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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 pre- and post-folic acid fortification among US adolescents, and associations with demographic, behavioral, and physiologic 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=2,445) in grades 8 (pre-folic acid fortification, mean age 14 y) and 12 (post-folic acid fortification).

Results—Average serum concentrations of tHcy, folate and vitamin B6 increased by 17%, 16% and 14%, respectively, while serum concentrations of vitamin B12 decreased by 11% post-fortification. Folic acid fortification provided, on average, an additional 118µg/day of folate intake. Male sex and white race were associated with significantly greater increases in tHcy concentration, while increases in BMI and serum folate concentration were associated with significant decreases in tHcys concentration. Female sex, non-smoking, use of multivitamins, and higher dietary intake of folate 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-value>0.50); whereas, serum folic acid concentration showed a

downward trend that incurred a discrete jump upward (17% higher; p-value <0.0001) with fortification. These trends differed significantly for males vs. females (p<0.001 for interaction).

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

Keywords

homocysteine; folate; adolescents

Introduction

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 cardiovascular diseases (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 B6 dependent conversion to cysteine or a folate and vitamin B12 dependent remethylation to methionine⁽¹³⁾. Serum folate, vitamin B6 and vitamin B12 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 United States Food and Drug Administration (FDA) mandated that cereal-grain products be fortified with 140 µg folic acid/100g of grain by January 1, 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, behavioral and physiologic factors.

Methods

Overview of the study

The subjects for this analysis were part of CATCH, a trial designed to evaluate the effectiveness of a multi-component school-based cardiovascular health promotion

intervention, described elsewhere in detail^(29–30). The main trial (1991–1994) was conducted among students in grades 3–5 attending 96 public elementary schools (56 intervention and 40 control schools) in California, Louisiana, Minnesota, and Texas. Following the main trial, two observational studies were conducted that measured physiologic and behavioral risk factors in study participants from grades 6 to 8 (1994–1997) (pre-fortification) and in grade 12 (post-fortification) (2000–2001)⁽³⁰⁾. This 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 5,106 third grade 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 B6 and vitamin B12 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 this 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 center 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, food frequency questionnaire, the Youth/Adolescent Questionnaire (YAQ)⁽³¹⁾, validated for use with children and adolescents ages 9 to 18 years old. 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. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters².

Non-fasting blood samples were collected from each student enrolled in the study as reported elsewhere⁽³⁰⁾. Briefly, blood was collected via venipuncture in red top serum separator tubes and allowed to clot for exactly 20 minutes at room temperature then placed directly on crushed ice until centrifugation. All samples were centrifuged 3–4 hours later at the study centers and serum was placed in 5 cc labeled Nalgene Cryule Vials (Nalgene Co. Rochester, NY) and refrigerated until shipment for processing at the Central Laboratory at Miriam Hospital, RI. Total serum tHcy was measured using the fluorimetric method of Vester and Rasmussen, except that 20% methanol was used in buffer B in the HPLC procedure⁽³²⁾. Serum folate and Vitamin B12 were measured using a solid-phase, no-boil radioimmunoassay in a commercial kit (Diagnostic Productions Corp, Los Angeles, CA)^(33–34). Serum vitamin B6 was measured using a radioassay kit (ALPCO, Windham, NH) that measures the conversion of titrated tyrosine to tyramine by the vitamin B6-dependent enzyme tyrosine decarboxylase⁽³⁵⁾. The same laboratory methods and assays were used at both time points.

Statistical analysis

Demographic characteristics and physiologic measures in the study sample were summarized pre-fortification (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 (mean and standard error of the mean 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 (body mass index and systolic blood pressure); and, for changes (between grade 8 and grade 12) in serum folate, vitamin B6 and vitamin B12. 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 body mass index; 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 modeled the inverse relation 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 nonlinear 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 -value >0.05). The change in the folate-tHcy relationship was therefore modeled as a simple translation of the junction point. Age trends in tHcy and folate concentrations were estimated by linear regression of log concentrations on age. Neither trend showed a significant change in slope between the two measurements (p -value >0.05). The grade 8 and grade 12 trends were therefore modeled 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. SAS software (version 9.1, Cary, NC) was used for all computations.

