# Prevalence of folate and vitamin B12 deficiency in Mexican children aged 1 to 6 years in a population-based survey

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## Abstract

**Objective**. To describe the magnitude and distribution of folate and vitamin B12 deficiency in Mexican children. **Materials and methods.** Folate and vitamin B12 serum concentrations were measured in a probabilistic sample of 2 099 children. Adjusted prevalence, mean concentrations and relevant associations were calculated based on series of logistic and linear regression models. **Results**. The overall prevalence of folate and vitamin B12 deficiency were 3.2% and 7.7%, respectively. The highest prevalence of folate was found in the 2-year-old (7.9%), and of vitamin B12 in the 1 year-old (9.1%) groups. Being a beneficiary of the fortified milk program Liconsa was protectively associated with serum folate (*p*=0.001) and daily Intake of milk with vitamin B12 (*p*=0.002) concentrations. **Conclusions**. We describe the magnitude of folate and vitamin B12 deficiencies in Mexican children. The deficiency of both vitamins in children under 2 years old is a moderate public health problem in Mexico.

Key words: Vitamin B12 deficiency; folate deficiency; children; probabilistic surveys; Mexico

**Cuevas-Nasu L, Mundo-Rosas V, Shamah-Levy T, Méndez-Gómez Humaran I, Ávila-Arcos MA, Rebollar-Campos MR, Villalpando S. Distribución de la prevalencia de deficiencia de folato y vitamina B12 en niños mexicanos de 1 a 6 años de edad. Encuesta con base poblacional. Salud Publica Mex 2012;54:116-124.**

## Resumen

**Objetivo**. Describir la magnitud de la deficiencia de folato y vitamina B12 en niños mexicanos. **Material y métodos.** Se midieron las concentraciones séricas de folatos y vitamina B12 en una muestra probabilística de 2 099 niños. Se calcularon prevalencias ajustadas, medias de las concentraciones y las asociaciones relevantes mediante una serie de modelos de regresiones logísticas y lineales. **Resultados**. La prevalencia global de deficiencia de folatos y vitamina B12 fueron 3.2% y 7.7%, respectivamente. La prevalencia más alta de folatos se encontró en el grupo de 2 años (7.9%) y de la vitamina B12 en los de 1 año de edad (9.1%). Ser beneficiario del programa de leche fortificada Liconsa estuvo asociado de manera protectora con el folato serico (*p*=0.001) y la ingestión diaria de leche con la de vitamina B12 (*p*=0.002). **Conclusiones**. La deficiencia de ambas vitaminas, en los niños menores de dos años, es un problema de salud pública de magnitud moderada en México.

Palabras clave: deficiencia de vitamina B12; deficiencia de folatos; niños; encuesta probabilística, México

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Deficiency of micronutrients is still one of the most frequent nutritional problems worldwide. The lack of access to a varied diet including animal foods, grains, fruits, and vegetables, is the recurrent cause of deficiency, given those are the most important sources of micronutrients.[1]

Folate deficiency is related mainly to a low intake of green leafy vegetables and legumes and meat.[2] This deficiency is associated with megaloblastic anemia.[3-5] During pregnancy, it is linked to an increased risk of low birth weight and neural tube defects,[6,7] and it is also associated with stunting.[8,9]

Vitamin B12 deficiency is more frequent in population with a poor or inadequate diet of animal foods.[10] Other causes of this deficiency include: intestinal parasitosis in low-income population,[11] *Helycobacter pylori* infections,[12] and atrophic gastritis in older adults (due to lack of intrinsic factor production).[13] Strict vegetarian diets may be associated with vitamin B12 deficiency, both, in the mother and in the newborn, because the demand for this vitamin increases during pregnancy and lactation.[14] Clinical manifestations of vitamin B12 deficiency include megaloblastic anemia which is undistinguishable from that produced by folate deficiency. Vitamin B12 is also crucially involved in the proliferation, maturation, and regeneration of neural cells[15] therefore, its deficiency causes memory loss, dementia, and depression[16] as well as cardiovascular disease[17,18] and cerebrovascular ischemia.[19]

