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Sana Syed Date

Determinants of Anemia among School-Aged Children
in Mexico, the United States and Colombia

By

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An abstract of
A thesis submitted to the Faculty of the
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Master of Science in Clinical Research

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Abstract

Determinants of Anemia among School-Aged Children
in Mexico, the United States and Colombia

By
Sana Syed

Background: Anemia affects half of the world's global population of pre-school children and a quarter of the world's school-aged children (SAC). Anemia is most commonly ascribed to iron deficiency (ID); however, other nutritional, infectious, and socio-economic determinants are often not concurrently measured. Data is lacking on reliable international estimates of anemia and its determinants in SAC. Such statistics are important to guide anemia prevention and treatment programs. Our study aims to measure, in three countries with varying anemia burdens, anemia prevalence in SAC and its association with anthropometric, biochemical, demographic and socio-economic determinants.

Methods: Data was obtained from three national cross-sectional surveys – 2005-6 Mexican National Nutrition Survey, 2010 Encuesta Nacional de Nutrición Situación Colombia and 2003-6 cycles of the US National Health and Nutrition Examination Survey. The prevalence of anemia and ID was determined. Associations were studied using survey regression methods with adjustment for inflammation, potential confounders and complex survey design effects.

Results: Data was analyzed for 3650 Mexican, 3543 US children and 8604 Colombian aged 5-14.99 years. Mexican data showed 60% females, 10% stunted, 1% wasted, 13% overweight, 4% obese. Prevalence of anemia was 12%; ID was 18%. US data showed 48% females, 15% black, 1% stunted, 1.2% wasted, 19% overweight, 5% obese. Prevalence of anemia was 0.5%; ID of 9% was assessed in children aged 5 years and 12 to 14.99yr. Colombian data showed 58% females, 12% black, 10% stunted, 2% wasted, 4% overweight, 0.5% obese. Prevalence of anemia was 4%; ID was 9%. In Mexico, anemia was associated with being overweight (OR 0.4, $p=0.007$). In the US, anemia was associated with age category 12.0-14.99y (OR 4.6, $p=0.03$) and being female (OR 3.4, $p=0.02$). In Colombia, anemia was associated with being black (OR1.8, $p<0.0001$), being in the poorest quintile (OR 1.8, $p<0.0001$), overweight (OR 0.5, $p<0.0001$) and with stunting (OR 1.5, $p<0.0001$).

Conclusion: There was low anemia prevalence in Mexico with extremely low prevalence of anemia in Colombia and the US. ID was associated with anemia with inconsistent associations with socioeconomic factors in very low prevalence countries.

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INTRODUCTION

Anemia affects approximately half of the global population of pre-school children and a quarter of the world's school-aged children (SAC) (1). Anemia in children is most often ascribed to iron deficiency; however, other nutritional, infectious, and socio-economic determinants are often not concurrently measured (2). Furthermore, there is also a lack of data on reliable international estimates of anemia and its determinants in SAC (3). Such information is important to guide anemia prevention and treatment programs.

When assessing the burden of anemia and its determinants, the effect of inflammation on hemoglobin (Hb) should be taken into consideration. Inflammation can cause changes in blood concentrations of acute-phase proteins (APP) (4) and thereby affect the assessment of some nutritional parameters and influence the prevalence estimates of a particular nutrient deficiency. The effect of inflammation (measured by APP namely C-reactive protein, [CRP] and α-1-acid glycoprotein, [AGP]) on Hb to assess for anemia in pre-school children has been previously reported (5). The children surveyed with elevated levels of CRP and AGP were noted to be significantly associated with a higher prevalence of anemia (66%) than those children surveyed with a normal CRP (40%).

To date, there is no expert group consensus on guidelines about how to account for markers of inflammation when presenting results on anemia prevalence. In our current study, we were interested in exploring the direction and magnitude of the relationship of anemia (as measured by Hb) and inflammation (as measured by CRP) in the SAC population. Our study included three countries (Mexico, the USA and Colombia) with

varying anemia burden. The study aimed to measure the prevalence of anemia in SAC and the association of anemia with an increase in CRP in each of the three countries. We also assessed the association of anemia with other known risk factors of anemia, including anthropometric indices of growth (including stunting, and overweight), micronutrient deficiencies (particularly iron, and vitamin A), demographic factors (like age, gender, and ethnicity) and socio-economic dynamics (as reflected in an asset index).

BACKGROUND

Anemia is a condition marked by a deficiency of red blood cells or of Hb in the blood, resulting in pallor and weariness. Anemia is characterized by reductions in Hb concentration, red-cell count, or packed-cell volume, with subsequent impairment in meeting the oxygen demands of tissues. The World Health Organization (WHO) has established thresholds to define anemia status by Hb concentration (Table 1) (2, 6). Hb concentration and thus anemia is affected by characteristics such as age, sex, and pregnancy status, as well as environmental factors such as smoking and altitude (6). Anemia is currently estimated to impact a quarter of the world's population (2). The 2010 Global Burden of Disease study (1) looking at the leading causes of global years lived with disability (YLDs) from 1990 to 2010 estimated that burden of anemia overall was very large at 68.2 million YLDs or almost a tenth (8.8%) of all YLDs worldwide. The most important contributor to this health loss was iron-deficiency anemia accounting for about 62% of anemia YLDs globally and is the third leading cause of all YLDs. Other main causes of anemia are: infectious diseases such as malaria, hookworm infections and schistosomiasis; deficiencies of other key micronutrients, including vitamin A, folate, and vitamin B12; or inherited conditions that affect red blood cells, such as thalassemia in addition to anemia of chronic disease (7). The prevalence of anemia varies significantly by gender and age with an estimated prevalence in the most vulnerable populations ranging from a high of 47% in pre-school children (PSC), 42% in pregnant women, 30% in non-pregnant women, and to a low of 25% in SAC (2). Across age groups, chronic anemia is associated with loss of productivity from impaired work capacity, cognitive impairment, and increased susceptibility to infection (8) – making it crucial to identify

the most effective population-level strategies to address this issue. Given that iron-deficiency is thought to be the main cause of anemia worldwide (9), most anemia interventions involve iron supplementation (10). Given the higher prevalence of anemia in PSC and pregnant women, most research has been focused on these two populations (2). There is a paucity of national level data estimates on the anemia status of SAC (3) making this age group a research priority. Furthermore, there is a need to refine the relative contribution of nutritional and other risk factors for anemia across different geographic settings to better guide national anemia reduction programs.

In assessing the burden of anemia and its determinants, the effect of inflammation on hemoglobin should be considered. This is because inflammation can cause changes in blood concentrations of acute-phase proteins (APP) (4) thereby affecting the assessment of some nutritional parameters and influencing the prevalence estimates of a particular nutrient deficiency. Inflammation can occur in response to infections, such as malaria and HIV, especially in developing countries (11). Since infection can be present along with micronutrient deficiencies, it is important to determine how the acute phase response of inflammation influences measurements of nutritional status. Inflammation is most commonly measured using two biomarkers: CRP and AGP. CRP levels increase within 10 hours (h) of the onset of acute inflammation and normalize rapidly (~1 week) (12). AGP levels begin to increase 24 h after the onset of inflammation but remain elevated well into convalescence (11). The effect of inflammation on hemoglobin (Hb) to assess for anemia in pre-school children (PSC) has been previously reported (5). In this cross-sectional study, the PSC studied with elevated CRP had an anemia prevalence of 66 % compared with PSC with normal CRP who had an anemia prevalence of 40 %.

