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Fortification of bakery and corn masa–based foods in Mexico and dietary intake of folic acid and folate in Mexican national survey data

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ABSTRACT

Background: In Mexico, wheat and corn flour fortification with folic acid (FA) was implemented in 2001 and mandated in 2008, but without direct enforcement. Current Mexican nutrient-content tables do not account for FA contained in bakery bread and corn masa–based foods, which are dietary staples in Mexico.

Objective: The objective of this study was to examine the impact of FA fortification of dietary staples on the proportion of the population consuming below the Estimated Average Requirement (EAR) for folate or above the Tolerable Upper Intake Level (UL) for FA.

Methods: We measured FA and folate content in dietary staples (bakery bread and tortillas) using microbial assays and MS, and we recalculated FA intake from 24-h recall dietary intake data collected in the 2012 Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición) utilizing estimates from our food measurements, using nutrient concentrations from tortillas to approximate nutrient content of other corn masa–derived foods. The revised FA intake estimates were used to examine population-level intake of FA and dietary folate equivalent (DFE) accounting for geographic differences in FA content with statistical models.

Results: FA content in dietary staples was variable, whereas use of FA-fortified flour in corn masa tortillas increased with population size in place of residence. Accounting for dietary staples' FA fortification increased population estimates for FA and DFE intake, resulting in a lower proportion with intake below the EAR and a higher proportion with intake above the UL. Despite accounting for FA-fortified staple foods, 9–33% of women of childbearing age still have intake below the EAR, whereas up to 12% of younger children have intake above the UL.

Conclusions: Unregulated FA fortification of dietary staples leads to unpredictable total folate intake without adequately impacting the intended target. Our findings suggest that monitoring, evaluation, and enforcement of mandatory fortification policies are needed. Without these, alternate strategies may be needed in order to reach women of childbearing age while avoiding overexposing children. *Am J Clin Nutr* 2019;00:1–15.

Keywords: dietary folate, folic acid, fortification of dietary staples, Mexican National Health and Nutrition Survey, bread and corn masa–based food measures, usual intake, inadequate intake, corn tortillas, fortified bread

Introduction

Adequate physiologic folate intake is critical during early development and rapid cell division, and may contribute to cerebral cortex synaptogenesis and neural reorganization (1). Adequate intake is considered key for preventing neural tube defects (NTDs) (1, 2). Physiologic folate is found naturally in some foods such as vegetables and fruits, whereas synthetic folic acid (FA) is the form of folate found in fortified foods and supplements. Use of FA supplements and FA fortification of wheat flour have been associated with a decrease in the incidence of NTDs in several countries (3, 4).

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Supplemental Tables 1–3 and Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: BSA, body surface area; cUMFA, circulating unmetabolized folic acid; DFE, dietary folate equivalent; EAR, Estimated Average Requirement; ENSANUT, Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición); FA, folic acid; INSP, National Institute of Public Health, Mexico; IOM, Institute of Medicine; NTD, neural tube defect; SFFQ, semiquantitative food frequency questionnaire; UL, Tolerable Upper Intake Level.

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The amount of folate intake necessary for essential functions at the population level is guided by the age-adjusted daily Estimated Average Requirement (EAR) (5). Excess intake of FA at or above the Tolerable Upper Intake Level (UL) may be detrimental (6–11) and is more frequent among those taking vitamin supplements, particularly in settings in which food is fortified (12, 13). In women aged 12–45 y, plasma folate (reflecting shorter-term exposure) increases with a daily intake of 8 tortillas made with fortified corn flour (3). Red cell folate concentrations have a more reliable increase in women taking supplements compared with women eating only fortified cereals (14).

Folate fortification is now mandated in 87 countries, although levels of fortification vary widely and the choice of grains to be fortified is based on usual dietary intake in target populations (15, 16). Mandatory wheat flour fortification (1400 μg FA/kg flour) was first introduced in the United States in 1998 to reduce the incidence of NTDs (17, 18). Fortification was designed to increase FA intakes by 100 μg/d. Due to simultaneous changes in US dietary patterns, median FA intake increased by 138 μg/d (162 μg/d in women of childbearing age) (19) based on data from NHANES, whereas in other adult US populations mean FA intake increased 190 μg/d after fortification (20–24). The population segment with daily folate intake below the EAR decreased from 50% to 7% after fortification (21). Red cell and plasma folate concentrations also increased in the United States after fortification (25).

In Mexico, voluntary wheat and corn flour fortification (2000 μg FA/kg flour) was implemented in 2001 and was made mandatory in 2008 (26, 27). However, there is no enforcement of the fortification, as evidenced in a recent survey of mills (28), nor is there a requirement to use fortified corn flour in products made with corn-based dough known as *masa*, which is used in making tortillas and other corn-based products in small local manufacturers and also in homes. Although the current Mexican nutrient content tables account for natural food folate as well as FA from industrialized products such as boxed breakfast cereals, the tables do not account for FA in dietary staples that are non-industrialized foods made with wheat flour (e.g., baked goods from bakeries), or those made using masa made with fortified corn flour (e.g., tamales and tortillas). The impact of fortification on dietary intake and on the proportion of the population consuming below the EAR has not been examined in Mexico. However, serum folate concentrations in the 2012 National Health and Nutrition Survey [Encuesta Nacional de Salud y Nutrición (ENSANUT) 2012] suggest that the prevalence of deficiency is low (29).

Few studies outside of the United States have reported FA or dietary folate equivalent (DFE) intake accounting for flour fortification and using nationally representative data (30). This study examines child and adult data from ENSANUT 2012 to estimate the population prevalence of dietary intake below the folate EAR and above the UL for FA and also to examine the impact of wheat and corn flour fortification of dietary staples on the distribution of FA and DFE intake in Mexico.

Methods

Our overarching study design had three parts. First, we measured folate (5-methyl-tetrahydrofolate) and FA (pteroyl glutamic acid) in Mexican dietary staples made with corn and wheat and typically purchased from small local manufacturers. Second, we next updated the nutrient content tables for the representative national survey ENSANUT 2012 for FA and folate using these measures. Third, we modeled the probability of tortilla FA fortification using key items from a national representative semiquantitative FFQ (SFFQ) from ENSANUT 2012 and population size of place of residence. We then used the food measures and tortilla fortification modeling combined with the 24-h recall data from ENSANUT 2012 to examine the distribution of usual intake of FA and total folate in Mexico after accounting for the contribution of bakery and corn masa–based FA-fortified foods. We used this distribution to examine our primary endpoint, the proportion of the population that had a dietary intake of folate below the age-specific EAR and dietary intake of FA above the UL. (Our predeclared primary endpoints did not change.)

Food samples

The nutrient content from flour-containing foods made by small manufacturers had not been measured or updated in nutrient content tables after fortification in Mexico. We measured FA concentrations in flour-containing foods (wheat and corn) that are dietary staples in Mexico and that are typically made by small local manufacturers or at home and are not sold in industrial packaging (31). We purchased flour-containing foods representative of Mexican dietary practice in 36 municipalities located in 13 states throughout Mexico and distributed across the 4 geographic regions represented in ENSANUT 2012 (**Supplemental Table 1**).

In accordance with FAO's guidelines for measuring nutrient content for a given food item, nutrient concentrations must be measured in a minimum of 2 samples of a food item (32). In order to improve the precision of our FA estimates, we measured the concentration of folate (33) as well as FA in triplicate in a total of 62 breads, of which 31 were white rolls and 31 were sweet breads. We also measured 5-methyl-tetrahydrofolate and FA concentrations in tortillas (a pancake-like flatbread made of corn or wheat) from *tortillerias* (shops where tortillas are made). White and sweet bread were collected separately because the sweet bread recipe usually contains eggs, a source of naturally occurring folate, in addition to wheat flour, sugar, salt, baking powder, shortening, and water, whereas white bread is generally made of only wheat flour, leavening, salt, shortening, and water. Breads are baked in an oven as in other areas of the world. Both white and sweet breads are generally baked as individual-portion-size rolls or buns. In central and southern Mexico, tortillas are primarily corn-based and are made from corn kernels processed with an alkali, then ground into a dough called "masa," or from a corn masa made from industrialized corn flour and water. Tortillas are the most highly consumed of the corn masa–based foods, and they are cooked on a metal sheet.

Breads and tortillas were weighed, and then pieces containing ~10 g (range: 9.5–13.7 g) were removed, weighed, and placed in small self-sealing bags made of thick plastic with barcoded labels linking the breads to their geographic origin (municipality and state). All breads and tortillas were purchased concurrent with dietary measures for ENSANUT 2012 and were frozen within 24 h of purchase at the bakery or tortilleria. All procedures for storing breads had been previously implemented by Poo-Prieto et al. (34) in measurements performed in the USDA laboratory.