Results

The mean age of participants was 14 years (range 10.9 to 16.8) at grade 8 (pre-fortification) and 18 years (range 15.2 to 20.7) 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 to non respondents (50.5% female vs. 46.1% female; $p=0.0015$ or 74.4% White vs 64.2% White; $p<0.001$, 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 B6 increased (17%, 16% and 14%, respectively) while serum concentrations of vitamin B12 decreased (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 /day of folate, for a total mean folate intake of 359 μg /day. We observed similar findings among the sub-cohort that is restricted to nonusers of multivitamins or folic acid supplements.

Average serum tHcy concentrations increased from 5.28 $\mu\text{mol/L}$ in grade 8 to 6.19 $\mu\text{mol/L}$ in grade 12 (Table 2). After adjusting for demographic, physiologic and behavioral variables, and changes in serum folate, vitamin B6, and vitamin B12, the increase in serum tHcy was significantly greater among males compared to females (1.24 $\mu\text{mol/L}$ vs. 0.59 $\mu\text{mol/L}$, respectively; p -value <0.0001). Similarly, the increase in serum tHcy was significantly greater among Whites compared to Blacks (1.01 $\mu\text{mol/L}$ vs. 0.43 $\mu\text{mol/L}$, respectively; p -value <0.0008). Change in BMI showed a small but significant inverse relationship with change in tHcys concentration (0.05 $\mu\text{mol/L}$ decrease in tHcys per 1kg/m^2 increase in BMI, p -value=0.006). Change in serum folate concentration was strongly and inversely associated with change in serum tHcy concentration (an average decrease of 0.72 $\mu\text{mol/L}$ decrease in tHcys for every 50 nmol/L increase in serum folate; p -value <0.0001). Changes in systolic blood pressure, serum vitamin B12 and B6 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 vs. 2.5 nmol/L in females, $p<0.0001$; 8.0 nmol/L in nonsmokers vs. 3.1 nmol/L in smokers, $p<0.0001$; and 13.2 nmol/L multivitamin users vs. 4.8 nmol/L in non-users, $p<0.001$). Dietary folate intake 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 $\mu\text{g/day}$ increase in folate intake; $p=0.001$). The differences in demographic and behavioral 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 relation of serum tHcy with serum folate in students not taking multivitamins or folic acid supplements is illustrated in Figure 1, as modeled by a segmented log-log relationship. In grade 8, serum tHcy concentrations leveled 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; $p<0.0001$) and upward by 0.4 $\mu\text{mol/L}$ of serum tHcy concentrations (8% higher, $p<0.003$). The upward age trend in serum tHcy (Figure 2) was uninterrupted in its course between the two measurement periods (p -value >0.50). In contrast, folate concentrations showed a downward trend but incurred a discrete jump upward (17% higher; p -value <0.0001) between grade 8 and 12. Sex-stratified analyses indicated more pronounced trends in males than in females ($p<0.001$ for interaction) for both tHcys concentration (+3.8%/year in males; $p=0.006$ vs. +1.4%/year in females; $p=0.19$) and folic acid concentration (−7.1%/year in males; $p=0.0001$ vs. −3.4%/year in females; $p=0.008$).

Discussion

In this 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 US adolescents and to a greater extent for females compared to males. Folate intakes at grade 12 were 118 $\mu\text{g/day}$ 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⁾ was not affected. The increase in tHcys was more marked for males than females.

The inverse relationship between serum folate and tHcy concentrations demonstrated a plateau that leveled off at higher serum folate concentrations post-fortification than pre-fortification (37.8 nmol/L vs. 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/day for folate, 1.5 mg/day for Vitamin B6 and about 6.1 µg/day for Vitamin B12) were comparable to the Recommended Dietary Allowance (RDA) for folate (400 µg/day), Vitamin B6 (1.3 mg/day) and Vitamin B12 (2.4 µg/day) for adolescents⁽³⁶⁾.

Folic acid fortification was introduced by the US Food and Drug Administration (FDA) 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 per 100g of cereal grain product and was estimated to increase the intake of folic acid by women of reproductive age by approximately 100 µg per day⁽³⁸⁾, similar to the 118µg/day that we observed among US adolescents in the current study. The post-fortification folic acid intake in the present study (359.1 µg/day) is much higher in comparison to countries where folic acid fortification has not been mandated. For instance, in Germany, Spain, Sweden, Denmark and the Netherlands, adolescents between 13 and 18 years have mean overall folate/folic acid intakes ranging between 203–295 µg/day for males and between 192–265 µg/day for females^(39–40). These values are similar to the mean folate intake we observed in the present study, pre-fortification (241.1 µg/day). 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 concentrations reported in our study⁽⁴²⁾.

