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Effect of different dosage and administration schedules of folic acid on blood folate levels in a population of Honduran women of reproductive age

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Abstract

Background: Observational studies and clinical trials have shown conclusive evidence that periconceptional folic acid supplementation prevents up to 70 % of neural tube defects (NTD). The Honduran government wanted to implement a supplementation programme of folic acid but needed to assess the relative effects of two dosages of folic acid.

Objective: To determine the effect of two dosages of folic acid on blood folate levels in Honduran female factory workers aged 18 to 49 years.

Design: This was a randomized, double-blind control supplementation trial conducted in Choloma, Honduras. A total of 140 eligible women were randomly assigned to two dosage groups and followed up for 12 weeks. One group received a daily dosage of 1 mg folic acid and the other a once weekly dosage of 5 mg. Serum folate and red blood cell folate levels were determined by radioassay at baseline, 6 weeks and 12 weeks.

Results: Serum folate levels increased from 6·3 (SE 0·2) to 14·9 (SE 0·6) ng/ml ($P < 0·0001$) in women assigned to the 1 mg/d group and from 6·9 (SE 0·3) to 10·1 (SE 0·4) ng/ml ($P < 0·0001$) in those assigned to the 5 mg/week group. Red blood cell folate concentrations also increased significantly in both groups, albeit more slowly. Educational level, age and BMI were not associated with the changes in serum and red blood cell folate levels during the supplementation period. However, a differential effect on serum folate levels by dosage group and time was observed.

Conclusions: Although both folate supplementation regimens increased serum and red blood cell folate levels significantly among the women studied, blood folate levels that are considered to be protective of NTD were reached faster with the daily dosage of 1 mg folic acid.

Keywords
Randomized clinical trial
Folic acid supplementation
Childbearing-age women
Honduras

Neural tube defects (NTD) are among the most frequent congenital malformations contributing to infant mortality and serious disability[1–4]. In Central America, information on the prevalence of NTD is scarce except for Costa Rica, Guatemala and Honduras, where prevalence rates between 6/10 000 and 26/10 000 have been reported[5–8]. In these countries, NTD coupled with congenital heart defects constitute 40 % of all causes of infant mortality[6].

Observational studies and clinical trials have shown conclusive evidence that periconceptional folic acid supplementation prevents up to 70 % of NTD[9–12]. The level of effectiveness likely depends on the population's folate levels and the local prevalence of NTD[13–15].

In 2002, Central American countries implemented a resolution to fortify wheat flour with 1·8 mg folic acid/kg[16]. Although fortification of wheat flour with folic acid has been mandatory in Honduras since January 2003, no monitoring system is in place to determine whether or not

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this fortification is done properly. In addition, consumption of wheat flour products in Honduras is low, as the main staples are rice, corn and beans[17]. Furthermore, although the Honduran Ministry of Health approved a periconceptional folic acid supplementation programme among women of childbearing age in February 2005, it has not been implemented. The Honduran government wanted to assess the efficacy of folic acid supplementation at a daily dose of 1 mg *v.* a weekly dose of 5 mg to revise the national health policy. To address this question, we conducted a randomized controlled trial to compare the efficacy of the two regimens.

Methods

Target population

The study was conducted in Choloma, a city in north-west Honduras with a population of 190 000 in 2005[18]. The city has several large assembly factories ('maquilas'). Staff held an information fair for all female workers aged 18 to 49 years in one of the maquilas willing to participate and, after explaining the purpose and content of the study, invited them to participate in the 12-week trial. The study protocol was approved by the Western Regional Institutional Review Board in Honduras and the Centers for Disease Control and Prevention (CDC) Institutional Review Board in Atlanta, Georgia, USA.

Screening procedures

Women who enrolled filled out a standardized questionnaire, which included the collection of demographic information, length of employment at the factory, place of origin, date of birth, level of education, reported medical history including pregnancy status and history, current medications and vitamin consumption. Women were excluded if they were younger than 18 years or older than 49 years, if they had been employed at the maquila for 6 months or less, if they were reported to be pregnant at the time of the interview, or if they had consumed a supplement containing folic acid during the three months prior to the study. Also excluded from the study were women who reported having had a previous pregnancy with a birth defect; those who had a chronic disease such as cancer, diabetes or chronic diarrhoea; and those who were taking medications that interfere with folic acid metabolism, including anticonvulsant drugs and folic acid antagonists. At the time of enrolment, blood was drawn to assess Hb levels and women with levels <12 mg/dl were excluded and referred to the maquila clinic for diagnosis and treatment. From the volunteers who remained in the study, we obtained a second blood sample to determine vitamin B_{12} and folate status. Women who were found to be vitamin B_{12}-deficient (<200 pg/ml) or who had folate levels <2 ng/ml were excluded from participation and referred to the factory physician for diagnosis, treatment and follow-up.

