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Quantifying the potential food fortification impact on nutrient intake and anemia prevalence among women of reproductive age in India

By

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Bachelor of Science
Indiana University
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An abstract of
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Abstract

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By Elizabeth Lambert

Anemia is a public health burden disproportionately affecting women of reproductive age (WRA). Food fortification, the addition of micronutrients to foods, may alleviate anemia caused by nutritional deficiencies. We analyzed the mean daily intake of nutrients (vitamin A, vitamin B12, vitamin B6, vitamin C, vitamin E, iron, riboflavin, thiamine, copper, folic acid, and zinc) involved in hemoglobin synthesis among WRA (15-49 years) in India in a no-fortification compared to a maximum-fortification scenario (at 100% coverage and compliance). We estimated additional nutrients contributed from fortified milk, oil, rice, salt, and wheat flour. We estimated the impact of fortification on nutrient intake, including the percent of nutrient Estimated Average Requirements (EAR) met by WRA. We estimated total change in nutritional anemia prevalence among WRA in both fortification scenarios.

We analyzed a 24-hour dietary recall from the National Nutrition Monitoring Bureau using a food composition table that we created with the 2017 Indian Food Composition and other tables to estimate no-fortification and maximum-fortification intake of the aforementioned nutrients. We calculated mean total intake in both scenarios. To the maximum fortification averages, we applied estimates generated from meta-regressions of daily iron intake to estimate average hemoglobin increase. We calculated the percent of WRA not meeting EARs for the scenarios using the EAR cut-point method. We analyzed hemoglobin values from WRA from another dataset (2015-2016 National Family Health Survey 4). We estimated anemia prevalence for the scenarios.
Compared to the no-fortification scenario, the maximum-fortification scenario increased daily folate intakes by 26-27%, iron by 70-106%, thiamine by 18-42%, vitamin B12 by 12-28%, and zinc by 39-59%. Percentage of women not meeting EARs decreased and 25th, 50th, and 75th percentile intakes increased from the no-fortification to maximum-fortification scenarios for iron, folate, vitamin B12, and zinc. Anemia prevalence decreased by 19-36% in the maximum-fortification scenario because of the additional iron consumed from fortified foods.

Our results suggest that fortification, where 100% of women consume foods that fully meet the nutrient levels stipulated in fortification standards, can improve nutrient intakes and reduce anemia prevalence among WRA. We recommend Indian policymakers consider these trends when crafting fortification policy.

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Our results suggest that fortification, where 100% of women consume foods that fully meet the nutrient levels stipulated in fortification standards, can improve nutrient intakes and reduce anemia prevalence among WRA. We recommend Indian policy-makers consider these trends when crafting fortification policy.

Introduction:

Anemia is a common disease among women of reproductive age (WRA) globally [1]. Anemia is defined as having an insufficient amount of hemoglobin in the body which reduces the body's ability to transport oxygen. Downstream factors associated with depleted oxygen levels cause an increase in maternal morbidity and mortality [2]. Globally, 20% of maternal mortality can be attributed to anemia [3]. The body's ability to produce hemoglobin is a complex mechanism and requires several nutrients to sustain its process. These nutrients are copper, folic acid, iron, riboflavin, thiamine, vitamin A, vitamin B12, vitamin B6, vitamin C, vitamin E, and zinc [4]. One of the primary types of anemia is nutritional anemia, defined as anemia due to a lack of one or more nutrients creating hemoglobin insufficiencies [5].

Anemia is particularly prevalent in Southeast Asia [3]. A recent study estimated that 53.1% of women in India have anemia [3]. The 2016-2018 Comprehensive National Nutrition Survey (CNNS) reported nutrient deficiencies and found that 15-40% of adolescent females and males aged 10-19 years are deficient in one or more of the nutrients needed for hemoglobin synthesis [6].

Food fortification is an intervention that, when properly implemented, can reduce nutritional anemia by increasing the amount of nutrients women are consuming in their everyday diet [5]. By improving diet and subsequent hemoglobin concentrations among WRA, anemia prevalence can be drastically reduced on a global scale [7]. Fortification of salt with iodine and fortification of oil with vitamin A is currently mandatory in India [8]. Fortification of three other staple foods (milk, rice, and wheat flour) with several nutrients is currently voluntary in India and recommended standards were made available in October 2018 by the Food Safety and Standards Authority of India [8]. Various studies have modeled the impact of food fortification on dietary outcome and anemia prevalence in India [9] [10] [11], yet only a few have focused on WRA [12] [13]. No studies conducted in India have investigated the combined effects of all nutrients involved in hemoglobin synthesis on anemia prevalence.

The primary objective for this study was to analyze the intake of nutrients involved in hemoglobin production among WRA in India. We modeled the impact of the mandatory fortification of milk, rice, salt, oil, and wheat flour on the dietary intake of the nutrients involved in hemoglobin synthesis in WRA in India. Additionally, we modeled hemoglobin levels from additional iron intake and subsequent anemia prevalence if fortification were to be mandatorily implemented. We also modeled the proportion of WRA not meeting the Estimated Average Requirements (EARs) and 25^{th}, 50^{th}, and 75^{th} percentile intakes of select nutrients for both the no-fortification and maximum-fortification scenarios. We hypothesize that hemoglobin levels will increase because of dietary iron increase and subsequently anemia prevalence among WRA in India will decrease due to mandatory food fortification at maximum fortification level.

Methods:

We followed the STROBE (strengthening the reporting of observational studies in epidemiology) checklist for observational and cross-sectional studies [14].

Settings and Data Sources

We conducted a secondary analysis which analyzed two publicly available and nationally representative cross-sectional datasets, National Nutrition Monitoring Bureau (NNMB) (2009-2012) and National Family Health Surveys 4 (NFHS-4) (2015-2016) [15] [16].

NNMB dataset was available for public download from the Global Dietary Database. Data are nationally representative with rural coverage and were collected using multistage stratified sampling. A single, 24-hour dietary recall was collected on 37,219 total participants using probability sampling without survey weights. For our analysis, participants were selected by sex (female), age (15-49 years), and non-pregnant non-lactation status. The details of the NNMB study have been previously described [17].