There is very little population-based information regarding folate and vitamin B12 deficiencies, although its prevalence could be very high worldwide.[20] Folate deficiency is among the five micronutrient deficiencies targeted for population-based interventions,[1] especially because of its role in the reduction of the incidence of neural tube defects.[21,22] Also, has been documented that about 40% of Latin American children and adults suffer from vitamin B12 deficiency or have marginally low serum concentrations. The prevalence of vitamin B12 depletion and marginal deficiency is even higher in Kenyan school children (70%) and Indian preschool children (80%).[23]

The recent review by WHO showed that the majority of data on the prevalence of folate and vitamin B12 deficiencies are derived from local surveys, but these and national survey data from a few countries suggest that deficiencies of both of these vitamins may be a public health problem.[24]

In Mexico, there are few studies documenting the deficiency of vitamin B12 and folate. ENN-99 (Encuesta Nacional de Nutrición 1999) was a probabilistic survey, representative of the national, regional, as well as urban and rural levels in Mexico. ENN-99 was conducted on a sample of almost 18 000 households.[25] Overall folate deficiency in ENN-99 was 7.3% in children under 12 years-of-age, the highest prevalence corresponding to children younger than 2 years and those aged 3 to 4 years (8.8% and 11.2%, respectively).[26] Besides in a sample of children younger than 2 years old, representative of beneficiaries of health care services provided by the Mexican Institute of Social Security (IMSS) the prevalence of folate deficiency in children was 10% in urban and 8% in rural area, respectively.[27]

Some studies have measured the magnitude of vitamin B12 deficiency in children under 5 years old, those had reported prevalence of 8% in six rural communities at central region in Mexico.[28] Others reported prevalence of 30% in a subsample of data from the National Nutrition Survey 1999 (ENN-99).[29] In both cases, these studies do not represent the national population.

The aim of this analysis is to describe the magnitude and distribution of folate and vitamin B12 deficiency in Mexican children aged 1 to 6 years, and to identify some associated sociodemographic and dietary determinants using data derived from the National Health and Nutrition Survey 2006 (ENSANUT-2006). Data from this study could help to plan public health strategies and policies focused on the control and reduction of these micronutrient deficiencies.

## Materials and methods

The National Health and Nutrition Survey conducted in Mexico (ENSANUT-2006) was a probabilistic, cross-sectional, stratified cluster sample study representative of the national, regional, and state levels, and of urban and rural areas. Sampling methodology was previously published.[30] The ENSANUT-2006 was more extensive than previous surveys. It covered more health and nutrition problems (including the deficiency of some nutrients such as vitamin B12) which broaden the populations studied in previous surveys. ENSANUT had the power to make distinctions between geographic regions, and urban and rural area of residence.

The information collected includes socio-demographic, health and nutrition conditions; anthropometry and food consumption in all participants, included information of 11 685 children aged 1 to 6 years. Blood samples were obtained from a systematic sub-sample of 2 099 children to document the vitamins deficiencies.

Statistical analysis and model based estimators were carried out to assess a possible selection bias, using reference variables like height, weight and the socioeconomic index. There were not differences compared the selected sub-sample and the overall sample of the survey.

### Blood sample collection, preparation, and preservation

Blood samples were drawn from the antecubital vein and collected in evacuated tubes (Vacutainer vacuum tubes, Beckton Dickinson Inc). Blood was centrifugated *in situ* at 2 500 g in a portable centrifuge (Hettich). Serum was stored in cryotubes covered with aluminum foil to preserve them from light and kept in liquid nitrogen to be transported to the Nutrition laboratory at the National Institute of Public Health in Cuernavaca, Mexico.

### Laboratory methods

Both serum folate and vitamin B12 were released from proteins using sodium hydroxide, dithiothreitol, and potassium cyanide, and transformed into cyanocobalamin and stable folates. Their concentrations were measured by competitive enzymatic immunoassays in a TOSOH automated immunoanalyzer.

