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Serum homocysteine and folate concentrations among a US cohort of adolescents before and after folic acid fortification

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Abstract

Objective: We assessed serum homocysteine (tHcy) and folate concentrations among US adolescents before and after fortification of cereal-grain products with folic acid, and associations with demographic, behavioural and physiological factors.

Design: Observational study conducted among participants of a randomized trial.

Setting: The Child and Adolescent Trial for Cardiovascular Health (CATCH) study.

Subjects: Adolescents (n 2445) in grades 8 (pre-fortification, mean age 14 years) and 12 (post-fortification, mean age 18 years).

Results: Average serum concentrations of tHcy, folate and vitamin B_6 increased by 17 %, 16 % and 14 %, respectively, while serum concentrations of vitamin B_{12} decreased by 11 % post-fortification. Folic acid fortification provided, on average, an additional intake of 118 μg folate/d. Male sex ($P<0{\cdot}0001$) and white race ($P=0{\cdot}0008$) were associated with significantly greater increases in tHcy concentration, while increases in BMI ($P=0{\cdot}006$) and serum folate concentration ($P<0{\cdot}0001$) were associated with significant decreases in tHcy concentration. Female sex ($P<0{\cdot}0001$), non-smoking ($P<0{\cdot}0001$), use of multivitamins ($P<0{\cdot}0001$) and higher dietary intake of folate ($P=0{\cdot}001$) were associated with significantly greater increases in serum folate concentrations. From grade 8 to grade 12, the upward age trend in serum tHcy concentration was uninterrupted in its course ($P>0{\cdot}50$); whereas serum folic acid concentration showed a downward trend that incurred a discrete jump upward (17 % higher; $P<0{\cdot}0001$) with fortification. These trends differed significantly for males *v.* females ($P<0{\cdot}001$ for interaction).

Conclusions: Fortification had a significant impact on improving folate status but not serum tHcy concentrations among US adolescents.

Keywords
Homocysteine
Folate
Adolescents

Cardiovascular risk factors in childhood and adolescence are related to disease risk in adults[1,2]. Thus, a better understanding of risk factor(s) relationships and disease pathogenesis in youth may aid in prevention and early treatment[1,2]. Observational studies and some randomized trials suggest that homocysteine (tHcy) is a continuous, independent and modifiable risk factor of CVD, especially stroke[3–7]. Elevation of tHcy as a result of dietary, genetic, metabolic and hormonal factors[8] may result in vascular wall damage[9,10], smooth muscle and connective tissue proliferation[9,10], procoagulant activity[9,10], inflammatory response[11] and oxidative stress[12].

An intermediate in methionine metabolism, tHcy is metabolized via a vitamin B_6-dependent conversion to cysteine or a folate- and vitamin B_{12}-dependent remethylation to methionine[13]. Serum folate, vitamin B_6 and

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vitamin B_{12} concentrations are inversely associated with serum tHcy concentrations[14,15], and dietary supplementation of these vitamins reduces tHcy concentrations[14,16]. In particular, folate appears to be the most important dietary determinant of tHcy concentration[17,18] and a high intake of dietary folate/folic acid has been associated with lower concentrations of tHcy in adults, independent of other dietary or lifestyle factors[19–22]. Furthermore, high blood concentration of folate or intake of folate/folic acid has also been associated with a reduced risk of CVD and stroke in some studies[23].

The US Food and Drug Administration mandated that cereal-grain products be fortified with 140 μg folic acid/100 g grain by 1 January 1998[24], providing a unique opportunity to examine the effect of folic acid fortification on tHcy concentrations in the general population. Numerous studies in adults have demonstrated that folic acid fortification resulted in increased folate intake, significantly improved serum folate concentrations and decreased circulating tHcy concentrations[14,16,25]. In contrast, few studies have investigated the effects of folic acid fortification of food in children and adolescents[26]. To date, most studies examining serum tHcy and folate concentrations in children and adolescents have been cross-sectional, based on data collected pre-fortification[27] or post-fortification[28], and compared to reference values. Among an ethnically diverse cohort of youth from the Child and Adolescent Trial for Cardiovascular Health (CATCH)[29,30], we therefore examined changes in serum tHcy and folate concentrations pre- and post-folic acid fortification and associations with demographic, behavioural and physiological factors.