Only seven nutrients were included in the NNMB data. Some nutrients involved in hemoglobin synthesis (e.g. vitamin B12) were missing. To accurately estimate food and nutrient intake, we created a food composition table (i.e., hereafter referred to as the food composition table) consisting of all foods and beverages consumed by NNMB participants using the 2017 Indian Food Composition Table [18]. Any missing foods (e.g. honey) or nutrients not available in the 2017 Indian FCT were obtained from USDA Food Data Central, the 1989 Indian Food Composition table, and the 2013 Bangladesh Food Composition Table [19] [20] [21]. For each food and beverage in the food composition table, we added a new variable that estimated the percent of food that is made with milk, oil, rice, salt, and/or wheat flour [22]. For example, buns contain 1.5% oil, 1% salt, and 67% wheat flour. We merged our own FCT with the 24-h dietary recall dataset and created a comprehensive dietary intake data set with intake of both key food and nutrients. For WRA, we used our food composition table to estimate total additional consumption of the nutrients involved in hemoglobin synthesis that are included in Indian fortification standards: folate, iron, riboflavin, thiamine, vitamin A, vitamin B6, vitamin B12, and zinc (**Appendix Table A)**.

We used the National Family Health Survey 4 (NFHS-4) for our hemoglobin analysis [16]. Data were available for public download from the Demographic and Health Surveys (DHS) Program. Data is representative at the national and state levels and is weighted by state and urban/rural and within major cities by slum/non-slum. Total sample size includes 699,686 women and data were collected between 2015 and 2016. Participants were selected by sex (female), age (15-49 years), and non-pregnant non-lactation status and those who had measured hemoglobin levels. Hemoglobin was measured on capillary blood samples using HemoCue® and adjusted for smoking and altitude. The details of the NFHS-4 study have been previously described [23].

Variables

Our variables of interest included daily consumption of the following nutrients: copper, folate, iron, riboflavin, thiamine, vitamin A, vitamin B6, vitamin B12, vitamin C, vitamin E, and zinc, and daily intake of fortified food including milk, rice, wheat flour, oil, and salt among WRA in the no-fortification and maximum-fortification scenarios. From the hemoglobin dataset, the variables of interest included WRA's hemoglobin levels and prevalence of anemia in two scenarios: no-fortification and maximum-fortification.

Modeling the effect of fortification and statistical analysis

We modeled the effect of fortification on diet inadequacy and hemoglobin changes among non-pregnant, non-lactating women of reproductive age (WRA) aged 15-49 years. All analyses were conducted using SAS® version 9.4 [24].

Modeling the effect of fortification on diet inadequacy

We utilized Simulating Intake of Micronutrients for Policy Learning and Engagement (SIMPLE) Macro [25] to calculate the percentage of WRA who met nutrient Estimated Average Requirements (EARs) in the no-fortification and maximum-fortification scenarios using the dietary dataset. EARs are the nutrient amount estimated to meet the requirements for half of a population. The values we used for the EARs came from the Indian Council of Medical Research

(ICMR) [26] for most nutrients. However, ICMR does not include EARs for vitamin E and copper; therefore, these were retrieved from the World Health Organization (WHO) [27]. Using the dietary dataset, we estimated the percentage of women meeting EARs for select nutrients (iron, folate, vitamin A, vitamin B12, and zinc) in the no-fortification and maximum-fortification scenarios.

Because we used a single-day 24-hour dietary recall, we could not model usual intake distributions from the proportion of within-individual variance to total variance. Therefore, we had to use an external adjustment factor to correct within-person variance (WIV) to total-person variance. We selected WIV:total proportions based on country, age, sex, and non-pregnant, non-lactating status based using data collected by French et al. [28]. The proportions are as follows: 0.82 for folate [29], 0.57 for iron [30], 0.65 for vitamin B12 [31], and 0.52 for zinc [30] (**Appendix Table B**).

1. No-fortification Scenario

We first estimated the intake averages and standard deviations for all the nutrients involved in hemoglobin synthesis (**Table 3**). We also estimated the usual intake distribution and inadequate intake of folate, iron, vitamin B12, and zinc using SIMPLE macro [25] (**Table 6**).

2. Maximum Fortification Scenario

The Food Safety and Standards Authority of India (FSSAI) created standards for wheat flour, oil, rice, salt, and milk fortification, which are summarized in **Appendix Table A**. For each nutrient, there is a range which indicates a lower concentration and higher concentration to be added per kilogram of food (e.g. 28-42.5 mg/kg of iron added to fortified wheat flour) [8].

The maximum fortification scenario assumes 100% population coverage and industry compliance; in other words, it assumes that 100% of women who consume a food that can be fortified (i.e. milk, oil, rice, salt, and wheat flour) consume a fortified version of the food, and it assumes that 100% of the food is fortified according to government standards.

For each nutrient, we calculated the nutrient contribution from fortification of the five staple foods (milk, oil, rice, salt, and wheat flour) in addition to the amount of the nutrient naturally occurring in the foods by adding no-fortification nutrient intake to fortification standards times the amount of food intake. Folic acid provided through fortification was converted to dietary folate equivalents (DFEs) by multiplying by 1.67 [32]. We generated descriptive statistics (mean and standard deviation) for each nutrient for both low and high concentration of maximum fortification (**Table 3**). We estimated the percentage of women with intakes below the EAR for folate, iron, vitamin B12, and zinc using the SIMPLE macro [25] (**Table 6**). We also calculated the 25th, 50th, and 75th percentile intakes for each nutrient and scenario.

Modeling the effect of fortification on hemoglobin

To calculate the potential hemoglobin increase based on WRA's iron intake, we used results from two meta-analyses to estimate increased hemoglobin from fortification iron intake [33] [34]: one for anemic women (who are expected to absorb more dietary iron than non-anemic women) and one for all women (regardless of anemia status).

Specifically, for anemic women, for each increase by 1 mg/day of fortification iron consumed, the mean change in hemoglobin increases by 0.569 g/L on average (95% Credibility Intervals: [0.169,0.961]) [33]. Whereas for all women, for each increase by 1 mg/day of fortification iron consumed, the mean change in hemoglobin increases by 0.194 g/L on average (95% Credibility Intervals: [0.009,0.446]) [34].