### Definition of variables

*a) Folate and vitamin B12 deficiencies*

Children with serum concentrations of vitamin B12 below 203pg/mL and of folate below 4ng/mL were categorized as deficient.[20]

*b) Sociodemographic variables*

A questionnaire administered to the mother or guardian of the child collected socioeconomic and household information. A socioeconomic status index (SES) was constructed through a main components analysis based on the household characteristics and family assets: floor covering, piped water, owning of radio, TV set, computer, telephone, refrigerator, washing machine, stove and automobile. The first component accounted for 46% of the total variance. The resulting standardized factor was divided into tertiles to categorize three socioeconomic status groups: low, medium and high.

*c) Dietary information*

Dietary data were obtained through a semiquantitative food frequency questionnaire for the seven days previous to the interview. The questionnaire included 102 food items and was administered by personnel trained and standardized on data collection.[31]

Data on food intake were transformed into energy and nutrients intake, using a food composition table compiled by INSP.[32] Details about the processing of dietary information were previously published.[33] Mean daily folate and vitamin B12 intakes were estimated and food groups that furnished those vitamins were identified and expressed as 100g of daily intake.

*d) Residence area*

In Mexico, the breakdown by size of locality (urban or rural) is directly related to the principle that smaller towns are reduced in development conditions. According to the National Institute of Statistics and Geography (INEGI, for its acronym in Spanish), localities with 2 500 inhabitants or more were considered as urban; otherwise were categorized as rural.[34]

*e) Region of residence*

According to the ENSANUT sample frame, the states of the country were divided into four regions: North, Center, Mexico City, and South.[30] Mexico City was excluded from this analysis because of the small number of available samples.

*f) Food assistance programs*

A child was defined as affiliated with a public food assistance program when the mother or guardian declared that he or she received food or micronutrient supplements from programs as such Oportunidades, Liconsa or others.

*g) Indigenous ethnicity*

Indigenous ethnicity was defined when a family member spoke an indigenous language.

### Statistical analysis

Data are described as frequencies and by confidence intervals (95%CI). Prevalence for folate and vitamin B12 deficiencies and the adjusted means of the serum concentration of both vitamins were estimated and stratified by age, sex, locality, geographic region, socioeconomic status and indigenous ethnicity. Linear regression models were constructed to assess the effect of nutrient intake, food assistance programs and indigenous ethnicity, adjusted by age sex and sociodemographic variables on folate and vitamin B12 serum concentrations. Interaction terms between folate and vitamin B12 were introduced in both vitamins models.

All analysis procedures were carried out using corrections for the sample. The original expansion factors of this survey were recalculated using a ratio calibration procedure, to improve the population representativeness for estimators at the national level.

The analysis was carried out using the Stata V10.1 software; adjustment for the sample design was performed using the module for complex samples SVY and the SPSS V15.0 software.

### Ethical aspects

The protocol was approved by the Research, Ethics and Biosecurity Committees. Written informed consent was signed-up by the mother or guardian of the child before participation in the survey.

# Results

This sample of 2 099 children aged 1 to 6 years with serum folate and vitamin B12 determinations represents approximately 6 million Mexican children.

### Folic acid deficiency

The overall mean of serum folate concentration was 12.5 ng/mL (95%CI 12.1,12.9) and the overall prevalence of folate deficiency was 3.2% (Table I). The highest prevalence was in children aged 1 to 2 years (4.3% and 7.9%, respectively) and it decreased with age down to 2.4% in children aged 6 years.