Two brands of industrialized corn flour dominate the corn flour market in Mexico: Maseca and Minsa. We purchased, stored, and then froze 10-g aliquots of each flour; we also prepared a tortilla from each flour by adding water and cooking the tortilla in the traditional manner, and froze a 10-g aliquot of each of the resulting tortillas. Bread and tortilla aliquots were stored initially at –20°C before transferring to –70°C, at which temperature they remained until analysis. Food samples were homogenized and analyzed in the Selhub laboratory (USDA/Tufts University) (35) for concentrations of 5-methyl-tetrahydroolate and FA using HPLC and microbial assays (34). All food measures were expressed in concentrations per 100 grams of food (34) (**Supplemental Table 2**). These food measures were then used to inform our estimates for food folate and FA concentrations for these food items.

National dietary intake data.

Estimates of food folate and FA intake reflecting the contribution from bakery breads and tortilleria products were carried out using the reported dietary intake data from participants who contributed 24-h recall data to ENSANUT 2012. The methodology of data collection for ENSANUT 2012 has been reported previously (36). Briefly, ENSANUT 2012 is a nationally representative survey carried out in Mexico between October 2011 and May 2012, and it included representation from 4 geographic regions of Mexico (north, center, Mexico City, south), as well as rural and urban populations. All dietary data were collected through in-person interviews. For the purpose of these analyses, we excluded children younger than age 1 y, pregnant women, and adults aged ≥80 y, and ages were grouped according to the age groupings used for micronutrients in the Dietary Reference Intake tables of the Institute of Medicine (IOM) (5, 27, 37) (**Supplemental Figure 1**).

A random representative subsample from the anthropometric sample of the ENSANUT 2012 study population (∼11%, n = 10,886) was selected for collection of dietary data using a 24-h dietary recall and a standardized multipass methodology adapted from that designed by the USDA (38). A representative subgroup (9%) of those selected for 24-h recalls was also selected for collection of a second 24-h recall obtained on nonconsecutive days after the first collection (2.4 ± 1.2 d) in order to estimate the component of day-to-day variance for energy and macronutrient intake (39). In addition, a previously validated SFFQ was applied to an independent but representative subsample (11.3%) of the anthropometric sample of ENSANUT 2012 (40). A representative sample of ∼10% of those with SFFQ was also selected to have 24-h recalls. Anthropometric measures for the population with 24-h recalls were used to calculate body surface area (BSA) using a standard formula (41).

Supplement intake.

The 24-h recall data included nutrients from food supplements distributed through the Mexican national social assistance program that were reported as ingested in the 24-h recall (e.g., Liconsa, NutriSano, or Nutrivida). Vitamin supplements were not collected as part of the 24-h recalls. Vitamin supplement intake was collected as part of the SFFQ in ENSANUT 2012. Intake of supplements containing FA was relatively infrequent and is documented in **Supplemental Table 3**. Although the prevalence of intake was captured in the FFQ, the dose, frequency, and duration of supplement use were not captured; thus we are unable to estimate the nutrient contribution to the diet.

Estimated nutrient intake.

The estimated nutrient intake was calculated from the 24-h recalls using nutrient content tables from the INSP (National Institute of Public Health, Mexico) (42). We included nutrients that were contained in fortified foods and food supplements (see "Supplement intake") distributed as part of national aid programs (e.g., Liconsa, NutriSano, or Nutrivida) if their intake was reported in the 24-h recall (43).

Food composition values

White and sweet bread.

The INSP nutrient content tables specified 0.0 μg of FA and 24.0 μg of food folate per 100 g of white bread (bolillo roll) and 68.0 μg of FA and 40.0 μg of food folate per 100 g of sweet bread (42). These FA and food folate content values were updated using the median statistic from the 31 laboratory measurements described previously: 112.0 μg of FA and 12.6 μg food folate for white bread and 82.6 μg of FA and 4.7 μg food folate for sweet bread (Supplemental Table 2).

Tortilla.

In the 24-h recall there were 3 tortilla food items that did not specify whether they were made with fortified flour (Minsa or Maseca) or unfortified corn masa (44); however, this information was available for SFFQ data and was used to generate prediction equations to inform our estimates from the 24-h recall. We used the SFFQ data on the type of tortilla consumed and modeled the probability of FA fortification of corn-based dough as a function of the population size of residential location (45). We estimated upper and lower limits of the probability of FA fortification of corn-based dough (masa) that cover a reasonable range of unobserved probabilities of FA fortification of corn-based dough.

To calculate the expected value of FA, we estimated individualized probabilities of fortification that depended on the population size of the location of residence (formulas are listed later and in **Supplemental Figure 2**) (46). For the 24-h recall data, we calculated the predicted probabilities of fortification applying these equations to the size of locality of residence for each subject.

Probability of fortification: corn-based dough (masa) or tortilla.

In the SFFQ questionnaire applied in ENSANUT 2012, the following nonmutually exclusive options were presented regarding the source of corn tortillas that participants reported consuming:

a. tortillas made at home from corn dough made with alkalinized ground corn (nixtamal)
b. tortillas made at home from Minsa or Maseca flour
c. tortillas purchased as premade in a tortilleria or made at home using ready-made corn dough (masa) purchased from a tortilleria

Within the tortilla consumers, intake was calculated in grams (gr) for each option, and the proportions of tortilla intake by type of tortilla (options a, b, or c) were calculated; for example, for homemade nixtamal (option a), the proportion of intake was calculated as

$$p_a = \frac{gr_a}{gr_a + gr_b + gr_c}$$

Fractional models were applied (47) with probit specification for each of the fractions p_a, p_b, and p_c. One limitation is that option c could be fortified, but we do not know the proportion of tortillerias that use FA-fortified corn flour. We used the fraction $p_b + p_c$ as an upper limit of the fortification probability. This is equivalent to assuming that all tortillas bought in tortillerias or made with purchased dough would be fortified. To define a lower fortification limit, we used the fraction in the households corresponding to the tortilla flour Minsa–Maseca: $p_m = p_b/(p_a + p_b)$. This fraction p_m is multiplied by the proportion reporting intake of purchased tortillas or purchased dough $p_m p_c$. This is equivalent to assuming that the probability of fortification in the tortillerias is equal to the probability of use of fortified flour in the households. Therefore, we define the lower limit as $p_b + p_m p_c$.

We applied a fractional-probit model for both the lower limit $p_{inf} = p_b + p_m p_c$ and the upper limit $p_{sup} = p_b + p_c$ of the fortification probability as a function of the natural logarithm of the population size of the localities of residence (*Population Size*) (45). The following prediction equations were obtained:

$$\text{Lower limit}: \Phi\left[-1.596 + 0.098 * ln\left(Population\ Size\right)\right]$$
$$\text{Upper limit}: \Phi\left[-1.447 + 0.239 * ln\left(Population\ Size\right)\right]$$

where $\Phi(z) = P(Z < z)$ is the standard normal cumulative distribution function. The previous equations showed that probability of fortification increased with increasing size of the population in the place of residence. Estimated probabilities are shown in Supplemental Figure 2.

Tortilla FA and food folate nutrient content values.

Once probabilities of fortification were determined, the individualized FA and food folate nutrient content values per 100 g of tortilla were calculated by multiplying the probability of fortification by the nutrient content. The nutrient content was determined from the laboratory measurements by averaging the Maseca and Minsa values, with a resulting average of 131.0 μg FA and 1.4 μg food folate per 100 g of tortilla made from industrialized corn flour.

For comparison, the original ENSANUT 2012 data for the 24-h recalls listed 87.3% of tortilla records as "generic" (i.e., there was no specification regarding whether the tortillas were made with fortified flour). These generic tortillas had been assigned 0 FA μg and 5 μg food folate per 100 g, whereas just 6.0% of tortilla records were identified explicitly as Maseca tortillas. For comparison, in the INSP nutritional content table, Maseca tortillas had previously been assigned 100 μg FA and 19 μg food folate per 100 g of tortilla.

Foods prepared with corn-based dough (masa).

The FA and food folate nutritional content of corn masa–based dough (used to make *sopes*, *tamales*, *gorditas*, and *tlacoyos*, collectively known as *antojitos*) was obtained using the same calculations made for tortillas.

Statistical analysis

We estimated the distributions of intake for FA and DFE by age and sex groups using values derived from the lower and upper limits of fortification probability described previously. Median and selected percentiles ± SEs of usual intake and prevalence of intake of DFE below the EAR and prevalence of FA intake above the UL were estimated using the Iowa State University method using the Software for Intake Distribution Estimation (PC-SIDE version 1.02). This method adjusted for the day-to-day (intraindividual) variations, which allowed obtaining estimates that more accurately reflect the prevalence of inadequate usual nutrient intake (48, 49).