Methods

Overview of the study

The subjects for the present analysis were part of CATCH, a trial designed to evaluate the effectiveness of a multicomponent school-based cardiovascular health promotion intervention, described elsewhere in detail[29,30]. The main trial (1991–1994) was conducted among students in grades 3 to 5 attending ninety-six public elementary schools (fifty-six intervention and forty control schools) in California, Louisiana, Minnesota and Texas. Following the main trial, two observational studies were conducted that measured physiological and behavioural risk factors in study participants from grades 6 to 8 (1994–1997; pre-fortification) and in grade 12 (2000–2001; post-fortification)[30]. The current report is based on analysis of data collected during the grade 8 and grade 12 follow-up assessments.

Study population

The CATCH study initially recruited 5106 grade 3 students to participate in a risk factor screening at baseline. Of these, 3645 (71 %) participated in risk factor screening at the end of grade 8 when serum tHcy, folate, vitamin B_6 and vitamin B_{12} were first assessed and 2909 (57 %) students participated in the follow-up risk factor screening in grade 12. Of these students, 2445 attended both grade 8 and grade 12 examinations and provided blood at both examinations. This group constituted the study population for the present report. Students were recruited through schools or direct mailing to their homes. Parental consent was obtained for those under 18 years of age. Written informed consent was obtained from all participants and their parents, respectively. Institutional Review Boards at all four study sites and the coordinating centre at the New England Research Institutes approved the study.

Data collection

To ensure comparability, data collection methods[29,30] were similar across all time points and during the initial trial. Information on family history of risk factors for CVD and smoking was collected by student completed questionnaires. Dietary history including information on dietary folate intake and dietary supplement use was assessed at grade 12 only using a 149-item self-administered FFQ, the Youth/Adolescent Questionnaire (YAQ)[31], validated for use with children and adolescents aged 9 to 18 years. Examinations were conducted primarily in schools and to a lesser extent in other central community settings and participants' homes (in mobile vans). These examinations were conducted by trained and certified CATCH study staff at the sites. BMI was calculated as weight in kilograms divided by the square of height in metres.

Non-fasting blood samples were collected from each student enrolled in the study as reported elsewhere[30]. Briefly, blood was collected via venepuncture in red-top serum separator tubes and allowed to clot for exactly 20 min at room temperature, then placed directly on crushed ice until centrifugation. All samples were centrifuged 3–4 h later at the study centres and serum was placed in 5 cm^3 labelled Nalgene Cryule Vials (Nalgene Co., Rochester, NY, USA) and refrigerated until shipment for processing at the Central Laboratory at Miriam Hospital (Providence, RI, USA). Total serum tHcy was measured using the fluorimeteric method of Vester and Rasmussen, except that 20 % methanol by vol. was used in buffer B in the HPLC procedure[32]. Serum folate and vitamin B_{12} were measured using a solid-phase, no-boil RIA in a commercial kit (Diagnostic Productions Corp., Los Angeles, CA, USA)[33,34]. Serum vitamin B_6 was measured using a radioassay kit (ALPCO, Windham, NH, USA) that measures the conversion of titrated tyrosine to tyramine by the vitamin B_6-dependent enzyme tyrosine decarboxylase[35]. The same laboratory methods and assays were used at both time points.

Statistical analysis

Demographic characteristics and physiological measures in the study sample were summarized pre-fortification

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(grade 8) and post-fortification (grade 12) by percentages, means and standard deviations as appropriate.

Serum tHcy and folate concentration changes between grade 8 and grade 12 were compared according to sex, race, smoking status and multivitamin use. These comparisons were made first without adjustment or statistical testing (means and their standard errors tabulated at grade 8 and grade 12), then by mixed-effects regression analyses. The regression models used change from grade 8 to grade 12 concentrations as the dependent variable. For serum tHcy, each model was adjusted for design factors (CATCH site, school, intervention group); for covariates (sex, race, age at grade 8, smoking status and multivitamin supplement use at grade 12); for changes (between grade 8 and grade 12) in cardiovascular risk factors (BMI and systolic blood pressure); and for changes (between grade 8 and grade 12) in serum folate, vitamin B_6 and vitamin B_{12}. For serum folate, each model was adjusted for design factors (CATCH site, school, intervention group); for covariates (sex, race, age at grade 8, smoking status and multivitamin supplement use at grade 12); for changes (between grade 8 and grade 12) in BMI; and for grade 12 intakes of dietary folate. These analyses were also conducted on a subset of the cohort that excluded multivitamin and folic acid supplement users. We obtained comparable results using log-transformed and untransformed values and therefore report the latter.