We added the additional hemoglobin from fortification to the adjusted hemoglobin levels for WRA in the no-fortification scenario in the hemoglobin dataset (NFHS-4). We also calculated anemia prevalence in the no-fortification scenario and maximum-fortification scenario. We estimated anemia prevalence among WRA using Indian-recommended cutoffs (<120 g/L) [26] for hemoglobin levels.

Bias

To estimate percentage of WRA not meeting EAR, we had to account for within-person variance in each population by nutrient [22]. When values for Indian WRA were not available (e.g. for folate and vitamin B12), we used values for WRA residing in Bangladesh or Japan.

We accounted for bias by using statistical clustering within the hemoglobin dataset.

Ethical Approval

We submitted the project protocol to the Emory University Institutional Review Board for consideration. Due to the de-identified nature of the secondary datasets used in the analyses, the board determined that the research does not constitute human subjects research, and no further review was required.

Results:

Table 1 shows estimated average requirements for women of reproductive age (WRA) based on Indian [26] or WHO guidelines [27].

Table 1: Estimated Average Requirements (EAR) for all nutrients for women of reproductive age (WRA).

Nutrient	EAR
Copper (mg/d)[1, 3]	1.1
Folate (mcg/d)[2]	180
Iron (mg/d)[2]	15
Riboflavin (mg/d)[2]	2.0
Thiamine (mg/d)[2]	1.4
Vitamin A (mcg/d)[2]	390
Vitamin B6 (mg/d)[2]	1.6
Vitamin B12 (mcg/d)[2]	2
Vitamin C (mg/d)[2]	55
Vitamin E (mg/d)[1, 3]	12
Zinc (mg/d)[2]	11

[1]WHO [27].
[2]Recommended Dietary Allowances and Estimated Average Requirements: Nutrient Requirements for Indians [26].
[3]India does not have dietary guidelines for copper and vitamin E.

Table 2 shows select sociodemographic characteristics among non-pregnant non-lactating women of reproductive age in the National Nutrition Monitoring Bureau (NNMB) Rural Survey [15] and NFHS-4 [16]. Women's mean age was similar in both datasets (~30 years). In the NFHS-4 dataset, 11.4% of the participants completed college, whereas in the NNMB dataset, only 5.9% of participants completed college.

Table 2: Select sociodemographic characteristics of the women of reproductive age from the NNMB Rural Survey dataset [15] and the NFHS-4 dataset [16] in India.

<table>
<tr><th>Characteristic</th><th>NNMB Rural Survey (N=11,625)</th><th>NFHS-4 2015-2016 (N=667,258)</th></tr>
<tr><td>Age (years), mean (SD)</td><td>31.2 (9.8)</td><td>30.1 (9.9)</td></tr>
<tr><td>Highest education completed, n (%)</td><td colspan="2"></td></tr>
<tr><td>Illiterate</td><td>4,128 (35.6%)</td><td rowspan="2">188,407 (28.2%)[1]</td></tr>
<tr><td>Can read & write[5]</td><td>88 (0.8%)</td></tr>
<tr><td>Lower primary education (grades 1-4)</td><td>891 (7.7%)</td><td rowspan="2">84,010 (12.6%)[2]</td></tr>
<tr><td>Upper primary education (grades 5-8)</td><td>2,404 (20.7%)</td></tr>
<tr><td>Secondary education (grades 9-12)</td><td>3,321 (28.6%)</td><td>338,853 (47.8%)[3]</td></tr>
<tr><td>College and above</td><td>777 (6.7%)</td><td>75,988 (11.4%)[4]</td></tr>
</table>

[1] No education.

[2] Primary school education.

[3] Secondary school education.

[4] Education higher than secondary school.

[5] It was assumed that women who can read and write had no formal education.

Table 3 shows the mean intake of the nutrients involved in hemoglobin synthesis in the no-fortification and maximum-fortification scenarios and the percentage change between the two scenarios. The range of outcomes within the maximum fortification scenario reflect modeling of the low concentration and high concentration of fortification for each staple food according to Food Safety and Standards Authority of India (FSSAI) [8] (**Appendix Table A)**. In the maximum fortification scenario, vitamin B6 increased the highest amount, by 240.2-254.5%. Vitamin B12 had the lowest increase, at 12.3-27.6%. Copper, vitamin C, and vitamin E are not included in standards for fortification of oil, wheat flour, salt, rice, and milk in India; therefore, they do not have values for maximum fortification.

Table 3: Mean (SD) daily intake among non-pregnant, non-lactating women of reproductive age in India (N=11,625) from the usual diet (no fortification)[1] and maximum fortification[2] scenarios for nutrients involved in hemoglobin synthesis: analysis of the National Nutrition Monitoring Bureau data [15].

Nutrient (units)	**No fortification Mean (SD)**	**Maximum fortification[3] Mean (SD)**	**Change[4]**
Copper (mg)	1.90 (0.80)	-[5]	-
Folate (DFE[6] mcg)	235.36 (419.60)	273.72 (419.84) - 299.29 (420.47)	16.3 - 27.16%
Iron (mg)	12.27 (7.43)	20.87 (9.10) - 25.32 (10.53)	70.1 - 106.4%
Riboflavin (mg)	0.66 (0.28)	1.04 (0.41) - 1.19 (0.47)	57.58 - 80.3%
Thiamine (mg)	1.09 (0.52)	1.39 (0.59) - 1.55 (0.64)	27.53 - 42.2%

Vitamin A (RAE[7] mcg)	190.69 (473.08)	208.12 (473.63) - 216.19 (473.97)	9.14 - 13.39%
Vitamin B6 (mg)	1.12 (0.45)	3.81 (1.19) - 3.97 (1.29)	240.2 - 254.5%
Vitamin B12 (mcg)	1.34 (3.26)	1.56 (2.58) - 1.71 (2.57)	12.3 - 27.6%
Vitamin C (mg)	37.77 (36.81)	-[5]	-
Vitamin E (mg)	3.34 (3.54)	-[5]	-
Zinc (mg)	7.77 (3.64)	10.83 (4.46) - 12.36 (4.99)	39.4 - 59.1%

[1] No fortification represents current intake from the NNMB dataset with no adjustments.
[2] Maximum fortification represents intake after 100% population coverage and 100% industrial compliance are applied to all nutrients in standards, in addition to nutrients occurring naturally in food. Nutrients with a dash do not have fortification standards in India.
[3] Range based on low concentration and high concentration of fortification standards issued by the Food Safety and Standards Authority of India (FSSAI) [8].
[4] Percent change from no fortification to maximum fortification ranges.
[5] Some nutrients do not have values because they are not included in the Indian food fortification standards [8].
[6] DFE, Dietary Folate Equivalents.
[7] RAE, Retinol Activity Equivalents.