Data from the National Health and Nutrition Examination Surveys have shown that folic acid fortification in the US is associated with significantly lower plasma tHcy concentrations when compared to 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 concentrations post folic acid fortification (1991–1994 versus 1999–2000) among male adolescents but not females, and, concentrations then showed small non significant increases during the post-fortification time points (1999–2000 versus 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 Bogalusa study⁽⁴³⁾ which showed a 15% average increase in serum tHcy concentrations from 12–14 years to 15–17 years. In addition, recent evidence from healthy subjects 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 B12 may become the main nutritional determinant of tHcy concentrations. In addition, increased tHcy has been shown to occur in children with low plasma vitamin B12⁽⁴²⁾. It is possible that our finding that tHcy concentrations increased post-fortification may in part be due to decreased vitamin B12.

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/day) 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 folate concentrations among males and females. Differences in re-methylation 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 this 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 concentrations remain 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 a 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 food frequency questionnaires were significantly correlated with folate nutritional status among adults⁽⁵⁰⁾. Finally, our study lacks a control 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.

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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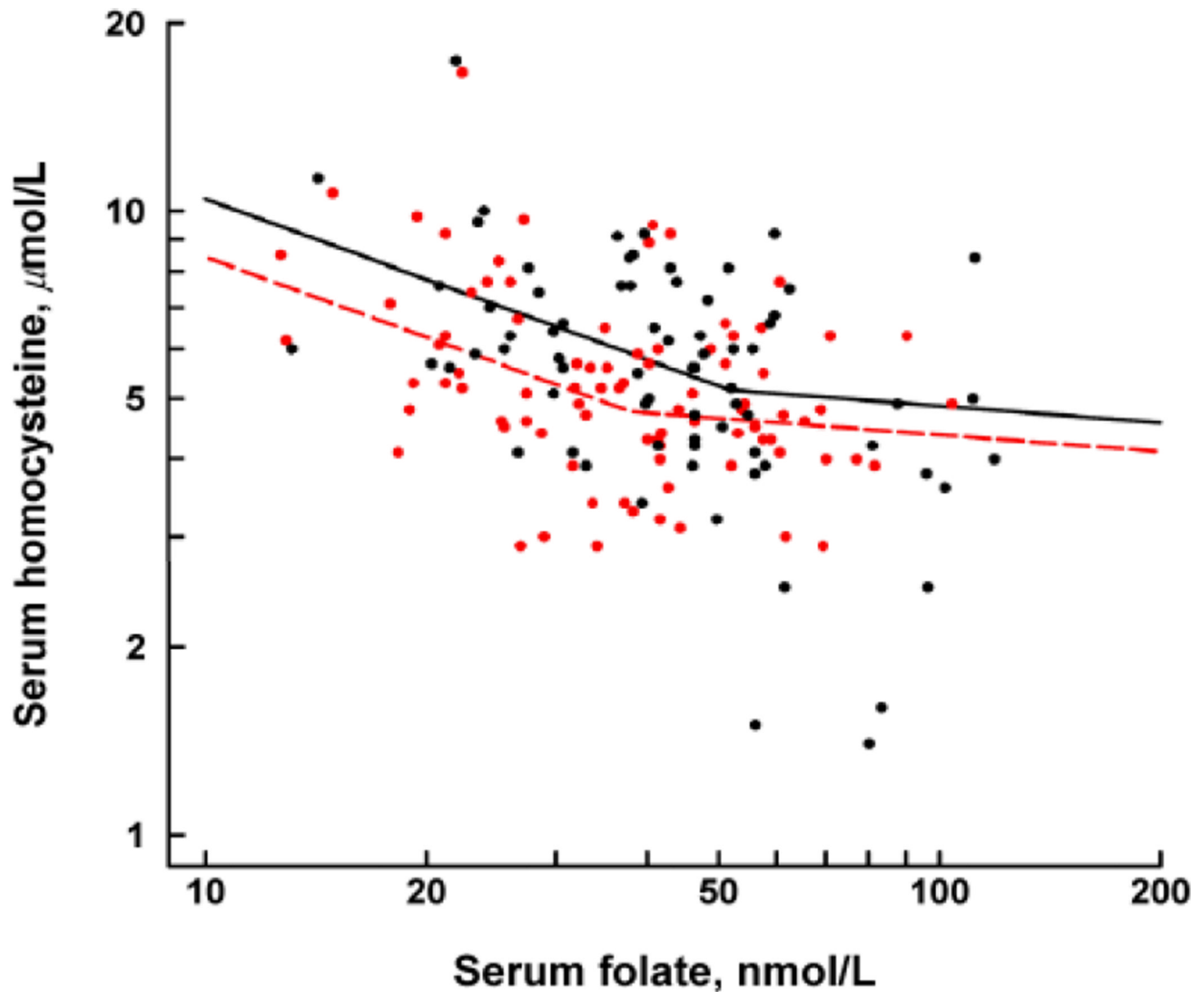


