

IZiNCG TECHNICAL BRIEF

Adjusting plasma or serum zinc concentrations for inflammation

Background on the issue

Plasma or serum zinc concentrations (PZC) are one of three indicators recommended for assessing the risk of zinc deficiency in a population (1). IZiNCG recommends that when the prevalence of low PZC is >20%, the risk of zinc deficiency is considered elevated and should be addressed through public health nutrition interventions (2). However, PZC may be depressed in the presence of inflammation (3-7). The acute phase response

can lead to a redistribution of zinc from the plasma or serum to the liver (8). In settings with a high burden of infection, this could lead to artificially high estimates of the prevalence of nutritional zinc deficiency. C-reactive protein (CRP) and alpha-1-acid glycoprotein (AGP) are 2 acute phase proteins most commonly assessed to measure the acute phase response (9). The BRINDA project has evaluated several approaches to address the issue of inflammation in the interpretation of micronutrient biomarkers (10); however, these have not been compared in the context of PZC.

What is BRINDA?

- BRINDA stands for Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia.
- BRINDA was formed in 2012 as a multi-agency and multi-country collaboration in an effort to improve micronutrient assessment.
- More information on the BRINDA project, including statistical macros for the BRINDA regression correction models can be found on the BRINDA website: <https://brinda-nutrition.org/>
- For more details on the adjustment of PZC for inflammation, please refer to the full manuscript: McDonald CM, Suchdev PS, Krebs NF, Hess SY, Wessells KR, Ismaily S, Rahman S, Wieringa FT, Williams AM, Brown KH, King JC. Adjusting plasma or serum zinc concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. *American Journal of Clinical Nutrition*. 2020;111(4):927–937.

Objectives of the analysis

As part of the BRINDA project, cross-sectional data from national surveys that measured PZC, CRP and/or AGP among preschool children (PSC) and/or nonpregnant women of reproductive age (WRA) were used to answer the following questions:

1. Is there a need to adjust PZC for inflammation to estimate the prevalence of nutritional zinc deficiency in PSC and WRA?
2. Is it necessary to adjust PZC for CRP, AGP or both?
3. How do the different adjustment approaches compare?

Surveys where PZC and CRP/AGP were available

Table 1 summarizes the key characteristics of the national surveys where PZC and CRP/AGP were available. A total of 18,964 PSC observations and 22,750 WRA observations were included. The datasets were restricted to include observations where PZC and CRP or AGP (or both) were available.

Table 1. Characteristics of the surveys included in the analysis

Country, year	PSC				WRA			
	N	Mean age, months	CRP > 5 mg/L, %	AGP > 1 g/L, %	N	Mean age, months	CRP > 5 mg/L, %	AGP > 1 g/L, %
Afghanistan, 2013	658	29.0	9.7	23.6	1044	30.8	12.8	11.6
Azerbaijan, 2013	1016	36.0	8.2	30.3	—	—	—	—
Bangladesh, 2012	309	36.0	9.2	27.8	728	29.8	5.2	13.1
Cambodia, 2014	534	37.4	11.0	39.7	693	30.2	9.5	33.5
Cameroon, 2009	776	30.8	37.5	38.7	746	27.2	17.8	7.0
Colombia, 2010	3573	37.9	18.8	—	—	—	—	—
Ecuador, 2012	2017	29.3	12.5	—	7267	30.2	17.2	—
Malawi, 2016	1071	32.3	23.8	55.8	760	760	7.7	11.3
Mexico, 2012	1164	41.4	9.8	—	1679	1679	23.8	—
Mongolia, 2006	240	20.1	—	24.6	—	—	—	—
Pakistan, 2011	7231	27.3	—	35.0	7490	7490	11.4	23.1
UK, 2014	—	—	—	—	862	862	15.8	—
Vietnam, 2010	375	37.2	12.3	—	1479	1479	6.6	—

Summary of the statistical methods

- CRP concentrations > 40 mg/L were not included in the calculation of deciles.
- Observations where the PZC was above the maximum value of 160.9 ug/dL reported in the 2013-2014 US NHANES (11) were also excluded, as these were considered to likely be biologically implausible values that reflected contamination.
- Descriptive statistics were calculated adjusting for the cluster, strata, and sampling weight of each survey.
- Spearman rank correlations were then calculated between PZC, CRP, and AGP concentrations, and the mean PZC and prevalence of zinc deficiency were estimated by CRP decile and AGP decile in each dataset.
- If the correlation and decile analyses indicated a significant negative association between PZC and either acute phase protein in a particular country/population group, the three adjustment approaches summarized in **Table 2** were applied and compared.