Study design

The study was designed as a randomized, double-blind controlled supplementation trial to assess in a 12-week period the impact of two dosage levels of folic acid on blood folate levels not at steady state among women of childbearing age. Selection of the duration of the study was based on taking into consideration comparability with other efficacy studies, working schedules for participating volunteers and the fact that longer periods might have decreased compliance. Sample size was determined using the criteria that a true difference of 5 ng/ml should be detected with a power of 95 % using a two-tailed test at a significance level of 0·05. Under these criteria, we determined that each final dosage group should be composed of fifty subjects. This sample size was increased to about seventy subjects to protect against attrition of up to 30 %. A randomization program was used to allocate volunteers into the two groups[19]. The trial was conducted between April and June 2005. During workdays, folic acid pills were administered to the study subjects by two factory nurses. Women assigned to the 5 mg/week group were given the folic acid supplement on Wednesdays and received a placebo on the other days of the week. Each Friday, participants took home the pills corresponding to Saturday and Sunday, and on Mondays the nurses documented if they had been consumed. There was no penalty for forgetting to take the pill. The 1 mg, 5 mg and placebo pills were of the same colour and size, and neither the authors nor the health staff or volunteers were aware of the subject's dose category until the study was completed.

Biochemical analyses

At baseline, midpoint (6 weeks) and endpoint (12 weeks), venous blood samples were collected from all participants after an overnight fast. Women receiving daily and weekly doses of folic acid had their blood samples drawn 1 d and 5 d after the last pill was taken, respectively. Blood samples were allowed to clot at room temperature for 30 min to 2 h; then serum was separated from the red cells by centrifugation and stored frozen at ≤−40°C until analysis. EDTA whole blood was diluted 1:11 with ascorbic acid (10 g/l) and stored frozen at ≤−40°C until analysis. Frozen samples were shipped on dry ice to the CDC laboratory where biochemical analyses were conducted. Total folate and vitamin B_{12} in serum and total folate in whole blood were measured by a radioassay[20]. An aliquot of serum or 1:11 diluted whole blood (with ascorbic acid 10 g/l) was combined with the tracers [^{125}I]folate and [^{57}Co]vitamin B_{12}, which were then boiled to inactivate endogenous folate-binding proteins, and the various forms of vitamin B_{12} were converted to cyanocobalamin. After cooling, the solution was combined with immobilized affinity-purified porcine intrinsic factor and folate-binding proteins, incubated for 1 h at room temperature, centrifuged, decanted, and the radioactivity associated with the pellet counted. Standard curves prepared by using

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the precalibrated folate/vitamin B_{12} standards in a human serum albumin base were used to determine the concentration of the folate and vitamin B_{12} in the patients' serum and whole blood.

Statistical analyses

Statistical analyses were conducted using the SAS for Windows statistical software package version 9·13 (SAS Institute Inc., Cary NC, USA)[19]. Baseline characteristics in the two groups were compared using the *t* test for continuous variables and the χ^2 test for categorical variables. We investigated the effects of the supplemental dosage regimen using a generalized linear regression model (PROC MIXED procedure in SAS)[21] of repeated measures analysis to determine if there were changes in serum and blood folate levels over time and (via interaction terms) whether over time trends were related to women's baseline age, education and BMI. All analyses were conducted based on the intention to treat, and *P* values were two-sided. For the generalized regression model, we assumed the correlation matrix across time within subjects to be unstructured. Due to the tendency of the distribution of the observed data to be skewed to the right, the serum and red blood cell folate levels were transformed using the natural log prior to fitting the longitudinal model. Results are presented without transformation because they supported the same outcomes.

