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Oluwaseun A. Odewole Date

Near elimination of folate deficiency anemia by mandatory folic acid fortification in older US adults: REasons for Geographic and Racial Differences in Stroke study (REGARDS) 2004-2007

By

Oluwaseun A. Odewole

Master of Public Health

Hubert Department of Global Health

____

Godfrey Oakley, MD, MSPM

Committee Chair

____

Amy Webb-Girard, PHD

Committee Member

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By

Oluwaseun A. Odewole

M.B.B.S

University of Ilorin, Nigeria

2008

Thesis Committee Chair: Godfrey P. Oakley Jr., MD, MSPM

An abstract of

A thesis submitted to the Faculty of the

Rollins School of Public Health of Emory University

in partial fulfillment of the requirements for the degree of

Master of Public Health

in Global Health

2012

ABSTRACT

Prevalence of Folate deficiency and Folate Deficiency Anemia in the REasons for Geographic and Racial Differences in Stroke study (REGARDS)

By: Oluwaseun A. Odewole

BACKGROUND: The US implemented mandatory folic acid fortification in 1998. While several studies have documented the resulting decrease in anemia and folate deficiency, none has determined the prevalence of folate deficiency anemia post-fortification.

OBJECTIVE: We aim to determine the prevalence of folate deficiency and folate deficiency anemia within a sample of the REGARDS cohort.

METHODS: REGARDS is a prospective cohort (collected from January 2003 to October 2007) comprised of 42% black and 58%white participants. We measured mean serum folate concentrations on a sample of 1,547 REGARDS participants who were 45 years and older and had baseline hemoglobin and red cell mean corpuscular volume (MCV) measurements. Folate deficiency was defined as serum folate concentrations <3.0 ng/mL and anemia was defined using both the WHO definition and age, sex, race specific criteria. Folate deficiency anemia was defined as the presence of both folate deficiency and anemia.

RESULTS: Mean hemoglobin level was 13.6g/dL and the prevalence of anemia by WHO definition in the sample was 15.9%. Mean serum folate was 14.6 ng/mL and two (0.13%) participants were found to be folate deficient. Both participants are African American females with markedly elevated C reactive protein levels and macrocytosis but had normal serum B12 levels. Of these two participants, only one had folate deficiency with anemia. Overall, the prevalence of folate deficiency anemia in the sample was 0.06%.

CONCLUSION: There is almost no folate deficiency anemia in our sample of REGARSDS participants from the post-fortification era. Thus, in addition to being instrumental in the decline of neural tube defects, our data suggests that folic acid fortification has also resulted in nearly eliminating the prevalence of folate deficiency anemia in the US in particular among those over age 50.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Godfrey Oakley my thesis advisor for giving me the opportunity to work on this project; I would also like to express my gratitude for his guidance, support and mentorship throughout this process. I would like to thank Amy Webb-Girard, PHD for her help and guidance throughout my MPH Degree and for agreeing to be on my thesis committee. Finally I would like to thank my family and friends for their continuous support of all my endeavors.

Table of Contents

BACKGROUND 1
MANUSCRIPT 4
Student contribution: 5
Abstract 6
Introduction: 8
Methods: 9
Results: 11
Discussion: 13
References: 16
Tables 20
Table 1: Characteristics of study participants, REGARDS 2003-2007 20
Table 2: Hematologic Parameters of study participants, REGARDS 2003-2007 21
Table 3: Characteristics of folate deficient participants, REGARDS 2003-2007 22
PUBLIC HEALTH IMPLICATIONS AND RECOMENDATIONS: 23
REFERENCES: 25

BACKGROUND

Anemia:

Anemia is defined by the World Health Organization(WHO) as hemoglobin levels less than 13.0g/dL in men and 12.0g/dL in women[1]. Anemia is usually viewed as an abnormal laboratory value with any associated morbidity and mortality being a result of underlying disease condition [2].