Corresponding estimates for AGP were 61% and 42 %, respectively. Currently, there is no clear expert group consensus on how to account for the effects of inflammation when reporting anemia prevalence. In this study, we wanted to quantify the association of anemia (as measured by Hb) with inflammation (as measured by CRP) in the SAC population.

In 2011, the Centers for Disease Control and Prevention (CDC), Global Alliance for Improved Nutrition (GAIN), and *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) formed a collaborative research group called Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA) (13). This group identified data from nationally and regionally representative surveys that included PSC (aged 6–59 months [mo]), SAC (aged 5–14.99 years [yr]) and women of reproductive age (WRA,15–49 years [yr]) that reported a minimum of the following biomarkers: (1) Hb, (2) a measure of inflammation (CRP and/or AGP) and (3) a measure of iron status. A total of 23 datasets were identified. Permission was received for 15 datasets from which SAC information was available in the following three countries which were included in our final analysis - Mexico, the USA and Colombia.

METHODS

Study Aims

In this study, we determined the prevalence of anemia and iron deficiency and investigated the degree to which anthropometric, biochemical, demographic and socio-economic determinants are associated with anemia in SAC in Mexico, the USA and Colombia. We hypothesized that elevated CRP (as a measure of inflammation) is associated with an increased prevalence of anemia in SAC in three countries of varying anemia prevalence (Mexico, Colombia and the US). Our secondary hypothesis was that other predictor variables such as age, sex, ethnicity, income level (measured by an asset index), being overweight and being stunted are also associated with anemia in SAC in the three studied countries. The goal of this study is to help inform policy changes and guidelines on the measurement and interpretation of anemia. These findings will also guide the development of a research agenda for future longitudinal studies.

Study Design

Cross-sectional survey.

Study Population

The study obtained exemption status from the Emory University institutional review board (IRB). Each of the individual country surveys had their own ethical and human subject approvals. We used data from three different national cross-sectional surveys for our study. For all three countries, the survey years were included for analysis based on availability of data on our selected study biomarkers of interest. Also, when possible, the most recent surveys available were utilized.

In Mexico, analyses were based on data from the Mexican National Health and

Nutrition Survey 2006 (ENSANUT 2006), which was designed to obtain information on the health and nutritional status of the Mexican population based on a nationally representative sample. The ENSANUT 2006 is a cross-sectional, multi-stage, stratified cluster sampling survey conducted between October 2005 and May 2006. It was constructed with sufficient sampling power to disaggregate the study sample into urban and rural areas by state. The study protocol was approved by the Ethics, Biosecurity, and Research Boards of the national institute of Public Health (INSP), Cuernavaca, Mexico. Anthropometric measurements and blood samples were obtained after participants had signed an informed consent letter. A detailed description of the design, sampling procedures and survey methodology of the ENSANUT 2006 has been published elsewhere (14). The overall sample size available from Mexico was 18,646 (Figure 2).

Data for the US was obtained from The National Health and Nutrition Examination Surveys (NHANES) which is a publicly available data set administered by the National Center for Health Statistics (NCHS), a part of the United States CDC. We used data from two cycles of the continuous NHANES 2003 to 2006. This is a complex, multistage probability sample of the US civilian, non-institutionalized population. Standardized protocols and calibrated equipment were used to obtain measurements of height and weight. A detailed description of the sampling procedures and survey methodology of the NHANES 2003-2006 has been published previously (15, 16). The overall sample size available from the US was 4,149 (Figure 2).

In Colombia, analyses were based on data from the 2010 Encuesta Nacional de la Situaci ón Nutricional en Colombia (ENSIN). This is a national nutritional survey looking at both rural and urban settings. Subsamples were randomly drawn to estimate

departmental-,subregional-, regional-, and/or national-level estimates of specific nutrition problems among individuals 0–64 yrs. Further sub analyses were done by gender, age, ethnicity and socio-economic level. The data is from a three year collection study from 2008-2010. The Colombia ENSIN 2010 was carried out together with the National Demographic and Health Survey by Profamilia, a nonprofit organization focusing on reproductive health. Profamilia collected anthropometric measurements and granted ethical approval for this module of the survey. The Instituto Colombiano de Bienestar Familiar (Colombian Institute of Family Wellbeing) and the Instituto Nacional de Salud (INS; National Institute of Health) coordinated the collection and analysis of blood samples and granted ethical approval. Written and verbal consents were obtained from the adult interviewee or parents of children for the blood draws, anthropometric measurements, and demographic information. A detailed description of the sampling procedures and survey methodology of the ENSIN 2010 has been published (17). The overall sample size available from Colombia was 11,274 (Figure 2).

Inclusion criteria for the current study:

From each country survey, we selected children who met the following criteria:

(1) Age of the participant ≥ 5yrs and ≤ 14.99 yrs;

(2) Availability of serum Hb level as a measure of anemia;

(3) Availability of CRP as a measure of inflammation.

Exclusion Criteria for the current study:

From each country survey, we excluded those participants who met the following criteria:

(1) Pregnancy.

Primary exposures:

The primary exposure of interest was inflammation as measured by CRP. To account for the effects of inflammation across the entire acute phase response, inflammation was defined as a categorical variable 'elevated CRP' with levels > 5mg/L noted to be elevated in the entire study population (18). CRP was also measured as a continuous variable.

Primary outcome:

The primary outcome for the study was anemia. The following thresholds for anemia were used as per WHO guidelines (2); Hb < 11.5g /mL for children aged 5–11.99 yrs and Hb < 12.0 g/mL for children aged 12–14.99 yrs. Anemia was adjusted based on the altitude of the cluster in where a participant resided and was based on subtracting published altitude adjustment Hb concentration (19) from the Hb values observed for each participant in Mexico and Colombia. Altitude information was not available in the US as this information was considered a possible identification source per the NHANES. Anemia in Colombia was adjusted for smoking based on subtracting published smoking adjustment Hb concentration (19). No smoking information for SAC was available in the US and Mexico.

Study Covariates

The following data on SAC was collected: demographics (age, gender and ethnicity information), socio-economic status as measured by a principal component index, ferritin and/or soluble transferrin receptor (sTFR) as measures of iron status, and retinol as a measure of vitamin A status. Principal component analysis (PCA) was used to classify respondents' socioeconomic status (SES) into a 5-quintile asset index based on

household durable goods in Mexico and Colombia. In the US, continuous poverty income ratio (PIR) was categorized into five quintiles to create an asset variable. The PIR, for the US, is a ratio of a family income to the poverty threshold based on the US National Census bureau (20). Low socioeconomic status was defined as the poorest quintile, therefore SES was categorized into two groups: poorest quintile versus all other quintiles. Ethnicity information was available in the US and Colombia and were categorized as black versus all other ethnicities. Age was categorized into two groups using a cut-off of 12.0 years – this was a biologically determined cut off for mean age of menarche in all three countries using prior literature (21-24).