We modelled the inverse relationship between serum tHcy and folate concentrations as a two-segment linear relationship[30], with both variables log-transformed to reduce the influence of extreme values. We used non-linear regression analysis to determine the segment slopes and their junction point separately at grade 8 and grade 12 measurements. Neither segment showed a significant change in slope between the two measurements ($P > 0{\cdot}05$). The change in the folate–tHcy relationship was therefore modelled as a simple translation of the junction point. Age trends in tHcy and folate concentrations were estimated by performing the linear regression of log concentrations *v.* age. Neither trend showed a significant change in slope between the two measurements ($P > 0{\cdot}05$). The grade 8 and grade 12 trends were therefore modelled as parallel, with separate intercepts allowing us to detect any discrete increase or decrease in tHcy and folate concentrations following fortification. These analyses were restricted to non-users of multivitamin and folic acid supplements and were stratified by sex.

All tests were conducted with type I error set at 0·05 for each pairing of dependent and independent variables. The SAS statistical software package version 9·1 was used for all computations.

Results

The mean age of participants was 14·1 years (range: 10·9 to 16·8 years) at grade 8 (pre-fortification) and 18·2 years (range: 15·2 to 20·7 years) at grade 12 (post-fortification) assessments (Table 1). Males and females were equally represented at both time points. Respondents were more likely to be female or of white race compared with non-respondents (50·5 % female *v.* 46·1 % female, $P = 0{\cdot}0015$ or 74·4 % white *v.* 64·2 % white, $P < 0{\cdot}001$, respectively). However, there was no significant difference in the distribution of respondents' and non-respondents' original school intervention condition. The proportion of respondents who reported smoking cigarettes increased from 12 % to 27 % from grade 8 to grade 12, and the proportion who reported taking a multivitamin remained the same at approximately 23 % of the sample. Average serum concentrations of tHcy, folate and vitamin B_6 increased (by 17 %, 16 % and 14 %, respectively) while serum concentrations of vitamin B_{12} decreased (by 11 %). When calculating folate intakes of students at grade 12, using the nutrient database values with and without fortification, fortification with folic acid provided, on average, an additional 118 μg folate/d, for a total mean folate intake of 359 μg/d. We observed similar findings among the sub-cohort that was restricted to non-users of multivitamins or folic acid supplements.

Average serum tHcy concentrations increased from 5·28 μmol/l in grade 8 to 6·19 μmol/l in grade 12 (Table 2). After adjusting for demographic, physiological and behavioural variables, and changes in serum folate, vitamin B_6 and vitamin B_{12}, the increase in serum tHcy was significantly greater among males compared with females (1·24 μmol/l *v.* 0·59 μmol/l, respectively; $P < 0{\cdot}0001$). Similarly, the increase in serum tHcy was significantly greater among whites compared with blacks (1·01 μmol/l *v.* 0·43 μmol/l, respectively; $P = 0{\cdot}0008$). Change in BMI showed a small but significant inverse relationship with change in tHcy concentration (0·05 μmol/l decrease in tHcy per 1 kg/m^2 increase in BMI, $P = 0{\cdot}006$). Change in serum folate concentration was strongly and inversely associated with change in serum tHcy concentration (an average decrease of 0·72 μmol/l in tHcy for every 50 nmol/l increase in serum folate, $P < 0{\cdot}0001$). Changes in systolic blood pressure, serum vitamin B_{12} and B_6 were not significantly associated with change in serum tHcy concentrations. These results were not substantially different when analyses were restricted to those who did not use multivitamins or folic acid supplements.