The US population is living longer in with the elderly population being projected to increase to about 50 million by 2050 [2-4]. The prevalence of anemia increases with advancing age, It has been estimated that about 10% of the US population aged 65 and older are anemic [5,6]. Estimates of nutritional anemia from before mandatory folate fortification of flour was instituted in the US show that nutritional anemia accounted for about one third of all anemia etiology in the elderly with folate deficiency alone accounting for 6.4 % of anemia and the combination of folate and vitamin B12 deficiency accounting for an additional 2% [6].

Folic acid and Mandatory Fortification:

Folate (Vitamin B9) is a water soluble vitamin which may occur naturally in food while folic acid is its synthetic form [7,8]. Folate is required for DNA synthesis and maintenance and is also a carbon atom source for other cellular pathways such as RNA synthesis and protein methylation [8-10]. It is also required for red blood cell synthesis and its deficiency can lead to megaloblastic anemia [9,11]. Folic acid deficiency in women has also been linked to neural tube defects prevention as demonstrated in the UK MRC study which showed a 70% risk reduction of neural tube defect recurrence in women who took 4000µg/day folic acid preconception [12]. Pre-conception folic acid use was later shown to prevent initial occurrence of neural tube defects [13].

The US Food and Drug Administration (FDA) required that all enriched cereal grains sold in the United States should have folic acid by January 1, 1998 [14]. This was in response to the Centers for Disease Control's (CDC) recommendation that women consume 400µ of folic acid daily in order to decrease the risk of neural tube defects [15]. Subsequently the incidence of neural tube defect affected pregnancies in the US has been estimated to have decreased by about 26% [16-19].

An added benefit from of fortification is the increase in circulating serum and red blood folate levels with the concomitant decrease in folate deficiency. Several studies have shown a decrease in the prevalence of folate deficiency in the US since fortification. In an analysis of the Framingham offspring cohort, Jacques et al found that among those who did not report using multi-vitamins the prevalence of folate deficiency (< 3 ng/ml) decreased from 22.0% in the pre-fortification era to 1.7% in the post-fortification era ($p < 0.001$) [20]. In their comparison of NHANES (1999 -2000) to NHANES III, Pfeiffer et al also found a similar decrease in the prevalence of folate deficiency (< 2.99ng/mL) from 16% to 0.5% [21] . Similar reductions were also reported in smaller non-population based studies [22-24].

Studies have also shown an increase in hemoglobin and hematocrit levels and a decrease in the prevalence of anemia since fortification. Ganji et al found a significant increase in mean hemoglobin levels from 15.1 to 15.4 g/dL (P < 0.0001) in men and from 13.3 to 13.6 g/dL (2.3%; P < 0.0001) in women, when pre and post fortification levels were compared. They also reported a 12% decrease in the prevalence of anemia in males (P=0.1758) and a 27.9% decrease in females (P=0.0005) when anemia prevalence was compared pre and post fortification [25].

While studies have been done to examine the prevalence of folate deficiency in the post fortification era, none has looked at the prevalence of folate deficiency anemia specifically. This study aims to determine the prevalence of folate deficiency, anemia, and folate deficiency anemia in the Reasons for Geographic and Racial Differences in Stoke (REGARDS) cohort.

MANUSCRIPT

Near elimination of folate deficiency anemia by mandatory folic acid fortification in older US adults: REasons for Geographic and Racial Differences in Stroke study (REGARDS) 2004-2007

Oluwaseun Odewole[1], MBBS, MPH Candidate;

Rebecca Williamson [1], MPH, (rswill6@emory.edu);

Neil A. Zakai [2], MD, MSc, (Neil.Zakai@uvm.edu);

R.J Berry[3], MD, MPHTM, (rjb1@cdc.gov);

Suzanne Judd[4], Ph.D., (sjudd@ms.soph.uab.edu);

Yan Ping Qi[3], MPH, (irv7@cdc.gov);

Demilade Adedinsewo[1], MBChB, (dadedin@emory.edu);

Godfrey P. Oakley Jr., MD, MSPM[1] (gpoakley@mindspring.com);

Affiliations: [1]Rollins School of Public health, Emory University

[2] University of Vermont College of Medicine, Departments of Medicine and pathology

[3]Centers for Disease Control, Atlanta

[4]University of Alabama School of Public Health

Corresponding Author: Godfrey P. Oakley Jr.