Results

The number of volunteers and participants are summarized in Fig. 1. Of the 288 women who volunteered, forty-seven were not eligible because they were pregnant, had been taking supplements with folic acid, suffered from chronic disease or had a previous pregnancy with a birth defect. An additional forty-six women were not eligible because they were found to be anaemic. Screening of the remaining 195 women for serum folate and vitamin B_{12} levels showed that an additional fifty were ineligible because of low folate or B_{12}. This decreased the number of eligible volunteers to 145. An additional five women were ineligible because they only worked 4 d/week. Thus, 140 women were enrolled and randomly assigned to a folic acid supplementation regimen of either 5 mg once weekly or 1 mg daily. Follow-up rates at the end of the study were fifty-eight out of seventy (82·9 %) for the 5 mg once weekly dosage group and fifty of out seventy (71·4 %) for the 1 mg daily dosage group. Eleven volunteers in the 5 mg/week group dropped out from the study because of the following reasons: four resigned from work, three became pregnant, one had low B_{12} and three did not want to continue in the trial. Among the women in the 1 mg/d group, eight dropped out because they resigned their positions, two were fired, five became pregnant, one had low B_{12}, two had burning and discomfort, and two did not give any specific reason.

Both groups were similar at baseline in terms of age, height, weight, BMI, educational level, and Hb and vitamin B_{12} levels (Table 1).

Effects on serum and red blood cell folate

Baseline serum and red blood cell folate levels did not differ significantly between the two groups (Table 2). During the 12-week supplementation period, both dosage supplementation groups (5 mg/week and 1 mg/d) resulted in a significant increase in serum folate; however, increases differed by dosage group. Among women assigned to the 5 mg/week group, serum folate levels increased from 6·9 (SE 0·4) to 10·1 (SE 0·3) ng/ml ($P<0.0001$). Women assigned to the daily supplementation group (1 mg/d) increased their serum folate levels from

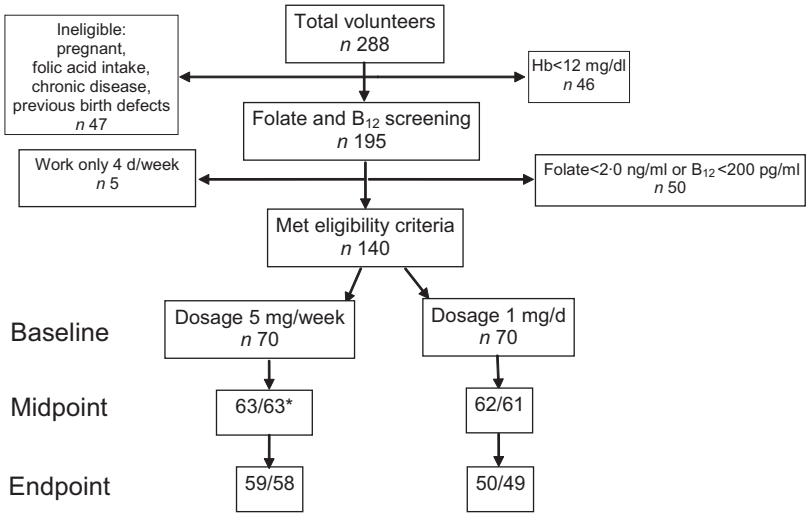

Fig. 1 Flow and follow-up of study participants: Honduras, 2005 (*number of women remaining in study/number of women with blood draws)

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Table 1 Characteristics of study participants by folic acid dosage and compliance status at baseline: Honduras, 2005