After obtaining the distributions of usual intake, we estimated the prevalence of intake below the EAR or above the UL using the age-specific threshold levels defined by IOM (5). The EAR ranges from 120 DFE in children aged 1–3 y to 320 DFE in women of childbearing age, and the UL for FA ranges from 300 μg in children aged 1–3 y to 1000 μg in adults (5). For calculating the EAR, we used DFE, defined as food folate plus FA, with FA multiplied by a correction factor of 1.7 to account for the higher bioavailability of FA.

Our main approach was based on usual intake; however, we estimated median FA intake directly from 24-h recall data (from 1 d) to examine how median FA intake values changed with the updates to the food composition reflecting FA fortification. We were unable to estimate usual intake distributions with the original food composition values due to a high density of points at zero (11.0% of the sample had intake of 0 μg). After updating the food composition values with the revised FA fortification estimates, only 1.6% of the sample had FA intake values at zero.

We estimated FA and DFE intake using the upper and lower probabilities of consuming FA-fortified corn tortilla. We then calculated population prevalence of FA above and DFE below the recommendations using the IOM UL and EAR threshold values, respectively (5). Point estimates of population sizes below the EAR or above the UL were obtained multiplying prevalence estimates by the total population represented in each sex and age group. We also estimated the food groups that contributed the most to observed FA intake (without usual intake modeling). To do so, we first identified single foods that were the main contributors to FA intake by age group. We then created additional food groups that were composed of boxed cereals, cookies, milk, pasta, chocolate powder, and sliced bread. Survey-weighted mean estimates were obtained for the total intake as well as for the intake of each specific group, and the ratio of

TABLE 1 Age distribution of ENSANUT 2012 participants for whom 24-h recalls were included in this analysis[1]

	Sample size (*n*)			Population size (*n*)	
Age group (y)	Only one 24-h recall	Two 24-h recalls (%)[2]	Total no. of subjects who have at least one 24-h recall	Thousands	Distribution of age groups (%)
Males					
1–3	794	80 (9.2)	874	3396.4	6.4
4–8	983	115 (10.5)	1098	6223.3	11.7
9–13	831	73 (8.1)	904	6760.6	12.7
14–18	585	57 (8.9)	642	5612.0	10.5
19–39	542	44 (7.5)	586	14,711.1	27.6
40–59	382	40 (9.5)	422	11,122.1	20.9
60–79	361	35 (8.8)	396	5441.9	10.2
Total	4478	444	4922	53,267.4	100
Females					
1–3	708	63 (8.2)	771	3053.8	5.5
4–8	1019	96 (8.6)	1115	6512.2	11.7
9–13	770	91 (10.6)	861	6658.1	11.9
14–18	591	57 (8.8)	648	4978.7	8.9
19–39	732	71 (8.8)	803	16,221.1	29.1
40–59	509	51 (9.1)	560	13,240.2	23.7
60–79	420	38 (8.3)	458	5159.9	9.2
Total	4749	467	5216	55,824.1	100

[1]ENSANUT 2012, 2012 Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición).
[2]Percentage of total subjects who had 2 recalls.

means from each food group source to the total mean intake was obtained as a postestimation.

For comparison purposes, we calculated survey-weighted median intake of FA and DFE before and after updating food composition tables using data from 1 d of the 24-h recalls (without usual intake modeling). Medians and SEs estimated directly from the 24-h recall data were obtained with the survey module in R version 3.5.1 (50, 51). We compared 4 scenarios: *1*) without updating food composition values; *2*) updating bread nutrient content values; *3*) updating bread and tortilla nutrient content values, the latter using the lower limit of FA fortification probability; and *4*) updating bread and tortilla nutrient content values, the latter using the upper limit of FA fortification probability. We performed these analyses for each sex and age group.

Results

We analyzed information from 9,227 subjects with dietary information from ENSANUT 2012. Age group distributions were similar for both sexes (**Table 1**). Our calculations on the proportions of tortillas made with industrialized flour based on the ENSANUT 2012 SFFQ showed that use of FA-fortified corn flour increased with population size in the residential location and was higher in urban areas (Supplemental Figure 2).

Estimated prevalences of usual intake of DFE < EAR are shown in **Table 2** along with estimated population median usual intake of DFE. The estimated prevalences of usual intakes of FA > UL and population sizes corresponding to these prevalences are shown in Table 2 along with estimated population medians for usual daily intake of FA.

Male distributions of both DFE and FA showed higher medians compared with those of females. Although the 2 FA fortification scenarios showed a large difference in the estimates for the median and prevalence of insufficient or excess intake, there was a considerable portion of the population with intake below the recommended DFE or with FA intake above the UL. The inadequate intake (below the EAR) occurred primarily in adolescent girls (aged 14–18 y) and women aged 19–39 y, whereas the excess intake (above the UL) occurred primarily in children <9 y and boys aged 9–13 y.

Our analyses demonstrated that after accounting for fortification of nonindustrialized foods such as bakery breads and corn, which are staples of the Mexican diet, the proportion of the population consuming DFE below the recommended intake varied by age and gender (**Figure 1**A and Table 2). Figure 1A shows the prevalence of the population with usual folate intake (52) below the EAR, as modeled from the 24-h recall data, both after updating the white and sweet bread nutrient content values and after accounting for heterogeneity in tortilla FA fortification. As children aged, the proportion below the EAR tended to increase: among 1- to 3-y-olds, the proportion with DFE < EAR was 3.2–6.3% in girls and 0.6–1.6% in boys, whereas in peripubertal boys aged 9–13 y, the proportion below the EAR was 0.9–4.8%, and it was slightly higher in girls aged 9–13 y at 4.0–11.5%. Among boys aged 14–18 y, the rate increased somewhat (3.5–12.6%), but it increased markedly to 9.3–32.7% in adolescent girls aged 14–18 y. In women aged 19–39 y, 8.9–28.1% consumed DFE < EAR. In women aged 40–59 and 60–79 y, the proportion was somewhat lower (7.1–21.5% and 3.7–28.6%, respectively).

Importantly, we also found that a small but still substantial proportion of children aged 1–3 y in Mexico had usual intakes above the age-adjusted UL of FA (300 μg), which has not previously been reported in ENSANUT. Figure 1B shows the prevalence of the population with usual FA intake (by PC-SIDE) above the UL as modeled from the 24-h recall data, both after updating the white and sweet bread nutrient content values and

TABLE 2 Population-level estimates of median daily usual intake of DFEs and estimated median daily usual intake of FA based on usual intake, and estimates of the population consuming less than the EAR or more than the UL, by sex and age group, for both lower and upper limit estimates of tortilla fortification with FA[1]