Department of Epidemiology

Rollins School of Public Health

Emory University

gpoakley@mindspring.com

Telephone: (404) 727-2656

Fax: 404-325-6918

Journal Intended for submission: JAMA

Word count: 1640

Number of Tables: 3

Student contribution:

I (Oluwaseun Odewole) conducted a secondary data analysis on the prevalence of folate deficiency and folate deficiency anemia in the REGARDS cohort from 2003-2007 in collaboration with my supervisor, Godfrey Oakley Jr., MD, Suzanne Judd, PhD and Neil Zakai, MD both REGARDS investigators, and other members of the research team from Emory University and Centers for Disease Control and Prevention who are listed as co-authors. While I was given a clean dataset, I was responsible for formulating a research question, developing a research protocol and analysis plan, securing Institutional Review Board (IRB) approval (which was determined to be exempt since the data had been earlier de-identified), variable creation including the use of standard measures in the definition of study outcome (anemia and folate deficiency) and covariates (Vitamin B-12 deficiency), and all subsequent data analyses for the results presented at the conclusion of the study. I conducted all analyses using SAS 9.3® (SAS Institute Inc. Cary, NC). Results were summarized in tables and presented to collaborators for review. I was responsible for writing this thesis and the forthcoming manuscript, analyzing and interpreting the results.

Abstract

Near elimination of folate deficiency anemia by mandatory folic acid fortification in older US adults: REasons for Geographic and Racial Differences in Stroke study (REGARDS) 2004-2007

CONTEXT: The US completed implementation of mandatory folic acid fortification of "enriched" cereal grains in 1998. While several studies have documented the resulting decrease in anemia and in folate deficiency, none has determined the prevalence of folate deficiency anemia post-fortification.

OBJECTIVE: We aim to determine the prevalence of folate deficiency and folate deficiency anemia within a sample of the REGARDS cohort.

DESIGN AND SETTING: REGARDS is a prospective cohort of 30,239 participants (collected from January 2003 to October 2007) comprised of 42% black and 58% white participants 45 years and older.

PARTICIPANTS: We measured serum folate concentrations in 1,547 REGARDS participants who were 50 years and older and had baseline hemoglobin and red cell mean corpuscular volume measurements. Folate deficiency was defined as serum folate concentrations <3.0 ng/mL and anemia was defined using both the WHO definition and Beutler's age, sex, race specific criteria. Folate deficiency anemia was defined as the presence of both folate deficiency and anemia.

RESULTS: Mean hemoglobin level was 13.6g/dL and the prevalence of anemia, by WHO definition, was 15.9%. The median serum folate concentration was 15.1ng/mL and only two (0.13%) participants were found to be folate deficient. Both participants are African American females with markedly elevated C-reactive protein levels and macrocytosis; but had normal serum cobalamin concentrations. Only one participant had folate deficiency anemia. Overall, the prevalence of folate deficiency anemia was 0.06%.

CONCLUSION: There is almost no folate deficiency anemia in older Americans post mandatory folic acid fortification. Thus, in addition to being responsible for the decline of neural tube defects, our data suggests that folic acid fortification has also resulted in

nearly eliminating folate deficiency anemia in the United States. Hence folate deficiency anemia may rarely be observed in clinical practice.

