11.8 (9.4, 14.3)	18.2 (17.4, 18.9)	30.3 (27.3, 33.2)
High	USA, 2006	1081	0.6 (0.5, 0.7)	9.1 (6.8, 11.5)	0.3 (0.3, 0.4)	--	6.1 (4.5, 7.8)	21.4 (20.4, 22.4)	22.9 (19.2, 26.6)

[1] CRP, AGP, and ferritin values presented as geometric mean (95% CI) due to non-normal distributions. All estimates account for survey design variables (cluster, strata, weight), except Mongolia which followed a simple random sampling design. '--' indicates the variable was unavailable in that survey. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, α-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CI, confidence interval; CIC, Country Income Classification; CRP, C-reactive protein; OWOB, overweight/obesity; WHZ, weight-for-height z-score.

[2]Country income classification (CIC) defined according to the World Bank definition for the year in which the survey was conducted (38).

[3]Overweight/obesity defined as a BMI-for-age z-score of ≥2 SD.

[4]Any inflammation defined as CRP >5 mg/L or AGP >1 g/L.

[5]Ferritin measured in plasma or serum, as reported in the survey.

[6]Iron deficiency defined as serum or plasma ferritin concentration <12 µg/L, adjusted for inflammation using the BRINDA regression correction approach (2).

Table 2.3. Relationships between ferritin and BMI as mediated by inflammation among women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

			WRA mediation analysis, adjusted[2]					
Country income classification[3]	**Country, survey year**	**n**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	571	-2.1 (-4.3, 0.1)	-2.7 (-4.7, - 0.8)	0.6 (-0.02, 1.3)	NM	--	--
	Burkina Faso, 2010	61	-2.9 (-15.9, 10.1)	5.1 (-6.1, 17.2)	-7.5 (-12.5, -3.2)	NM	--	--
	Cambodia, 2014	609	3.8 (2.0, 5.5)	2.3 (0.4, 4.1)	1.4 (0.2, 2.6)	38%	17%	21%
	Côte d'Ivoire, 2007	706	0.5 (-1.1, 2.1)	-0.8 (-2.3, 0.7)	1.3 (0.7, 1.8)	NM	--	--
	Laos, 2006	690	11.9 (8.3, 14.3)	9.1 (5.5, 11.9)	2.6 (1.2, 3.9)	23%[4]	17%	3%
	Malawi, 2016	594	-0.5 (-2.5, 1.6)	-1.1 (-3.2, 0.1)	0.6 (0.04, 1.2)	NM	--	--
Low-middle	Cameroon, 2009	594	0.1 (-1.2, 1.4)	-0.6 (-1.9, 0.7)	0.7 (0.4, 1.0)	NM	--	--
	Georgia, 2009	1605	1.2 (0.6, 1.8)	0.8 (0.2, 1.4)	0.4 (0.1, 0.6)	31%	31%	--
	India, 2011	147	7.5 (0.07, 15.4)	3.7 (-2.1, 9.4)	3.6 (1.1, 6.2)	NM[5]	--	--
	Nigeria, 2012	506	-1.5 (-3.2, 0.3)	-1.5 (-3.2, 0.2)	0.003 (-0.04, 0.5)	NM	--	--
	Pakistan, 2011	5004	1.0 (0.4, 1.7)	0.8 (0.2, 1.5)	0.2 (0.1, 0.3)	21%	9%	11%
	Vietnam, 2010	1178	2.4 (-0.2, 4.9)	-0.5 (-3.2, 2.2)	2.9 (2.0, 3.8)	NM	--	--
Upper-middle	Azerbaijan, 2013	2528	3.5 (2.7, 4.3)	1.4 (0.1, 2.3)	2.1 (1.6, 2.6)	60%	45%	15%
	Colombia, 2010	8300	2.0 (1.4, 2.6)	1.6 (1.0, 2.2)	0.4 (0.2, 0.5)	19%	19%	--
	Mexico, 2006	2910	1.2 (0.1, 2.3)	0.4 (-0.7, 1.5)	0.9 (0.3, 1.5)	71%	71%	--
	Mexico, 2012	3540	2.7 (1.3, 4.1)	0.8 (-1.0, 2.4)	1.9 (1.2, 2.6)	71%	71%	--
High	United Kingdom, 2014	862	1.9 (0.5, 3.3)	1.3 (-1.0, 2.9)	0.6 (-0.04, 1.2)	NM	--	--
	United States, 2006	3024	1.3 (0.6, 2.0)	-0.03 (-1.0, 0.08)	1.3 (1.0, 1.7)	100%	100%	--

[1]Ferritin, CRP, and AGP variables were natural-log transformed for analysis due to non-normal distributions. Mediation estimates were exponentiated, and results are presented as percent change (95% confidence interval) in ferritin for every 1-unit change in BMI. Ferritin concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. AGP, alpha-1-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; NM, no mediation; WRA, women of reproductive age.

[2]Model for mediation analysis: $ln\text{Ferritin} = \beta_0 + \beta_1(\text{BMI}) + M_1(ln\text{CRP}) [+M_2(ln\text{AGP})]$ where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BMI on ferritin; Direct Effect = the effect of BMI on ferritin controlling for inflammation; Indirect Effect = the effect of BMI on ferritin as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (41). Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an

improved toilet, and urban/rural residence. Covariates were included in the mediation model if they were associated with the outcome variable at p<0.1 in bivariate models (Supplemental Table 6). Unadjusted mediation estimates are presented in Supplemental Table 11.

[3]Country income classification defined according to the World Bank definition for the year in which the survey was conducted (38).

[4]In the survey from Laos, 23% of the relationship between BMI and ferritin was mediated by inflammation, with 17% of the mediated effect through CRP, 3% of the mediated effect through AGP, and 3% of the mediated effect unexplained.

[5]For the survey from India, the confidence interval for the total effect appears significant, however as the p-value was 0.063 mediation was not considered present.

Table 2.4. Relationships between ferritin BMI-for-age z-score as mediated by inflammation among preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

			PSC mediation analysis, adjusted[2]					
Country income classification[3]	**Country, survey year**	**n**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	595	-2.8 (-11.7, 6.0)	-1.9 (-10.8, 7.0)	-0.9 (-2.8, 1.1)	NM	--	--
	Bangladesh, 2010	1179	-6.5 (-11.0, -2.3)	-5.7 (-10.1, -1.6)	-0.8 (-2.2, 0.5)	NM	--	--
	Bangladesh, 2012	368	-4.8 (-15.3, 5.5)	-2.7 (-12.6, 7.1)	-2.2 (-6.1, 1.7)	NM	--	--
	Burkina Faso, 2010	63	-10.5 (-41.9, 19.8)	-10.6 (-37.8, 15.4)	0.1 (-7.5, 7.7)	NM	--	--
	Cambodia, 2014	599	-6.2 (-16.6, 3.8)	-7.0 (-16.6, 2.0)	0.9 (-3.3, 5.0)	NM	--	--
	Côte d'Ivoire, 2007	435	1.4 (-7.2, 10.0)	-0.6 (-8.6, 7.4)	2.0 (-2.2, 6.2)	NM	--	--
	Kenya, 2007	665	-7.3 (-16.8, 1.6)	-7.2 (-16.0, 1.1)	-0.1 (-2.7, 2.5)	NM	--	--
	Kenya, 2010	551	-5.0 (-13.4, 3.2)	-7.3 (-15.0, -0.2)	2.5 (-1.8, 6.7)	NM	--	--
	Laos, 2006	443	-5.8 (-18.2, 6.2)	-4.5 (-17.2, 7.9)	-1.3 (-5.0, 2.3)	NM	--	--
	Liberia, 2011	956	-1.6 (-6.9, 3.7)	-1.7 (-7.2, 3.8)	0.1 (-1.9, 2.1)	NM	--	--
	Malawi, 2016	748	-3.0 (-16.4, 10.4)	-1.5 (-8.5, 5.4)	1.3 (-1.5, 4.0)	NM	--	--
	Mongolia, 2006	239	-0.2 (-7.2, 6.7)	-1.9 (-15.0, 11.2)	-1.1 (-3.9, 1.7)	NM	--	--
	Nicaragua, 2005	946	-9.0 (-17.2, -1.7)	-9.5 (-16.8, -3.1)	0.5 (-1.4, 2.4)	NM	--	--
	Zambia, 2009	330	-8.6 (-20.1, 2.0)	-9.2 (-20.0, 0.8)	0.6 (-4.1, 5.4)	NM	--	--
Low-middle	Cameroon, 2009	556	12.9 (4.6, 19.7)	12.5 (4.9, 18.7)	0.3 (-1.8, 2.5)	NM	--	--
	Georgia, 2009	2064	0.3 (-2.5, 3.1)	0.3 (-2.5, 3.1)	-0.02 (-0.1, 0.1)	NM	--	--
	Nigeria, 2012	303	-2.6 (-10.8, 5.5)	-3.8 (-13.2, 5.5)	1.2 (-2.2, 4.7)	NM	--	--
	Pakistan, 2011	5824	-3.0 (-5.6, -0.5)	-3.1 (-5.8, -0.6)	0.1 (-0.1, 0.3)	NM	--	--
	Philippines, 2011	1656	-6.2 (-12.6, -0.1)	-4.3 (-10.7, 1.9)	-2.0 (-3.7, -0.2)	31%	8%	23%
	Vietnam, 2010	344	-11.8 (-19.4, -5.8)	-11.1 (-19.1, -4.3)	-0.9 (-2.5, 0.7)	NM	--	--
Upper-middle	Azerbaijan, 2013	987	-2.1 (-6.9, 2.7)	-0.1(-4.6, 4.3)	-2.0 (-4.2, 0.2)	NM	--	--
	Colombia, 2010	7753	-4.8 (-7.9, -1.9)	-4.7 (-7.8, -1.8)	-0.2 (-0.6, 0.3)	NM	--	--
	Mexico, 2006	1562	-3.2 (-9.0, 2.5)	-3.4 (-9.3, 2.4)	0.2 (-1.3, 1.8)	NM	--	--
	Mexico, 2012	2454	-5.8 (-9.6, -2.3)	-6.5 (-10.3, -3.2)	0.8 (-0.3, 1.8)	NM	--	--
High	USA, 2006	1081	-5.8 (-9.6, -2.3)	-6.5 (-10.3, -3.2)	0.8 (-0.3, 1.8)	NM	--	--

[1]Ferritin, CRP, and AGP variables were natural-log transformed for analysis due to non-normal distributions. Mediation estimates were exponentiated, and results are presented as percent change (95% confidence interval) in ferritin for every 1-unit change in BAZ. Ferritin concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which used simple random sampling. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, alpha-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; NM, no mediation; PSC, pre-school age children; WHZ, weight-for-height z-score.

[2]Model for mediation analysis: *ln*Ferritin = $\beta_0 + \beta_1$(BAZ) + M_1(*ln*CRP) [+M_2(*ln*AGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BAZ on ferritin; Direct Effect = the effect of BAZ on ferritin controlling for inflammation; Indirect Effect = the effect of BAZ on ferritin as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (41). Covariates available for adjustment were: age, education level (respondent or maternal education level), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the mediation model if they were associated with the outcome variable at $p<0.1$ in bivariate models (Supplemental Table 6). Unadjusted mediation estimates are presented in Supplemental Table 11.
[3]Country income classification defined according to the World Bank definition for the year in which the survey was conducted (38).

Figures

Figure 2.1 Mediation analysis path diagram

Total Effect: Effect of BMI on ferritin (c)

Direct Effect: Effect of BMI on ferritin controlling for inflammation (**c'**)

Indirect Effect: Effect of BMI on ferritin as mediated by the effect of CRP (a*b) or of AGP (a_2*b_2)

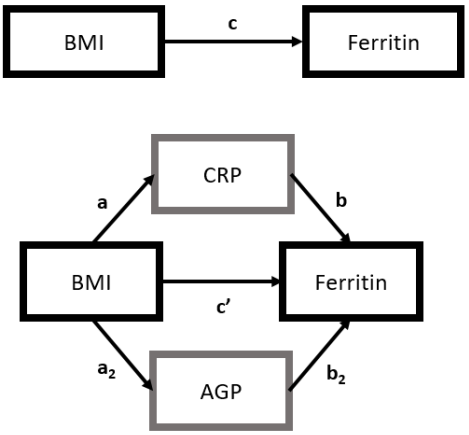

Figure 2.1 Legend: Ferritin, CRP and AGP were analyzed as their *natural-log* equivalents and as continuous variables. Ferritin refers to either serum or plasma ferritin concentration. AGP, α-1-acid glycoprotein; BMI, body mass index; CRP, C-reactive protein (41).

Figure 2.2 Percent of adjusted relationship between ferritin concentration and BMI mediated by CRP and/or AGP among WRA with normal weight to overweight/obesity in 18 surveys in order of ascending mean BMI (left to right): BRINDA Project

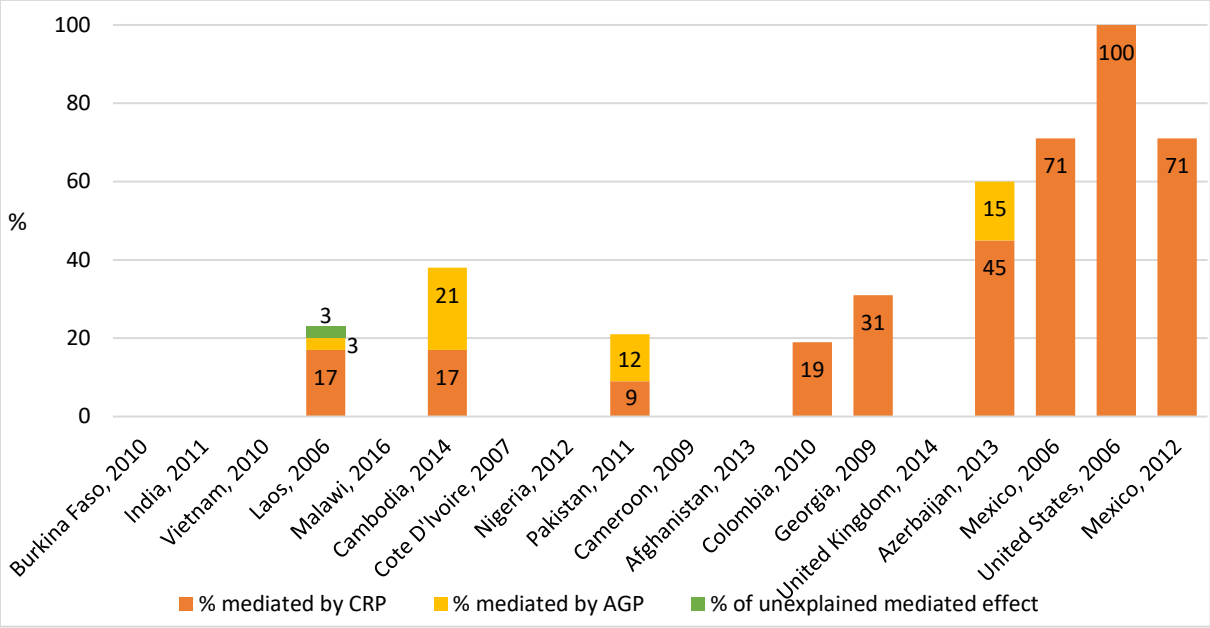

Figure 2.2 Legend: Surveys with mediated effects are displayed with the percent indicating the proportion mediated by CRP, AGP, or unexplained (3% in Laos). Surveys without data indicate no mediation was observed. Both CRP and AGP were measured in 4 of the 10 surveys with mediation: Laos, Cambodia, Pakistan, and Azerbaijan. Ferritin concentration was measured in serum or plasma, as reported in the survey. Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in mediation models if they were associated with the outcome variable at $p<0.1$ in bivariate models (Supplemental Table 2.6). Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. AGP, α-1-acid glycoprotein; BMI; body mass index. BRINDA, Biomarkers Reflecting Nutritional Determinants of Anemia; CRP, C-reactive protein; WRA, women of reproductive age (15-49 years).

Supplemental Material

Supplemental Table 2.1 Number and proportion of observations excluded due to exclusion criteria for WRA, by survey: BRINDA project[1]

		Exclusion Criteria[2]										
Survey, year	**Total obs. in dataset**	**BMI <18.5 kg/m²**	**Implausible Height**	**Implausible Weight**	**Pregnant**	**Positive Malaria Result**	**Missing BMI**	**Missing SF**	**Missing CRP**	**Missing AGP**	**Total observations excluded**	**Analytical sample size & proportion of total**
	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Afghanistan, 2013	23875	954 (4.0)	0 (0)	0 (0)	0 (0)	n/a	13991 (58.6)	22825 (95.6)	22825 (95.6)	22825 (95.6)	23304 (97.6)	571 (2.4)
Burkina Faso, 2010	484	60 (12.4)	0 (0)	0 (0)	0 (0)	36 (7.4)	70 (14.5)	355 (73.3)	355 (73.3)	355 (73.3)	423 (87.4)	61 (12.6)
Cambodia, 2014	724	94 (13.0)	2 (0.3)	1 (0.1)	0 (0)	n/a	0 (0)	19 (2.6)	19 (2.6)	19 (2.6)	115 (15.9)	609 (84.1)
Côte D'Ivoire, 2007	863	81 (9.4)	11 (1.3)	2 (0.2)	n/a	39 (4.5)	14 (1.6)	29 (3.4)	29 (3.4)	29 (3.4)	157 (18.2)	706 (81.1)
Laos, 2006	863	123 (14.3)	0 (0)	0 (0)	n/a	n/a	36 (4.2)	47 (5.5)	47 (5.5)	47 (5.5)	173 (20.1)	690 (79.9)
Malawi, 2016	804	70 (8.7)	1 (0.1)	0 (0)	0 (0)	116 (14.4)	17 (2.1)	28 (3.5)	28 (3.5)	28 (3.5	210 (26.1)	594 (73.9)
Cameroon, 2009	787	67 (8.5)	0 (0)	0 (0)	n/a	108 (13.7)	4 (0.5)	27 (3.4)	27 (3.4)	27 (3.4)	193 (24.5)	594 (75.5)
Georgia, 2009	1846	86 (4.7)	0 (0)	0 (0)	n/a	n/a	18 (1.0)	158 (8.6)	158 (8.6)	n/a	241 (13.1)	1605 (86.9)
India, 2011	972	529 (54.4)	2 (0.2)	0 (0)	0 (0)	n/a	6 (0.6)	645 (66.4)	647 (66.6)	647 (66.6)	825 (84.9)	147 (15.1)
Nigeria, 2012	620	55 (8.9)	0 (0)	1 (0.2)	n/a	56 (9.0)	10 (1.6)	0 (0)	0 (0)	0 (0)	114 (18.4)	506 (81.6)
Pakistan, 2011	22278	3024 (15.6)	3 (0.01)	4 (0.02)	0 (0)	n/a	361 (1.6)	14186 (63.7)	14381 (64.6)	14017 (63.0)	17274 (77.5)	5004 (22.5)
Vietnam, 2010	1492	305 (20.4)	0 (0)	0 (0)	n/a	n/a	1 (0.1)	4 (0.3)	9 (0.6)	n/a	314 (21.1)	1178 (78.9)
Azerbaijan, 2013	2910	138 (4.7)	0 (0)	0 (0)	0 (0)	n/a	73 (2.5)	254 (8.7)	254 (8.5)	254 (8.7)	382 (13.1)	2528 (86.9)
Colombia, 2010	9697	573 (5.9)	0 (0)	0 (0)	n/a	n/a	281 (2.9)	614 (6.3)	614 (6.3)	n/a	1397 (14.4)	8300 (85.6)
Mexico, 2006	3050	88 (2.9)	0 (0)	1 (0.03)	n/a	n/a	22 (0.7)	26 (0.9)	18 (0.6)	n/a	140 (4.6)	2910 (95.4)
Mexico, 2012	4176	97 (2.3)	0 (0)	0 (0)	n/a	n/a	32 (0.8)	564 (13.5)	545 (13.1)	n/a	636 (15.2)	3540 (84.8)
United Kingdom, 2014	2050	69 (3.7)	0 (0)	0 (0)	0 (0)	n/a	93 (4.5)	1123 (54.8)	1108 (54.1)	n/a	1188 (58.0)	862 (42.0)

United States, 2006	3456	143 (4.1)	0 (0)	1 (0.03)	0 (0)	n/a	47 (1.4)	273 (7.9)	259 (7.5)	n/a	432 (12.5)	3024 (87.5)

[1]Criteria for exclusion from analyses were: BMI <18.5 kg/m^2; height or weight outside the ranges of 101.6-219.9 cm and 22.7-222.2 kg;(1) pregnancy; positive test result for malaria; or missing values for ferritin, CRP, AGP, or BMI (due to missing values for weight or height). Some observations may be excluded from multiple categories. Abbreviations: α, alpha-1-glycoprotein; BRINDA, Biomarkers Reflecting Nutritional Determinants of Anemia; CRP, C-reactive protein; WRA, women of reproductive age (15-49 years).

[2]Exclusion criteria percentages are proportions of total observations in the individual datasets.

Supplemental Table 2.2 Number and proportion of observations excluded due to exclusion criteria for PSC, by survey: BRINDA project[1]

		Exclusion Criteria[2]										
Survey, year	**Total obs. in dataset**	**BAZ <-2 SD**	**WHZ <-2 SD**	**Implausible BAZ**	**Implausible WHZ**	**Positive Malaria Result**	**Missing BAZ**	**Missing SF**	**Missing CRP**	**Missing AGP**	**Total obs. excluded**	**Analytical sample size & proportion of total**
	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Afghanistan, 2013	19896	1598 (8.0)	1823 (9.2)	744 (3.8)	424 (2.1)	n/a	341 (1.7)	19231 (96.7)	19231 (96.7)	19231 (96.7)	19301 (97.0)	595 (3.0)
Bangladesh, 2010	1561	305 (19.5)	277 (17.7)	12 (0.8)	8 (0.5)	n/a	18 (1.2)	68 (4.4)	68 (4.4)	68 (4.4)	382 (24.5)	1179 (75.5)
Bangladesh, 2012	1108	134 (12.1)	161 (14.5)	26 (2.3)	19 (1.7)	n/a	85 (7.7)	640 (57.8)	637 (57.5)	637 (57.5)	740 (33.2)	368 (33.2)
Burkina Faso, 2010	482	5 (1.1)	8 (1.7)	0 (0)	0 (0)	9 (1.9)	73 (15.1)	357 (74.1)	357 (74.1)	357 (74.1)	419 (86.9)	63 (13.1)
Cambodia, 2014	874	60 (6.9)	69 (7.9)	5 (0.6)	1 (0.1)	n/a	0 (0)	209 (23.9)	209 (23.9)	209 (23.9)	275 (31.5)	599 (68.5)
Côte D'Ivoire, 2007	864	104 (12.0)	116 (13.4)	29 (3.4)	16 (1.9)	214 (24.8)	37 (4.3)	118 (13.7)	118 (13.7)	118 (13.7)	429 (49.7)	435 (50.3)
Kenya, 2007	1056	47 (4.5)	51 (4.8)	5 (0.5)	5 (0.5)	196 (18.6)	40 (3.8)	160 (15.2)	160 (15.2)	160 (15.2)	391 (37.0)	665 (63.0)
Kenya, 2010	896	26 (2.9)	29 (3.2)	4 (0.5)	3 (0.3)	276 (30.8)	31 (3.5)	47 (5.3)	47 (5.3)	47 (5.3)	345 (38.5)	551 (61.5)
Laos, 2006	514	25 (4.9)	40 (7.8)	1 (0.2)	1 (0.2)	n/a	8 (1.6)	32 (6.2)	32 (6.2)	33 (6.4)	71 (13.8)	443 (86.2)
Liberia, 2011	1476	129 (8.7)	152 (10.3)	3 (0.2)	3 (0.2)	358 (24.3)	10 (0.7)	42 (2.9)	42 (2.9)	42 (2.9)	520 (35.2)	956 (64.8)
Malawi, 2016	1233	48 (3.9)	52 (4.2)	12 (1.0)	8 (0.7)	310 (25.1)	24 (2.0)	131 (10.6)	131 (10.6)	131 (10.6)	485 (39.3)	748 (60.7)
Mongolia, 2006	242	0 (0)	0 (0)	0 (0)	0 (0)	n/a	1 (0.4)	2 (0.8)	n/a	2 (0.8)	239 (1.2)	239 (98.8)
Nicaragua, 2005	1424	11 (0.8)	10 (0.8)	2 (0.1)	2 (0.1)	n/a	4 (0.3)	467 (32.8)	n/a	0 (0)	478 (33.6)	946 (66.4)
Zambia, 2009	885	5 (0.6)	6 (0.7)	1 (0.1)	0 (0)	n/a	2 (0.2)	473 (53.5)	473 (53.4)	474 (53.4)	555 (62.7)	330 (37.3)
Cameroon, 2009	853	16 (1.9)	29 (3.4)	1 (0.1)	0 (0)	195	22.9	61 (7.2)	61 (7.2)	61 (.7.2	297 (34.8)	556 (65.2)
Georgia, 2009	2489	31 (1.2)	20 (0.8)	57 (2.3)	43 (1.7)	n/a	103 (4.1)	347 (13.9)	347 (13.9)	n/a	425 (17.1)	2064 (82.9)
Nigeria, 2012	640	61 (9.5)	63 (9.8)	30 (4.7)	25 (3.9)	198 (30.9)	13 (2.0)	93 (14.5)	93 (14.5)	93. (14.5)	337 (52.7)	303 (47.3)
Pakistan, 2011	10689	1365 (12.8)	1543 (14.4)	361 (3.4)	280 (2.6)	n/a	524 (4.9)	3467 (32.4)	n/a	3132 (29.3)	4865 (45.5)	5824 (54.5)
Philippines, 2011	1784	80 (4.5)	98 (5.5)	6 (0.3)	4 (0.2)	n/a	6 (0.3)	17 (1.0)	17 (1.0)	17 (1.0)	128 (7.2)	1656 (92.8)

Vietnam, 2010	395	23 (5.8)	25 (6.3)	2 (0.5)	1 (0.2)	n/a	3 (0.8)	15 (3.8)	17 (4.3)	n/a	51 (12.9)	344 (87.1)
Azerbaijan, 2013	1404	41 (2.9)	39 (2.8)	49 (3.5)	39 (2.8)	n/a	49 (3.5	351 (25.0)	351 (25.0)	351 (25.0)	417 (29.7)	987 (70.3)
Colombia, 2010	7753	66 (0.9)	76 (1.0)	11 (0.1)	7 (0.1)	n/a	161 (2.1)	3453 (44.5)	3887 (50.1)	n/a	3973 (51.2)	3780 (48.8)
Mexico, 2006	6618	93 (1.4)	90 (1.4)	10 (0.2)	6 (0.1)	n/a	538 (8.1)	5028 (76.0)	5026 (76.0)	n/a	5056 (76.4)	1562 (23.6)
Mexico, 2012	8528	101 (1.2)	102 (1.2)	0 (0)	0 (0)	n/a	482 (5.7)	5904 (69.2)	5989 (70.2)	n/a	6074 (71.2)	2454 (28.8)
United States, 2006	2665	21 (0.8)	20 (0.8)	11 (0.4)	7 (0.3)	n/a	131 (4.9)	1512 (56.8)	1350 (50.7)	n/a	1584 (59.4)	1081 (40.6)

[1]Criteria for exclusion from analyses were: BAZ or WHZ <-2 SD; BAZ or WHZ less than -5 SD or greater than 5 SD;(2) positive test result for malaria; or missing values for ferritin, CRP, AGP, or BAZ (due to missing values for weight or height/length). Some observations may be excluded from multiple categories. Abbreviations: α, alpha-1-glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Nutritional Determinants of Anemia; CRP, C-reactive protein; PSC, preschool-age children (6-59 months); WHZ, weight-for-height z-score.

[2]Exclusion criteria percentages are proportions of total observations in the individual datasets.

Supplemental Table 2.3 Available covariates in each survey: BRINDA project[1]

Survey	Age	Rural/Urban Setting	SES	Water	Sanitation	Respondent/Maternal Education Level	Household Head Education Level
Afghanistan	PSC, WRA	--	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Azerbaijan	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	WRA only	--
Bangladesh, 2010	PSC, WRA	--	--	PSC only	PSC only	--	--
Bangladesh, 2012	PSC, WRA	PSC only	PSC only	PSC only	PSC only	PSC only	--
Burkina Faso	PSC, WRA	--	PSC, WRA	PSC, WRA	PSC, WRA	--	PSC, WRA
Cambodia	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Cameroon	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Colombia	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	WRA only	PSC only
Côte d'Ivoire	PSC, WRA	PSC, WRA	PSC, WRA	WRA only	PSC, WRA	PSC, WRA	--
Georgia	PSC, WRA	PSC, WRA	--	--	--	WRA only	--
India	PSC, WRA	WRA only	--	WRA only	WRA only	WRA only	--
Kenya, 2007	PSC, WRA	PSC only	PSC only	PSC only	PSC only	PSC only	--
Kenya, 2010	PSC, WRA	PSC only	PSC only	PSC only	PSC only	PSC only	--
Laos	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Liberia	PSC, WRA	PSC only	PSC only	PSC only	PSC only	--	--
Malawi	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Mexico, 2006	PSC, WRA	PSC, WRA	PSC, WRA	--	PSC, WRA	--	PSC, WRA
Mexico, 2012	PSC, WRA	PSC, WRA	PSC, WRA	--	--	--	--
Mongolia	PSC, WRA	PSC only	--	--	--	PSC only	--
Nicaragua	PSC, WRA	PSC only	--	PSC only	PSC only	PSC only	--
Nigeria	PSC, WRA	PSC, WRA	--	--	--	--	--
Pakistan	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	PSC, WRA	--
Philippines	PSC, WRA	PSC only	PSC only	--	PSC only	PSC only	--
United Kingdom	PSC, WRA	--	WRA only	--	--	WRA only	--
United States	PSC, WRA	--	PSC, WRA	--	--	WRA only	PSC only
Vietnam	PSC, WRA	PSC, WRA	--	--	--	--	--
Zambia	PSC, WRA	PSC only	--	--	--	--	--

[1]Covariate definitions: age in years (WRA) or months (PSC); sex (male/female; PSC only); urban or rural residence; household socioeconomic status (SES) categorized as low SES versus high SES (variable created from the ordinal 3-category SES variable from the harmonized BRINDA dataset (3), which was then dichotomized into a binary variable of low SES versus high SES, where high SES included both medium and high categories); water defined as access to an improved water source (compared to no access or access only to an unimproved water source); sanitation defined as access to an improved toilet (compared to no access or access only to an unimproved toilet); and education level defined as either none or primary school only versus secondary school or more and measured as either the respondent (WRA) or maternal (PSC) education level, except in surveys that reported household head education level. BRINDA: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; PSC, preschool-age children (6-59 months); SES, socio-economic status; WRA, women of reproductive age (15-49 years).

Supplemental Table 2.4 Age and household characteristics for women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

Country Income Class-ification[2]	Survey, year	n	Age, yr median [IQR]	Urban residence % (95% CI)	High SES[3] % (95% CI)	Improved Water Source[4] % (95% CI)	Improved Toilet[5] % (95% CI)	High Education[6] % (95% CI)
Low	Afghanistan, 2013	571	29.2 [24.3, 29.8]	--	93.1 (89.6, 96.6)	81.5 (75.7, 87.4)	56.6 (47.8, 65.3)	10.2 (6.1, 14.4)
	Burkina Faso, 2010	61	30.2 [25.3, 37.7]	--	76.5 (57.9, 95)	31.3 (5.8, 56.7)	13.0 (0.9, 25.1)	2.1 (0.0, 7.6)
	Cambodia, 2014	609	29.8 [25.1, 33.6]	13.1 (9.9, 16.3)	60.5 (53.7, 67.3)	53.1 (45.7, 60.6)	52.1 (46.6, 57.7)	31.7 (26.5, 36.9)
	Côte d'Ivoire, 2007	706	26.3 [21.1, 31.9]	56.1 (51.9, 60.3)	63.9 (58.1, 69.8)	88.0 (82.6, 93.3)	89.9 (85.9, 93.8)	15.5 (11.5, 19.4)
	Laos, 2006	690	28.5 [20.4, 37.2]	33.1 (19.2, 46.9)	61.9 (51.2, 72.5)	42.4 (33.5, 51.3)	90.6 (84.1, 97.1)	34.8 (25.4, 44.1)
	Malawi, 2016	594	27.8 [20.8, 36.8]	10.3 (1.9, 18.7)	61.0 (52.9, 69.0)	83.6 (75.7, 91.4)	83.8 (76.4, 91.2)	22.1 (15.7, 28.5)
Low-middle	Cameroon, 2009	594	26.2 [22.0, 31.8]	62.8 (52.5, 73.2)	64.1 (56.7, 71.6)	75.0 (68.9, 81.0)	67.0 (61.5, 72.6)	37.5 (33.3, 41.7)
	Georgia, 2009	1605	32.2 [24.2, 41.0]	50.3 (43.2, 57.3)	--	--	--	95.3 (93.8, 96.8)
	India, 2011	147	25.2 [21.6, 31.2]	0[7]	--	90.5 (83.1, 97.9)	23.1 (12.6, 33.6)	59.9 (42.8, 77.0)
	Nigeria, 2012	506	26.3 [21.5, 31.1]	0[7]	--	--	--	--
	Pakistan, 2011	5004	29.6 [25.8, 34.6]	32.0 (28.8, 35.3)	61.2 (58.6, 63.8)	93.0 (91.7, 94.4)	84.5 (82.7, 86.4)	30.3 (28.0, 32.5)
	Vietnam, 2010	1178	33.4 [25.9, 40.9]	50.5 (48.2, 52.8)	--	--	--	--
Upper-middle	Azerbaijan, 2013	2528	31.7 [23.9, 41.4]	45.7 (39.4, 51.9)	68.6 (65.1, 72.2)	77.1 (72.2, 82.1)	93.7 (90.9, 96.5)	95.0 (93.5, 96.5)
	Colombia, 2010	8300	28.6 [19.4, 39.2]	77.5 (76.5, 78.6)	62.0 (60.6, 63.4)	87.0 (85.4, 88.6)	97.2 (96.3, 98.0)	49.1 (46.9, 51.3)
	Mexico, 2006	2910	31.6 [23.0, 40.1]	71.3 (67.0, 75.6)	53.0 (49.1, 57.0)	--	87.9 (85.3, 90.5)	41.6 (38.0, 45.3)
	Mexico, 2012	3540	33.5 [27.4, 40.6]	79.2 (77.4, 80.9)	69.8 (67.0, 72.6)	--	--	--
High	United Kingdom, 2014	862	33.4 [23.3, 41.9]	--	63.0 (57.9, 68.2)	--	--	91.4 (88.7, 94.1)
	United States, 2006	3024	34.7 [24.7, 42.5]	--	74.2 (71.0, 77.4)	-	--	100.0 (100,100)

[1]Estimates account for complex survey design (cluster, strata) with survey weights applied. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. '--' indicates the variable was unavailable in that survey. BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; SES, socioeconomic status.

[2]Country income classification defined according to the World Bank definition for the year the survey took place (4).

[3]A binary SES variable (low/high where high includes both medium and high categories) was created from the 3-level ordinal SES variable available from the harmonized BRINDA dataset,(3) which was created from survey-specific asset scores (quintiles) of household ownership or composition.

[4]'Improved Water Source' (compared to no access or access only to an unimproved water source) was defined as having access to: piped water in a dwelling/yard; a communal/public tap; a borehole/tube well, owned or shared; a protected well/spring; a protected open dug well; or rain water.
[5]'Improved Toilet' (compared to no access or access only to an unimproved toilet) was defined as have access to: a flush toilet/pit latrine flush to piped sewer; a ventilated improved pit/latrine/Sanplat; or a flush to pit/latrine.
[6]'High Education' (compared to none or primary school only) was measured as respondent education level or head of household education level in surveys in which respondent education was not measured (Burkina Faso and Mexico 2006).
[7]Surveys from India and Nigeria contained only observations from rural areas.

Supplemental Table 2.5 Age, sex, and household characteristics for preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

Country Income Class-ification[2]	Country, survey year	n	Age, mo median [IQR]	Male %	Urban residence % (95% CI)	High SES[3] % (95% CI)	Improved Water Source[4] % (95% CI)	Improved Toilet[5] % (95% CI)	High Education[6] % (95% CI)
Low	Afghanistan, 2013	595	27.8 [17.0, 39.9]	51.9	--	94.9 (92.5, 97.3)	81.5 (74.1, 89.0)	68.1 (60.1, 76.0)	9.8 (5.3, 14.4)
	Bangladesh, 2010	1179	7.7 [6.3, 9.3]	49.1	--	--	98.4 (95.3, 100.0)	25.2 (16.9, 33.5)	--
	Bangladesh, 2012	368	37.9 [26.8, 48.0]	54.6	26.9 (19.1, 34.7)	44.3 (33.1, 55.5)	99.0 (97.5, 100.0)	63.3 (49.5, 77.1)	47.1 (36.0, 58.2)
	Burkina Faso, 2010	63	48.3 [43.6, 53.5]	44.4	--	80.1 (63.3, 96.8)	29.6)8.5, 50.6)	12.2 (0, 27.7)	2.1 (0, 7.1)
	Cambodia, 2014	599	37.0 [24.6, 48.3]	54.4	11.6 (8.5, 14.7)	58.4 (50.0, 66.7)	53.4 (44.4, 62.3)	53.9 (46.7, 61.1)	27.7 (22.5, 32.9)
	Côte d'Ivoire, 2007	435	29.6 [17.3, 44.2]	54.7	58.2 (52.0, 64.5)	67.7 (62.1, 73.4)	--	66.6 (58.0, 75.3)	12.4 (9.3, 15.4)
	Kenya, 2007	665	18.6 [12.1, 26.4]	51.1	0[7]	60.0 (54.3, 65.8)	52.6 (42.9, 62.4)	0.2 (0.0, 0.5)	14.7 (11.7, 17.7)
	Kenya, 2010	551	21.7 [13.4, 27.0]	50.5	0[7]	61.7 (56.1, 67.2)	58.2 (49.0, 67.3)	1.1 (0.1, 2.2)	16.4 (12.5, 20.3)
	Laos, 2006	443	33.4 [20.7, 45.6]	49.4	14.3 (5.7, 22.8)	38.9 (28.7, 49.2)	43.9 (32.3, 55.5)	28.5 (17.9, 39.1)	15.8 (8.1, 23.6)
	Liberia, 2011	956	17.3 [11.2, 26.0]	47.6	43.6 (39.0, 48.2)	68.3 (61.6, 75.0)	83.2 (61.6, 75.0)	43.5 (37.0, 50.0)	--
	Malawi, 2016	748	31.4 [18.5, 44.7]	51.3	13.7 (0, 28.0)	56.5 (49.6, 63.3)	87.2 (81.0, 93.4)	82.6 (77.5, 87.6)	24.0 (13.1, 34.9)
	Mongolia, 2006	239	18.9 [12.3, 26.7]	51.9	49.0 (42.5, 55.5)	--	--	--	80.3 (74.7, 85.2)
	Nicaragua, 2005	946	34.0 [19.0, 47.0]	50.1	62.2 (51.0, 73.3)	--	89.1 (82.9, 95.2)	36.4 (28.9, 43.9)	45.8 (37.4, 54.2)
	Zambia, 2009	330	37.0 [23.9, 48.0]	57.6	21.8 (15.9, 27.7)	--	--	--	--
Low-middle	Cameroon, 2009	556	29.0 [18.8, 38.9]	50.7	61.8 (51.5, 72.1)	65.8 (57.9, 73.7)	77.4 (71.4, 83.3)	67.2 (61.3, 73.1)	37.7 (32.8, 42.6)
	Georgia, 2009	2064	36.3 [23.5, 50.2]	53.7	47.3 (40.5, 54.0)	--	--	--	--
	Nigeria, 2012	303	29.5 [20.9, 35.7]	50.5	0[7]	--	--	--	--
	Pakistan, 2011	5824	25.6 [14.8, 39.3]	51.2	30.9 (27.8, 34.0)	58.8 (56.2, 61.4)	94.9 (93.7, 96.0)	91.6 (90.4, 92.7)	20.5 (18.7, 22.4)
	Philippines, 2011	1656	15.4 [10.7, 19.0]	49.5	9.1 (8.4, 9.8)	16.0 (12.8, 19.1)	44.6 (40.0, 49.3)	92.6 (87.5, 97.7)	66.6 (61.3, 71.9)
	Vietnam, 2010	344	37.5 [25.9, 49.2]	52.3	47.4 (42.0, 52.8)	--	--	--	--
Upper-middle	Azerbaijan, 2013	987	36.5 [23.6, 47.1]	55.0	45.3 (38.1, 52.5)	68.6 (64.1, 73.1)	77.4 (71.8, 83.0)	93.0 (89.9, 96.2)	--

	Colombia, 2010	3780	37.6 [25.7, 49.0]	52.7	69.9 (68.6, 71.2)	48.6 (46.5, 50.7)	86.6 (83.8, 89.5)	88.5 (86.2, 90.9)	42.8 (39.8, 45.8)
	Mexico, 2006	1562	43.4 [32.6, 52.5]	52.4	52.0 (47.7, 56.3)	34.6 (30.6, 38.6)	--	74.4 (70.4, 78.3)	37.1 (33.2, 41.0)
	Mexico, 2012	2454	37.0 [35.2, 38.8]	49.3	72.2 (69.3, 75.2)	53.5 (49.7, 57.3)	--	--	--
High	USA, 2006	1081	40.0 [25.2, 50.0]	50.6	--	59.4 (54.1, 64.7)	--	--	93.3 (91.4, 95.3)

[1]Estimates account for the complex survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which followed a simple random sampling design. Inclusion criteria were: BMI-for-age z-score or weight-for-height z-score ≥-2 SD and a negative malaria test result. '--' indicates the variable was unavailable in that survey. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; SES, socioeconomic status.