	DFE[2]						FA					
	Lower limit of tortilla fortification			Upper limit of tortilla fortification			Lower limit of tortilla fortification			Upper limit of tortilla fortification		
Age group (y)	Median DFE (μg)	Percentage of population below the EAR	Estimated population size with DFE < EAR (n)	Median DFE (μg)	Percentage of population below the EAR	Estimated population size with DFE < EAR (n)	Median FA (μg)	Percentage of population above the UL	Estimated population size with FA > UL (n)	Median FA (μg)	Percentage of population above the UL	Estimated population size with FA > UL (n)
Males												
1–3	329 ± 8.7	1.6 ± 2.5	52,644.9	389 ± 10.7	0.6 ± 1.3	19,037.1	103 ± 3.2	6.3 ± 5.1	212,685.4	135 ± 4.0	10.3 ± 6.0	349,494.2
4–8	400 ± 8.9	4.2 ± 2.5	263,493.8	496 ± 9.5	2.4 ± 1.4	151,101.3	159 ± 4.1	4.8 ± 4.2	301,393.6	216 ± 4.7	11.9 ± 4.0	743,060.1
9–13	510 ± 12.5	4.8 ± 5.2	324,916.6	676 ± 14.6	0.9 ± 1.8	61,731.5	207 ± 5.7	2.3 ± 3.0	155,630.1	303 ± 6.7	5.035 ± 5.2	340,398.5
14–18	511 ± 15.3	12.6 ± 10.7	709,351.0	705 ± 19.3	3.5 ± 4.5	195,745.0	203 ± 8.2	0.6 ± 1.9	34,524.7	316 ± 9.6	3.57 ± 4.1	200,346.8
19–39	474 ± 11.1	18.0 ± 5.3	2,645,053.1	702 ± 17.5	3.6 ± 4.8	534,012.4	200 ± 5.8	0 ± 0	0.0	331 ± 9.4	0 ± 0	0.0
40–59	506 ± 16.9	9.9 ± 15.7	1,095,860.1	747 ± 22.6	2.4 ± 5.7	264,261.0	172 ± 5.8	0 ± 0	0.0	319 ± 10.3	0 ± 0	0.0
60–79	446 ± 14.1	17.0 ± 17.9	925,662.9	686 ± 22.4	0.9 ± 6.9	46,887.2	161 ± 6.6	0 ± 0	0.0	288 ± 9.6	0 ± 0	0.0
Females												
1–3	275 ± 8.2	6.3 ± 4.2	193,735.7	327 ± 9.3	3.2 ± 2.9	96,196.0	100 ± 3.4	3.9 ± 3.1	118,825.0	129 ± 3.8	6.9 ± 3.7	212,119.9
4–8	393 ± 8.5	4.1 ± 4.3	267,653.3	498 ± 10.2	1.3 ± 2.0	81,533.3	160 ± 4.1	4.4 ± 5.1	284.845.6	220 ± 5.0	9.2 ± 7.3	599,517.3
9–13	426 ± 9.6	11.5 ± 4.5	764,353.6	573 ± 11.8	4.0 ± 2.6	264,261.3	173 ± 4.4	0.2 ± 0.5	10,613.1	260 ± 6.1	1.1 ± 2.0	75,902.7
14–18	402 ± 13	32.7 ± 7.6	1,627,023.8	549 ± 15.2	9.3 ± 10.5	462,516.9	151 ± 5.5	0.6 ± 1.2	27,701.2	238 ± 7.2	0.3 ± 1.0	15,090.3
19–39	408 ± 10.1	28.1 ± 5.4	4,551,641.2	575 ± 12.6	8.9 ± 4.9	1,436,540.8	142 ± 3.6	0.1 ± 0.2	18,119.0	238 ± 5.4	0.2 ± 0.4	33,707.5
40–59	425 ± 12.2	21.5 ± 11.4	2,849,300.9	587 ± 15.1	7.2 ± 6.7	946,677.6	144 ± 4.4±	0 ± 0	0.0	238 ± 6.9	0 ± 0	0.0
60–79	398 ± 13.5	28.6 ± 14.2	1,477,796.2	565 ± 18.3	3.7 ± 12.3	188,904.0	132 ± 5.1	0 ± 0	0.0	221 ± 8.1	0 ± 0	0.0

[1]Values are means or percentages ± SEs unless otherwise indicated. EARs and ULs are from the Institute of Medicine recommendations. Modeled using PC-SIDE. DFE, dietary folate equivalent; EAR, Estimated Average Requirement; FA, folic acid; UL, Tolerable Upper Intake Level.

[2]DFEs calculated as DFE = 1.7 FA + food folate.

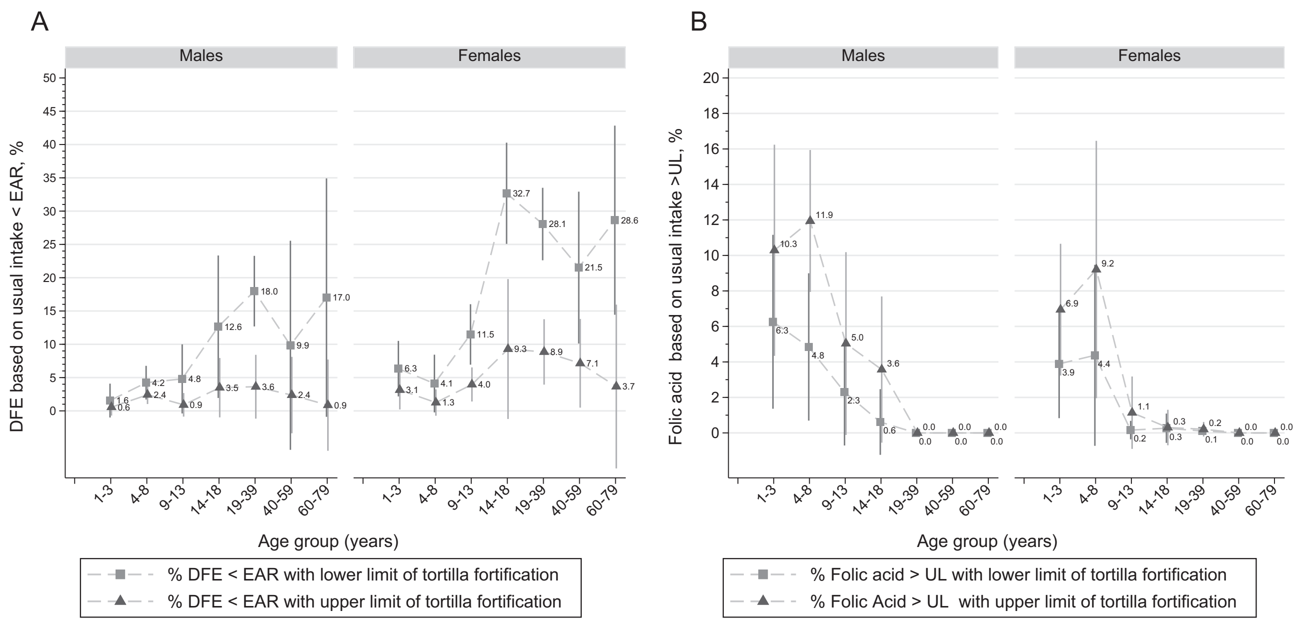

FIGURE 1 Prevalence of dietary intake of (A) DFEs below the EAR based on usual dietary intake (PC-SIDE) and of (B) FA above the UL based on usual dietary intake (PC-SIDE), both by age group and sex based on 24-h dietary recall data. Error bars represent SEs. EAR and UL levels are from the Institute of Medicine. DFEs calculated as DFE = 1.7 FA + food folate. DFE, dietary folate equivalent; EAR, Estimated Average Requirement; FA, folic acid; UL, Tolerable Upper Intake Level.

after accounting for heterogeneity in tortilla FA fortification. We found that 6.3–10.3% of boys and 3.9–6.9% of girls aged <4 y and 4.8–11.9% of boys and 4.4–9.2% of girls aged 4–8 y consumed FA > UL. In peripubertal boys aged 9–13 y, 2.3–5.0% had a usual intake of FA > UL; in girls at these ages, the prevalence was lower at 0.2–1.1%. **Figure 2** shows the distribution of FA intake for select population percentiles. For girls aged 4–8 y, their intake appears the same or even higher as that of older teens and adult women of childbearing age (19–39 y) at nearly all percentages. This is the case at both upper and lower limits of tortilla fortification. BSA estimates demonstrated median BSA in the 4- to 8-y-olds of 0.82 m^2 in girls compared with 1.74–1.78 m^2 in adult women (>18 y), suggesting an ∼2-fold higher BSA-to-FA intake ratio in the 4- to 8-y-old girls compared with that in adult women. In boys, the pattern is somewhat different, with intake increasing in later childhood and adolescence, particularly in the modeling with the upper limit of tortilla fortification.

Food groups contributing to FA intake are shown in **Tables 3** and **4**. Tortillas represented the single food with the greatest source of FA intake at all ages for both genders. In children aged 1–13 y, foods other than bread and tortillas represent between 28% and 64% of FA intake. Among the larger contributors to FA intake in children aged <13 y are tortillas, boxed cereals, bakery sweet bread, cookies, and crackers, although for children aged 1–3 y, FA-fortified boxed cereals, milk, and pasta are the main contributors, along with bakery bread and tortillas. However, even among children aged 1–3 y, 18–34% of FA intake is attributed to tortillas alone, which is more than any other single food. It is notable that the largest proportion of the population that is ingesting above the UL for FA is the 1- to 8-y-olds.

Heterogeneity in FA fortification of tortillas had a major impact in the FA intake (Table 3) at a national level, with up to 65% of folate/FA intake attributable to tortillas alone (with the largest proportion of FA intake among adults aged 40–59 y). Bakery bread (combining sweet and white bread) also comprises a large proportion of FA and DFE intake (Tables 3 and 4). Among children aged 1–3 y, 13–17% of intake derives from fortified bakery bread. Together, tortillas and bread represent 34–47% of FA intake in this age group.

Figure 3 illustrates how the survey-weighted median intake for DFE and FA estimated directly from the ENSANUT 2012 24-h recall data changed after incorporating the adjustments made to tortilla and bakery bread nutrient content. The estimates for both DFE and FA intake increased markedly for all age groups and for both sexes using the updated values for FA content, especially when the heterogeneity of tortilla FA fortification was taken into account. The increases were particularly striking for adolescents and adults. Of note, when comparing the nutrient estimates for FA intake between the original ones prior to adjusting the values for nutrient composition and those including the adjustments for tortilla and bakery bread FA content, the proportion of the population sample whose reported FA intake was zero decreased from 11% to only 1.6% of the sample.