Serum folate concentrations increased from 42·6 nmol/l in grade 8 to 49·3 nmol/l in grade 12 (Table 3). In analyses simultaneously adjusted for age, sex, race, current smoking, current multivitamin use and change in BMI, females, current non-smokers and multivitamin users had significantly greater increases in serum folate concentration than males, smokers and multivitamin non-users, respectively (10·8 nmol/l in males *v.* 2·5 nmol/l in females, $P < 0{\cdot}0001$; 8·0 nmol/l in non-smokers *v.* 3·1 nmol/l in smokers, $P < 0{\cdot}0001$; 13·2 nmol/l in multivitamin users *v.* 4·8 nmol/l in non-users, $P < 0{\cdot}001$). Dietary folate intake

Table 1 Characteristics of the CATCH (Child and Adolescent Trial for Cardiovascular Health) cohort; 2445 students examined at both grade 8 (1996–1997) and grade 12 (2000–2001)

	Grade 8		Grade 12	
	n	%	*n*	%
Sex				
Male	1210	49·5	–	–
Female	1235	50·5	–	–
Race/ethnicity				
White	1820	74·4	–	–
Black	286	11·7	–	–
Hispanic	250	10·2	–	–
Others	89	3·6	–	–
Current smoker	294	12·1	669	27·4
Multivitamin use	524	22·9	550	22·5
Folic acid supplement use	–†		118	4·8
	Mean	SD	Mean	SD
Age (years)	14·1	0·5	18·2	0·5
BMI (kg/m^2)	21·9	4·5	24·4	5·2
Systolic blood pressure (mmHg)	114	8	116	10
Diastolic blood pressure (mmHg)	56	7	57	8
Serum concentration, all				
Homocysteine (μmol/l)	5·28	1·86	6·19	2·39
Folate (nmol/l)	42·6	21·5	49·3	25·7
Vitamin B_6 (nmol/l)	48·5	49·8	55·1	63·6
Vitamin B_{12} (pmol/l)	398	168	356	153
Serum concentration, supplement non-users*				
Homocysteine (μmol/l)	5·34	1·89	6·28	2·45
Folate (nmol/l)	39·8	17·7	45·2	21·8
Vitamin B_6 (nmol/l)	43·1	32·9	47·2	47·3
Vitamin B_{12} (pmol/l)	387	154	349	151
Dietary intake, all				
Folate (μg/d; with fortification)	–†	–	359	165
Folate (μg/d; without fortification)	–	–	241	125
Vitamin B_6 (mg/d)	–	–	1·50	0·68
Vitamin B_{12} (μg/d)	–	–	6·15	4·66
Dietary intake, supplement non-users*				
Folate (μg/d; with fortification)	–†	–	348	161
Folate (μg/d; without fortification)	–	–	232	121
Vitamin B_6 (mg/d)	–	–	1·47	0·67
Vitamin B_{12} (μg/d)	–	–	6·20	4·76

*Non-users of multivitamins or folic acid supplements, *n* 1530.
†Not determined at grade 8.

was strongly and positively associated with change in serum folic acid concentration (1·01 nmol/l increase in serum folic acid concentration for every 100 μg/d increase in folate intake, $P = 0{\cdot}001$). The differences in demographic and behavioural factors were similar and remained significant after adjusting for total folate intake (which included folic acid from fortification) in grade 12. These results were not substantially different when analyses were restricted to those who did not use multivitamins or folic acid supplements.

The inverse relationship of serum tHcy with serum folate in students not taking multivitamins or folic acid supplements is illustrated in Fig. 1, as modelled by a segmented log–log relationship. In grade 8, serum tHcy concentrations levelled off above a junction point located at a serum folate concentration of 37·8 nmol/l. In grade 12, the junction point shifted rightward, to a higher serum folate concentration of 51·9 nmol/l (37% higher, $P < 0{\cdot}0001$) and upward by a serum tHcy concentration of 0·4 μmol/l (8% higher, $P < 0{\cdot}003$). The upward age trend in serum tHcy (Fig. 2) was uninterrupted in its course between the two measurement periods ($P > 0{\cdot}50$). In contrast, folate concentrations showed a downward trend but incurred a discrete jump upward (17% higher, $P < 0{\cdot}0001$) between grade 8 and 12. Sex-stratified analyses indicated more pronounced trends in males than in females ($P < 0{\cdot}001$ for interaction) for both tHcy concentration (+3·8%/year in males, $P = 0{\cdot}006$ *v.* +1·4%/year in females, $P = 0{\cdot}19$) and folic acid concentration (−7·1%/year in males, $P = 0{\cdot}0001$ *v.* −3·4%/year in females, $P = 0{\cdot}008$).