The following thresholds (18) were used to define abnormal values for these biochemical indicators:

1) Ferritin<12mg/L
2) sTFR - males > 5.0 mg/dL, females >4.4 mg/dL
3) Retinol < 0.7mmol/L

Ferritin was used to measure iron deficiency because it has the highest sensitivity and specificity to detect iron deficiency in those without inflammation in comparison to bone marrow biopsy (18). As ferritin (positive) and retinol (negative) are acute phase proteins, iron deficiency and vitamin A deficiency were defined excluding those patients with elevated CRP. Iron deficiency anemia was defined as the presence of anemia along with low ferritin among those with CRP $\leq$ 5mg/L (25, 26).

General methods

Measures of anthropometrics included measurements of weight and height using standardized techniques by trained health workers for all three surveys. Details of the

laboratory analyses from each country have previously been described (14-17) and are summarized in Table 2.

Data management and statistical methods:

Statistical analyses were done using SAS 9.3 (SAS Institute Inc., Cary, NC) and R 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set at a 2-sided alpha of 0.05. All analyses were adjusted for Complex Survey design effects (using cluster, strata and weight statements). Participant descriptive statistics were presented as means (standard error, SE) and as percent (95% confidence interval, CI) for continuous and categorical outcomes. Crude prevalence and 95% CIs for anemia in each of the study population, was examined using SAS PROC SURVEYFREQ to account for complex survey design effects.

We used the WHO Child Growth Standards (WHO Anthro, Geneva, Switzerland) (27) to calculate z-scores, and categorized stunting as a height-for-age z-score of less than −2, wasting as a weight for-height z-score of less than −2, overweight as a BMI for-age z-score of greater than 2 and obesity as a BMI for-age z-score of greater than 3.

Anemia population attributable fractions (PAF) were used to assess for the characteristics that were most associated with anemia and have the highest prevalence in the population. Anemia PAFs were calculated as follows, where $P_{E/D}$ is the prevalence of exposure given disease e.g. the prevalence of overweight SAC amongst all SAC who have anemia.

$$PAF = P_{E/D}\frac{(OR-1)}{OR}$$

The adjusted odds ratio (OR) for anemia was used in the formula for each factor that was significantly associated (P<0.05) with.

Multivariable modeling approach

The following covariates were considered based on theory for the multivariable model to evaluate the association of CRP with anemia: categorized age, sex, asset score, low ferritin (in Mexico, Colombia), overweight, stunting, ethnicity (in USA and Colombia) and Vitamin A deficiency (in USA). Our multivariable modeling approach was informed by the hypothesized conceptual causal diagram (Figure 1) for anemia determinants. Statistical interactions were assessed for the primary predictor of interest (CRP) with age and gender. These were both not significant and were therefore not included in the final logistic regression model.

RESULTS

Demographic and health characteristics

Data was analyzed for 3660 Mexican, 3543 US children and 8573 Colombian children aged 5 to 14.99 yrs (Figure 2). The original numbers of SAC surveyed in each country were 18646 in Mexico, 4149 in the US and 11274 in Colombia – these were reduced to the sample sizes stated above due to lack of data available for Hb and CRP/AGP. We looked at basic demographic characteristics (age, height, weight and sex) among SAC survey participants present in the original datasets as well as the final datasets (Table 3). For Mexico and the USA age, height, weight and sex were similar in original and final datasets, whereas for Colombia age, height and weight were similar in original and final datasets but the percentage of females was higher in the final dataset. Since our primary outcome variable anemia was based off Hb, we examined Hb distribution curves for each country. These are shown in Figure 3 with anemia cut-offs per the WHO.

Mexican data consisted of a higher proportion of females (59.5%), and overall low anthropometric measures of stunting (10.2%), wasting (1.3%), being overweight (13.3%) and being obese (3.9%). Prevalence of anemia using Hb adjusted for altitude was low (11.6%) as was iron deficiency (18.0%). The US data had about equal gender distribution (48.2% females), with overall very low anthropometric measures of stunting (0.9%), wasting (1.2%), being overweight (18.9%) and being obese (4.7%). There was a small proportion of SAC with black ethnicity (15.2%) in our US SAC population. Prevalence of anemia was very low (1.5%). Iron deficiency data was assessed in children (of both genders) aged 5 yrs and in girls aged12 to 14.99 yrs and was estimated to be low at 8.4%.

Colombian data consisted of a higher proportion of females (57.4%), with overall low anthropometric measures of stunting (9.8%), wasting (2.0%), being overweight (4.3%) and being obese (0.5%). Prevalence of anemia using Hb adjusted for altitude and smoking was low (4.2%) and the prevalence of iron deficiency was very low (9.2%).

Characteristics associated with anemia:

Crude odds ratios for anemia in SAC using bivariate analysis are shown in Table 5. In each of the three countries, anemia was associated with different factors in school-aged children. In Mexico, low ferritin was associated with an increased odds for anemia and being overweight showed an association with a decreased odds for anemia. In the US, having elevated CRP, being in age category 12-14.99 yrs, being female, being of black ethnicity, being in the poorest SES quintile and having Vitamin A Deficiency was associated with an increased odds for anemia. In Colombia, being of black ethnicity, being in the poorest SES quintile, having low ferritin, and being stunted was associated with an increased odds for anemia, while being overweight was associated with a decreased odds for anemia.

In multivariable analysis, SAC characteristics associated with having anemia in each country (Table 6) included: in Mexico having low ferritin (OR: 1.46; 95% CI: 1.06, 2.02), of note being overweight was strongly and inversely associated with anemia (60% reduction in Odds, OR 0.41; 95% CI: 0.21, 0.78); in the US being in age category 12-14.99 yrs (OR: 1.46; 95% CI: 1.06, 2.02), being female (OR: 2.30; 95% CI: 1.43, 3.68), being of black ethnicity (OR: 18.53; 95% CI: 9.03, 38.01) and having Vitamin A Deficiency (OR: 4.84; 95% CI: 1.28, 18.28); and in Colombia being of black ethnicity (OR: 1.62; 95% CI: 1.15, 2.27), being in the poorest SES quintile (OR: 1.77; 95% CI:

1.28, 2.45), having low ferritin (OR: 2.72; 95% CI: 1.96, 3.78) and being stunted (OR: 1.56; 95% CI: 1.07, 2.32).

Inflammation as measured by CRP was initially used in the logistic model as a categorical predictor but was not found to be significantly associated with anemia. In the final multivariable analysis, CRP was included as a continuous predictor and was not found to be significant. We explored using different CRP cut-offs (instead of greater than 5ng/mL being considered elevated) by creating CRP quintiles as a predictor in a logistic regression model (with lowest CRP quintile as the reference category). No CRP quintile was found to be significantly associated with anemia at a 5% alpha significance level in Mexico and the US. We were unable to create quintiles in the Colombian dataset given unequal distribution of the CRP variable among SAC.