**Table I**

**Prevalence of folic acid deficiency in children aged 1 to 6 years* by age group, sex, area of residence, region, and indigenous status. Ensanut 2006, Mexico**

| | Folic acid | | | | | |
|---|---|---|---|---|---|---|
| *Variables* | *Sample* | *Expanded* | *Deficiency (<4ng/mL)* | | *Serum concentration* | |
| | *N* | *(thousands) N* | *%* | *95%CI* | *Medium* | *95%CI* |
| National | 2099 | 6039.5 | 3.2 | (1.4 , 4.9) | 12.50 | (12.1 , 12.9) |
| From 1 to 5 years of age | 1051 | 3628.6 | 3.6 | (0.9 , 6.2) | 12.65 | (12.1 , 13.2) |
| 1 | 156 | 726.6 | 4.3 | (0.0 , 8.6) | 12.79 | (11.5 , 14.1) |
| 2 | 221 | 738.8 | 7.9 | (0.0 , 19.2) | 12.17 | (10.8 , 13.5) |
| 3 | 309 | 1030.0 | 0.7 | (0.0 , 1.6) | 12.74 | (11.6 , 13.8) |
| 4 | 365 | 1133.2 | 2.8 | (0.5 , 5.2) | 12.77 | (11.9 , 13.6) |
| 5 | 534 | 1237.1 | 2.9 | (0.9 , 4.9) | 12.24 | (11.6 , 12.9) |
| 6 | 514 | 1173.8 | 2.4 | (0.8 , 3.9) | 12.30 | (11.6 , 13.0) |
| Sex | | | | | | |
| Boys | 1064 | 3202.5 | 4.1 | (1.1 , 7.1) | 12.19 | (11.6 , 12.8) |
| Girls | 1035 | 2837.0 | 2.1 | (1.0 , 3.3) | 12.83 | (12.3 , 13.3) |
| Area of residence | | | | | | |
| Urban | 931 | 3574.2 | 3.5 | (0.8 , 6.2) | 12.48 | (11.9 , 13.1) |
| Rural | 1168 | 2465.3 | 2.8 | (1.0 , 4.6) | 12.51 | (12.0 , 13.1) |
| Region | | | | | | |
| Northern | 376 | 1328.9 | 0.9 | (0.0 , 2.0) | 13.58 | (12.7 , 14.4) |
| Center | 678 | 1797.8 | 2.9 | (1.2 , 4.6) | 12.43 | (11.8 , 13.1) |
| Southern | 1045 | 2912.8 | 4.4 | (1.0 , 7.8) | 12.04 | (11.4 , 12.7) |
| Indigenous ethnicity | 206 | 555.8 | 4.5 | (0.6 , 8.3) | 11.53 | (10.4 , 12.6) |

* Sample size 2 099, weighted cases: (thousands=6039.5)

Prevalence of folate deficiency was higher in boys (4.1%) than in girls (2.1%); in urban (3.5%) than in rural (2.8%), and in the Southern (4.4%) than in the Northern (0.9%) region. Folate deficiency was also higher in indigenous children (4.5%) compared with the national prevalence (3.2%). However, none of these differences were significant.

In a multiple linear regression model having folate concentrations as the dependent variable, the intake of vegetables (more than 100 g/day) (coefficient 0.79, *p*=0.09) and being beneficiary of the Fortified Milk program (Liconsa) (coefficient 1.72, *p*=0.004) were positively associated with serum folate concentration. Living in the Center or Southern regions was negatively associated with serum folate concentration (coefficients 1.37 and 1.7, p=0.014 and p=0.004, respectively). The interaction between folate intake and vitamin B12 was not significant (Table III).

## Vitamin B12 deficiency

The overall mean concentration of vitamin B12 was 510.2 pg/mL (95%CI 487.9, 532.6), and overall prevalence deficiency was 7.7%. The prevalence of vitamin B12 deficiency varied in a non-systematic manner between 5.5% in children aged 3 years and 10.6% in those aged 6 years. The prevalence was highest in children from the Southern region (12.1%) and in children of indigenous ethnicity (14.2%), and lowest in the Northern region (3.4%), and non-indigenous children and compared with the national prevalence (7.7%) (Table II).