Introduction:

The global prevalence of anemia in the elderly (age >65 years) is estimated to be 23.9%, resulting in approximately 164 million elderly people being anemic worldwide [26]. Nutritional deficiencies (including folate, cobalamin, and iron), chronic inflammation, and renal insufficiency are some of the common causes of anemia in the elderly [6]. Nutritional anemia accounted for about one third of all anemia etiology in the elderly US population prior to the implementation of folic acid fortification [6]. In an analysis of the Third National Health and Nutrition Examination Survey (NHANES III phase 2, 1991-1994), folate deficiency alone accounted for 6.4% of anemia and the combination of folate and cobalamin deficiency accounted for an additional 2% [6]. These estimates represent prevalence of folate deficiency anemia in the pre-folic acid fortification era.

In March 1996 the US Food and Drug Administration required mandatory fortification of "enriched" cereal grains with folic acid to be implemented by January 1998 [14]. This requirement has resulted in Americans consuming an average of 138 µg/day of folic acid from mandatory fortification [27].

The CDC recently called mandatory folic acid fortification and the resulting decrease in the prevalence of neural tube defects one of the ten greatest public health achievements of the decade [28]. There has also been a decrease in the prevalence of both folate deficiency and anemia [20-25]; however, the effect of fortification on the prevalence of folate deficiency anemia has yet to be determined. This study aims to determine the prevalence of folate deficiency, anemia, and folate deficiency anemia in a sample of the Reasons for Geographic and Racial Differences in Stoke (REGARDS) cohort.

Methods:

Study population and Participants:

REGARDS is a prospective, longitudinal study of 30,239 subjects aged 45 and older recruited between January 2003 and October 2007 to better understand geographic and racial differences in stroke incidence in the US. Study design and inclusion criteria for REGARDS have been previously published [29]. Briefly, physical measurements, a resting electrocardiogram, medication inventory, phlebotomy and urine collection were performed during an in-home visit at baseline. Participants also completed self-administered questionnaires, including a Food Frequency Questionnaire (FFQ), at baseline and are contacted by telephone at 6 month intervals about stroke symptoms, hospitalizations and general health status. We selected a random sample of 1,547 REGARDS participants aged ≥50 years with baseline complete blood count and measured their serum folate and cobalamin concentrations. One participant had insufficient stored sample for serum folate testing and was excluded from the analysis. Our final analytic sample size was 1,546 participants. We secured Emory's Institutional Review Board (IRB) approval, however our study was determined to be exempt since the data had been earlier de-identified.

Measurements:

A complete blood count was performed the day after sample collection by automated cell counting on a Beckman Coulter LH 755 Hematology Workcell (Beckman Coulter, Inc. Fullerton, CA). Serum folate levels were measured retrospectively on stored serum samples collected at baseline using the Cobas Folate II assay (Roche Diagnostics, Indianapolis, IN) in the Laboratory for Clinical Biochemistry Research at the University of Vermont (www.med.uvm.edu/lcbr/). The range of the folate assay is 0.6–20 ng/mL. Serum cobalamin concentrations were measured retrospectively using the Elecsys cobalamin assay (Roche Diagnostics, Indianapolis, IN) at the clinical lab. The range of the cobalamin assay is 22–1,476 pmol/L.

Outcome Definitions

Folate deficiency was defined as serum folate concentrations <3 ng/mL [30]. Anemia was defined using both the World Health Organization (WHO) criteria (<12 g/dL for women and <13 g/dL for men) and age, sex, race specific criteria defined by Beutler [1,30]. Folate deficiency anemia was defined as the presence of both folate deficiency and anemia using both anemia definitions. We provide a descriptive overview of the participants with folate deficiency.

Analysis:

The statistical software SAS 9.3 (SAS Institute Inc. Cary, NC) was used to determine the descriptive statistics and the prevalence of anemia, folate deficiency, and folate deficiency anemia. For the purpose of our analysis, we assigned participants with serum folate concentrations >20 ng/mL (the upper limit of quantification for the assay) a value of 20 ng/mL. Thus we report median serum folate concentrations for our sample. For those with folate deficiency, we provide observed demographic, clinical and laboratory values.

Results:

We included 1,546 REGARDS participants in our analysis. The mean age of our sample was 65.4 years (SD = 8.8), 54.1% were white, and 60.0% were female. A summary of the demographic characteristics of study participants can be seen in Table 1.