Anemia population attributable fractions:

To identify the characteristics that had the highest prevalence and strongest association with anemia, we calculated prevalence fractions for anemia in each country (Figure 3). The characteristics that were associated with the largest percentage of cases of anemia were as follows: in Mexico having low ferritin (10%), being overweight was protective (-5%); in the US being in the age category of 12-14.99y (57%), being female (45%), being of black ethnicity (94%) and having Vitamin A deficiency (11%); and in Colombia being of black ethnicity (11%), being in the poorest SES quintile (31%) and being stunted (25%).

DISCUSSION

Based on the WHO classification (2), the results of our study show lower anemia prevalence in the school-aged population than that seen in pre-schoolers and pregnant women in all three countries analyzed. Anemia prevalence in Mexico, the US, and Colombia was 11.55%, 1.48%, and 4.19%, respectively. On a population level this low prevalence still translates into millions of SAC who are an easily accessible population for public health interventions. Not only is this one of the first studies to assess the determinants of anemia in SAC at the national level, this also showed cross country comparisons using standardized methods to account for inflammation and adjusted for altitude/smoking where possible. We found that CRP as a measure of inflammation was not associated with anemia in SAC in any of the countries included in the study. The modifiable factors associated with anemia identified in this study were iron deficiency in Mexico and Colombia and vitamin A deficiency in the US. Iron deficiency (as measured by ferritin) has been previously described as a modifiable determinant of anemia in children, both in the pre-school age group (28, 29) and in SAC (30, 31). In girls, anemia was thought to be secondary to blood loss (and subsequent iron deficiency). This blood loss was due to infections such as malaria and schistosomiasis in younger girls (12–13 y), and menstruation in older girls (14–18 yrs). Poor dietary intake of iron in resource-poor countries is also considered as a reason for low body iron and resultant anemia. Being overweight was found to be inversely associated with anemia in Mexican SAC. In our study we found the following non-modifiable characteristics to be associated with anemia: being female, of black ethnicity, and in age category 12-14.99y in the US; and being of black ethnicity and in the poorest SES quintile in Colombia. An important point

to note is that while the final analysis datasets consisting of SAC with available Hb and CRP information were smaller than those originally available to us, our demographic descriptive statistics of age, height, weight and sex (Table 3) were similar in all three countries except for a higher percentage of females in Colombia.

Our anemia prevalence estimates concur with previously published data from the ENSAUT 2006 (Mexico), NHANES 2004-6 (the US) and the ENSIN 2010 (Colombia) (32-34). Inflammation has a known association with anemia. A study from Papau New Guinea (5) in pre-school children showed a negative effect of inflammation on Hb and therefore increased inflammation being associated with higher anemia prevalence. Our finding of no association between CRP as a measure of inflammation and anemia was thought to be secondary to the low prevalence of inflammation in the SAC population. Alternatively, it could be suggested that the best measure of inflammation is not CRP given that its levels increase within 10 h of the onset of acute inflammation and normalize rapidly (~1 week) (12). Alternatively, a preferred inflammation biomarker could be AGP which is now being used more widely now in population-based studies as its levels begin to increase 24 h after the onset of inflammation but remain elevated well into convalescence (11).

Given the rapidity with which traditional diets and lifestyles are changing in many resource-poor countries, it is not surprising that food insecurity and under-nutrition persist in the same countries where chronic diseases such as obesity are emerging as a major epidemic (35). This double burden of diseases in low- and middle-income countries is well recognized. Our descriptive analysis of Mexico and Colombia (both upper-middle-income economies) showed them to be in different stages of health

transition as reflected in their anthropometrics. In Mexico, we noted the presence of a double burden of disease with stunting (10.24%) reflective of malnutrition in early life present along with almost equal prevalence of overweight (13.25%) with excessive intake of calories being one of the main common factor behind this conditions and risk factors, along with other lifestyle choices and genetic predisposition. Colombia had a disease burden more indicative of lower income economies with its SAC population having a higher burden of stunting (9.83%) relative to those who were overweight (4.33%).

The strengths of our study include that we provided updated anemia and iron deficiency estimates (for Mexico and Colombia), along with showing a cross-country comparison using nationally representative data. Additionally, we applied standard criteria across multiple surveys in adjusting Hb for altitude and using similar age cut-offs to define SAC. We hope that findings from this study will provide guidance to governments and other stakeholders about health priorities for SAC.

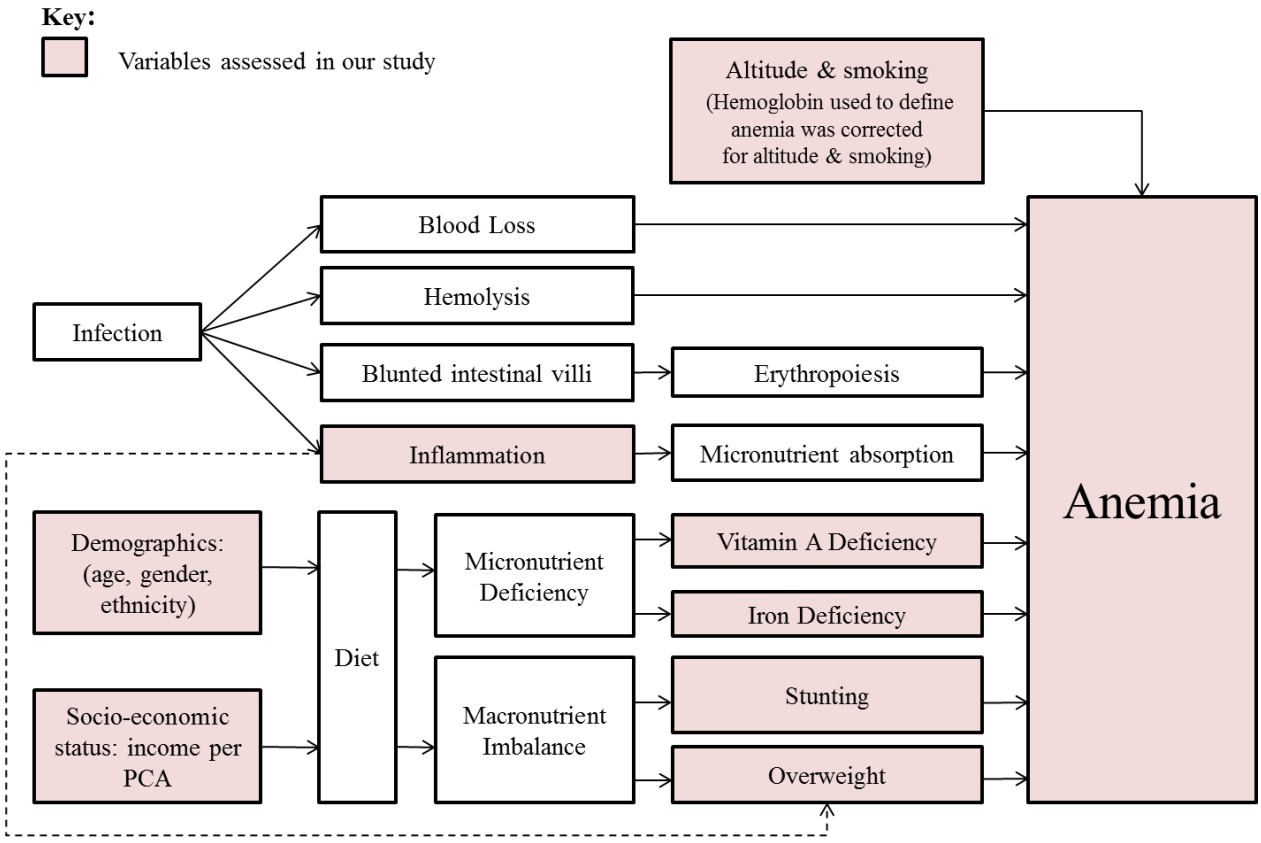
Our study had several limitations. The cross-sectional study designs of all three country surveys prevented us from establishing a temporal sequence with regards to the presence of inflammation and development of anemia. Our anemia variable was defined using Hb which (as shown in Figure 3) had a different standard deviations (SD) in each country with the Hb SD being the smallest in the US. Higher standard deviations in Mexico and Colombia could have been secondary to the different methods of Hb measurement used in each country. In Colombia and Mexico, hemocue utilized finger prick blood samples. The Coulter, which is a more precise method, was utilized in the US which required venous blood samples As outlined in Table 2, each country had differences in research lab methodology which also limited cross country comparisons.