Table 2. Comparison of the adjustment approaches

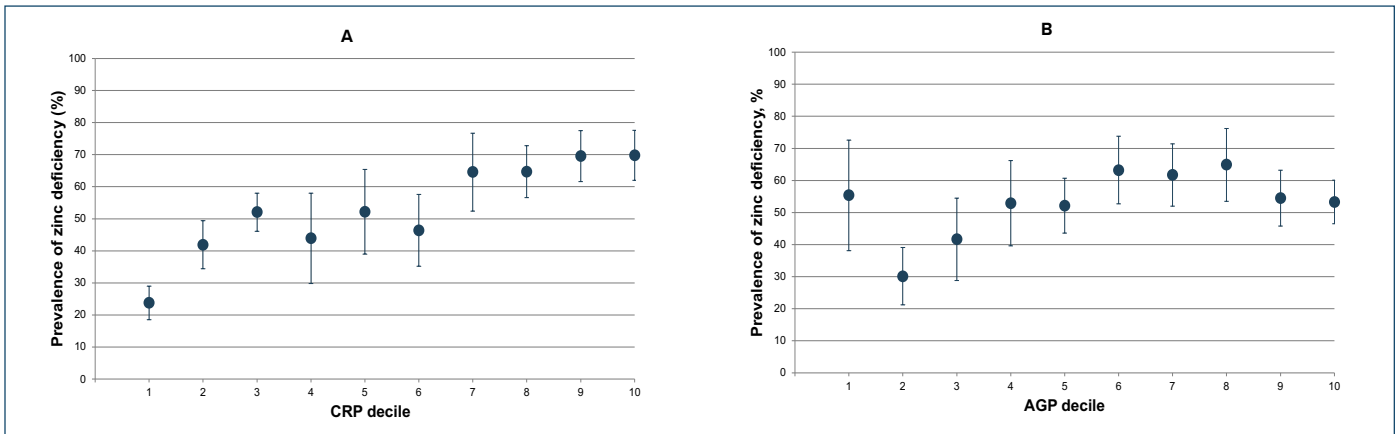
Adjustment approach	Description	Strengths	Limitations
1. Exclusion	<ol style="list-style-type: none"> Excludes observations in which either CRP is > 5 mg/L, AGP is > 1 g/L, or both. Mean PZC and prevalence of zinc deficiency is estimated using remaining observations. 	<ul style="list-style-type: none"> Easy to implement. 	<ul style="list-style-type: none"> Potentially, a large loss in sample size and precision. Possible introduction of bias.
2. Correction factor	<ol style="list-style-type: none"> Four categories of inflammation are defined: <ol style="list-style-type: none"> Reference: CRP < 5 mg/L & AGP < 1 g/L Incubation: CRP > 5 mg/L & AGP < 1 g/L Early convalescence: CRP > 5 mg/L & AGP > 1 g/L Late convalescence: CRP < 5 mg/L & AGP > 1 g/L Survey-specific (i.e. internal) correction factors (ICFs) are calculated by dividing the geometric mean PZC of the reference category by the geometric mean PZC of each inflammatory category. PZC is multiplied by the appropriate ICF & the prevalence of zinc deficiency is re-estimated. 	<ul style="list-style-type: none"> Accounts for the inflammation profile of the population. 	<ul style="list-style-type: none"> Requires CRP and AGP. Precision of the adjusted prevalence estimate is dependent on the size of the reference group.
3. BRINDA regression correction	<ol style="list-style-type: none"> Uses linear regression to adjust PZC by CRP, AGP or both on a continuous scale using the following equation: $PZC_{R\text{adjusted}} = PZC_{\text{unadjusted}} - \beta_1(\ln_CRP_{\text{obs}} - \ln_CRP_{\text{ref}}) - \beta_2(\ln_AGP_{\text{obs}} - \ln_AGP_{\text{ref}})$ Prevalence of zinc deficiency is re-estimated. 	<ul style="list-style-type: none"> Adjusts for CRP and AGP concentrations on a linear scale. Uses survey-specific slopes. 	<ul style="list-style-type: none"> More complicated to implement; however, a statistical macro is available.

Key findings of the analysis

PZC tended to be weakly but negatively associated with CRP and AGP concentrations in PSC. The relation between the estimated prevalence of zinc deficiency and CRP decile followed a positive linear pattern in the six PSC

datasets that measured both CRP and AGP concentrations. However, there was considerable variation in the nature of the association across PSC data sets (**Figure 1**).