	Total population						
	5 mg/week (*n* 70)			1 mg/d (*n* 70)			
Characteristic	Mean	SE	Median	Mean	SE	Median	*P* for difference
Age (years)	26·7	0·9	26·8	26·8	0·5	26·0	NS
Education (years)	7·1	0·4	6·0	7·2	0·3	6·0	NS
Weight (kg)	57·7	1·3	56·4	58·7	1·5	58·0	NS
Height (m)	1·5	0·8	1·5	1·5	0·9	1·5	NS
BMI (kg/m^2)	25·0	1·3	23·7	25·3	0·9	24·7	NS
Hb (mg/dl)	13·2	0·3	13·1	13·3	0·3	13·2	NS
Vitamin B_{12} (pg/ml)	319·3	13·8	289·5	355·2	17·3	326·6	NS
	Compliers						
	5 mg/week (*n* 58)			1 mg/d (*n* 50)			
	Mean	SE	Median	Mean	SE	Median	
Age (years)	26·8	0·9	26·0	27·6	0·9	26·7	NS
Education (years)	7·1	0·5	6·0	6·9	0·6	6·0	NS
Weight (kg)	57·7	1·6	56·3	58·7	1·5	56·8	NS
Height (m)	1·5	0·8	1·5	1·6	0·9	1·5	NS
BMI (kg/m^2)	25·3	0·7	23·7	25·2	0·6	24·7	NS
Hb (mg/dl)	13·1	0·8	13·0	13·3	1·2	13·2	NS
Vitamin B_{12} (pg/ml)	345·3	17·3	323·5	319·2	18·3	305·6	NS
	Non-compliers						
	5 mg/week (*n* 12)			1 mg/d (*n* 20)			
	Mean	SE	Median	Mean	SE	Median	
Age (years)	25·6	1·1	25·8	24·5	1·5	24·7	NS
Education (years)	6·8	0·6	6·0	6·2	0·3	6·0	NS
Weight (kg)	58·0	3·9	55·2	57·8	2·4	57·7	NS
Height (m)	1·5	0·1	1·5	1·5	0·1	1·5	NS
BMI (kg/m^2)	25·8	1·6	24·2	24·8	1·1	24·2	NS
Hb (mg/dl)	12·9	2·4	12·8	13·0	2·3	13·2	NS
Vitamin B_{12} (pg/ml)	281·0	26·7	236·8	333·2	31·9	319·6	NS

Table 2 Serum folate and red blood cell folate levels of study participants at baseline, midpoint (6 weeks) and endpoint (12 weeks): Honduras, 2005

		Baseline			Midpoint			Endpoint			
Dosage group	*n*	Mean	SE	Median	Mean	SE	Median	Mean	SE	Median	% change*
					Serum folate (ng/ml)						
5 mg/week†	58	6·9	0·3	6·6	8·7	0·3	8·0	10·1	0·4	9·1	46
1 mg/d†	50	6·3	0·2	6·0	12·2	0·3	12·0	14·9	0·6	14·1	136
P‡		0·4			<0·0001			<0·0001			
					Red blood cell folate (ng/ml)						
5 mg/week	58	200·8	8·4	189·7	226·5	9·4	210·0	250·8	9·7	244·0	25
1 mg/d	50	199·4	11·9	170·9	220·7	9·7	205·0	294·4	12·3	270·0	48
P		0·6			0·1			<0·0001			

*Percentage increase from baseline to endpoint.
†Mean values were significantly different at each time point (test for trend): $P < 0·0001$.
‡Cross-sectional significance level of difference between mean values of 5 mg and 1 mg dosage at each time point.

6·3 (SE 0·2) to 14·9 (SE 0·6) ng/ml ($P < 0.0001$). These folate levels at the endpoint were 46 % and 136 % higher than at baseline, respectively. Red blood cell folate concentrations also increased significantly in both groups, but at a slower rate: 25 % and 48 %, respectively.

The magnitude of the increase in serum and red blood cell folate was influenced by the baseline quartile of serum and red blood cell folate levels and the folic acid dosage. Subjects in the lowest quartile of baseline serum and red blood cell folate (<25th percentile) and receiving 1 mg folic acid/d showed a larger increase in serum (230 %) and red blood cell folate (89 %) than did subjects receiving a single weekly dose of 5 mg folic acid (92 % and 33 %, respectively) (Table 3). The difference in red

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Table 3 Serum folate and red blood cell folate levels of study participants by baseline quartile of serum and red blood cell folate levels and folic acid dosage regimen: Honduras, 2005