In a multiple linear regression model with vitamin B12 serum concentration as dependent variable, the dairy foods intake (more than 100 mL per day) was found to be positively associated with the serum concentration of vitamin B12 (coefficient 70.0, p=0.002). In addition, belonging to medium or high socioeconomic

**Table II**

**Prevalence of vitamin B12 deficiency in children aged 1 to 6 years by age group, sex, area of residence, region, and indigenous status. Ensanut 2006, Mexico**

| *Variables* | *Sample N* | *Expanded (thousands) N* | *Deficiency (<203pg/mL) %* | *Deficiency (<203pg/mL) 95%CI* | *Serum concentration Medium* | *Serum concentration 95%CI* |
|---|---|---|---|---|---|---|
| National | 2040 | 5950.6 | 7.7 | (5.3 , 10.0) | 510.22 | (487.9 , 532.6) |
| From 1 to 5 years of age | 1007 | 3568.9 | 7.3 | (4.2 , 10.4) | 516.29 | (489.3 , 543.3) |
| 1 | 151 | 720.3 | 9.1 | (5.0 , 22.1) | 481.88 | (427.8 , 535.9) |
| 2 | 208 | 719.9 | 5.9 | (4.2 , 14.1) | 550.62 | (493.4 , 607.8) |
| 3 | 296 | 1003.8 | 5.5 | (3.2 , 12.7) | 524.89 | (485.7 , 564.1) |
| 4 | 352 | 1124.8 | 8.6 | (3.9 , 14.2) | 508.67 | (453.0 , 564.3) |
| 5 | 528 | 1224.1 | 6.0 | (4.4 , 11.5) | 542.55 | (485.9 , 599.2) |
| 6 | 505 | 1157.7 | 10.6 | (6.9 , 15.6) | 457.36 | (422.1 , 492.6) |
| Sex | | | | | | |
| Boys | 1042 | 3178.2 | 6.9 | (3.7 , 10.0) | 519.12 | (488.5 , 549.8) |
| Girls | 998 | 2772.4 | 8.6 | (5.3 , 11.9) | 500.03 | (469.6 , 530.5) |
| Area of residence | | | | | | |
| Urban | 916 | 3543.5 | 5.9 | (2.6 , 9.2) | 536.27 | (503.9 , 568.6) |
| Rural | 1124 | 2407.1 | 10.2 | (7.1 , 13.3) | 471.89 | (444.0 , 499.8) |
| Region | | | | | | |
| Northern | 373 | 1323.1 | 3.4 | (0.7 , 6.2) | 563.79 | (513.3 , 614.2) |
| Center | 641 | 1747.3 | 3.6 | (1.6 , 5.7) | 535.95 | (506.2 , 565.8) |
| Southern | 1026 | 2880.2 | 12.1 | (7.7 , 16.4) | 470.01 | (436.4 , 503.7) |
| Indigenous ethnicity | 203 | 548.8 | 14.2 | (7.2 , 21.1) | 364.18 | (330.3 , 398.1) |

* Sample size 2 099, weighted cases: (thousands=6039.5)

**Table III**

**MULTIPLE LINEAR REGRESSION MODEL WITH FOLATE CONCENTRATION AS DEPENDENT VARIABLE,* ADJUSTING FOR SOCIODEMOGRAPHIC AND FOOD INTAKE VARIABLES IN MEXICAN CHILDREN AGED 1 TO 6 YEARS. ENSANUT 2006, MEXICO**