The mean hemoglobin concentration of the entire sample was 13.6 g/dL. The lowest mean hemoglobin levels were found in black females (12.7 g/dL) while the highest were found in white males (14.6 g/dL) [Table 2]. The prevalence of anemia in our sample using the WHO criteria was 16.0%; however, when anemia was classified by age, gender and race cutoffs (Beutler's definition) the prevalence was 13.1% [1]. The highest prevalence of anemia (by WHO definition) in our sample was found in black females (25.3%) while the lowest was found in white males (7.7%) [See Table 2].

Overall, 18.2% (n=282) of our sample had serum folate levels above the upper limit of the assay (20ng/mL). The median serum folate was 15.1ng/mL and the prevalence of folate deficiency in our sample was 0.13% (2/1,546).

Using the WHO definition of anemia in combination with folate deficiency, there was only one participant with folate deficiency anemia (1/1,546; 0.06%). There were no cases of folate deficiency anemia using the combination of folate deficiency and anemia by Beulter's definition [Table 2].

Table 3 shows the characteristics of the two folate deficient REGARDS participants. Both participants were African American females, had normal serum cobalamin levels (280.2

pmol/L, 208.6 pmol/L), markedly elevated high sensitivity C-reactive protein levels (43.8 mg/L, 17.4 mg/L) and macrocytosis (MCV : 113 fL , 99 fL).

Discussion:

After mandatory folic acid fortification in the United States, there is almost no folate deficiency or folate deficiency anemia. In our study there were two individuals with folate deficiency while only one had concurrent anemia. Hence, the prevalence of folate deficiency was 0.13% and that of anemia associated with folate deficiency was 0.06%. Our findings in this cohort suggest that mandatory folic acid fortification has improved the health of the older Americans by virtually eliminating folate deficiency and folate deficiency anemia.

While the contribution of mandatory flour fortification in North America to the reduction in neural tube defects has been documented [18,19,28,31-33]; not enough has been said about its benefits in reducing folate deficiency anemia in the older population. Our finding of a folate deficiency anemia prevalence of 0.06% is over a hundred fold less than pre-fortification estimates of 6.4% in the elderly US population [6,34]. We found a folate deficiency prevalence of 0.13% which is in keeping with other population based analysis of serum folate levels after fortification. For example, in the Framingham offspring cohort (which was composed of middle aged and elderly individuals) among non-supplement users, Jacques et al. found a folate deficiency prevalence of 1.7% [20]. Using data from NHANES, Pfeiffer et al. also observed a low prevalence of folate deficiency (0.5%) post-fortification [21].

Other countries that have instituted mandatory folate fortification have corroborated the findings in the US. For instance, in the Canadian health measures survey, a population-based cohort, Macfarlane et al. found a less than 1% prevalence of folate deficiency post-fortification [35,36].

A hospital laboratory based study from Australia also showed a 77% decrease (from 9.3% to 2.1%) in the incidence of low serum folate levels after mandatory folic acid fortification when compared to pre-fortification [37]. Hospital based studies have also documented low to non-existent folate deficiency and folate deficiency anemia post mandatory fortification so much so that questions have been raised about the utility of screening for folate deficiency in hospitalized patients with anemia [22,38,39].

One of the strengths of this study is that our sample is obtained from a population-based cohort. The clinical snapshot we have of participants with folate deficiency/folate deficiency anemia based on information collected by REGARDS is also strength of our study. Our study is limited by the fact that only white and black races are represented in the REGARDS cohort which may affect its generalizability. Because the REGARDS data was collected only after mandatory folic acid fortification in the US, we do not have pre-fortification estimates from the same population for comparison. Also, since we defined folate deficiency anemia as the concurrent appearance of low serum folate with anemia, we cannot be certain that the anemia is due to folate deficiency. Given the participant(s) elevated C - reactive protein levels, chronic inflammation and anemia of chronic disease cannot be ruled out as potential causes of anemia. The two participants in our sample with folate deficiency also had a prior history of stroke on entry to the study. Additionally they both had low caloric intake hence poor diet/under-nutrition may have played a role in the etiology of folate deficiency/folate deficiency anemia in them. Thus, our estimate of the prevalence of anemia associated with folate deficiency post mandatory folic acid fortification as 0.06 percent may be an over estimate.