[2]Country income classification defined according to the World Bank definition for the year the survey took place (4).

[3]A binary SES variable (low/high) was created from the 3-level ordinal SES variable available harmonized BRINDA dataset (3), which was created from survey-specific asset scores (quintiles) of household ownership or composition.

[4]'Improved Water Source' (compared to no access or access only to an unimproved water source) was defined as having access to: piped water in a dwelling/yard; a communal/public tap; a borehole/tube well, owned or shared; a protected well/spring; a protected open dug well; or rain water.

[5]'Improved Toilet' (compared to no access or access only to an unimproved toilet) was defined as have access to: a flush toilet/pit latrine flush to piped sewer; a ventilated improved pit/latrine/Sanplat; or a flush to pit/latrine.

[6]'High Education' (compared to none or primary school only) was measured as maternal education level or head of household education level in surveys in which maternal education was not measured (Burkina Faso, Colombia, Mexico 2006, and the United States).

[7]Surveys from Kenya (2007 and 2010) and Nigeria contained only observations from rural areas.

Supplemental Table 2.6 Bivariate and multivariable percent change associations between ferritin, CRP, AGP, and BMI among women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

				Bivariate (unadjusted) and multivariable (adjusted) linear regression				
Country Income Classification	**Country, survey year**	**n**		**Ferritin regressed on BMI**	**CRP regressed on BMI**	**AGP regressed on BMI**	**Ferritin regressed on *ln*CRP**	**Ferritin regressed on *ln*AGP**
Low-income	Afghanistan, 2013	571	B	-2.1 (-4.3, 0.2)	4.9 (-2.2, 12.5)	0.6 (-0.2, 1.4)	7.7 (2.3, 13.4)	123.1 (54.1, 222.9)
			M	-2.1 (-4.3, 0.2)	4.4 (-2.7, 12.1)	0.6 (-0.2, 1.4)	7.7 (2.3, 13.4)	123.1 (54.1, 222.9)
	Burkina Faso, 2010	61	B	-2.9 (-17.6, 14.4)	-21.8 (-34.3, -7.0)	-3.5 (-9.9, 3.3)	29.9 (9.0, 54.7)	118.0 (14.9, 313.6)
			M	-1.3 (-13.9, 13.1)	-22.3 (-35.2, -6.9)	-4.0 (-9.8, 2.2)	28.8 (12.3, 47.7)	115.8 (9.9, 323.7)
	Cambodia, 2014	609	B	3.7 (2.0, 5.5)	17.1 (13.4, 20.9)	2.5 (0.5, 4.6)	14.7 (9.5, 20.1)	43.0 (30.2, 57.2)
			M	3.4 (1.7, 5.1)	16.7 (12.9, 20.5)	2.9 (0.8, 5.0)	13.9 (8.9, 19.2)	42.9 (30.5, 56.6)
	Côte d'Ivoire, 2007	706	B	0.2 (-1.4, 1.9)	7.1 (4.7, 9.5)	1.2 (0.6, 1.7)	16.4 (10.5, 22.6)	95.7 (57.2, 143.6)
			M	0.7 (-1.1, 2.4)	6.8 (4.3, 9.2)	1.2 (0.6, 1.7)	16.9 (11.1, 23.0)	97.4 (57.8, 146.8)
	Laos, 2006	690	B	11.9 (8.6, 15.4)	22.6 (18.4, 26.9)	1.9 (1.3, 2.6)	19.1 (12.5, 26.1)	58.5 (6.3, 136.2)
			M	5.5 (2.4, 8.7)	18.4 (14.2, 22.8)	1.9 (1.3, 2.6)	12.7 (7.5, 18.1)	60.6 (20.1, 114.8)
	Malawi, 2016	594	B	-0.5 (-2.5, 1.6)	10.8 (5.7, 16.1)	2.9 (1.8, 4.1)	2.6 (-2.2, 7.5)	22.4 (5.8, 41.7)
			M	-0.5 (-2.5, 1.6)	10.7 (5.8, 15.9)	3.2 (1.9, 4.4)	2.6 (-2.2, 7.5)	22.4 (5.8, 41.7)
Low-middle	Cameroon, 2009	594	B	0.0 (-1.3, 1.3)	6.5 (3.7, 9.4)	0.5 (0.2, 0.9)	10.4 (5.3, 15.7)	102.9 (50.3, 174.1)
			M	0.2 (-1.6, 1.2)	6.8 (3.9, 9.9)	0.6 (0.3, 1.0)	8.6 (3.1, 14.4)	82.4 (33.1, 149.8)
	Georgia, 2009	1605	B	1.2 (0.7, 1.8)	8.3 (6.6, 10.0)	--	6.1 (2.7, 9.6)	--
			M	1.2 (0.7, 1.8)	7.6 (5.7, 9.5)	--	6.1 (2.7, 9.6)	--
	India, 2011	147	B	7.5 (-0.4, 16.0)	20.5 (6.4, 22.9)	2.4 (1.2, 3.7)	27.7 (13.4, 43.9)	228.8 (65.7, 552.4)
			M	7.5 (-0.4, 16.0)	13.6 (5.5, 22.4)	2.4 (1.2, 3.7)	27.7 (13.4, 43.9)	228.8 (65.7, 552.4)
	Nigeria, 2012	506	B	-1.5 (-3.3, 0.4)	-0.6 (-3.5, 2.3)	0.05 (-0.6, 0.7)	13.3 (7.0, 20.1)	111.2 (69.8, 162.6)
			M	-1.5 (-3.3, 0.4)	-0.6 (-3.5, 2.3)	0.05 (-0.6, 0.7)	13.3 (7.0, 20.1)	111.2 (69.8, 162.6)
	Pakistan, 2011	5004	B	1.0 (0.4, 1.7)	3.0 (2.0, 4.1)	1.0 (0.7, 1.2)	3.9 (1.5, 6.4)	16.7 (7.3, 26.9)
			M	0.8 (0.1, 1.5)	2.9 (1.8, 3.9)	1.0 (0.7, 1.2)	4.2 (1.8, 6.6)	17.5 (7.6, 28.4)
	Vietnam, 2010	1178	B	4.1 (1.6, 6.7)	17.8 (15.1, 20.5)	--	20.8 (15.2, 26.7)	--
			M	2.3 (-0.4, 5.0)	17.1 (14.1, 20.1)	--	18.9 (13.4, 24.7)	--
Upper-middle	Azerbaijan, 2013	2528	B	3.5 (2.7, 4.3)	13.6 (12.3, 15.0)	1.5 (1.3, 1.8)	20.5 (16.8, 24.4)	143.5 (102.3, 193.1)
			M	3.5 (2.5, 4.4)	11.7 (10.3, 13.3)	1.5 (1.2, 1.8)	20.8 (16.8, 25.0)	136.3 (95.1, 186.1)
	Colombia, 2010	8300	B	2.0 (1.4, 2.6)	13.1 (11.6, 14.6)	--	3.9 (2.7, 5.1)	--
			M	2.1 (1.4, 2.7)	13.0 (11.3, 14.8)	--	3.8 (2.6, 5.0)	--
	Mexico, 2006	2910	B	1.8 (0.8, 2.8)	10.0 (8.2, 11.9)	--	13.2 (7.7, 19.0)	--
			M	1.1 (0.02, 2.2)	8.5 (6.6, 10.4)	--	10.2 (4.0, 16.7)	--
	Mexico, 2012	3540	B	2.7 (1.3, 4.2)	11.4 (9.7, 13.2)	--	21.6 (15.7, 27.7)	--
			M	2.7 (1.2, 4.2)	11.4 (9.7, 13.1)	--	21.3 (15.5, 27.4)	--
High	United Kingdom, 2014	862	B	1.9 (0.5, 3.3)	6.5 (5.4, 7.6)	--	14.2 (4.8, 24.4)	--
			M	1.6 (0.2, 3.1)	6.5 (5.4, 7.6)	--	14.2 (4.7, 24.5)	--
	United States, 2006	3024	B	1.3 (0.6, 2.0)	11.5 (10.9, 12.1)	--	12.9 (10.4, 15.4)	--
			M	1.0 (0.3, 1.7)	11.0 (10.4, 11.5)	--	11.0 (8.5, 13.5)	--

[1]Ferritin, CRP and AGP variables were *natural-log* transformed for analysis due to non-normal distributions. Regression estimates were exponentiated, and results are presented as the percent change (95% confidence interval) in the dependent variable for every 1-unit change in the independent variable. Note that

for the values presented for 'Ferritin regressed on *ln*CRP' and 'Ferritin regressed on *ln*AGP', the percent changes in ferritin concentration are for every 1-unit change in *natural-log* transformed CRP or AGP, and the units differ (CRP, mg/L; AGP, g/L). See Table 2 of main manuscript for geometric mean CRP and AGP values by survey. All estimates account for the complex survey design (cluster, strata) with survey weights applied. Ferritin was measured in either serum or plasma, as reported by the survey. Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the multivariable regression model if they were associated with the outcome variable at $p<0.1$ in the bivariate model. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. Country income classification was defined according to the World Bank definition for the year in which the survey was conducted (4). '--' indicates the variable was unavailable in that survey. AGP, α-1-acid glycoprotein; B, bivariate model; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; M, multivariable model.

Supplemental Table 2.7 Unadjusted and adjusted associations between ferritin, CRP or AGP, and BMI stratified by age among women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

			Stratified analysis, unadjusted		**Stratified analysis, adjusted**	
			β (95% confidence interval)		β (95% confidence interval)	
Survey, year	**n**	**Model**	**Ages 15-29 years**	**Ages 30-49 years**	**Ages 15-29 years**	**Ages 30-49 years**
Afghanistan, 2013	571	No effect modification	--	--	--	--
Burkina Faso, 2010	61	*ln*AGP=BMI	0.06 (-0.09, 0.2)	-0.05 (-0.09, -0.02)	0.05 (-0.09, 0.19)	-0.05 (-0.10, -0.02)
Cambodia, 2014	609	No effect modification	--	--	--	--
Côte d'Ivoire, 2007	706	*ln*Ferritin=*ln*CRP	0.19 (0.13, 0.26)	0.07 (-0.01, 0.15)	0.19 (0.13, 0.26)	0.08 (0.002, 0.16)
Laos, 2006	690	*ln*Ferritin=BMI	0.03 (-0.03, 0.1)	0.13 (0.09, 0.16)	0.05 (0.01, 0.1)	0.09 (0.04, 0.13)
		*ln*AGP=BMI	0.01 (-0.001, 0.03)	0.13 (0.09, 0.16)	no covariates to test	no covariates to test
		*ln*Ferritin=*ln*CRP	0.11 (0.04, 0.18)	0.2 (0.12, 0.29)	0.11 (0.05, 0.17)	0.15 (0.07, 0.24)
		*ln*Ferritin=*ln*AGP	0.03 (-0.44, 0.5)	0.93, 0.43, 1.43)	0.19 (-0.19, 0.57)	0.79, 0.37, 1.20)
Malawi, 2016	594	No effect modification	--	--	--	--
Cameroon, 2009	594	*ln*CRP=BMI	0.04 (0.01, 0.08)	0.09 (0.06, 0.13)	-0.01 (-0.03, 0.01)	0.0006 (-0.02, 0.02)
Georgia, 2009	1605	No effect modification	--	--	--	--
India, 2011	147	*ln*Ferritin=*ln*CRP	0.15 (-0.001, 03)	0.37 (0.22, 0.53)	no covariates to test	no covariates to test
		*ln*Ferritin=*ln*AGP	0.6 (-0.14, 1.34)	2.12 (1.38, 2.86)	no covariates to test	no covariates to test
Nigeria, 2012	506	No effect modification	--	--	--	--
Pakistan, 2011	5004	*ln*Ferritin=BMI	0.02 (0.01, 0.03)	0.01 (-0.004, 0.01)	0.02 (0.01, 0.03)	0.003 (-0.01, 0.01)
		*ln*Ferritin=*ln*AGP	0.24 (0.12, 0.36)	0.1 (-0.01, 0.2)	0.27 (0.14, 0.39)	0.1 (-0.02, 0.21)
Vietnam, 2010	1178	No effect modification	--	--	--	--
Azerbaijan, 2013	2528	*ln*CRP=BMI	0.17 (0.14, 0.19)	0.1 (0.08, 0.11)	0.16 (0.14, 0.18)	0.1 (0.08, 0.11)
		*ln*AGP=BMI	0.02 (0.02, 0.03)	0.01 (0.01, 0.02)	0.02 (0.02, 0.03)	0.01 (0.02, 0.03)
Colombia, 2010	8300	No effect modification	--	--	--	--
Mexico, 2006	2910	*ln*Ferritin=BMI	0.005 (-0.02, 0.03)	0.02 (0.002, 0.03)	no covariates to test	no covariates to test
		*ln*CRP=BMI	0.11 (0.08, 0.13)	0.07 (0.05, 0.1)	no covariates to test	no covariates to test
Mexico, 2012	3450	No effect modification	--	--	--	--
United Kingdom, 2014	862	No effect modification	--	--	--	--
United States, 2006	3024	No effect modification	--	--	--	--

[1]Results presented as the unexponentiated β (95% confidence interval). Effect modification was evaluated through stratified analyses in each of the bivariate models listed above by testing for a significant interaction between the predictor (i.e., BMI, *ln*CRP, or *ln*AGP) and the effect modifier variable of age. Unadjusted stratified analyses were completed for all models where $p<0.1$ for the interaction. Adjusted stratified analyses were then completed for any marginally significant covariates ($p<0.1$) found in bivariate analyses. All estimates account for the complex survey design (cluster, strata) with survey weights applied. Covariates available for adjustment were: education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. AGP, α-1-acid glycoprotein; BMI; body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein.

Supplemental Table 2.8. Bivariate and multivariable percent change associations between ferritin, CRP, AGP, and BAZ among preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

				Bivariate (unadjusted) and multivariable (adjusted) linear regression				
Country Income Classification[3]	**Country, survey year**	**n**		**Ferritin regressed on BAZ**	**CRP regressed on BAZ**	**AGP regressed on BAZ**	**Ferritin regressed on *ln*CRP**	**Ferritin regressed on *ln*AGP**
Low	Afghanistan, 2013	595	B	-1.4 (-10.2, 8.1)	-11.6 (-27.4, 7.5)	-3.9 (-6.1, -1.6)	8.0 (3.6, 12.5)	27.0 (-5.7, 71.1)
			M	-2.8 (-0.4, 1.9)	-11.6 (-27.4, 7.5)	-3.9 (-6.1, -1.6)	7.9 (3.6, 12.3)	28.0 (-3.9, 70.4)
	Bangladesh, 2010	1179	B	-6.5 (-10.7, -2.0)	-3.8 (-11.9, 5.1)	-1.2 (-3.2, 0.9)	10.9 (8.6, 13.3)	113.9 (83.0, 149.9)
			M	-6.7 (-11.0, -2.2)	-3.4 (-11.9, 5.8)	-0.9 (-2.9, 1.2)	10.5 (8.2, 12.9)	113.8 (81.5, 151.8)
	Bangladesh, 2012	368	B	-4.8 (-14.3, 5.7)	-4.7 (-14.8, 6.6)	-2.6 (-6.5, 1.5)	27.6 (18.3, 37.5)	148.5 (76.2, 250.3)
			M	2.9 (-6.7, 13.5)	-3.6 (-13.8, 7.8)	-1.8 (-5.3, 1.9)	25.0 (17.4, 33.0)	121.0 (64.1, 197.7)
	Burkina Faso, 2010	63	B	-10.5 (-38.5, 30.3)	-6.7 (-43.7, 54.8)	0.1 (-9.2, 10.4)	12.7 (-5.2, 34.0)	184.4 (59.1, 408.4)
			M	-9.8 (-35.7, 26.6)	-6.6 (-43.7, 54.9)	0.3 (-8.1, 9.6)	9.9 (-11.0, 35.8)	173.0 (62.5, 358.4)
	Cambodia, 2014	599	B	-6.3 (-15.6, 4.0)	10.5 (-3.4, 26.4)	1.7 (-7.0, 11.1)	16.2 (12.4, 20.1)	62.2 (54.5, 70.4)
			M	-3.7 (-11.3, 4.4)	8.3 (-4.9, 23.4)	--	19.2 (15.2, 23.3)	65.0 (57.3, 73.0)
	Côte d'Ivoire, 2007	435	B	1.4 (-7.2, 10.7)	8.1 (-7.5, 26.4)	-0.04 (-3.1, 3.2)	31.9 (25.3, 39.0)	132.9 (74.0, 211.7)
			M	2.9 (-5.5, 12.0)	6.9 (-8.8, 25.3)	-1.1 (-4.2, 2.0)	28.8 (22.6, 35.4)	113.8 (57.8, 189.6)
	Kenya, 2007	665	B	-7.3 (-15.6, 1.8)	4.5 (-7.6, 18.2)	-1.1 (-3.9, 1.7)	19.9 (15.1, 24.8)	135.9 (89.0, 194.5)
			M	-6.1 (-14.4, 2.9)	6.9 (-6.1, 21.7)	-1.0 (-3.9, 2.0)	20.0 (15.1, 25.1)	143.8 (94.2, 206.1)
	Kenya, 2010	551	B	-5.0 (-12.7, 3.4)	7.7 (-9.5, 28.3)	1.6 (-1.2, 4.5)	25.2 (18.6, 32.1)	324.2 (223.8, 455.8)
			M	-6.1 (-13.7, 2.1)	7.3 (-9.9, 27.9)	1.3 (-1.6, 4.3)	25.3 (18.7, 32.2)	323.5 (224.5, 452.7)
	Laos, 2006	443	B	-5.8 (-16.8, 6.6)	-6.0 (-30.1, 26.5)	-2.0 (-6.7, 2.8)	8.7 (2.7, 15.1)	96.4 (29.1, 198.8)
			M	-2.4 (-12.9, 9.3)	-5.3 (-27.0, 22.7)	-2.2 (-6.8, 2.6)	12.1 (6.1, 18.5)	133.6 (63.8, 233.0)
	Liberia, 2011	956	B	-1.6 (-6.8, 3.9)	3.0 (-7.0, 14.1)	-0.6 (-2.6, 1.5)	18.1 (12.7, 23.8)	135.7 (74.2, 218.9)
			M	-1.6 (-6.8, 3.9)	1.8 (-8.0, 12.7)	-0.7 (-2.8, 1.4)	18.1 (12.7, 23.8)	135.7 (74.2, 218.9)
	Malawi, 2016	748	B	-0.2 (-7.0, 7.0)	18.6 (3.5, 35.8)	3.8 (-2.5, 10.6)	7.1 (3.0, 11.3)	18.3 (0.9, 38.7)
			M	2.9 (-2.7, 8.9)	17.2 (2.4, 34.1)	3.5 (-2.9, 10.3)	11.2 (7.1, 15.4)	31.2 (10.4, 56.0)
	Mongolia, 2006	239	B	-3.0 (-15.2, 11.1)	--	-1.6 (-5.4, 2.4)	--	101.1 (30.8, 209.2)
			M	-2.9 (-14.4, 10.1)	--	-1.9, -5.6, 2.0)	--	70.5 (13.2, 156.6)
	Nicaragua, 2005	946	B	-9.0 (-15.9, -1.6)	--	0.7 (-1.9, 3.3)	--	113.3 (73.6, 162.2)
			M	-8.9 (-15.9, -1.2)	--	0.4 (-2.1, 3.0)	--	116.6 (76.6, 165.7)
	Zambia, 2009	330	B	-8.6 (-18.7, 2.7)	-10.2 (-32.4, 19.3)	1.4 (-3.1, 6.2)	8.8 (5.7, 11.9)	169.4 (101.7, 259.9)
			M	-8.6 (-18.7, 2.7)	-10.2 (-32.4, 19.3)	1.4 (-3.1, 6.2)	8.8 (5.7, 11.9)	169.4 (101.7, 259.9)
Low-middle	Cameroon, 2009	556	B	12.9 (4.6, 21.9)	9.3 (-5.1, 25.9)	-0.4 (-2.3, 1.6)	13.7 (8.8, 18.9)	172.6 (100.9, 269.9)
			M	6.7 (-1.4, 15.4)	10.7 (-4.4, 28.2)	-0.2 (-2.2, 1.8)	16.9 (11.8, 22.1)	203.1 (132.9, 294.5)
	Georgia, 2009	2064	B	0.3 (-2.5, 3.2)	2.1 (-7.9, 13.2)	--	-0.7 (-2.7, 1.3)	--
			M	0.7 (-2.1, 3.5)	3.1 (-7.0, 14.4)	--	-0.8 (-2.7, 1.2)	--

	Nigeria, 2012	303	B	-2.6 (-10.6, 6.1)	7.9 (-5.5, 23.1)	0.2 (-2.6, 3.1)	27.0 (18.1, 36.6)	262.6 (163.1, 399.8)
			M	0.4 (-6.5, 7.8)	7.9 (-5.5, 23.1)	0.2 (-2.6, 3.1)	26.3 (18.2, 35.0)	246.6 (153.5, 373.9)
	Pakistan, 2011	5824	B	-3.0 (-5.5, -0.5)	--	0.6 (-0.4, 1.6)	--	22.5 (12.4, 33.6)
			M	-3.5 (-6.2, -0.8)	--	0.7 (-0.4, 1.9)	--	23.2 (12.2, 35.4)
	Philippines, 2011	1656	B	-6.2 (-11.9, -0.02)	-11.0 (-20.1, -0.9)	-2.1 (-3.8, -0.3)	14.0 (10.0, 18.2)	142.1 (97.6, 196.6)
			M	-9.9 (-15.6, -3.8)	-9.3 (-18.7, 1.3)	-1.3 (-3.2, 0.5)	15.7 (11.6, 20.0)	163.6 (115.8, 222.0)
	Vietnam, 2010	344	B	-11.8 (-17.9, -5.3)	-7.5 (-19.8, 6.6)	--	12.7 (5.7, 20.2)	--
			M	-11.8 (-17.9, -5.3)	-7.5 (-19.8, 6.6)	--	12.7 (5.7, 20.2)	--
Upper-middle	Azerbaijan, 2013	987	B	-2.1 (-6.7, 2.7)	-9.3 (-19.8, 2.5)	-1.9 (-3.9, 0.2)	15.7 (12.7, 18.7)	178.7 (137.2, 227.4)
			M	-0.4 (-5.0, 4.4)	-8.4 (-18.6, 3.2)	-1.5 (-3.6, 0.5)	14.7 (11.9, 17.6)	163.7 (125.5, 208.3)
	Colombia, 2010	3780	B	-4.8 (-7.6, -1.9)	-4.2 (-11.7, 4.0)	--	6.1 (4.3, 8.0)	--
			M	1.2 (1.0, 1.4)	-5.2 (-12.7, 2.8)	--	6.9 (5.1, 8.8)	--
	Mexico, 2006	1562	B	-3.2 (-8.6, 2.5)	1.5 (-8.8, 12.9)	--	15.8 (10.9, 21.0)	--
			M	-3.3 (-8.5, 2.2)	1.2 (-8.9, 12.4)	--	15.6 (10.8, 20.6)	--
	Mexico, 2012	2454	B	-5.8 (-9.2, -2.2)	8.0 (-2.7, 19.9)	--	10.4 (7.8, 13.0)	--
			M	-5.4 (-8.8, -1.9)	8.1 (-2.6, 20.0)	--	10.3 (8.0, 12.8)	--
High	USA, 2006	1081	B	-5.9 (-10.0, -1.6)	20.3 (8.1, 34.0)	--	13.1 (10.1, 16.3)	--
			M	-5.4 (-9.5, -1.2)	21.6 (9.6, 34.9)	--	13.5 (10.4, 16.7)	--

[1]Ferritin, CRP and AGP variables were *natural-log* transformed for analysis due to non-normal distributions. Regression estimates were exponentiated, and results are presented as the percent change (95% confidence interval) in the dependent variable for every 1-unit change in the independent variable. Note that for the values presented for 'Ferritin regressed on *ln*CRP' and 'Ferritin regressed on *ln*AGP', the percent changes in ferritin concentration are for every 1-unit change in *natural-log* transformed CRP or AGP. All estimates account for cluster survey design (cluster, strata) with survey weights applied, except in the Mongolia survey which followed a simple random sampling design. Ferritin was measured in either serum or plasma, as reported by the survey. Covariates available for adjustment were: age, education level (maternal or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the multivariable regression model if they were associated with the outcome variable at $p<0.1$ in the bivariate model. Inclusion criteria were: BAZ or WHZ ≥ -2 SD and a negative malaria test result. Country income classification was defined according to the World Bank definition for the year in which the survey was conducted (4). '--' indicates the variable was unavailable in that survey. AGP, α-1-acid glycoprotein; B, bivariate model; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; M, multivariable model; WHZ, weight-for-height z-score.

Supplemental Table 2.9. Unadjusted and adjusted associations between ferritin, CRP or AGP, and BAZ stratified by age among preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

			Stratified analysis, unadjusted		Stratified analysis, adjusted	
			β (95% confidence interval)		β (95% confidence interval)	
Survey, year	**n**	**Model**	**Ages 6-23 months**	**Ages 24-59 months**	**Ages 6-23 months**	**Ages 24-59 months**
Afghanistan, 2013	595	No effect modification	--	--	--	--
Bangladesh, 2010	1179	Survey only included children 6-24 mo. No stratification possible.	--	--	--	--
Bangladesh, 2012	368	No effect modification	--	--	--	--
Burkina Faso, 2010	63	All children older than 24 months No stratification possible.	--	--	--	--
Cambodia, 2014	599	*ln*CRP = BAZ	-0.23 (-0.5, 0.05)	0.22 (0.03, 0.41)	-0.23 (-0.5, 0.04)	0.22 (0.03, 0.41)
Côte d'Ivoire, 2007	435	*ln*CRP = BAZ	-0.15 (-0.43, 0.12)	0.27 (0.1, 0.44)	-0.15 (-0.43, 0.12)	0.26 (0.1, 0.41)
		*ln*AGP = BAZ	-0.04 (-0.08, -0.01)	0.03 (-0.02, 0.08)	-0.05 (-0.09, -0.01)	0.02 (-0.02, 0.07)
Kenya, 2007	665	*ln*Ferritin = BAZ	-0.14 (-0.25, -0.03)	0.05 (-0.08, 0.17)	-0.12 (-0.23, 0.01)	0.04 (-0.1, 0.18)
Kenya, 2010	551	No effect modification	--	--	--	--
Laos, 2006	443	*ln*Ferritin = BAZ	0.23 (0.02, 0.44)	-0.17 (-0.30, -0.40)	0.19 (-0.01, 0.40)	-0.14 (-0.25, -0.02)
Liberia, 2011	956	*ln*Ferritin = *log*AGP	1.07 (0.75, 1.39)	0.48 (0.05, 0.92)	No covariates to test.	
Malawi, 2016	748	No effect modification	--	--	--	--
Mongolia, 2006	239	No effect modification	--	--	--	--
Nicaragua, 2005	946	*ln*AGP = BAZ	-0.04 (-0.09, 0.01)	0.03 (0.005, 0.05)	-0.04 (-0.09, 0.01)	0.03 (0.002, 0.05)
		*ln*Ferritin = *ln*AGP	1.02 (0.59, 1.45)	0.63 (0.44, 0.82)	No covariates to test.	
Zambia, 2009	330	No effect modification	--	--	--	--
Cameroon, 2009	556	*ln*Ferritin = *ln*AGP	1.49 (0.96, 2.02)	0.89 (0.5, 1.27)	1.31 (0.8, 1.81)	0.87 (0.48, 1.27)
Georgia, 2009	2065	No effect modification	--	--	--	--
Nigeria, 2012	303	*ln*AGP = BAZ	-0.01 (-0.09, 0.07)	0.004 (-0.02, 0.03)	No covariates to test	
		*ln*Ferritin= *ln*CRP	0.24 (0.11, 0.37)	0.24 (0.19, 0.29)	No covariates to test	
Pakistan, 2011	5824	No effect modification	--	--	--	--
Philippines, 2011	1656	All children older than 24 months No stratification possible.	--	--	--	--
Vietnam, 2010	344	*ln*Ferritin=*ln*CRP	0.24 (0.11, 0.38)	0.11 (0.04, 0.17)	No covariates to test	
Azerbaijan, 2013	987	*ln*Ferritin=BAZ	-0.09 (-0.18, -0.0006)	0.01 (-0.04, 0.07)	-0.09 (-0.18, 0.001)	0.01 (-0.04, 0.07)
		*ln*Ferritin=*ln*CRP	0.08 (0.02, 0.14)	0.16 (0.14, 0.19)	0.08 (0.02, 0.14)	0.16 (0.13, 0.18)
Colombia, 2010	3780	*ln*Ferritin = BAZ	-0.12 (-0.18, -0.05)	-0.02 (-0.05, 0.02)	No covariates to test	
Mexico, 2006	1562	*ln*Ferritin=*ln*CRP	0.04 (-0.07, 0.15)	0.16 (0.11, 0.20)	No covariates to test	
Mexico, 2012	2454	No effect modification	--	--	--	--
United States, 2006	1081	*ln*Ferritin=*ln*CRP	0.21 (0.15, 0.26)	0.1 (0.06, 0.13)	0.21 (0.15, 0.27)	0.1 (0.06, 0.13)

[1]Results presented as the unexponentiated β (95% confidence interval). Effect modification was evaluated through stratified analyses in each of the bivariate models listed in the table by testing for a significant interaction between the predictor (i.e., BAZ, *ln*CRP, or *ln*AGP) and the effect modifier variable of age. Unadjusted stratified analyses were completed for all models where $p<0.1$ for the interaction. Adjusted stratified analyses were then completed for any marginally significant covariates ($p<0.1$) found in bivariate analyses. All estimates account for the complex survey design (cluster and strata) with survey weights applied, except in the survey from Mongolia which followed a simple random sampling design. Covariates available for adjustment were: education level (maternal or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, α-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; WHZ, weight-for-height z-score.

Supplemental Table 2.10 Unadjusted and adjusted associations between ferritin, CRP or AGP, and BAZ stratified by sex among preschool age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

			Stratified analysis, unadjusted		Stratified analysis, adjusted	
			β (95% confidence interval)		β (95% confidence interval)	
Survey, year	**n**	**Model**	**Male**	**Female**	**Male**	**Female**
Afghanistan, 2013	595	*ln*AGP = BAZ	-0.07 (-0.10, -0.03)	-0.01 (0.61, -0.05)	-0.07 (-0.10, -0.03)	-0.01 (0.61, -0.05)
Bangladesh, 2010	1179	No effect modification	--	--	--	--
Bangladesh, 2012	368	*ln*Ferritin=*ln*CRP	0.18 (0.08, 0.28)	0.3 (0.22, 0.37)	0.2 (0.12, 0.29)	0.25 (0.17, 0.33)
Burkina Faso, 2010	63	No effect modification	--	--	--	--
Cambodia, 2014	599	No effect modification	--	--	--	--
Côte d'Ivoire, 2007	435	No effect modification	--	--	--	--
Kenya, 2007	665	*ln*CRP = BAZ	-0. 1 (-0.26, 0.05)	0.19 (-0.04, 0.43)	-0.06 (-0.21, 0.08)	0.2 (-0.04, 0.44)
Kenya, 2010	551	*ln*AGP = BAZ	-0.01 (-0.04, 0.03)	0.04 (-0.002, 0.09)	-0.01 (-0.05, 0.03)	0.04 (-0.01, 0.08)
Laos, 2006	443	No effect modification	--	--	--	--
Liberia, 2011	956	No effect modification	--	--	--	--
Malawi, 2016	748	*ln*CRP = BAZ	0.05 (-0.15, 0.24)	0.36 (0.14, 0.57)	0.05 (-0.14, 0.24)	0.34 (0.13, 0.56)
Mongolia, 2006	239	No effect modification	--	--	--	--
Nicaragua, 2005	946	*ln*AGP = BAZ	-0.02 (-0.06, 0.02)	0.03 (-0.003, 0.06)	-0.02 (-0.06, 0.02)	0.03 (-0.004, 0.06)
		*ln*Ferritin=*ln*AGP	0.73 (0.4, 1.06)	0.79 (0.52, 1.06)	0.73 (-0.4, 1.07)	0.82 (0.56, 1.08)
Zambia, 2009	330	No effect modification	--	--	--	--
Cameroon, 2009	556	No effect modification	--	--	--	--
Georgia, 2009	2065	*ln*Ferritin=*ln*CRP	-0.02 (-0.04, 0.004)	0.004 (-0.02, 0.03)	-0.02 (-0.04, 0.002)	0.004 (-0.02, 0.03)
Nigeria, 2012	303	No effect modification	--	--	--	
Pakistan, 2011	5824	*ln*Ferritin=*ln*AGP	0.19 (0.07, 0.30)	0.22 (0.09, 0.34)	0.19 (0.07, 0.32)	0.22 (0.08, 0.35)
Philippines, 2011	1656	No effect modification	--	--	--	--
Vietnam, 2010	344	No effect modification	--	--	--	--
Azerbaijan, 2013	987	No effect modification	--	--	--	--
Colombia, 2010	3780	No effect modification	--	--	--	--
Mexico, 2006	1562	No effect modification	--	--	--	--
Mexico, 2012	2454	No effect modification	--	--	--	--
United States, 2006	1081	No effect modification	--	--	--	--

[1]Results presented as the unexponentiated β (95% confidence interval). Effect modification was evaluated through stratified analyses in each of the bivariate models listed in the table by testing for a significant interaction between the predictor (i.e., BAZ, *ln*CRP, or *ln*AGP) and the effect modifier variable of sex. Unadjusted stratified analyses were completed for all models where $p<0.1$ for the interaction. Adjusted stratified analyses were then completed for any marginally significant covariates ($p<0.1$) found in bivariate analyses. All estimates account for the complex survey design (cluster and strata) with survey weights applied, except in the survey from Mongolia which followed a simple random sampling design. Covariates available for adjustment were: education level (maternal or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural

residence. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, α-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; WHZ, weight-for-height z-score.

Supplemental Table 2.11. Unadjusted relationships between ferritin and BMI or BAZ as mediated by inflammation among women of reproductive age (15-49 years) and preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

			WRA mediation analysis, unadjusted[2]					
Country income classification[3]	**Country, survey year**	**n**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	571	-2.1 (-4.3, 0.1)	-2.7 (-4.7,- 0.8)	0.6 (-0.02, 1.3)	NM	--	--
	Burkina Faso, 2010	61	-2.9 (-15.9, 10.1)	5.1 (-6.1, 17.2)	-7.5 (-12.5, -3.2)	NM	--	--
	Cambodia, 2014	609	3.8 (2.0, 5.5)	2.3 (0.4, 4.1)	1.4 (0.2, 2.6)	38%	17%	21%
	Côte d'Ivoire, 2007	706	0.2 (-1.4, 1.8)	-1.0 (-2.6, 0.5)	1.3 (0.8, 1.8)	NM	--	--
	Laos, 2006	690	11.9 (8.6, 15.3)	9.1 (5.7, 12.7)	2.6 (1.2, 4.0)	23%[4]	17%	3%
	Malawi, 2016	594	-0.5 (-2.5, 1.6)	-1.1 (-3.2, 0.1)	0.6 (0.04, 1.2)	NM	--	--
Low-middle	Cameroon, 2009	594	-0.01 (-1.3, 1.3)	-0.7 (-1.9, 0.5)	0.7 (0.4, 1.0)	NM	--	--
	Georgia, 2009	1605	1.2 (0.6, 1.8)	0.8 (0.2, 1.4)	0.4 (0.1, 0.6)	31%	31%	--
	India, 2011	147	7.5 (0.07, 15.4)	3.7 (-2.1, 9.4)	3.6 (1.1, 6.2)	NM[5]	--	--
	Nigeria, 2012	506	-1.5 (-3.2, 0.3)	-1.5 (-3.2, 0.2)	0.003 (-0.04, 0.5)	NM	--	--
	Pakistan, 2011	5004	1.0 (0.4, 1.7)	0.8 (0.2, 1.5)	0.2 (0.1, 0.3)	21%	9%	11%
	Vietnam, 2010	1178	4.1 (1.7, 6.7)	1.1 (-1.4, 3.7)	3.0 (2.1, 3.9)	73%	73%	--
Upper-middle	Azerbaijan, 2013	2528	3.5 (2.7, 4.3)	1.4 (0.1, 2.3)	2.1 (1.6, 2.6)	60%	45%	15%
	Colombia, 2010	8300	2.0 (1.4, 2.6)	1.6 (1.0, 2.2)	0.4 (0.2, 0.5)	19%	19%	--
	Mexico, 2006	2910	1.8 (0.8, 2.8)	0.8 (-0.3, 1.8)	1.0 (0.4, 1.6)	58%	58%	--
	Mexico, 2012	3540	2.7 (1.3, 4.2)	0.8 (-1.0, 2.5)	2.0 (1.3, 2.6)	71%	71%	--
High	United Kingdom, 2014	862	1.9 (0.5, 3.3)	1.3 (-1.0, 2.9)	0.6 (-0.04, 1.2)	NM	--	--
	United States, 2006	3024	1.3 (0.6, 2.0)	-0.03 (-1.0, 0.08)	1.3 (1.0, 1.7)	100%	100%	--
			PSC mediation analysis, unadjusted[2]					
Low	Afghanistan, 2013	595	-0.9 (-2.8, 1.1)	-0.6 (-9.9, 8.7)	-1.4 (-10.5, 7.6)	NM	--	--
	Bangladesh, 2010	1179	-6.5 (-11.0, -2.3)	-5.7 (-10.1, -1.6)	-0.8 (-2.2, 0.5)	NM	--	--
	Bangladesh, 2012	368	-4.8 (-15.3, 5.5)	-2.7 (-12.6, 7.1)	-2.2 (-6.1, 1.7)	NM	--	--
	Burkina Faso, 2010	63	-10.5 (-41.9, 19.8)	-10.6 (-37.8, 15.4)	0.1 (-7.5, 7.7)	NM	--	--
	Cambodia, 2014	599	-6.2 (-16.6, 3.8)	-7.0 (-16.6, 2.0)	0.9 (-3.3, 5.0)	NM	--	--
	Côte d'Ivoire, 2007	435	1.4 (-7.2, 10.0)	-0.6 (-8.6, 7.4)	2.0 (-2.2, 6.2)	NM	--	--
	Kenya, 2007	665	-7.3 (-16.8, 1.6)	-7.2 (-16.0, 1.1)	-0.1 (-2.7, 2.5)	NM	--	--
	Kenya, 2010	551	-5.0 (-13.4, 3.2)	-7.3 (-15.0, -0.2)	2.5 (-1.8, 6.7)	NM	--	--
	Laos, 2006	443	-5.8 (-18.2, 6.2)	-4.5 (-17.2, 7.9)	-1.3 (-5.0, 2.3)	NM	--	--
	Liberia, 2011	956	-1.6 (-6.9, 3.7)	-1.7 (-7.2, 3.8)	0.1 (-1.9, 2.1)	NM	--	--
	Malawi, 2016	748	-3.0 (-16.4, 10.4)	-1.5 (-8.5, 5.4)	1.3 (-1.5, 4.0)	NM	--	--
	Mongolia, 2006	239	-0.2 (-7.2, 6.7)	-1.9 (-15.0, 11.2)	-1.1 (-3.9, 1.7)	NM	--	--
	Nicaragua, 2005	946	-9.0 (-17.2, -1.7)	-9.5 (-16.8, -3.1)	0.5 (-1.4, 2.4)	NM	--	--
	Zambia, 2009	330	-8.6 (-20.1, 2.0)	-9.2 (-20.0, 0.8)	0.6 (-4.1, 5.4)	NM	--	--
Low-middle	Cameroon, 2009	556	12.9 (4.6, 19.7)	12.5 (4.9, 18.7)	0.3 (-1.8, 2.5)	NM	--	--
	Georgia, 2009	2064	0.3 (-2.5, 3.1)	0.3 (-2.5, 3.1)	-0.02 (-0.1, 0.1)	NM	--	--

	Nigeria, 2012	303	-2.6 (-10.8, 5.5)	-3.8 (-13.2, 5.5)	1.2 (-2.2, 4.7)	NM	--	--
	Pakistan, 2011	5824	-3.0 (-5.6, -0.5)	-3.1 (-5.8, -0.6)	0.1 (-0.1, 0.3)	NM	--	--
	Philippines, 2011	1656	-6.2 (-12.6, -0.1)	-4.3 (-10.7, 1.9)	-2.0 (-3.7, -0.2)	31%	8%	23%
	Vietnam, 2010	344	-11.8 (-19.4, -5.8)	-11.1 (-19.1, -4.3)	-0.9 (-2.5, 0.7)	NM	--	--
Upper-middle	Azerbaijan, 2013	987	-2.1 (-6.9, 2.7)	-0.1(-4.6, 4.3)	-2.0 (-4.2, 0.2)	NM	--	--
	Colombia, 2010	3780	-4.8 (-7.9, -1.9)	-4.7 (-7.8, -1.8)	-0.2 (-0.6, 0.3)	NM	--	--
	Mexico, 2006	1562	-3.2 (-9.0, 2.5)	-3.4 (-9.3, 2.4)	0.2 (-1.3, 1.8)	NM	--	--
	Mexico, 2012	2454	-5.8 (-9.6, -2.3)	-6.5 (-10.3, -3.2)	0.8 (-0.3, 1.8)	NM	--	--
High	USA, 2006	1081	-5.8 (-9.6, -2.3)	-6.5 (-10.3, -3.2)	0.8 (-0.3, 1.8)	NM	--	--

[1]Mediation effects were exponentiated and results are presented as percent change (95% confidence interval) in ferritin for every 1-unit change in BMI (WRA) or BAZ (PSC). Ferritin concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which used simple random sampling. Inclusion criteria were: BMI ≥18.5 kg/m2 (WRA) or BAZ or WHZ ≥-2 SD (PSC), not pregnant (WRA only), and a negative malaria result. AGP, alpha-1-acid glycoprotein; BAZ, BMI-for-age z-score; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; NM, no mediation; PSC, pre-school age children; WHZ, weight-for-height z-score; WRA, women of reproductive age.