| *Covariables* | *Coefficient* | *Standard error* | *p value* | *95%CI* |
|---|---|---|---|---|
| SES Medium‡ | -0.28 | 0.50 | 0.581 | (-1.26 , 0.71) |
| SES High‡ | -0.04 | 0.62 | 0.945 | (-1.25 , 1.17) |
| Beneficiary of Oportunidades | 0.35 | 0.50 | 0.480 | (-0.63 , 1.33) |
| Beneficiary of Liconsa | 1.72 | 0.52 | 0.001 | (0.71 , 2.73) |
| Center Region§ | -1.37 | 0.56 | 0.014 | (-2.46 , -0.27) |
| Southern Region§ | -1.70 | 0.59 | 0.004 | (-2.85 , -0.55) |
| Area Rural | 0.53 | 0.47 | 0.262 | (-0.40 , 1.45) |
| Sex Boy | -0.59 | 0.35 | 0.090 | (-1.28 , 0.09) |
| Age (years) | -0.19 | 0.12 | 0.128 | (-0.43 , 0.05) |
| Indigenous status | -0.80 | 0.67 | 0.228 | (-2.11 , 0.50) |
| Folate intake | 0.00 | 0.00 | 0.357 | (-0.004 , 0.010) |
| Vitamin B12 intake | 0.02 | 0.38 | 0.952 | (-0.72 , 0.76) |
| Folate X Vitamin B12 | 0.00 | 0.00 | 0.710 | (0 , 0) |
| Grains intake (>100g/d) | 0.69 | 0.53 | 0.188 | (-0.34 , 1.73) |
| Legumes intake (>100g/d) | 0.20 | 0.48 | 0.673 | (-0.74 , 1.14) |
| Vegetables intake (>100g/d) | 0.79 | 0.47 | 0.092 | (-0.13 , 1.72) |
| Constant | 13.03 | 0.89 | 0.000 | (11.29 , 14.77) |

* n=2011

‡ Dummy variable: SES Low

§ Dummy variable: Northern Region

status was positively associated with the serum concentration of vitamin B12 (coefficients 98.7 and 88.9, respectively, $p$=0.001).

Being of indigenous ethnicity was negatively associated with the serum concentrations of vitamin B12 (coefficient 48.2, $p$=0.04) (Table IV).

## Discussion

We describe herein the magnitude and distribution of the prevalence of folate and vitamin B12 deficiency in a population sample of Mexican children younger than 6 years.

The observed national prevalence of 7.7% for vitamin B12 and 3.2% for folate deficiencies represents more than 456 000 and 193 000 children younger than 6 years, suffering from these vitamin deficiencies in Mexico. The national overall prevalence of folate deficiency was relatively low, although the groups of 1 and 2-year-old children had the highest prevalence (4.3% and 7.9%, respectively).

While the national prevalence of vitamin B12 was always above 5%. Such prevalence, close to 5%, should be considered as a public health problem according to the WHO Technical Consultation on folate and vitamin B12.[20]

Results also indicate that the highest prevalence of folate and vitamin B12 deficiency have seen in the most vulnerable population: children younger than 2 years old, from indigenous origin, and living in the Southern region, known for its poor economic development in Mexico.

The low serum concentrations of vitamin B12 and folate, might be associated with the low body reserves of newborns at birth. In developing countries, maternal deficiency of vitamin B12 is frequent during pregnancy due to poor or inadequate intakes of animal foods. For small children, dietary intake of both vitamins is limited during the weaning period because, frequently, weaning foods offered is reduced to a limited variety of foods with a low content of micronutrients. This, in time, critically reduces their body stores and serum levels.

Also, the high prevalence of B12 deficiency in school children might be associated with an inadequate intake or a low absorption of the vitamin because of parasitic or recurrent bacterial infections.[35-37]

**Table IV**

**MULTIPLE LINEAR REGRESSION MODEL WITH VITAMIN B12 CONCENTRATION AS DEPENDENT VARIABLE,* ADJUSTING FOR SOCIODEMOGRAPHIC AND FOOD INTAKE VARIABLES IN MEXICAN CHILDREN AGED 1 TO 6 YEARS. ENSANUT 2006, MEXICO**