			Baseline		Midpoint		Endpoint			
Dosage group	Baseline quartile	*n*	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	% change*	*P*†
					Serum folate (ng/ml)					
5 mg/week	1st quartile‡	14	3·8	3·5, 4·1	6·5	5·9, 7·1	7·3	6·4, 8·1	92	<0·0001
1 mg/d		12	4·3	3·9, 4·6	11·1	9·6, 12·7	14·2	9·9, 18·4	230	<0·0001
P				0·06‖		0·0005		0·004		
5 mg/week	2nd quartile	15	5·6	5·2, 5·9	7·9	6·8, 8·9	8·8	7·9, 9·8	57	<0·0001
1 mg/d		13	5·6	5·5, 5·8	11·6	10·5, 12·7	13·7	12·2, 15·2	145	<0·0001
P				0·71		0·002		0·0005		
5 mg/week	3rd quartile	14	7·4	7·1, 7·8	9·3	8·4, 10·1	10·8	9·3, 12·2	46	0·0008
1 mg/d		12	6·6	6·4, 6·8	12·0	10·7, 13·3	16·3	13·6, 19·0	147	<0·0001
P				0·81		0·006		0·005		
5 mg/week	4th quartile	15	10·5	9·5, 11·4	11·1	9·6, 12·5	13·5	11·7, 15·3	29	0·027
1 mg/d		13	8·5	7·9, 9·0	14·0	12·8, 15·2	15·4	13·8, 17·1	81	<0·0001
P				0·003		0·019		0·15		
					Red blood cell folate (ng/ml)					
5 mg/week	1st quartile§	15	132·4	122·4, 142·4	156·7	143·3, 170·2	176·3	162·2, 190·5	33	0·0004
1 mg/d		12	125·5	114·1, 136·9	168·1	152·7, 183·5	237·3	211·1, 263·4	89	<0·0001
P				0·55		0·36		0·002		
5 mg/week	2nd quartile	14	175·7	170·5, 181·0	200·6	187·3, 214·0	226·3	213·2, 239·4	29	<0·0001
1 mg/d		13	160·1	157·0, 163·3	193·6	177·9, 209·3	254·4	228·3, 280·5	59	<0·0001
P				0·011		0·34		0·09		
5 mg/week	3rd quartile	15	208·5	201·9, 215·1	248·7	221·9, 275·4	259·1	243·8, 274·4	24	<0·0001
1 mg/d		13	198·4	189·2, 207·6	218·2	205·1, 231·4	296·6	276·1, 317·1	49	<0·0001
P				0·14		0·11		0·02		
5 mg/week	4th quartile	14	290·8	264·8, 316·9	305·3	271·6, 339·0	339·4	297·9, 381·0	17	0·29
1 mg/d		12	298·1	246·3, 349·9	304·2	258·8, 349·6	397·0	341·8, 452·2	33	0·0193
P				0·38		0·86		0·13		

*Percentage increase from baseline to endpoint.
†Kruskal–Wallis test to compare dosage groups by baseline quartile and time point.
‡Serum folate (ng/ml), 5 mg/week dosage group: 1st quartile, <4·62; 2nd quartile, ≥4·62 and <6·64; 3rd quartile, ≥6·64 and <8·53; 4th quartile, ≥8·53. Serum folate (ng/ml), 1 mg/d dosage group: 1st quartile, <5·08; 2nd quartile, ≥5·08 and <5·99; 3rd quartile, ≥5·99 and <7·28; 4th quartile, ≥7·28.
§Red blood cell folate (ng/ml), 5 mg/week dosage group: 1st quartile, <162·00; 2nd quartile, ≥162·00 and <189·76; 3rd quartile, ≥189·76 and <229·71; 4th quartile, ≥229·71. Red blood cell folate (ng/ml), 1 mg/d dosage group: 1st quartile, <150·73; 2nd quartile, ≥150·73 and <168·59; 3rd quartile, ≥168·59 and <219.03; 4th quartile ≥219·03.
‖Wilcoxon–Mann–Whitney test to compare dosage groups by baseline quartile and time point.

blood cell folate levels for other quartiles showed similar patterns, although the change from baseline to final levels was smaller. Subjects who received 1 mg/d consistently showed significantly higher increases across all quartiles compared with those receiving 5 mg/week.

Serum and red blood cell folate levels during the supplementation period did not vary by age and education level (data not shown). However, when we stratified serum folate levels by dosage group, BMI and time (Table 4), we observed significant increases in serum folate levels in dosage group and time for each category of BMI. Serum folate levels were significantly different between dosages only among those women with BMI < 30 kg/m^2. Similar trends were observed in red blood cell folate levels between dosage groups and time but no significant differences within BMI groups.

Discussion

The results of the present randomized clinical trial indicate that both the daily and the weekly folic acid supplementation regimens significantly increased serum and red blood cell folate levels among the women studied. However, supplementation with a daily dose of 1 mg led to a larger and more rapid increase in both serum and red blood cell folate levels from baseline to endpoint.