Discussion

Our analyses demonstrated that accounting for FA fortification in staple foods made from wheat and corn, such as bakery bread and tortillas, changed population-level estimates for the adequacy of folate intake in Mexico. Given the critical role of folate in multiple biologic processes, changes in estimates of population-level adequacy may impact multiple public health indicators. However, our findings show that variability in estimates is large. Lack of enforcement of mandatory fortification policies may also

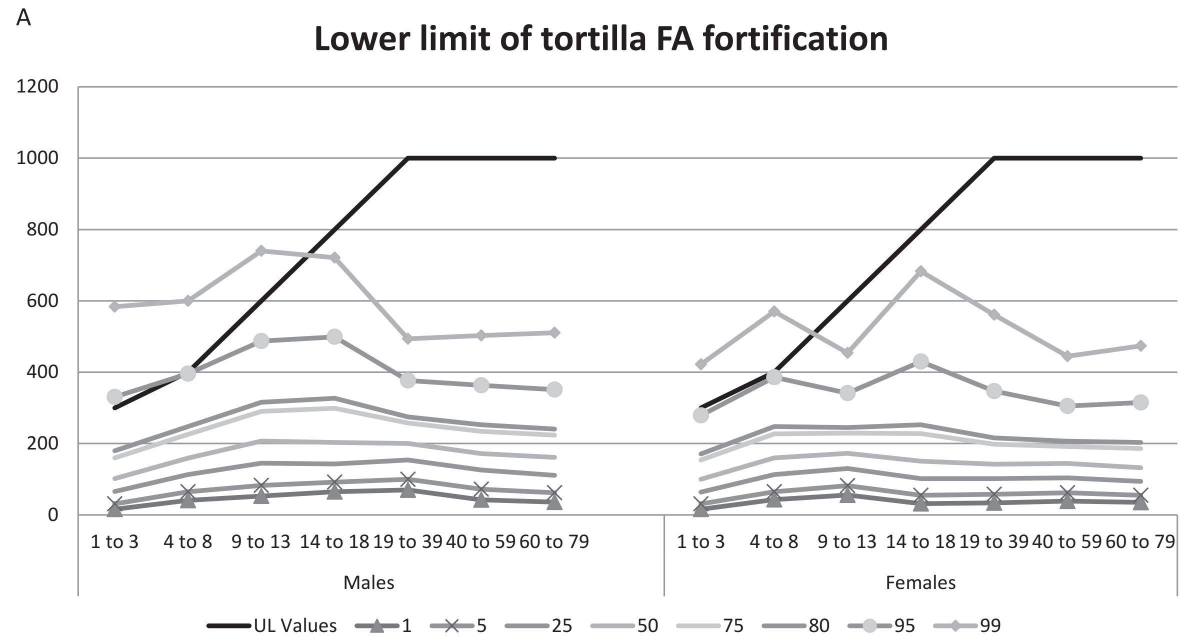

FIGURE 2 Median and selected percentiles of usual FA intake in micrograms by sex and age groups in ENSANUT 2012. (A) Estimates are percentiles from the distribution of usual intake (PC-SIDE) in micrograms, using values derived from the lower limit of fortification probability. (B) Estimates are percentiles from the distribution of usual intake (PC-SIDE) in micrograms, using values derived from the upper limit of fortification probability. ENSANUT, Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición); FA, folic acid; UL, Tolerable Upper Intake Level.

impact public health indicators. Our findings suggest a need for monitoring, evaluation, and enforcement of mandated policies. Our findings may be relevant for other Latin American countries with similar FA fortification policies, such as northern Andean and Southern Cone countries, in which foods made with wheat or corn flour and manufactured by small manufacturers also represent dietary staples.

Overall, our food measurements and analytic models suggest the need to account for the imprecision in FA fortification in nation-level estimates of folate intake. Bread and tortillas together account for a majority of FA intake in multiple age groups in our estimates for Mexico. The unrecognized variability in FA content may have public health implications. Current lack of enforcement in fortification (31) may contribute to unrecognized exposure to insufficient folate as well as to excess FA (53), both of which may have detrimental health consequences. Our measurements suggest that although flour fortification has been mandatory for the past 10 y, there is

TABLE 3 The contribution of intake of select foods to the total FA intake by age group and sex in ENSANUT 2012[1]

Food group	Males (y) 1–3	Males (y) 4–8	Males (y) 9–13	Males (y) 14–18	Males (y) 19–39	Males (y) 40–59	Males (y) 60–79	Females (y) 1–3	Females (y) 4–8	Females (y) 9–13	Females (y) 14–18	Females (y) 19–39	Females (y) 40–59	Females (y) 60–79
Lower limit of tortilla FA fortification, %														
Bakery white bread	5.8 ± 0.9	9.2 ± 1.0	13.6 ± 1.6	11.5 ± 2.1	16.1 ± 1.7	13.7 ± 1.9	17.0 ± 4.8	4.0 ± 0.7	11.6 ± 1.6	14.3 ± 1.4	8.9 ± 1.2	11.2 ± 1.4	15.4 ± 2.2	9.0 ± 1.8
Bakery sweet bread	12.1 ± 1.6	10.6 ± 1.1	9.6 ± 1.1	8.9 ± 1.3	13.8 ± 1.5	14.1 ± 1.7	13.2 ± 2.0	12.5 ± 1.6	10.9 ± 1.2	11.2 ± 1.3	9.6 ± 1.3	11.0 ± 1.1	13.4 ± 1.5	15.3 ± 2.1
Tortillas	18.5 ± 1.6	22.9 ± 1.8	31.8 ± 2.4	30.5 ± 4.8	48.4 ± 2.4	53.2 ± 2.6	52.3 ± 5.2	17.9 ± 1.7	20.6 ± 1.6	29.0 ± 2.0	29.2 ± 2.7	39.5 ± 2.4	41.9 ± 2.5	41.8 ± 4.3