[2]Model for mediation analysis: lnFerritin = $\beta_0 + \beta_1$(BMI or BAZ) + M_1(lnCRP) [+M_2(lnAGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BMI (or BAZ) on ferritin; Direct Effect = the effect of BMI (or BAZ) on ferritin controlling for inflammation; Indirect Effect = the effect of BMI (or BAZ) on ferritin as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (5).

[3]Country income classification defined according to the World Bank definition for the year in which the survey was conducted (4).

[4]In the survey from Laos (WRA), 23% of the relationship between BMI and ferritin was mediated by inflammation, with 17% of the mediated effect through CRP, 3% of the mediated effect through AGP, and 3% of the mediated effect unexplained.

[5]For the survey from India (WRA), the confidence interval for the total effect appears significant, however as the p-value was 0.063 mediation was not considered present.

Supplemental Table 2.12. Sensitivity analysis: mediation analysis (unadjusted) assessing the relationship between ferritin, BMI or BAZ and inflammation including and excluding observations that tested positive for malaria for WRA and PSC with normal weight to overweight/obesity: BRINDA project[1]

	Including positive malaria observations (sensitivity analysis)		**Excluding positive malaria observations (original analysis)**	
Survey, year	n	β (95% CI)	n	β (95% CI)
WRA				
Cameroon, 2009	691	0.005 (0.002, 0.008)	594	0.01 (0.004, 0.01)
Côte d'Ivoire, 2007	742	0.01 (0.01, 0.02)	706	0.01 (0.01, 0.02)
Malawi, 2016	693	0.007 (0.003, 0.01)	594	0.01 (0.0004, 0.01)
Nigeria, 2012	555	-0.0002 (-0.005, 0.004)	506	0.00003 (-0.005, 0.005)
PSC				
Cameroon, 2009	740	-0.005 (-0.04, 0.03)	556	0.003 (-0.02, 0.02)
Côte d'Ivoire, 2007	606	0.01 (-0.03, 0.06)	435	0.02 (-0.02, 0.06)
Kenya, 2007	825	0.01 (-0.02, 0.04)	665	-0.001 (-0.03, 0.03)
Kenya, 2010	813	0.01 (-0.03, 0.06)	551	0.03 (-0.02, 0.07)
Liberia, 2011	1268	0.01 (-0.02, 0.05)	956	0.001 (-0.02, 0.02)
Malawi, 2016	1027	0.04 (0.004, 0.07)	748	0.01 (-0.02, 0.04)
Nigeria, 2012	452	0.02 (-0.02, 0.06)	303	0.01 (-0.02, 0.05)
Zambia, 2009	406	-0.006 (-0.06, 0.05)	330	0.01 (-0.04, 0.05)

[1]Estimates of the mediated effect are presented as the unexponentiated β coefficient of *ln*Ferritin (95% confidence intervals). β represents the indirect effect, that is the effect of BMI or BAZ on ferritin concentration as mediated by the effect of CRP or AGP. Malaria status was evaluated by survey-specific diagnostic tests that have been previously described (3). While Burkina Faso (2010) measured malaria status, all observations for both WRA and PSC were excluded as part of the criteria to exclude observations with underweight/wasting, thus the survey is not included in this sensitivity analysis. BAZ, BMI-for-age z-score; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinant of Anemia; PSC, preschool-age children (6-59 months); WRA, women of reproductive age (15-49 years).

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CHAPTER 3: Inflammation does not mediate the relationship between overweight or obesity and retinol or retinol-binding protein among adult women or young children: an analysis of 24 surveys

Abstract

Background: In the presence of inflammation, serum or plasma concentrations of retinol or retinol-binding protein (RBP) transiently decrease, confounding their interpretation as biomarkers of vitamin A status. The extent to which inflammation associated with overweight or obesity may influence interpretation of retinol or RBP is uncertain.

Objective: To describe relationships between weight status, inflammation, and retinol or RBP among non-pregnant women of reproductive age (15-49 years, WRA) and preschool-age children (6-59 months, PSC) with normal weight to overweight or obesity in differing geographic settings.

Methods: Cross-sectional data were separately analyzed from n=13 surveys (WRA) and n=22 surveys (PSC) (24 total surveys) from the Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project, excluding observations with underweight, wasting, pregnancy, or malaria. Relationships were assessed between BMI (WRA) or BMI-for-age z-score (BAZ, PSC), inflammatory biomarkers C-reactive protein (CRP) and/or α-1-acid glycoprotein (AGP), and retinol or RBP by linear regression, and potential mediation by CRP and/or AGP in relationships between BMI or BAZ and retinol or RBP with structural equation modeling. Regression and mediation models accounted for complex survey designs.

Results: Among WRA, greater BMI was positively associated with retinol or RBP in 5 of 13 surveys, BMI was positively associated with CRP and/or AGP in 10 of 13 surveys, but associations between biomarkers of inflammation and retinol or RBP were inconsistent. Among PSC, BAZ was not associated with retinol, RBP, CRP, or AGP, but biomarkers of inflammation were consistently negatively associated with retinol or RBP. In 3 of 13 surveys among WRA and 1 of 22 surveys among PSC, inflammation partially mediated the relationship between BMI or BAZ and retinol or RBP, however the direction of association varied.

Conclusion: In these surveys, inflammation associated with overweight and obesity does not appear to impact vitamin A assessment when measured with retinol or RBP; however, inflammation should continue be measured to interpret vitamin A status among PSC.

Introduction

Vitamin A deficiency affects approximately 30% of children under 5 years (1) and 15% of pregnant women globally (2), making addressing this deficiency an ongoing public health priority (3). The most likely cause of VAD is insufficient diet, that is, chronically low intakes of vitamin A-containing foods that result in the body's inability to meet its physiological vitamin A needs, including normal cellular and metabolic functions, tissue growth, and infection resistance (3). Historically, vitamin A deficiency has been more widespread in low- and middle-income countries where diets low in vitamin A are common, and deficiency may exacerbate the severity of infectious illnesses common to these areas, such as diarrhea and measles (2–4). Reliable monitoring of population vitamin A status is necessary to track progress toward reducing vitamin A deficiency and inform future interventions.

Concentrations of serum or plasma retinol and retinol-binding protein (RBP) are frequently measured biomarkers of vitamin A status in epidemiological studies, and both are affected by infection and inflammation (3). RBP is synthesized in the liver, the principal storage site of vitamin A, and is the primary retinol transporter in the bloodstream. RBP is also a negative acute-phase protein, meaning that measurement of vitamin A status may be confounded under inflammatory conditions as the liver downregulates secretion of RBP and the kidneys upregulate its excretion (3). Thus, identifying the presence of inflammation may aid interpretation of retinol and RBP values for population vitamin A assessment (5–7).

The Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project previously established that adjusting for inflammation with a regression correction among children consistently decreased the estimated prevalence of vitamin A deficiency; however, the association between inflammation and vitamin A biomarkers among adult women was less consistent, and adjusting for inflammation was not recommended (6–8). It is possible that the relationship between

inflammation and vitamin A biomarkers differs by the source of inflammation (e.g., acute infection vs chronic inflammation). While literature on vitamin A biomarkers and inflammation among children has focused mostly on infectious illnesses, a growing body of literature on vitamin A biomarkers and chronic disease has reported associations between retinol or RBP and cardiovascular disease, obesity, and insulin resistance (9–13). However, it is unclear if the inflammation associated with these conditions influences the interpretation of retinol and RBP, and to what extent.

Previous BRINDA research also examined the prevalence and independence of intra-individual overweight or obesity and micronutrient deficiencies among children and adult women (14,15), and reported that overweight or obesity was generally not associated with vitamin A deficiency based on inflammation-adjusted retinol or RBP for either group. But these analyses did not explore weight status as a source of inflammation, which could potentially influence vitamin A status assessment. Therefore, using data from the BRINDA project, we aimed to: 1) to determine the relationships between weight status (BMI), inflammation (CRP and/or AGP), and vitamin A biomarkers (retinol or RBP) among adult women and young children with normal weight to overweight or obesity in differing geographic settings; and 2) examine whether inflammation mediates the relationship between BMI (women) or BMI-for-age z-score (BAZ, children) and vitamin A biomarkers in these same settings.

Methods

Data source and inclusion criteria

The BRINDA project includes a harmonized dataset of nationally- or regionally representative surveys. The methods of the BRINDA project, including criteria for survey inclusion and methodology of anthropometric calculations and biochemical collection, have been previously documented (16,17). For these secondary analyses, we analyzed 13 datasets from 13 countries with data on non-pregnant women of reproductive age (WRA, 15-49 years) and 22 datasets from 20 countries with data on

preschool-aged children (PSC, 6-59 month). Our inclusion and exclusion criteria matched that of two previous BRINDA analyses (14,15), with the exception that for our analyses the surveys must have measured serum or plasma retinol or RBP, and included a marker of inflammation (CRP and/or AGP). Observations were excluded with: BMI <18.5 kg/m^2 (WRA); BAZ or weight-for-height z-score (WHZ) <-2 SD (PSC); height or weight outside the ranges of 101.6-219.9 cm and 22.7-222.2 kg, respectively (WRA) (15); BAZ less than -5 SD or greater than 5 SD (PSC) (14); zero values for retinol or RBP; pregnancy (WRA); or a positive malaria result (n=5 [WRA] and n=9 [PSC] surveys measured malaria). Observations that tested positive for malaria were excluded to minimize the influence of inflammation from infectious disease, however observations with other morbidity symptoms (i.e., reported fever or diarrhea) were not excluded because the variables were not reported uniformly across surveys, and prior BRINDA analyses showed that reported morbidity was not consistently associated with inflammation (18).

After application of exclusion criteria, the analytical sample in n=10 of 13 WRA surveys and n=18 of 22 PSC surveys included at least 40% of the original observations. As in previous analyses, we did not apply a sample size cutoff for excluding surveys, but did ensure that all surveys met the minimum analytical sample size required for mediation analysis: a minimum of 10 observations per linear relation (19). In this dataset, the smallest survey sample size was Burkina Faso for both WRA (n=61) and PSC (n=60). Both surveys included CRP and AGP as inflammatory markers, meaning there were five linear relations, necessitating a minimum sample size of n=50. The proportion of observations excluded overall and by individual exclusion criterion are presented in **Supplemental Tables 3.1 and 3.2**.

Variable definitions

The outcome variables were unadjusted retinol (µmol/L) or RBP (µmol/L), CRP (mg/L), and AGP (g/L); the predictor variables were BMI (kg/m^2, WRA) or BAZ (PSC), CRP (mg/L), and AGP (g/L). For consistency with prior BRINDA analyses (14), we applied BAZ to all age groups, though WHZ is

recommended for use for children <24 months (20). Among WRA, n=10 surveys measured retinol and n=8 measured RBP, including n=4 surveys that measured both biomarkers. Among PSC, retinol and RBP were each measured in n=13 surveys, including n=4 surveys that measured both. For surveys that measured both retinol and RBP, we selected the biomarker with the greater number of observations. CRP was measured in all WRA and n=19 PSC surveys, AGP in n=10 WRA and n=19 PSC surveys, and both CRP and AGP were measured in n=10 WRA and n=16 PSC surveys. For the mediation analysis, the outcome variable was either retinol or RBP, the predictor variable was BMI or BAZ, and the mediating variables were CRP and/or AGP.

Covariates selected and available for analysis are listed by survey in **Supplemental Table 3.3**. We selected covariates based on expected relationships with vitamin A, as well as potential confounders of the relationship between BMI, inflammation, and vitamin A. Covariates were defined for each survey as reported by the survey representative, unless otherwise indicated (16): age (years [WRA] or months [PSC]); sex (PSC only); urban or rural residence; socio-economic status (SES, an ordinal 3-category variable of low, medium, and high SES categories from the harmonized BRINDA dataset (16)); water and sanitation (defined as access to an improved or unimproved water source or toilet facility according to the WHO/UNICEF Joint Monitoring Program (21,22)), highest level of education level completed by the respondent (WRA) or mother (PSC) except in surveys that reported household head education level (Burkina Faso and Colombia [WRA and PSC]), and whether or not vitamin A supplementation was received in the last six months (PSC only, n=9 surveys).

The following categorical variables were defined to describe the nutrition and health status of the survey populations: BMI (WRA) categorized as normal (18.5-24.9 kg/m^2) and overweight or obesity (≥25.0 kg/m^2); BAZ (PSC) categorized as normal (-2 to 2 SD) and overweight or obesity (>2 SD); and any inflammation defined as CRP >5 mg/L and/or AGP >1 g/L. For both WRA and PSC, vitamin A deficiency was defined as retinol or RBP <0.7 µmol/L (23), and the ratio of retinol to RBP was assumed to be 1:1,

which is consistent with prior BRINDA publications (6,7). Among WRA only, vitamin A insufficiency (<1.05 µmol/L (24)) is also presented as prior BRINDA analyses noted a small proportion of the WRA population were deficient (6). After applying our exclusion criteria, ≤3% of WRA in 10 of 13 surveys were vitamin A deficient.

As inflammation related to infectious illnesses has historically been more common in low- and low-middle income countries (LIC and LMIC, and inflammation related to non-communicable disease has been evaluated more commonly in upper-middle and high-income countries (UMIC and HIC) (25,26), we present results grouped by World Bank country income-level classifications (LIC, LMIC, UMIC, HIC) according to their ranking at the time the survey was conducted (27). The country income groups are presented for qualitative interpretation only (i.e., no formal statistical testing was completed between country groupings).

Statistical analyses

Data were analyzed using SAS version 9.4 (SAS Institute, Cary, North Carolina, USA) and STATA version 16 (StataCorp, College Station, Texas, USA). All analyses accounted for complex survey designs (cluster and strata) by calculating variance estimates and applying survey weights, except Mongolia which used simple random sampling. Analyses were conducted separately by survey and by population using continuous variables. P-values were considered statistically significant at $p<0.05$.

Means (95% confidence interval [CI]) are presented for continuous variables, except CRP, AGP, retinol, and RBP are presented as geometric means (95% CI) due to their non-normal distributions, and age is presented as the median (interquartile range); percent (95% CI) are presented for categorical variables. For descriptive analyses among PSC, retinol, RBP and vitamin A deficiency were adjusted for inflammation following the BRINDA regression correction approach (16).

Bivariate and multivariable analyses

In surveys for which >30% of the analytical sample values had a single low value for CRP (representing the lower limit of detection in the lab analysis), we generated and applied a random number between 0 and the lowest value (Afghanistan, Colombia, and Zambia [PSC surveys]). For example, 65% (n=2418) of the CRP values in the survey from Colombia were reported as 0.2; for these observations we generated and applied a random value between 0 and 0.2 (28,29). For all surveys, retinol, RBP, CRP, and AGP were natural log (*ln*) transformed to achieve normal distributions and the residuals visually examined. For any surveys where the distribution of residuals appeared abnormal after transformation of the outcome variables, a sensitivity analysis compared the Spearman's rank correlation coefficient with the bivariate linear regression estimate, and the results examined to determine the appropriateness of continuing with linear regression analyses. All regression analyses in PSC surveys were conducted with the unadjusted retinol or RBP variable.

We assessed the following bivariate linear regression models in each survey:

1. $Y(ln\text{Retinol or RBP}) = B_0 + B_1(\text{BMI})^*$
2. $Y(ln\text{CRP}) = B_0 + B_1(\text{BMI})^*$
3. $Y(ln\text{AGP}) = B_0 + B_1(\text{BMI})^*$
4. $Y(ln\text{Retinol or RBP}) = B_0 + B_1(ln\text{CRP})$
5. $Y(ln\text{Retinol or RBP}) = B_0 + B_1(ln\text{AGP})$

*PSC models used BAZ in place of BMI.

Relationships between the previously defined covariates and outcome variables were assessed in separate bivariate linear regression analyses, with marginally significant covariates (at least $p<0.10$) included in multivariable adjusted models. Models were assessed for collinearity with variance inflation factors (>5) and tolerance (>0.1). CRP and AGP variables were analyzed in separate models, rather than combined into a single variable, in order to examine the individual associations between CRP or AGP with retinol or RBP. Regression results are presented as percent change in the dependent variable for

every 1-unit change in the independent variable, with their specific units of measurement (retinol or RBP: µmol/L; CRP: mg/L; AGP: g/L).

Mediation analysis

We used mediation analysis to test the hypothesis that inflammation mediates part or all of the relationship between BMI or BAZ and retinol or RBP among WRA or PSC with normal to elevated BMI or BAZ. Mediation analyses were conducted using Structural Equation Modeling (SEM) procedures in STATA according to the path diagram, with the path through CRP or AGP eliminated in surveys without the variable (**Supplemental Figure 3.1**). Interpretation of mediation analysis is as follows: total effect = the effect of BMI on retinol or RBP; direct effect = the effect of BMI on retinol or RBP controlling for inflammation; and indirect effect = the effect of BMI on retinol or RBP as mediated by the effect of CRP or AGP. Mediation was considered present if both the total effect and the indirect effect were significant at $p<0.05$ (30,31). Linearized standard errors and 95% CI were generated and adjusted for the complex survey design by calculating variance estimates and applying survey weights. Final mediation estimates were exponentiated, and adjusted mediation results are presented as percent change in retinol or RBP concentration for every 1-unit change in BMI or BAZ. Survey-specific SEM models were adjusted for the same marginally significant covariates found in bivariate models. All mediation analyses met the minimum sample size requirement of at least 10 observations per linear relation (19).

Sensitivity analyses

We conducted sensitivity analyses by including values for BMI <18.5 kg/m^2 (WRA) or BAZ/WHZ <-2 SD (PSC) for surveys where greater than 50% of the observations were excluded due to our pre-specified exclusion criteria (n=4 surveys WRA; n=7 surveys PSC). Sensitivity mediation analyses were also conducted in datasets with available malaria data to examine the inflammation-mediated relationship between BMI or BAZ and retinol or RBP among all individuals versus those without a positive malaria result (n=4 surveys WRA and n=8 surveys PSC; surveys from Burkina Faso were not included as all

observations that tested positive for malaria were excluded due to our exclusion criteria). An additional sensitivity mediation analysis was conducted in a subset of surveys to assess the effect of excluding observations with reported morbidity symptoms (fever or diarrhea in the past 24 hours [Kenya 2010, PSC] or past 2 weeks [Liberia, PSC; Côte d'Ivoire and Malawi, WRA]; results were compared qualitatively.

Ethical approval and role of the funding source

The BRINDA protocol was reviewed by the institutional review boards of the National Institute of Health and was deemed to be non-human-subjects research. The funder was not involved in the study design, analysis, interpretation, or decision to publish.

Results

Participant characteristics

Among WRA, after application of our exclusion criteria the analytical sample size ranged from n=61 (Burkina Faso) to n=4946 (Pakistan), with a total sample size of 16,771 (median [IQR] 692 [583, 1833]). The median age was approximately 30 years. The proportion of the analytical sample residing in urban areas ranged from 10.3% (Malawi) to 62.8% (Cameroon); the survey from Nigeria contained observations from rural areas only. In surveys with SES data (n=11 of 13) and after applying the exclusion criteria, more than 60% of the population was classified as high SES (**Supplemental Table 3.4**).

Among PSC, after application of our exclusion criteria the analytical sample size ranged from n=60 (Burkina Faso) to n=5896 (Pakistan), with a total sample size of 24,707 (median [IQR] 632 [352, 1235]). Most surveys (n=12 of 22) included children 6-59 months of age, while other surveys included the following age ranges: Bangladesh (2010), 6-24 months; Kenya (2007, 2010), Liberia, and Mongolia, 6-35 months; Vietnam, 10-59 months; Cameroon and Mexico (2012), 12-59 months; and Burkina Faso and the Philippines, 24-59 months. The proportion residing in urban areas ranged from 9.1% (Philippines) to

72.2% Mexico; the surveys from Kenya (2007, 2010) and Nigeria contained observations from rural areas only. Among surveys that measured SES (n=16), the proportion of the analytical sample classified as high SES ranged from 16.0% (Philippines) to 95.1% (Afghanistan) (**Supplemental Table 3.5**).

Prevalence of overweight and obesity, vitamin A deficiency, and inflammation among WRA

Among WRA, the median prevalence of overweight and obesity among surveys from UMIC and HIC was more than double the prevalence of overweight and obesity in surveys from LIC and LMIC (56.3% vs. 25.2%), with the greatest prevalence of overweight and obesity from the survey in Azerbaijan (UMIC) (57.3% (95% CI: 54.7, 59.8). The prevalence of vitamin A insufficiency among WRA was less than 8% in UMIC and HIC, and ranged from 5.8% (Vietnam) to 63.7% (Pakistan) in LIC and LMIC. The median prevalence of inflammation (elevated CRP and/or AGP) was similar among surveys from UMIC and HIC (26.3%) and LIC and LMIC (24.7%) **(Table 3.1).**

Prevalence of overweight and obesity, iron deficiency, and inflammation among PSC

Among PSC, prevalence of overweight and obesity was <10% across all surveys, except in Mongolia (10.5%, LIC), Nigeria (11.7%, LMIC), and Azerbaijan (14.5%, UMIC). The prevalence of inflammation-adjusted vitamin A deficiency among PSC varied across surveys, ranging from <1% in Nicaragua (LIC) and the Philippines (LMIC) to 42.8% in Zambia (LIC) and 51.0% in Pakistan (LMIC). The median prevalence of any inflammation in UMIC and HIC was 18.9% and 38.5% in LIC and LMIC. **(Table 3.2).**

Associations between BMI, vitamin A (retinol or RBP), and inflammation among WRA

Among WRA, results from adjusted linear models that examined the relationship between BMI and vitamin A (retinol or RBP) and BMI and inflammation (CRP and/or AGP) were similar across country income classifications (**Figure 3.1**, **Supplemental Table 3.6**). In 4 of 10 surveys from LIC and LMIC (Côte d'Ivoire, Papua New Guinea, Cameroon, and Vietnam) and 1 of 3 surveys from UMIC and HIC

(Azerbaijan), greater BMI was significantly and positively associated with retinol or RBP concentration; the percent increase in retinol or RBP for every 1-unit increase in BMI ranged from 0.3% (0.02, 0.5) (Vietnam) to 1.0% (0.2, 1.7) (Papua New Guinea) and 1.0% (0.8, 1.2) (Azerbaijan). In the US survey (HIC), greater BMI was negatively associated with retinol concentration (-0.4% [-0.6, -0.1]). Greater BMI was associated with greater inflammation in 10 of 13 surveys. The relationship between inflammation and retinol or RBP concentration was inconsistent across surveys and income levels, as well as between inflammatory markers. For example, in Nigeria, the association between CRP and RBP concentration was negative and significant while the relationship with AGP was positive and significant.

Associations between BAZ, vitamin A (retinol or RBP), and inflammation among PSC

Among PSC, the majority of relationships between BAZ and retinol or RBP, and BAZ and inflammation, were nonsignificant with varied directions of associations in adjusted models (Figure 3.1, **Supplemental Table 3.7**). Surveys from Bangladesh (2010, LIC), the Philippines (LMIC), and Colombia (UMIC) had significant positive associations between BAZ and retinol or RBP, and the percent increase in retinol or RBP concentration for every 1-unit increase in BAZ in these surveys ranged from 1.5% (0.5, 2.6) (Bangladesh, 2010) to 2.9% (1.0, 4.9) (Philippines). The survey from Nigeria (LMIC) showed a significant negative association between BAZ and RBP concentration (-9.9% [18.4, -0.4]). Only the survey from Malawi showed a significant (positive) relationship between BAZ and inflammation in the adjusted model. In contrast, retinol or RBP concentration was significantly negatively associated with inflammation (CRP and/or AGP) in 19 of 22 surveys.

Mediation analysis between BMI, vitamin A (retinol or RBP), and inflammation

Among WRA, inflammation partially mediated the relationship between BMI and retinol or RBP in 3 of 13 surveys in adjusted models: Papua New Guinea (LIC), Cameroon (LMIC), and the US (HIC) (**Figure 3.2, Table 3.3).** All three mediated relationships were inconsistent (i.e., opposite directions of

association between the direct and indirect effects (32)), and in each case of mediation there was a positive relationship between an inflammation biomarker and the vitamin A biomarker. In the survey from the US, the percent change in retinol concentration for every 1-unit increase in BMI was 0.2% (0.1, 0.4), and CRP mediated -69.2% of the relationship between BMI and retinol (i.e., CRP reduced the association between BMI and retinol by 69.2%). In Papua New Guinea and Cameroon, RBP concentration changed by -0.2% for every 1-unit increase in BMI in both surveys, and inflammation mediated -19.0% and -17.8% (respectively) of the relationship between BMI and RBP. Compared to AGP, CRP mediated the majority of the total inflammation proportion in both Papua New Guinea and Cameroon (77.0% CRP vs 21.0% AGP, Papua New Guinea; 82.6% CRP vs 17.4% AGP, Cameroon); the survey from the US did not measure AGP. Post-hoc mediation analyses that included the SES covariate in the adjusted mediation model in all surveys where the variable was available and not already included did not change the final mediation results.

Among PSC, mediation was present in 1 of 22 surveys. In the Philippines, inflammation positively mediated 24.3% of the relationship between BAZ and RBP, a 0.8% increase in RBP for every 1-unit change in BAZ (p=0.05 for the indirect effect). When compared to AGP, CRP mediated the majority of the total inflammation proportion (89.6% CRP vs 9.1% AGP) (**Table 3.4**). Unadjusted mediation results for both WRA and PSC are presented in **Supplemental Table 3.8.**

Sensitivity analyses

Among surveys that measured malaria status (n=4 WRA, n=8 PSC), results from the sensitivity mediation analysis that included all observations regardless of malaria test result were similar to the main mediation results that excluded observations with a positive malaria test result (**Supplemental Table 3.9**), as were results from a sensitively analysis that among surveys that additionally excluded observations with morbidity data of reported fever or diarrhea (**Supplemental Table 3.10**). Results were

also similar to the main results in sensitivity analyses that included all values for BMI <18.5 kg/m^2 (WRA) or BAZ/WHZ <-2 SD (PSC) in surveys where greater than 50% of the observations were excluded due to our pre-specified exclusion criteria (data not shown).

Discussion

We examined relationships between weight status, inflammation, and concentrations of vitamin A biomarkers (serum or plasma retinol or RBP) in national and regional surveys in 13 datasets from 13 countries with data on WRA, and 22 datasets from 20 countries with data on PSC. Among WRA with BMI ≥18.5 kg/m^2, we did not observe consistent relationships in the strength or direction of association between the inflammatory markers CRP or AGP and retinol or RBP concentration, even though BMI was generally positively and significantly associated with biomarkers of vitamin A and inflammation. Conversely, among PSC with BAZ ≥-2 SD, relationships between inflammation and retinol or RBP concentration were consistently significant and negative, while relationships between BAZ and biomarkers of vitamin A and inflammation were not observed. With mediation analysis we observed that accounting for inflammation reduced the relationship between BMI and vitamin A biomarkers in 3 WRA surveys, and increased the relationship in one PSC survey, though the magnitude of association was small in all cases (i.e., the percent change in retinol or RBP concentration for every 1-unit increase in BMI/BAZ ranged from -0.2% to 0.8%). No patterns emerged among World Bank country income classifications.

Our findings confirm observations from previous BRINDA analyses that inflammation was not consistently associated with vitamin A biomarkers among WRA, but that among PSC, biomarkers of association were strongly and consistently associated with biomarkers of vitamin A (6–8). Based on these observations, Larson et al. (7,8) and Namaste et al. (6) recommended to adjust for inflammation with the regression correction among PSC but not among WRA when assessing vitamin A status with

retinol or RBP. Of the 24 surveys we analyzed, 20 overlapped with those analyzed by Larson et al. and Namaste et al. We applied different inclusion and exclusion criteria, which shaped our datasets differently; however, the similarity of our finding reflects the robustness of their recommendation. We further examined the role of weight status, and found that this is unlikely to play a large role in population vitamin A assessment among both WRA and PSC. While our findings identified BMI as a correlate of inflammation in many surveys among WRA, these associations did not change the conclusion that inflammation had weak and inconsistent relationships with WRA vitamin A biomarkers.

We observed that inflammation partially mediated the relationship between BMI and retinol or RBP in 3 surveys among WRA (Cameroon, Papua New Guinea, and the US), but the mediation was inconsistent (i.e., the direct and indirect effects had opposite signs (32)), suppressing the total effect. Through examination of the mediation path analysis (Figure 3.2 and Table 3.3), we understand that in the US survey, the positive association between BMI and CRP weakened (suppressed) the association of BMI with retinol. In the surveys from Cameroon and Papua New Guinea, the relationship between AGP and RBP was positive, but the sum of the indirect effects was negative, meaning that, overall, it appears that inflammation had a suppressant effect on the relationship between BMI and RBP. Though this is inconsistent with biological relationships, the mediated effect is of such a small magnitude (-0.2% Cameroon, Papua New Guinea; 0.2% US), that this would not influence conclusions about population vitamin A status.

In adjusted linear regression analyses, BMI was associated with retinol or RBP in 6 of 13 surveys among WRA, and in general, greater BMI was associated with greater retinol or RBP. While the highly regulated circulating concentrations of RBP (and by association, retinol) appear to be primarily liver-derived (33–35), RBP (or RBP4) is also expressed in adipose tissue and has been identified as an adipokine (33,36). Because of the hypothesized role as an adipokine, researchers have found differing levels of circulating RBP associated with cardiovascular disease, insulin resistance, obesity, and

hypertension in adults (9,12,13,33,36,37), and obesity and metabolic syndrome in children and adolescents (38,39), though the direction of association varies and causal relationships have not been established. Additionally, it is unknown whether and how much of circulating RBP in states of chronic disease are adipocyte-derived (33). Thus, while current understanding of biological pathways supports a negative relationship between inflammation and circulating concentrations of RBP (and retinol), which in some cases may complicate assessment of vitamin A status, additional research is needed to clarify the relationship between chronic disease and RBP and/or retinol, including establishing temporal relationships and measurement of vitamin A stores. Given that global prevalence of overweight and obesity is rising, including among children (40), it will be useful to better understand how measurement and use of retinol and RBP may be influenced by overweight and obesity.

Among WRA, we observed consistent positive and significant relationships between BMI and CRP and/or AGP, which aligns with the literature that inflammation is often elevated in overweight and obesity (41–43), as well as previous BRINDA analyses (18). Both CRP and AGP tend be correlated with BMI, but literature examining the relationship between BMI and CRP (44–49) is more common than literature examining the relationship between BMI and AGP (50,51), even though AGP is generally thought of as a longer-term inflammatory biomarker (5). Furthermore, some studies suggest CRP may be more strongly associated with retinol and RBP than AGP (52), or that the influence of AGP on retinol or RBP may be inconsistent (53,54). The lesser influence of AGP on the relationship between BMI and retinol or RBP may be observed in our mediation results where the proportion mediated by AGP ranged from 9-21% compared to the proportion mediated by CRP (77-90%) in the three surveys that measured both CRP and AGP (Papua New Guinea [WRA], Cameroon [PSC], and The Philippines [PSC]). As CRP and AGP are often the only inflammatory biomarkers available for measurement in epidemiological surveys, incorporating a broader range of acute phase proteins would help understand which markers would best inform micronutrient assessment.

Among PSC, inflammation was significantly associated with lower retinol or RBP in all but three surveys (Bangladesh 2012, Cameroon, and Pakistan). We speculate that the inflammation driving these associations may be due to helminth or parasitic infections, diarrhea, or other common inflammatory illnesses among children that were not part of our exclusion criteria, but have been found to be associated in other BRINDA analyses (18,55). Though elevated CRP is associated with elevated BMI in studies among children and adolescents (46,56), the low median BAZ (0.2 SD) among PSC in our analyses may explain why we observed few associations between BAZ and inflammation, and between BAZ and retinol or RBP.

Strengths of this study include the diversity of geographic regions and country income classifications represented in our analyses. While no obvious trends emerged between LIC, LMIC, UMIC, and HIC, it is clear that greater BMI and greater inflammation are common worldwide, and that inflammation among children impacts vitamin A status assessment, regardless of exposure to infectious or noninfectious illnesses. Furthermore, the consistency of our findings with prior research with regard to vitamin A and inflammation biomarkers strengthens the plausibility of our results (6–8).

A limitation of our analyses is the proportion of observations excluded due to our exclusion criteria, as >70% of observations were excluded in the surveys from Afghanistan (WRA and PSC), Burkina Faso (WRA and PSC), Mexico (PSC), and Pakistan (WRA). While our analytical plan and exclusion criteria were developed prior to completing any analyses, and sensitivity analyses that included the observations excluded for malaria, or with BMI <18.5 kg/m^2 or BAZ <-2 SD, did not change the main results, we interpreted the results of the above surveys with caution. Additionally, in three surveys (Afghanistan, Colombia, and Zambia), we generated and imputed random CRP values between 0 and the lowest CRP value as >30% of the analytical sample had a single value for the lower limit of detection. The random imputation makes it difficult to assess the relationships with CRP in these surveys, however we

confirmed that the direction and strength of association did not differ with the randomly generated values by re-running all regression models with multiple random seeds.

Our analyses were also limited by the selected measurement parameters. For example, overweight and obesity were defined using BMI among adults, which is not a direct measure of adiposity, though BMI has been found to be highly correlated with waist circumference and predicting cardiometabolic risk (57). Additionally, although BAZ is not recommended for use with children <24 months (20), our use of BAZ was based on prior BRINDA analyses where use of BAZ rather than WHZ did not affect the conclusions (14). Measurement of inflammation was limited to CRP and AGP, and though there were no other inflammatory biomarkers available for analysis in these datasets, many epidemiological studies measure inflammation with CRP and AGP, and also use them for interpreting retinol and RBP concentrations; thus their inclusion here may enhance the comparability of our findings with other studies.

Additionally, the differing laboratory practices and calibration techniques employed by laboratories involved in sample analyses across the 24 individual surveys may have introduced random error, affecting our biomarker interpretation (28,58). However, the fact that we observed consistent relationships between BMI and inflammation for WRA, and for inflammation and vitamin A biomarkers for PSC, suggest that the null results observed elsewhere were not solely attributable to variation lab methods. Alternatively, we cannot rule out that the variation we observed in significant relationships among both PSC and WRA could be the influence of residual confounding, or the relationships simply occurred by chance. Finally, though neither accurately reflect vitamin A total body stores (3), both retinol and RBP are commonly measured biomarkers of vitamin A status and therefore useful to examine with respect to methodological factors that may affect their interpretation. Although we assumed an imprecise biological 1:1 ratio of retinol to RBP (59) for descriptive analyses, this would not affect the mediation results.

Conclusion

It has been established that inflammation affects assessment of vitamin A when measured with retinol or RBP, particularly among children, but as research on vitamin A has largely concentrated on relationships with conditions of undernutrition, the role of inflammation associated with overweight and obesity in vitamin A assessment has been less studied. Our findings suggest that, among WRA and PSC, inflammation associated with overweight and obesity does not impact vitamin A assessment when measured with retinol or RBP, though relationships were present among WRA in some settings. We also confirmed prior findings that inflammation consistently impacts vitamin A assessment with retinol and RBP among PSC, and inconsistently among WRA. When planning and implementing population vitamin A surveillance, considering the context, including possible sources of inflammation and common health conditions, should be prioritized.

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Tables

Table 3.1. Biological and nutritional characteristics for women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

CIC[2]	Country, survey year	n	BMI, kg/m^2, mean (95% CI)	Overweight/ obesity[3], % (95% CI)	CRP, mg/L, mean (95% CI)	AGP, g/L, mean (95% CI)	Any inflammation[4] % (95% CI)	RBP or SR[5], µmol/L mean (95% CI)	Vit A Deficiency[6] % (95% CI)	Vit A Insufficiency[6] % (95% CI)
Low	Afghanistan, 2013	571	25.1 (24.6, 25.6)	42.1 (36.0, 48.1)	0.7 (0.5, 0.8)	0.7 (0.7, 0.7)	18.3 (14.0, 22.5)	1.1 (1.0, 1.2)	11.0 (7.5, 14.5)	42.2 (35.0, 49.3)
	Burkina Faso, 2010	61	20.8 (20.0, 21.7)	3.1 (0.0, 7.8)	1.8 (1.3, 2.2)	1.2 (1.1, 1.3)	74.5 (63.5, 85.5)	0.9 (0.8, 1.1)	18.7 (9.8, 27.6)	63.4 (41.9, 85.0)
	Cambodia, 2014	609	22.9 (22.5, 23.3)	22.5 (17.1, 27.9)	0.8 (0.7, 0.9)	0.7 (0.7, 0.8)	35.9 (25.9, 45.8)	2.0 (1.8, 2.1)	3.0 (1.6, 4.3)	9.2 (6.6, 11.8)
	Côte D'Ivoire, 2007	706	23.5 (23.1, 23.8)	26.0 (22.3, 29.8)	1.5 (1.3, 1.7)	0.8 (0.8, 0.8)	31.9 (27.9, 35.9)	1.5 (1.4, 1.5)	0.7 (0.1, 1.3)	13.2 (10.2, 16.1)
	Malawi, 2016	595	22.8 (22.2, 23.4)	17.7 (13.3, 22.0)	0.7 (0.6, 0.8)	0.6 (0.6, 0.6)	11.7 (8.4, 15.1)	1.4 (1.3, 1.4)	2.7 (0.7, 4.7)	14.2 (10.5, 17.8)
	Papua New Guinea, 2005	692	23.3 (22.9, 23.7)	24.4 (19.6, 29.2)	0.4 (0.3, 0.5)	1.6 (1.5, 1.6)	23.6 (20.0, 27.3)	1.6 (1.5, 1.6)	0.4 (0.0, 1.0)	6.7 (4.5, 8.8)
Low-middle	Cameroon, 2009	594	24.7 (24.3, 25.1)	39.0 (34.4, 43.6)	0.9 (0.8, 1.1)	0.7 (0.7, 0.8)	16.3 (13.2, 19.4)	1.4 (1.3, 1.4)	1.4 (0.5, 2.3)	12.8 (9.7, 15.9)
	Nigeria, 2012	506	24.2 (23.6, 24.7)	35.2 (29.5, 40.9)	1.6 (1.4, 1.8)	0.7 (0.7, 0.8)	25.7 (21.6, 29.8)	1.6 (1.6, 1.7)	1.0 (0.8, 2.0)	8.7 (5.6, 11.8)
	Pakistan, 2011	4946	24.4 (24.2, 24.5)	36.5 (34.3, 38.3)	1.0 (0.9, 1.0)	0.8 (0.8, 0.8)	32.6 (36.0, 40.8)	0.8 (0.7, 0.8)	38.4 (36.0, 40.8)	63.7 (61.3, 66.2)
	Vietnam, 2010	1138	21.7 (21.6, 21.9)	10.2 (8.3, 12.0)	0.9 (0.8, 0.9)	--	7.1 (5.9, 8.4)	1.7 (1.6, 1.7)	1.1 (0.6, 1.7)	5.8 (4.4, 7.2)
Upper-middle	Azerbaijan, 2013	2528	26.8 (26.5, 27.1)	57.3 (54.7, 59.8)	1.1 (1.1, 1.2)	0.9 (0.8, 0.9)	34.9 (32.5, 37.4)	1.5 (1.5, 1.5)	0.4 (0.1, 0.7)	7.8 (6.5, 9.2)
High	United Kingdom, 2014	836	26.4 (25.8, 27.0)	49.0 (43.9, 54.2)	2.2 (2.0, 2.4)	--	15.5 (12.2, 18.9)	1.6 (1.5, 1.7)	1.1 (0.0, 2.2)	7.3 (4.1, 10.5)
	USA, 2006	2989	27.9 (27.4, 28.5)	56.3 (52.7, 59.9)	1.8 (1.7, 1.9)	--	26.3 (24.1, 28.5)	1.8 (1.7, 1.8)	0.3 (0.1, 0.6)	3.0 (2.2, 3.7)

[1]CRP, AGP, RBP, and SR values presented as geometric mean (95% CI) due to non-normal distributions. All estimates account for survey design variables (cluster, strata, weight). '--' indicates the variable was unavailable in that survey. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria

test result. AGP, α-1-acid glycoprotein; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CI, confidence interval; CIC, country income classification; CRP, C-reactive protein; RBP, retinol binding protein; SR, serum retinol.