Altitude adjustment was done for our Hb measurements in Mexico and Colombia. We were unable to adjust for altitude in our US data as this is not published in the NHANES data. Even though SES was based on PCA in each country, it was, however, more subjective in Mexico and Colombia where the PCA was derived from house-hold possessions and durable goods. In comparison, the US used a more objective measure such as the poverty income ratio. Lastly, we were unable to account for other known determinants of anemia in our multivariable analysis such as information about disease chronicity, menstruating females, inherited hemoglobinopathies, and other hematologic/ oncologic disorders as this information was not available in these national nutrition datasets.

In conclusion, anemia is not a public health problem of large magnitude in the school aged populations in Mexico, the US and Colombia. But in each country, we identified modifiable risk factors (iron and vitamin A deficiency) which were strongly associated with anemia. These can be considered as possible areas for intervention nationally for example using school based programs. Our study also highlights at risk SAC belonging to specific age groups, SES class and ethnicity which could be particularly targeted.

APPENDIX: 4 Figures and 6 Tables

Figure 1: Important causal pathways for Anemia among School Aged Children aged 5 – 14.99 years



Legend: Dashed line represents a possible pathway for anemia

Figure 2: Sample selection for analysis of anemia in School Aged Children 5 – 14.99 years in Mexico, USA and Colombia

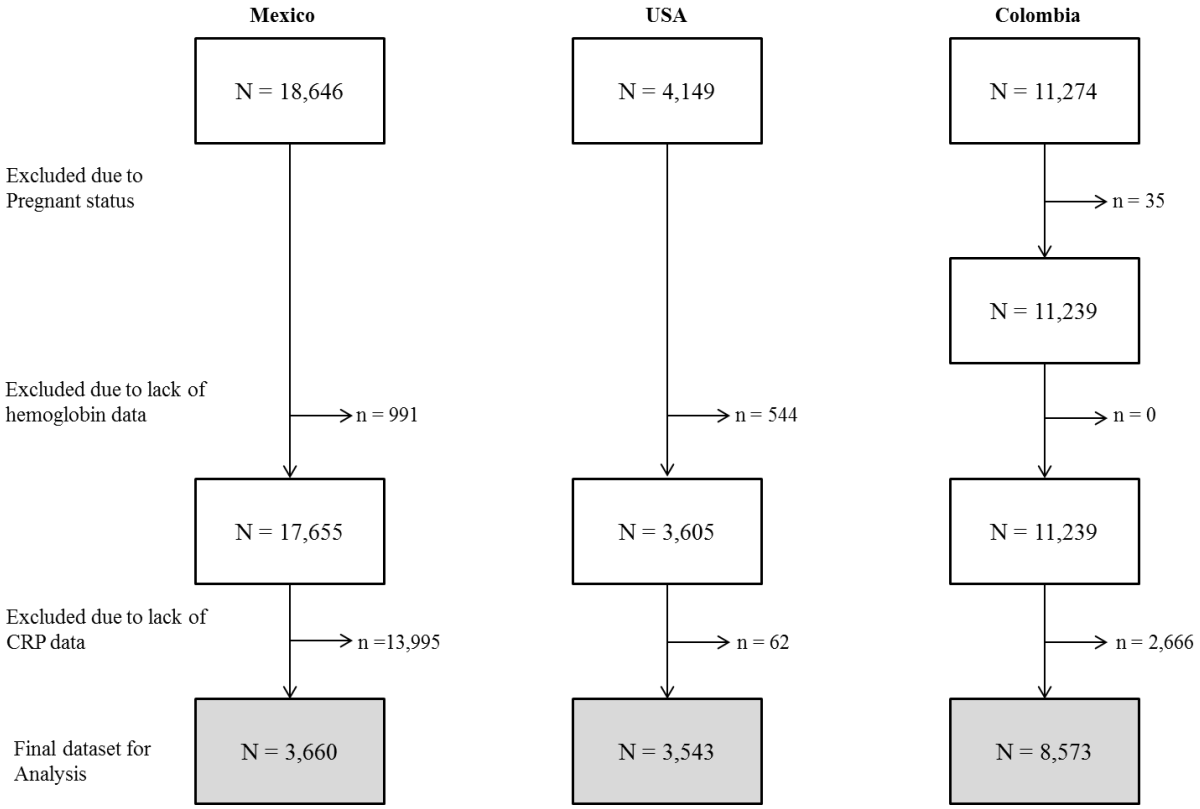


Figure 3: Hemoglobin Distribution among School Aged Children in Mexico, USA and Colombia with Anemia cut-offs