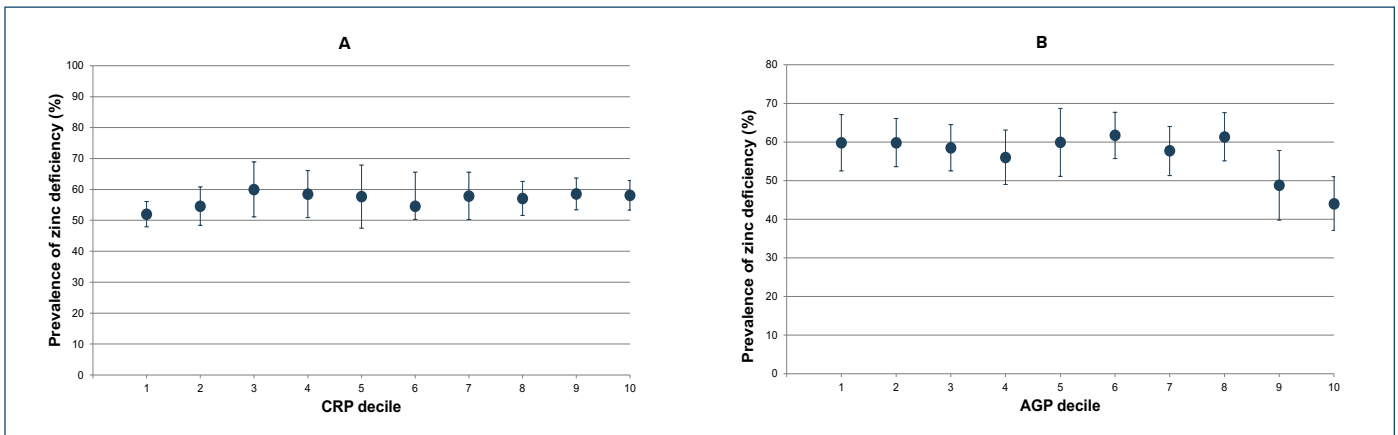
Figure 1. Prevalence of zinc deficiency in PSC by CRP (A) and AGP (B) deciles



Associations between PZC the two acute phase proteins were weak and inconsistent in WRA. No clear pattern could be identified between the prevalence of zinc deficiency and either CRP

decile or AGP decile in the pooled analysis of the five WRA data sets that measured both CRP and AGP concentrations (**Figure 2**).

Figure 2. Prevalence of zinc deficiency in WRA by CRP (A) and AGP (B) deciles



Based on the results of the correlation and decile analyses, it was appropriate to pursue and compare adjustment methods for PZC in the following PSC data sets: Cameroon, Malawi, Ecuador, Afghanistan, Cambodia, and Azerbaijan. Application of the adjustment methods was not warranted in WRA given the lack of a strong or consistent association between PZC and either CRP or AGP. Reasons for the inconsistent associations between PSC and CRP/AGP among

PSC data sets, and the lack of a strong or consistent association across WRA datasets are not fully known, but could be related to varying degrees of infection severity.