Discussion

In the present study we found that folic acid fortification was associated with 16% higher mean serum folate concentrations. It significantly prevented the age-related downward trend in serum folate concentrations among

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Table 2 Serum homocysteine concentration in the CATCH (Child and Adolescent Trial for Cardiovascular Health) cohort before and after folate fortification

	Serum homocysteine (μmol/l)								
	Grade 8		Grade 12		Change from grade 8 to grade 12*				
Predictor	Mean†	SE	Mean†	SE	Mean	SE	Subgroup difference	95 % CI	*P*
All	5·28	0·04	6·19	0·05	0·91	0·06			
Sex									
Female	5·12	0·05	5·60	0·06	0·59	0·08	–‡		
Male	5·46	0·05	6·79	0·08	1·24	0·08	0·65	0·43, 0·86	<0·0001
Race/ethnicity									
White	5·20	0·04	6·22	0·06	1·01	0·07	–		
Black	5·96	0·14	6·38	0·13	0·43	0·16	–0·58	−0·92, −0·24	0·0008
Hispanic	5·03	0·10	5·80	0·15	0·86	0·17	−0·15	−0·51, 0·21	0·42
Smoking									
Non-smoker	5·27	0·04	6·09	0·05	0·88	0·07	–		
Smoker	5·31	0·08	6·41	0·10	0·99	0·10	0·11	−0·11, 0·33	0·33
Multivitamin use									
Non-user	5·30	0·04	6·26	0·06	0·94	0·06	–		
Users	5·23	0·08	5·88	0·09	0·81	0·11	−0·13	−0·37, 0·10	0·27
Per indicated change in predictor									
BMI, 1 kg/m^2					−0·05	0·02			0·006
Systolic blood pressure, 5 mmHg					0·04	0·03			0·20
Serum folate, 50 nmol/l					−0·72	0·11			<0·0001
Serum vitamin B_6, 50 nmol/l					0·03	0·04			0·48
Serum vitamin B_{12}, 1000 pmol/l					−0·15	0·35			0·67

*From mixed-effects regression analysis, adjusting for age, sex, race, CATCH site, intervention group, random variation among schools, multivitamin supplement use and smoking in grade 12, and changes between grade 8 and 12 in BMI, systolic blood pressure and serum concentrations of folate, vitamin B_6 and vitamin B_{12}. *P* tests for non-zero difference in mean serum homocysteine change between indicated subgroup and reference subgroup, or for non-zero change in serum homocysteine per indicated change in predictor.
†Unadjusted.
‡Reference group.

Table 3 Serum folate concentration in the CATCH (Child and Adolescent Trial for Cardiovascular Health) cohort before and after folate fortification

	Serum folate (nmol/l)								
	Grade 8		Grade 12		Change from grade 8 to grade 12*				
Predictor	Mean†	SE	Mean†	SE	Mean	SE	Difference	95 % CI	*P*
All	42·6	0·4	49·3	0·5	6·7	0·6			
Sex									
Female	39·7	0·6	50·7	0·8	10·8	0·8	–‡		
Male	45·6	0·6	47·9	0·7	2·5	0·8	−8·4	−10·4, −6·3	<0·0001
Race/ethnicity									
White	45·0	0·5	51·4	0·6	6·7	0·7	–		
Black	30·7	0·7	38·9	1·2	7·2	1·7	0·5	−3·1, 4·0	0·79
Hispanic	39·4	1·0	45·7	1·1	4·6	1·8	−2·1	−5·8, 1·6	0·27
Smoking									
Non-smoker	43·0	0·5	51·3	0·6	8·0	0·7	–		
Smoker	41·6	0·8	44·2	0·8	3·1	1·0	−5·0	−7·3, −2·7	<0·0001
Multivitamin use									
Non-user	41·2	0·5	45·7	0·5	4·8	0·6	–		
Users	47·5	1·1	62·0	1·3	13·2	1·1	8·4	6·0, 10·9	<0·0001
Per indicated increment in predictor									
Change in BMI, grade 8 to grade 12, 1 kg/m^2					−0·17	0·19			0·38
Dietary folate intake, grade 12, 100 μg/d					1·01	0·32			0·001