Predicted mean hemoglobin increase after applying the meta-regression equations is shown in **Table 4** for anemic women and for all women, regardless of anemia status. Estimated iron added through fortification was 8.60 - 13.05 mg/d in the maximum fortification scenario. We estimated the mean (SD) increase in hemoglobin for all women to be 4.90 (2.39) - 7.43 (3.62) g/L, and for anemic women, we estimated the mean intake to be 1.67 (0.81) - 2.53 (1.23) g/L.

Table 4: Predicted mean increase in hemoglobin (g/L) in women of reproductive age (15-49 y) in India for maximum fortification scenario based on iron intake: analysis of the National Nutrition Monitoring Bureau (NNMB) dataset [15].[1]

Anemia Status	**Iron added through fortification (mg/d)[2]**	**Hb (g/L) Mean (SD)**
Anemic women	8.60 - 13.05	4.90 (2.39) - 7.43 (3.62)
All women	8.60 - 13.05	1.67 (0.81) - 2.53 (1.23)

[1] For anemic women: for each increase by 1 mg/day of fortification iron consumed, the mean change in hemoglobin increases by 0.569 g/L on average (95% Credibility Intervals: [0.169,0.961]). For all women: for each increase by 1 mg/day of fortification iron consumed, the mean change in hemoglobin increases by 0.194 g/L on average (95% Credibility Intervals: [0.009,0.446]).
[2]Range based on low and high nutrient concentration for each nutrient in fortified foods (milk, oil, rice, salt, and wheat flour) from the Food Safety and Standards Authority of India (FSSAI) **(Appendix Table A)**.

Mean hemoglobin levels and prevalence of anemia are shown in **Table 5** among the women of reproductive age (15-49 y) in the NFHS-4 dataset for the no- and maximum-fortification scenarios. Before fortification, anemia prevalence was 52.6%. After maximum fortification, anemia prevalence decreased to between 35.6% and 42.7%.

Table 5: Hemoglobin and anemia in women of reproductive age (15-49 y) in India in the no-fortification and maximum fortification scenario: analysis of the NFHS-4 dataset.

Indicator	**No fortification**	**Maximum fortification[1]**
Total sample, N	667,258	667,258
Hemoglobin (g/L), mean (SD)	116.37 (16.20)	120.68 (15.21) - 122.4 (14.62)
Anemia, n (%)	350,961 (52.6%)	284,652 (42.7%) - 237,657 (35.6%)

[1] Range based on low concentration and high concentration of maximum fortification from the Food Safety and Standards Authority of India (FSSAI) [8].

The comparison of dietary intake to dietary guidelines for the no- and maximum-fortification scenarios is presented for select nutrients in **Table 6**. For each nutrient, the percent of participants not meeting the Indian or WHO Estimated Average Requirement (EAR) before and after fortification is shown. The 25th, 50th, and 75th percentiles are presented in both scenarios. The percent of women with iron intakes below the EAR for iron decreased from 76% in the no-fortification scenario to 15% and 4% for low concentration and high concentration of maximum fortification, respectively. The percent of women with iron intakes below the EAR for Vitamin B12 only decreased from 72% to 59%-66% in the maximum fortification scenario. For all nutrients, all intake quartiles were higher in the high concentration of the maximum fortification scenario than the no-fortification scenarios.

Table 6: Percent of women of reproductive age (15-49 y) not meeting Indian or WHO Estimated Average Requirements (EARs) for nutrients for the no- and maximum-fortification scenarios and 25th, 50th, and 75th percentile intake values.[1]

Nutrient	**Statistic**	**No fortification**	**Maximum fortification[2]**

			Low Concentration	High Concentration
Iron	WRA with intakes < EAR, %	76	15	4
	Percentile of nutrient intakes (mg/d)			
	25th	8.93	16.69	20.43
	50th	11.51	20.21	24.63
	75th	14.77	24.29	29.70
Folate	WRA with intakes < EAR, %	38	23	15
	Percentile of nutrient intakes (mcg/d)			
	25th	161	183.92	198.92
	50th	197.78	222.33	238.70
	75th	242.98	268.79	286.43
Vitamin B12	WRA with intakes < EAR, %	72	66	59
	Percentile of nutrient intakes (mcg/d)			
	25th	0.64	1.09	1.24
	50th	1.18	1.59	1.77
	75th	2.16	2.32	2.52
Zinc	WRA with intakes < EAR, %	88	55	38
	Percentile of nutrient intakes (mg/d)			
	25th	6.11	8.68	9.89
	50th	7.62	10.59	12.04
	75th	9.43	12.77	14.47

[1] We were unable to model vitamin A using SIMPLE [25] due to the low amount of vitamin A consumed by participants in the NNMB [15].
[2] Range based on low concentration and high concentration of maximum fortification from the Food Safety and Standards Authority of India (FSSAI) [8].

Figures 1-4 visualize the modeled 25th percentile, 50th percentile, and 75th percentile intake of iron, folate, vitamin B12, and zinc, respectively. Results for no-fortification, the low concentration and high concentration of maximum fortification, and percent of women with intakes below the Estimated Average Requirement (EAR) for each nutrient are presented. The 25th, 50th, and 75th percentiles of iron intake exceed the EAR for WRA in the maximum fortification scenario. For iron and zinc, the 75th percentile still fell below the EAR in the no-fortification scenarios. For folate and vitamin B12, the 75th percentile was above the EAR in all three scenarios.

Figure 1: Iron intake among women of reproductive age (15-49 y) at the 25th, 50th, and 75th percentile.