Figure 1.

Inverse relation between serum homocysteine (tHcy) and serum folate concentrations in the CATCH cohort before and after national folic acid fortification. From Grade 8 (red, 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 3,152 available joint measurements of homocysteine and folate concentrations, excluding users of multivitamins or supplements. Symbols represent a 5% random sample of the fitted data.

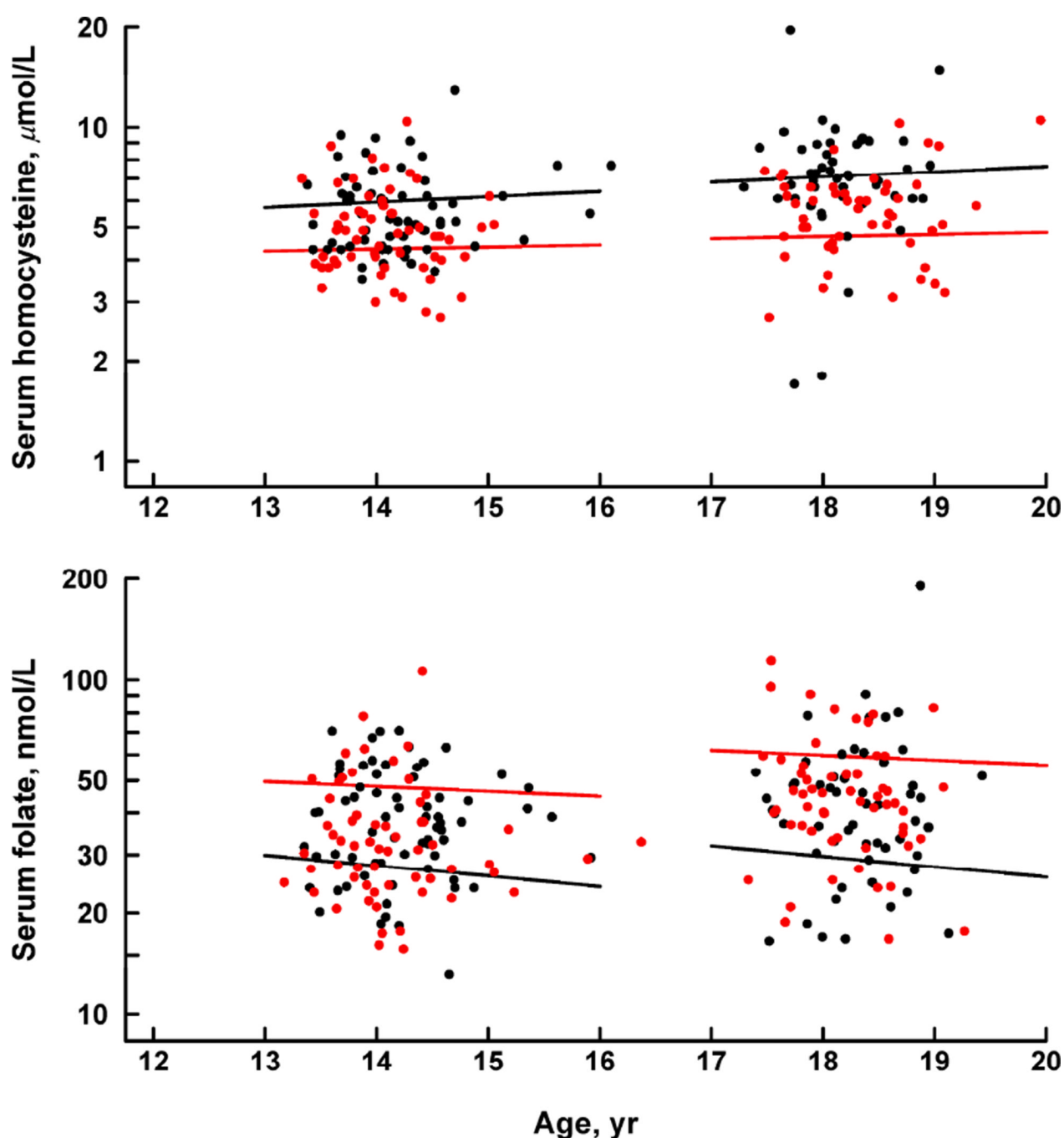


Figure 2.

Trends in serum homocysteine and serum folate concentration in the CATCH cohort, before and after national folate fortification. *Upper panel:* For both males (black) and females (red), the increase in homocysteine levels from Grade 8 to Grade 12 was a continuation of the within-grade age trend. *Lower panel:* For both males (black) and females (red), post-fortification folate levels (Grade 12) were significantly higher than would be predicted by continuation of the within-grade age trend at Grade 8 ($p < 0.0001$ 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 (upper panel) and folate (lower panel), the age trend was significantly steeper for males than females ($p < 0.0001$ for age $\times$ sex interaction).

Table 1

Characteristics of the CATCH cohort (Child and Adolescent Trial for Cardiovascular Health). N=2445 students examined at both grade 8 (1996–97) and grade 12 (2000–01).

	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	—	—
Hispanics	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
	<i>Mean</i>	<i>SD</i>	<i>Mean</i>	<i>SD</i>
Age, yr	14.1	0.5	18.2	0.5
Body mass index, 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, μ mol/L	5.28	1.86	6.19	2.39
Folate, nmol/L	42.6	21.5	49.3	25.7
Vitamin B6, nmol/L	48.5	49.8	55.1	63.6
Vitamin B12, pmol/L	398	168	356	153
Serum concentration, supplement non-users [†]				
Homocysteine, μ mol/L	5.34	1.89	6.28	2.45
Folate, nmol/L	39.8	17.7	45.2	21.8
Vitamin B6, nmol/L	43.1	32.9	47.2	47.3
Vitamin B12, pmol/L	387	154	349	151
Dietary intake, all				
Folate, μ g/day (with fortification)	—*	—	359	165
Folate, μ g/day (without fortification)	—	—	241	125
Vitamin B6, mg/day	—	—	1.50	0.68