Corn flour–based foods[2]	1.5 ± 0.4	2.5 ± 0.4	3.5 ± 0.9	1.7 ± 0.4	2.5 ± 0.6	3.8 ± 0.9	1.9 ± 0.6	1.9 ± 0.4	2.0 ± 0.3	2.7 ± 0.6	3.8 ± 0.8	3.6 ± 0.7	3.4 ± 0.8	3.4 ± 0.9
Pasta	13.8 ± 2.2	7.8 ± 1.6	4.1 ± 0.7	2.6 ± 1.0	2.2 ± 0.6	2.3 ± 0.7	8.1 ± 4.9	11.2 ± 1.3	9.1 ± 1.3	6.7 ± 1.1	5.3 ± 1.8	6.6 ± 1.9	4.9 ± 1.1	9.6 ± 2.3
Chocolate-flavored powder	7.9 ± 1.3	6.5 ± 0.9	5.1 ± 1.1	1.2 ± 0.3	3.9 ± 1.6	0.4 ± 0.2	0.1 ± 0.1	9.1 ± 1.6	4.1 ± 0.6	6.2 ± 1.1	2.4 ± 0.7	2.5 ± 1.0	3.3 ± 1.5	1.9 ± 0.9
Boxed cereals	23.0 ± 4.0	22.4 ± 2.7	16.1 ± 2.5	24.8 ± 7.5	4.3 ± 1.3	4.8 ± 2.3	2.8 ± 1.4	21.4 ± 2.9	27.9 ± 3.4	19.5 ± 4.0	21.0 ± 4.6	15.8 ± 3.7	11.4 ± 3.1	13.2 ± 5.3
Milk	8.4 ± 1.2	1.2 ± 0.2	0.7 ± 0.2	1.3 ± 0.7	0.3 ± 0.1	0.4 ± 0.2	1.5 ± 1.1	10.7 ± 2.5	2.0 ± 0.4	0.8 ± 0.2	0.4 ± 0.1	0.8 ± 0.3	0.7 ± 0.2	1.1 ± 0.3
Cookies[3]	7.3 ± 1.6	12.9 ± 3.6	12.4 ± 3.6	14.1 ± 6.1	4.3 ± 1.3	3.3 ± 0.9	1.6 ± 0.5	8.2 ± 1.3	8.1 ± 2.4	6.1 ± 1.3	15.9 ± 5.9	5.5 ± 1.2	4.3 ± 2.4	1.9 ± 0.4
Sliced bread[4]	0.6 ± 0.2	3.0 ± 0.4	2.3 ± 0.6	2.6 ± 0.7	2.5 ± 0.8	2.7 ± 0.9	0.6 ± 0.4	2.0 ± 0.7	2.7 ± 0.4	2.6 ± 0.5	2.5 ± 0.5	2.4 ± 0.7	0.5 ± 0.2	1.6 ± 0.5
Other foods	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.5 ± 0.2	1.3 ± 0.4	0.9 ± 0.2	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
Upper limit of tortilla FA fortification, %														
Bakery white bread	4.6 ± 0.7	6.9 ± 0.7	9.2 ± 1.0	7.8 ± 1.2	9.6 ± 1.1	7.5 ± 1.1	9.8 ± 3.0	3.1 ± 0.5	8.7 ± 1.2	9.7 ± 1.0	6.1 ± 0.8	7.1 ± 0.9	9.3 ± 1.4	5.6 ± 1.1
Bakery sweet bread	9.6 ± 1.2	8.0 ± 0.8	6.5 ± 0.7	6.0 ± 0.7	8.2 ± 0.9	7.7 ± 0.9	7.6 ± 1.1	9.8 ± 1.2	8.2 ± 0.9	7.5 ± 0.8	6.5 ± 0.8	6.9 ± 0.7	8.1 ± 0.9	9.6 ± 1.3
Tortillas	33.7 ± 2.2	39.1 ± 2.2	51.0 ± 2.4	51.0 ± 5.4	67.1 ± 2.1	70.8 ± 2.1	70.8 ± 4.2	32.7 ± 2.1	37.6 ± 2.1	48.8 ± 2.3	47.5 ± 3.2	58.5 ± 2.2	61.5 ± 2.2	60.6 ± 3.9
Corn flour–based foods[2]	3.1 ± 0.7	4.8 ± 0.8	5.3 ± 1.0	3.3 ± 0.8	3.7 ± 0.8	5.6 ± 1.3	2.8 ± 0.9	4.3 ± 1.0	4.3 ± 0.7	5.0 ± 1.0	7.0 ± 1.2	5.8 ± 0.9	5.3 ± 1.2	5.2 ± 1.3
Pasta	10.9 ± 1.7	5.8 ± 1.2	2.8 ± 0.5	1.8 ± 0.6	1.3 ± 0.4	1.3 ± 0.4	4.6 ± 2.9	8.8 ± 1.0	6.9 ± 1.0	4.5 ± 0.8	3.6 ± 1.2	4.1 ± 1.2	3.0 ± 0.7	6.0 ± 1.5
Chocolate-flavored powder	6.3 ± 1.0	4.9 ± 0.7	3.4 ± 0.8	0.8 ± 0.2	2.3 ± 1.0	0.2 ± 0.1	0.1 ± 0.1	7.2 ± 1.3	3.1 ± 0.4	4.2 ± 0.8	1.6 ± 0.5	1.6 ± 0.6	2.0 ± 0.9	1.2 ± 0.6
Boxed cereals	18.2 ± 3.4	16.8 ± 2.1	10.8 ± 1.8	16.7 ± 5.7	2.6 ± 0.8	2.6 ± 1.3	1.6 ± 0.8	16.8 ± 2.4	21.0 ± 2.7	13.2 ± 2.9	14.2 ± 3.3	9.9 ± 2.5	7.0 ± 1.9	8.2 ± 3.5
Milk	6.6 ± 1.0	0.9 ± 0.1	0.5 ± 0.1	0.9 ± 0.5	0.2 ± 0.1	0.2 ± 0.1	0.9 ± 0.6	8.4 ± 2.0	1.5 ± 0.3	0.6 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
Cookies[3]	5.8 ± 1.2	9.7 ± 2.8	8.4 ± 2.6	9.5 ± 4.3	2.6 ± 0.8	1.8 ± 0.5	0.9 ± 0.3	6.5 ± 1.1	6.1 ± 1.8	4.1 ± 0.9	10.8 ± 4.2	3.4 ± 0.7	2.6 ± 1.5	1.2 ± 0.2
Sliced bread[4]	0.5 ± 0.1	2.3 ± 0.3	1.5 ± 0.4	1.8 ± 0.4	1.5 ± 0.5	1.5 ± 0.5	0.4 ± 0.2	1.5 ± 0.5	2.0 ± 0.3	1.7 ± 0.3	1.7 ± 0.3	1.5 ± 0.4	0.3 ± 0.1	1.0 ± 0.4
Other foods	0.9 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1