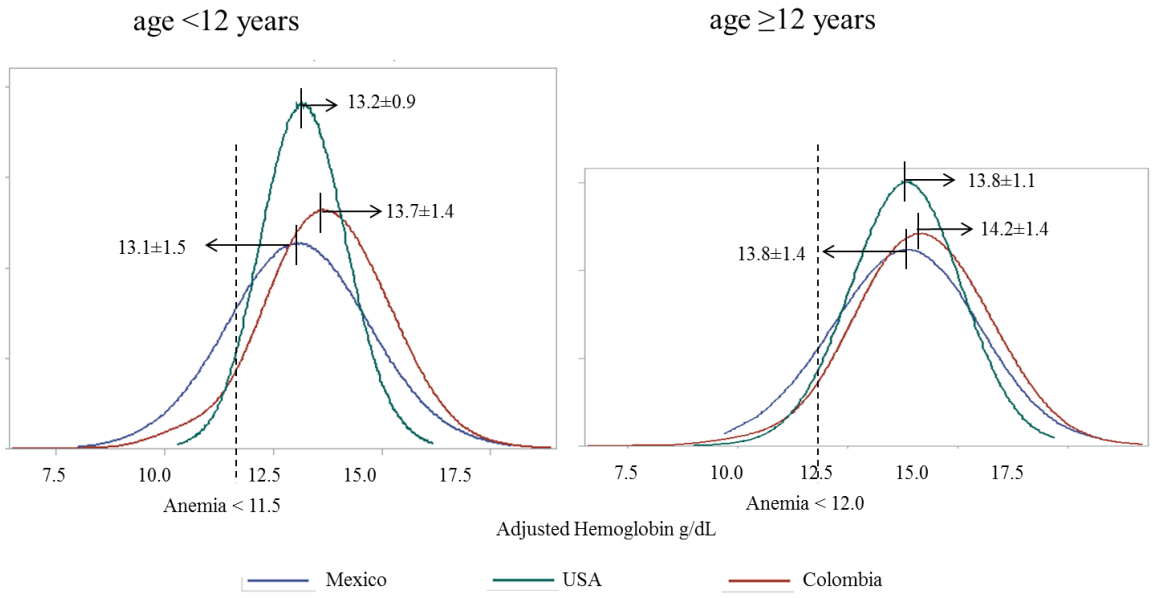


Figure 4: Anemia population attributable fractions for associated factors in School Aged Children in Mexico, USA and Colombia

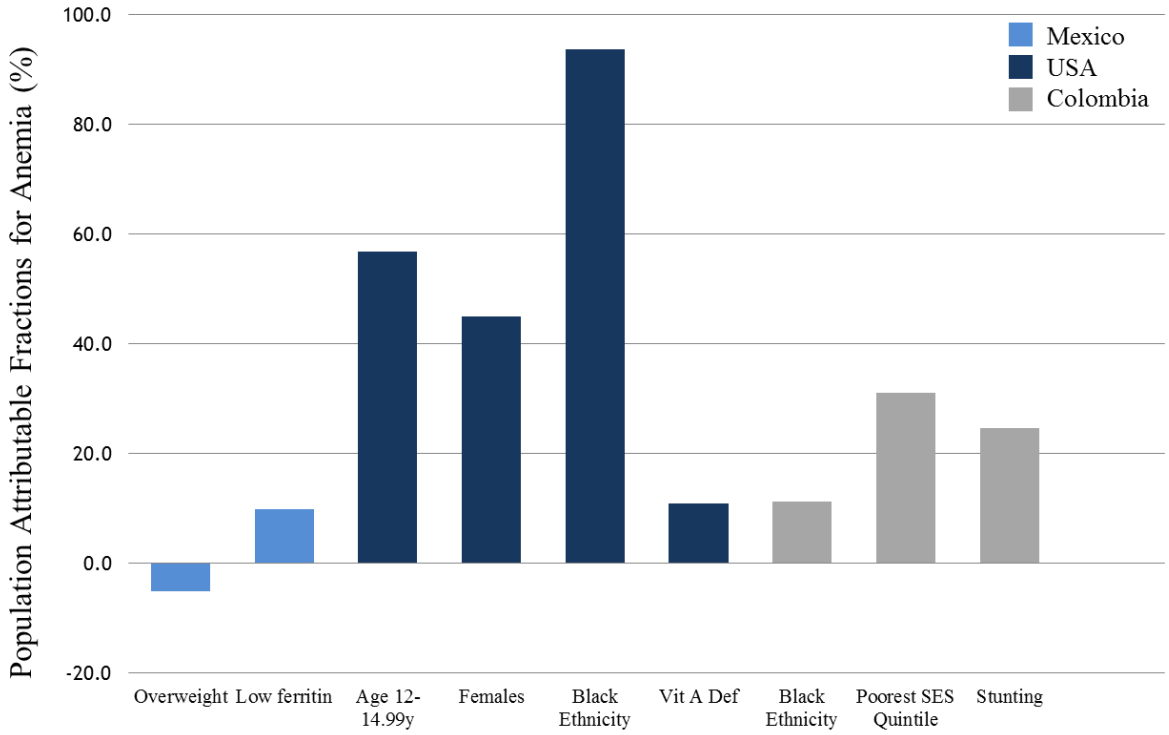


Table 1. WHO thresholds of hemoglobin used to define anemia in different subpopulations, at sea level

	Hemoglobin concentration (g/mL)
Non-pregnant women (>15 years)	<12.0
Pregnant women	<11.0
Children (0.5-4.9 years)	<11.0
Children (5.0-11.9 years)	<11.5
Children (12.0-14.9 years)	<12.0
Men (>15 years)	<13.0

Table 2. Summary of laboratory analytical methods for biomarkers of interest in Mexico, USA and Colombia.

	Countries		
Biomarkers	**Mexico**	**USA**	**Colombia**
Hemoglobin	HemoCue analyzer (HemoCue)	Beckman Coulter	HemoCue analyzer (HemoCue)
Ferritin	Plasma optical emission spectrometer (Tietz)	Immuno-radiometry In 2003: BioRad assay Immuno-turbidimetry in 2004-6: Roche/ Hitachi 912 clinical analyzer	Chemoluminescence (ADVIA Centaur, Siemens) assay
Soluble transferrin receptor	N/A	Immuno-turbidimetry (Roche/ Hitachi 912 clinical analyzer)	N/A
Retinol	N/A	High Performance Liquid Chromatography (Isocratic HPLC)	N/A
CRP	Nephelometry	Latex-enhanced nephelometry (Behring Nephelometer)	Turbidimetric (ASC-180, Bayer Diagnostics) assay

Table 3. Basic demographic characteristics of original & final (after applying exclusion criteria) datasets in Mexico, USA and Colombia

Characteristics	Countries											
	Mexico				USA				Colombia			
	Original Dataset		Final Dataset		Original Dataset		Final Dataset		Original Dataset		Final Dataset	
	n*	% or mean (SE)	n*	% or mean (SE)	n*	% or mean (SE)	n*	% or mean (SE)	n*	% or mean (SE)	n*	% or mean (SE)
Age (years)	3708	9.12 (0.08)	3660	9.11 (0.08)	4149	10.04 (0.06)	3543	10.25 (0.06)	11239	10.47 (0.04)	8573	9.92 (0.04)
Height (cm)	3706	129.56 (0.46)	3658	129.52 (0.47)	4127	140.55 (0.36)	3529	141.96 (0.33)	11058	136.31 (0.23)	8465	133.18 (0.24)
Weight (kg)	3706	33.88 (1.08)	3658	33.70 (1.08)	4128	40.17 (0.56)	3529	41.37 (0.51)	11058	34.16 (0.17)	8465	32.03 (0.17)
Sex % (Females)	2147/ 3708	59.79% (1.61)	2115/ 3660	59.50% (1.62)	2106/ 4149	48.53% (0.87)	1790/ 3543	48.21% (1.08)	5485/ 11239	48.32% (0.63)	4944/ 8573	57.36% (0.70)