Vitamin B12, μ g/day	—	—	6.15	4.66
Dietary intake, supplement non-users [†]				
Folate, μ g/day (with fortification)	—*	—	348	161
Folate, μ g/day (without fortification)	—	—	232	121
Vitamin B6, mg/day	—	—	1.47	0.67
Vitamin B12, μ g/day	—	—	6.20	4.76

* Not determined at Grade 8.

[†] Non-users of multivitamins or folic acid supplements, N=1530.

Table 2
Serum homocysteine concentration in the CATCH cohort before and after folate fortification.

Predictor	Serum homocysteine, $\mu\text{mol/L}$							
	Grade 8		Grade 12		Change from Grade 8 to Grade 12 [†]			
	Mean*	SE	Mean*	SE	Mean	SE	Subgroup Difference	95% CI
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
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
Hispanic	5.03	0.10	5.80	0.15	0.86	0.17	−0.15	−0.51, 0.21
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
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
Per indicated change in predictor:								
Body-mass index, 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 B6, 50 nmol/L					0.03	0.04		0.48
Serum vitamin B12, 1000 pmol/L					−0.15	0.35		0.67

* Unadjusted.

[†] 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 body mass index, systolic blood pressure, and serum concentrations of folate, vitamin B6, and vitamin B12. 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.

§ Reference group.

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Table 3

Serum folate concentration in the CATCH cohort before and after folate fortification.

Predictor	Serum folate, nmol/L							
	Grade 8		Grade 12		Change from Grade 8 to Grade 12 ^f			
	Mean*	SE	Mean*	SE	Mean	SE	Difference	95% CI
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
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
Hispanic	39.4	1.0	45.7	1.1	4.6	1.8	-2.1	-5.8, 1.6
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
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
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 folate, Grade 12, 100 µg					1.01	0.32		0.001

* Unadjusted.

^f 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 body mass index. 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.

[§] Reference group