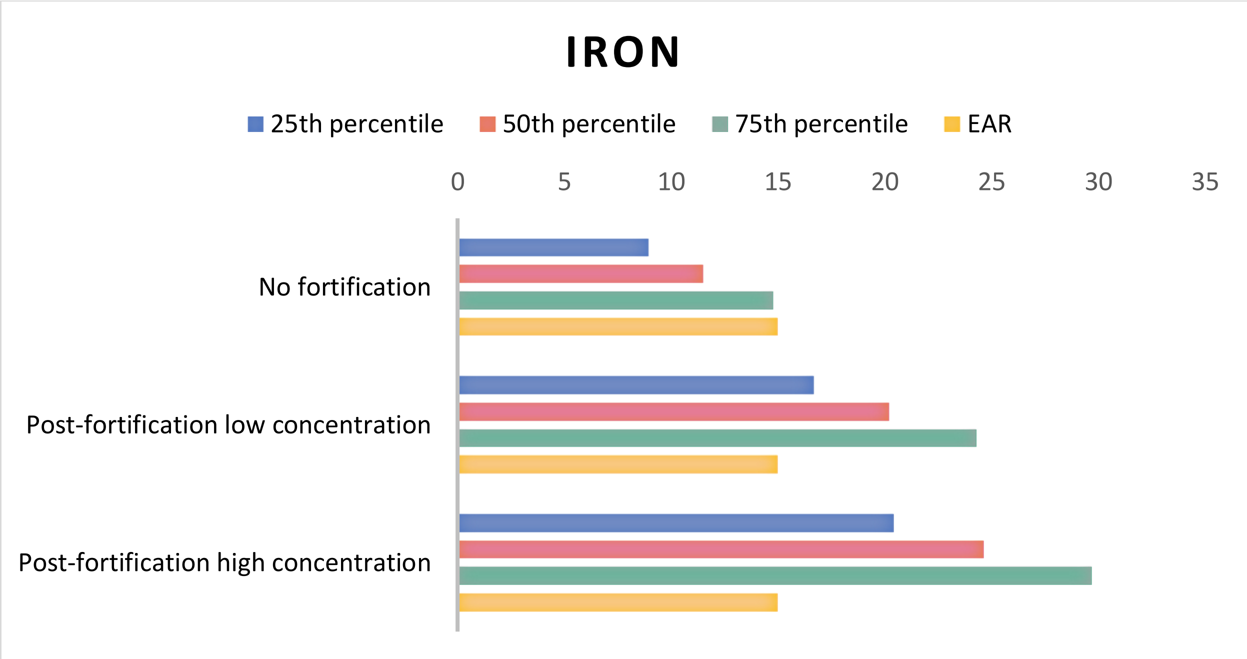


Figure 2: Folate intake among women of reproductive age (15-49 y) at the 25th, 50th, and 75th percentile.

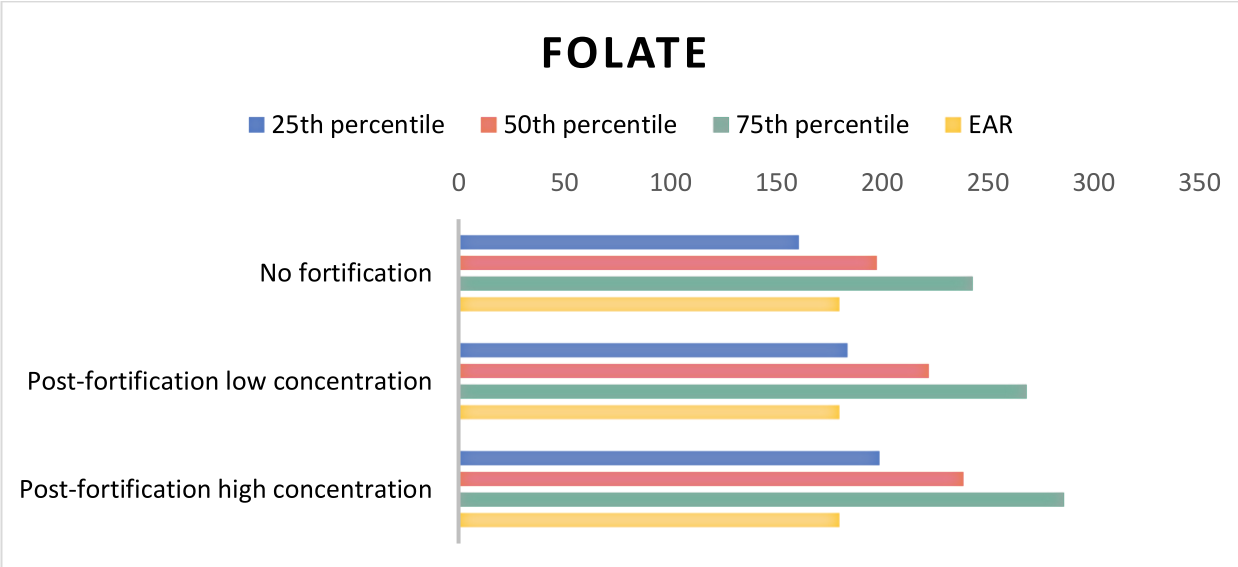


Figure 3: Vitamin B12 intake among women of reproductive age (15-49 y) at the 25th, 50th, and 75th percentile.