*From mixed-effects regression analysis, adjusting for age, sex, race, CATCH site, intervention group, random variation among schools, multivitamin supplement use and smoking in grade 12, dietary folate intake at grade 12 and change between grade 8 and 12 in BMI. *P* tests for non-zero difference in mean serum folate change between indicated subgroup and reference subgroup, or for non-zero change in serum folate per indicated increment in predictor.
†Unadjusted.
‡Reference group.

US adolescents and to a greater extent for females compared with males. Folate intakes at grade 12 were 118 μg/d higher from fortified foods. However, despite the increase in serum folate concentrations, tHcy concentrations increased by 17 % and the age-related increase in serum tHcy concentration reported in other studies[26]

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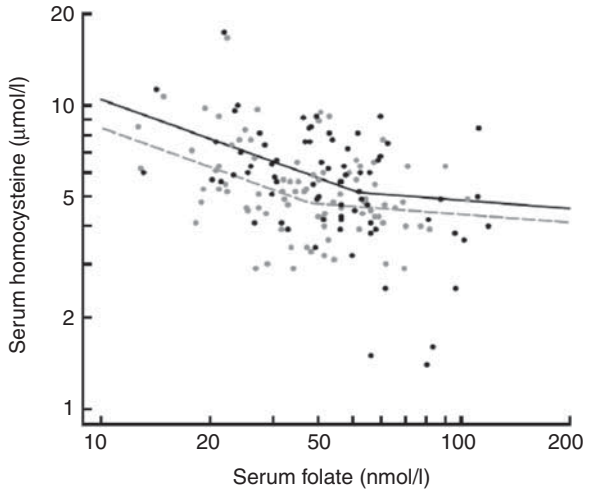

Fig. 1 Inverse relationship between serum homocysteine and serum folate concentrations in the CATCH (Child and Adolescent Trial for Cardiovascular Health) cohort before and after national folic acid fortification. From grade 8 (grey, pre-fortification) to grade 12 (black, post-fortification) the breakpoint of the fitted segmented regression curve shifted rightward, indicating higher folate levels, while the entire curve shifted upward, indicating higher homocysteine levels for a given folate level. Segmented regression curves were fitted to 3152 available joint measurements of homocysteine and folate concentrations, excluding users of multivitamins or supplements. Symbols represent a 5 % random sample of the fitted data

was not affected. The increase in tHcy was more marked for males than females. The inverse relationship between serum folate and tHcy concentrations demonstrated a plateau that levelled off at higher serum folate concentrations post-fortification than pre-fortification (37·8 nmol/l *v.* 51·9 nmol/l, respectively). We also observed encouraging dietary intakes of folate and vitamins in our adolescent population. Mean intakes observed in our study (359 μg/d for folate, 1·5 mg/d for vitamin B_6 and about 6·1 μg/d for vitamin B_{12}) were comparable to the RDA for folate (400 μg/d), vitamin B_6 (1·3 mg/d) and vitamin B_{12} (2·4 μg/d) for adolescents[36].

Folic acid fortification was introduced by the US Food and Drug Administration in 1998 with the intention of reducing the occurrence of neural tube birth defects in women of childbearing age[37]. The level of folic acid fortification was set at 140 μg/100 g cereal-grain product and was estimated to increase the intake of folic acid by women of reproductive age by approximately 100 μg/d[38], similar to the 118 μg/d that we observed among US adolescents in the current study. The post-fortification folic acid intake in the present study (359·1 μg/d) is much higher in comparison with countries where folic acid fortification has not been mandated. For instance, in Germany, Spain, Sweden, Denmark and the Netherlands, adolescents aged 13–18 years have mean overall folate/folic acid intakes ranging between 203 and 295 μg/d for males and between 192 and 265 μg/d for females[39,40]. These values are similar to the mean folate intake we observed in the present study, pre-fortification (241·1 μg/d). The higher folate intake post-fortification we observed is also consistent with reports from Canada, where folic acid fortification has been mandated[41]. In European countries where folic acid fortification has not been mandated, serum folate concentrations in adolescents have been nearly one-third lower than the concentrations reported in our study[42].