[2]Country income classification defined according to the World Bank definition for the year in which the survey was conducted (27).

[3]Overweight/obesity defined as a BMI ≥25.0 kg/m^2.

[4]Any inflammation defined as CRP >5 mg/L or AGP >1 g/L.

[5]RBP or SR measured in plasma or serum, as reported in the survey. Surveys that measured RBP: Cambodia, Côte D'Ivoire, Malawi, Papua New Guinea, Cameroon, Nigeria, and Azerbaijan. Surveys that measured SR: Afghanistan, Burkina Faso, Pakistan, Vietnam, United Kingdom, and the USA.

[6]Vitamin A deficiency defined as serum or plasma RBP or SR concentration <0.7 µmol/L. Vitamin A insufficiency defined as serum or plasma RBP or SR <1.05 µmol/L (23,24).

Table 3.2. Biological and nutritional characteristics for preschool-aged children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

CIC[2]	Country, survey year	n	BAZ mean (95% CI)	Overweight/ Obesity[3] % (95% CI)	CRP, mg/L mean (95% CI)	AGP, g/L mean (95% CI)	Any inflammation[4] % (95% CI)	RBP or SR, µmol/L[5] mean (95% CI)	VA Deficiency[6] % (95% CI)	VAS[7] % (95% CI)
Low	Afghanistan, 2013	586	0.2 (0.1, 0.3)	7.0 (3.7, 10.3)	0.3 (0.2, 0.3)	0.8 (0.8, 0.8)	26.5 (21.5, 31.6)	0.7 (0.7, 0,8)	37.8 (32.5, 43.0)	43.3 (35.5, 51.2)
	Bangladesh, 2010	1179	-0.6 (-0.7, -0.5)	2.1 (1.3, 2.9)	0.8 (0.6, 0.9)	0.9 (0.9, 0.9)	34.4 (30.8, 38.1)	1.0 (1.0, 1.0)	5.3 (3.4, 7.2)	--
	Bangladesh, 2012	360	-0.4 (-0.6, -0.3)	3.8 (0.7, 6.9)	0.7 (0.6, 0.9)	0.8 (0.8, 0.9)	27.6 (19.9, 35.4)	1.0 (0.9, 1.0)	5.6 (2.2, 8.9)	78.3 (69.5, 87.1)
	Burkina Faso, 2010	60	-0.1 (-0.5, 0.3)	1.6 (0, 5.4)	5.2 (3.0, 7.4)	1.6 (1.5, 1.7)	91.9 (86.0, 97.8)	1.0 (0.9, 1.2)	13.4 (4.4, 22.5)	--
	Cambodia, 2014	599	-0.4 (-0.5, -0.4)	0.8 (0, 1.7)	0.6 (0.5, 0.7)	0.8 (0.7, 0.9)	38.5 (30.5, 46.5)	1.4 (1.3, 1.4)	6.3 (4.1, 8.5)	--
	Côte d'Ivoire, 2007	435	0.1 (0.0, 0.2)	6.2 (3.7, 8.6)	2.0 (1.6, 2.4)	1.1 (1.1, 1.1)	59.3 (54.3, 64.3)	1.3 (1.2, 1.3)	2.0 (0.7, 3.4)	--
	Kenya, 2007	665	0.2 (0.2, 0.3)	4.2 (2.5, 5.9)	1.0 (0.8, 1.2)	1.1 (1.1, 1.1)	59.4 (54.6, 64.2)	1.1 (1.0, 1.1)	5.6 (3.9, 7.2)	--
	Kenya, 2010	551	0.3 (0.2, 0.3)	4.5 (3.0 (6.1)	0.9 (0.7, 1.1)	1.0 (0.9, 1.0)	47.9 (42.0, 53.8)	1.1 (1.0, 1.1)	7.8 (5.7, 10.0)	--
	Liberia, 2011	956	-0.1 (-0.2, 0.01)	2.5 (1.4, 3.7)	1.4 (1.2, 1.6)	1.0 (0.9, 1.0)	50.4 (45.9, 54.8)	1.1 (1.0, 1.1)	5.5 (4.0, 7.0)	90.9 (88.4, 93.4)
	Malawi, 2016	748	0.1 (-0.1, 0.2)	5.3 (2.9, 7.8)	1.0 (0.8, 1.2)	1.0 (1.0, 1.1)	49.0 (42.5, 55.6)	1.0 (1.0, 1.1)	8.0 (5.1, 10.8)	70.1 (62.9, 77.2)
	Mongolia, 2006	202	0.8 (0.7, 0.9)	10.5 (6.9, 15.1)	--	0.8 (0.8, 0.9)	24.7 (19.4, 30.7)	0.8 (0.7, 0.8)	26.2 (20.2, 32.3)	--
	Nicaragua, 2005	1403	0.5 (0.4, 0.7)	6.2 (4.2, 8.2)	--	0.8 (0.8, 0.8)	24.1 (20.6, 27.6)	1.3 (1.3, 1.3)	0.7 (0.3, 1.1)	68.6 (64.2, 73.0)
	Philippines, 2011	802	0.2 (0.1, 0.3)	5.1 (2.7, 7.5)	1.3 (1.1, 1.6)	1.0 (1.0, 1.1)	56.3 (51.9, 60.8)	1.0 (1.0, 1.0)	10.1 (7.8, 12.5)	33.3 (23.4, 43.2)
	Zambia, 2009	313	0.4 (0.3, 0.6)	5.4 (2.4, 8.5)	1.6 (0.9, 2.2)	1.0 (0.9, 1.0)	71.6 (67.4, 75.7)	0.7 (0.7, 0.8)	42.8 (34.7, 51.0)	--
Low-middle	Cameroon, 2009	556	0.4 (0.3, 0.5)	4.2 (2.0, 6.3)	1.3 (1.1, 1.5)	0.9 (0.9, 0.9)	37.7 (32.7, 42.7)	1.0 (1.0, 1.0)	11.1 (8.3, 14.0)	27.0 (22.1, 32.0)
	Nigeria, 2012	283	0.4 (0.2, 0.7)	11.7 (6.1, 17.2)	2.0 (1.6, 2.5)	1.0 (0.9, 1.0)	56.2 (48.8, 63.6)	1.0 (1.0, 1.1)	11.7 (7.8, 12.5)	--
	Pakistan, 2011	5896	-0.1 (-0.1, -0.02)	5.4 (4.7, 6.1)	--	0.9 (0.9, 0.9)	35.3 (33.7, 36.9)	0.6 (0.6, 0.6)	51.0 (48.6, 53.3)	--

	Philippines, 2011	1656	-0.1 (-0.2, -0.01)	2.1 (1.0, 3.3)	0.7 (0.6, 0.9)	0.8 (0.8, 0.8)	25.9 (22.3, 29.4)	1.2 (1.2, 1.2)	0.8 (0.3, 1.3)	62.8 (58.8, 66.9)
	Vietnam, 2010	329	-0.1 (-0.3, -0.02)	4.3 (2.0, 6.5)	0.7 (0.6, 0.8)	--	12.5 (9.7, 15.3)	1.2 (1.2, 1.3)	5.5 (2.9, 8.0)	--
Upper-middle	Azerbaijan, 2013	987	0.9 (0.8, 1.0)	14.5 (11.7, 17.3)	0.3 (0.3, 0.4)	0.8 (0.8, 0.9)	30.8 (27.0, 34.6)	1.1 (1.0, 1.1)	6.2 (3.5, 8.8)	2.1 (1.0, 3.3)
	Colombia, 2010	3711	0.4 (0.3, 0.4)	3.7 (2.9, 4.4)	0.3 (0.3, 0.4)	--	18.9 (17.2, 20.7)	0.9 (0.9, 0.9)	19.8 (18.0, 21.7)	--
	Mexico, 2012	2430	0.6 (0.5, 0.6)	8.5 (6.7, 10.3)	0.5 (0.4, 0.6)	--	11.8 (9.3, 14.2)	1.0 (1.0, 1.0)	7.0 (5.4, 8.6)	--

[1] CRP, AGP, SR, and RBP values presented as geometric mean (95% CI) due to non-normal distributions. All estimates account for survey design variables (cluster, strata, weight), except Mongolia which followed a simple random sampling design. '--' indicates the variable was unavailable in that survey. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, α-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; CRP, C-reactive protein; RBP, retinol-binding protein; SR, serum retinol; VA, vitamin A; VAS, vitamin A supplementation; WHZ, weight-for-height z-score.

[2]CIC defined according to the World Bank definition for the year in which the survey was conducted (27).

[3]Overweight/obesity defined as a BMI-for-age z-score of ≥2 SD.

[4]Any inflammation defined as CRP >5 mg/L or AGP >1 g/L.

[5]RBP or SR measured in plasma or serum, as reported in the survey. Surveys that measured RBP: Bangladesh 2010, Cambodia, Cote d'Ivoire, Kenya 2007 and 2010, Liberia, Malawi, Papua New Guinea, Cameroon, Philippines, Azerbaijan; surveys that measured SR: Afghanistan, Bangladesh 2012, Burkina Faso, Mongolia, Nicaragua, Zambia, Nigeria, Pakistan, Vietnam, Colombia, Mexico.

[6]VA deficiency defined as RBP or SR concentration <0.7 µmol/L (23), adjusted for inflammation using the BRINDA regression correction approach (6,7).

[7]Vitamin A supplementation received by the child in the past 6 months, as reported in the survey.

Table 3.3. Relationships between vitamin A (retinol binding protein or serum retinol) and BMI as mediated by inflammation among women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

				WRA mediation analysis, adjusted[2]					
CIC[3]	**Country, survey year**	**n**	**RBP or SR**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	571	SR	0.2 (-1.3, 1.8)	0.1 (-1.5, 1.8)	0.1 (-0.1, 0.2)	NM	--	--
	Burkina Faso, 2010	61	SR	-0.2 (-4.5, 4.3)	-0.03 (-5.5, 5.7)	-0.1 (-2.7, 2.4)	NM	--	--
	Cambodia, 2014	609	RBP	-0.9 (-2.2, 0.4)	-2.4 (-3.7, -1.1)	1.5 (0.2, 2.8)	NM	--	--
	Côte d'Ivoire, 2007	706	RBP	0.7 (0.2, 1.3)	0.9 (0.3, 1.5)	-0.2 (-0.3, 0.0003)	NM	--	--
	Malawi, 2016	595	RBP	0.9 (-0.1, 1.9)	1.2 (-0.04, 2.4)	-0.3 (-0.5, 1.3)	NM	--	--
	Papua New Guinea, 2005	692	RBP	**1.0 (0.3, 1.7)**	**1.2 (0.4, 1.9)**	**-0.2 (0.4, -0.01)**	-19.0%	77.0%	21.0%
Low-middle	Cameroon, 2009	594	RBP	**0.8 (0.3, 1.4)**	**1.0 (0.4, 1.5)**	**-0.2 (-0.3, -0.03)**	-17.8%	82.6%	17.4%
	Nigeria, 2012	506	RBP	-0.1 (-0.7, 0.5)	-0.1 (-0.7, 0.5)	0.1 (-0.2, 0.3)	NM	--	--
	Pakistan, 2011	4946	SR	0.3 (-0.3, 0.8)	0.3 (-0.3, 0.8)	-0.02 (-0.1, 0.1)	NM	--	--
	Vietnam, 2010	1138	SR	0.9 (-0.03, 1.9)	0.9 (-0.2, 1.9)	0.1 (-0.3, 0.5)	NM	--	--
Upper-middle	Azerbaijan, 2013	2528	RBP	1.0 (0.7, 1.2)	0.9 (0.7, 1.2)	0.1 (-0.1, 0.2)	NM	--	--
High	United Kingdom, 2014	836	SR	-0.3 (-0.6, -0.1)	-0.3 (-0.9, 0.3)	-0.03 (-0.3, 0.3)	NM	--	--
	United States, 2006	2989	SR	**-0.3 (-0.5, -0.1)**	**-0.6 (-0.8, -0.3)**	**0.2 (0.1, 0.4)**	-69.2%	100%	--

[1]RBP, SR, CRP, and AGP variables were natural-log transformed for analysis due to non-normal distributions. Mediation estimates were exponentiated, and results are presented as percent change (95% confidence interval) in vitamin A (RBP or SR) for every 1-unit change in BMI, adjusted for available covariates. RBP or SR concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. AGP, alpha-1-acid glycoprotein; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; CRP, C-reactive protein; NM, no mediation; RBP, retinol binding protein; SR, serum retinol; WRA, women of reproductive age.

[2]Model for mediation analysis: *ln*Vitamin A [RBP or SR] = $\beta_0 + \beta_1$(BMI) + M_1(*ln*CRP) [+M_2(*ln*AGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BMI on Vitamin A; Direct Effect = the effect of BMI on Vitamin A controlling for inflammation; Indirect Effect = the effect of BMI on Vitamin A as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (30,31). Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the mediation model if they were associated with the outcome variable at p<0.1 in bivariate models (Supplemental Table 6). Unadjusted mediation estimates are presented in Supplemental Table 8.

[3]CIC defined according to the World Bank definition for the year in which the survey was conducted (27).

Table 3.4. Relationships between vitamin A (retinol or retinol-binding protein) and BMI-for-age z-score as mediated by inflammation among preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

				PSC mediation analysis, adjusted[2]					
CIC[3]	**Country, survey year**	**n**	**RBP or SR**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	306	SR	1.4 (-3.0, 5.7)	-0.3 (-4.0, 3.4)	1.7 (-0.3, 3.7)	NM	--	--
	Bangladesh, 2010	579	RBP	1.6 (0.5, 2.5)	1.3 (0.4, 2.1)	0.3 (-0.3, 0.8)	NM	--	--
	Bangladesh, 2012	196	SR	3.6 (-0.3, 7.3)	3.8 (0.2, 7.3)	-0.2 (-1.0, 0.5)	NM	--	--
	Burkina Faso, 2010	26	SR	-9.9 (-18.5, -2.3)	-10.7 (-19.7, -3.0)	1.0 (-4.3, 6.2)	NM	--	--
	Cambodia, 2014	326	RBP	-2.0 (-8.5, 4.4)	-1.5 (-4.6, 1.5)	-0.5 (-5.7, 4.6)	NM	--	--
	Côte d'Ivoire, 2007	238	RBP	-0.2 (-2.9, 2.5)	0.3 (-1.8, 2.4)	-0.5 (-1.8, 0.7)	NM	--	--
	Kenya, 2007	340	RBP	0.9 (-2.0, 3.7)	1.0 (-1.6, 3.7)	-0.2 (-1.0, 0.6)	NM	--	--
	Kenya, 2010	278	RBP	-2.0 (-4.8, 0.9)	-1.3 (-4.0, 1.4)	-0.7 (-1.8, 0.5)	NM	--	--
	Liberia, 2011	455	RBP	1.9 (-0.1, 3.8)	1.9 (0.03, 3.8)	-0.1 (-0.7, 0.6)	NM	--	--
	Malawi, 2016	384	RBP	1.5 (-1.1, 4.1)	2.8 (0.4, 5.0)	-1.3 (-2.3, -0.3)	NM	--	--
	Mongolia, 2006	107	SR	-0.9 (-9.2, 7.5)	-1.6 (-9.9, 6.6)	0.8 (-0.7, 2.2)	NM	--	--
	Nicaragua, 2005	703	SR	0.9 (-1.8, 3.5)	0.8 (-2.0, 3.5)	0.1 (-0.3, 0.5)	NM	--	--
	Papua New Guinea	430	RBP	2.5 (0.1, 4.7)	1.7 (-0.5, 3.9)	0.7 (-0.2, 1.7)	NM	--	--
	Zambia, 2009	182	SR	0.03 (-3.3, 3.4)	-0.02 (-3.4, 3.4)	0.1 (-1.1, 1.2)	NM	--	--
Low-middle	Cameroon, 2009	282	RBP	-1.9 (-4.2, 0.3)	-1.5 (-3.6, 0.6)	-0.5 (-1.3, 0.4)	NM	--	--
	Nigeria, 2012	146	SR	1.6 (-1.3, 4.4)	1.9 (-0.8, 4.6)	-0.3 (-1.3, 0.6)	NM	--	--
	Pakistan, 2011	3035	SR	-1.6 (-3.6, 0.4)	-1.6 (-3.6, 0.4)	-0.02 (-0.1, 0.1)	NM	--	--
	Philippines, 2011	819	RBP	**3.1 (1.2, 4.9)**	**2.4 (0.5, 4.2)**	**0.8 (-0.01, 1.5)[4]**	24.3%	89.6%	9.1%
	Vietnam, 2010	171	SR	2.6 (-1.0, 6.1)	2.2 (-1.3, 5.7)	0.4 (-0.2, 1.0)	NM	--	--
Upper-middle	Azerbaijan, 2013	543	RBP	-0.1 (-1.9, 1.6)	-0.8 (-2.2, 0.7)	0.7 (-0.1, 1.4)	NM	--	--
	Colombia, 2010	1960	SR	2.2 (0.7, 3.6)	2.1 (0.6, 3.5)	0.1 (-0.1, 0.3)	NM	--	--
	Mexico, 2012	1203	SR	1.3 (-0.4, 2.9)	1.7 (0.03, 3.4)	-0.5 (-1.1, 0.2)	NM	--	--

[1]SR, RBP, CRP, and AGP variables were natural-log transformed for analysis due to non-normal distributions. Mediation estimates were exponentiated, and results are presented as percent change (95% confidence interval) in vitamin A (SR or RBP) for every 1-unit change in BAZ, adjusted for available covariates. SR or RBP concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which used simple random sampling. Inclusion criteria were: BAZ or WHZ ≥-2 SD and a negative malaria test result. AGP, alpha-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; CRP, C-reactive protein; NM, no mediation; PSC, preschool-age children; WHZ, weight-for-height z-score.

[2]Model for mediation analysis: lnVitamin A [RBP or SR] = $\beta_0 + \beta_1$(BAZ) + M_1(lnCRP) [+M_2(lnAGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BAZ on vitamin A; Direct Effect = the effect of BAZ on vitamin A controlling for inflammation; Indirect Effect = the effect of BAZ on vitamin A as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (30,31). Covariates available for adjustment were: age, education level (head of

household or maternal education level), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the mediation model if they were associated with the outcome variable at p<0.1 in bivariate models (Supplemental Table 6). Unadjusted mediation estimates are presented in Supplemental Table 8.

[3]CIC defined according to the World Bank definition for the year in which the survey was conducted (27).

[4]P-value for indirect effect = 0.05.

Figures

Figure 3.1. Patterns of association among WRA and PSC between Vitamin A (RBP or SR), BMI or BAZ, and inflammation biomarkers (CRP or AGP) in 24 surveys: BRINDA Project[1]

			Adjusted linear regression models (dependent variable = independent variable)									
			VA = BMI/BAZ		**CRP = BMI/BAZ**		**AGP = BMI/BAZ**		**VA = CRP**		**VA = AGP**	
CIC	Survey, year	RBP or SR	WRA	PSC	WRA	PSC	WRA	PSC	WRA	PSC	WRA	PSC
Low	Afghanistan, 2013	SR	+	+	+	-	+	-	+	**-**	-	**-**
	Bangladesh, 2010	RBP		**+**		-		-		**-**		**-**
	Bangladesh, 2012	SR		+		+		-		-		-
	Burkina Faso, 2010	SR	-	**-**	**-**	-	-	+	+	**-**	+	-
	Cambodia, 2014	RBP	-	-	**+**	+	**+**	+	**+**	**+**	**+**	**+**
	Côte d'Ivoire, 2007	RBP	**+**	-	**+**	+	**+**	-	-	**-**	-	**-**
	Kenya, 2007	RBP		+		+		-		**-**		**-**
	Kenya, 2010	RBP		-		+		+		**-**		**-**
	Liberia, 2011	RBP		+		+		-		**-**		**-**
	Malawi, 2016	RBP	+	+	**+**	**+**	**+**	+	**-**	**-**	-	**-**
	Mongolia, 2006	SR		-				-				**-**
	Nicaragua, 2005	SR		+				-				**-**
	Papua New Guinea, 2005	RBP	**+**	+	**+**	-	+	-	**+**	**-**	-	**-**
	Zambia, 2009	SR		-		-		+		**-**		**-**
Low-middle	Cameroon, 2009	RBP	**+**	-	**+**	+	**+**	-	**-**	**-**	-	**-**
	Nigeria, 2012[2]	RBP/SR	-	+	-	+	+	-	**-**	**-**	**+**	**-**
	Pakistan, 2011	SR	+	-	**+**		**+**	+	-		+	-
	Philippines, 2011	RBP		**+**		-		-		**-**		**-**
	Vietnam, 2010	SR	**+**	+	**+**	-			+	**-**		
Upper-middle	Azerbaijan, 2013	RBP	**+**	-	**+**	-	**+**	-	**-**	**-**	**+**	**-**
	Colombia, 2010	SR		**+**		-				**-**		
	Mexico, 2012	SR		+		+				**-**		
High	United Kingdom, 2014	SR	-		**+**				-			
	United States, 2006	SR	**-**		**+**				+			

Figure 3.1 Footnotes:

[1]Associations from adjusted linear regression models are presented. Green cells with bolded '**+**' indicate a significant positive association; orange cells with a bolded '**-**' indicate a significant negative association ($p<0.05$). Grey cells with '+' or '-' indicate the direction of non-significant associations. Blank cells indicate the variable was not available in that dataset. See Supplemental Tables 6 and 7 for regression estimates for both unadjusted and adjusted models. All estimates accounted for the complex survey design (cluster, strata) with survey weights applied. VA (RBP or SR) was measured in either serum or plasma, as reported by the survey. Covariates available for adjustment were: age, education level (respondent, maternal, or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in adjusted regression models if they were associated with the outcome variable at $p<0.1$ in the unadjusted model. Inclusion criteria for analysis were: BMI ≥18.5 kg/m^2 (WRA), BAZ ≥-2 SD or WHZ ≥-2 SD (PSC), not pregnant (WRA), and a negative malaria test result (WRA and PSC). CIC was defined according to the World Bank definition for the year in which the survey was conducted (27). Abbreviations: AGP, alpha-1 acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; CRP, C-reactive protein; PSC, preschool-age children (6-59 months); RBP, retinol-binding protein; SR, serum retinol; VA, vitamin A; WRA, women of reproductive age (15-49 years).

[2]In the Nigeria survey for WRA, RBP was used for analysis as there were fewer missing values; for PSC, retinol had fewer missing values and was used for analysis.

Figure 3.2. Examples of inconsistent mediation present in the WRA surveys from Cameroon, Papua New Guinea, and the United States: BRINDA project

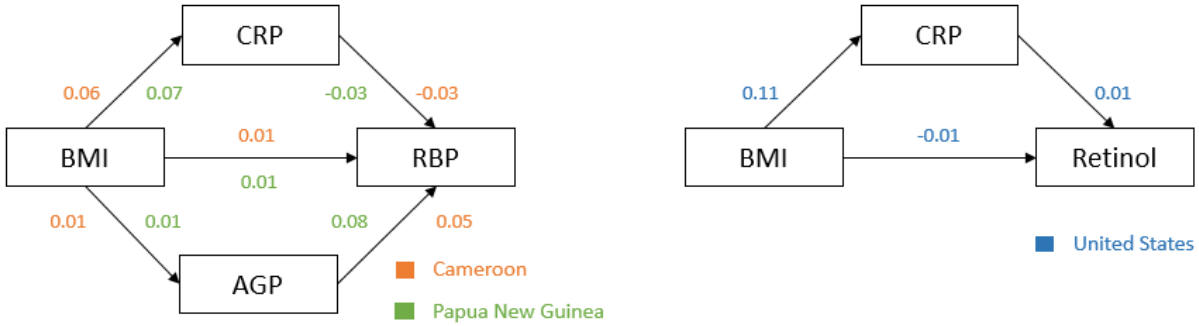
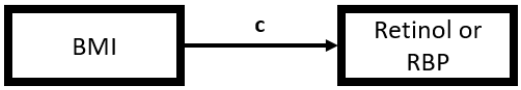


Figure 3.2 Footnote: The model for mediation analysis was *ln*Retinol/RBP = β_0 + β_1(BMI) + M_1(*ln*CRP) [+M_2(*ln*AGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Mediation was considered present when both the total and indirect effects were significant at $p<0.05$ (Table 3) (30,31). Inconsistent mediation is defined as the indirect and direct effects having opposite directions of associations (32). Interpretation is as follows: Total Effect = the effect of BMI on retinol/RBP (value between 'BMI' and 'Retinol/RBP'); Direct Effect = the effect of BMI on retinol/RBP controlling for inflammation (value not shown); Indirect Effect = the effect of BMI on retinol/RBP as mediated by the effect of CRP or AGP (values between 'BMI', 'CRP/AGP' and 'Retinol/RBP'). Indirect effects are calculated by multiplying the values between the independent variable (BMI) and the mediator (CRP) by the value between the mediator (CRP) and the dependent variable (retinol/RBP), and then summing that value with the second indirect effect when there is a second mediator (AGP) (see Supplemental Figure 1). Covariates included in the specified models were: age and education level (Cameroon); age, education level, and SES (Papua New Guinea); and age and SES (United States). Age was defined in years, education level as the highest level completed by the respondent, and SES was a 3-level ordinal variable of low, medium and high SES from the harmonized BRINDA project dataset (16). Covariates were included in the mediation model if they were associated with the outcome variable at $p<0.1$ in bivariate models (see Supplemental Table 6). AGP, alpha-1 acid glycoprotein; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CRP, C-reactive protein; ln, natural log; SES, socioeconomic status; WRA, woman of reproductive age (15-49 years).

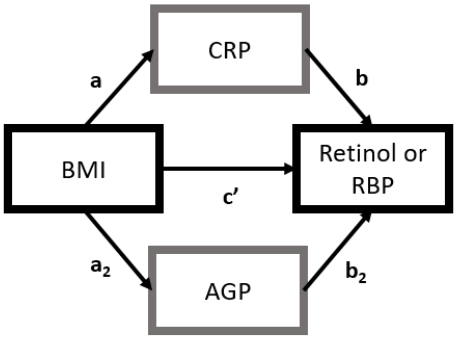
Supplemental Figure 3.1. Mediation analysis model

Total Effect: Effect of BMI on retinol or RBP (c)



Direct Effect: Effect of BMI on retinol or RBP controlling for inflammation (**c'**)

Indirect Effect: Effect of BMI on retinol or RBP as mediated by the effect of CRP (a*b) or of AGP (a_2*b_2)



Supplemental Figure 3.1 Footnote. Abbreviations: AGP, alpha-1 acid glycoprotein; BMI, body mass index; CRP, C-reactive protein; RBP, retinol-binding protein. References for mediation model: (1,2)

Supplemental Table 3.1. Number and proportion of observations excluded due to exclusion criteria for WRA, by survey: BRINDA project[1]

		Exclusion Criteria										
Survey, year	**Total obs. in dataset**	**BMI <18.5 kg/m²**	**Implausible Height**	**Implausible Weight**	**Pregnant**	**Positive Malaria Result**	**Missing BMI**	**Missing SR or RBP**	**Missing CRP**	**Missing AGP**	**Total obs. excluded**	**Analytical sample size & proportion of total**
	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Afghanistan, 2013	23875	954 (4.0)	0 (0)	0 (0)	0 (0)	n/a	13991 (58.6)	22829 (95.6)	22825 (95.6)	22825 (95.6)	23304 (97.6)	571 (2.4)
Burkina Faso, 2010	484	60 (12.4)	0 (0)	0 (0)	0 (0)	36 (7.4)	70 (14.5)	340 (70.2)	355 (73.3)	355 (73.3)	423 (87.4)	61 (12.6)
Cambodia, 2014	724	94 (13.0)	2 (0.3)	1 (0.1)	0 (0)	n/a	0 (0)	19 (2.6)	19 (2.6)	19 (2.6)	115 (15.9)	609 (84.1)
Côte D'Ivoire, 2007	863	81 (9.4)	11 (1.3)	2 (0.2)	n/a	39 (4.5)	14 (1.6)	29 (3.4)	29 (3.4)	29 (3.4)	157 (18.2)	706 (81.8)
Malawi, 2016	804	70 (8.7)	1 (0.1)	0 (0)	0 (0)	116 (14.4)	17 (2.1)	28 (3.5)	28 (3.5)	28 (3.5	210 (26.1)	595 (74.0)
Papua New Guinea, 2005	779	51 (6.5)	4 (0.5)	0 (0)	n/a	n/a	14 (1.8)	30 (3.9)	30 (3.9)	30 (3.9)	87 (11.2)	692 (88.8)
Cameroon, 2009	787	67 (8.5)	0 (0)	0 (0)	n/a	108 (13.7)	4 (0.5)	27 (3.4)	27 (3.4)	27 (3.4)	178 (22.6)	609 (77.4)
Nigeria, 2012	620	55 (8.9)	0 (0)	1 (0.2)	n/a	56 (9.0)	10 (1.6)	0 (0)	0 (0)	0 (0)	114 (18.4)	506 (81.6)
Pakistan, 2011	22278	3024 (15.6)	3 (0.01)	4 (0.02)	0 (0)	n/a	361 (1.6)	14265 (64.0)	14381 (64.6)	14017 (63.0)	17332 (77.8)	4946 (22.2)
Vietnam, 2010	1492	305 (20.4)	0 (0)	0 (0)	n/a	n/a	1 (0.1)	52 (3.5)	9 (0.6)	n/a	354 (23.7)	1138 (76.3)
Azerbaijan, 2013	2910	138 (4.7)	0 (0)	0 (0)	0 (0)	n/a	73 (2.5)	254 (8.7)	254 (8.5)	254 (8.7)	382 (13.1)	2528 (86.9)
United Kingdom, 2014	2050	69 (3.7)	0 (0)	0 (0)	0 (0)	n/a	93 (4.5)	1153 (56.2)	1108 (54.1)	n/a	1214 (59.2)	836 (40.8)
United States, 2006	3456	143 (4.1)	0 (0)	1 (0.03)	0 (0)	n/a	47 (1.4)	311 (9.0)	259 (7.5)	n/a	467 (13.5)	2989 (86.5)

[1]Criteria for exclusion from analyses were: BMI <18.5 kg/m^2; height or weight outside the ranges of 101.6-219.9 cm and 22.7-222.2 kg;(3) pregnancy; positive test result for malaria; or missing values for RBP, SR, CRP, AGP, or BMI (due to missing values for weight or height). Some observations may be excluded from multiple categories. Exclusion criteria percentages are proportions of total observations in the individual datasets. Abbreviations: AGP, α-1-acid glycoprotein; BMI, body mass index; BRINDA, Biomarkers Reflecting Nutritional Determinants of Anemia; CRP, C-reactive protein; obs., observations; RBP, retinol-binding protein; SR, serum retinol; WRA, women of reproductive age (15-49 years).

Supplemental Table 3.2. Number and proportion of observations excluded due to exclusion criteria for PSC, by survey: BRINDA project[1]

		Exclusion Criteria										
Survey, year	**Total obs. in dataset**	**BAZ <-2 SD**	**WHZ <-2 SD**	**Implausible BAZ**	**Implausible WHZ**	**Positive Malaria Result**	**Missing BAZ**	**Missing SR or RBP**	**Missing CRP**	**Missing AGP**	**Total obs. excluded**	**Analytical sample size & proportion of total**
	n	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Afghanistan, 2013	19896	1598 (8.0)	1823 (9.2)	744 (3.8)	424 (2.1)	n/a	341 (1.7)	19239 (96.7)	19231 (96.7)	n/a	19310 (97.0)	586 (3.0)
Bangladesh, 2010	1561	305 (19.5)	277 (17.7)	12 (0.8)	8 (0.5)	n/a	18 (1.2)	68 (4.4)	68 (4.4)	68 (4.4)	382 (24.5)	1179 (75.5)
Bangladesh, 2012	1108	134 (12.1)	161 (14.5)	26 (2.3)	19 (1.7)	n/a	85 (7.7)	235 (21.2)	637 (57.5)	637 (57.5)	748 (67.5)	360 (32.5)
Burkina Faso, 2010	482	5 (1.1)	8 (1.7)	0 (0)	0 (0)	9 (1.9)	73 (15.1)	357 (74.1)	357 (74.1)	357 (74.1)	422 (87.6)	60 (12.4)
Cambodia, 2014	874	60 (6.9)	69 (7.9)	5 (0.6)	1 (0.1)	n/a	0 (0)	209 (23.9)	209 (23.9)	209 (23.9)	275 (31.5)	599 (68.5)
Côte D'Ivoire, 2007	864	104 (12.0)	116 (13.4)	29 (3.4)	16 (1.9)	214 (24.8)	37 (4.3)	118 (13.7)	118 (13.7)	118 (13.7)	429 (49.7)	435 (50.3)
Kenya, 2007	1056	47 (4.5)	51 (4.8)	5 (0.5)	5 (0.5)	196 (18.6)	40 (3.8)	160 (15.2)	160 (15.2)	160 (15.2)	391 (37.0)	665 (63.0)
Kenya, 2010	896	26 (2.9)	29 (3.2)	4 (0.5)	3 (0.3)	276 (30.8)	31 (3.5)	47 (5.2)	47 (5.3)	47 (5.3)	345 (38.5)	551 (61.5)
Liberia, 2011	1476	129 (8.7)	152 (10.3)	3 (0.2)	3 (0.2)	358 (24.3)	10 (0.7)	42 (2.8)	42 (2.9)	42 (2.9)	520 (35.2)	956 (64.8)
Malawi, 2016	1233	48 (3.9)	52 (4.2)	12 (1.0)	8 (0.7)	310 (25.1)	24 (2.0)	131 (10.6)	131 (10.6)	131 (10.6)	485 (39.3)	748 (60.7)
Mongolia, 2006	242	0 (0)	0 (0)	0 (0)	0 (0)	n/a	1 (0.4)	40 (16.5)	n/a	2 (0.8)	40 (16.5)	202 (83.5)
Nicaragua, 2005	1424	11 (0.8)	10 (0.8)	2 (0.1)	2 (0.1)	n/a	4 (0.3)	4 (0.3)	n/a	0 (0)	21 (1.5)	1403 (98.5)
Papua New Guinea, 2005	934	37 (4.0)	41 (4.4)	12 (1.3)	6 (0.6)	n/a	37 (4.0)	62 (6.6)	63 (6.7)	62 (6.6)	132 (14.1)	802 (85.9)
Zambia, 2009	885	5 (0.6)	6 (0.7)	1 (0.1)	0 (0)	n/a	2 (0.2)	494 (55.8)	473 (53.4)	474 (53.4)	572 (64.6)	313 (35.4)
Cameroon, 2009	853	16 (1.9)	29 (3.4)	1 (0.1)	0 (0)	195	22.9	61 (7.2)	61 (7.2)	61 (.7.2	297 (34.8)	556 (65.2)

Nigeria, 2012	640	61 (9.5)	63 (9.8)	30 (4.7)	25 (3.9)	198 (30.9)	13 (2.0)	63 (9.8)	93 (14.5)	93. (14.5)	357 (55.8)	283 (44.2)
Pakistan, 2011	10689	1365 (12.8)	1543 (14.4)	361 (3.4)	280 (2.6)	n/a	524 (4.9)	3370 (31.5)	n/a	3132 (29.3)	4793 (44.8)	5896 (55.2)
Philippines, 2011	1784	80 (4.5)	98 (5.5)	6 (0.3)	4 (0.2)	n/a	6 (0.3)	17 (1.0)	17 (1.0)	17 (1.0)	128 (7.2)	1656 (92.8)
Vietnam, 2010	395	23 (5.8)	25 (6.3)	2 (0.5)	1 (0.2)	n/a	3 (0.8)	32 (8.1)	17 (4.3)	n/a	66 (16.7)	329 (83.3)
Azerbaijan, 2013	1404	41 (2.9)	39 (2.8)	49 (3.5)	39 (2.8)	n/a	49 (3.5	351 (25.0)	351 (25.0)	351 (25.0)	417 (29.7)	987 (70.3)
Colombia, 2010	7753	66 (0.9)	76 (1.0)	11 (0.1)	7 (0.10)	n/a	161 (2.1)	3415 (44.0)	3887 (50.1)	n/a	4042 (52.1)	3711 (47.9)
Mexico, 2012	8528	101 (1.2)	102 (1.2)	0 (0)	0 (0)	n/a	482 (5.7)	5932 (69.6)	5989 (70.2)	n/a	6098 (71.5)	2430 (28.5)

[1]Criteria for exclusion from analyses were: BAZ or WHZ <-2 SD; BAZ or WHZ less than -5 SD or greater than 5 SD;(4) positive test result for malaria; or missing values for SR, RBP, CRP, AGP, or BAZ (due to missing values for weight or height/length). Some observations may be excluded from multiple categories. Exclusion criteria percentages are proportions of total observations in the individual datasets. Abbreviations: AGP, alpha-1-acid glycoprotein; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Nutritional Determinants of Anemia; CRP, C-reactive protein; PSC, preschool-age children (6-59 months); RBP, retinol-binding protein; SR, serum retinol; WHZ, weight-for-height z-score.

Supplemental Table 3.3. Available covariates in each survey: BRINDA project[1]

Survey	Age	Rural/Urban Setting	SES	Water	Sanitation	Respondent/Maternal Education Level	Household Head Education Level
WRA							
Afghanistan	x	--	x	x	x	x	--
Azerbaijan	x	x	x	x	x	x	--
Burkina Faso	x	--	x	x	x	x	x
Cambodia	x	x	x	x	x	x	--
Cameroon	x	x	x	x	x	x	--
Côte d'Ivoire	x	x	x	x	x	x	--
Malawi	x	x	x	x	x	x	--
Nigeria	x	x	--	--	--	--	--
Pakistan	x	x	x	x	x	x	--
Papua New Guinea	x	x	x	--	--	x	--
Vietnam	x	x	--	--	--	--	--
United Kingdom	x	--	x	--	--	x	--
United States	x	--	x	--	--	x	--
PSC							
Afghanistan	x	--	x	x	x	x	--
Azerbaijan	x	x	x	x	x	x	--
Bangladesh, 2010	x	--	--	x	x	--	--
Bangladesh, 2012	x	x	x	x	x	x	--
Burkina Faso	x	--	x	x	x	x	x
Cambodia	x	x	x	x	x	x	--
Cameroon	x	x	x	x	x	x	--
Colombia	x	x	x	x	x	--	x
Côte d'Ivoire	x	x	x	x	x	x	--
Kenya, 2007	x	x	x	x	x	x	--
Kenya, 2010	x	x	x	x	x	x	--
Liberia	x	x	x	x	x	--	--
Malawi	x	x	x	x	x	x	--
Mexico, 2012	x	x	x	--	--	--	--
Mongolia	x	x	--	--	--	x	--
Nicaragua	x	x	--	x	x	x	--
Nigeria	x	x	--	--	--	--	--
Pakistan	x	x	x	x	x	x	--
Papua New Guinea	x	x	x	x	x	--	--
Philippines	x	x	x	x	x	x	--
Vietnam	x	x	--	--	--	--	--
Zambia	x	x	--	--	--	--	--

[1]Covariate definitions: age in years (WRA) or months (PSC); sex (male/female; PSC only); urban or rural residence (surveys from Kenya and Nigeria contained observations from rural areas only); household socioeconomic status (SES, variable created from the ordinal 3-category SES variable from the harmonized BRINDA dataset (5)); water and sanitation sources defined as access to an improved or unimproved water source or toilet facility as defined by the WHO/UNICEF Joint Monitoring Program (6,7); and education level defined as the highest level completed by either the respondent/caregiver or head of household. BRINDA: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; PSC, preschool-age children (6-59 months); SES, socio-economic status; WRA, women of reproductive age (15-49 years).