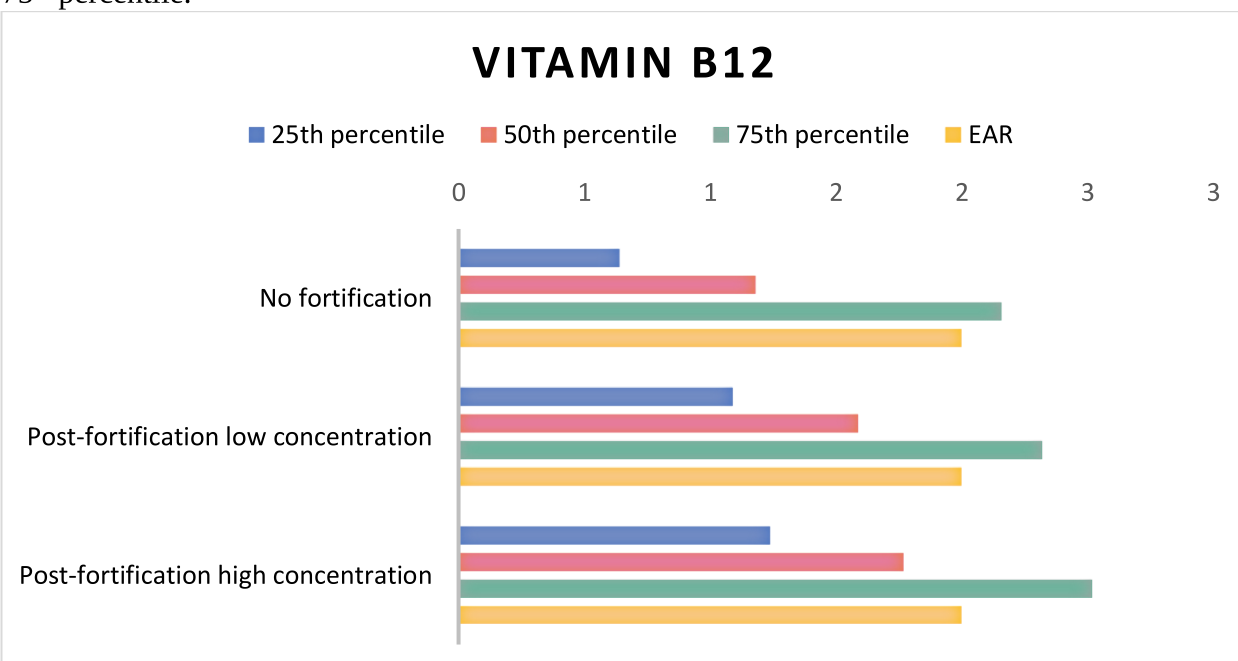


Figure 4: Zinc intake among women of reproductive age (15-49 y) at the 25th, 50th, and 75th percentile.

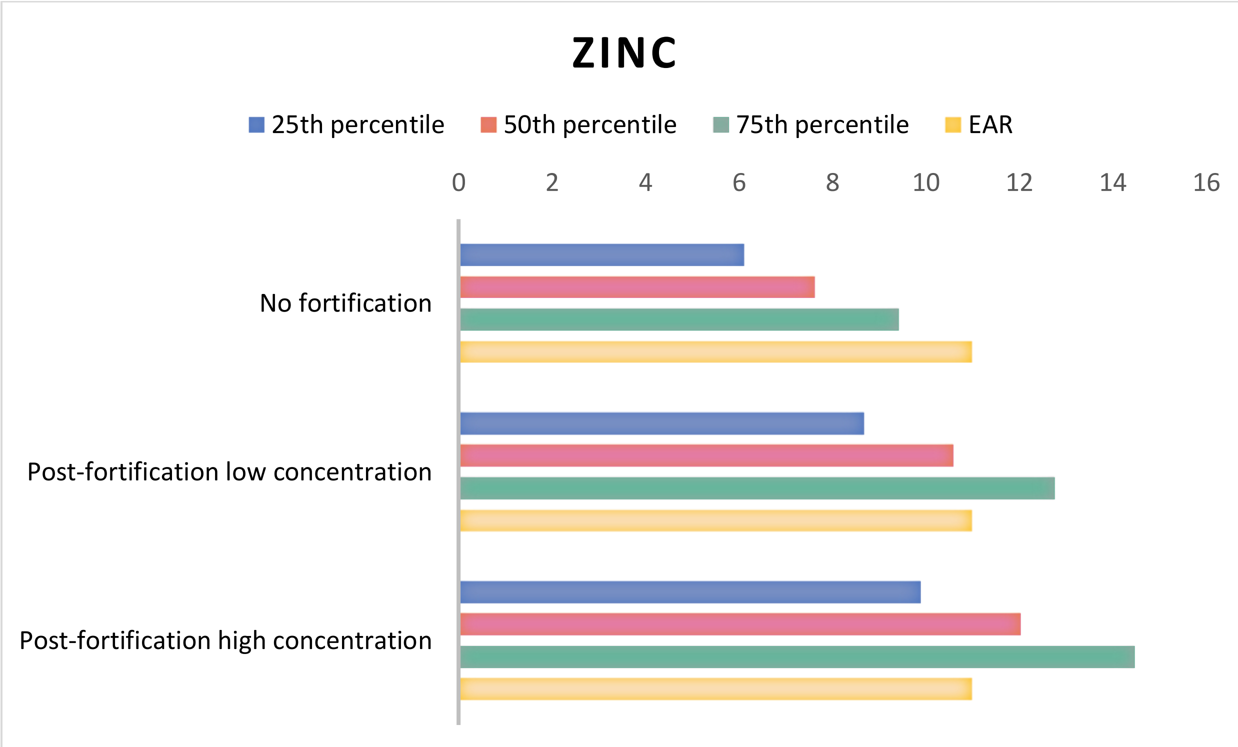
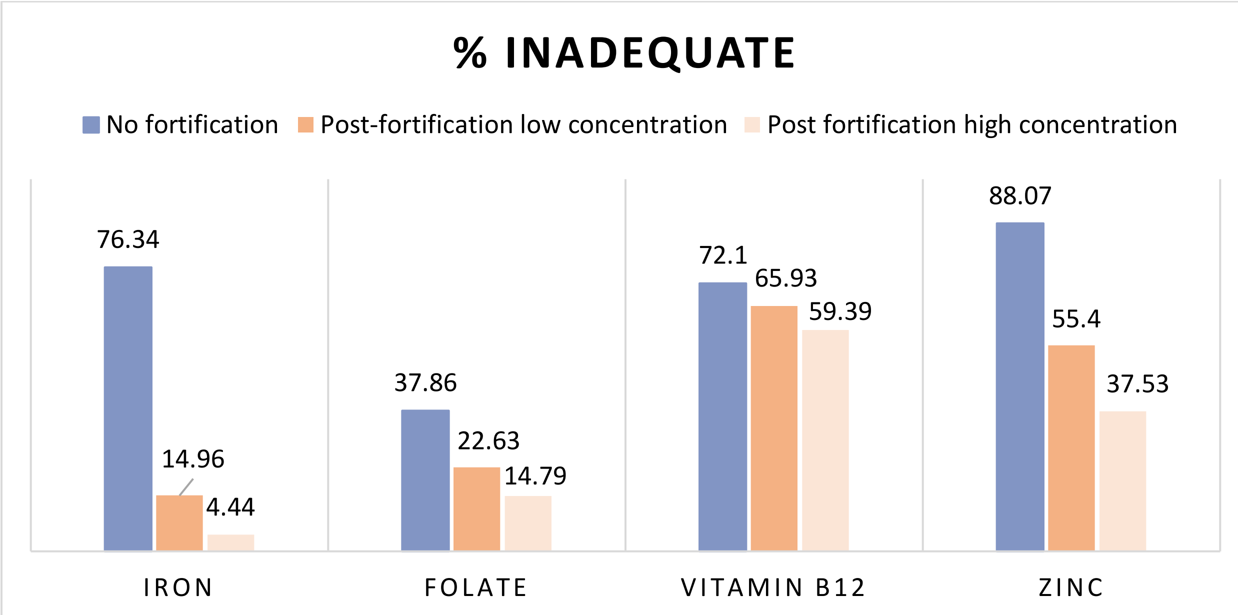