*Stated n of those SAC with age, height, weight and sex information available

Table 4: Anthropometric and biochemical characteristics of School Aged Children 5 – 14.99 y in Mexico, USA and Colombia

Characteristics	Mexico/N=3660 n*	Mexico/N=3660 % (SE of %) or mean (SE)	USA/N=3543 n*	USA/N=3543 % (SE of %) or mean (SE)	Colombia/N=8573 n*	Colombia/N=8573 % (SE of %) or mean (SE)
Demographics						
Age in years	3660	9.11 (0.08)	3543	10.25 (0.06)	8573	9.92 (0.04)
Age						
< 12.0y	3083/3660	78.74% (1.22)	2063/3543	66.69% (1.03)	6223/8573	72.52% (0.62)
12.0 y-14.99y	577/3660	21.26% (1.22)	**1480/3543**	**33.31% (1.03)**	2350/8573	27.48% (0.62)
Sex (Females) %	2115/3660	59.50% (1.62)	**1790/3543**	**48.21% (1.08)**	4944/8573	57.36% (0.70)
Ethnicity						
Black	N/A	N/A	**1173/3543**	**15.21% (1.81)**	**958/8573**	**11.62% (0.54)**
Non-Black	N/A	N/A	2370/3543	84.79% (1.81)	7615/8573	88.38% (0.54)
Asset						
Poorest	1191/3656	31.07 (1.84)%	831/3419	15.97% (1.26)	**3299/8573**	**25.99% (0.78)**
All Other	2465/3656	68.93 (1.84)%	2588/3419	84.03% (1.26)	5274/8573	74.00% (0.78)
Nutrition/Growth						
Stunting (HAZ <-2) %	396/3658	10.24% (0.91)	36/3507	0.89% (0.19)	**963/8390**	**9.83% (0.48)**
Wasting (BAZ <-2) %	54/3658	1.32% (0.25)	42/3507	1.22% (0.29)	160/8390	2.01% (0.20)
Overweight (BAZ >2) %	**450/3658**	**13.25% (1.26)**	740/3507	18.92% (1.42)	339/8390	4.33% (0.33)
Obese (BAZ >3) %	106/3658	3.87% (0.84)	224/3507	4.71% (0.55)	40/8390	0.45% (0.09)
Biochemical markers						
Hemoglobin (g/dL)	3660	13.76 (0.61)	3543	13.59 (0.48)	8573	14.51 (0.31)
[1]Hemoglobin (g/dL) adjusted for altitude &/or [2]smoking	3660	13.30 (0.48)	N/A	N/A	8573	14.09 (0.27)
C-Reactive Protein (ng/mL)	3660	2.16 (0.19)	3543	1.65 (0.12)	8573	2.46 (0.11)
[3]Iron Deficiency %	626/3302	18.09% (1.19)	‡104/971	8.77% (0.97)	736/7450	9.17% (0.43)
[4]Low Ferritin%	663/3650	17.25% (1.15)	‡110/1056	8.38% (0.95)	**831/8573**	**9.17% (0.40)**
[5]High sTFR%	664/3645	19.61% (1.29)	‡90/1038	7.80% (1.22)	N/A	N/A
[6]Vitamin A Deficiency %	N/A	N/A	‡**25/3086**	**0.59% (0.11)**	N/A	N/A
[7]Elevated C-Reactive Protein (ng/mL) %	353/3660	9.39% (0.84)	268/3543	6.56% (0.63)	1123/8573	13.23% (0.55)
[8]Anemia % not using adjusted Hb (altitude/ smoking/)	337/3660	8.03% (0.71)	106/3543	1.48% (0.24)	425/8573	3.57% (0.27)
[9]Anemia % using adjusted Hb	454/3660	11.55% (0.89)	N/A	N/A	452/8573	4.19% (0.32)
[10]Iron Deficiency Anemia using adjusted Hb%	103/408	22.79% (2.69)	‡18/50	44.84% (7.05)	77/372	17.58% (2.64)

* Reported n is of actual sampled population. Reported % are weighted per the survey design

[1] Hemoglobin values for Mexico & Colombia adjusted for altitude as follows: < 1000m no change (0.0 g/dL), >=1000m to <1500m, subtract 0.2 g/dL, >=1500m to <2000m subtract 0.5 g/dL, >=2000m to <2500m subtract 0.8 g/dL, >=2500m to <3000m subtract 1.3 g/dL, >=3000m to <3500 subtract 1.9 g/dL, >=3500m to <4000m subtract 2.7 g/dL, >=4000m to <4500m subtract 3.5 g/dL, for >=4500m subtract 4.5 g/dL. [2]Hemoglobin values for Colombia adjusted for smoking by subtracting 0.3 g/dL [2]No smoking information for SAC available for the US and MX. [3]Iron Deficiency % assessed using serum ferritin (SF) in Mexico, USA & Colombia, corrected for inflammation excluding CRP >5.0 ng/mL; Age>=5y, SF <15 µg/L [4, 5, 6] Low Ferritin%, High sTFR%, Vitamin A Deficiency% uncorrected for inflammation. [4] Low Ferritin defined as: Age>=5y, SF <15 µg/L [4] High sTFR defined as: sTFR> 8.3 mg/L [5] Vitamin A Deficiency assessed using serum retinol <0.70 µmol/L [7] Elevated CRP >5.0 ng/mL [7] Anemia definition: Age <11.99y, Hb (g/dL) <11.5; Age>=12y, Hb (g/dL) <12.0 [9] Anemia in Mexico using Hb adjusted for altitude &/or smoking, Anemia in the US using Hb adjusted for AA extraction, Anemia in Colombia using Hb adjusted for altitude &/or smoking and AA extraction [10] Iron Deficiency Anemia definition %: % children with ferritin[2] (adjusted for inflammation) amongst those with anemia using adjusted hemoglobin[9]; ‡Ferritin (n=1056) and sTFR (n=1038) information available in the US for children aged 5 years both genders and females aged 12 years and older. Vit A information (n= 3086) available in the US for both males and females aged 6 years and older

Table 5: Crude associations of known Predictors of Anemia in School Aged Children aged 5 – 14.99 y in Mexico, USA and Colombia