Supplemental Table 3.4. Age and household characteristics for women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

CIC[2]	Survey, year	n	Age, yr median [IQR]	Urban residence % (95% CI)	High SES[3] % (95% CI)	Improved Water Source[4] % (95% CI)	Improved Toilet[5] % (95% CI)	High Education[6] % (95% CI)
Low	Afghanistan, 2013	571	29.2 [24.3, 29.8]	--	92.9 (89.2, 96.6)	81.5 (75.7, 87.4)	56.6 (47.8, 65.3)	10.2 (6.1, 14.4)
	Burkina Faso, 2010	61	30.2 [25.3, 37.7]	--	76.5 (57.9, 95.0)	31.3 (5.8, 56.7)	13.0 (0.9, 25.1)	2.1 (0.0, 7.6)
	Cambodia, 2014	609	29.8 [25.1, 33.6]	13.1 (9.9, 16.3)	60.5 (53.7, 67.3)	53.1 (45.7, 60.6)	52.1 (46.6, 57.7)	31.7 (26.5, 36.9)
	Côte d'Ivoire, 2007	706	26.3 [21.1, 31.9]	56.1 (51.9, 60.3)	63.9 (58.1, 69.8)	88.0 (82.6, 93.3)	89.9 (85.9, 93.8)	15.5 (11.5, 19.4)
	Malawi, 2016	595	27.7 [20.7, 36.7]	10.3 (1.9, 18.7)	61.0 (52.9, 69.0)	83.6 (75.7, 91.4)	83.8 (76.4, 91.2)	22.0 (15.6, 28.4)
	Papua New Guinea, 2005	692	27.6 (20.5, 35.3)	21.3 (11.1, 31.4)	61.5 (49.6, 73.5)	--	--	24.4 (19.6, 29.2)
Low-middle	Cameroon, 2009	594	26.2 [22.0, 31.8]	62.8 (52.5, 73.2)	64.1 (56.7, 71.6)	75.0 (68.9, 81.0)	67.0 (61.5, 72.6)	37.5 (33.3, 41.7)
	Nigeria, 2012	506	26.3 [21.5, 31.1]	0[7]	--	--	--	--
	Pakistan, 2011	4946	29.6 [25.8, 34.6]	32.7 (29.4, 36.0)	61.7 (59.1, 64.3)	92.9 (91.5, 94.2)	84.5 (82.7, 86.4)	30.8 (28.5, 33.1)
	Vietnam, 2010	1138	33.5 [26.0, 41.0]	49.8 (47.2, 52.5)	--	--	--	--
Upper-middle	Azerbaijan, 2013	2528	31.7 [23.9, 41.4]	45.7 (39.4, 51.9)	68.6 (65.1, 72.2)	77.1 (72.2, 82.1)	93.7 (90.9, 96.5)	95.0 (93.5, 96.5)
High	United Kingdom, 2014	836	33.9 [24.1, 42.0]	--	63.7 (58.3, 69.0)	--	--	91.4 (88.7, 94.1)
	United States, 2006	2989	34.7 [24.7, 42.5]	--	74.1 (70.9, 77.3)	-	--	100.0 (100,100)

[1]Estimates account for complex survey design (cluster, strata) with survey weights applied. Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. '--' indicates the variable was unavailable in that survey. BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; SES, socioeconomic status.

[2]CIC defined according to the World Bank definition for the year the survey took place (8).

[3]SES variables was a 3-level ordinal SES variable available from the harmonized BRINDA dataset (5), which was created from survey-specific asset scores (quintiles) of household ownership or composition. 'High SES' includes the medium and high levels.

[4]'Improved Water Source' was defined as having access to: piped water in a dwelling/yard; a communal/public tap; a borehole/tube well, owned or shared; a protected well/spring; a protected open dug well; or rain water (6).

[5]'Improved Toilet' was defined as have access to: a flush toilet/pit latrine flush to piped sewer; a ventilated improved pit/latrine/Sanplat; or a flush to pit/latrine (7).

[6]'High Education' was defined as completing at least secondary school, and was measured as the respondent education level or head of household education level (Burkina Faso).

[7]The survey from Nigeria only contained observations from rural areas.

Supplemental Table 3.5. Age, sex, and household characteristics for preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

CIC[2]	Country, survey year	n	Age, mo median [IQR]	Male %	Urban residence % (95% CI)	High SES[3] % (95% CI)	Improved Water Source[4] % (95% CI)	Improved Toilet[5] % (95% CI)	High Education[6] % (95% CI)
Low	Afghanistan, 2013	586	27.9 [17.0, 39.9]	52.2	--	95.1 (92.9, 97.4)	81.4 (73.8, 88.9)	68.1 (60.1, 76.2)	9.9 (5.3, 14.4)
	Bangladesh, 2010	1179	7.7 [6.3, 9.3]	49.1	--	--	98.4 (95.3, 100.0)	25.2 (16.9, 33.5)	--
	Bangladesh, 2012	360	37.9 [26.8, 48.0]	54.4	26.3 (18.5, 34.0)	44.7 (33.3, 56.0)	99.0 (97.5, 100.0)	63.9 (50.0, 77.9)	47.0 (35.7, 58.2)
	Burkina Faso, 2010	60	48.3 [43.6, 53.5]	43.3	--	81.2 (63.9, 98.4)	31.2 (8.7, 53.6)	11.3 (0.0, 26.1)	2.2 (0, 7.4)
	Cambodia, 2014	599	37.0 [24.6, 48.3]	54.4	11.6 (8.5, 14.7)	58.4 (50.0, 66.7)	53.4 (44.4, 62.3)	53.9 (46.7, 61.1)	27.7 (22.5, 32.9)
	Côte d'Ivoire, 2007	435	29.6 [17.3, 44.2]	54.7	58.2 (52.0, 64.5)	67.7 (62.1, 73.4)	--	66.6 (58.0, 75.3)	12.4 (9.3, 15.4)
	Kenya, 2007	665	18.6 [12.1, 26.4]	51.1	0[7]	60.0 (54.3, 65.8)	52.6 (42.9, 62.4)	0.2 (0.0, 0.5)	14.7 (11.7, 17.7)
	Kenya, 2010	551	21.7 [13.4, 27.0]	50.5	0[7]	61.7 (56.1, 67.2)	58.2 (49.0, 67.3)	1.1 (0.1, 2.2)	16.4 (12.5, 20.3)
	Liberia, 2011	956	17.3 [11.2, 26.0]	47.6	43.6 (39.0, 48.2)	68.3 (61.6, 75.0)	83.2 (61.6, 75.0)	43.5 (37.0, 50.0)	--
	Malawi, 2016	748	31.4 [18.5, 44.7]	51.3	13.7 (0, 28.0)	56.5 (49.6, 63.3)	87.2 (81.0, 93.4)	82.6 (77.5, 87.6)	24.0 (13.1, 34.9)
	Mongolia, 2006	202	18.9 [12.3, 26.7]	53.0	46.5 (39.7, 53.4)	--	--	--	80.3 (74.7, 85.2)
	Nicaragua, 2005	1403	34.3 [20.2, 46.2]	50.1	56.4 (44.1, 68.7)	--	90.2 (85.9, 94.5)	27.1 (20.4, 33.7)	37.2 (30.0, 44.4)
	Papua New Guinea, 2005	802	31.5 [19.6, 44.4]	53.6	19.7 (10.2, 29.1)	59.2 (47.9, 70.6)	67.3 (57.7, 76.8)	8.6 (3.4, 13.7)	--
	Zambia, 2009	313	36.6 [24.1, 48.0]	58.1	21.9 (15.8, 28.0)	--	--	--	--
Low-middle	Cameroon, 2009	556	29.0 [18.8, 38.9]	50.7	61.8 (51.5, 72.1)	65.8 (57.9, 73.7)	77.4 (71.4, 83.3)	67.2 (61.3, 73.1)	37.7 (32.8, 42.6)
	Nigeria, 2012	283	29.6 [22.0, 35.8]	51.6	0[7]	--	--	--	--
	Pakistan, 2011	5896	25.5 [14.8, 39.2]	51.5	31.0 (27.9, 34.1)	58.5 (55.9, 61.1)	94.7 (93.5, 95.8)	91.3 (90.2, 92.5)	20.7 (18.8, 22.6)
	Philippines, 2011	1656	15.4 [10.7, 19.0]	49.5	9.1 (8.4, 9.8)	16.0 (12.8, 19.1)	44.6 (40.0, 49.3)	92.6 (87.5, 97.7)	66.6 (61.3, 71.9)
	Vietnam, 2010	329	37.5 [25.9, 49.2]	52.0	46.2 (40.5, 51.9)	--	--	--	--
Upper-middle	Azerbaijan, 2013	987	36.5 [23.6, 47.1]	55.0	45.3 (38.1, 52.5)	68.6 (64.1, 73.1)	77.4 (71.8, 83.0)	93.0 (89.9, 96.2)	--
	Colombia, 2010	3711	38.3 [26.2, 49.4]	52.8	70.7 (69.4, 72.0)	48.4 (46.3, 50.5)	86.3 (83.2, 89.4)	87.9 (85.1, 90.7)	42.3 (39.2, 45.4)
	Mexico, 2012	2430	37.1 [26.3, 49.7]	49.5	72.2 (69.3, 75.2)	53.5 (49.7, 57.3)	--	--	--

[1]Estimates account for the complex survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which followed a simple random sampling design. Inclusion criteria were: BMI-for-age z-score or weight-for-height z-score ≥-2 SD and a negative malaria test result. '--' indicates the variable was unavailable in that survey. BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; SES, socioeconomic status.

[2]CIC defined according to the World Bank definition for the year the survey took place (8).

[3]SES variables was a 3-level ordinal SES variable available from the harmonized BRINDA dataset (5), which was created from survey-specific asset scores (quintiles) of household ownership or composition. 'High SES' includes the medium and high levels.

[4]'Improved Water Source' was defined as having access to: piped water in a dwelling/yard; a communal/public tap; a borehole/tube well, owned or shared; a protected well/spring; a protected open dug well; or rain water (6).

[5]'Improved Toilet' was defined as have access to: a flush toilet/pit latrine flush to piped sewer; a ventilated improved pit/latrine/Sanplat; or a flush to pit/latrine (7)

[6]'High Education' was defined as completing at least secondary school, and was measured as maternal education level or head of household education level in surveys in which maternal education was not measured (Burkina Faso and Colombia).

[7]The surveys from Kenya (2007 and 2010) and Nigeria only contained observations from rural areas.

Supplemental Table 3.6. Bivariate and multivariable percent change associations between Vitamin A, CRP, AGP, and BMI among women of reproductive age (15-49 years) with normal weight to overweight/obesity by survey: BRINDA project[1]

					Bivariate (unadjusted) and multivariable (adjusted) linear regression				
CIC	**Country, survey year**	**n**	**RBP or SR**		**Vitamin A regressed on BMI**	**CRP regressed on BMI**	**AGP regressed on BMI**	**Vitamin A regressed on *ln*CRP**	**Vitamin A regressed on *ln*AGP**
Low-income	Afghanistan, 2013	571	SR	B	0.2 (-1.4, 1.8)	5.1 (-2.0, 12.7)	0.4 (-0.4, 1.2)	1.4 (-0.4, 3.3)	-1.0 (-8.8, 7.5)
				M	0.2 (-1.4, 1.8)	4.0 (-3.5, 12.1)	0.4 (-0.4, 1.2)	1.4 (-0.4, 3.3)	-1.0 (-8.8, 7.5)
	Burkina Faso, 2010	61	SR	B	-0.1 (-5.5, 5.7)	**-21.8 (-34.3, -7.0)**	-3.5 (-9.9, 3.3)	0.4 (-9.2, 11.1)	6.3 (-19.7, 40.7)
				M	-0.2 (-5.6, 5.6)	**-22.3 (-35.2, -6.9)**	-4.0 (-9.8, 2.2)	0.3 (-9.8, 11.5)	5.4 (-13.3, 53.3)
	Cambodia, 2014	609	RBP	B	-0.9 (-2.3, 0.5)	**17.1 (13.4, 20.9)**	**2.5 (0.5, 4.6)**	**10.5 (6.0, 15.2)**	**62.5 (52.7, 73.0)**
				M	-0.7 (-2.1, 0.7)	**16.9 (13.3, 20.6)**	**2.9 (0.8, 5.0)**	**10.4 (5.9, 15.1)**	**62.1 (52.0, 72.9)**
	Côte d'Ivoire, 2007	706	RBP	B	**0.8 (0.3, 1.4)**	**7.3 (4.8, 9.9)**	**2.3 (0.9, 3.8)**	-1.3 (-3.2, 0.7)	-4.7 (-13.1, 4.5)
				M	**0.7 (0.1, 1.3)**	**7.1 (4.5, 9.8)**	**2.3 (0.9, 3.8)**	-1.3 (-3.3, 0.7)	-4.7 (-13.0, 4.5)
	Malawi, 2016	595	RBP	B	1.0 (-0.1, 2.1)	**8.6 (3.1, 14.4)**	**2.9 (1.8, 4.1)**	**-2.7 (-5.0, -0.3)**	-4.6 (-14.8, 6.9)
				M	0.9 (-0.1, 2.0)	**8.6 (3.1, 14.4)**	**2.3 (0.8, 3.7)**	**-3.0 (-5.3, -0.6)**	-3.7 (-14.2, 8.0)
	Papua New Guinea, 2005	692	RBP	B	0.7 (-0.002, 1.5)	**7.1 (2.8, 11.6)**	0.4 (-0.2, 1.1)	**-2.6 (-3.9, -1.3)**	-4.5 (-16.0, 8.6)
				M	**1.0 (0.2, 1.7)**	**7.1 (2.8, 11.6)**	0.5 (-0.1, 1.1)	**0.3 (0.04, 0.5)**	-4.4 (-15.9, 8.8)
Low-middle	Cameroon, 2009	594	RBP	B	**1.0 (0.4, 1.5)**	**6.5 (3.7, 9.4)**	**0.5 (0.2, 0.9)**	**-1.8 (-3.3, -0.3)**	-2.0 (-16.0, 14.3)
				M	**0.8 (0.3, 1.3)**	**6.7 (3.7, 9.8)**	**0.7 (0.3, 1.0)**	**-1.9 (-3.4, -0.4)**	-2.7 (-16.8, 13.8)
	Nigeria, 2012	506	RBP	B	-0.1 (-0.7, 0.6)	-0.6 (-3.5, 2.3)	0.1 (-0.6, 0.7)	**-4.1 (-6.1, -2.2)**	**14.6 (4.6, 25.6)**
				M	-0.1 (-0.7, 0.6)	-0.6 (-3.5, 2.3)	0.1 (-0.6, 0.7)	**-4.1 (-6.1, -2.2)**	**14.6 (4.6, 25.6)**
	Pakistan, 2011	4946	SR	B	**0.9 (0.3, 1.4)**	**3.0 (1.9, 4.1)**	**1.0 (0.7, 1.3)**	-0.9 (-2.6, 0.9)	3.6 (-3.7, 11.4)
				M	0.2 (-0.3, 0.8)	**2.9 (1.8, 4.0)**	**1.0 (0.7, 1.3)**	-1.3 (-3.0, 0.4)	2.5 (-4.6, 10.1)
	Vietnam, 2010	1138	SR	B	**1.2 (0.3, 2.2)**	**17.4 (14.8, 20.1)**	--	1.4 (-0.8, 3.7)	--
				M	**0.3 (0.02, 0.5)**	**16.6 (13.8, 19.6)**	--	1.0 (-1.2, 3.3)	--
Upper-middle	Azerbaijan, 2013	2528	RBP	B	**1.2 (1.0, 1.4)**	**13.6 (12.3, 15.0)**	**1.5 (1.3, 1.8)**	**2.7 (1.9, 3.6)**	**21.6 (15.5, 28.2)**
				M	**1.0 (0.8, 1.2)**	**11.7 (10.2, 13.2)**	**1.5 (1.2, 1.8)**	**1.7 (0.8, 2.6)**	**18.1 (26.2, 39.9)**
High	United Kingdom, 2014	836	SR	B	-0.2 (-0.7, 0.3)	**6.5 (5.4, 7.6)**	--	-1.3 (-5.1, 2.7)	--
				M	-0.3 (-0.8, 0.1)	**6.6 (5.1, 8.0)**	--	-1.4 (-5.1, 2.5)	--
	United States, 2006	2989	SR	B	**-0.3 (-0.5, -0.1)**	**11.5 (10.9, 12.1)**	--	0.9 (-0.2, 1.9)	--
				M	**-0.4 (-0.6, -0.1)**	**10.9 (10.3, 11.5)**	--	0.6 (-0.5, 1.7)	--

[1]Vitamin A (RBP or SR), CRP and AGP variables were *natural-log* transformed for analysis due to non-normal distributions. Regression estimates were exponentiated, and results are presented as the percent change (95% confidence interval) in the dependent variable for every 1-unit change in the independent variable. Note that for the values presented for 'Vitamin A regressed on *ln*CRP' and 'Vitamin A regressed on *ln*AGP', the percent changes in Vitamin A concentration are for every 1-unit change in *natural-log* transformed CRP or AGP, and the units differ (CRP, mg/L; AGP, g/L). See Table 1 of main manuscript for geometric mean CRP and AGP values by survey. All estimates account for the complex survey design (cluster, strata) with survey weights applied. Vitamin A (as RBP or SR) was measured in either serum or plasma, as reported by the survey. Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the multivariable regression model if they were associated with the outcome variable at $p<0.1$ in the bivariate model.

Inclusion criteria were: BMI ≥18.5 kg/m^2, not pregnant, and a negative malaria test result. CIC was defined according to the World Bank definition for the year in which the survey was conducted (8). '--' indicates the variable was unavailable in that survey. AGP, α-1-acid glycoprotein; B, bivariate model; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC; country income classification; CRP, C-reactive protein; M, multivariable model; RBP, retinol binding protein; SR, serum retinol.

Supplemental Table 3.7. Bivariate and multivariable percent change associations between Vitamin A, CRP, AGP, and BAZ among preschool-aged children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

					Bivariate (unadjusted) and multivariable (adjusted) linear regression				
CIC	**Country, survey year**	**n**	**RBP or SR**		**Vitamin A regressed on BMI**	**CRP regressed on BAZ**	**AGP regressed on BAZ**	**Vitamin A regressed on *ln*CRP**	**Vitamin A regressed on *ln*AGP**
Low-income	Afghanistan, 2013	586	SR	B	3.7 (-0.3, 7.8)	-12.3 (-28.1, 7.0)	**-4.0 (-6.2, -1.6)**	**-5.3 (-7.4, -3.1)**	**-31.5 (-38.7, -23.4)**
				M	1.4 (-2.9, 6.0)	-12.3 (-28.1, 7.0)	-3.5 (-7.4, 0.6)	**-5.3 (-8.8, -1.6)**	**-31.2 (-45.3, -13.5)**
	Bangladesh, 2010	1179	RBP	B	**1.4 (0.2, 2.7)**	-3.8 (-11.9, 5.1)	-1.2 (-3.2, 0.9)	**-5.4 (-6.3, -4.5)**	**-22.5 (-27.9, -16.8)**
				M	**1.5 (0.5, 2.6)**	-3.3 (-11.8, 6.1)	-1.0 (-3.0, 1.1)	**-5.3 (0.3, 2.5)**	**-22.0 (-27.2, -16.5)**
	Bangladesh, 2012	360	SR	B	**4.4 (0.4, 8.5)**	-3.1 (-13.6, 8.8)	-2.2 (-6.3, 2.1)	-4.2 (-11.5, 3.8)	-9.7 (-28.1, 13.4)
				M	3.6 (-0.3, 7.7)	3.8 (-9.6, 19.2)	-1.4 (-5.2, 2.5)	-2.4(-10.2, 6.0)	-3.4 (-22.5, 20.3)
	Burkina Faso, 2010	60	SR	B	**-9.9 (-18.1, -0.8)**	-6.4 (-42.0, 51.2)	0.1 (-9.5, 10.6)	**-9.8 (-18.5, -0.1)**	1.1 (-40.2, 71.0)
				M	**-9.9 (-18.4, -0.4)**	-5.1 (-41.1, 53.1)	0.7 (-7.3, 9.3)	**-10.2 (-19.0, -0.5)**	-2.5 (-43.3, 67.6)
	Cambodia, 2014	599	RBP	B	-2.0 (-8.3, 4.6)	10.3 (-3.6, 26.3)	1.7 (-7.0, 11.1)	**3.5 (0.6, 6.5)**	**62.1 (54.4, 70.1)**
				M	-2.0 (-8.3, 4.6)	8.2 (-5.1, 23.2)	1.7 (-7.0, 11.1)	**3.5 (0.6, 6.5)**	**62.1 (54.4, 70.1)**
	Côte d'Ivoire, 2007	435	RBP	B	-0.3 (-3.0, 2.5)	8.1 (-7.5, 26.4)	-0.04 (-3.1, 3.2)	**-7.9 (-9.8, -6.0)**	**-25.5 (-32.2, -18.1)**
				M	-0.01 (-2.7, 2.8)	6.3 (-9.1, 24.2)	-1.4 (-4.4, 1.7)	**-7.7 (-9.6, -5.8)**	**-25.2 (-32.2, -17.5)**
	Kenya, 2007	665	RBP	B	1.0 (-1.7, 3.8)	4.5 (-7.6, 18.2)	-1.1 (-3.9, 1.7)	**-5.8 (-6.9, -4.7)**	**-22.1 (-26.2, -17.7)**
				M	0.8 (-2.0, 3.8)	6.9 (-6.1, 21.7)	-1.0 (-3.9, 2.0)	**-5.7 (-6.9, -4.5)**	**-20.6 (-25.3, -15.6)**
	Kenya, 2010	551	RBP	B	-1.8 (-4.6, 1.0)	7.7 (-9.5, 28.3)	1.6 (-1.2, 4.5)	**-6.0 (-7.4, -4.7)**	**-26.9 (-32.3, -21.2)**
				M	-2.0 (-4.9, 1.0)	7.7 (-9.5, 28.3)	1.3 (-1.6, 4.4)	**-6.0 (-7.4, -4.6)**	**-26.7 (-32.6, -20.3)**
	Liberia, 2011	956	RBP	B	1.1 (-0.9, 3.1)	3.0 (-7.0, 14.1)	-0.6 (-2.6, 1.4)	**-5.0 (-6.4, -3.6)**	**-21.9 (-29.4, -13.7)**
				M	1.8 (-0.2, 3.8)	2.0 (-7.8, 12.8)	-0.6 (-2.6, 1.5)	**-5.2 (-6.5, -3.8)**	**-22.5 (-29.4, -14.8)**
	Malawi, 2016	748	RBP	B	1.4 (-1.3, 4.2)	**18.6 (3.5, 35.8)**	3.8 (-2.5, 10.6)	**-7.0 (-8.2, -5.7)**	**-12.5 (-18.5, -6.1)**
				M	1.5 (-1.1, 4.2)	**17.2 (2.4, 34.1)**	3.5 (-2.9, 10.3)	**-6.9 (-8.2, -5.6)**	**-12.4 (-18.3, -6.0)**
	Mongolia, 2006	202	SR	B	-0.9 (-8.9, 7.8)	--	-2.6 (-6.7, 1.7)	--	**-24.9 (-42.7, -1.7)**
				M	-0.9 (-8.9, 7.8)	--	-2.9 (-6.9, 1.4)	--	**-24.9 (-42.7, -1.7)**
	Nicaragua, 2005	1403	SR	B	0.9 (-1.8, 3.6)	--	-0.4 (-2.6, 1.8)	--	**-17.3 (-21.9, -12.4)**
				M	0.9 (-1.8, 3.6)	--	-0.4 (-2.5, 1.8)	--	**-17.3 (-21.9, -12.4)**
	Papua New Guinea, 2005	802	RBP	B	**2.9 (0.5, 5.4)**	-11.7 (-25.3, 4.4)	-2.1 (-4.5, 0.4)	**-6.0 (-7.1, -4.8)**	**-24.7 (-31.3, -17.5)**
				M	2.2 (-0.1, 4.6)	-7.1 (-21.3, 9.6)	-0.8 (-4.2, 2.7)	**-5.8 (-7.0, -4.6)**	**-23.6 (-30.3, -16.3)**
	Zambia, 2009	313	SR	B	-34.5 (-3.5, 3.7)	-13.0 (-37.4, 20.9)	1.6 (-3.5, 7.0)	**-2.1 (-3.7, -0.4)**	**-15.0 (-23.6, -5.4)**
				M	-34.5 (-3.5, 3.7)	-13.0 (-37.4, 20.9)	1.6 (-3.5, 7.0)	**-2.1 (-3.7, -0.4)**	**-15.0 (-23.6, -5.4)**
Low-middle	Cameroon, 2009	556	RBP	B	-1.9 (-4.2, 0.4)	9.3 (-5.1, 25.9)	-0.4 (-2.3, 1.6)	**-5.7 (-7.0, -4.3)**	**-25.5 (-33.4, -16.6)**
				M	-1.9 (-4.2, 0.4)	12.2 (-2.6, 29.3)	-0.1 (-1.9, 1.7)	**-5.7 (-7.0, -4.3)**	**-25.5 (-33.4, -16.6)**
	Nigeria, 2012	283	SR	B	1.6 (-1.4, 4.7)	5.5 (-8.3, 21.4)	-0.2 (-2.8, 2.4)	**-7.0 (-9.1, -4.8)**	**-27.2 (-35.5, -17.7)**
				M	1.6 (-1.4, 4.7)	5.5 (-8.3, 21.4)	-0.2 (-2.8, 2.4)	**-7.0 (-9.1, -4.8)**	**-27.2 (-35.5, -17.7)**
	Pakistan, 2011	5896	SR	B	-1.5 (-3.4, 0.4)	--	0.8 (-0.2, 1.9)	--	-1.9 (-6.9, 3.4)

				M	-1.6 (-3.6, 0.4)	--	1.0 (-0.2, 2.1)	--	-1.6 (-6.9, 4.1)
	Philippines, 2011	1656	RBP	B	**3.6 (1.6, 5.6)**	**-11.0 (-20.1, -0.9)**	**-2.1 (-3.8, -0.3)**	**-6.9 (-7.7, -6.2)**	**-27.6 (-31.3, -23.8)**
				M	**2.9 (1.0, 4.9)**	-9.2 (-18.7, 1.4)	-1.8 (-3.6, 0.1)	**-6.4 (-7.2, -5.6)**	**-26.0 (-30.1, -21.7)**
	Vietnam, 2010	329	SR	B	2.6 (-1.3, 6.6)	-8.4 (-20.8, 5.8)	--	**-4.4 (-7.0, -1.6)**	--
				M	2.6 (-1.3, 6.6)	-8.4 (-20.8, 5.8)	--	**-4.4 (-7.0, -1.6)**	--
Upper-middle	Azerbaijan, 2013	987	RBP	B	-0.4 (-2.1, 1.4)	-9.3 (-19.8, 2.5)	-1.9 (-3.9, 0.2)	**-5.8 (-6.8, -4.7)**	**-21.8 (-27.6, -15.6)**
				M	-0.2 (-1.9, 1.6)	-8.4 (-18.6, 3.2)	-1.5 (-3.6, 0.5)	**-5.8 (-6.9, -4.7)**	**-22.5 (-28.1, -16.5)**
	Colombia, 2010	3711	SR	B	**2.2 (0.7, 3.8)**	-4.0 (-14.0, 7.1)	--	**-2.2 (-2.8, -1.6)**	--
				M	**2.2 (0.7, 3.7)**	-5.5 (-15.2, 5.4)	--	**-2.1 (-2.7, -1.5)**	--
	Mexico, 2012	2430	SR	B	1.2 (-0.4, 3.0)	7.2 (-3.4, 19.0)	--	**-6.4 (-7.3, -5.4)**	--
				M	1.3 (-0.4, 2.9)	7.2 (-3.4, 19.0)	--	**-6.3 (-7.3, -5.4)**	--

[1]Vitamin A (RBP or SR), CRP and AGP variables were *natural-log* transformed for analysis due to non-normal distributions. Regression estimates were exponentiated, and results are presented as the percent change (95% confidence interval) in the dependent variable for every 1-unit change in the independent variable. Note that for the values presented for 'Vitamin A regressed on *ln*CRP' and 'Vitamin A regressed on *ln*AGP', the percent changes in Vitamin A concentration are for every 1-unit change in *natural-log* transformed CRP or AGP, and the units differ (CRP, mg/L; AGP, g/L). See Table 2 of main manuscript for geometric mean CRP and AGP values by survey. All estimates account for the complex survey design (cluster, strata) with survey weights applied. Vitamin A (as RBP or SR) was measured in either serum or plasma, as reported by the survey. Covariates available for adjustment were: age, education level (maternal or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the multivariable regression model if they were associated with the outcome variable at $p<0.1$ in the bivariate model. Inclusion criteria were: BAZ ≥-2 SD, WHZ ≥-2 SD, and a negative malaria test result. CIC was defined according to the World Bank definition for the year in which the survey was conducted (8). '--' indicates the variable was unavailable in that survey. AGP, α-1-acid glycoprotein; B, bivariate model; BAZ, BMI-for-age z-score; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC; country income classification; CRP, C-reactive protein; M, multivariable model; RBP, retinol binding protein; SR, serum retinol.

Supplemental Table 3.8. Unadjusted relationships between vitamin A (retinol or RBP) and BMI or BAZ as mediated by inflammation among women of reproductive age (15-49 years) and preschool-age children (6-59 months) with normal weight to overweight/obesity by survey: BRINDA project[1]

				WRA mediation analysis, unadjusted[2]					
CIC[3]	**Country, survey year**	**n**	**SR or RBP**	**Total Effect**	**Direct Effect**	**Indirect Effect**	**% Mediated**	**% Mediated by CRP**	**% Mediated by AGP**
Low	Afghanistan, 2013	571	SR	0.2 (-1.4, 1.8)	0.1 (-1.45, 1.8)	0.1 (-0.1, 0.2)	NM	--	--
	Burkina Faso, 2010	61	SR	-0.1 (-4.4, 4.5)	0.1 (-5.1, 5.6)	-0.2 (-2.5, 2.2)	NM	--	--
	Cambodia, 2014	609	RBP	-0.9 (-2.4, 0.4)	-2.4 (-3.7, -1.1)	1.5 (0.2, 2.8)	NM	--	--
	Côte d'Ivoire, 2007	706	RBP	0.8 (0.3, 1.4)	1.0 (0.4, 1.6)	-0.2 (-0.30, -0.001)	NM	--	--
	Malawi, 2016	595	RBP	1.0 (-0.1, 2.1)	1.3 (0.003, 2.6)	-0.3 (-0.7, 0.1)	NM	--	--
	Papua New Guinea, 2005	692	RBP	**0.7 (0.01, 1.5)**	**0.9 (0.2, 1.6)**	**-0.2 (-0.4, -0.02)**	-26.5	82.6	17.4
Low-middle	Cameroon, 2009	594	RBP	**1.0 (0.5, 1.5)**	**1.1 (0.6, 1.6)**	**-0.2 (-0.3, -0.03)**	-15.5	81.3	18.7
	Nigeria, 2012	506	RBP	-0.1 (-0.7, 0.5)	-0.1 (-0.7, 0.5)	0.1 (-0.2, 0.3)	NM	--	--
	Pakistan, 2011	4946	SR	0.9 (0.3, 1.4)	0.9 (0.3, 1.4)	-0.01 (-0.1, 0.1)	NM	--	--
	Vietnam, 2010	1138	SR	1.2 (0.3, 2.2)	1.2 (0.1, 2.2)	0.1 (-0.3, 0.5)	NM	--	--
Upper-middle	Azerbaijan, 2013	2528	RBP	1.2 (1.0, 1.4)	1.1 (-1.1, 3.4)	0.1 (-0.1, 0.2)	NM	--	--
High	United Kingdom, 2014	836	SR	-0.2 (-0.7, 0.3)	-0.2 (-0.8, 0.4)	-0.1 (-0.4, 0.3)	NM	--	--
	United States, 2006	2989	SR	**-0.3 (-0.5, -0.1)**	**-0.5 (-0.8, -0.3)**	**0.3 (0.1, 0.4)**	-90.7	100	--
				PSC mediation analysis, unadjusted[2]					
Low	Afghanistan, 2013	306	SR	3.7 (-0.2, 7.5)	2.2 (-2.0, 6.3)	1.5 (0.3, 2.6)	NM	--	--
	Bangladesh, 2010	579	RBP	1.4 (0.3, 2.6)	1.2 (0.3, 2.1)	0.3 (-0.3, 0.8	NM	--	--
	Bangladesh, 2012	196	SR	4.4 (0.5, 8.1)	4.3 (0.6, 7.8)	0.1 (-0.5, 0.8	NM	--	--
	Burkina Faso, 2010	26	SR	-9.9 (-18.3, -2.5)	-10.7 (-19.7, -3.0)	1.0 (-4.4, 6.3)	NM	--	--
	Cambodia, 2014	326	RBP	-2.0 (-8.5, 4.4)	-1.5 (-4.6, 1.5)	-0.5 (-5.7, 4.6)	NM	--	--
	Côte d'Ivoire, 2007	238	RBP	-0.3 (-3.0, 2.4)	0.2 (-1.8, 2.3)	-0.5 (-1.8, 0.7)	NM	--	--
	Kenya, 2007	340	RBP	1.0 (-1.7, 3.7)	1.1 (-1.5, 3.6)	-0.04 (-0.8, 0.8)	NM	--	--
	Kenya, 2010	278	RBP	-1.9 (-4.6, 0.9)	-1.3 (-3.9, 1.3)	-0.6 -1.7, 0.5)	NM	--	--
	Liberia, 2011	455	RBP	1.1 (-0.8, 3.1)	1.2 (-0.7, 3.0)	-0.1 (-0.7, 0.6)	NM	--	--
	Malawi, 2016	384	RBP	1.4 (-1.2, 4.1)	2.7 (0.4, 5.0)	-1.3 (-2.7, 0.2)	NM	--	--
	Mongolia, 2006	107	SR	-0.9 (-9.2, 7.5)	-1.6 (-9.9, 6.6)	0.8 (-0.7, 2.2)	NM	--	--
	Nicaragua, 2005	703	SR	0.9 (-1.8, 3.5)	0.8 (-2.0, 3.5)	0.1 (-0.3, 0.5)	NM	--	--
	Papua New Guinea, 2005	430	RBP	2.9 (0.6, 5.2)	2.2 (-0.03, 4.3)	0.7 (-0.3, 1.7)	NM	--	--
	Zambia, 2009	182	SR	0.03 (-3.3, 3.4)	-0.02 (-3.4, 3.4)	0.1 (-1.1, 1.2)	NM	--	--
	Cameroon, 2009	282	RBP	-1.9 (-4.2, 0.3)	-1.5 (-3.6, 0.6)	-0.5 (-1.3, 0.4)	NM	--	--

Low-middle	Nigeria, 2012	146	SR	1.6 (-1.3, 4.4)	1.9 (-0.8, 4.6)	-0.3 (-1.3, 0.6)	NM	--	--
	Pakistan, 2011	3035	SR	-1.5 (-3.4, 0.4)	-1.5 (-3.4, 0.4)	-0.01 (-0.1, 0.03)	NM	--	--
	Philippines, 2011	819	RBP	3.6 (1.6, 5.5)	-11.0 (-22.3, -1.1)	0.9 (0.1, 1.7)	24.3	92.5	7.4
	Vietnam, 2010	171	SR	2.6 (-1.0, 6.1)	2.2 (-1.3, 5.7)	0.4 (-0.2, 1.0)	NM	--	--
Upper-middle	Azerbaijan, 2013	543	RBP	-0.4 (-2.1, 1.3)	-1.0 (-2.4, 0.4)	0.6 (-0.1, 1.3)	NM	--	--
	Colombia, 2010	1960	SR	2.2 (0.7, 3.7)	2.2 (0.7, 3.6)	0.1 (-0.2, 0.3)	NM	--	--
	Mexico, 2012	1203	SR	1.2 (-0.4, 2.9)	1.7 (0.004, 3.4)	-0.5 (-1.2, 0.2)	NM	--	--

[1]RBP, SR, CRP, and AGP variables were natural-log transformed for analysis due to non-normal distributions. Mediation effects were exponentiated and results are presented as percent change (95% confidence interval) in ferritin for every 1-unit change in BMI (WRA) or BAZ (PSC). SR or RBP concentration measured in serum or plasma, as reported in the survey. All estimates account for cluster survey design (cluster, strata) with survey weights applied, except in the survey from Mongolia which used simple random sampling. Inclusion criteria were: BMI ≥18.5 kg/m2 (WRA) or BAZ or WHZ ≥-2 SD (PSC), not pregnant (WRA only), and a negative malaria result. AGP, alpha-1-acid glycoprotein; BAZ, BMI-for-age z-score; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia; CIC, country income classification; CRP, C-reactive protein; NM, no mediation; PSC, pre-school age children; SR, serum retinol; RBP, retinol-binding protein; WHZ, weight-for-height z-score; WRA, women of reproductive age.

[2]Model for mediation analysis: *ln*Vitamin A [RBP or SR] = $\beta_0 + \beta_1$(BMI/BAZ) + M_1(*ln*CRP) [+M_2(*ln*AGP)] where all values were continuous and AGP was included as a mediator only in analyses for which it was available in the dataset. Interpretation is as follows: Total Effect = the effect of BMI/BAZ on SR/RBP; Direct Effect = the effect of BMI/BAZ on SR/RBP controlling for inflammation; Indirect Effect = the effect of BMI/BAZ on SR/RBP as mediated by the effect of CRP or AGP. Mediation was considered present when both the total and indirect effects were significant (1,2). Covariates available for adjustment were: age, education level (respondent or household head), household socioeconomic status, access to an improved water source, access to an improved toilet, and urban/rural residence. Covariates were included in the mediation model if they were associated with the outcome variable at $p<0.1$ in bivariate models (Supplemental Table 3.6).

[3]CIC defined according to the World Bank definition for the year in which the survey was conducted (8).

Supplemental Table 3.9. Malaria sensitivity analysis: mediation analysis (unadjusted) assessing the relationship between Vitamin A (RBP or SR), BMI or BAZ and inflammation including and excluding observations that tested positive for malaria for WRA and PSC with normal weight to overweight/obesity: BRINDA project[1]

	Including positive malaria observations (*sensitivity analysis*)		**Excluding positive malaria observations (*original analysis*)**	
Survey, year	n	β (95% CI)	n	β (95% CI)
WRA				
Cameroon, 2009	691	-0.002 (-0.003, -0.0006)	594	-0.002 (-0.003, -0.0003)
Côte d'Ivoire, 2007	742	-0.001 (-0.003, 0.0003)	706	-0.002 (-0.003, -0.000008)
Malawi, 2016	694	-0.003 (-0.006, -0.0002)	595	-0.003 (-0.007, 0.0006)
Nigeria, 2012	555	0.0004 (-0.002, 0.003)	506	0.001 (-0.002, 0.003)
PSC				
Cameroon, 2009	740	-0.002 (-0.01, 0.01)	556	-0.01 (-0.01, 0.004)
Côte d'Ivoire, 2007	606	-0.004 (-0.02, 0.01)	435	-0.005 (-0.02, 0.01)
Kenya, 2007	825	-0.003 (-0.01, 0.006)	665	-0.0004 (-0.01, 0.01)
Kenya, 2010	813	-0.004 (-0.02, 0.01)	551	-0.01 (-0.02, 0.01)
Liberia, 2011	1268	-0.003 (-0.01, 0.004)	956	-0.001 (-0.01, 0.01)
Malawi, 2016	1027	-0.02 (-0.03, -0.01)	748	-0.01 (-0.03, 0.002)
Nigeria, 2012	426	-0.006 (-0.02, 0.03)	283	-0.003 (-0.01, 0.01)
Zambia, 2009	386	0.004 (-0.01, 0.02)	313	0.001 (-0.01, 0.01)

[1]Estimates of the mediated effect are presented as the unexponentiated β coefficient of *ln*Vitamin A (RBP or SR) (95% confidence intervals). β represents the indirect effect, that is the effect of BMI or BAZ on Vitamin A (RBP or SR) concentration as mediated by the effect of CRP or AGP. Malaria status was evaluated by survey-specific diagnostic tests that have been previously described (5). While Burkina Faso (2010) measured malaria status, all observations for both WRA and PSC were excluded as part of the criteria to exclude observations with underweight/wasting, thus the survey is not included in this sensitivity analysis. BAZ, BMI-for-age z-score; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinant of Anemia; PSC, preschool-age children (6-59 months); RBP, retinol binding protein; SR, serum retinol; WRA, women of reproductive age (15-49 years).