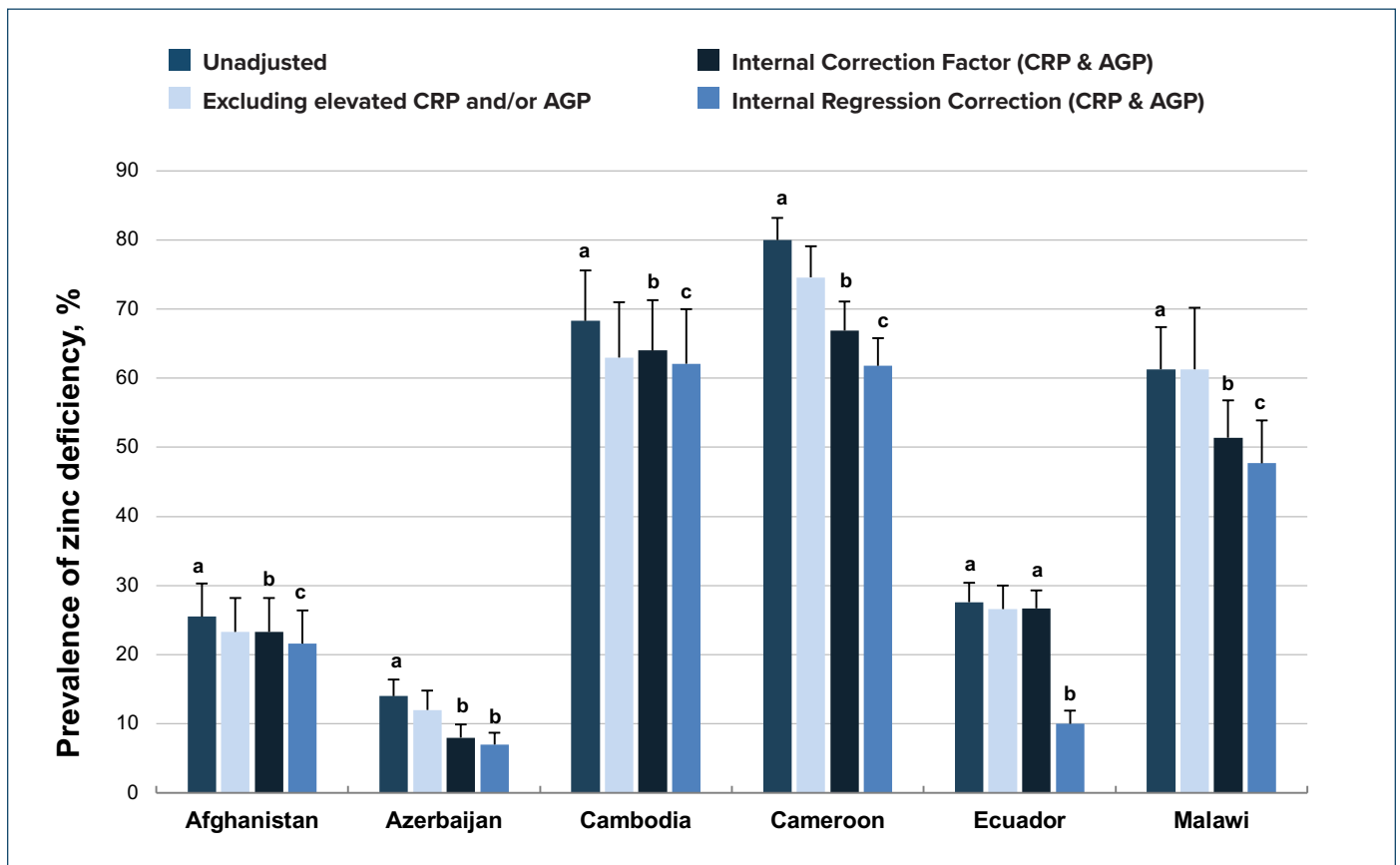
Comparisons across adjustment approaches were made using the variations that accounted for both CRP and AGP for the following reasons:

1. With the exception of Ecuador, which only had data available for CRP, both variables were available in the surveys that were analyzed;

- The greatest reduction in the prevalence of zinc deficiency was usually seen when both variables were accounted for;
- Each variable represents a different phase of the acute phase response and is important from a biological perspective.

As shown in **Figure 3**, in all six surveys, application of the BRINDA regression correction approach, adjusting for both CRP and AGP when available, resulted in the largest reduction from the unadjusted prevalence of zinc deficiency (median decrease of 7.1 percentage points).

Figure 3. Estimated prevalence of zinc deficiency with the use of different adjustment approaches in 6 PSC data sets¹



¹Bars within a given survey without a common letter are significantly different, $P < 0.05$ (adjusted for multiple comparisons). Statistical comparisons were not made with the prevalence of zinc deficiency under the exclusion approach, because the population of PSC is different.

Recommendations for future analyses

- When conducting descriptive statistics, perform correlation and decile analyses to understand the strength and direction of the association between PZC, CRP and AGP.
- If the results of the correlation and decile analysis indicate a clear, negative association between PZC and CRP and/or AGP, the BRINDA regression correction approach should be applied.
- Both CRP and AGP concentrations should be included in the regression model, if possible.

References

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Other IZiNCG Technical Briefs on PZC

Available from <https://www.izincg.org/technical-briefs>

No. 1, 2007

Quantifying the risk of zinc deficiency: Recommended indicators.

No. 2, 2012, 2nd edition

Assessing population zinc status with serum zinc concentration.

No. 6, 2018

How to deal with hemolysis for plasma/serum zinc analysis.

No. 9, 2018

The value of measuring plasma or serum zinc concentrations in national surveys.

No. 12, 2020

Comparison of laboratory instrument types for analysis of plasma or serum zinc concentration.

About IZiNCG

IZiNCG is the International Zinc Nutrition Consultative Group whose primary objectives are to promote and assist efforts to reduce global zinc deficiency through interpretation of nutrition science, dissemination of information, and provision of technical assistance to national governments and international agencies. IZiNCG focuses on identification, prevention and treatment of zinc deficiency in the most vulnerable populations of low-income countries.

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