Figure 5 visualizes the percent of the women of reproductive age (15-49 y) not meeting EARs for iron, folate, vitamin B12, and zinc. Results are presented for the no-fortification scenario and maximum-fortification scenarios for the low and high concentration levels. The percent of WRA not reaching EAR decreases between no-fortification and maximum-fortification results for each nutrient modeled, and most significantly for iron and zinc.

Figure 5: Percent of women of reproductive age (15-49 y) with intakes less than Estimated Average Requirements for iron, folate, vitamin B12, and zinc.



<u>Discussion</u>:

This study modeled the potential impact of the fortification of milk, oil, rice, salt, and wheat flour compared with no fortification on dietary intake of nutrients involved in hemoglobin synthesis and percent not meeting the Estimated Average Requirements (EAR) among women of reproductive age (15-49 y) in India. In addition, we estimated maximum hemoglobin levels and anemia prevalence based on additional iron from fortification among WRA. Our results suggest that in a maximum fortification scenario, folate intake may increase by about 26-27%, iron by 70-106%, thiamine by 18-42%, vitamin A by 9-13%, vitamin B12 by 12-28%, and zinc by 39-59%. Percentage of women not meeting EARs decreased from the no-fortification scenario to maximum-fortification scenarios for iron, folate, vitamin B12, and zinc. Twenty-fifth, fiftieth, and seventy-fifth percentile intake amounts increased for iron, folate, vitamin B12, and zinc. Anemia prevalence may decrease by 19-32% in the maximum fortification scenario as a result of the fortification of wheat flour, salt, and rice with iron. These results agree with our initial hypothesis that if 100% of women consume milk, oil, rice, salt, and wheat flour that are fortified to Government of India standards, this will raise levels of intake for the nutrients involved in hemoglobin production and reduce overall anemia prevalence.

Upon our review of the literature, we were unable to find any studies which model the intake of foods fortified with the nutrients involved in hemoglobin synthesis on subsequent hemoglobin changes among WRA in India. Thus, we have included a discussion of evidence from other interventions, including randomized controlled trials, cross-sectional studies, and other secondary analyses analyzing food fortification of several staples and hemoglobin levels among various populations collected in India.

In a secondary analysis, Swaminathan et al. modeled nationally representative dietary iron intake and anemia data among WRA and stratified across geographic regions of India [12]. They found the relationship between iron intake and anemia to be weak and found adverse effects (reducing gut bacteria and causing systemic inflammation) associated with high iron intake among WRA if iron fortification of salt, wheat flour, and rice is increased. Iron fortification was added at the following levels: 850-1100 ppm for salt, 20 mg/kg for wheat flour, 20 mg/kg for rice, whereas our study estimated the addition of 850-1100 ppm iron for salt, 28-42.5 mg/kg iron for wheat flour, and 28-42.5 mg/kg iron for rice. The discrepancy in results may be due to difference in sampling source because they used dietary data from a household consumer expenditure study. They also stratified their analyses using additional demographic characteristics, including body weight, income, and education, and found that some geographic regions may differ in their association of anemia from iron fortification. This study highlights the need to compare results across regions of India where different iron fortification vehicles are most often consumed.

Wirth et al. modelled estimated nutrient intake from fortified rice provided through the public distribution system in one Indian state in children 6-59 months and female caretakers [13]. They added 60 mg/kg iron, 30 mg/kg zinc, 1500 mcg/kg RE vitamin A, 100 mg/kg vitamin C, 3.5 mg/kg thiamine, 4 mg/kg riboflavin, 5mg/kg vitamin B6, 250 mcg/kg folic acid, and 2.5 mcg/kg vitamin B12. These levels of fortification are slightly different than ours. We modelled lower levels for all nutrients: 28 mg-24.5 mg/kg iron, 10-15 mg/kg zinc, 500 mcg/kg RE vitamin A, 1-1.5 mg/kg thiamine, 1.25-1.75 mg/kg riboflavin, 1.5-2.5 mg/kg vitamin B6, 75-125 mcg/kg folic acid, and 0.75-1.25 mcg/kg vitamin B12. They found that consumption of fortified rice would increase amount of population meeting RDAs by at least 80% for iron, zinc, thiamin, riboflavin, niacin, vitamin A, vitamin B6, vitamin B12, and vitamin C. They also modeled 100% coverage of fortified rice within different districts in India but primarily focused their findings on the state of Telangana. They discuss that many households in India may be producing their own rice, and full coverage of fortified rice via public distribution may not be feasible for all districts.

Haas et al. conducted a randomized control trial the effect of double fortified salt (DFS) on anemia among Indian tea-pickers in West Bengal [9]. Women were provided with DFS fortified with 47 mg/kg of iodine provided as potassium iodate and 3.3 mg iron provided as ferrous fumarate per gram of salt, which is slightly different than our study which added 20-30 ppm of iodine and 850-1100 ppm of iron. Anemia prevalence declined by approximately 25%, which is similar to the anemia reduction estimated in our study. They primarily focused on DFS, whereas our study took into consideration all possible fortification vehicles. While their study also looked at women in India, they had a different study group which included women aged 18-55 years and included pregnant women.