We can conclude that both folate deficiency and anemia associated with folate deficiency have essentially been eliminated post-fortification. In addition to reducing the occurrence of neural tube defects and anencephaly, reductions in both the prevalence of folate deficiency and folate deficiency anemia are an added benefit of mandatory flour fortification. Policy discussions on whether or not to require mandatory fortification should not only consider the well demonstrated role of mandatory folic acid fortification in preventing spina-bifida and anencephaly, but should also include the virtual elimination of folate deficiency and folate deficiency anemia among those ages 50 years and older.

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Tables

Table 1: Characteristics of study participants, REGARDS 2003-2007

Characteristic (N=1,547)	Number (n)	Prevalence (%)	Mean	Standard deviation
Age	--	--	65.37	8.81
White	837	54.14	--	--
Female	928	60.03	--	--
Education				
College	550	35.67	--	--
Some college	419	27.17	--	--
High school	381	24.71	--	--
Less than high School	192	12.45	--	--
Income *(1000's)*				
<$20	272	17.59	--	--
$20 - $34	350	22.64	--	--
$35 - $74	490	34.69	--	--
$ > $75	229	14.81	--	--
Not reported	205	13.26	--	--
Multivitamin use based on medication inventory	559	36.13	--	--
Comorbidity				
Mean C-reactive protein	--	--	4.51	7.87
Prior stroke	93	6.03	--	--
Prior transient ischemic attacks	59	4.08	--	--

Table 2: Hematologic Parameters of study participants, REGARDS 2003-2007

Parameter	All N = 1,546	White males N = 363	White females N = 474	Black males N =255	Black females N = 454
Serum folate (ng/mL)					
Median	15.09	15.65	16.73	13.21	13.87
Folate deficiency[1], n (%)	2 (0.13)	0.00	0.00	0.00	2 (0.44)
Serum cobalamin (pmol/L)					
Mean (SD)	661.70 (353.82)	559.51 (292.07)	632.39 (331.76)	699.53 (374.28)	752.72 (383.33)
Cobalamin deficiency[2], n (%)	41 (2.65)	12 (3.31)	15 (3.16)	4 (1.57)	10(2.20)
Corpuscular volume (fL)					
Mean (SD)	89.71 (6.04)	91.88 (4.51)	91.00 (4.93)	88.71 (6.85)	87.20 (6.64)
Macrocytosis[3], n (%)	282 (18.24)	92 (25.34)	93 (18.62)	46 (18.04)	51 (11.23)
Hemoglobin (mg/dL)					
Mean (SD)	13.57 (1.43)	14.64 (1.23)	13.44 (1.17)	13.84 (1.39)	12.70 (1.22)
Anemia by WHO definition, n (%)	247 (15.97)	28 (7.71)	42 (8.90)	62 (24.31)	115 (25.33)
Anemia by Beutler's definition [4], n (%)	202 (13.07)	39 (10.74)	52 (10.97)	51 (20)	60 (13.22)
Folate deficiency anemia					
WHO criteria, n (%)	1 (0.06)	0.00	0.00	0.00	1(0.87)
Beutler's criteria, n (%)	0.00	0.00	0.00	0.00	0.00