| *Covariables* | *Coefficient* | *Standard error* | *p value* | *95%CI* |
|---|---|---|---|---|
| SES Medium‡ | 98.71 | 28.40 | 0.001 | (42.94 , 154.47) |
| SES High‡ | 88.88 | 26.93 | 0.001 | (35.99 , 141.78) |
| Beneficiary of Oportunidades | -23.73 | 23.91 | 0.321 | (-70.69 , 23.24) |
| Beneficiary of Liconsa | 38.40 | 39.67 | 0.333 | (-39.50 , 116.31) |
| Center Region§ | -12.61 | 25.41 | 0.620 | (-62.51 , 37.28) |
| Souther Region§ | -25.71 | 33.72 | 0.446 | (-91.92 , 40.50) |
| Area Rural | 13.41 | 18.56 | 0.470 | (-23.03 , 49.85) |
| Sex Boy | 27.80 | 17.12 | 0.105 | (-5.83 , 61.42) |
| Age (years) | -0.06 | 5.86 | 0.992 | (-11.58 , 11.46) |
| Indigenous status | -48.28 | 23.49 | 0.040 | (-94.4 , -2.15) |
| Vitamin B12 intake | 25.50 | 15.09 | 0.092 | (-4.14 , 55.14) |
| Folate intake | -0.03 | 0.13 | 0.853 | (-0.29 , 0.24) |
| Folate X Vitamin B12 | 0.01 | 0.04 | 0.760 | (-0.07 , 0.10) |
| Grains intake (>100g/d) | -35.01 | 23.84 | 0.142 | (-81.83 , 11.81) |
| Milk intake (>100g/d) | 70.07 | 22.60 | 0.002 | (25.70 , 114.45) |
| Legumes intake (>100g/d) | -28.38 | 22.52 | 0.208 | (-72.61 , 15.85) |
| Meat intake (>100g/d) | -4.41 | 62.86 | 0.944 | (-127.87 , 119.05) |
| Eggs intake (>100g/d) | -38.64 | 33.90 | 0.255 | (-105.22 , 27.94) |
| Constant | 411.74 | 46.32 | 0.000 | (320.78 , 502.71) |

* n=1953
‡ Dummy variable: SES Low
§ Dummy variable: Northern Region

The presence of fortification programs, population group, indicators and cut-off points used to assess folate and vitamin B12 status in countries with similar levels of development as such Guatemala,[38] Costa Rica[39] and Venezuela[40] were not homogeneous and precluded us to make comparisons with the findings reported. Comparability with other population-based surveys in Mexico and the estimation of trends are difficult as well. In the 1999 National Nutrition Survey (ENN-1999)[26] folate was measured in red blood cells using a microbiological assay, whereas in the ENSANUT-2006, folate concentration was measured in serum using an immune assay. On these grounds the comparison is not valid because red blood cell folate is an indicator of body stores while serum values indicate recent dietary intake. However, repeated plasma folate measurements over time reflect trends in folate status.[41] In 1999, the prevalence of low folate stores in children under 5y of age was low (10%), but we are not able to assess whether the 2006 prevalence of low serum folate in the same age group (3.6%) represent a change.

Is also difficult to quantify the change in the magnitude of the deficiency of vitamin B12 because published studies are based on specific groups or populations who do not represent the national population. However, the prevalence reported by Allen L. in the preschool age group from a subsample of the National Nutrition Survey 1999 (ENN-99) was 30%[29] compared with 7% in the present study, suggests a decrease in the magnitude of this problem.

For more than a decade, Mexico has implemented targeted interventions in addressing nutrient deficiencies in children: The voluntary fortification of maize and wheat flour with iron and folic acid;[42] Oportunidades Human Development Program that distributes a nutritional supplement (suplemento Nutrisano) and The Social Milk Supply Program (LICONSA) that provides fortified milk containing nutrients deficient in the Mexican diet, including folic acid and vitamin B12. Without a doubt, these interventions have a positive effect on reducing these vitamins deficiencies in children, however, the impact of these interventions in reducing folate and vitamin B12 deficiencies has not been evaluated. We found in this study that being beneficiary of the Liconsa program was associated with higher serum concentrations of folate, and the intake of more than 100mL of dairy products was positively associated with serum concentrations of vitamin B12.

This study contributes to generate information on the magnitude of the deficiency of both vitamins, since there are very few available publications documenting folate and vitamin B12 deficiencies in children at preschool age, one of the most affected group.[43] The current strategies for reducing and controlling micronutrient deficiencies should thus be reexamined. Particular attention must be paid to the content of folic acid and vitamin B12 of the food or supplements distributed by food assistance programs like Oportunidades and Liconsa.

*Declaration of conflict of interests.* The authors declare that they have no conflict of interests.

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