In a program evaluation, Chakrabarti et al. estimated the impact of a 4-year food safety net program which provided wheat flour fortified with 50 mg/kg of iron to pregnant women, which is slightly higher than our study which estimated an additional 28-42.5 mg/kg of iron per person in the maximum fortification scenario [10]. They did not find a reduction in anemia prevalence in Northern India but did find one in Southern India, where demand for wheat flour was higher. The reason that hemoglobin is not impacted by intake of wheat flour in Northern India may be because the program only reached nonpoor households which were not at risk for anemia. They argue that widespread implementation is necessary to reach the populations most at risk for anemia. Their findings were different than ours because they restricted the study group to pregnant women, who have different dietary needs than all WRA.

Madhari et al. modeled the dietary intake of several nutrients, including vitamin B12, iron, and folate among school children (6-17 y) in South India before and after fortification and supplementation [11]. They modeled intake of fortified foods and iron-folic acid supplements. They added nutrient fortification at the same levels as our study: for salt, 850-1100 ppm iron, for rice and wheat flour, 28-42.5 mg/kg iron, 75-125 mcg/kg folic acid, 0.75-1.25 vitamin B12, for oil 6-9.9 mcg/kg vitamin A, and for milk 270-450 mcg/L vitamin A. Iron inadequacy, defined as percent of women with intakes below the EAR, decreased; however, deficient intakes among vitamin B12 and folate remained high. They also found that there is a potential for excess intake among iron, which we did not measure. This could be due to the addition of iron supplementation in their analysis. The population analyzed was children, which have different dietary needs than WRA.

Our study had several strengths. In the NNMB dataset, nutrient values for foods consumed by participants were included for folate, iron, riboflavin, thiamine, vitamin A, vitamin

C, and zinc. Utilization of the SIMPLE macro reduced the intra-person variation for estimating the percentage of women not meeting EARs. Very few studies have used this method of estimating dietary intake, and no studies with Indian dietary data have used this method. By creating our own food composition table, we were able to assign nutrient profiles for copper, vitamin B6, vitamin B12, and vitamin E to all of the 394 foods in the NNMB dataset that were consumed by participants.

Our study also had several limitations. The meta-regressions we used were dependent on literature reviews where one of the nutrients involved in hemoglobin synthesis was administered to WRA and the impact of hemoglobin was measured; however, the only nutrient added through the interventions was iron. Thus, we were only able to estimate increased hemoglobin levels from the addition of fortification iron. Because we only received meta-regression data on hemoglobin increase from iron intake, we cannot speak to the efficacy of fortification with additional nutrients to reducing anemia. We had to use two separate datasets to analyze diet and hemoglobin, with two different participant groups. Therefore, we were only able to provide approximate values for increased hemoglobin. Another limitation of the study is the estimation of food intake through the NNMB data. Because the NNMB broke down food items into ingredients, it is possible that oil intake was underestimated. The small amount of oil consumed made it impossible to estimate EAR values for vitamin A, as oil is the primary staple fortified with vitamin A. Additionally, the 2017 Indian Food Composition table did not have every food consumed by the NNMB participants; therefore, we had to draw on additional sources which could have created variance in nutrient values. Additionally, the NNMB dataset was only representative of the rural population.

In the context of previous studies, our study suggests that the fortification of wheat flour, rice, and salt with iron may be an effective method of decreasing nutritional anemia among WRA in India. There are also dietary benefits to fortification of other vehicles, including oil and milk. However, assuming a 100% coverage and compliance for fortification implementation may be highly unrealistic. Further research is needed to demonstrate the effectiveness of fortification on preventing anemia via other micronutrients besides iron. Policymakers may want to evaluate the benefits of food fortification when considering making fortification mandatory in India.

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Appendix:

Table A. Summary of the fortification standards for India [8].

Food	**Nutrient**	**Amount**	**Comment**
Wheat flour	Iron	28 mg – 42.5 mg/Kg	Added at a higher level to account for less bioavailability
	Folic acid	75 mcg –125 mcg/Kg	
	Vitamin B12	0.75 mcg – 1.25 mcg/Kg	
	Zinc	10 mg –15 mg/Kg	
	Vitamin A	500 mcg RE – 750 mcg RE/Kg	
	Thiamine	1 mg – 1.5 mg/Kg	
	Riboflavin	1.25 mg - 1.75/Kg	
	Niacin	12.5 mg - 20 mg/Kg	
	Vitamin B6	1.5 mg – 2.5 mg/Kg	
Rice	Iron	28 mg – 42.5 mg/Kg	Added at a higher level to account for less bioavailability
	Folic acid	75 mcg – 125 mcg/Kg	
	Vitamin B12	0.75 mcg – 1.25 mcg/Kg	
	Zinc	10 mg – 15 mg/Kg	
	Vitamin A	500 mcg RE – 750 mcg RE/Kg	
	Thiamine	1 mg – 1.5 mg/Kg	
	Riboflavin	1.25 mg – 1.75 mg/Kg	
	Niacin	12.5 mg – 20 mg/Kg	
	Vitamin B6	1.5 mg – 2.5 mg/Kg	
Double Fortified Salt	Iodine	20 – 30 parts per million	

	Iron	850 – 1100 parts per million	
Edible Oil	Vitamin A	6 mcg RE – 9.9 mcg RE/g	
	Vitamin D	0.11 mcg - 0.16 mcg/g	
Milk	Vitamin A	270 mcg RE – 450 mcg RE/L	
	Vitamin D	5mcg – 7.5 mcg/L	

Table B. Summary of within-person variance proportions used for SIMPLE macro [28].

Nutrient	Country	Age (y) (SD)	Proportion	Source
Folate	Japan	30-49	0.82	Fukumoto [29]
Iron	India	31.4 (7.7)	0.57	Hebert et al. [30]
Vitamin B12	Bangladesh	15-49	0.65	Arimond et al. [31]
Zinc	India	43.2 (13.6)	0.52	Hebert et al. [30]