The increases and patterns attained in serum and red blood cell folate by both folic acid dosage regimens in the present trial are in line with previous studies[22–24], but direct comparisons on absolute values cannot be made because of differences in laboratory procedures. However, in 2001 Martinez *et al.*[23] carried out a supplementation trial with a weekly dosage of 5 mg folic acid among non-pregnant women of childbearing age in Nuevo León, Mexico, using similar laboratory tests. These authors found after 12 weeks that serum and red blood cell folate levels increased from 5·93 to 7·0 ng/ml (18 %) and from 150·5 to 184·0 ng/ml (22 %), respectively. These absolute and relative increases in folate levels were significantly lower than those attained in our study. Some of these differences could be explained by smaller attrition and better compliance in our study.

Comparison of blood folate levels observed in the present study with other cross-sectional studies at pre-fortification and post-fortification levels are important

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Table 4 Serum folate and red blood cell folate levels of study participants by folic acid dosage group, BMI and time: Honduras, 2005

			Baseline		Midpoint		Endpoint			
Dosage group	BMI category (kg/m^2)	*n*	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI	% change*	*P*†
					Serum folate (ng/ml)					
5 mg/week	<25‡	33	6·1	5·3, 6·9	8·1	7·3, 9·0	9·4	8·4, 10·3	54	<0·0001
1 mg/d	<25	27	6·3	5·7, 6·9	12·4	11·5, 13·2	15·5	13·4, 17·7	146	<0·0001
P				0·46		<0·0001		<0·0001		
5 mg/week	≥25 and <30	20	7·5	6·2, 8·7	9·3	8·1, 10·4	10·6	8·9, 12·4	41	<0·0001
1 mg/d	≥25 and <30	17	6·6	5·7, 7·4	12·0	10·7, 13·4	15·4	14·1, 16·7	133	<0·0001
P				0·45		0·002		<0·0001		
5 mg/week	≥30	5	9·5	7·0, 12·0	10·2	8·0, 12·4	13·2	10·1, 16·3	39	0·0004
1 mg/d	≥30	6	5·5	3·9, 7·1	12·2	9·8, 14·6	10·7	8·3, 13·1	95	0·002
P				0·005		0·28		0·24		
					Red blood cell folate (ng/ml)					
5 mg/week	<25	33	191·0	169·0, 213·0	220·9	195·0, 247·0	240·5	212·0, 269·0	26	<0·0001
1 mg/d	<25	27	193·7	163·0, 225·0	214·0	193·0, 235·0	293·0	269·0, 317·0	51	<0·0001
P				0·97		0·94		0·002		
5 mg/week	≥25 and <30	20	214·1	184·0, 245·0	235·4	205·0, 265·0	266·6	237·0, 296·0	25	<0·0001
1 mg/d	≥25 and <30	17	203·5	164·0, 243·0	233·1	192·0, 274·0	309·0	255·0, 363·0	52	<0·0001
P				0·50		0·78		0·17		
5 mg/week	≥30	5	211·6	187·0, 237·0	233·6	205·0, 263·0	237·0	212·0, 262·0	12	0·013
1 mg/d	≥30	6	175·8	132·0, 220·0	222·6	173·0, 272·0	268·5	195·0, 342·0	53	0·008
P				0·27		0·74		0·60		

*Percentage increase from baseline to endpoint.
†Test for trend.
‡BMI < 25 kg/m^2, normal weight; BMI ≥ 25 and <30 kg/m^2, overweight; BMI ≥ 30 kg/m^2, obese.

because they can provide guidance on how populations or subgroups of populations are standing compared with other groups or populations that have reached higher folate levels. The baseline results from our study were similar to reported folate levels in a sample of volunteers among non-pregnant Honduran women[24]. However, mean baseline serum folate levels in our study were significantly higher than pre-fortification levels of US non-pregnant women of childbearing age studied in the 1994 NHANES (National Health and Nutrition Examination Survey) (6·9 and 6·3 *v.* 4·6 ng/ml, $P < 0{\cdot}01$)[25]. Serum folate levels after 6 weeks of intervention in our study subjects receiving a daily folic acid supplement of 1 mg were similar to those found in 1999–2000 NHANES non-pregnant women after fortification (12·2 *v.* 12·6 ng/ml)[26]. Furthermore, by the 12th week of intervention, serum folate levels were significantly higher in our study subjects (14·9 *v.* 12·6 ng/ml, $P < 0.001$) than in 1999–2000 NHANES. In contrast, among women receiving a weekly 5 mg dose, mean serum folate levels were significantly lower than in NHANES non-pregnant women post-fortification (8·7 and 10·1 *v.* 12·6 ng/ml, $P < 0{\cdot}001$).