Supplemental Table 3.10. Morbidity sensitivity analysis: mediation analysis (unadjusted) assessing the relationship between Vitamin A (RBP or SR), BMI or BAZ and inflammation excluding and including observations that reported fever and/or diarrhea in the past 2 weeks (WRA) and in the past 24 hours (PSC) with normal weight to overweight/obesity: BRINDA project[1]

	Excluding morbidity observations (*sensitivity analysis*)		**Including morbidity observations (*original analysis*)**	
Survey, year	n	β (95% CI)	n	β (95% CI)
WRA				
Côte d'Ivoire, 2007	484	-0.002 (-0.004, 0.0001)	706	-0.002 (-0.003, -0.000008)
Malawi, 2016	451	-0.002 (-0.005, 0.002)	595	-0.003 (-0.007, 0.0006)
PSC				
Kenya, 2010	302	-0.003 (-0.02, 0.01)	551	-0.01 (-0.02, 0.01)
Liberia	221	0.002 (-0.01, 0.01)	956	-0.001 (-0.01, 0.01)

[1]Estimates of the mediated effect are presented as the unexponentiated β coefficient of *ln*Vitamin A (RBP or SR) (95% confidence intervals). β represents the indirect effect, that is the effect of BMI or BAZ on Vitamin A (RBP or SR) concentration as mediated by the effect of CRP or AGP. Morbidity status was self-reported by the participants (WRA) and reported by caregivers of PSC or measured directly (i.e., fever) as indicated by the survey (5). BAZ, BMI-for-age z-score; BMI, body mass index; BRINDA, Biomarkers Reflecting Inflammation and Nutritional Determinant of Anemia; PSC, preschool-age children (6-59 months); RBP, retinol binding protein; SR, serum retinol; WRA, women of reproductive age (15-49 years).

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CHAPTER 4: Estimated consumption of discretionary salt and salt from bouillon among households, women, and young children in northern Ghana: A mixed-methods study

Abstract

Background: Salt consumption ≥5 g/d is associated with increased risk of noncommunicable diseases. Developing salt reduction strategies requires understanding salt usage and consumption patterns.

Objectives: 1) Estimate consumption of salt, including salt from bouillon, among households, women, and children, and compare to global recommendations; 2) Estimate the proportion of salt consumed from bouillon; 3) Identify factors, including knowledge, attitudes, and practices (KAP), associated with household salt consumption in 2 districts in Northern Region, Ghana.

Methods: Employing mixed-methods methodology, households were enrolled from 14 urban and 14 rural clusters from Tolon and Kumbungu districts in a pilot survey and focus group discussions (FGDs, n=20). Using the Fortification Assessment Coverage Toolkit, households (n=369) reported most recent purchases of discretionary salt (DS, 'table salt') and bouillon cubes. From purchase data, median (IQR) household consumption (g/d) of DS and total salt (TS, DS + salt from bouillon, assumed to be 55% salt) were calculated, including the proportion of salt from bouillon. DS and TS consumption for women (15-49 y) and children (2-5 y) were estimated with the Adult Male Equivalent method and compared to global recommendations. Salt intake from urinary sodium excretion was predicted with the INTERSALT equation (women only). Associations between DS and TS consumption and household and individual (women's) characteristics, including KAP, were tested with mixed effects ANOVA. Minimally-adjusted and multivariable models included district, setting (urban/rural), household size, and participant type (non-lactating or lactating woman) as fixed effects, and the random effect of cluster. Qualitative themes were generated from FGDs using the Framework Method.

Results: From reported household purchase data, estimated consumption of DS and TS appeared to exceed global recommendations for many children (TS: 2.9 [1.9, 5.2] g/d) and the majority of women (TS: 6.0 [4.0, 10.2] g/d). Women's mean urinary sodium excretion also suggested high sodium exposure

(7.1 g/d). Bouillon contributed <25% to households' daily TS consumption. Household salt consumption was greater among households in 3rd-5th (highest) asset quintiles and those with severe food insecurity. Few other characteristics were associated with household salt consumption. Salient qualitative themes included salt's ubiquity as a seasoning, and how intra-household dynamics, taste preferences, and perceptions about salt and health shaped salt usage and consumption.

Conclusion: Household salt and bouillon purchase data suggest that salt consumption among women and children in this area exceeds recommendations; food prepared outside the home may further contribute to salt consumption. Salt reduction interventions may be warranted in this context.

Introduction

Salt consumption is high worldwide with adults regularly consuming ≥10 grams per day (g/d), double the recommended limit of <5 g/d by both the World Health Organization (WHO) and the American Heart Association (AHA) (1–3). Consuming >5 g/d of salt increases the risk of developing hypertension, heart disease, and other noncommunicable diseases (NCDs) (4–6), and salt reduction strategies are recommended to reduce NCD morbidity and mortality (7,8). Sustainable Development Goal 3.4 aims to reduce premature deaths from NCDs by one-third by 2030 (relative to 2015 levels); as of 2020, only 17 countries worldwide had made progress towards this goal (9). In Ghana, >30% of adults report experiencing hypertension (10), and NCD mortality increased 2010-2016 (9).

Few studies have assessed salt consumption in Ghana. In studies among adults, salt consumption ranged from approximately 6-12 g/d using data from 24-h urinary sodium excretion (11–14), and was 12.5 g/d from analyses of household salt inventories (15). Among children (5-12 y), salt consumption was approximately 6.4 g/d from 24-h urine data (16). Despite the variation in these estimates, and the challenges of measuring dietary salt (17,18), the studies suggest that salt consumption in Ghana exceeds global recommendations. However, as most studies focused primarily on older populations (≥40 y), with limited data on children, further examination of salt consumption patterns and dietary sources among younger populations is needed.

Universal salt iodization is implemented globally to control iodine deficiency disorders, and has been mandatory in Ghana since 1996 (19). Since implementation, iodine deficiency disorders have reduced and intakes of iodized salt have increased (20,21), though Ghana's goal of reaching 90% of the population with adequately iodized salt (15+ ppm) has yet to be achieved (10). Some of the success with raising awareness of iodized salt's health benefits and household use of iodized over non-iodized salt may be attributed to mass media campaigns (22,23). Studies examining the influence of knowledge,

attitudes, and practices (KAP) on salt consumption suggest that KAP data are useful for informing salt reduction strategies (24–26), particularly when combined with multipronged strategies that include the food environment, such as labeling high-sodium foods and increasing access to affordable, lower-sodium foods (27,28).

Development of salt reduction strategies also requires information on dietary sources of salt. In Ghana, important sources of dietary salt include discretionary salt, which includes salt added at the table or during cooking, and salt from condiments such as bouillon cubes (24,29,30). While salt, bouillon cubes, and other condiments such as tomato paste are produced with iodized salt (29,31), there are no national or regional standards in Ghana for fortifying salt and condiments with additional micronutrients. Given that micronutrient deficiencies persist in Ghana (32) and globally (33), salt reduction efforts must consider that salt and salt-containing condiments like bouillon are also vehicles for micronutrient fortification (34). Balancing salt reduction strategies with the promotion of iodized salt, or potential new interventions such as multiple-micronutrient fortified bouillon cubes, is a critical undertaking for policymakers and public health programs.

To help inform nutrition policy discussions related to salt in Ghana, we conducted a mixed-methods study, including a pilot survey and focus group discussions, in two districts in the Northern Region. Our objectives were to: 1) estimate consumption of salt, including salt from bouillon, among households, women, and children, and compare to global recommendations; 2) estimate the proportion of salt consumed from bouillon; and 3) identify factors, including knowledge, attitudes, and practices, associated with household salt consumption.

Methods

Additional detailed methods can be found in Supplemental Material (**Supplemental Methods**).

Study design, participants and sample size

This study took place in the Northern Region of Ghana in 14 urban and 14 rural clusters in the districts of Tolon and Kumbungu. Our mixed-methods approach employed a convergent parallel research design, such that quantitative and qualitative collection methods were developed and implemented in parallel with the intention that qualitative data would explain and expand upon quantitative findings (35). The primary method of data collection was quantitative as a cross-sectional pilot survey conducted November 2020-March 2021, and recruited non-pregnant non-lactating women of reproductive age (WRA, 15-49 y), non-pregnant lactating women (LW, 4-18 months post-partum, 15-49 y), and preschool-age children ('children', 2-5 y). Qualitative data were collected through focus group discussions (FGDs) conducted October-November 2020, and recruited WRA, adult men, and women >49 y who had knowledge of or made decisions about household food procurement and cooking practices. Potential participants were excluded if they suffered from any illnesses that precluded participation in research activities, or failed the COVID-19 screening (fever and/or recent [within 14 d] exposure to COVID-19). All participants provided written informed consent.

The findings from this mixed-methods study informed a planned randomized controlled trial (RCT) investigating the effects of a multiple micronutrient fortified bouillon cube on the nutrition status of adult women and young children in this region (clinicaltrials.gov registry NCT05178407; CoMIT Project, Condiment Micronutrient Innovation Trial). This study was approved by the Ghana Health Services (GHS) Ethical Review Committee and the Institutional Review Board of the University of California, Davis. WHO and GHS protocols to prevent COVID-19 were followed by all survey personnel and participants during all research activities (36).

For the present analyses, WRA and LW groups were eligible (n=487), and we were able to detect a strength of correlation in bivariate associations with household salt consumption of at least 0.14 (80%

power, α=0.05). For the FGDs, we aimed to recruit 120 participants (n=60 WRA, n=30 men, n=30 woman >49 y).

Data collection procedures – Quantitative

On the day of recruitment, trained fieldworkers administered pilot survey questionnaires in the preferred language of the participant (English or Dagbani, the primary local dialect). Questionnaires were programmed onto electronic tablets using the software program SurveyCTO®. Household heads completed a household roster and household questionnaire that collected demographic characteristics, including an inventory of household assets and a food insecurity questionnaire validated for use in this population at the household level (37). The household questionnaire also included the Fortification Assessment Coverage Toolkit (FACT) which collected information on household purchases and frequency of consumption of fortifiable foods, including bouillon and salt (38). Participating WRA and LW then completed the WHO STEPwise Approach Surveillance (STEPS) Instrument for Noncommunicable Disease Risk Factor Surveillance (version 3.2) (39) and a KAP questionnaire developed specifically for the pilot survey that collected information on usage and consumption patterns related to bouillon, salt, and *dawadawa* (a condiment made from fermented locust beans and used to flavor many common dishes (40)).

One to three days following recruitment, participants presented at a location central to each cluster. There, anthropometric measurements (height and weight) were completed in triplicate (WRA and children only) by trained and standardized anthropometrists on equipment calibrated daily. Blood pressure was measured in triplicate with measures one minute apart among WRA and children using a Riester® RBP-100 automatic portable upper-arm blood pressure monitor. One spot urine sample was collected from each WRA to measure urinary sodium, potassium, and creatinine. Urine samples were processed in the field and stored at -20°C for transport to the University of California, San Francisco,

Children's Hospital Oakland Research Institute for analysis. Urinary sodium and potassium concentrations were determined by inductively-coupled plasma spectrometry, and urine creatinine was measured with the Cayman creatinine colorimetric assay kit (#500701). For sodium and potassium, a random selection of samples (n=24, 10%) were run as technical replicates at the beginning, middle, and end of the analysis period (coefficients of variation: 7.9% and 3.2%, respectively). Creatinine samples were analyzed in duplicate (coefficient of variation: -4.8%).

Data collection procedures – Qualitative

The FGDs were held in community spaces, such as schools or health centers, central to each cluster. FGD participants completed a demographic questionnaire and COVID-19 screening. Trained facilitators (n=4) conducted the FGDs in Dagbani following a semi-structured FGD guide (see **Supplemental Appendix**) that primarily focused on the usage, purchasing, and consumption of bouillon, with a subset of questions gathering perceptions on salt usage and relationships to health. FGDs (n=20) were conducted among groups of 5-6 participants, and included a note-taker who used a written form to systematically gather participant responses and reactions during the FGDs. The FGDs were audio-recorded and lasted approximately 2 hours. A debriefing with the facilitator, note-taker, and a supervisor was held after each FGD to record key insights with a written debriefing form. Audio-recordings were translated and transcribed to English by a fieldworker not involved with the FGDs. A random subset (n=7 total: n=3 WRA, n=2 men, n=2 women >49 y from both rural and urban clusters) of the audio-recordings were translated and transcribed by a second independent transcriptionist. Discrepancies between the notes and transcriptions were resolved through discussion and consultations with fieldworkers.

Data analysis

For this mixed-methods study, quantitative and qualitative analyses were performed separately with the qualitative data used to explain the quantitative data, and to help understand incongruent or contradictory results between the two types of data (41). Quantitative data were analyzed using Stata (16.1, StataCorp LLC, College Station, TX); qualitative data were analyzed using NVivo (QSR International Pty Ltd., released March 2020); the intercoder reliability (ICR) score for the qualitative data was calculated using Stata. All testing was two-sided with values considered significant at $p<0.05$. Statistical analysis plans (quantitative and qualitative) are publically available at: https://osf.io/t3zrn/.

Quantitative analyses

Descriptive statistics were calculated for all available variables separately among households and individuals (children, WRA, LW). Household food insecurity was calculated using the Household Food Insecurity Access Scale (HFIAS) (37). Among WRA, Body Mass Index (BMI) categories were defined according to standard categories (42); among children, anthropometric z-scores were calculated according to WHO standard (43). For WRA, elevated blood pressure and hypertension were defined according to three organizations to capture a broader picture of hypertension risk: the American Heart Association (44), the International Society of Hypertension (45), and WHO (46). For children, 'at risk' blood pressure thresholds were defined by American Academy of Pediatrics (47). Blood pressure definitions and thresholds are listed in **Supplemental Methods Tables 4.1 and 4.2**. Sodium:potassium ratios were calculated by dividing mean urinary sodium (mmol/L) by mean urinary potassium (mmol/L); a ratio ≤1 indicated a diet potentially lower in sodium or protective against hypertension, though evidence is still insufficient to conclusively determine these correlations (48).

Estimating household and individual salt consumption from purchase data

Estimated median (IQR) daily consumption of household discretionary salt ('table salt') and total salt (discretionary salt plus salt from bouillon) was calculated from purchase data collected with the Household FACT Questionnaire (38). Although other contributors to total dietary salt exist, such as processed foods or restaurant meals, for simplicity we refer to the combination of discretionary salt and salt from bouillon as total salt. Household daily discretionary salt consumption was estimated by dividing the quantity of salt last purchased (grams) by the total number of days that quantity was reported to last (i.e., grams of salt / number of days; g/d) (49,50). This calculation was repeated for bouillon, with the total multiplied by 55% to estimate salt consumption from bouillon (29). Prior to estimating household daily discretionary and total salt consumption, the variables' distributions were examined and sensitivity analyses conducted that truncated to the next nearest value any observations three times less than the 25th percentile or three times greater than the 75th percentile of the calculated IQR (49). As the median values did not change, all observations were retained. Estimated median (IQR) daily consumption of discretionary and total salt of individuals (WRA and children) was calculated using the Adult Male Equivalent (AME) method (49,50). Because measurement of salt is at the household level, oftentimes reporting of estimated individual consumption with the AME method is referred to as 'apparent intake' or 'apparent consumption'(49); for ease of presentation, we will hereafter refer to it as 'consumption.'

Estimating WRA daily total salt consumption from spot urine samples

Among WRA only, daily total salt consumption was estimated from spot urine samples. We used the INTERSALT equation to predict 24-h urinary sodium excretion (51), and then estimated daily total salt consumption (g/d) by dividing the predicted 24-h urinary sodium excretion (mg/d) by 390 as there are 390 mg sodium in 1 g sodium chloride ('table salt') (14). The INTERSALT equation was selected as it

has separate equations for males and females and has been evaluated in populations of African descent (51–53). Use of spot urine samples to predict 24-h sodium excretion (and subsequent daily salt consumption) are recommended only for estimations of population salt consumption above or below a threshold, such as the maximum salt consumption of 5 g/d recommended by the WHO (54). Use of this method to estimate individual salt consumption, or predict clinical outcomes such as hypertension, are not recommended due to within-person variability in urinary sodium excretion, measurement error in urine collection, and bias built into the predictive equations (52,54,55).

We then examined estimations of salt consumption in relation to international recommendations. We defined high salt consumption for WRA as ≥5 g/d, according to WHO recommendations (7). We defined high salt consumption for children 2-3 y as ≥3 g/d and as ≥3.75 g/d for children 4-5 y, according to the National Academies of Sciences 2019 Chronic Disease Risk Reduction recommendations (48).

Factors associated with estimated household salt consumption from purchase data

Using mixed effects analysis of variance (ANOVA), we examined factors associated with estimated household salt consumption from the purchase data. Factors were selected based on variable availability in the dataset (**Supplemental Table 4.3**), and we explored relationships between household salt consumption and household and individual characteristics, including those related to KAP, based on a conceptual model of hypothesized expected relationships (**Supplemental Figure 4.1**). Models included potential predictors at both household-level and individual-level (WRA and LW) as the perceptions of WRA and LW may have influenced their household's salt consumption. All potential predictors were selected *a priori* according to our analysis plan. Observations from children (n=244) were not included as only WRA and LW responded to both the STEPS and KAP questionnaires.

Outcome variables included estimated household discretionary salt consumption and estimated household total salt consumption (continuous, g/d). Due to extreme outliers in the outcome variables, we truncated values that were less than the 2.5 percentile (n=8, 1.7% discretionary salt; n=9, 1.9% total salt) or greater than the 97.5 percentile (n=9, 1,9% discretionary salt; n=9, 1.9% total salt) to the value at the percentile thresholds (**Supplemental Figure 4.2**); sensitivity analyses were conducted with the original values included. Mixed effects models included district (Tolon/Kumbungu), setting (urban/rural), household size, and participant type (WRA or LW) as separate fixed effects, and cluster as the random effect. We tested each categorical predictor in separate minimally-adjusted mixed effects models with each outcome variable. Then, marginally significant predictors ($p<0.1$) were included in multivariable mixed-effects models. Collinearity between predictors was assessed with variance inflation factors (>5) and tolerance (<0.1). To inform model interpretation, we constructed a correlation matrix among all predictors, including variables of social desirability from the Marlowe-Crowne Social Desirability Scale (56) (**Supplemental Figure 4.3**).

Qualitative analyses

A codebook was developed based on the FGD guide and followed a format of segments, structural codes, and content codes (57–59). Segments were defined as the primary questions from the FGD guide. Segments were then subdivided into structural codes, defined as the sub-questions and all relevant probes within each primary question, and then multiple content codes were applied to each structural code. The notes and debriefings forms from the FGDs were used to develop the initial list of content codes; content codes were updated and refined through discussions between two coders (JND and SK) throughout the coding process.

Using the codebook, two researchers independently coded n=7 (35%) FGD transcripts: one cultural insider (emic, SK) and one cultural outsider (etic, JND) (60,61). Coders applied a line-by-line

directed content coding strategy, meaning that each line of text or phrase could be applied to multiple content codes (58). Double-coded transcripts were compared and consistency between the coders assessed through calculating an intercoder reliability (ICR) score (Cohen's Kappa) and percent agreement. The ICR was calculated for each transcript segment as defined in the codebook (57,62). Segments with an ICR score <0.7 (ICR ≥0.7 indicates substantial agreement (62)) were discussed, independently recoded, and the ICR score recalculated. Average final ICR score was 0.95 and percent agreement was 96.5% (**Supplemental Table 4.4**). Double-coded and revised transcripts were uploaded into NVivo, and the remaining transcripts (n=13) were coded in NVivo directly by JND.

Content analysis and thematic selection followed the Framework Method (61). Framework matrices were generated with NVivo wherein the coded data were reduced and summarized. JND, SK, and RES reviewed the data matrices, and discussed salient major themes and subthemes. While the FGD guide provided structure for a deductive qualitative analysis approach, review of the data matrices and discussions among co-authors also allowed for inductive inquiry. Final themes and subthemes were agreed upon after triangulation with data summarized from the note-taking and debriefing forms, as well as discussions with fieldworkers involved with the FGDs.

Results

Characteristics of participating households, women, and children in the pilot survey

Fieldworkers visited n=375 households, n=371 provided consent, and n=369 households participated in the pilot survey (**Supplemental Figure 4.4**); the median (IQR) household size was 10 (8, 14) members. Households were primarily (99%) from the Mole-Dagbani ethnic group and practiced Islam. Household heads (58%) had completed secondary school, 84% of households had electricity, 55% had access to an improved water source, and 29% had access to an improved toilet. Three-quarters of households were moderately to severely food insecure (**Table 4.1**).

Participating households included n=487 adult women (n=244 WRA and n=243 LW) and n=246 children (Supplemental Figure 3). Among adult women, the median (IQR) age was 30 (24, 35) years. The majority of women had not completed primary school (71%), were married (86%), and self-employed (70%) (Table 1). Among WRA only, mean (SD) BMI was 22.4 (3.9) kg/m^2, and hypertension affected 17% (AHA definition) and 6% (ISH/WHO definition). Mean (SD) sodium:potassium ratio was 5.7 (4.0) (**Table 4.2**). Among children, median (IQR) age was 3 (2, 4) y, mean (SD) BAZ was -0.4 (1.0), and 32% were stunted. The proportion of children with blood pressure measurements considered 'At Risk' were 19% male and 12% female (Tables 4.1 and 4.2).

Estimated salt consumption among women, children, and households

Using the AME method to disaggregate the household salt purchase data, the median (IQR) estimated discretionary salt consumption among WRA was 5.1 (3.1, 8.9) g/d, estimated total salt consumption was 6.0 (4.0, 10.2) g/d, and predicted total salt intake calculated from urinary sodium excretion was 7.1 (6.2, 7.9) g/d (**Table 4.3**). Among children, estimated median (IQR) consumption of discretionary salt was 2.4 (1.6, 4.2) g/d, and total salt was 2.9 (1.9, 5.2) g/d (Table 4.3). The majority of the population (WRA and children) appeared to exceed global recommendations for daily salt consumption **(Figure 4.1)**. Bouillon contributed approximately 23% to household total salt consumption.

Factors associated with daily household salt consumption

Household characteristics associated with both household discretionary and total salt consumption in minimally-adjusted and multivariable models included household size and food insecurity, though the significance was marginal for food insecurity in the discretionary salt multivariable model (p=0.05). Setting (urban vs. rural) and household assets (marginally significant) were positively associated with both outcome variables in minimally-adjusted models but not multivariable models. No associations were found with other household characteristics (district, education level of the household

head, and household consumption of *dawadawa*). No significant associations were found with demographic characteristics of WRA or LW, nor weekly consumption of salty snacks or processed foods. Weekly consumption of vegetables, fruits, and foods prepared with bouillon (marginally significant) were positively associated with household discretionary and total salt consumption in minimally-adjusted models, but only vegetable consumption remained associated in the multivariable model with discretionary salt consumption (p=0.03) (**Figure 4.2; Tables 4.4 and 4.5**). Results from sensitivity analyses with all values (i.e., not truncated to the 2.5 and 97.5 percentiles) were similar.

Associations between KAP predictors and household salt consumption varied (Tables 4.4 and 4.5; Figure 4.2). The knowledge that dietary salt causes health problems was positively associated with household discretionary and total salt consumption in minimally-adjusted models, but not multivariable models. The attitude that it is not important to lower dietary salt was positively associated with household discretionary and total salt consumption in both minimally-adjusted and multivariable models, while the attitude that it is very important to lower dietary salt was negatively associated with household discretionary and total salt consumption in all models. The practices of never or rarely adding salt to food at the table and doing at least one action regularly to control dietary salt (total salt models only) were negatively associated with household discretionary and total salt consumption in all models. In correlation analyses with social desirability variables (Supplemental Figure 4.3), the practices of adding salt at the table and doing at least one action regularly to control salt intake were strongly correlated with social desirability, as was weekly vegetable consumption (moderate correlation).

In exploratory analyses of the effect of groups of knowledge, attitude, or practice predictors on daily household discretionary and total salt consumption, we found similar trends in the direction and significance of association as the previously described models (Supplemental Methods and **Supplemental Table 4.5**).

We conducted post-hoc analyses to better understand associations between household assets, food insecurity, and salt consumption. We found that discretionary salt consumption increased with greater food insecurity status, except in the 3rd asset quintile, which, upon examination, contained an outlier (outlier defined as >60% larger than the value at the 99th percentile) (**Supplemental Table 4.6**). Sensitivity analyses examining associations between food insecurity or asset quintiles and household discretionary salt consumption with the outlier truncated to the 99th percentile did not change the direction or strength of the associations. We also found that households in the lowest (1st and 2nd) asset quintiles last purchased smaller quantities of salt when compared to other quintiles (**Supplemental Table 4.7**).

Focus group discussions (FGDs)

We further explored the factors influencing household salt consumption through FGDs (n=20 FGDs) with WRA (n=10 FGDs; n=56 participants), men (n=5; n=29), and women >49 years (n=5; n=29). Characteristics of FGD participants are presented in **Supplemental Table 4.8**. We found that the influencing factors fell into three overarching themes: 1) salt's ubiquitous role as a primary seasoning; 2) the influence of intra-household dynamics and taste preferences of key household members; and 3) perceived relationships between salt and health.

Theme 1: Salt's ubiquitous role as a primary seasoning

When participants spoke of salt, they spoke of it in terms of its ubiquity in the local cuisine. While other seasonings or ingredients were also commonly added to household foods, such as fish flakes, *dawadawa* (fermented locust beans), bouillon ('maggi'), or hot peppers, salt was used in cooking all the time, including when other seasonings were unavailable or not preferred. Participants also discussed reducing or eliminating the purchase of bouillon, *dawadawa,* and fish when household finances were low, however salt was never mentioned as a seasoning to eliminate.

When you cook in the afternoon you will use a little salt, dawadawa and bouillon to make the food tasty. [WRA, rural]

> **Note:** Cooking in 'the afternoon' refers to daily cooking as most households in this region cook 1-2 times a day.

When you do not like maggi you can use salt and dawadawa alone. [WRA, urban]

I add the local dawawa, fish, salt, and add the maggi small. We don't do that all the time. We only add the maggi when we have money. [woman >49 y, rural]

Participants also reported that food would be inedible if prepared without salt, further illustrating the perceived necessity of salt:

With regards to cooking, you cannot use only bouillon to food and it will be edible. We as Dagombas have pepper, salt, and even the fish…the soup cannot be soup if these things are absent. You cannot eat it. [man, urban]

> **Note:** 'Dagombas' refers to members of the predominate ethnic group of the region.

It is beneficial. Some people say that salt makes the soup because if there is no salt in food you cannot eat it. [WRA, urban]

It is even salt that is beneficial. Just a few people do not eat salt. Once you cook without salt you cannot tell what the soup has become. No matter how sweet the soup is, without salt it is not tasty. [woman >49 years, rural]

> **Note:** The term 'sweet' is a translation from Dagbani, and generally refers to the degree of flavor, of 'tastiness,' of the food. Sweet is also a preferred taste, so participants may describe food they enjoy as 'sweet' or 'tasty'. A 'sweet' food does not necessarily indicate a sugary or sweetened food item.

Despite salt's ubiquity, salt was not perceived as the primary flavoring of common dishes. We asked FGD participants what was the flavor they perceived most in foods prepared with bouillon. Many (n=61, 54%) reported that *dawadawa* or fish were perceived, or tasted, more than other flavorings; others reported that bouillon was the main flavoring (n=41, 36%); three participants (3%) reported salt as the main flavoring.

Theme 2: Influence of intra-household dynamics and taste preferences of key household members

We asked participants which household members decided which dishes to cook daily, and the responses exemplify how household dynamics, including household finances, dictated food procurement and preparation:

> *The amount provided as housekeeping money by the landlord* (household head) *determines what they will cook. If he provides an amount that can cook rice, that is what they will prepare. If it is one [an amount] that can cook fufu or tuo zaafi that is what they will prepare.* [man, urban]
>
> > **Note:** *Fufu* is a dough made from pounded West African yam; *tuo zaafi* is boiled dough made from cereal flour (mainly maize in the study area). They are served with soups or stews.
>
> *The man of the house only gives the money for the food but the wife then decides what to cook, be it rice, fufu, or tuo zaafi. It is up to the wife to cook something good for the house.* [WRA, rural]
>
> *The household head will provide and the cooks will make the decision on the food to be cooked.* [WRA, rural]
>
> > **Note:** 'Cooks' refers to the multiple housewives or female household members who share cooking duties.

The taste preferences of the household head and children came through as influences on household salt consumption as these family members dictated how household foods should taste (e.g., saltiness). The food preferences of WRA and women >49 years appeared to be less influential than other household members.

> *[Salt's] benefit lies in when they add it to food and all of the house can eat to your satisfaction. If they are not able to add the amount that will be enough for the food, then the whole house cannot enjoy it.* [man, rural]
>
> *Aside that it is the children who decide [what] to eat because you cannot cook what they will not eat.* [WRA, urban]
>
> *We do not cook what we desire to eat. It is what the man provides that you cook. If he gives you rice or maize or yam, that is what you will cook with.* [woman >49 years, rural]

Theme 3: Perceived relationships between salt and health

In the FGDs, we asked participants what health problems were common in the area, which of these health problems may be related to nutrition, and what could be done to address these health problems. The majority of participants (n=81, 71%), and at least one participant in each of the 20 FGDs, reported that high blood pressure or stroke was a common affliction within their community (**Supplemental Table 4.9**).

Participants commented that high blood pressure and stroke could be addressed through changes in dietary patterns, such as reducing consumption of salt or bouillon, while other participants reported different causes or consequences of high blood pressure or stroke, such as stress or chewing *kola nut* (a tree nut that contains caffeine). Still others reported confusion with differing messages of how salt may relate to health outcomes, including messaging that consuming iodized salt is preferred.

> *We can protect ourselves by changing our food routine. When there is a particular food that has an effect on you when you take it you would have to stop, otherwise you would end up in the hospital. So it is up to us to stop consuming food that affects us.* [man, rural]
>
> *Some people say the high blood pressure is as a result of eating too much salt and others say it is as a result of the maggi. But with the stroke I don't know what causes it.* [man, rural]
>
> *Blood pressure is caused by talking too much but not from the foods we eat* [WRA, urban].
>
> > **Note:** The phrase 'talking too much' indicates that a person leads a stressful life or is under stress.
>
> *Sometimes you are advised to take Annapurna instead of the solid salt.* [WRA, rural]
>
> > **Note:** *Annapurna* is a brand of iodized salt; solid salt is the local salt produced on a small scale.
>
> *Sometimes you will be advised not to take salt but other times you will again be advised to take salt solution.* [WRA, rural]
>
> > **Note:** In this context, 'salt solution' refers to oral rehydration salt solution which is often used to treat diarrhea.

Participants discussed perceptions that salt is healthy because it is natural (i.e., not made with unknown ingredients) and because it is part of their dietary tradition. Participants also commented that

salt was healthy as it helps food become palatable, which was thought to increase the likelihood that a person will eat and be better nourished. However, many participants discussed that salt must not be overconsumed, both to protect one's health and to protect the palatability of the food.

> *Salt is healthy because we have been using it all along.* [WRA, rural]
>
> *Dawadawa and salt are healthy. When you add dawadawa to soup but there is no salt, you cannot swallow it. Once you cannot swallow, you cannot eat to your satisfaction nor drink water. It is food that you eat and the water that you drink that makes you healthy*. [woman >49 y, rural]
>
> *There is no food that has no benefit, it is when they overuse it that it exceeds its benefits. Salt does not need to be a lot for you to consume.* [man, urban]

Participants appeared to determine whether or not salt (or other food) was healthy based on how their bodies felt after consumption. If a bad effect was discovered, a person may reduce or stop their consumption.

> *How will we protect ourselves from these diseases that are cause by the foods we take? We can do so by not consuming food that have effects on us when we take them.* [WRA, urban]
>
> *That is the way it is. When you notice that you do not feel healthy anytime you eat a particular food you will forget about that food.* [woman >49 y, urban]
>
> *The only way we can protect ourselves from these diseases is staying away from food we are not supposed to eat. For me, I don't take maggi because it used to affect me until I stopped, and I am now okay. When you take foods that have effects on you need to stay away from them.* [man, rural]

Discussion

In this mixed-methods study from the Northern Region, Ghana, we analyzed quantitative data from a pilot survey and qualitative data from focus group discussions. From reported household salt purchase data, we found that estimated consumption of both discretionary salt ('table salt') and salt from bouillon ('total salt') appeared to exceed global recommendations for many children and the majority of WRA. We also estimated that bouillon contributed less than 25% to households' daily total salt consumption. While consumption of salt from processed foods or from foods prepared outside the home was not included in our estimates, mean urinary sodium excretion and the mean

sodium:potassium ratio among WRA also suggested high sodium exposure. We identified a limited number of characteristics associated with household salt consumption, which is consistent with qualitative data emphasizing the ubiquity of salt as a household seasoning. Other salient qualitative themes illustrated how household salt usage and consumption were shaped by intra-household dynamics, taste preferences of key household members, and perceived relationships between salt and health.

Achieving global recommendations to consume <5 g/d of salt for adults and <3-3.75 g/d for children 2-5 y of age will require the attention of multiple stakeholders, including policymakers, private industry, and public health officials (65). Our estimates of salt consumption among adults and children in Northern Ghana align with findings from other studies that salt consumption in Ghana is above recommended thresholds (13,16). Though use of the AME method may overestimate individual apparent consumption for women and children as the method uses adult males as the control population and assumes that intrahousehold food sharing is proportional to individual energy requirements (49,66), our estimates likely underestimated both household and individual apparent consumption as the purchase data did not take into account contributions from processed foods or foods eaten outside the home. Consumption of these items may be lower in northern Ghana compared to larger urban settings, such as Accra, though consumption of any salty processed foods is >80% across urban and rural settings and wealth quintiles according to the most recent Ghana Demographic and Health Survey (10). Among WRA, comparison of consumption data (estimated mean total salt: 6.0 g/d) versus urinary sodium excretion data (7.1 g/d) also suggest that the additional contribution of processed foods may be low in this sample, but further work is required to confirm this given the sources of error in the data sources (17,18,67).

Understanding sources of dietary salt is necessary to inform salt consumption reduction programs. In our study, bouillon contributed a small proportion to household daily salt consumption

compared to discretionary salt consumption. In fact, the results suggest that even if bouillon were eliminated as a dietary source much of this population would still exceed daily salt recommendations. Given that discretionary salt, and specifically salt added during cooking, appears to be the main source of dietary salt in this population (13,24), examining the implications of targeting different sources of dietary salt for salt reduction programs is needed. For example, discretionary salt's ubiquity as a household seasoning and the importance of taste preferences in determining household cooking practices may make it the hardest to reduce in the general diet, whereas a package of ultra-processed food or a cube of bouillon may be easier to 'count', and therefore target. However, if consumption of a fortification vehicle (e.g., salt or bouillon) is reduced there are implications for the impact of a fortification program on micronutrient status; to address this, ideally the fortification program design (fortification levels) would be adjusted accordingly (68).

Salt reduction programs aimed at discretionary salt usage and consumption must also consider whether to target program activities to specific subsets of the population, and if so, which, if any, population characteristics are "targetable." In our analyses, both greater food insecurity and greater household assets were associated with greater household discretionary and total salt consumption. In post-hoc analyses, mean per capita discretionary salt consumption increased as food insecurity increased in all but the 3rd asset quintile. These contradictory findings could be a result of a relatively homogenous population in terms of assets (69), measurement error in estimating household salt consumption (including differential measurement error by purchasing amount and/or frequency), or reporting bias, though we reviewed correlations between household total salt consumption and social desirability variables (Marlowe-Crowne Social Desirability Scale (56)) and found no correlations. But they may also reflect the influences of households' taste preferences and intra-household dynamics, or simply salt's universal and essential role in cooking, as suggested by the FGDs.

Few KAP predictors were associated with household salt consumption, and some, specifically practice predictors, were correlated with social desirability variables, suggesting reporting bias in participants' responses. Given our observation that high salt consumption is common and that relatively few factors were associated with household salt consumption, this may indicate that the majority of this population could benefit from salt reduction behavioral change communication (BCC), without necessarily targeting specific population subgroups. BCC that broadly encourages reductions in salt usage and consumption has been urged by global experts (65,70), and a review of behavior change salt reduction interventions found that targeting specific population characteristics in low- and middle-income countries did little to influence the effectiveness of the interventions (71).

While our observational analyses are limited for investigating causality, the KAP responses could be a starting point for BCC message development, though further work is needed to develop an impact pathway with hypothesized causal links between BCC efforts and behavior change. Promoting the relationship between salt consumption and health may be important to include in BCC. While prevalence of hypertension was lower among WRA than national estimates (6% vs 12.9%, WHO hypertension definition (10)), it may be greater among older populations (72). Additionally, many FGD participants reported awareness of the presence of stroke and hypertension in their community, and reported that their knowledge of salt's contribution to ill health influenced decisions to reduce salt and bouillon consumption. These perceptions may have been biased due to the focus of the FGDs (bouillon and salt). But, in our pilot survey one-third of participants believed dietary salt was "bad", 87% of whom stated this was because salt could cause hypertension, heart attack, and stroke, which corroborates our conclusion. Thus, incorporating frameworks such as the Health Belief Model, where behavior change depends on perceived benefits or consequences of health outcomes, into culturally-tailored BCC may be an important salt reduction strategy (24,71).

Strengths of our study include the use of mixed-methods methodology that explained and supported convergent and divergent quantitative and qualitative findings (57). The face validity and reliability of the qualitative data were enhanced through triangulation with notes from the FGDs and discussions with fieldworkers, independent transcriptions and translation of FGD transcripts, independent double-coding of transcripts verified with Cohen's Kappa, and the inclusion of exemplar quotes to illustrate and support each salient FGD theme (35,57). Additionally, our estimates of salt consumption align with prior estimates of salt intake in Ghana and provide some of the only estimates of salt consumption for children aged 2-5 y, though our estimates are limited by the use of purchase data and do not include the contribution of salt from processed foods or foods eaten outside the home. Another limitation was the use of a salt prediction equation to estimate salt intake from urinary sodium excretion as prediction equations introduce bias and likely underestimated salt intake (52,55); however, the WHO recommends the use of spot urine samples to predict salt intake above or below the 5g threshold at the population level (54). Our analyses were also limited by lack of data on intra-household food distribution and women's decision-making, though we were able to elucidate some of women's roles in cooking decisions and food procurement through the FGDs. Finally, though we were limited by the cross-sectional nature of our data, our findings are similar to other studies in Ghana and sub-Saharan Africa in the amount of salt consumed, the main dietary source, and salt usage and consumption behaviors (13,24,25,73).

Conclusion

In this mixed-methods study in the Northern Region, Ghana, using quantitative data from a pilot survey and qualitative data from focus group discussions, we found that estimated daily consumption of salt, including discretionary salt and salt from bouillon, appeared to exceed global recommendations for many children 2-5 y and the majority of adult women; however, the contribution of bouillon to daily

household salt consumption was less than 25%. Multi-pronged, population-level salt reduction strategies that include behavior change communication linking salt consumption to health outcomes may be useful salt reduction approaches for this population.