Data from the National Health and Nutrition Examination Surveys have shown that folic acid fortification in the USA is associated with significantly lower plasma tHcy concentrations compared with concentrations before fortification. One study found that folic acid fortification resulted in, on average, a 1·0 μmol/l decrease in tHcy in US children[28]. Pfeiffer and colleagues[26] found a small significant decrease in tHcy concentration post-folic acid fortification (1991–1994 *v.* 1999–2000) among male adolescents but not females, and concentrations then showed small non-significant increases during the post-fortification time points (1999–2000 *v.* 2003–2004). It is possible that once serum folate reaches an optimal concentration, its effect on tHcy concentrations plateau and tHcy concentrations are subsequently determined by other factors such as increasing age. Our findings are comparable to the age-related increase found in the Bogalusa Heart Study[43] which showed an average increase in serum tHcy concentration of 15 % from 12–14 years to 15–17 years. In addition, recent evidence from healthy individuals supplemented with folic acid[44] and studies examining factors contributing to elevations in tHcy post-fortification[45] suggest that once folate status is optimal, vitamin B_{12} may become the main nutritional determinant of tHcy concentrations. In addition, increased tHcy has been shown to occur in children with low plasma vitamin B_{12}[42]. It is possible that our finding that tHcy concentrations increased post-fortification may in part be due to decreased vitamin B_{12}.

Folic acid supplementation and fortification have been shown to increase folate intake and serum folate concentrations while decreasing serum tHcy concentrations in different populations[14,46,47]. Among adults in the Framingham Offspring study, folate intake increased (approximately 190 μg/d) and serum tHcy concentrations decreased (approximately 50 %) post-fortification[46]. Dose–response relationships between serum tHcy and folate concentrations that level off at higher folate concentrations have also been demonstrated. Among children, plasma folate was negatively associated with plasma tHcy in a dose-dependent fashion only until folate concentrations reached 20 nmol/l[32]. A plateau was observed in our study for the relationship between folate and tHcy at both pre- and post-fortification. Post-fortification, this plateau appeared to be reached at higher concentrations of folate and tHcy in our study, which may be related to age trends of increasing tHcy.

Our study findings indicate differences in age-related trends of increase in serum tHcy and decrease in serum

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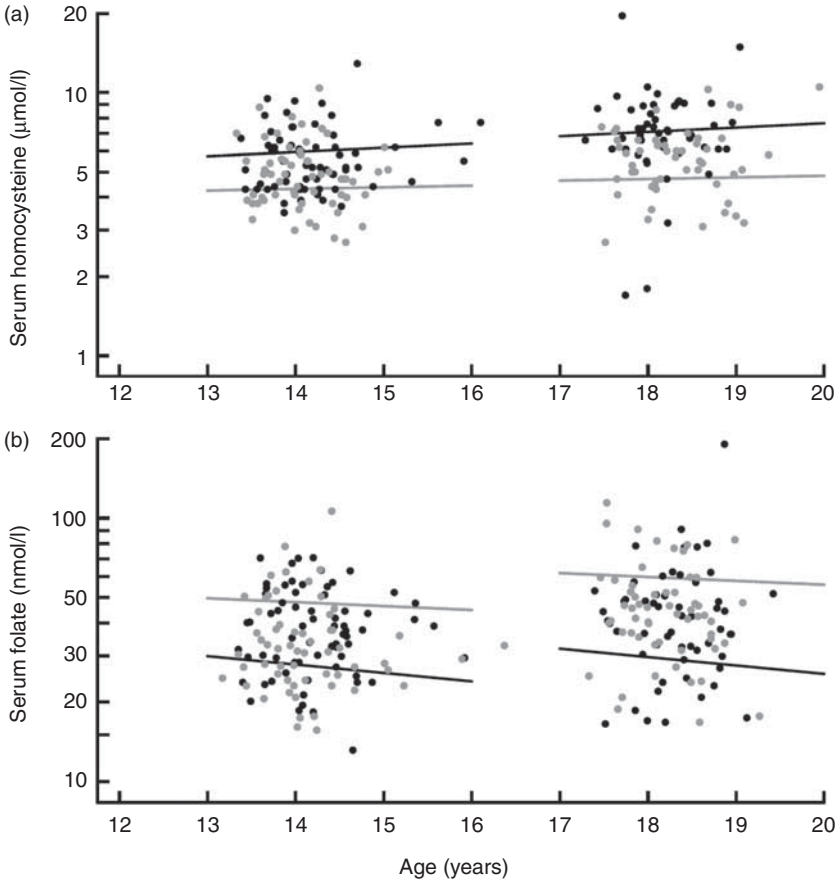