[1]Estimates are column percentages ± SEs. Survey-weighted mean estimates were obtained for the total intake as well as for the intake of each specific group. The ratio of means from each food group source to the total mean intake was obtained as a postestimation. ENSANUT 2012, 2012 Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición); FA, folic acid.

[2]"Corn flour" refers to foods made with FA-fortified corn flour.

[3]Cookies includes both cookies and crackers.

[4]Sliced bread includes white and whole wheat.

TABLE 4 The contribution of intake of select foods to the total folate (DFE) intake by age group and sex in ENSANUT 2012[1]

	Males (y)							Females (y)						
Food group	1–3	4–8	9–13	14–18	19–39	40–59	60–79	1–3	4–8	9–13	14–18	19–39	40–59	60–79
Lower limit of tortilla FA fortification, %														
Bakery white bread	5.5 ± 0.8	9.3 ± 0.9	13.5 ± 1.5	11.9 ± 2.0	16.5 ± 1.7	13.4 ± 1.9	16.6 ± 4.6	3.9 ± 0.6	11.6 ± 1.6	14.2 ± 1.4	8.8 ± 1.2	10.7 ± 1.3	14.7 ± 2.1	8.4 ± 1.6
Bakery sweet bread	11.3 ± 1.4	10.3 ± 1.0	9.3 ± 1.0	8.9 ± 1.2	13.7 ± 1.5	13.4 ± 1.6	12.5 ± 1.9	11.7 ± 1.5	10.5 ± 1.2	10.7 ± 1.3	9.2 ± 1.3	10.1 ± 1.0	12.4 ± 1.4	13.9 ± 1.9
Tortillas	16.0 ± 1.4	20.2 ± 1.6	28.6 ± 2.2	27.6 ± 4.0	43.0 ± 2.3	47.3 ± 2.3	47.2 ± 4.8	16.0 ± 1.6	18.1 ± 1.4	26.0 ± 1.8	25.8 ± 2.4	34.3 ± 2.3	37.2 ± 2.2	36.7 ± 3.7
Corn flour–based foods[2]	1.5 ± 0.3	2.5 ± 0.4	3.7 ± 0.9	2.0 ± 0.4	3.2 ± 0.6	4.0 ± 0.8	2.1 ± 0.6	1.9 ± 0.4	2.1 ± 0.3	2.8 ± 0.6	3.7 ± 0.7	4.1 ± 0.7	3.6 ± 0.8	3.4 ± 0.8
Pasta	14.0 ± 2.1	8.1 ± 1.6	4.4 ± 0.7	3.2 ± 1.1	2.6 ± 0.6	2.6 ± 0.7	8.2 ± 4.9	11.9 ± 1.2	9.5 ± 1.3	7.1 ± 1.1	5.6 ± 1.8	6.6 ± 1.8	5.0 ± 1.0	9.5 ± 2.2
Chocolate-flavored powder	7.2 ± 1.2	6.2 ± 0.9	4.8 ± 1.0	1.1 ± 0.3	3.7 ± 1.5	0.4 ± 0.1	0.1 ± 0.1	8.4 ± 1.5	3.9 ± 0.5	5.8 ± 1.1	2.2 ± 0.7	2.3 ± 0.9	3.0 ± 1.3	1.7 ± 0.8
Boxed cereals	21.1 ± 3.8	21.6 ± 2.6	15.4 ± 2.4	24.6 ± 7.8	4.3 ± 1.3	4.5 ± 2.2	2.6 ± 1.3	19.9 ± 2.8	26.8 ± 3.3	18.7 ± 3.8	19.9 ± 4.3	14.5 ± 3.4	10.5 ± 2.8	11.8 ± 4.8
Milk	10.4 ± 1.2	3.2 ± 0.2	2.5 ± 0.2	2.7 ± 0.8	2.0 ± 0.2	1.8 ± 0.2	2.1 ± 0.3	12.2 ± 2.2	3.4 ± 0.4	2.6 ± 0.2	2.1 ± 0.2	4.9 ± 2.8	2.4 ± 0.2	3.4 ± 0.4
Cookies[3]	5.6 ± 1.4	9.4 ± 3.4	9.6 ± 3.4	10.3 ± 6.2	1.5 ± 0.3	1.5 ± 0.4	1.3 ± 0.5	4.7 ± 0.9	6.2 ± 2.3	3.4 ± 0.8	13.4 ± 5.6	3.1 ± 1.0	3.9 ± 2.1	1.8 ± 0.4
Sliced bread[4]	0.9 ± 0.2	4.0 ± 0.5	3.0 ± 0.7	3.6 ± 0.8	3.6 ± 1.1	3.5 ± 1.1	0.8 ± 0.4	2.5 ± 0.8	3.5 ± 0.5	3.5 ± 0.7	3.3 ± 0.7	3.2 ± 0.8	0.9 ± 0.3	2.4 ± 0.7
Other foods	6.6 ± 0.6	5.3 ± 0.5	5.3 ± 0.9	4.0 ± 0.6	5.8 ± 0.6	7.6 ± 0.9	6.5 ± 0.8	6.8 ± 0.8	4.4 ± 0.4	5.2 ± 0.4	5.9 ± 1.5	6.2 ± 0.8	6.3 ± 0.5	7.1 ± 1.0
Upper limit of tortilla FA fortification, %														
Bakery white bread	4.5 ± 0.7	7.0 ± 0.7	9.3 ± 1.0	8.0 ± 1.2	9.9 ± 1.1	7.6 ± 1.1	9.8 ± 3.0	3.1 ± 0.5	8.8 ± 1.2	9.7 ± 1.0	6.1 ± 0.8	7.0 ± 0.9	9.2 ± 1.3	5.5 ± 1.1
Bakery sweet bread	9.1 ± 1.1	7.8 ± 0.8	6.4 ± 0.7	6.0 ± 0.7	8.2 ± 0.9	7.6 ± 0.9	7.4 ± 1.1	9.4 ± 1.2	8.0 ± 0.8	7.4 ± 0.8	6.3 ± 0.8	6.6 ± 0.6	7.8 ± 0.8	9.0 ± 1.2
Tortillas	30.6 ± 1.9	36.7 ± 2.1	48.2 ± 2.3	48.9 ± 4.8	63.7 ± 2.1	66.8 ± 2.0	67.1 ± 4.2	30.4 ± 2.0	35.2 ± 2.0	46.1 ± 2.2	44.7 ± 3.0	54.0 ± 2.3	57.7 ± 2.1	56.0 ± 3.6
Corn flour–based foods[2]	2.9 ± 0.7	4.8 ± 0.8	5.3 ± 1.0	3.4 ± 0.7	4.0 ± 0.8	5.7 ± 1.2	2.9 ± 0.8	4.2 ± 0.9	4.3 ± 0.7	4.9 ± 0.9	6.8 ± 1.2	6.0 ± 0.9	5.3 ± 1.1	5.0 ± 1.2
Pasta	11.2 ± 1.7	6.2 ± 1.2	3.0 ± 0.5	2.2 ± 0.7	1.6 ± 0.4	1.5 ± 0.4	4.8 ± 3.0	9.5 ± 1.0	7.2 ± 1.0	4.9 ± 0.8	3.8 ± 1.2	4.3 ± 1.2	3.1 ± 0.7	6.2 ± 1.5
Chocolate-flavored powder	5.8 ± 0.9	4.7 ± 0.7	3.3 ± 0.7	0.8 ± 0.2	2.2 ± 0.9	0.2 ± 0.1	0.1 ± 0.1	6.7 ± 1.2	2.9 ± 0.4	4.0 ± 0.7	1.5 ± 0.5	1.5 ± 0.6	1.9 ± 0.9	1.1 ± 0.5
Boxed cereals	17.0 ± 3.2	16.3 ± 2.1	10.5 ± 1.7	16.7 ± 5.8	2.6 ± 0.8	2.6 ± 1.3	1.5 ± 0.8	15.9 ± 2.3	20.3 ± 2.7	12.8 ± 2.8	13.7 ± 3.1	9.4 ± 2.3	6.6 ± 1.8	7.7 ± 3.3
Milk	8.4 ± 1.0	2.4 ± 0.2	1.7 ± 0.1	1.8 ± 0.5	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.2	9.8 ± 1.8	2.6 ± 0.3	1.8 ± 0.1	1.5 ± 0.1	3.2 ± 1.9	1.5 ± 0.2	2.2 ± 0.3
Cookies[3]	4.5 ± 1.1	7.1 ± 2.6	6.6 ± 2.4	7.0 ± 4.3	0.9 ± 0.2	0.9 ± 0.2	0.8 ± 0.3	3.7 ± 0.7	4.7 ± 1.8	2.4 ± 0.6	9.2 ± 4.0	2.0 ± 0.6	2.4 ± 1.4	1.1 ± 0.3
Sliced bread[4]	0.7 ± 0.2	3.0 ± 0.4	2.0 ± 0.5	2.4 ± 0.6	2.2 ± 0.7	2.0 ± 0.6	0.4 ± 0.2	2.0 ± 0.7	2.7 ± 0.4	2.4 ± 0.5	2.3 ± 0.4	2.1 ± 0.5	0.6 ± 0.2	1.6 ± 0.5
Other foods	5.3 ± 0.5	4.0 ± 0.3	3.7 ± 0.6	2.7 ± 0.3	3.5 ± 0.3	4.3 ± 0.5	3.9 ± 0.5	5.4 ± 0.6	3.3 ± 0.3	3.6 ± 0.3	4.1 ± 1.0	4.0 ± 0.5	3.9 ± 0.3	4.6 ± 0.6

[1]Estimates are column percentages ± SEs. DFE, dietary folate equivalent; ENSANUT 2012, 2012 Mexican National Health and Nutrition Survey (Encuesta Nacional de Salud y Nutrición); FA, folic acid.
[2]"Corn flour" refers to foods made with FA-fortified corn flour.
[3]Cookies includes both cookies and crackers.
[4]Sliced bread includes white and whole wheat.