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Tables

Table 4.1. Characteristics of households, adult women, and children who participated in the pilot survey in Tolon and Kumbungu districts, northern Ghana: CoMIT Project[1]

Characteristics	**Overall**	**Urban**	**Rural**
	n (%)	*n (%)*	*n (%)*
Household			
Total households	369 (100)	172 (47)	197 (53)
Household size (median [IQR])	10 (8,14)	11 (8,14)	10 (7,14)
Education level of household head			
None	100 (27)	22 (13)	78 (39)
Primary school	56 (15)	21 (12)	35 (18)
Secondary or higher	213 (58)	129 (75)	84 (43)
Has electricity	308 (84)	172 (100)	136 (70)
Access to an improved water source[2]	203 (55)	134 (78)	69 (35)
Access to an improved toilet[2]	108 (29)	80 (47)	28 (14)
Food insecurity status[3]			
Food secure	20 (5)	18 (10)	2 (1)
Mild insecurity	66 (18)	24 (14)	42 (21)
Moderate insecurity	193 (52)	86 (50)	107 (54)
Severe insecurity	90 (25)	44 (26)	46 (24)
Adult women			
Total	487 (100)	238 (49)	249 (51)
Age, y (median [IQR])	30 (24, 35)	30 (23, 35)	30 (24, 36)
Highest education level completed			
None	345 (71)	145 (61)	200 (80)
Primary school	55 (11)	30 (13)	25 (10)
Secondary or higher	86 (18)	62 (26)	24 (10)
Marital status			
Never married	56 (11)	31 (13)	25 (10)
Currently married	416 (86)	198 (84)	218 (88)
Widowed or separated	13 (3)	8 (3)	5 (2)
Employment status			
Self-employed	337 (70)	170 (72)	167 (67)
Homemaker	79 (16)	33 (14)	46 (18)
Student	34 (7)	20 (8)	14 (6)
Other	35 (7)	14 (6)	21 (9)
Children			
Total	246 (100)	123 (50)	123 (50)
Age, y (median [IQR])	3 (2, 4)	3 (2, 4)	3 (2,4)
Female	116 (47)	53 (43)	63 (51)

[1] Adult women includes non-pregnant, non-lactating women of reproductive age (15-49 y; n=244) and non-pregnant lactating women (15-49 y, 4-18 mos post-partum; n=243). Children are aged 2-5 y. Improved toilet defined as having access to one of the following: flush/pour flush to septic tank or pit/latrine, ventilated improved pit latrine, or pit latrine with slab (63). Improved water defined as having access to one of the following: piped water into dwelling, piped water into yard/plot compound, water piped to a neighbor's dwelling/yard/plot, public tap or standpipe, protected tube well or borehole, protected dug well, cart with small tank/drum (64). Food insecurity calculated with the Household Food Insecurity Asset Scale score (37). 'Other' employment status includes those who were non-paid (n=1), government employees (n=3), or unemployed but able to work (n=31). Abbreviations: CoMIT, Condiment Micronutrient Innovation Trial; IQR, interquartile range.

Table 4.2. Biological characteristics for non-pregnant non-lactating women of reproductive age (15-49 y) and children (2-5 y) who participated in the pilot survey in Tolon and Kumbungu districts, northern Ghana: CoMIT Project[1]

Characteristics	*n*	Overall	*n*	Urban	*n*	Rural
Women						
BMI, kg/m^2	*222*	22.4 ± 3.9	*106*	22.8 ± 4.5	*116*	22.1 ± 3.1
BMI category,[2] n (%)						
Underweight		21 (9)		13 (12)		8 (7)
Normal weight		157 (71)		69 (65)		88 (76)
Overweight		34 (15)		17 (16)		17 (15)
Obese		10 (5)		7 (7)		3 (3)
Blood pressure, mmHg[3]						
Systolic	*223*	110.8 ± 16.1	*106*	110.8 ± 15.3	*117*	110.9 ± 16.9
Diastolic	*223*	69.8 ± 10.8	*106*	69.7 ± 9.3	*117*	69.8 ± 12.1
AHA elevated blood pressure,[3] n (%)		13 (6)		7(7)		6 (5)
AHA hypertension,[3] n (%)		38 (17)		16 (15)		22 (19)
ISH high-normal blood pressure,[3] n (%)		14 (6)		9 (9)		5 (4)
ISH and WHO hypertension,[3] n (%)		13 (6)		5 (5)		8 (7)
Urinary sodium excretion,[4] mmol/L	*219*	142.9 ± 72.4	*103*	135.8 ± 72.1	*116*	149.2 ± 72.3
Urinary potassium excretion,[4] mmol/L	*219*	35.0 ± 26.2	*103*	33.7 ± 26.2	*116*	36.2 ± 26.2
Urinary creatinine excretion,[4] mg/dL	*220*	130.0 ± 84.6	*104*	133.2 ± 92.1	*116*	127.2 ± 77.6
Sodium:Potassium ratio	*219*	5.7 ± 4.0	*103*	5.7 ± 4.1	*116*	5.6 ± 3.9
Predicted urinary sodium excretion,[4] mg/d	*219*	2756.4 ± 558.2	*103*	2795.5 ± 547.9	*116*	2712.3 ± 569.1
Children						
BAZ	*232*	-0.4 ± 1.0	*114*	-0. 5 ± 1.0	*118*	-0.4 ± 1.0
BAZ category,[2] n (%)						
Underweight		9 (4)		3 (3)		6 (5)
Normal		219 (94)		109 (96)		110 (93)
At risk of overweight		4 (2)		2 (2)		2 (2)
Stunted, [2] n (%)		73 (32)		27 (24)		46 (39)
Wasted, [2] n (%)		11 (5)		3 (3)		8 (7)
Blood pressure, mmHg						
Systolic	*184*	90.3 ± 10.8	*96*	90.7 ± 10.2	*88*	89.9 ± 11.5
Diastolic	*184*	55.0 ± 6.7	*96*	55.6 ± 6.6	*88*	54.3 ± 6.8
Blood Pressure 'At Risk', [3] n (%)						
Female		22 (12)		11 (12)		11 (13)
Male		35 (19)		21 (22)		14 (16)

[1] Results are presented as mean ± SD unless otherwise indicated. AHA, American Heart Association; APA, American Pediatric Association; BAZ, BMI-for-age z-score; BMI, Body Mass Index; CoMIT, Condiment Micronutrient Innovation Trial; DBP; diastolic blood pressure; HAZ, height-for-age z-score; ISH, International Society of Hypertension; SBP, systolic blood pressure; WHO, World Health Organization; WHZ, weight-for-height z-score.

[2] Women BMI categories: underweight, BMI <18.5 kg/m^2; normal weight, BMI ≥18.5-24.9 kg/m^2; overweight, BMI 24.0-25.9 kg/m^2; obese BMI ≥30.0 kg/m^2. Child BAZ categories: underweight, BAZ <-2 SD; normal weight, BAZ -2 ≥ SD ≤ 2; and at risk of overweight, BAZ >2 SD. Stunted, HAZ <-2 SD; wasted, WHZ <-2 SD.

[3] Blood pressure measurements for both women and children are the average of 3 measurements taken in the same sitting using an automatic portable upper-arm blood pressure monitor. Bood pressure guidelines and thresholds: AHA adult elevated blood pressure: systolic 120-129 and diastolic <80 mmHg; AHA adult hypertension: systolic ≥130 or diastolic ≥80 mmHg (44). ISH high-normal blood pressure: systolic 130-139 and/or diastolic 85-89 mmHg; ISH and WHO hypertension: systolic ≥140 and/or diastolic ≥90 mmHg (45,46). 'At risk' blood pressure for children listed in Supplemental Table 2 (47).

[4] Analysis of urinary sodium, potassium, and creatinine completed from a single spot urine sample from each participant. Predicted urinary sodium excretion (g/d) calculated using the INTERSALT formula (51).

Table 4.3. Apparent intake of salt of women (15-49 years) and children (2-5 years), and estimated salt consumption of households, based on household estimates or urinary sodium excretion among those who participated in the pilot survey in the Tolon and Kumbungu districts, northern Ghana: CoMIT Project[1]

		Estimated discretionary salt consumption from purchase data (g/d)		Estimated total salt consumption from purchase data (g/d)	Proportion of total salt consumption from bouillon		Predicted total salt intake from urinary sodium excretion (g/d)	
	n	median (IQR)	n	median (IQR)	%	n	median (IQR)	mean (SD)
Women (15-49 y)	239	5.1 (3.1, 8.9)	237	6.0 (4.0, 10.2)	14.3%	219	7.1 (6.2, 7.9)	7.1 (1.4)
Urban	116	4.3 (2.8, 7.3)	115	5.4 (3.6, 8.9)	20.8%	103	7.0 (6.0, 7.8)	7.0 (1.5)
Rural	123	6.3 (3.6, 10.2)	122	7.1 (4.2, 11.5)	11.2%	116	7.2 (6.3, 8.1)	7.2 (1.4)
Children (2-5 y)	241	2.4 (1.6, 4.2)	239	2.9 (1.9, 5.2)	16.7%	--	--	--
Urban	120	2.2 (1.4, 3.8)	119	2.6 (1.3, 4.2)	17.0%	--	--	--
Rural	121	2.8 (1.7, 4.8)	120	3.3 (2.1, 5.4)	14.7%	--	--	--
Households	363	43.1 (32.1, 106.9)	360	56.2 (40.3, 116.2)	23.2%	--	--	--
Urban	168	41.0 (28.6, 85.7)	167	52.3 (39.2, 102.2)	21.7%	--	--	--
Rural	195	55.0 (33.3, 106.9)	193	66.5 (41.2, 117.9)	17.3%	--	--	--

[1] Estimated discretionary salt and total salt (discretionary salt + salt from bouillon where bouillon was assumed to be 55% salt) calculated from purchase data using the FACT questionnaire (38). Estimations for women and children presented as grams per day per Adult Male Equivalent. Predicted urinary sodium excretion (mg/d) was estimated using the INTERSALT equation (51), and then predicted total salt intake (g/d) was calculated by dividing the estimate of urinary sodium excretion (g/d) by 390 as there are 390 mg sodium in 1 g sodium chloride ('table salt') (14). CoMIT, Condiment Micronutrient Innovation Trial; IQR, interquartile range; SD, standard deviation.

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Table 4.4: Factors associated with household daily discretionary salt consumption among households who participated in the pilot survey in Tolon and Kumbungu districts, northern Ghana: CoMIT Project[1]

Variable	Category	Hh discretionary salt consumption, g/d		Minimally adjusted analyses[2]		Multivariable analyses[2]	
		n	mean (SD)	*β* (95% CI)	*p*	*β* (95% CI)	*p*
Household-level factors							
Size, # of members	1-8	118	53.1 (52.7)	ref.	***<0.0001***	ref.	***0.0005***
	9-11	135	60.9 (45.4)	6.9 (-6.8, 20.4)		-1.1 (-14.9, 12.7)	
	12-15	112	74.8 (55.7)	25.6 (10.9, 40.3)		7.7 (-8.7, 24.2)	
	16+	112	104.4 (70.1)	49.9 (35.4, 64.5)		32.3 (14.9, 49.6)	
District	Tolon	242	76.1 (64.8)	ref.	*0.10*	ref.	*0.60*
	Kumbungu	235	68.6 (52.7)	-10.0 (-21.9, 1.9)		3.5 (-9.7, 16.8)	
Setting	Urban	232	64.7 (53.4)	ref.	***0.006***	ref.	*0.10*
	Rural	245	79.7 (63.5)	16.6 (4.7, 28.5)		11.0 (-2.1, 24.2)	
Participant type	WRA	239	71.4 (57.8)	ref.	*0.76*	ref.	*0.22*
	Lactating women	238	73.5 (60.7)	1.5 (-8.3, 11.3)		6.7 (-4.0, 17.5)	
Asset quintiles	1st (lowest)	88	52.6 (44.2)	ref.	***0.06***	ref.	*0.52*
	2nd	81	73.9 (61.2)	15.4 (-1.4, 32.3)		13.4 (-4.0, 30.7)	
	3rd	95	86.0 (66.3)	23.2 (6.5, 39.8)		14.1 (-3.2, 31.4)	
	4th	107	74.1 (58.3)	7.2 (-9.8, 24.3)		8.1 (-10.2, 26.4)	
	5th (highest)	106	73.8 (59.7)	13.5 (-4.6, 31.6)		9.4 (-9.7, 28.6)	
Food insecurity	None to mild	113	63.7 (46.4)	ref.	***<0.0001***	ref.	*0.05*
	Moderate	250	62.7 (51.4)	3.1 (-8.9, 15.0)		-2.6 (-16.8, 11.5)	
	Severe	114	102.5 (79.9)	37.3 (23.5, 51.1)		17.3 (-1.4, 36.1)	
Hh head education	None	110	69.9 (61.9)	ref.	*0.36*		
	Preschool or primary	73	81.4 (64.5)	11.2 (-5.4, 27.8)			
	Secondary or greater	294	71.2 (56.8)	2.1 (-11.1, 15.3)			
Dawadawa consumption	Low	104	63.6 (43.4)	ref.	*0.72*		
	Medium	286	76.5 (63.3)	4.5 (-8.3, 17.3)			
	High	58	61.9 (62.6)	-0.4 (-18.5, 17.6)			

Demographic factors (individual-level)							
Women age, y	15-24 y	129	71.7 (61.9)	ref.	*0.96*		
	25-34 y	206	75.0 (61.8)	-1.5 (-13.9, 10.9)			
	35-49 y	138	70.4 (53.1)	0.1 (-13.2, 13.4)			
History of heart disease	No	438	71.5 (59.0)	ref.	*0.65*		
	Yes	37	86.2 (61.3)	4.3 (-14.3, 23.0)			
Current hypertension	No	203	73.1 (60.5)	ref.	*0.64*		
	Yes	12	70.6 (38.5)	7.8 (-24.7, 40.2)			
BMI	Normal	154	77.0 (59.7)	ref.	*0.99*		
	Underweight	20	71.3 (59.1)	-0.7 (-26.2, 24.8)			
	Overweight/obese	43	58.8 (57.2)	-14.2 (-32.7, 4.2)			
Dietary patterns (individual-level)							
Vegetable consumption	1-2 d/wk	57	50.6 (49.9)	ref.	***<0.0001***	ref.	***0.03***
	3-5 d/wk	215	61.8 (51.3)	10.2 (-5.4, 25.7)		9.7 (-9.2, 28.5)	
	6-7 d/wk	203	90.2 (64.7)	39.1 (22.4, 55.8)		24.6 (-6.8, 34.5)	
Fruit consumption	None	126	54.8 (48.3)	ref.	***0.0003***	ref.	*0.35*
	1-2 d/wk	285	74.2 (60.0)	14.9 (3.4, 26.4)		1.5 (-12.3, 15.4)	
	≥3 d/wk	64	100.8 (64.2)	34.3 (17.3, 51.2)		13.9 (-6.8, 34.5)	
Salty snacks consumption	None	165	66.1 (49.5)	ref.	*0.99*		
	1-2 d/wk	225	74.8 (66.5)	-1.1 (-15.2, 12.9)			
	≥3 d/wk	85	79.5 (55.0)	-0.9 (-17.2, 15.5)			
Consumption of foods made with bouillon	0-6 d/wk	27	40.6 (24.3)	ref.	***0.05***	ref.	*0.35*
	7 d/wk	311	71.3 (58.4)	21.3 (0.2, 42.4)		9.4 (-10.4, 29.2)	
Knowledge factors (individual-level)							
Quantity of salt consumed	Just the right amount	368	72.7 (57.3)	ref.	*0.83*		
	Too much	25	76.2 (50.7)	1.3 (-21.1, 23.7)			
	Too little	82	71.0 (70.1)	4.2 (-9.2, 17.5)			
Think dietary salt causes health problems	No	43	51.0 (39.9)	ref.	***0.048***	ref.	*0.19*
	Yes	377	76.6 (60.9)	22.0 (4.4, 39.6)		18.6 (-2.7, 40.0)	

	Don't know	55	61.9 (56.1)	16.9 (-5.1, 38.8)		21.5 (-4.1, 47.1)	
Attitude factors (individual-level)							
Importance of lowering salt in the diet	Somewhat important	170	69.1 (56.9)	ref.	***<0.0001***	ref.	***0.03***
	Very important	190	56.7 (44.3)	-12.9 (-24.0, -1.9)		-3.07 (-16.8, 10.6)	
	Not at all important	115	104.2 (71.4)	26.45 (13.7, 39.3)		20.9 (3.6, 38.2)	
Perception of dietary salt	No effect	91	63.7 (42.2)	ref.	*0.32*		
	Good	144	77.5 (62.6)	7.1 (-7.1, 21.3)			
	Bad	103	61.3 (59.0)	-3.3 (-18.4, 11.3)			
Perception of dietary dawadawa	No effect	32	64.2 (49.4)	ref.	*0.84*		
	Good	306	69.3 (57.8)	2.1 (-18.3, 22.5)			
	Bad	0	--	--			
Perception of dietary bouillon	No effect	48	76.3 (57.0)	ref.	*0.23*		
	Good	231	67.1 (58.4)	-14.1 (-30.8, 2.6)			
	Bad	59	69.4 (51.8)	-7.6 (-28.0, 12.7)			
Practice factors (individual-level)							
Add salt at the table	Always/often/sometimes	196	85.2 (61.9)	ref.	***0.0006***	ref.	*0.41*
	Rarely/never	279	63.8 (55.8)	-14.2 (-24.4, -4.0)		-5.0 (-17.0, 7.0)	
Add salt during cooking	Always/often/sometimes	454	72.4 (60.0)	ref.	*0.51*		
	Rarely/never	21	77.6 (40.2)	8.1 (-16.0, 32.3)			
Eat processed foods	Always/often/sometimes	233	69.6 (62.8)	ref.	*0.40*		
	Rarely/never	242	75.5 (55.6)	4.4 (-5.8, 14.5)			
Do at least 1 action regularly to control dietary salt	No	91	74.2 (48.6)	ref.	*0.12*		
	Yes	384	72.2 (61.6)	-10.4 (-23.1, 2.2)			

[1] Discretionary salt refers to 'table salt' and estimates of Hh salt consumption were calculated from purchase data using the Fortification Assessment Coverage Toolkit (38). Hh discretionary salt consumption (g/d) presented as unadjusted mean (SD). Total households included: n=363. Hh head education defined as the highest education level completed. Hh consumption of dawadawa categories defined as: low, 0-10 g/d; med, 10.01-48.9 g/d; high >49 g/d, where the cutoffs

for each category were defined by the natural cutoffs present in the variable's distribution. History of heart disease was self-reported. Hypertension defined according to the WHO definition: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mm Hg (46). BMI categories defined as: normal, 18.5-24.9 kg/m^2; underweight, <18.5 kg/m^2; overweight/obese, ≥25.0 kg/m^2. Salty snacks defined as salty crisps, chips, nuts, or salty fried foods eaten between main meals. BMI, body mass index; Hh, household; CoMIT, Condiment Micronutrient Innovation Trial; ref., reference group; WHO, World Health Organization; wk, week.

[2] Minimally adjusted and multivariable analyses were linear mixed effects regression models that controlled for Hh size, district (Tolon/Kumbungu), area (urban/rural), and participant type (woman of reproductive age or lactating woman) as fixed effects, and cluster as a random effect. The outcome variable (Hh discretionary salt consumption in g/d) was tested as a continuous variable and all predictors were categorical variables. Predictors were included from both the household-level and individual-level (from data from non-pregnant, non-lactating women of reproductive age, 15-49 y, and non-pregnant lactating women, 6-18 mos post-partum, 15-49 y; n=487 total). After being tested separately in minimally adjusted models, marginally significant predictors ($p<0.1$, **bolded**) were included in the multivariable model.

Table 4.5: Factors associated with household daily total salt consumption among households who participated in the pilot survey in Tolon and Kumbungu districts, northern Ghana: CoMIT Project[1]

Variable	Category	Hh total salt consumption, g/d		Minimally adjusted analyses[2]		Multivariable analyses[2]	
		n	mean (SD)	*β* (95% CI)	*p*	*β* (95% CI)	*p*
Household-level factors							
Size, # of members	1-8	117	62.5 (54.3)	ref.	***<0.0001***	ref.	***0.0001***
	9-11	131	71.4 (47.3)	7.9 (-6.2, 22.0)		0.9 (-13.4, 15.2)	
	12-15	112	85.8 (57.5)	27.1 (12.0, 42.1)		9.1 (-7.7, 25.8)	
	16+	112	117.8 (70.2)	53.9 (38.9, 68.8)		37.4 (19.7, 55.0)	
District	Tolon	238	87.3 (66.3)	ref.	*0.12*	ref.	*0.68*
	Kumbungu	234	79.9 (54.9)	-9.8 (-22.1, 2.5)		2.9 (-10.8, 16.7)	
Setting	Urban	230	76.1 (55.5)	ref.	***0.0097***	ref.	*0.09*
	Rural	242	90.8 (65.1)	16.2 (3.9, 28.5)		11.6 (-2.0, 25.1)	
Participant type	WRA	237	82.3 (59.9)	ref.	*0.73*	ref.	*0.26*
	Lactating women	235	62.1 (84.9)	1.8 (-8.3, 11.9)		6.4 (-4.7, 17.5)	
Asset quintiles	1st (lowest)	87	62.5 (47.5)	ref.	***0.07***	ref.	*0.36*
	2nd	81	85.2 (63.4)	16.3 (-1.1, 33.6)		17.0 (-0.7, 34.7)	
	3rd	95	96.7 (66.9)	23.2 (6.1, 40.3)		14.9 (-2.6, 32.5)	
	4th	107	85.5 (59.9)	7.7 (-9.8, 25.2)		12.7 (-6.1, 31.4)	
	5th (highest)	102	86.1 (61.1	14.2 (-4.7, 33.1)		16.1 (-5.3, 37.4)	
Food insecurity	None to mild	113	74.7 (50.4)	ref.	***<0.0001***	ref.	***0.01***
	Moderate	247	73.5 (53.2)	3.4 (-8.9, 15.8)		-3.9 (-18.7, 11.0)	
	Severe	114	114.2 (74.8)	38.1 (24.0, 52.3)		28.3 (8.3, 48.3)	
Hh head education	None	109	81.2 (64.9)	ref.	*0.45*		
	Preschool or primary	71	92.3 (66.8)	10.1 (-7.1, 27.3)			
	Secondary or greater	282	82.4 (57.9)	1.5 (-12.1, 15.2)			
Dawadawa consumption	Low	104	73.9 (44.7)	ref.	*0.59*		
	Medium	282	88.4 (65.1)	5.6 (-7.6, 18.8)			
	High	57	72.0 (65.0)	-1.1 (-19.7, 17.5)			

Demographic factors (individual-level)							
Women age, y	15-24 y	126	82.9 (63.8)	ref.	*0.98*		
	25-34 y	204	86.6 (63.1)	-1.4 (-14.2, 11.)			
	35-49 y	138	81.0 (55.4)	-0.3 (-14.0, 13.5)			
History of heart disease	No	434	82.4 (60.5)	ref.	*0.50*		
	Yes	36	100.3 (65.8)	6.7 (-12.7, 26.0)			
Current hypertension	No	202	83.8 (62.6)	ref.	*0.58*		
	Yes	12	81.8 (42.2)	9.5 (-23.9, 42.8)			
BMI	Normal	153	87.8 (62.0)	ref.	*0.35*		
	Underweight	20	81.9 (58.9)	-0.6 (-26.9, 25.6)			
	Overweight/obese	43	69.6 (59.6)	-14.0 (-33.0, 5.0)			
Dietary patterns (individual-level)							
Vegetable consumption	1-2 d/wk	56	61.6 (53.0)	ref.	***<0.0001***	ref.	*0.44*
	3-5 d/wk	213	72.2 (53.9)	9.3 (-6.7, 25.4)		6.1 (-13.4, 25.7)	
	6-7 d/wk	201	102.2 (65.2)	40.7 (23.6, 57.9)		14.3 (-8.7, 37.3)	
Fruit consumption	None	125	66.1 (50.9)	ref.	***0.0007***	ref.	*0.25*
	1-2 d/wk	282	85.2 (61.8)	14.1 (2.2, 25.9)		1.4 (-12.8, 15.6)	
	≥3 d/wk	63	112.7 (64.8)	33.7 (16.1, 51.2)		16.3 (-5.1, 37.7)	
Salty snacks consumption	None	161	77.6 (52.1)	ref.	*0.97*		
	1-2 d/wk	224	85.7 (67.8)	-1.9 (-16.6, 12.8)			
	≥3 d/wk	85	90.6 (57.2)	-1.7 (-18.7, 15.3)			
Consumption of foods made with bouillon	0-6 d/wk	24	51.3 (28.0)	ref.	***0.08***	ref.	*0.25*
	7 d/wk	311	82.0 (60.0)	20.3 (-2.7, 43.3)		12.7 (-8.9, 34.3)	
Knowledge factors (individual-level)							
Quantity of salt consumed	Just the right amount	363	84.2 (59.0)	ref.	*0.92*		
	Too much	25	88.3 (53.0)	2.0 (-21.0, 24.9)			
	Too little	82	80.7 (71.6)	2.6 (-11.0, 16.3)			
Think dietary salt causes health problems	No	42	62.6 (43.4)	ref.	***0.06***	ref.	*0.30*
	Yes	374	87.8 (62.3)	22.0 (3.7, 40.3)		16.9 (-4.9, 38.8)	

	Don't know	54	72.8 (59.7)	17.1 (-5.7, 39.8)		17.5 (-9.2, 44.1)	
Attitude factors (individual-level)							
Importance of lowering salt in the diet	Somewhat important	168	80.4 (59.4)	ref.	***<0.0001***	ref.	***0.01***
	Very important	187	67.2 (47.7)	-13.7 (-25.1, -2.3)		-2.8 (-16.8, 11.2)	
	Not at all important	115	115.8 (70.3)	26.3 (13.2, 39.5)		24.4 (6.6, 42.1)	
Perception of dietary salt	No effect	91	75.3 (44.5)	ref.	*0.32*		
	Good	141	88.9 (64.8)	6.1 (-8.6, 20.7)			
	Bad	103	71.3 (60.1)	-4.9 (-20.4, 10.5)			
Perception of dietary dawadawa	No effect	32	75.8 (51.0)	ref.	*0.98*		
	Good	303	80.2 (59.7)	0.3 (-20.7, 21.4)			
	Bad	0	--	--			
Perception of dietary bouillon	No effect	48	87.8 (57.9)	ref.	*0.12*		
	Good	231	77.3 (60.4)	-16.0 (-33.2, 1.2)			
	Bad	56	83.3 (53.0)	-5.6 (-26.7, 15.6)			
Practice factors (individual-level)							
Add salt at the table	Always/often/sometimes	194	96.2 (62.2)	ref.	***0.01***	ref.	*0.43*
	Rarely/never	276	75.0 (58.7)	-13.7 (-24.2, -3.2)		-4.9 (-17.2, 7.3)	
Add salt during cooking	Always/often/sometimes	449	83.5 (61.8)	ref.	*0.45*		
	Rarely/never	21	90.0 (42.6)	9.5 (-15.3, 34.2)			
Eat processed foods	Always/often/sometimes	232	79.8 (63.6)	ref.	*0.26*		
	Rarely/never	238	87.8 (58.3)	6.0 (-4.4, 16.5)			
Do at least 1 action regularly to control dietary salt	No	91	86.1 (49.9)	ref.	***0.07***	ref.	***0.02***
	Yes	379	83.2 (63.4)	-12.1 (-25.1, 0.9)		-18.5 (-34.5, -2.5)	

[1] 'Total salt' defined as discretionary salt ('table salt') plus the proportion of salt from bouillon, where bouillon was assumed to be 55% salt (29). Estimates of Hh total salt consumption were calculated from purchase data using the Fortification Assessment Coverage Toolkit (38). Hh total salt consumption (g/d) presented as unadjusted mean (SD). Households with total salt data: n=360. Hh head education defined as the highest education level completed. Hh

consumption of dawadawa categories defined as: low, 0-10 g/d; med, 10.01-48.9 g/d; high >49 g/d, where the cutoffs for each category were defined by the natural cutoffs present in the variable's distribution. History of heart disease was self-reported. Hypertension defined according to the WHO definition: systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mm Hg (46). BMI categories defined as: normal, 18.5-24.9 kg/m^2; underweight, <18.5 kg/m^2; overweight/obese, ≥25.0 kg/m^2. Salty snacks defined as salty crisps, chips, nuts, or salty fried foods eaten between main meals. BMI, body mass index; CoMIT, Condiment Micronutrient Innovation Trial; Hh, household; ref., reference group; WHO, World Health Organization.; wk, week.

[2] Minimally adjusted and multivariable analyses were linear mixed effects regression models that controlled for Hh size, district (Tolon/Kumbungu), area (urban/rural), and participant type (woman of reproductive age or lactating woman) as fixed effects, and cluster as a random effect. The outcome variable (Hh total salt consumption in g/d) was tested as a continuous variable and all predictors were categorical variables. Predictors were included from both the household-level and individual-level (from data from non-pregnant, non-lactating women of reproductive age, 15-49 y, and non-pregnant lactating women, 6-18 mos post-partum, 15-49 y; n=487 total). After being tested separately in minimally adjusted models, marginally significant predictors (p<0.1, **bolded**) were included in the multivariable model.

Figures

Figure 4.1. Distributions of estimated salt consumption among women and children participating in the pilot survey in the Tolon and Kumbungu districts, northern Ghana: CoMIT Project

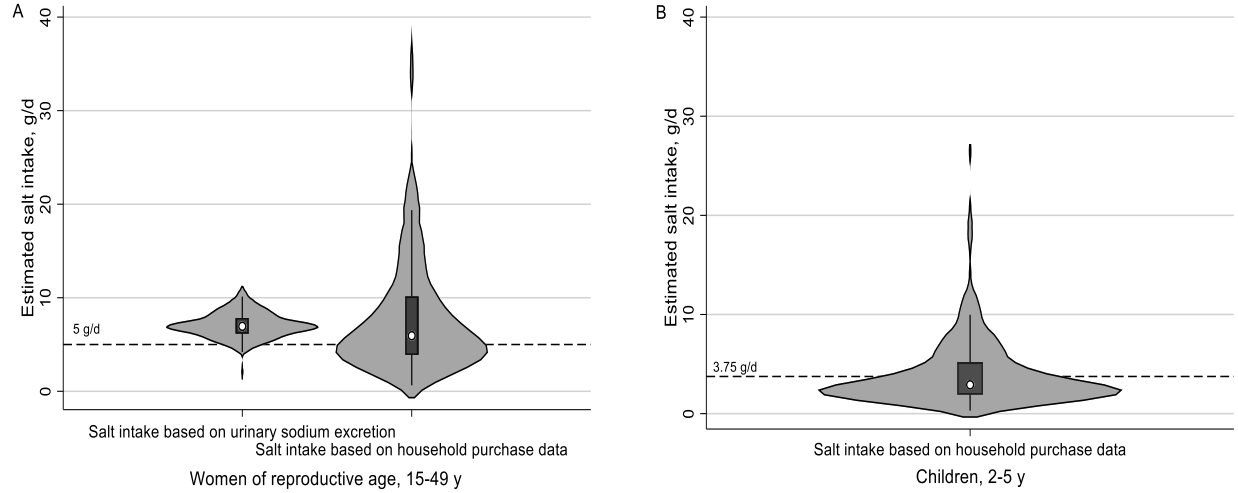


Figure 4.1 Legend: A. Among women of reproductive age (15-49 y), 'Salt intake based on urinary sodium excretion' indicates predicted salt intake calculated from urinary sodium excretion data using the INTERSALT equation (n=219) (51). 'Salt intake based on household purchase data' indicates estimated total salt consumption (discretionary salt + salt from bouillon) disaggregated from household purchase data collected using the FACT tool (n=237) (38). Dashed line represents the global recommended threshold to consume <5/g of salt (7) . B. Among children 2-5 y (n=239), 'Salt intake based on household purchase data' defined as indicated above. Dashed line indicates the threshold recommended in the 2019 guidelines from the National Academies of Sciences for children 4-5 y to consume <3.75 g/d; children 2-3 y are recommended to consume <3 g/d (48).

Figure 4.2. Factors associated with household salt consumption among women in the pilot survey from mixed effect ANOVA analyses: CoMIT Project

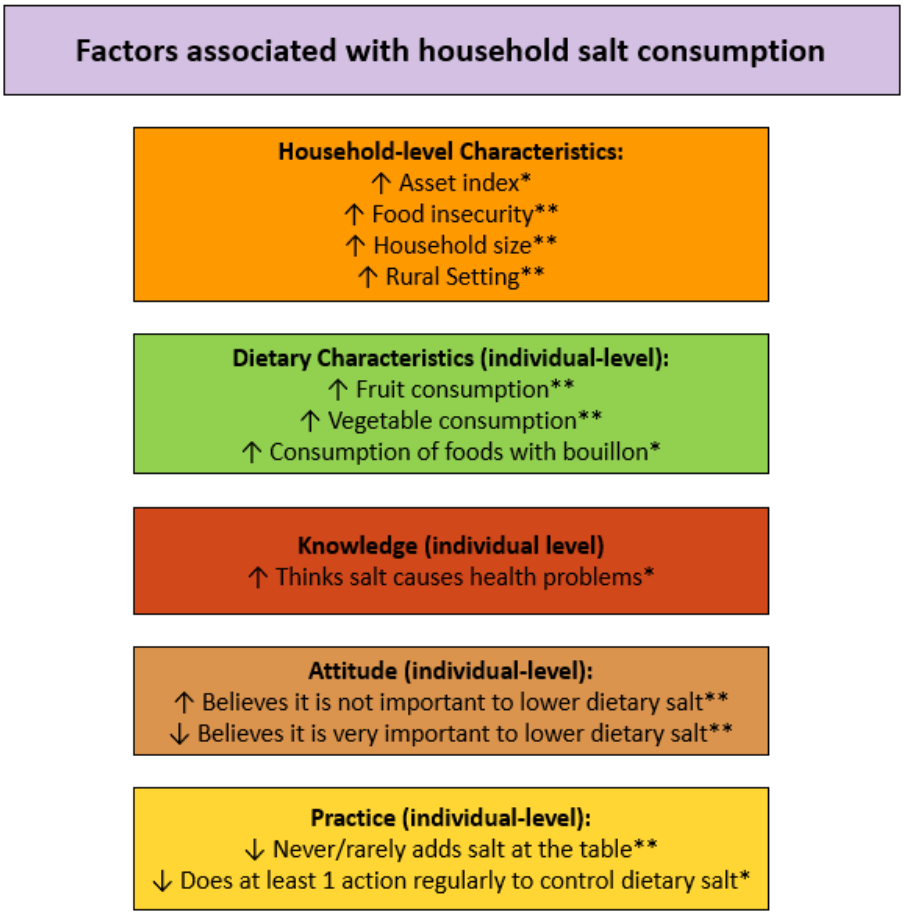


Figure 4.2 Legend: *p<0.1; **p<0.05. Factors included are those that were associated with both household discretionary salt ('table salt') and total salt (including salt from bouillon) consumption, except "Does at least 1 action regularly to control dietary salt" was only associated in model with household total salt. CoMIT, Condiment Micronutrient Innovation Trial.

Supplemental Material

Supplemental Methods

The main objective of the pilot survey was to evaluate the micronutrient status and assess intake and usage patterns of fortified foods among 3 priority populations: non-pregnant non-lactating women of reproductive age (WRA, 15-49 y), non-pregnant lactating women (LW, 4-18 months post-partum, 15-49 y), and preschool-age children ('children', 2-5 y).

Participants and eligibility

The 3 priory groups were chosen due to their greater physiological demands for micronutrients and greater risk of micronutrient deficiency (1), and because these same physiologic groups were planned for the RCT. For the pilot survey, potential participants were excluded if they suffered from a chronic severe medical condition, were ill (i.e., fever, diarrhea) ≤24-h prior to any research activities, or screened negative for COVID-19. Informed written (or thumbprint) consent was obtained from eligible participants, or their parent/guardian or caregiver (children and women 15-17 years of age and not married, divorced, separated, or living with a partner), with community members acting as witnesses for participants unable to read or write their informed consent. FGD participants were eligible if they provided written informed consent and screened negative for COVID-19. All participants received a bar of Key Soap as an incentive.

COVID-19 screening protocol

At the time of any initial interaction with participants (i.e., at recruitment or data collection if they occurred on different days), participants were screened for COVID-19 following GHS and WHO protocols current at the time. The protocol included checking each participant's temperature twice, and asking if they were aware of any close contact with a COVID-19 positive person (CPP) in the prior 2

weeks. Close contact was defined as: 1) having lived or stayed overnight with the CPP; 2) had intimate relations with the CPP; 3) took care of the CPP or the CPP took care of the participant; 4) stayed within 2 meters of the CPP for more than 10 minutes with the CPP not wearing a mask; 5) were exposed to direct contact with body fluids of the CPP) (2). During all research activities, fieldworks, participants, and any accompanying family members were required to sanitize their hands and wear mask (adults only) that completely covered their nose and mouth. Masks were provided to anyone in need.

Sampling and recruitment

Within the 2 districts, clusters (e.g., villages, towns, communities) were selected through consultation with district maps. Within the selected clusters, fieldworkers identified households with potential participants through a random walk method with door-to-door recruitment starting from a random central location. Selected households in which 1 or more members of the 3 physiological groups resided were eligible for recruitment, or one eligible individual was randomly chosen based on a Kish Table if a household contained more than 1 eligible participant per physiologic group. Recruitment of FGD participants followed the same sampling protocol except that only 1 group (WRA, men, or women >49 y) was recruited per household.

For the pilot survey, sample size was determined based on the main outcome of micronutrient status, with an assumed estimated prevalence of micronutrient deficiency of 50% for any one micronutrient included in the proposed multiple micronutrient fortified bouillon cubes (iron, folic acid, zinc, vitamin A, and vitamin B12). Per this calculation, 250 participants per physiological group allowed for analysis precision up to ±7%, including 20% potential loss to attrition.

Data collection procedures – Quantitative

Questionnaires

In addition to the household questionnaire, the following 2 questionnaires were administered to participating WRA and lactating women: 1) The World Health Organization (WHO) STEPwise Approach Surveillance (STEPS) Instrument for Noncommunicable Disease Risk Factor Surveillance (version 3.2) asked participants how often they added salt during cooking or at the table, frequency of consumption of processed foods, perceptions about salt use and health, and a limited health history that included history of raised blood pressure, heart attack, or stroke; this questionnaire was modified to also ask about the frequency of consumption of salty snacks, such as salty crisps or salty fried foods, in number of days per week and number of servings per day (3). 2) A Knowledge, Attitudes, and Practices (KAP) questionnaire was developed specifically for the pilot survey that gathered data on usage and consumption patterns related to bouillon, salt, and *dawadawa* (a local condiment made from fermented locust beans and used to flavor many common dishes (4)). In addition to WRA and lactating women, the KAP questionnaire was administered to caregivers of participating children if the caregiver herself was not a participant.

Anthropometry

Anthropometric measurements (height and weight) were completed in triplicate (WRA and children only) by trained and standardized anthropometrists on equipment calibrated daily. Height was measured by Seca® stadiometers to the nearest 0.1 cm; children were measured standing as all were at least 2 years of age (5). Weight was measured to the nearest 0.1 kg with battery-operated Seca® scales. Women were weighed without shoes, headwear and outer garments; children were weighed nude or lightly clothed.

Blood pressure

Blood pressure was measured in triplicate with measures one minute apart among WRA and children using a Riester® RBP-100 automatic portable upper-arm blood pressure monitor. To decrease

measurement error, participants were required to sit quietly for 5 minutes prior to the first measurement and during all measurements, to sit with a straight back with arms and legs uncrossed, and to not speak. The circumference of participants' upper arms was measured to ensure selection of the appropriate blood pressure cuff size (6).

Urine sample collection and processing

One spot urine sample was collected from WRA to measure urinary sodium, potassium, and creatinine. Upon receipt of urine samples, fieldworkers placed the samples in a cooler with cold packs, then transported them to the study laboratory for same-day processing. Urine (1.5 mL) was aliquoted into microtubes and stored at -20°C until the frozen samples were transported to the University of California, San Francisco, Children's Hospital Oakland Research Institute for analysis.

Urine laboratory analysis

Urinary sodium and potassium concentrations were determined by inductively-coupled plasma spectrometry (ICP-OES). Urine samples were thawed, vortexed, and 0.5ml was transferred into trace metal-free tubes. Urine samples were then dissolved into 0.25ml OmniTrace 70% HNO3 and digested overnight at 60°C. The acid lysates were then diluted to 5% HNO3 with OmniTrace water before analysis with an Agilent 5100 SVDV ICP-OES calibrated with National Institute of Standards and Technology (NIST)–traceable elemental standards and routinely validated with Seronorm Trace Element Serum Levels 1 and 2. A random selection of samples (n=24, 10%) were run as technical replicates at the beginning, middle, and end of the analysis period (coefficient of variation for sodium: 7.9%; potassium: 3.2%). Urine creatinine was measured with the Cayman creatinine colorimetric assay kit (#500701). The urine samples were diluted 20-fold and analyzed in duplicate (coefficient of variation: -4.8%). High and low controls were included on each plate, and values were within reported ranges. The relative difference for all analytes was <1%.

Data collection procedures - Qualitative

FGD guide development

The semi-structured FGD guide was developed with in-country collaborators and fieldworkers with consideration of the study setting, context, and culture (see **Supplemental Appendix**). Fieldworkers site-translated the guide into Dagbani and held group discussions to agree upon appropriate translations. The guide was piloted in the field, after which it was revised and refined before initiating the study FGDs.