Comparison of red blood cell folate levels at the end of 12 weeks showed that levels in NHANES non-pregnant women of childbearing age after folic acid fortification were similar to those observed in women receiving a weekly folic acid dose of 5 mg in our study (253·3 *v.* 250·8 ng/ml, P = NS). In contrast, women receiving a daily supplementation dose of 1 mg reached significantly higher red blood cell folate levels than NHANES women post-fortification (294·4 *v.* 253·3 ng/ml, $P < 0.01$) and were above the Healthy People 2010 target objectives (220 ng/ml)[27]. In addition, our data showed that women below the 25th percentile of baseline serum and red blood cell folate were the ones who benefited the most from the intervention.

Also, our study does not support the association of obesity with low serum and red blood cell folate levels[28]. The percentage increase in blood levels attained in both intervention groups was similar among all BMI groups. This is noteworthy, as obese women have been shown to have lower folate levels[29,30].

The present study was a double-blind, randomized clinical trial with a high degree of regimen adherence and supervision. Our method of determining serum and red blood cell folate levels was carried out by the same laboratory that assessed folate levels for NHANES, ensuring comparability of the methods with other populations. The drop-out rates among supplementation groups ranging from 18 % to 30 % could have introduced some bias, but we believe it was minimal because the baseline characteristics of drop-outs and participants were similar in all aspects. Furthermore, in the majority of cases the cause for dropping out was not linked to the trial per se. Therefore, folate levels may not have been overestimated for participating women.

Important limitations of the present study were: (i) the two groups had different time lags between last pill taken and blood draw, which could have biased the group receiving the daily dosage to observed higher levels in serum and red blood cell folate; (ii) we were not able to observe the long-term effect of the two dosage regimens as the intervention and measurement of folic acid levels spanned only 12 weeks; and (iii) we did not assess the unmetabolized folic acid in serum[31,32] nor the dietary

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intake of the participants. Recent research has demonstrated that, with higher dosages of folic acid, one can expect amounts of unmetabolized folic acid to be found in blood. Our study was not designed to evaluate unmetabolized folic acid.

The results of the present study are a first step helping the Honduran government to revise the current folic acid supplementation policy to provide women of childbearing age with enough folic acid to ensure adequate levels of folate before and during pregnancy. In a country where the underemployment rate is 29·6% and 69·5% of the population lives in poverty[33], lack of access to processed foods prevents the impact that food fortification has had in other countries[5,33–36]. For these reasons Honduras would benefit more from the implementation of a cost-effective preconceptional folic acid supplementation programme that would reach the poor urban and rural high-risk populations. Recently, the regional office of the WHO in South Asia (Philippines, Cambodia and Vietnam) published results on community trials assessing the effectiveness of micronutrient supplementation to the population with a pill containing both Fe and folic acid[37–42]. The strategy tailored the supplementation intervention and market strategy to each country's culture and values. The adherence to weekly and daily pill use was high; however, while changes were observed in Fe measurements, changes in folate levels were not provided. These studies suggest that before embracing country-wide supplementation programmes it is important to evaluate the marketing strategies, the type of supplementation programmes and the cost-effectiveness of the interventions.

In our study, a daily dose of 1 mg folic acid quickly elevated blood folate to levels that are believed to be protective against NTD[15]. While the blood folate levels of women consuming 5 mg weekly did attain levels in keeping with the Healthy People 2010 for red blood cell folate, the protective effect of folic acid in the prevention of NTD has been demonstrated previously. Therefore, if pre- and periconceptional folic acid use nears 100% and reaches high blood folate levels, we could expect a substantial decrease in the number of NTD cases. Establishing an NTD surveillance system coupled with periodic monitoring of blood folate could assess the impact from supplementation. Therefore, it is important that all women are made aware of the importance of periconceptional folic acid and that all women of reproductive age are taking it daily. Fortification of flour in Honduras has been in place since 2003. However, despite being an effective way of increasing folate intake, the penetration of this fortified food among high-risk populations remains very low because people do not have access to fortified products. Therefore, any national health policy strategy will require a combination of approaches including periconceptional supplementation with folic acid and fortification of staples, coupled with the promotion of vitamin use and monitoring of food fortification and levels of serum and red blood cell folate in the target population, and the establishment of a surveillance system to monitor the impact of these interventions on the prevalence of NTD.

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