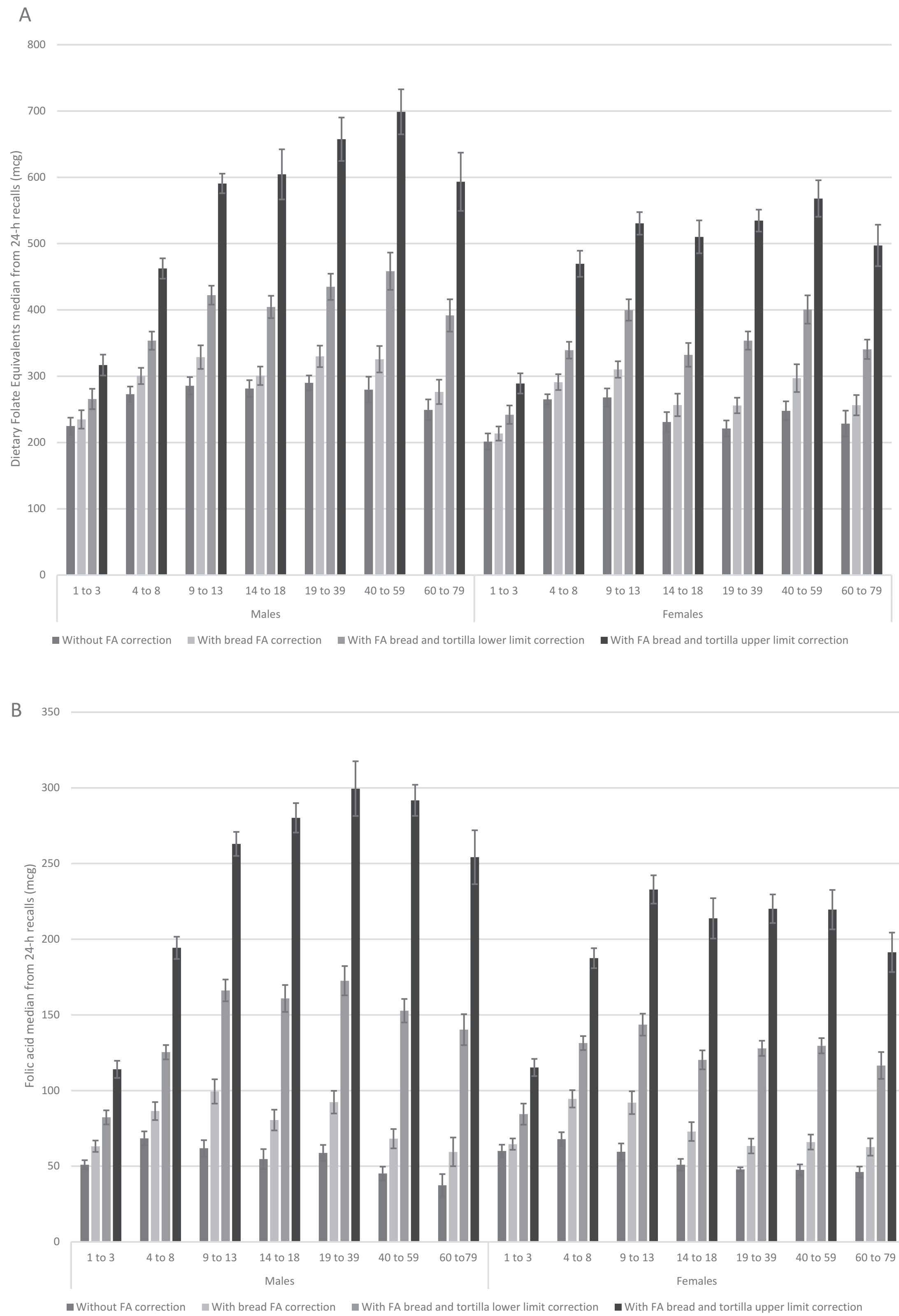

FIGURE 3 Median DFEs and FA intake, by type of nutrient content correction, age group, and sex, based on 1 day's 24-h dietary recall data. (A) DFEs and (B) FA. DFEs calculated as DFE = 1.7 FA + food folate. The upper limit was estimated using information from the adult SFFQ sample ($n = 2087$) reporting intake of corn tortillas from any source, including those homemade (whether from alkalinized ground corn or using industrialized flour or made with corn-based dough bought from tortillerias) and also including those ready-made from a tortilleria. The lower limit was estimated using information from the adult SFFQ sample ($n = 731$) reporting intake of homemade tortillas regardless of the source of corn dough. Survey-weighted median levels were calculated for each age/sex group. DFE, dietary folate equivalent; FA, folic acid; SFFQ, semiquantitative FFQ.

great variability in the use of fortified flours. Our findings are consistent with those of recent reports on zinc and iron content in wheat and corn flour that similarly found variable content, minimal oversight, and documented that although fortification is mandatory, adherence to the WHO recommendations for fortification is recommended but not required (28, 31, 54).

After updating FA and food folate nutrient content with our food measurement-based estimates, and analytic models reflecting the heterogeneity of tortilla FA fortification, the level of folate intake insufficiency for children aged 1–13 y, adolescents aged 14–18 y, and adults aged 19–59 y decreased appreciably. However, our data show that despite the improvement in the sufficiency of folate intake (intake above the EAR), a large proportion of women aged 19–39 y (as well as 14- to 18-y-old girls) and those aged 60–79 y continue to have intakes that are below the recommended levels. This suggests that the current fortification and enforcement policy may not have sufficiently addressed the goal of increasing intake in women of childbearing age because our calculations suggest that after accounting for the fortification in Mexican flour used in the preparation of commonly eaten foods made by small manufacturers, the proportion of women aged 19–39 y who have intakes below the recommended level remains high.

As shown in Figure 3, our estimates of intake demonstrate increased DFE and FA intake at all ages compared with data that do not account for fortification in bakery bread and corn masa–based foods. As such, our estimates suggest that the proportion of children and women of childbearing age ingesting fewer DFE than the EAR may be considerably lower than previously estimated (55). Our revised intake estimates are also more consistent with published reports on plasma concentrations of folate because serologic data suggest a modest decrease in the prevalence of folate deficiency among women aged 19–39 y, from 5% in the 1999 National Nutrition Survey (the earliest available for Mexico) prior to fortification to 1.9% in ENSANUT 2012 (29).

Because the threshold for deficiency in plasma is quite low, it is not unexpected that our proportion of women with intake below the EAR is much higher than the proportion with deficiency. Importantly, we found that children aged 1–8 y in Mexico are at risk of ingesting FA at levels above the age-specific UL of FA. Some studies have shown that excess intake of FA may be detrimental when at or above the UL (6–11). Our estimates of intake above the UL in 1–8-y-olds suggest that a large number of these children (4.8–11.9%) in Mexico, representing 917,749–1,904,191 children, have intakes above the UL. The NHANES-based study that found elevated circulating unmetabolized FA (cUMFA) in 41–45% of US children aged <11 y suggests physiologically excessive intake at these ages (56, 57) among individuals with smaller BSA. Furthermore, a 200-μg increase in intake of FA was associated with a doubling of the risk for elevated cUMFA (56). Chronic exposure to FA as can occur with a flour fortification program can lead to the appearance of unmetabolized FA in circulation (58, 59). Because higher FA intake has been associated with an increased risk for carcinogenesis (7–9, 11) and both elevated FA intake and cUMFA have been associated with impaired natural killer cell function and cytotoxicity (57, 60), concerns have been raised that higher FA intake may have adverse effects. However, the impact of elevated FA intake in young children is not known. In addition, because age-specific UL levels in children were not derived based on child-specific data but, rather, were calculated using adult data, intake above the UL may not represent a true risk but instead may be an artifact of the UL value (61). However, the similarity in FA intake between Mexican children aged 4–8 y and adults aged 19–39 y (Figure 2) suggests an elevated FA to BSA intake in younger children (more marked in girls), similar to the pattern seen in NHANES (57). Taken together, our results suggest that the variability of fortification of tortillas and bread in Mexico may contribute to chronically elevated FA intake in younger children.

Our results also suggest that intake below the EAR may occur predominantly in regions where tortillas and corn masa–based foods are less likely to be made with fortified flour, suggesting that women living in areas that are less populated might be more likely to have intake below the EAR.

Overall, these findings highlight the importance of following WHO/FAO recommendations for food fortification in which implementation could be structured to minimize overexposure while reaching the target population considering enforceable minimal and maximal levels that account for both EAR and UL of population segments likely to ingest the fortified foods (5, 62).

Strengths and limitations

Our data are based on dietary intake from a nationally representative survey in Mexico. Limitations of our approach include measurement of a relatively limited number of food samples, although we measured more than would normally be measured for determining content for nutrient tables. In contrast to standard food content measurement practices, we measured concentrations in a large number of food samples representing all 4 ENSANUT regions, including multiple states in central and southern Mexico where corn masa–based products dominate food staples, and included samples from both rural and urban settings, as well as from the most populated states in Mexico. Our food samples represented foods eaten by large portions of the population, with >50% and >44% of the ENSANUT 2012 participants reporting consuming sweet and white bread, respectively, and ∼66% consuming tortillas (44).

Although our data included the nutrient content from national social programs distributing dietary supplements in Mexico, they did not include the use of other supplements. Given that supplement intake is extremely low—<2% in nearly all age categories and up to 4% in women of childbearing age (Supplemental Table 3)—we do not think our estimates of proportions above the UL or below the EAR would change substantially with inclusion of FA intake from supplements in the form of pills or tablets. In addition, we did not include the variability of the FA content in breads in our estimates. However, our analyses of the source of FA in diet intake showed that the FA attributable to intake of foods made with corn-based dough (masa), including tortillas, was generally far greater than that attributable to intake of bakery bread (Table 3). Furthermore, analyses on how median FA intake changed after updating bread and tortilla FA content showed that the larger change in FA intake was observed when we updated FA content of corn masa–based foods (including tortillas) (Figure 2). Estimation accuracy could be improved by simply recording the type of tortilla eaten into the

24-h recall data collection and also if data on the use of fortified flour at the tortilla manufacturers (tortillerias) were available. In addition, our data are limited by the lack of cUMFA measures to provide information on relative abilities to metabolize ingested FA.

Surprisingly few studies have reported total folate and specifically FA intake after fortification. Our study is 1 of few such studies at a global level and the first such report for Mexico. The amount of variability in the total folate and FA content contained in the foods that we measured highlights the challenges faced in estimating adequacy of population-level exposure to folate and FA. Our data are consistent with those of a Chilean study (4), which found that flour was variably fortified with FA, although the authors did not measure foods and did not examine impact using national survey data. Although FA flour fortification policies vary globally (16, 20–24, 27, 37, 63–65), several Latin American countries fortify wheat and corn with FA at concentrations similar to those used in Mexico and may have similar patterns of intake of nonindustrialized wheat and corn-based foods. There are limited data from this region documenting the effects of FA flour fortification on folate intake.

In summary, our analyses demonstrated that accounting for FA fortification in staple foods made from wheat and corn, such as bakery bread and tortillas, changed population-level estimates for the adequacy of folate intake in Mexico. Our approach highlights the importance of accounting for the heterogeneity of food additives considering the demographic characteristics of a population. Our models showed that exposure to fortified flour increased considerably with increasing population size in the location of residence. Failure to account for such variation results in an underestimation of the population exceeding the UL and an overestimation of the population with intake below the EAR. Our results suggest the need to implement more representative assessment of FA intake in the segments of the population at potentially greater risk of intake above the UL or below the EAR (i.e., children and adolescents, women of childbearing age, and elders). In addition, our findings suggest that more regulation and oversight (including monitoring and evaluation of fortification) (31) in the implementation of national policies for fortifying corn and wheat flour may be beneficial and may avoid possible overexposure of vulnerable segments of the population or insufficient intake in a large segment of the population initially targeted by this public health intervention.

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