	Mexico				USA				Colombia			
Predictors	***Anemia (%)**	****Crude OR**	**95%CI**	**p-value‡**	***Anemia (%)**	****Crude OR**	**95%CI**	**p-value‡**	***Anemia (%)**	****Crude OR**	**95%CI**	**p-value‡**
[1]Elevated CRP%¥												
No	11.59				1.39				4.03			
Yes	11.14	0.96	0.61–1.49	0.843	2.68	**1.95**	**1.03–3.72**	**0.027**	5.06	1.27	0.89–1.81	0.183
[2]Age (years)												
< 12.0 y	11.96				1.02				4.39			
12.0 y-14.99y	10.13	0.83	0.53–1.29	0.411	2.39	**2.38**	**1.47–3.87**	**<0.0001**	3.67	0.83	0.62–1.12	0.219
Sex												
Male	10.95				1.00				4.26			
Female	12.20	1.13	0.82–1.56	0.451	1.98	**1.99**	**1.40–2.84**	**<0.0001**	4.14	0.97	0.75–1.26	0.818
[3]Ethnicity												
Non Black					0.46				3.81			
Black	N/A	N/A	N/A	N/A	7.14	**16.71**	**7.54–37.02**	**<0.0001**	7.08	**1.92**	**0.39–2.66**	**<0.0001**
[4]Asset												
All Other	11.09				1.28				3.29			
Poorest	12.96	1.19	0.85–1.69	0.313	2.51	**1.99**	**1.03–3.83**	**0.024**	6.74	**2.12**	**1.56–2.88**	**<0.0001**
[5]Low Ferritin%												
No	10.89								3.68			
Yes	14.76	**1.42**	**1.03–1.95**	**0.030**	N/A	N/A	N/A	N/A	9.28	**2.68**	**1.93–3.70**	**<0.0001**
[6]Vit A Deficiency %												
No					1.38							
Yes	N/A	N/A	N/A	N/A	8.11	**6.31**	**1.67–23.92**	**0.0011**	N/A	N/A	N/A	N/A
[7]Overweight												
No	12.60				1.49				4.28			
Yes	5.53	**0.41**	**0.21–0.78**	**0.004**	1.48	0.99	0.57–1.73	0.981	1.69	**0.39**	**0.15–0.96**	**0.034**
[8] Stunting												
No	11.66				1.50				3.93			
Yes	11.58	0.99	0.64-1.54	0.973	--†	---	---	---	6.31	**1.65**	**1.13–2.39**	**0.0083**

*Percentage of SAC with anemia in each predictor group of interest ‡ null hypothesis for p-value is that OR=1 **Weighted Crude anemia Odds Ratio (OR) for categorical predictors: elevated CRP %, age, sex, ethnicity, asset, low ferritin %, Vitamin A deficiency %, overweight (Y/N), stunting (Y/N) ¥CRP: C-Reactive Protein [1]Grouped for analysis with Elevated CRP >5.0 ng/mL [2]Age categories based on 12 years as the average of menarche for girls in each of the three countries, grouped for analysis as Age <12.0 and Age 12.0-14.99y [3]Grouped for analysis as Black and Non-Black. Original categories in each country as follows: Mexico – no ethnicity information available. US - Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race; including Multi-racial. Colombia - Indigenous, Gypsy or Roma, Raizal Archipelago, Palenquero San Bacillus, Black/Mulatto/Afro-Colombian/Afro, None of the above. [4]Grouped for analysis as Poorest and All Other. Original categories per PCA Asset Index – Poorest, Poor, Average, Rich, Richest. [5] Low Ferritin defined as: Age>=5y, SF <15 µg/L [6]Vitamin A Deficiency assessed using serum retinol <0.70 µmol/L [7]Grouped for analysis with Overweight = BAZ >2 (Y/N) [8]Grouped for analysis with Stunting = HAZ <-2 (Y/N) N/A: No ethnicity information in MX, no Vit A information in MX and Co, no ferritin information in SAC in the US. † There were no stunted children with anemia in the US.

Table 6: Adjusted estimates for associations of known Predictors of Anemia in School Aged Children aged 5 – 14.99 y in Mexico, USA and Colombia

	Mexico				USA				Colombia			
Predictors	***Anemia (%)**	****Adj OR**	**95%CI**	**p-value‡**	***Anemia (%)**	****Adj OR**	**95%CI**	**p-value‡**	***Anemia (%)**	****Adj OR**	**95%CI**	**p-value‡**
Continuous												
[1]CRP (ng/mL)	---	1.01	0.99–1.03	0.162	---	0.99	0.98–1.02	0.779	---	1.01	0.99–1.01	0.225
Categorical												
[2]Age (years)												
< 12.0 y	11.96				1.02				4.39			
12.0 y-14.99y	10.13	0.73	0.46–1.16	0.185	2.39	**3.12**	**1.67–5.82**	**0.0004**	3.67	0.75	0.54–1.04	0.079
Sex												
Male	10.95				1.00				4.26			
Female	12.20	1.19	0.86–1.65	0.296	1.98	**2.30**	**1.43–3.68**	**0.0006**	4.14	0.98	0.74–1.30	0.901
[2]Ethnicity												
Non Black					0.46				3.81			
Black	N/A	N/A	N/A	N/A	7.14	**18.53**	**9.03–38.01**	**<0.0001**	7.08	**1.62**	**1.15–2.27**	**0.005**
[4]Asset												
All Other	11.09				1.28				3.29			
Poorest	12.96	1.11	0.78–1.59	0.548	2.51	1.07	0.68–1.68	0.779	6.74	**1.77**	**1.28–2.45**	**0.0005**
[5]Low Ferritin%												
No	10.89								3.68			
Yes	14.76	**1.46**	**1.06–2.02**	**0.020**	N/A	N/A	N/A	N/A	9.28	**2.72**	**1.96–3.78**	**<0.0001**
[6]Vit A Deficiency %												
No					1.38							
Yes	N/A	N/A	N/A	N/A	8.11	**4.84**	**1.28–18.28**	**0.019**	N/A	N/A	N/A	N/A
[7]Overweight												
No	12.60				1.49				4.28			
Yes	5.53	**0.41**	**0.21–0.78**	**0.007**	1.48	0.91	0.51–1.61	0.736	1.69	0.47	0.19–1.19	0.113
[8] Stunting												
No	11.66				1.50				3.93			
Yes	11.58	0.86	0.54–1.37	0.514	--[†]	---	---	---	6.31	**1.56**	**1.07–2.32**	**0.022**

*Percentage of SAC with anemia in each predictor group of interest ‡ null hypothesis for p-value is that OR=1

**Adjusted anemia Odds Ratio (OR) is presented for a multivariable logistic model (outcome=anemia) that accounted for cluster study design: sample size available in each country for multivariable model - Mexico (N=3644), USA (N=2975), Colombia (N=8390) [1]CRP: C-Reactive Protein; used as a continuous variable [2]Age categories based on 12 years as the average of menarche for girls in each of the three countries, grouped for analysis as Age <12.0 and Age 12.0-14.99y [3]Grouped for analysis as Black and Non-Black. Original categories in each country as follows: Mexico – no ethnicity information available. US - Mexican American, Other Hispanic, Non-Hispanic White, Non-Hispanic Black and Other Race; including Multi-racial. Colombia - Indigenous, Gypsy or Roma, Raizal Archipelago, Palenquero San Bacillus, Black/Mulatto/Afro-Colombian/Afro, None of the above. [4]Grouped for analysis as Poorest and All Other. Original categories per PCA Asset Index – Poorest, Poor, Average, Rich, Richest. [5] Low Ferritin defined as: Age>=5y, SF <15 µg/L [6]Vitamin A Deficiency assessed using serum retinol <0.70 µmol/L [7]Grouped for analysis with Overweight = BAZ >2 (Y/N) [8]Grouped for analysis with Stunting = HAZ <-2 (Y/N). N/A: No ethnicity information in MX, no Vit A information in MX and Co, no ferritin information in SAC in the US. [†] There were no stunted children with anemia in the US.

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