Additional FGD procedures

Eligible FGD participants completed a demographic questionnaire at the time of recruitment. On the day of the FGD, participants were screened for COVID-19 and participated in the FGD. Following the FGD, participants completed a 'Willingness-to-Pay' activity that assessed how much participants might be willing to pay for a new bouillon product: a multiple-micronutrient fortified bouillon cube.

Data analysis

Quantitative analyses

Among WRA, Body Mass Index (BMI) categories were defined as underweight (BMI <18.5 kg/m^2), normal weight (BMI ≥18.5-24.9 kg/m^2), overweight (BMI ≥25.0-29.9 kg/m^2), or obesity (BMI ≥30.0 kg/m^2) (5). Among children, BMI for age z-score (BAZ) categories were defined as underweight (BAZ <-2 SD), normal weight (BAZ -2 ≥ SD ≤ 2), or at risk of overweight (BAZ >2 SD). Also among children, stunting was defined as height-for-age z-score <-2 SD, and wasting as weight-for-height z-score <-2 SD. Anthropometric z-scores were calculated according to WHO standard (7).

Among WRA, elevated blood pressure and hypertension were defined according to three organizations to capture a broader picture of hypertension risk: the American Heart Association (8), the

International Society of Hypertension (9), and the WHO (10). For children, 'at risk' blood pressure thresholds were defined by American Academy of Pediatrics (11).

Definitions of normal, elevated, and hypertension thresholds for WRA and children:

Supplemental Table 4.1. Blood pressure and hypertension guidelines for women of reproductive age (15-49 y)

Organization	Normal blood pressure (mmHg)	Elevated blood pressure (mmHg)	Hypertension (mmHg)
American Heart Association	SBP <120 and DBP <80	SBP 120-129 and DBP <80	SBP ≥130 or DBP ≥80
International Society of Hypertension	SBP <130 and DBP <85	SBP 130-139 and/or DBP 85-89	SPB ≥140 and/or DBP ≥90
World Health Organization	SBP <120 and DBP <80		SPB ≥140 and/or DBP ≥90

Supplemental Table 4.1 Footnotes: International Society of Hypertension classifies 'Elevated blood pressure' as 'High-normal blood pressure'. References: American Heart Association (8); International Society of Hypertension (9); World Health Organization (10). DBP, diastolic blood pressure; SBP, systolic blood pressure.

Supplemental Table 4.2. 'At risk' blood pressure guidelines for preschool-aged children (2-5 y)

Age, y	Males	Females
2	SBP 100 or DBP 55-57	SBP 101 or DBP 58-59
3	SBP 101 or DBP 58-60	SBP 102 or DBP 60-61
4	SBP 102 or DBP 60-62	SBP 103 or DBP 62-63
5	SBP 103 or DBP 63-65	SBP 104 or DBP 64-66

Supplemental Table 4.2 Footnotes: 'At risk' refers to the screening threshold at which children aged 2-5 y should undergo further evaluation for elevated blood pressure by a qualified professional, according to the American Academy of Pediatrics (11). DPB, diastolic blood pressure; SBP, systolic blood pressure.

Adult Male Equivalent (AME) method

The AME method assigns an adjustment factor (AME unit) to each member of a household based on sex- and age-specific energy expenditure and energy requirements, where males 19-30 y have an AME value of 1.0 and are the reference category (i.e., AME=1 for adult males aged 19-30 y, AME=0.7 for adult females aged 19-30 y, and AME=0.5 for children). To estimate individual daily consumption of discretionary salt, we 1) calculated a household AME value by summing all AME units from all household members; 2) calculated the individual AME fraction attributed to WRA and children by dividing each

individual AME value by the household AME value; and 3) calculated the individual daily salt consumption by multiplying the individual AME fraction by the household discretionary salt consumption (g/d) (12). This process was repeated for estimating individual consumption of total salt with an additional step of multiplying the final answer by 55%. Final estimates for WRA and children are reported as g/d/AME.

Estimating WRA daily total salt consumption from spot urine samples

From spot urine samples (WRA only), averages of replicate urinary sodium, creatinine, and potassium values were calculated with 1 value per participant included for analysis. The distributions of each analyte were examined and all observations retained. To estimate daily total salt consumption from spot urine samples, we first used the INTERSALT equation to predict 24-h urinary sodium excretion (13):

$$23 \{5.07 + [0.34 * \text{spot Sodium (mmol/L)}] - [2.16 * \text{spot Creatinine (mmol/L)}] - [0.09 * \text{spot Potassium (mmol/L)}] + [2.39 * \text{BMI (kg/m}^2\text{)}] + [2.35 \times \text{age (years)}] - [0.03 * \text{age}^2 \text{ (years)}]\}$$

To estimate daily total salt consumption (g/d), we then divided the predicted 24-h urinary sodium excretion (mg/d) by 390 as there are 390 mg sodium in 1 g sodium chloride ('table salt') (14).

Exploratory quantitative analyses

A second set of multivariable mixed-effects models was built to test associations with groups of KAP-specific predictors. Selected predictors were put into three distinct groups (knowledge, attitudes, or practices) that were determined based on the intent of the question (15). Each of the three groups of KAP predictors was tested separately in minimally-adjusted, mixed-effects models with the two outcome variables. Marginally significant ($p<0.1$) KAP predictors were then included in multivariable, mixed-effects models.

Supplemental Tables

Supplemental Table 4.3. List of potential predictors of household salt consumption

Predictor short name	Full description of predictor
Household-level predictors	
Size	Household size (number of members)
District	District of Tolon or Kumbungu
Setting	Urban or rural setting
Participant type	Woman of reproductive age (15-49 y, WRA) or lactating woman (LW)
Asset quintiles	Asset quintiles (1st lowest, 5th highest)
Food insecurity	Food insecurity status: None, mild, moderate, severe
Education level	Highest level of education completed by the household head (none, primary school, secondary+)
Cons. of DD	Household consumption of dawadawa
Individual-level predictors	*[among WRA and LW only]*
Age	Age in years of WRA or LW
Hx of heart dis.	History of heart disease (yes/no)
Hypertension	Current hypertension (yes/no)
BMI	BMI categories: underweight, normal weight, overweight, obesity
Vegetable cons.	Frequency of consuming vegetables, days per week
Fruit cons.	Frequency of consuming fruit, days per week
Salty snacks	Frequency of consuming salty snacks such as crisps, days per week
Bouillon cons.	Frequency of consuming foods prepared with bouillon, days per week
Knowledge predictors	*[individual-level: WRA and LW]*
Qty salt	How much salt do you think you consume? (too little, just right, too much)
Salt as a problem	Do you think that too much salt in your diet could cause a health problem? (yes/no)
Attitude predictors	*[individual-level: WRA and LW]*
Lower salt	How important to you is lowering salt in your diet? (not important, somewhat important, very important)
Benefits of salt	Do you think that having salt in your diet is good, bad, or has no effect?
Benefits of DD	Do you think that having dawadawa in your diet is good, bad, or has no effect?
Benefits of bouillon	Do you think having bouillon in your diet is good, bad, or has no effect?
Practice predictors	*[individual-level: WRA and LW]*
Add salt at table	Do you add salt to your food at the table always, sometimes, or never?
Add salt in cooking	Do you add salt to your food while cooking always, sometimes, or never?
Eat proc. foods	Do you eat salty processed foods always, sometimes or never?
Cntl salt intake	Do you do at least 1 action to control your salt intake? (yes/no)
Social desirability	*[individual-level: WRA and LW]*
SD1	Social desirability 1: Do you occasionally give up doing something because you don't think you have the ability?
SD2	Social desirability 2: Do you occasionally feel like not listening to people even if you know they are right?
SD3	Social desirability 3: Are you sometimes irritated/annoyed by people who ask you to do something for them?
SD4	Social desirability 4: Are you always courteous, even to people who are disagreeable/not pleasant?
SD5	Social desirability 5: When you make a mistake, are you always willing to admit it?

Supplemental Table 4.3 Footnotes: All analyses were completed with categorical predictors. See main text and supplemental methods for references and further descriptions of predictors. Social desirability questions taken from the Marlowe-Crowne social desirability scale and are yes/no responses (16). All predictors were selected *a priori* according to our statistical analysis plan.

Supplemental Table 4.4. Intercoder reliability score (ICR) calculated as Cohen's Kappa and percent agreement

Segment	**Average ICR**	**Percent Agreement**
1. Household makeup; household members; meal sharing	0.94	95.9%
2. Cooking decisions and cooking duties; sharing of cooking during	0.97	97.8%
3. Bouillon preferences and habits	0.94	96.1%
4. Bouillon usage and consumption	0.92	95.0%
5. Other flavorings and seasonings, including salt and *dawadawa*	0.93	96.4%
6. Bouillon purchasing	0.95	96.8%
7. Health and nutritional problems	1.00	100%
8. Description of fortified bouillon cubes	0.88	91.4%
9. Knowledge and usage of fortified bouillon cubes	1.00	100%
10. Beliefs about fortified bouillon cubes	0.91	95.1%
11. Perceptions of salt, *dawadawa*, and MSG	1.00	100%
Average total	**0.95**	**96.5%**

Supplemental Table 4.4 Footnotes: ICR score calculated in Stata 16. Average ICR represents the average final ICR of n=7 focus group discussion (FGD) transcripts that were independently coded by 2 coders. See FGD guide in Supplemental Appendix for full descriptions of FGD questions (segments).

Supplemental Table 4.5. Knowledge, attitude, and practice factors associated with household daily discretionary salt and total salt consumption among households who participated in the pilot survey: CoMIT Project

		Outcome: Hh discretionary salt consumption, g/d		Outcome: Hh total salt consumption, g/d	
Variable	Categories	KAP group minimally-adjusted model[2]	KAP multivariable model[*]	KAP group minimally-adjusted model[*]	KAP multivariable model[*]
		β (95% CI)	*β* (95% CI)	*β* (95% CI)	*β* (95% CI)
Knowledge group					
Quantity of salt consumed	Just the right amount	ref.		ref.	
	Too much	0.6 (-21.7, 22.8)		1.3 (-21.5, 24.2)	
	Too little	3.1 (-10.3, 16.5)		1.6 (-12.1, 15.4)	
Think dietary salt can cause health problems	No	ref.	ref.	ref.	ref.
	Yes	**21.8 (4.1, 39.4)**	**27.1 (7.2, 47.0)**	**21.9 (3.6, 40.2)**	**26.2 (5.7, 46.7)**
	Don't know	17.2 (-4.7, 39.2)	19.8 (-5.2, 44.8)	17.3 (-5.5, 40.1)	19.2 (-6.7, 45.1)
Attitude group					
Importance of lowering salt in the diet	Somewhat important	ref.	ref.	ref.	ref.
	Very important	-10.5 (-22.9, 1.8)	-12.4 (-25.2, 0.4)	-10.4 (-23.2, 2.3)	-12.2 (-25.4, 1.0)
	Not at all important	**37.0 (21.4, 52.5)**	**37.8 (21.4, 54.1)**	**37.6 (21.6, 53.6)**	**38.7 (21.9, 55.6)**
Perception of dietary salt	No effect	ref.	ref.	ref.	ref.
	Good	**21.3 (5.4, 37.1)**	**25.3 (9.2, 41.5)**	**21.7 (5.3, 38.1)**	**25.7 (9.0, 42.4)**
	Bad	3.6 (-12.4, 19.6)	8.7 (-7.8, 25.1)	2.4 (-14.1, 18.8)	7.4 (-9.5, 24.3)
Perception of dietary dawadawa	No effect	ref.		ref.	
	Good	-1.9 (-23.9, 20.1)		-2.4 (-25.1, 20.2)	
	Bad	No obs.		No obs.	
Perception of dietary bouillon	No effect	ref.	ref.	ref.	ref.
	Good	**-23.8 (-43.1, -4.5)**	-15.1 (-34.4, 4.1)	**-25.4 (-45.2, -5.5)**	-16.8 (-36.6, 3.0)
	Bad	-17.9 (-38.9, 3.1)	-12.3 (-32.9, 8.4)	-15.2 (-37.0, 6.6)	-10.0 (-31.5, 11.5)

Practice group					
Add salt at the table	Always/often/ sometimes	ref.	ref.	ref.	ref.
	Rarely/never	**-16.1 (-26.5, -5.7)**	-5.5 (-17.5, 6.4)	**-15.8 (-26.5, -5.1)**	-5.0 (-17.3, 7.3)
Add salt during cooking	Always/often/ sometimes	ref.		ref.	
	Rarely/never	1.3 (-22.9, 25.4)		2.3 (-22.5, 27.0)	
Eat processed foods	Always/often/ sometimes	ref.		ref.	
	Rarely/never	4.4 (-5.6, 14.5)		6.0 (-4.4, 16.3)	
Do at least 1 action regularly to control dietary salt	No	ref.	ref.	ref.	ref.
	Yes	**-13.1 (-25.8, -0.4)**	**-19.2 (-36.5, -2.0)**	**-14.6 (-27.6, -1.5)**	**-19.4 (-37.3, -1.6)**

Supplemental Table 4.5 Footnotes: 'Discretionary salt' defined as 'table salt' and 'total salt' defined as discretionary salt plus the proportion of salt from bouillon, which was assumed to be 55% salt (17). Estimates of Hh discretionary salt and total salt consumption calculated from purchase data using the Fortification Assessment Coverage Toolkit (18). Total households with salt data: n=363; total households with discretionary salt data: n=360. Individual-level factors include data from non-pregnant, non-lactating women of reproductive age (15-49 years, n=239 salt only; n=237 discretionary salt) and non-pregnant lactating women (n=238 salt only; n=235 discretionary salt). KAP predictors were selected *a priori* according to our analysis plan. KAP questions were selected from the World Health Organization (WHO) STEPwise Approach Surveillance (STEPS) Instrument for Noncommunicable Disease Risk Factor Surveillance (version 3.2) (3) and from a KAP questionnaire developed specifically for the pilot survey. The category of KAP attributed to the question was based on the intent of the question (15). CoMIT, Condiment Micronutrient Innovation Trial; Hh, household; KAP, knowledge, attitude, practice; ref., reference group.

* KAP group regression models were linear mixed effects models where each group of predictors that corresponded to Knowledge, Attitudes, or Practices was first tested separately in minimally adjusted model that controlled for Hh size, district (Tolon/Kumbungu), area (urban/rural), and participant type (woman of reproductive age or lactating woman) as fixed effects, and cluster as a random effect. Then, marginally significant KAP predictors ($p<0.1$, **bolded**) were included in a multivariable model ($p<0.5$) that controlled for the same variables as the minimally adjusted models.

Supplemental Table 4.6. Estimated per capita discretionary salt consumption (g/d) by asset quintiles and food insecurity status among households participating in the pilot survey: CoMIT Project

	Asset quintiles									
	n	**1st**	***n***	**2nd**	***n***	**3rd**	***n***	**4th**	***n***	**5th**
None or Mild FI	*12*	5.3 (4.7)	*7*	5.3 (4.1)	*14*	10.8 (10.4)	*22*	5.4 (3.9)	*28*	4.7 (3.0)
Moderate FI	*44*	6.0 (4.6)	*48*	6.5 (5.7)	*32*	6.7 (9.5)	*38*	5.0 (5.5)	*29*	6.1 (4.9)
Severe FI	*18*	9.2 (8.1)	*19*	13.2 (15.1)	*25*	9.6 (7.7)	*16*	10.2 (9.9)	*11*	7.2 (7.5)
Total n	*74*		*74*		*71*		*76*		*68*	

Supplemental Table 4.6 Footnotes: Per capita discretionary salt consumption presented as mean (SD). Household food insecurity status based on a calculated food insecurity score. 3rd asset quintile and None/Mild Food Insecurity contained an outlier (outlier defined as >60% larger than the value at the 99th percentile; sensitivity analyses examining ANOVA associations between food insecurity or asset quintiles and household discretionary salt consumption with the outlier truncated to the 99th percentile did not change the direction or strength of the associations. CoMIT, Condiment Micronutrient Innovation Trial; FI, food insecurity.

Supplemental Table 4.7. Salt quantity (g) last purchased by asset quintiles among households participating in the pilot survey: CoMIT Project

	Asset Quintiles					
	1st	**2nd**	**3rd**	**4th**	**5th**	**Total**
	n (%)	*n (%)*	*n (%)*	*n (%)*	*n (%)*	*n (%)*
Last purchased 0-299 g salt	35 (47.3)	29 (39.2)	20 (28.2)	25 (32.9)	19 (28.0)	128 (25.3)
Last purchased 300-999 g salt	27 (36.5)	22 (29.7)	21 (29.6)	26 (34.2)	25 (36.8)	121 (33.3)
Last purchased 1000+ g salt	12 (16.2)	23 (31.1)	30 (42.3)	25 (32.9)	24 (35.3)	114 (31.4)
Total	74 (100)	74 (100)	71 (100)	76 (100)	68 (100)	363 (100)

Supplemental Table 4.7 Footnote: CoMIT, Condiment Micronutrient Innovation Trial.

Supplemental Table 4.8. Characteristics of focus group participants: CoMIT Project

		Total	**WRA 15-49 yrs**	**Women >49 yrs**	**Men**
		n (%)	n (%)	n (%)	n (%)
Total	n	114	56 (48)	29 (25)	29 (25)
Age, y		38 (16,79)	31 (16,48)	60 (50, 79)	35 (20,74)
Household size	# household members	12 (4, 45)	13 (4, 40)	12 (4, 36)	15 (5, 45)
Tribe	Mole-Dagbani	111 (97)	55 (48)	29 (25)	27 (24)
	Other	3 (3)	1 (1)	0 (0)	2 (2)
Education	None	82 (72)	40 (35)	27 (24)	15 (13)
	Primary	14 (12)	8 (7)	2 (2)	4 (3)
	Secondary	15 (13)	8 (7)	0 (0)	7 (6)
	More than secondary	2 (2)	0 (0)	0 (0)	2 (2)
	Refused	1 (1)	0 (0)	0 (0)	1 (1)
Marital Status	Single	16 (14)	9 (8)	0 (0)	7 (6)
	Married	81 (71)	47 (41)	12 (11)	22 (19)
	Divorced	1 (1)	0 (0)	1 (1)	0 (0)
	Widowed	16 (14)	0 (0)	16 (14)	0 (0)
Employment Status	Government employee	2 (1)	0 (0)	(0)0 (0)	2 (1)
	Non-government employee	0 (0)	0 (0)	0 (0)	0 (0)
	Self-employed	78 (68)	37 (32)	21 (18)	20 (17)
	Non-paid employment	1 (1)	0 (0)	1 (1)	0 (0)
	Student	7 (6)	4 (3)	1 (1)	2 (2)
	Homemaker	5 (4)	5 (4)	0 (0)	0 (0)
	Retired	0 (0)	0 (0)	0 (0)	0 (0)
	Unemployed (able to work)	13 (11)	8 (7)	3 (3)	2 (2)
	Unemployed (unable to work)	10 (8)	4 (3)	4 (3)	2 (2)
	Refused	1 (1)	0 (0)	0 (0)	1 (1)

Supplemental Table 4.8 Footnotes: There were a total of 20 focus groups, with 5-6 participants per focus group. Age and household size are presented as median (range). CoMIT, Condiment Micronutrient Innovation Trial.

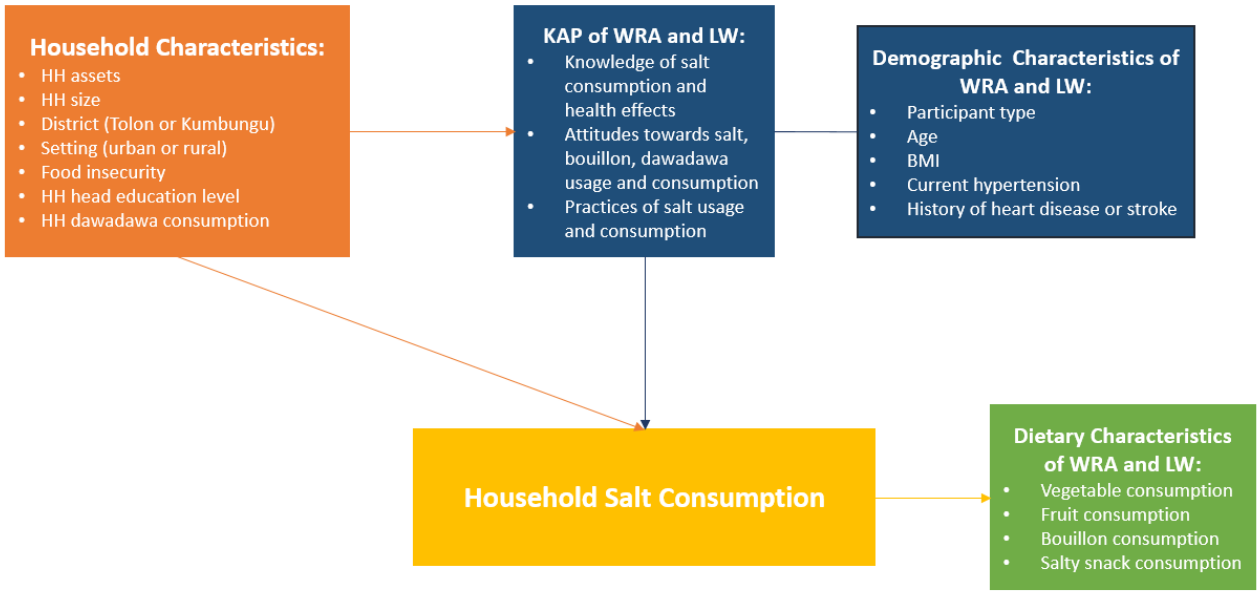
Supplemental Table 4.9. Focus group participant responses to FGD guide questions regarding health problems

FGD Guide Question	FGD Participant Response	WRA	Women >49 y	Men
		n (%)	*n (%)*	*n (%)*
1. Common health problems within community	High blood pressure or stroke	44 (79)	16 (55)	21 (72)
2. Health problems related to nutrition	High blood pressure or stroke	18 (32)	2 (7)	17 (59)
3. How to address health problems	Change dietary patterns	22 (40)[1]	2 (7)	17 (59)

Supplemental Table 4.9 Footnotes: Some of the WRA that responded to question 3 (How to address health problems?) did not respond to question 2 (What health problems were related to nutrition?). Total FGD WRA participants: n=46; women >49 years: n=29, men: n=29. FGD, Focus Group Discussion; WRA, women of reproductive age (15-49 y).

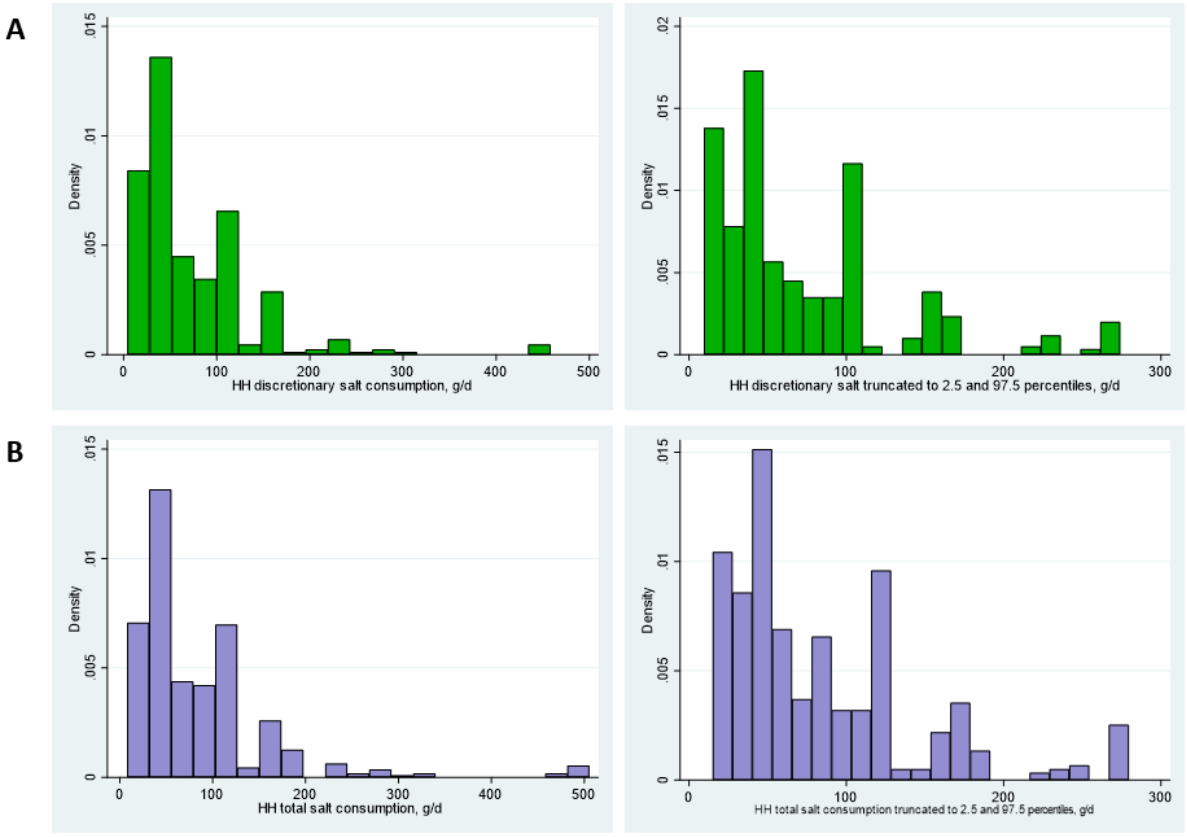
Supplemental Figures

Supplemental Figure 4.1. Conceptual model of hypothesized relationships influencing household salt consumption from participants in the pilot survey: CoMIT Project



Supplemental Figure 4.1 Legend: Variables depicted in the in conceptual model are those that were available in the dataset. 'Dawadawa' is a local condiment made of fermented locust beans used to flavor foods. CoMIT, Condiment Micronutrient Innovation Trial; HH, Household; KAP, Knowledge, Attitudes, and Practices; LW, Lactating women; WRA, women of reproductive age.

Supplemental Figure 4.2. Distributions of household discretionary salt and total salt consumption (g/d) before and after truncating values below 2.5 percentile and above 97.5 percentile among participating households in the pilot survey: CoMIT Project



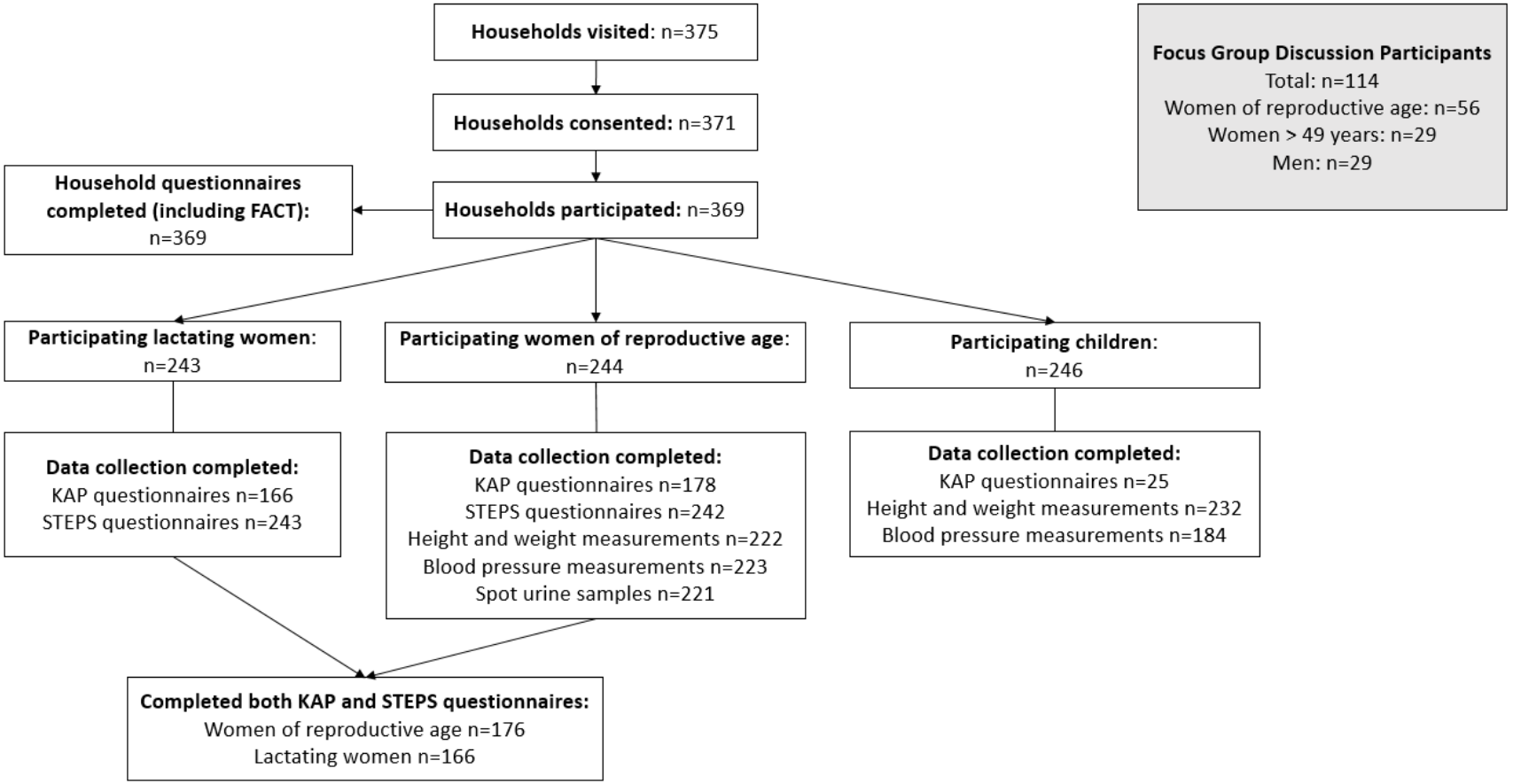
Legend for Supplemental Figure 4.2: Data is from reported household purchase data collected using the FACT tool (18). Values below the 2.5 percentile were truncated to the value at the 2.5 percentile; values above the 97.5 percentile were truncated to the value at the 97.5 percentile. A: Discretionary salt ('table salt') distribution with all values (left) and after truncation (right). B: Total salt (discretionary salt + salt from bouillon) distribution with all values (left) and after truncation (right). G/d, grams per day; HH, household; CoMIT, Condiment Micronutrient Innovation Trial.

Supplemental Figure 4.3. Correlations between potential predictors of household salt consumption, including social desirability (SD) variables, among participants in the pilot survey: CoMIT Project

	Lower salt	Add salt at table	Add salt in cooking	Eat proc. Food	Qty salt	Benefits of salt	Salt as a problem	Benefits of DD	Benefits of bouillon	Fruit cons.	Vegetable cons.	Bouillon cons.	Salty snacks	Cntl salt intake	Hx. of heart disease	Hypertension	BMI	Age	Assets	Food insec.	Education	HH DD
Lower salt	1.00																					
Add salt at table	-0.31	1.00																				
Add salt in cooking	0.22	-0.03	1.00																			
Eat proc. Food	-0.15	0.19	0.09	1.00																		
Qty salt	0.10	0.08	0.09	0.00	1.00																	
Benefits of salt	0.11	-0.09	0.16	-0.29	0.30	1.00																
Salt as a problem	-0.34	0.16	-0.09	-0.14	-0.17	-0.16	1.00															
Benefits of DD	0.28	-0.39	0.94	0.05	0.26	0.57	-0.64	1.00														
Benefits of bouillon	0.28	0.03	0.29	-0.03	-0.01	0.42	-0.27	0.21	1.00													
Fruit cons.	0.24	-0.31	-0.04	-0.11	-0.05	0.08	-0.04	0.56	0.19	1.00												
Vegetable cons.	0.02	-0.17	0.31	-0.24	-0.19	-0.21	0.24	-0.52	0.00	0.12	1.00											
Bouillon cons.	0.11	-0.50	.	0.18	0.15	-0.23	-0.18	-0.94	-0.10	0.00	0.48	1.00										
Salty snacks	0.38	-0.18	0.10	-0.14	-0.16	0.02	0.07	-0.14	0.03	-0.02	0.08	0.32	1.00									
Cntl salt intake	0.44	-0.43	.	-0.01	0.35	0.73	-0.22	0.69	0.14	0.30	-0.51	-0.02	0.24	1.00								
Hx. of heart disease	-0.02	0.09	0.36	0.42	-0.10	0.07	0.19	.	-0.01	-0.23	-0.19	.	0.21	0.95	1.00							
Hypertension	-0.06	0.10	0.51	0.22	-0.23	-0.20	-0.08	-0.06	-0.01	0.08	0.15	0.94	-0.18	-0.05	0.29	1.00						
BMI	0.07	0.08	-0.01	-0.10	0.11	0.15	-0.06	0.31	0.13	0.02	0.14	0.09	0.10	0.32	0.18	0.49	1.00					
Age	-0.10	0.33	-0.18	0.16	0.12	-0.11	-0.24	0.10	-0.01	0.01	-0.04	0.04	-0.06	-0.22	0.14	.	0.24	1.00				
Assets	0.10	-0.16	-0.10	-0.10	0.02	0.14	-0.09	0.42	0.13	0.32	0.19	-0.21	-0.10	0.12	-0.29	0.06	0.14	-0.07	1.00			
Food insec.	0.30	-0.08	-0.07	-0.50	0.11	0.05	-0.06	-0.05	0.11	0.09	0.10	0.19	0.23	0.11	-0.08	-0.14	0.05	0.00	-0.08	1.00		
Education	0.09	0.04	0.15	-0.23	0.14	0.12	-0.14	0.37	0.24	0.16	-0.01	0.05	-0.01	0.17	-0.03	-0.09	0.41	0.00	0.36	0.23	1.00	
HH DD	0.12	0.12	-0.16	-0.27	0.32	0.35	-0.11	0.30	-0.05	0.12	-0.31	-0.21	0.07	0.39	0.08	-0.02	0.14	0.18	-0.10	0.23	-0.01	1.00
SD1	-0.08	-0.17	-0.18	0.27	0.02	0.49	0.12	.	0.01	-0.01	-0.42	-0.16	-0.21	0.77	0.09	-0.28	-0.10	-0.14	-0.04	-0.36	-0.21	0.03
SD2	0.07	-0.54	0.37	0.10	0.10	0.33	-0.17	0.55	0.05	0.21	-0.22	-0.05	0.21	.	0.30	-0.27	-0.05	-0.31	0.15	-0.03	0.08	0.02
SD3	0.19	-0.61	0.37	0.10	0.18	0.26	-0.16	0.55	0.08	0.25	-0.04	0.20	0.12	0.76	0.30	0.04	0.03	-0.17	0.09	-0.03	-0.20	-0.07
SD4	-0.19	-0.21	-0.04	0.25	-0.38	-0.28	-0.14	.	0.10	0.03	0.43	0.30	0.15	-0.96	0.95	0.95	-0.05	0.02	0.20	-0.10	-0.08	-0.19
SD5	0.01	0.11	.	0.19	-0.53	-0.58	-0.19	.	0.02	0.23	0.47	0.22	0.03	.	.	.	-0.05	-0.03	0.26	0.21	-0.24	-0.40

Supplemental Figure 4.3 Legend: Correlation analysis was conducted in Stata 16 using the 'polychoric' command for correlations among ordinal variables. Correlations were considered weak at <0.15 (no color), moderate between 0.2 and 0.4 (orange), or strong at >0.5 (blue). Social desirability (SD) variables were from the Marlowe-Crowne social desirability scale (16). See Supplemental Table 3 for variable definitions. "." Indicates that a correlation estimate could not be generated due to missing values. CoMIT, Condiment Micronutrient Innovation Trial.

Supplemental Figure 4.4. Flow chart of participating households, participants, and data collected in the pilot survey and focus group discussions: CoMIT Project



Legend for Supplemental Figure 4: In the pilot survey, one KAP questionnaire was completed per household (n=369 total), including n=25 KAP questionnaires completed by caregivers of participating children where the caregiver herself was not a participant. Each participating woman of reproductive age and lactating woman completed the STEPS questionnaire (n=485 total). Abbreviations: CoMIT, Condiment Micronutrient Innovation Trial; FACT, Fortification Assessment Coverage Tool; KAP, Knowledge, Attitudes and Practices; STEPS, WHO noncommunicable disease surveillance tool.

Supplemental Appendix Focus Group Discussion (FGD) Guide

1. **A household is defined a group of people who recognize the same head of household and who live together and share living expenses and meals. If a man has two or more wives and they and their children live and eat together (even if they eat together only sometimes), they form one household. If the wives and their children do not live together in the same compound and always eat separately, they will form more than one household. Members are included if they have lived with the household at least 6 out of the past 12 months. The following exceptions are always household members: the head of household, any child under 9 months of age, and those who intend to stay in the household for at least 6 months.**
    a. Do you think this definition correctly describes a household? Why or why not?
    b. In your home, who usually eats together at meals?
    c. How do you determine if someone is <u>not</u> a household member?

2. **Which household member is the main household cook?**
    a. If a man has multiple wives, how are cooking duties shared among wives?
    b. Who decides what dishes the cook should make in your household?
    c. How is food shared with other members of a compound, such as co-wives or other family members not living under the same roof?

3. **Now, let's talk more specifically about bouillon. Could someone please describe for me what bouillon is?**
    a. How often does your household cook with bouillon?
    b. Does anyone live in a household that does not cook with bouillon?
    c. What are the different types of bouillon products your household cooks with?
    d. With which type of bouillon does your household cook with MOST OFTEN?
    e. Thinking of the type of bouillon you just mentioned, why does your household choose to cook with this type most often?
    f. If this type or brand of bouillon isn't available, what other types or brands of bouillon would you choose?
    g. During different times of the year, such as during the lean season, do you change how you use bouillon?
    h. Does your household ever use bouillon for purposes other than cooking?

4. **Now let's talk about bouillon cubes specifically. What are some typical dishes that your household makes with bouillon cubes, that is, dishes your household makes every day or every week?**
    a. Why do you (or the cook of your household) add bouillon cubes to these dishes?
    b. How do you (or the cook of your household) decide how many bouillon cubes to add to these dishes?
    c. Do you always put the entire bouillon cube into the cooking pot or do you save part of it for later use?
    d. If your household doesn't have any bouillon cubes to add to a dish that calls for bouillon cubes, what do you do?
    e. Why might your household not have any bouillon cubes on hand?

f. For what other reasons might you not add bouillon cubes to a dish?

5. **When you (or the cook of your household) add bouillon cubes to a dish, how often do you also add salt, dawa dawa, or other seasonings?**
    a. When you also add salt, dawa dawa, or other seasonings to a dish made with bouillon, which one is the main flavoring??
    b. What dishes do you make every day or every week that use salt, dawa dawa, or other seasonings instead of bouillon?
    c. What are some reasons you would add salt, dawa dawa, or other seasonings instead of bouillon to these dish?

6. **How often does your household typically purchase bouillon cubes?**
    a. What quantity of bouillon cubes does your household typically purchase at one time?
    b. Who in the household decides how often to purchase bouillon?
    c. Who in the household decides how much bouillon to purchase at one time?
    d. Are the purchased bouillon cubes typically shared between different households on the compound?
    e. If your household has less money one week than normal, does your household purchase more, less, or the same amount of bouillon cubes?

7. **What major health problems do you see in this community?**
    a. Which, if any, of these health problems is due to a nutritional problem?
    b. What do you think could be done to address these health and nutritional problems in your community?

8. **Now we are going to discuss fortified bouillon cubes. What does it mean to have a bouillon cube that is fortified?** *Before continuing with the discussion, assess the group and ensure that each participant understands what 'fortified' means.*

9. **What have you heard about fortified bouillon cubes?**
    a. If you had to use a fortified bouillon cube in all the dishes where you typically use bouillon cubes, what would be some of your concerns?

10. **If you and your household were consuming dishes made with fortified bouillon cubes, that is bouillon cubes fortified with nutrients like iron, zinc, vitamin A, folic acid, and B12, what, if any, do you think the effects would be on a person's body?**
    a. Who, if anyone, in your household might benefit from consuming fortified bouillon cubes?
    b. How might fortified bouillon cubes benefit your or members of your household?
    c. Why do you think fortified bouillon cubes might benefit you or your household members?
    d. Why might you not want to consume fortified bouillon cubes, or feed dishes with fortified bouillon cubes to your household?

11. **What, if any, impact do you think salt, MSG or dawa dawa has on your body? What impact does it have on the bodies of your children or other household members?**
    a. Why do you think these seasonings have these effects on your body and/or that of your children or other household members?

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