1. Folate deficiency is serum folate <3ng/mL

2. Cobalamin deficiency is serum cobalamin ≤148 pmol/L

3. Macrocytosis is defined as MCV >95 fL

4. Beutler's criteria for anemia:

- White males: age 20-59 years = 13.7mg/dL; age 60+=13.2mg/dL

-White females: age 20-50+ =12.2mg/dL

-Black Males: age 20-59=12.9 mg/dL; age 60+ 12.7 mg/dL

-Black females: age 20 to 50+ =11.5ng/d

Table 3: Characteristics of folate deficient participants, REGARDS 2003-2007

Characteristic	Participant I	Participant II
Demographics		
Age	54	66
Sex	Female	Female
Race	African American	African American
Region	Buckle[1]	Belt[2]
Education level	Some college	High school
BMI	33.5	25.8
Smoking	Current	Never
Alcohol use	Past	Never
Caloric Intake		
Daily caloric intake (KCALs)	752	1,056.4
Estimated folate intake from fortified foods(FFQ)	50.3	169.9
Estimated folate intake from natural foods(FFQ)	97.29	67.5
Multivitamin Use		
Multivitamin use in medication inventory	No	No
Multivitamin use in FFQ	No	Yes
Hematologic Parameters		
Serum folate (ng/mL)	2.66	1.94
Serum cobalamin(pmol/L)[3]	280.2	208.6
Hematocrit (%)	36.3	36.1
Hemoglobin levels (g/dL)	12.6	11.8
MCV [4](fL)	113	99
Anemia by WHO definition	No	Yes
Anemia by Beutler's definition[1]	No	No
C-reactive protein levels(mg/L)[5]	43.8	17.4
Past medical History		
Participant reported stroke at baseline	Yes	Yes
History of cancer diagnosis	No	-
Self-reported health	Good	Good
Self-reported hypertension	Yes	No
Self-reported diabetes	No	No

1. The stroke Buckle is a region within the stroke belt that has a higher stroke mortality rate than the rest of the stroke belt. It includes the coastal plains of Georgia, North Carolina and South Carolina 29.

2. The Stroke Belt is a region in the Southeastern part of the US with high stroke mortality. It is comprised of 8 states: Georgia, North Carolina, South Carolina, Mississippi, Tennessee, Alabama, Louisiana, and Arkansas 29.

3. Cobalamin deficiency is defined as serum cobalamin ≤148 pmol/L

4. MCV >=95fL used as cutoff for macrocytosis.

5. High sensitivity C- reactive protein elevation is defined as >10mg/L.

PUBLIC HEALTH IMPLICATIONS AND RECOMENDATIONS:

Mandatory flour fortification with folic acid illustrates how a single public health intervention can have multiple health benefits in various populations. Fifty three countries currently have regulations for mandatory folate fortification in place [8]. However most developing countries are yet to make the decision to make folate fortification mandatory. This deprives the populations of these countries the opportunity to benefit from a proven public health intervention [8,28,40]. In our study we found a folate deficiency prevalence of 0.13% and folate deficiency anemia prevalence of 0.06% leading us to conclude that both folate deficiency and folate deficiency anemia have essentially been eliminated post-fortification. In addition to the documented reduction in the occurrence of neural tube defects and anencephaly, reductions in both the prevalence of folate deficiency and folate deficiency anemia are an added benefit of mandatory flour fortification. Policy discussions on whether or not to require mandatory fortification should not only consider the well demonstrated role of mandatory folic acid fortification in preventing Spina-bifida and anencephaly but should also include the virtual elimination of folate deficiency and folate deficiency anemia among those ages 50 years and older.

While concerns have been raised about the safety of mandatory folic acid fortification, the food safety organizations of several countries contemplating fortification have carried out systematic reviews and met-analysis on the benefits and adverse effects of folate fortification have recommended based on current evidence that their countries should fortify with folic acid [8,40].

Mandatory folic acid supplementation of flour and enriched grain products is a "sustainable, passive and inexpensive way passive public health intervention that can prevent human disease" [9]. However it is necessary to continuously monitor and evaluate existing programs in order to properly address new and existing concerns regarding safety of fortification [40]. This process may also contribute sound scientific evidence on the benefits of fortification, especially in countries that have yet to decide on mandatory folic acid fortification of flour.

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