Fig. 2 Trends in serum homocysteine and serum folate concentration in the CATCH (Child and Adolescent Trial for Cardiovascular Health) cohort before and after national folate fortification. (a) For both males (black) and females (grey), the increase in homocysteine levels from grade 8 to grade 12 was a continuation of the within-grade age trend. (b) For both males (black) and females (grey), post-fortification folate levels (grade 12) were significantly higher than would be predicted by continuation of the within-grade age trend at grade 8 ($P < 0{\cdot}0001$ for discontinuity between grades). Regression lines were fitted to 4620 measurements for the combined grades, excluding users of multivitamins or supplements. Symbols represent a 5% random sample of the fitted data. For both homocysteine (a) and folate (b), the age trend was significantly steeper for males than females ($P < 0{\cdot}0001$ for age $\times$ sex interaction)

folate concentrations among males and females. Differences in remethylation rates[36], lean body mass[37] and hormone-related metabolism[38,39] may account for observed male/female differences in relationships between tHcy, folate and other vitamins. Inconsistencies[16,34,40] in reports of sex-related differences in the literature may be related to differences in vitamin nutritional status, ethnicity composition and other related risk factor differences between study populations.

Some limitations of our study deserve mention. Only 53% of the original cohort was included in the present study, which may lead to bias. Analyses suggested some significant demographic differences between respondents and non-respondents that may have resulted in inaccurate estimates of changes in tHcy and folate status for gender and race subgroups. Our blood samples were non-fasting. However, an earlier study[27] reported that fasting did not appear to affect tHcy concentrations in participants after adjusting for age and race. Single measurements of tHcy at particular time points do not accurately reflect tHcy variability over time. However, it has been previously demonstrated that an individual's tHcy concentration remains relatively constant over a 30-month period (reliability coefficients: 0·66–0·82)[27]. It is also important to note differences in folate equivalents between folic acid used for fortification and dietary folate, as well as limitations in food databases used to determine folate intake. Folic acid taken with food is 85% bioavailable while food folate is only 50% bioavailable; thus, folic acid taken with food has 1·7-fold (85/50) higher folate equivalents compared with dietary folate (polyglutamate)[48]. Current food databases, the majority of which were generated prior to current applications of trienzyme methods, underestimate actual folate content[49]. Further, incomplete food tables and seasonal variation in nutrient content or variation in preparation methods may either under- or overestimate actual folate intake[49]. However, investigators have reported that estimates of folate intake derived from FFQ are significantly correlated with folate nutritional status among adults[50]. Finally, our study lacks a control

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group parallel to the post-folic acid fortification group, making a direct inference not possible. However, our attempt to disentangle age and fortification effects was facilitated by the considerable variability in age among the study population at the two time points. The 2-year spread within grade, coupled with the large sample size, enabled us to establish the age trend precisely within grade and demonstrate with high statistical significance that the trend was interrupted after fortification for folate but not for tHcy.

Conclusion

In summary, we have shown that among US adolescents, fortification substantially improved serum folate concentrations and attenuated the age-related decrease in serum folate concentrations, with a greater impact on females than males. Furthermore, fortification resulted in dietary intakes of folate that approached or exceeded the RDA. Future studies are warranted to investigate the significance of these improvements in folate status on clinical outcomes, in the post-fortification era.

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